Relationship between Gluten Strength and Free Asparagine Content of Canadian Wheat Varieties

by

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Abstract

The free amino acid asparagine is the main precursor to formation of acrylamide, a probable carcinogen, in bakery products. Therefore, it is critical to understand if reducing free asparagine in wheat will be detrimental to dough viscoelasticity, and consequently, to the quality of the bread made from the dough. Thus, this study aimed to investigate how factors affecting free asparagine levels in wheat, i.e., environment, genotype, and fertilization treatments, affected dough rheological properties. Because good gluten strength is necessary for good bread quality, this study sought to understand the relationship between wheat free asparagine concentration and gluten strength.

Eight commercial Canadian wheat varieties were grown at four site-years under four fertilization treatments, i.e., combinations of two levels of nitrogen and two levels of sulfur. Wheat grain samples were milled into whole-wheat and straight grade flour. Free asparagine concentration was quantified using whole-wheat flours and dough rheological properties were measured using straight grade flours. Empirical dough rheological properties were evaluated using the Brabender Farinograph and the Brabender Extensograph. Using a subset of 10 selected samples, shear rheometry and low-intensity ultrasound (LIU) tests were performed on yeasted and non-yeasted doughs.

From empirical tests, it was observed that environment, genotype and their interaction were the main factors affecting gluten strength, with little effect from fertilization. Additionally, gluten strength parameters were negatively correlated to wheat free asparagine concentration. When evaluated under linear shear oscillatory tests, non-yeasted dough rheology significantly changed over time, while prominent dough softening was observed during fermentation of yeasted doughs. When tested under creep-recovery and stress relaxation, a strong negative correlation was observed between wheat free asparagine concentration and gluten strength. Finally, LIU provided meaningful information on dough rheological properties, with similar outcomes compared to shear tests. LIU results showed bubble disproportionation happened to a greater extent in samples with higher free asparagine concentration and protein content, samples that were hypothesized to have higher gliadin-to-glutenin ratios.

In conclusion, selecting wheat varieties with low levels of free asparagine and applying commercial levels of nitrogen fertilization are good strategies to produce safe and high-quality wheat, with no detrimental effects on gluten strength.

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Dedication

Dedicated to my loving parents, Regina and Claudio, to my inspiring brother, Henrique, and to my husband and greatest supporter, Jelther.

Thesis structure and publication status

This thesis is a grouped manuscript thesis containing seven chapters, with chapters three to five being each written and formatted as a research manuscript.

Chapter 1 presents a general introduction for the work presented in this thesis.

Chapter 2 contains a thorough review of the literature, providing necessary information for the studies that were carried out throughout this thesis. A review paper comprising two thirds of this chapter and titled "Relationship between nitrogen functionality and wheat flour dough rheology: extensional and shear approaches" was submitted to Food Research International. **Authors:** Susane Trevisan, Ali Salimi Khorshidi, Martin G. Scanlon.

Chapter 3 is the manuscript titled "Relationship between free asparagine concentration and gluten strength in Canadian hard spring wheat" which is under preparation to be submitted to Cereal Chemistry's 2023 Focus Issue on Wheat Grain Quality for publication as two separate papers: "Asparagine and dough quality: Gluten strength factors in commercial hard red spring wheat" and "Asparagine and dough quality: Gluten strength relationships in hard red spring wheat". **Authors:** Susane Trevisan, Ali Salimi Khorshidi, Elaine Sopiwnyk, Yi Xie, Zhaoxian Zhou, James D. House, Martin G. Scanlon.

Chapter 4 is the manuscript titled "The relationship between wheat free asparagine concentration and dough rheological properties: A dynamic and static shear rheology approach".

Chapter 5 is the manuscript titled "Low intensity ultrasound: a novel method to study the relationship between dough rheological properties and free asparagine concentration".

Chapter 6 comprises a general discussion of the thesis.

Chapter 7 contains the final conclusion of the thesis and suggestions for future work.

Contribution of Authors

For the present thesis, the contribution of authors is described below:

For the review paper comprising two thirds of Chapter 2 and titled “Relationship between nitrogen functionality and wheat flour dough rheology: extensional and shear approaches”, Susane Trevisan conducted the literature review, the writing, and responded to required revisions from co-authors. Ali Salimi Khorshidi and Martin G. Scanlon conducted the editing of the paper.

For the remaining chapters 3, 4, and 5, Susane Trevisan was the exclusive author, responsible for conducting the experiments, writing, and responding to required revisions. Advisors James D. House and Martin G. Scanlon, as well as Ali Salimi Khorshidi who also was one of Susane Trevisan’s advisors during the majority of her master’s program, conducted the editing of the chapters. None of these chapters were submitted for publication at the stage of thesis submission, except that two different research papers were written and subsequently submitted to Cereal Chemistry’s 2023 Focus Issue on Wheat Grain Quality using data and analyses reported in Chapter 3.

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List of Abbreviations

Description	Abbreviation
Apparent extensional viscosity	AEV
Brabender Units	BU
Crop heat units	CHU
Dough development time	DDT
Dough stability	DS
Euclidian distance	D^2_{vu}
Extensibility	E
Extensibility at 135 min of resting	E_{135}
Extensibility at 45 min of resting	E_{45}
Extensograph Einheit	*EE*
Elongational stress	*ES*
Frequency	f
Farinograph optimum water absorption	*FAB*
Final relaxation modulus from fitting	G_∞
Initial relaxation modulus	G_0
Final relaxation modulus	G_e
Magnitude of the complex shear moduli at time t	G^*_t
Shear storage modulus	G'
Shear loss modulus	G''
Shear storage moduli for non-yeasted doughs	G'_{NY}
Shear storage modulus for yeasted doughs	G'_{SY}
Relaxation modulus	$G(t)$
Magnitude of the complex shear moduli at frequency f	G^*_f
Magnitude of the complex shear moduli for non-yeasted dough	G^*_{NY}
Magnitude of the complex shear modulus for yeasted dough	G^*_{SY}
Magnitude of the complex shear modulus	$G^*(\omega)$
Gliadins	Gli
Gliadin-to-glutenin ratio	Gli/Glu

Glutenins	Glu
Relaxation spectra at long time	H_{long}
Relaxation spectra at short time	H_{short}
Relaxation spectrum	$H(t)$
High molecular weight	HMW
i^{th} Maxwell element	i
Relative elastic part of compliance	J_{el}
Compliance	$J(t)$
Maximum creep compliance	J_{max}
Low-intensity ultrasound	LIU
Low molecular weight	LMW
Loaf volume	LV
Linear viscoelastic region	LVR
Magnitude of the complex longitudinal modulus	$M^*(\omega)$
Longitudinal storage modulus	M'
Longitudinal loss modulus	M''
Magnitude of complex longitudinal moduli at time t	M^*_t
Mixing tolerance index	MTI
Mixing time to peak	MTP
Power-law exponent	n
Power-law exponent for the shear storage modulus	$n(G')$
Power-law exponent for the magnitude of the complex shear modulus	$n(G^*)$
Power-law exponent for the shear loss tangent	$n(\tan \delta)$
Non-yeasted dough	NY
Maximum power at peak	$Power$
Maximum resistance to extension at 135 min of dough resting	R_{135}
Resistance-to-extension ratio at 135 min of dough resting	R_{135}/E_{135}
Maximum resistance to extension at 45 min of dough resting	R_{45}
Resistance-to-extensibility ratio at 45 min of dough resting	R_{45}/E_{45}
Maximum resistance to extension	R_{max}
Resistance-to-extensibility ratio	R_{max}/E

Gel strength	S
Slope of the shear storage modulus curve for yeasted dough	$S_{const.}\, G'_{SY}$
Slope of the magnitude of complex shear modulus curve for yeasted dough	$S_{const.}\, G^*_{SY}$
Slope of the shear storage modulus curve for non-yeasted dough	$S\, G'_{NY}$
Slope of the magnitude of complex shear modulus curve for non-yeasted dough	$S\, G^*_{NY}$
Slope of the magnitude of complex longitudinal modulus curve	$S\, M^*$
Slope of the shear loss tangent curve for yeasted dough	$S \tan \delta_{SY}$
Slope of the longitudinal loss tangent	$S \tan \delta_M$
Slope of the shear loss tangent for non-yeasted dough	$S \tan \delta_{NY}$
Slope of the phase velocity curve	$S\, v_p$
Slope of the phase velocity curve for yeasted dough	$S\, v_{p,SY}$
Slope of the attenuation coefficient curve for yeasted dough	$S\, \alpha_{SY}$
Sehn-Steel dimensionless number	*Sehn-Steel*
Specific loaf volume	*SLV*
Yeasted dough	SY
Time	t
Loss tangent	$\tan \delta$
Shear loss tangent at frequency f	$\tan \delta_f$
Longitudinal loss tangent	$\tan \delta_M$
Longitudinal loss tangent at time t	$\tan \delta_{M,t}$
Shear loss tangent for non-yeasted dough	$\tan \delta_{NY}$
Shear loss tangent for yeasted dough	$\tan \delta_{SY}$
Loss tangent at time t	$\tan \delta_t$
Shear loss tangent at frequency f	$\tan \delta_f$
Phase velocity for yeasted dough	$v_{p,SY}$
Phase velocity at time t	$v_{p,t}$
Phase velocity	v_p
Water absorption	*WA*
Attenuation coefficient	α
Final attenuation coefficient obtained from fitting	α_∞
Attenuation coefficient for the i^{th} Maxwell element	α_i

Attenuation coefficient for yeasted dough	α_{SY}
Attenuation coefficient at time *t*	α_t
Strain	γ
Constant shear strain	γ_0
Phase angle	δ
Ultrasonic wavelength	λ
Dough density	ρ
Stress	σ
Relaxation time	τ
Relaxation time for the i^{th} Maxwell element	$\tau_{\alpha,i}$
Relaxation time for yeasted doughs for the i^{th} Maxwell element	$\tau_{\alpha,SY,i}$
Relaxation time i^{th} Maxwell element	τ_i
Relaxation times from stress relaxation test	τ_{SR}
Angular frequency	ω
Hencky strain	ε_H

1 Introduction

Acrylamide is a chemical compound commonly used to improve the water solubility and cross-linking of polymers, resulting in polyacrylamide, which has been widely applied in various processes, e.g., wastewater treatment, textiles, and paints (IARC, 1994). However, in 2002, a Swedish research group published the first study reporting the presence of acrylamide in heated carbohydrate-rich products, such as potatoes and bread (Tareke et al., 2002). Since then, awareness regarding acrylamide content in food products has increased, leading to global efforts to gather information on acrylamide levels in foodstuffs and the development of regulations to control acrylamide in food (Raffan & Halford, 2019).

Acrylamide is classified as a probable carcinogen to humans (group 2A) (IARC, 1994). For this reason, the European Commission and the European Food Safety Authority (EFSA) have been working to decrease consumer exposure to dietary acrylamide (Raffan & Halford, 2019). Recent data collected by EFSA has shown that soft bread contains about 42 µg kg^{-1} of acrylamide (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015), but levels as high as 102 µg kg^{-1} have also been reported (Abt et al., 2019). Although the acrylamide levels in bread are not as high as in other food products, such as in potato fried products (308 µg kg^{-1}), concern is rather related to bread's high consumption in the average diet (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015). Thus, aiming to provide further guidance to the food industry, the European Commission set acrylamide benchmark levels for various food products, including bread (50 µg kg^{-1}) (European Commission, 2017). As a result, the baking industries and wheat growers must cooperate and implement strategies to mitigate acrylamide formation in grain-based food products.

To target low levels of acrylamide in food, one must first know how this compound is formed in the food matrix. Acrylamide is one of various compounds formed during the Maillard Reaction, which occurs between free amino acids and reducing sugars at temperatures higher than 120ºC (Tareke et al., 2002). Among the free amino acids naturally present in wheat, free asparagine has been shown to be the major precursor to acrylamide formation (Curtis et al., 2016; Mottram et al., 2002; Stadler et al., 2002). According to a review from Maan et al. (2020), various acrylamide mitigation strategies have been studied, such as changing the processing conditions, i.e., time-temperature profile, and introducing additives such as L-Asparaginase. However, these strategies are accompanied by changes in product appearance and flavor formation as well as by additional manufacturing expenditures (Capuano & Fogliano, 2011; Maan et al., 2020).

One effective way of mitigating acrylamide formation in bread is to reduce the free asparagine concentration naturally present in wheat (Friedman, 2003). Accordingly, the European Commission advised cereal producers to take measures that would reduce free asparagine levels in wheat (European Commission, 2014), an approach which was also recommended by the Acrylamide Toolbox 2019 (Food Drink Europe, 2019). Studies conducted worldwide have shown that three main factors significantly affect free asparagine in wheat: growth environment (Corol et al., 2016; Navrotskyi et al., 2018; Xie et al., 2021), wheat genotype (Curtis et al., 2009; Ohm et al., 2017), and agronomic practices, i.e., adequate nitrogen and sulfur fertilization treatments (Claus et al., 2006; Lea et al., 2007; Randall et al., 1981; Weber et al., 2008). All of these factors also affect protein content and composition in wheat. Therefore, it is extremely necessary in formulating advice on free asparagine reduction protocols to investigate and take into account the effect of these three factors on wheat protein content and composition.

Environmental conditions have been reported to affect wheat protein content and composition (Johansson et al., 2013). Some examples of environmental conditions that affect protein content and composition are drought (Uthayakumaran et al., 2012; Zhou et al., 2018), soil texture (Nyiraneza et al., 2012), and growth temperature (DuPont et al., 2006). Wheat genotype has also been shown to play an important role on protein content and composition (Mastilović et al., 2018; Panozzo & Eagles, 2000; X. Wang et al., 2018). Nitrogen is commonly applied in the field to increase wheat productivity (Ladha et al., 2016) and protein content (Abedi et al., 2011). Furthermore, sulfur-containing wheat proteins are positively affected when adequate sulfur is available in the soil (Steinfurth et al., 2011; Wieser et al., 2004). However, excess nitrogen application and insufficient sulfur supplementation has been shown to increase free asparagine concentration in wheat (Lea et al., 2007; Stockmann et al., 2018).

As a staple food, bread has its guaranteed place in the diet of humans. The worldwide market volume of bread and other bakery products was estimated to be about 91.4 million tonnes in 2017 with an expected increase to 135 million tonnes by 2025 (Wunsch, 2020). In 2021, the worldwide revenue for bread is expected to be about US$ 437 million, with an average consumption of 26.6 kg per person (Statista, 2022). In Canada, the average consumption of bread is 27.7 kg per person in 2021, slightly higher than the global average (Statista, 2021a). In other countries, e.g., the United Kingdom, bread is a key food product, with an estimated average consumption of 34.5 kg per person in 2021 (Statista, 2021b). In this same region, about 2.1 million

tonnes of bread were sold in 2019, with a volume of about 2.2 million tonnes expected by 2025 (Statista, 2021d).

Wheat flour is the principal component of bread. Wheat is one of the food crops of utmost importance in the world (Igrejas & Branlard, 2020) and one of the major sources of energy to humankind (D'Odorico et al., 2014). The forecast for world wheat production is 785.8 million tonnes for 2021, with Canada being the fifth country among the top ten wheat exporters (FAO, 2021). In addition, when compared to the 2018-20 period, there is expected to be an increase of 12% in the world's wheat consumption by 2030. Also, by 2030, the world's wheat exports are forecast to increase by up to 220 million tonnes, with Canada expected to contribute 13% of the global share in wheat trade, becoming the third largest wheat exporter (OECD/FAO, 2021). Among the destinations for Canadian wheat, Western Europe, including the United Kingdom, is a critical region that has received about 1.67 million tonnes of wheat between the years of 2019 and 2021 (Canadian Grain Commission, 2021). In the marketing of wheat, protein content is a critical parameter for wheat pricing (PDQ, 2020).

A singular adjective that can be attributed to wheat is "unique". The reason for the uniqueness of wheat lies in the wheat protein. The wheat grain can contain from 7 up to 20% of protein (Dobraszczyk, 2001). The wheat protein content dictates the final wheat application, with bread production requiring wheat with higher protein content (12-15%) than for production of pastries (< 11%) (Carson & Edwards, 2009). Among these proteins, about 85% are gluten proteins, i.e., gliadins and glutenins (Dobraszczyk et al., 2001; Veraverbeke & Delcour, 2002). The gluten proteins are responsible for the unparalleled viscoelastic characteristic of wheat flour dough. Optimal dough viscoelasticity is an essential attribute when targeting high quality and palatable bread production (Delcour & Hoseney, 2010c). Thus, mitigation of free asparagine accumulation in wheat is important, but any concomitant changes in protein content and composition associated with that mitigation are of concern for the baking industries.

Specifically for the breadmaking industry, the gluten proteins are of singular importance. The gliadins are known to confer extensibility and the glutenins confer elasticity (also reported as strength) to the wheat flour dough. Therefore, an adequate protein composition, i.e., gliadin-to-glutenin ratio, is essential to guarantee both sufficient dough expansion and gas-holding capacity during fermentation to produce bread with good volume and crumb appearance (Delcour & Hoseney, 2010c; Veraverbeke & Delcour, 2002). An inadequate protein composition will provide

wheat flour doughs with an unbalanced gluten strength, i.e., extensibility-to-elasticity characteristic (Uthayakumaran et al., 2000), which is detrimental to good bread production.

The investigation of how gluten strength changes, when targeting for low acrylamide forming potential in wheat, can be approached through rheological measurements. These rheological measurements are of enormous importance for the investigation of wheat flour dough physical properties (Belton, 2012). Rheological tests can be performed through two main categories of tests: empirical and fundamental (Dobraszczyk & Morgenstern, 2003). Examples of empirical tests, that are widely known in the baking industry, are the farinograph and the extensograph (T. D. Hadnadev et al., 2011). Fundamental tests, on the other hand, are more commonly known in the scientific community. These tests allow one to apply a wide range of stresses or strains, providing the researcher with dough properties measured in well-defined physical units. Examples of these tests are shear oscillatory, creep-recovery, and stress relaxation (Dobraszczyk & Morgenstern, 2003). Among fundamental tests, low-intensity ultrasound is a novel technique that has also been employed to evaluate dough rheological properties (Koksel et al., 2016; Scanlon & Page, 2015).

Although rheological measurements have been widely applied in the literature, there is a lack of their use to study the effect of free asparagine mitigation strategies on the gluten strength of Canadian wheat. To our knowledge, only Malunga et al. (2019) has briefly reported correlation values between free asparagine concentration and some dough rheological parameters. Thus, this thesis will be the first to provide an understanding of the relationship between gluten strength and free asparagine concentration using empirical and fundamental rheological measurements.

In summary, this study has a great deal of importance to Canada. Canadian farmers need to show efforts to reduce free asparagine concentration in wheat that will produce baked goods meeting current food safety demands, since they are providing grains to international markets. Therefore, it is of the utmost importance to understand if the reduction of free asparagine concentration in wheat negatively affects gluten strength, since good final bread quality is essential to consumer acceptance. This important research question must be investigated in order to provide Canadian farmers with optimal strategies to grow wheat with low acrylamide forming potential that also produces high quality products for the baking industry.

This thesis is an extensive research study that seeks to evaluate the gluten strength of eight western Canadian wheat varieties grown under four fertilization treatments and four

environmental-time conditions. The overall goal of this thesis is to examine if strategies to reduce wheat free asparagine concentration will have a negative impact on the gluten strength of flour produced from Canadian wheat varieties. The relationship between rheological parameters from empirical tests and free asparagine concentration will be investigated. Complementary fundamental rheological tests will be performed for a deeper understanding of this relationship. The specific objectives that drive this research are:

1. To understand the relationship between wheat free asparagine concentration, as affected by environment, genotype and fertilization treatments, and gluten strength using empirical measurements.
2. To understand the relationship between wheat free asparagine concentration and gluten strength using fundamental rheology and low-intensity ultrasound.

The respective hypotheses of this thesis are:

1. As free asparagine in wheat increases, wheat flour dough will present a higher extensibility and lower gluten strength.
2. The interaction between free asparagine concentration and yeast activity will manifest itself in the fundamental rheological properties of dough, i.e., greater yeast activity will be observed in samples with higher free asparagine concentration.

2 Literature Review

2.1 Acrylamide, a global health concern

Acrylamide (C_3H_5NO) is a vinyl monomer that is used, for example, to produce polymers with improved cross-linking and aqueous solubility (IARC, 1994). The health concerns associated with this compound are related to its neurotoxicity and probable carcinogenicity to humans (Group 2A) (IARC, 1994; Karimi & Rashedinia, 2014; World Health Organization, 2006).

The Maillard reaction, an essential process for flavor and aroma formation in foods (Fayle & Gerrard, 2002), is also responsible for acrylamide formation. Acrylamide was first hypothesized to form in foods in 2000, when Tareke et al. (2000), in an animal study aimed at measuring exposure to acrylamide, found higher levels of acrylamide in a fried feed compared to a control unfried diet. The authors found a direct impact of acrylamide levels in the fried feed on the rats' adduct levels, i.e., increased levels of N-(2-carbamoylethyl) valine (CEV), which is an adduct formed when acrylamide reacts with the N-terminus of hemoglobin (Tareke et al., 2000). The CEV levels found in rats were similar to CEV levels found in non-smoking humans, which led the authors to conclude that humans are exposed to acrylamide through their diet (Tareke et al., 2000). Moreover, Johnson et al. (1986) studied 900 Fischer 344 rats receiving water with acrylamide levels (0 to 2.0 mg acrylamide kg^{-1} body weight per day) over two years. The authors found that in the last three months of the study, the accumulative mortality of rats was significantly higher for the animals receiving water with 2.0 mg acrylamide kg^{-1} body weight per day. Tissue evaluation of the rats showed that acrylamide ingestion resulted in an increased incidence of tumors. Among the tumor types, the scrotal mesothelioma in male rats was greater when the animals received 0.1 mg acrylamide kg^{-1} body weight per day compared to the control group. Moreover, other types of tumors, such as tumors in the mammary gland, uterus, and oral tissues, appeared in female rats receiving 2.0 mg acrylamide kg^{-1} body weight per day (Johnson et al., 1986).

Later, Tareke et al. (2002) proved that acrylamide could be formed in carbohydrate-rich foods when cooked at high temperatures (>120 ºC). They also showed that acrylamide formation was temperature-dependent, as shown by increasing levels of acrylamide in french-fried potatoes when temperatures increased from 120 to 220 ºC (Tareke et al., 2002). Several research studies have since been conducted over the past 15-20 years to quantify and investigate acrylamide formation in foods in order to evaluate possible mitigation strategies (Arvanitoyannis &

Dionisopoulou, 2014; Capuano et al., 2009, 2010; Claeys et al., 2005; Claus et al., 2008; Lingnert et al., 2016; Stadler & Scholz, 2004).

Among foodstuff, wheat-based products are susceptible to acrylamide formation because wheat naturally contains free asparagine (Mottram et al., 2002), which is the main amino acid precursor to acrylamide formation (Mottram et al., 2002; Stadler et al., 2002; Yaylayan et al., 2003; Zyzak et al., 2003). Furthermore, as the flour extraction rate increases, the free asparagine content also increases (Capuano et al., 2010). In 2012, a report from the European Food Safety Authority (EFSA) showed that a variety of food products contained acrylamide, such as French fries, potato crisps, breakfast cereals, biscuits, coffees, and soft breads. As published by Raffan & Halford (2019), who evaluated acrylamide content in a variety of food products, data from the EFSA Panel on Contaminants in the Food Chain (CONTAM) (2015) indicated that soft breads had about 42 µg kg^{-1} of acrylamide. It was estimated that adults are exposed to acrylamide (0.2-1.0 µg kg^{-1} of body weight per day) (FAO/WHO, 2011) and, although the concentration in soft bread was not as high as for French fries in the same year (338 µg kg^{-1}), because soft bread is an important food product in European diets, it is one of the main sources of acrylamide for adults (European Food Safety Authority, 2012). Additionally, due to its high consumption, bread is the second major source of acrylamide for humans, with potato fries being the main source (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015).

Therefore, recent acrylamide benchmark levels set by the European Commission (2017) urged food industries to apply mitigation strategies to reduce acrylamide formation in their products. Reduction of free asparagine in raw materials is one of the strategies to produce food products with low levels of acrylamide (Friedman, 2003). In 2014, a report from the European Commission on the main outcomes from the acrylamide workshop held in Brussels highlighted the necessity for cereal producers worldwide to reduce free asparagine levels in the wheat they grow (European Commission, 2014). The report advised the development or selection of wheat varieties with low levels of free asparagine and implementation of good agronomic practices, such as avoiding sulfur deficiency and controlling nitrogen fertilization (European Commission, 2014). Acrylamide benchmark levels for soft bread was set to 50 µg kg^{-1} of bread by the European Commission in 2017 (European Commission, 2017). Recent studies showed that there is still much to do in order to meet acrylamide benchmarks in bread (Andačić et al., 2020; Esposito et al., 2020; Mencin et al., 2020). Therefore, it is of utmost importance for the breadmaking industry to assure

that acrylamide mitigation strategies are being applied by wheat producers, such as good agronomic practices associated with fertilization (European Commission, 2017).

2.2 Free asparagine accumulation in wheat and mitigation strategies

Wheat grain contains a variety of free amino acids; however, the free asparagine is the main focus when acrylamide mitigation in bread is desired (Curtis et al., 2009). Asparagine is an important amino acid for nitrogen storage and transport in plants due to its high N:C ratio (2:4) (Lea et al., 2007). Asparagine transports nitrogen from plant roots to the leaves through the xylem, and from leaves to the seeds through the phloem (Lea et al., 2007). Ammonium or nitrate present in the soil can be absorbed by the roots. In order to be assimilated by the plant into amino acids, nitrate needs to be reduced to ammonium (Masclaux-Daubresse et al., 2010). Ammonium is assimilated through the glutamine synthetase cycle, i.e., conversion of ammonium into glutamine, which is then converted into asparagine by the enzyme asparagine synthetase (Masclaux-Daubresse et al., 2010). Gao et al. (2016) identified four asparagine synthetase genes in wheat: *TaASN1*, *TaASN2*, *TaASN3*, and *TaASN4*. This information was later updated by Xu et al. (2018) with the observation of two copies of *TaASN3* (*TaASN3.1* and *TaASN3.2*) and single copies of the other genes (*TaASN1*, *TaASN2*, and *TaASN4)*.

Free asparagine may accumulate in plants under a variety of stresses, such as water stress, diseases, and nutrient availability, e.g., soils with sulfur deficiency and excessive nitrogen (Claus et al., 2006; Corol et al., 2016; Granvogl et al., 2007; Lea et al., 2007; Muttucumaru et al., 2006; Weber et al., 2008). This accumulation under excess of nitrogen and deficiency in sulfur might happen as a stress-induced asparagine accumulation mechanism. Because free asparagine serves as a nitrogen storage source for the plant, stress situations, e.g., nutrient limitation, lead to an upregulation of asparagine synthetase in order to produce more asparagine for nitrogen storage (Oddy et al., 2020). The genes that regulate asparagine synthesis in wheat are expressed differently, for example, according to plant growing stage or nutrient availability. The genes *TaASN1* and *TaASN2* were shown to be expressed under sulfur deficiency, with the former expressed during early development and the latter expressed at the late development stage in the endosperm (Curtis et al., 2019; R. Gao et al., 2016; Xu et al., 2018).

The mechanisms that underpin asparagine functions and accumulation in wheat are still being studied. However, factors found to influence free asparagine accumulation in wheat have been explored in several studies (Oddy et al., 2020). Over a decade ago, a research group found

significant correlation between wheat genotype and free asparagine accumulation in wheat (Curtis et al., 2009). A few years later, Corol et al. (2016) found that not only genotype, but also environment and the interaction between these two factors can highly affect free asparagine accumulation in wheat. Therefore, with the goal of promoting the production of food with low acrylamide content, the European Commission highlighted the necessity of cereal producers to select wheat varieties and apply fertilization treatments in order to produce wheat with low free asparagine content (European Commission, 2014).

However, cereals producers and the stakeholders involved in the baking industry must bear in mind that environment and genotype also affect the protein content and its composition in wheat (Johansson et al., 2013). In addition to environment and genotype, the amount of nitrogen and sulfur available to the plant can also significantly impact the wheat protein content and composition (Godfrey et al., 2010; Park et al., 2014; Wilson et al., 2020).

Nitrogen is an important nutrient for wheat plant growth and is an essential factor in protein synthesis in the wheat grain (Hawkesford, 2014). Nitrogen is mostly delivered to the crop through fertilization treatments (Hawkesford, 2014), which mainly involves the administration of urea or ammonium nitrate (Gooding, 2009). Amino acids are the major transport form for nitrogen that are delivered into the wheat endosperm, and these are then the building blocks for the grain's wheat proteins. Nitrogen absorbed by the wheat plant contributes mainly to the formation of storage proteins in the mature grain, although a small portion of it (~5%) is present in the form of free amino acids, e.g., free asparagine (Corol et al., 2016).

Since the middle of the twentieth century, farmers have substantially increased the use of nitrogen fertilizers for cereal crops as an agronomic practice to achieve higher productivity (Hawkesford, 2014; Ladha et al., 2016). In 2016, the world's demand of nitrogen for fertilizer use was around 105.1 million tonnes, with an expected consumption of 111.6 million in 2022 (FAO, 2019). In the 2014-2015 period, the wheat crop was the principal destination (18.2%) of nitrogen fertilizer worldwide (Heffer et al., 2017). The application of nitrogen for wheat production is of paramount importance as it increases grain yield (Y. Zhang et al., 2016) and protein content (Abedi et al., 2011). Varying from 6% to 20% in wheat grains, protein content is a critical factor for wheat classification and marketing since protein content requirements vary depending on the product made from the wheat, e.g., higher protein content is required for bread but not for cakes and cookies (biscuits).

In addition to its effects on protein content, nitrogen fertilization can affect wheat protein composition (Godfrey et al., 2010; Wieser & Seilmeier, 1998). The strong relationship between nitrogen fertilization and wheat protein quality arises from nitrogen's vital role in the formation and balance of amino acids in the wheat grain (M. Zhang et al., 2016). Wheat protein is composed of metabolic non-gluten protein fractions (albumins and globulins) as well as the gluten storage protein fractions - prolamins and glutelins (Veraverbeke & Delcour, 2002). Gluten is the major fraction in wheat protein, comprising about 85% of the total protein, with its prolamins referred to as gliadins and its glutelin proteins as glutenins (Dobraszczyk et al., 2001; Veraverbeke & Delcour, 2002). Since gluten protein composition affects the protein quality of the wheat, the gliadin-glutenin balance is an important factor in the quality of wheat-based products (Carson & Edwards, 2009).

It is the nature of the gliadins and the glutenins that make wheat a unique cereal (Veraverbeke & Delcour, 2002). These proteins are the functional components in the flour that, when optimally mixed with water, form a viscoelastic material with desirable characteristics (Carson & Edwards, 2009). As an example, for the bread production industries, an optimal gliadin-glutenin balance must provide enough gliadins to confer fluidity to the dough (so as to ensure desired dough expansion), as well as enough glutenins to ensure desired gas retention (so as to allow bubble growth during fermentation) (Delcour & Hoseney, 2010a). Deviation from the optimal balance changes the wheat flour dough's physical properties, and this can impact the production process and the quality attributes of the final product, such as loaf volume, crumb texture and overall appearance. Thus, by altering protein content and protein composition, wheat nitrogen fertilization alters the rheological properties of the wheat flour dough (Uthayakumaran et al., 2000).

Moreover, sulfur-containing amino acids are also present in gluten proteins, therefore, sulfur availability in the soil can affect protein composition, which can also affect the quality of the wheat's final products (Flæte et al., 2005; Wilson et al., 2020; F. J. Zhao, Salmon, et al., 1999). In summary, the content and composition of gluten proteins are essential to determine wheat suitability for a final product, such as bread or biscuit (P. R. Shewry et al., 2003). Therefore, a comprehensive understanding of the effects of environment, genotype and fertilization is required to guarantee that the quality of the final product will not be impaired by asparagine mitigation strategies.

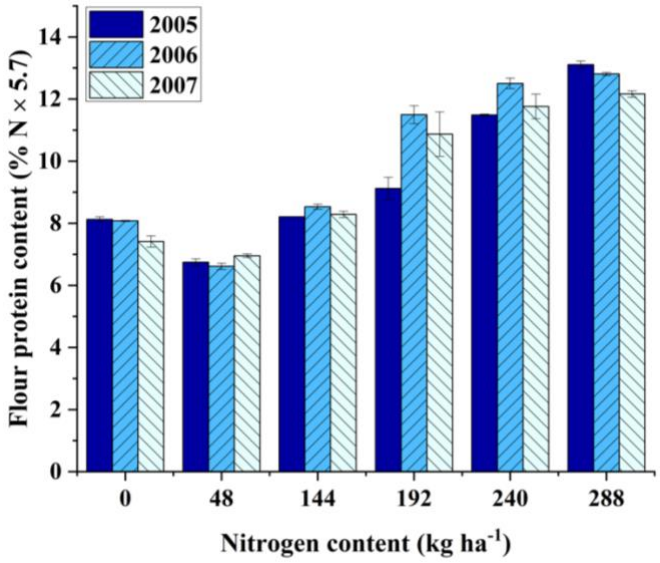
2.3 Wheat proteins and free asparagine

2.3.1 Effect on protein content

About 50 to 95% of the nitrogen in wheat grain is supplied by the wheat plant through remobilization of the nitrogen absorbed by the plant (Gaju et al., 2014). This grain nitrogen is utilized in the biosynthesis of amino acids, the building blocks for wheat proteins (metabolic and storage). Thus, when nitrogen fertilization is promoted, wheat grain protein content increases. Only the storage proteins, i.e., the gluten proteins, are significantly affected by additional nitrogen fertilization (Fuertes-Mendizábal et al., 2010).

Field experiments have been carried out by numerous research groups on wheat plant growth under nitrogen fertilization treatments, and all have reported a positive relationship between nitrogen fertilization (0-350 kg N ha^{-1}) and grain protein content (Chope et al., 2014; Fuertes-Mendizábal et al., 2010; X. Gao et al., 2012; Giuliani et al., 2011; Godfrey et al., 2010; Kharel et al., 2011; M. Li et al., 2019; Nyiraneza et al., 2012; Park et al., 2014; Rossmann et al., 2019; Y. Zhang et al., 2016). Godfrey et al. (2010) evaluated wheat protein content variability for a breadmaking wheat cultivar grown for three years in the United Kingdom under various nitrogen fertilization treatments, i.e., 0-288 kg ha^{-1}. They reported that overall, the flour protein content increased as the quantity of nitrogen fertilizer increased (Figure 2.1). More recently, the application of nitrogen fertilization (135 kg N ha^{-1}) was shown to significantly increase the whole meal flour protein content when compared to lower levels of nitrogen (35 and 80 kg N ha^{-1}) (Guerrini et al., 2020). The effect of nitrogen fertilization on grain protein content was also investigated in pot experiments, and an increase in grain protein content with increasing nitrogen supply through fertilization was observed (Fuertes-Mendizábal et al., 2013; Xue et al., 2016).

Figure 2.1. Variation of flour protein content for the years 2005, 2006, and 2007 for different nitrogen fertilization treatments. Graph is created using data provided in Table S3 of the Supporting Information from Godfrey et al. (2010).



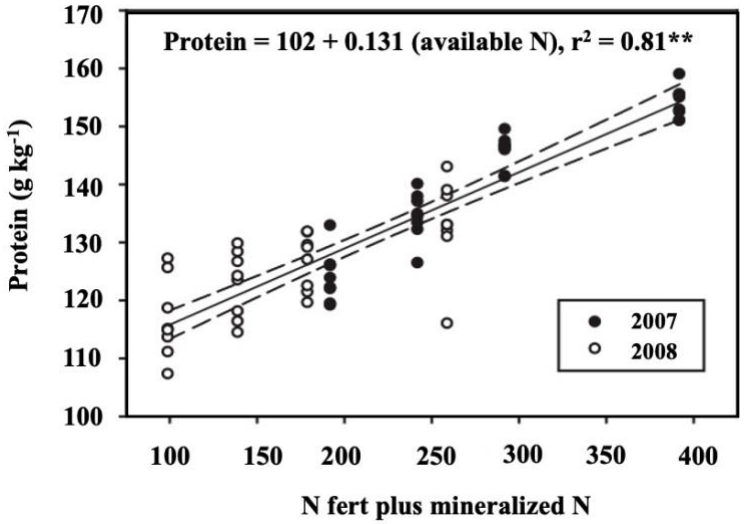
Not only is the amount of nitrogen supplied to the wheat plant important, but fertilization timing is a factor that improves wheat grain protein content (Fuertes-Mendizábal et al., 2010). Late application and splitting of nitrogen fertilization at different plant growth stages have been shown to be advantageous in increasing wheat grain protein content compared to early fertilization (Fuertes-Mendizábal et al., 2010, 2013; Nyiraneza et al., 2012; Xue et al., 2016). As an example, Rossmann et al. (2019) studied nitrogen fertilization timing on three wheat varieties in a two year-field experiment, and showed that late nitrogen fertilization positively affected grain protein content for all wheat varieties.

Variation in weather and soil conditions also affect the conversion of nitrogen applied into grain protein content. Nyiraneza et al. (2012) demonstrated that some soils permit an increase in wheat grain protein content for all fertilization levels, while some allow nitrogen absorption up to a certain nitrogen level, with grain protein content decreasing at higher levels of nitrogen fertilization. Adequate rainfall and soil moisture content are reported to enhance nitrogen uptake by the plant, thus permitting protein content to increase in the wheat grain (X. Gao et al., 2012; Giuliani et al., 2011).

The effects of climatic conditions, i.e., temperature and precipitation, during the production season on wheat grain protein content has also been studied. Mastilović et al. (2018) demonstrated that more precipitation during the 2008 production season resulted in grains with higher protein content compared to those grown under less precipitation (2010) at the same growing location (Mastilović et al., 2018). Similarly, for two Australian wheat varieties, Catalina and Peake, grown at the same location in 2008 and 2009, variation in grain protein content according to field conditions was observed (Hasniza, Wilkes, et al., 2014). The main difference was the anomalous temperature during grain filling in 2009, with temperatures above 40ºC for three days, while maximum temperatures during the same period in 2008 were less than 35ºC. The rainfall during the grain filling period in 2009 was 50% less than that in 2008, and the nitrogen content in the soil before planting was over 50% lower in 2009 compared to that in 2008 (Hasniza, Copeland, et al., 2014). These conditions resulted in significant variations in grain protein content, e.g., for wheat grains grown at one of the sites, the protein content decreased from 14.6% in 2008 to 10.3% in 2009 for Catalina, and from 14.9% in 2008 to 11.5% in 2009 for Peake (Hasniza, Wilkes, et al., 2014). No strong correlations were found between rainfall during grain filling and grain protein content (Hasniza, Wilkes, et al., 2014).

Interestingly, the results of Uthayakumaran et al. (2012) and Godfrey et al. (2010) contradicted those of Mastilović et al. (2018) and Hasniza, Wilkes, et al. (2014). In the study conducted by Uthayakumaran et al. (2012) for three wheat varieties grown at four different locations in Australia, the location with lowest rainfall during the grain filling period yielded wheat flours with the highest protein content compared to other locations. Water content in the soil might be a confounding factor. Higher protein content in grains grown in Wagga Wagga was surmised to be due to the significantly higher nitrogen content of its soil compared to the other locations. Therefore, the negative effect of low rainfall on the protein content of grains from Wagga Wagga was compensated by high soil nitrogen content. An illustration of the importance of nitrogen availability was the study of Park et al. (2014), who accounted for the mineralized nitrogen in the soil in order to study the effect of nitrogen fertilization on wheat grain protein content. As a result, very good correlations between grain protein content and nitrogen absorbed by the plant were observed (Figure 2.2).

Figure 2.2. Effect of nitrogen fertilization on grain protein content for two years. Figure 1 from Hyejin Park, David E. Clay, Robert G. Hall, Jai S. Rohila, Tulsi P. Kharel, Sharon A. Clay & Sanghun Lee (2014) Winter Wheat Quality Responses to Water, Environment, and Nitrogen Fertilization, Communications in Soil Science and Plant Analysis, 45:14, 1894-1905, DOI: 10.1080/00103624.2014.909833 (Open Access article).



Although wheat genotype is an important element in grain protein content, studies conducted with two or more wheat varieties found that grain protein content showed a stronger relationship with nitrogen fertilization than with wheat variety (Giuliani et al., 2011; M. Li et al., 2019; Rossmann et al., 2019). This outcome agrees with earlier studies, where nitrogen fertilization was the main, or one of the main, factors enhancing wheat grain protein content (Flæte et al., 2005; Triboi et al., 2000).

Besides nitrogen, sulfur availability in the soil is also of great importance. Reductions of sulfur dioxide (SO_2) emissions have been discussed since 1970s, with the Convention on Long-range Transboundary Air Pollution being established in 1979 to enforce reductions of SO_2 emissions (Vestreng et al., 2007). According to a complete analysis of the sulfur emissions in Europe from 1980 to 2004, a reduction of about 73% in anthropogenic SO_2 emissions was reported (Vestreng et al., 2007). As a consequence of the reduced SO_2 emissions, as well as the increased application of fertilizers that were depleted of sulfur, soil sulfur deficiency has become an issue around the world (Blake-Kalff et al., 2000). Sulfur is an essential nutrient for the growth of the wheat plant and plays an important role in improving the quality of the final wheat-based products

(F. J. Zhao, Hawkesford, et al., 1999). The wheat plant absorbs inorganic sulphate that is present in the soil and uses the reduced form of sulphate, i.e., sulphide, to build sulfur-rich amino acids, such as cysteine (Scheerer et al., 2010; F. J. Zhao, Hawkesford, et al., 1999). Cysteine is then used by the plant to produce the tripeptide glutathione, which is used to build sulfur-rich proteins (K. Wang et al., 2015; F. J. Zhao, Hawkesford, et al., 1999). The cysteine present in wheat gluten proteins is of utmost importance for the structure of the wheat flour dough because cysteine residues are sites for intra and inter-polypeptide disulfide bonds (K. Wang et al., 2015; F. J. Zhao, Hawkesford, et al., 1999). These disulfide bonds present between gluten proteins create structure that allow the gluten to be stronger, i.e., more elastic-like (K. Wang et al., 2015; F. J. Zhao, Hawkesford, et al., 1999). Therefore, various studies have evaluated sulfur fertilization effects on wheat protein content (Järvan et al., 2017; Steinfurth et al., 2011; Wieser et al., 2004; Wilson et al., 2020; F. J. Zhao, Salmon, et al., 1999; Zörb et al., 2009).

Sulfur fertilization has been shown to have opposing effects on protein content in the literature. On the one hand, some studies have reported a non-significant effect of sulfur fertilization on wheat protein content (Steinfurth et al., 2011; Wieser et al., 2004; F. J. Zhao, Salmon, et al., 1999; Zörb et al., 2009). On the other hand, a study has reported that sulfur application decreased protein content (Järvan et al., 2017). More recently, another study showed that sulfur fertilization caused wheat protein content to either increase or decrease, depending on the climatic conditions of the specific year (Wilson et al., 2020). As an example of a non-significant effect on protein content, Wieser et al. (2004) studied a single wheat variety grown in a greenhouse under four levels of sulfur fertilization (0 to 160 mg per pot) using two different soil types that varied in nutrient composition. The authors observed that nitrogen present in the grain was highly correlated to flour protein content, with sulfur fertilization playing little or no effect on the flour protein content (Wieser et al., 2004). Zörb et al. (2009) studied two winter wheat varieties, selected based on their similarity regarding protein concentration and baking quality. The wheat plants were grown in pots with three different sulfur fertilization rates (0, 0.1, and 0.2 g of sulfur per pot), with a fourth treatment where 0.1 g was applied at sowing followed by late fertilization where 0.1 g was applied at head development (Zörb et al., 2009). The authors observed that, with the exception of plants receiving no sulfur fertilization, the protein content, for both wheat varieties, did not show significant variations among the sulfur fertilization treatments (Zörb et al.,

2009). Therefore, different rates of sulfur fertilization did not affect protein content (Zörb et al., 2009).

Conversely, field studies conducted by Järvan et al. (2017) showed a different effect associated with sulfur fertilization. The authors evaluated two wheat varieties, i.e., the variety Lars grown in a field in northern Estonia and the variety Ada grown in a field in southern Estonia, under constant nitrogen fertilization of 100 kg ha^{-1} and varying sulfur fertilizations (Järvan et al., 2017). Overall, the authors observed a decrease in protein content as sulfur was applied to the plant, which was hypothesized to be due to a protein dilution effect because yield significantly increased with sulfur application (Järvan et al., 2017). More recently, a field study conducted over two years (four wheat varieties in 2017 and five varieties in 2018) by Wilson et al. (2020) showed that sulfur fertilization (22 kg ha^{-1}) decreased the grain protein content in 2017, with some genotypes being affected to a greater extent than others. Similarly to Järvan et al. (2017), Wilson et al.(2020) also hypothesized that a decrease in protein content was likely due to the protein dilution effect caused by the high yields. In 2018, the same study showed that when nitrogen was applied in the presence of 22 kg ha^{-1} of sulfur, the grain protein content was positively affected (Wilson et al., 2020). However, the authors attributed the increase in protein content to the increased heat and drought conditions in 2018, when compared to the weather in 2017, which alleviated the protein dilution effect, thus decreasing kernel weight and increasing protein content (Wilson et al., 2020).

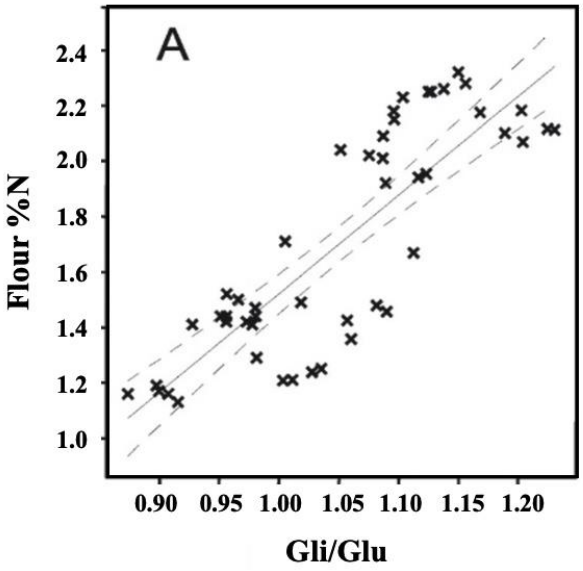
2.3.2 Effect on protein composition

In addition to wheat protein content, protein composition is also affected by nitrogen fertilization (Chope et al., 2014; Fuertes-Mendizábal et al., 2010, 2013; Godfrey et al., 2010; González-Torralba et al., 2011; Kharel et al., 2011; M. Li et al., 2019; Park et al., 2014; Rossmann et al., 2019; Xue et al., 2016; Y. Zhang et al., 2016). Wheat protein quality is mainly related to gluten protein composition, which in turn is affected by variation in the content of gluten's constituent protein fractions (Veraverbeke & Delcour, 2002). As the rate of nitrogen fertilization increases, the amounts of gliadins (Gli) and glutenins (Glu) increase while non-gluten proteins remain constant (Fuertes-Mendizábal et al., 2010). The Gli/Glu ratio is an important criterion for defining protein composition because it affects the overall viscoelasticity of the dough, and thus, its rheological properties (Carson & Edwards, 2009; Delcour & Hoseney, 2010a).

A number of recent research studies have indicated that nitrogen fertilization treatments increase the amount of gliadins to a greater extent than the glutenins, leading to an increase in the

Gli/Glu ratio (Chope et al., 2014; Fuertes-Mendizábal et al., 2013; Godfrey et al., 2010; M. Li et al., 2019; Xue et al., 2016). These observations substantiate earlier findings for different wheat varieties (Triboi et al., 2000; Wieser & Seilmeier, 1998), and the classic work of Finney et al. (1957), where it was shown that the specific grain protein components changed according to nitrogen fertilization levels. Finney et al. (1957) also showed the implications of this for bread quality: although loaf volume continued to increase as protein content increased, quality enhancements with increasing protein content at higher protein content did not occur at the same rate as at lower protein content. The strong positive relationship between grain nitrogen and the Gli/Glu ratio was illustrated well by the study conducted over 3 years by Godfrey et al. (2010), which is reproduced as Figure 2.3.

Figure 2.3. Relationship between nitrogen concentration in wheat flour (Flour %N) and the Gli/Glu ratio determined from Size Exclusion HPLC. Reprinted (adapted) with permission from Godfrey, D., Hawkesford, M. J., Powers, S. J., Millar, S., & Shewry, P. R. (2010). Effects of crop nutrition on wheat grain composition and end use quality. *Journal of Agricultural and Food Chemistry*, *58*(5), 3012–3021. https://doi.org/10.1021/jf9040645. Copyright (2010) American Chemical Society.



Nevertheless, the relationship between the Gli/Glu ratio in the grain and nitrogen fertilization of the wheat plant is not always this straightforward. In addition to variations in

protein composition due to nitrogen fertilization being affected by genetic factors (Wieser & Seilmeier, 1998), González-Torralba et al. (2011) reported that when nitrogen fertilization amounts exceeded those necessary for maximum yield, the interaction between nitrogen fertilization and environment influenced the Gli/Glu values. No clear trend was observed between nitrogen fertilization and the Gli/Glu ratio, and the highest Gli/Glu was only observed in the year when the greatest amount of nitrogen was applied (up to 340 kg N ha^{-1}) (González-Torralba et al., 2011).

In a two-year study, Y. Zhang et al. (2016) found a slight increase in the Gli/Glu ratio with application of nitrogen fertilizer, but only in the second year of the study. Fuertes-Mendizábal et al. (2010) applied nitrogen to the wheat variety Soissons grown in Spain and observed an increase in both gliadins and glutenins as the nitrogen concentration in the grain increased, with the gliadins increasing slightly (but not significantly) more than the glutenins (Fuertes-Mendizábal et al., 2010).

With the same variety, Soissons, grown in the United Kingdom in a two-year study, nitrogen fertilization increased the amounts of the gliadin and glutenin fractions, with a greater rise in the second year (Rossmann et al., 2019). The Gli/Glu calculated for the samples from the first year showed no variation in response to nitrogen fertilization (Rossmann et al., 2019). In the UK study, the Gli/Glu ratio was always greater than 1.00, meaning that more gliadins were present in the grain than glutenins (Rossmann et al., 2019), contrasting with the results for Soissons grown in Spain where the maximum Gli/Glu was 0.76 (Fuertes-Mendizábal et al., 2010). Although the maximum amount of nitrogen fertilizer applied in the study of Rossmann et al. (2019) was slightly higher compared to that in the study by Fuertes-Mendizábal et al. (2010), the difference in environmental conditions of the two studies is likely the reason for the contrasting Gli/Glu values for the same variety. For example, water availability in the soil post-anthesis can significantly affect the accumulation of gluten proteins in the grain (C. X. Zhao et al., 2009).

Besides analysing the Gli/Glu ratio to study differences in protein composition, the gliadins and glutenins can also be separated into different fractions. The gliadins are classified into α-, β-, γ- and ω-gliadins based on their gel electrophoresis mobility at pH 3 (Wrigley et al., 2006). The α-, β- and γ-gliadins, which are known as sulfur-rich proteins, have methionine and cysteine in their structure, with the cysteine residue providing the site for intra-polypeptide disulfide bonds (P. R. Shewry et al., 1986). The ω-gliadins have no cysteine in their structure (Grosch & Wieser,

1999; Wieser, 2007) and thus are known as sulfur-poor proteins (P. R. Shewry & Tatham, 1997). The gliadins act as a plasticizer in the dough structure, conferring extensibility to the dough's viscoelastic matrix (Sapirstein & Fu, 2000; Wieser, 2007).

Glutenins are known to be composed of different subunits, linked mainly through disulfide bonds, varying in size from 500,000 to 10 million (Wieser et al., 2006). The largest subunits are termed as glutenin macropolymer, which is known to affect the gluten strength to a greater extent when compared to gliadins (Wieser, 2007). The two major components of glutenin macropolymers are the low molecular weight (LMW) glutenin subunits and the high molecular weight (HMW) glutenin subunits, which are linked with each other through inter-polypeptide disulfide bonds (Veraverbeke & Delcour, 2002; Wrigley, 1996). Overall, together with the α-, β-, γ-gliadins, the LMW-glutenin subunits are classified into sulfur-rich proteins due to the amount of cysteine residues in their structure (P. R. Shewry & Tatham, 1990, 1997).

Finally, the HMW-glutenin subunits can be divided into x-type and y-type, with the latter having more cysteine residues than the former. The HMW-glutenin subunits stand as a group, because they contain less sulfur compared to sulfur-rich proteins, while having more sulfur content than ω-gliadins (P. R. Shewry & Tatham, 1990, 1997). The glutenin macropolymer is the main component responsible for conferring elasticity to wheat flour dough, thus giving strength to the dough structure. An adequate gluten strength is desirable for doughs prepared for breadmaking because it increases the gas-holding capacity of the dough (Delcour et al., 2012), and this is essential for high-quality pan bread.

Therefore, it is important to understand how nitrogen and sulfur fertilizations affect the gliadin and glutenin fractions. For example, Zheng et al. (2018) evaluated the gluten composition of four wheat varieties grown at two locations in China, a sub-humid region and a semi-arid region, over two growing seasons and under four fertilization treatments (0, 75, 150, and 225 kg ha^{-1} of nitrogen). The authors observed that wheat samples that were grown in both regions had their HMW and LMW glutenin subunits significantly increased with nitrogen fertilization (Zheng et al., 2018). Moreover, all the gliadin fractions also significantly increased with nitrogen fertilization (Zheng et al., 2018). For the sub-humid region, nitrogen fertilization was the main factor affecting HMW and LMW glutenin subunits, α/β-gliadins, and the Gli/Glu ratio (Zheng et al., 2018). On the other hand, for the semi-arid region, nitrogen fertilization was the main factor affecting the

α/β-gliadins, with genotype being the main factor for the other gluten protein fractions (Zheng et al., 2018).

Results from Triboi et al. (2000) also showed that both HMW and LMW glutenin subunits increased with nitrogen fertilization. The authors studied two bread wheat varieties (Bancal and Rinconada), grown at two locations over two growing seasons and supplied with three nitrogen fertilization levels (0, 100 and 200 kg ha^{-1}) (Triboi et al., 2000). After performing protein extraction and protein fractionation by RP-HPLC, the authors reported a significant increase in the HMW-glutenin subunits when wheat was supplied with 200 kg ha^{-1} of nitrogen. The LMW-glutenin subunits were also affected, with a significant increase when wheat was supplied with 100 and 200 kg ha^{-1} of nitrogen (Triboi et al., 2000). By studying a single wheat variety, i.e., Soissons, grown in Northern Spain, Fuertes-Mendizábal et al. (2010) also observed a significant effect of nitrogen fertilization on HMW-glutenin subunits. The authors applied nitrogen fertilization at four levels (0, 100, 140 and 180 kg ha^{-1}) with splitting at two development stages, and with the two highest levels being also split over three development stages, thus having in total six different treatments (Fuertes-Mendizábal et al., 2010). After performing HMW-glutenin subunit separation by SDS-PAGE, the authors observed a significant increase in both x- and y-type as well as a significant increase in the total HMW-glutenin subunits with increasing nitrogen fertilization (Fuertes-Mendizábal et al., 2010). More recently, Landolfi et al. (2021) studied the effect of nitrogen fertilization on the protein content and composition of two soft wheat varieties (landrace and modern) and a tritordeum variety. The field study reported by the authors included the growth of the three varieties under the same environmental condition and receiving two nitrogen fertilization levels (80 and 160 kg ha^{-1}) (Landolfi et al., 2021). From this study, the Gli/Glu ratio did not significantly change for the landrace and tritordeum varieties, but significantly decreased for the modern wheat variety as fertilization increased (Landolfi et al., 2021). Moreover, as opposed to results from Fuertes-Mendizábal et al. (2010), Triboi et al. (2000) and Zheng et al. (2018) did not observe significant changes in the HMW/LMW-GS ratio (Landolfi et al., 2021).

In summary, nitrogen fertilization influences the wheat protein content positively, and this is reflected mainly in an increase in the quantity of gluten proteins. Although there are confounding factors, the majority of studies confirm a significant positive correlation between the Gli/Glu ratio and nitrogen fertilization treatments. Therefore, nitrogen treatments will impact wheat protein

quality, thus potentially changing the dough's rheological properties. Although Fuertes-Mendizábal et al. (2010), Landolfi et al. (2021), Triboi et al. (2000) and Zheng et al. (2018) did not mention the sulfur content available to the wheat plants in their studies, as mentioned before, sufficient sulfur availability to the plant is essential to produce wheat flour doughs with adequate strength (Steinfurth et al., 2011).

Studies have been conducted to evaluate the effects of sulfur fertilization on wheat protein composition (Z. Dai et al., 2015; Uthayakumaran et al., 2007; Wieser et al., 2004; Yu et al., 2021; Zheng et al., 2018). By conducting a pot experiment, Wieser et al. (2004) studied the effect of different levels of sulfur, i.e., 0, 40, 80, and 160 mg S per pot, using two different soils, on the protein composition of a single wheat variety. The authors observed, for both soils, that as sulfur levels increased, the Gli/Glu ratio decreased (Wieser et al., 2004). Moreover, gliadin composition also changed as sulfur levels increased, with a decrease in the sulfur-poor fraction, i.e., ω-gliadins, and an increase in the sulfur-rich fraction, i.e., α- and γ-gliadins, with a greater effect on the γ-gliadins (Wieser et al., 2004). Uthayakumaran et al. (2007) studied a single wheat variety grown in a sulfur-deficient field under two levels of nitrogen (100 and 200 kg ha^{-1}) and three levels of sulfur (0, 10, and 40 kg ha^{-1}). The authors observed that the S/N ratio in the grain increased as sulfur increased when applying nitrogen levels of 100 kg ha^{-1} (Uthayakumaran et al., 2007). The authors also observed a decrease in the ω-gliadin content as the S/N ratio increased, but with a sharp increase in the levels of ω-gliadins when the S/N ratio decreased to a value of 0.06 or lower (Uthayakumaran et al., 2007), a value that indicates sulfur deficiency in the grain (Randall et al., 1981).

In a glasshouse experiment, Z. Dai et al. (2015) studied a single wheat variety grown under four fertilization treatments, changing the levels of nitrogen and sulfur. Agreeing with previous studies, Z. Dai et al. (2015) reported an increase in sulfur-poor gliadins and a decrease in sulfur-rich gliadins and LMW-glutenin subunits when wheat plants were submitted to low sulfur levels and optimum levels of nitrogen. More recently, Yu et al. (2021) published a research study where field and glasshouse experiments were conducted. In the field trial, four Australian wheat varieties were grown under three levels of sulfur fertilization (0, 30 and 50 kg ha^{-1}) (Yu et al., 2021). For the glasshouse experiments, two Australian wheat varieties where grown under the same three levels of sulfur fertilization (0, 30 and 50 kg ha^{-1}) (Yu et al., 2021). The authors reported a significant effect ($p < 0.001$) of sulfur fertilization on the Gli/Glu ratio and on the gliadin and

glutenin subunits for both field and glasshouse experiments (Yu et al., 2021). The Gli/Glu ratio was reported to decrease as sulfur levels increased (Yu et al., 2021). Overall, sulfur application caused a decrease in the gliadin content, with ω-gliadins being the most affected (Yu et al., 2021), in agreement with previous studies. Therefore, sulfur fertilization is of utmost importance to guarantee the presence of sulfur-rich proteins in wheat that will participate in the intra and inter-polypeptide disulfide bonds that lead to the formation of strong wheat flour doughs.

In order to evaluate how environment, genotype, and fertilization treatments affect wheat flour dough properties, the formation of dough is an important step that must be properly followed prior to most rheological studies. Dough formation includes a number of processes, of which dough development is the most important (Cauvain, 2015b). In the dough development process, shear and extensional forces, created by the movement of the blades during mixing, act on the dough to create a fully developed, soft viscoelastic network material (Cauvain, 2015b). During subsequent processes that are required for bread production, the dough piece is manipulated multiple times and it must be able to deform and flow (Anderssen et al., 2004; Dobraszczyk & Morgenstern, 2003). The most common means of inducing flow are through shear and elongation. In evaluating the flow properties of viscoelastic materials such as dough, tests utilizing pure shear and uniaxial extension are frequently employed. These tests must also operate at various rates of testing because a broad range of strain rates are used to induce dough flow, from 10 s^{-1} during mixing to a more genteel 10^{-4} s^{-1} during proofing (Bloksma, 1990). These same tests can be conducted within dough's linear viscoelastic region (LVR); the dough is therefore not permanently deformed, so that insights on how the dough's native state affects the rheological response are obtained (Dobraszczyk & Morgenstern, 2003).

2.4 Wheat flour dough formation

In order to link the effect of environment, genotype and fertilization treatments on protein content and composition to dough rheology, the changes in wheat protein content and composition must be considered in the context of the dough formation process. Dough formation is comprised of three main outcomes: the hydration and homogenization of ingredients; the occlusion of air in the dough; and the development of the dough (Cauvain, 2015b; Scanlon & Zghal, 2001; Sluimer, 2005b).

At the beginning of mixing, wheat flour particles and the water interact. The movement of the mixer blades or pins enables the flour particles to hydrate, promoting contact between these

particles and exposing dry flour particles to the water phase. By repeating this process, the flour particles attain a point of full hydration, forming a mass with evenly distributed moisture and a maximum resistance to mixing (Delcour & Hoseney, 2010a). The correct amount of water that results in an optimally hydrated dough varies according to the desired final product, e.g., a flour suitable for cookies absorbs less water than a flour destined for bread production (Cauvain, 2015b). In a study on the effects of nitrogen and sulfur fertilization on the quality characteristics of bread dough made from four New Zealand wheat cultivars, Wooding et al. (2000) observed a significant increase in water absorption (*WA*) with increasing nitrogen fertilization levels from 0 to 225 kg ha^{-1}. This increase in the WA of wheat flour was consistently observed regardless of whether the wheat flour dough was produced by a mechanical dough development (*MDD*) mixer or by a mixograph (Wooding et al., 2000). The increase in the amount of water required for optimal hydration has been repeatedly attributed to an increase in flour protein content (Finney, 1945; Sapirstein et al., 2018), which was also the case in the study of Wooding et al. (2000). Wilson et al. (2020) reported that farinograph water absorption and the protein content of hard winter wheat varieties responded similarly to the application of nitrogen fertilizer (from 56 to 145 kg ha^{-1}), confirming the critical role of protein content in the water absorption of wheat flour.

Wheat flour dough is a composite material. Two phases are distinguishable: a viscoelastic dough matrix phase and a dispersed gas phase (Campbell & Martin, 2012; Scanlon & Page, 2015; Scanlon & Zghal, 2001; Sluimer, 2005b). Air entrapment occurs during mixing, mainly after full hydration of the flour particles (Cauvain, 2015b). Therefore, the flour components, such as protein, affect not only the overall rheological properties of the dough, but also the amount and distribution of air bubbles that are part of this composite material (Scanlon & Page, 2015). The role of wheat gluten components, i.e., glutenins and gliadins, in the inclusion of air and retention of gas within the viscoelastic structure of wheat flour dough is widely accepted (MacRitchie, 2016; Ooms et al., 2018). Sang et al. (2020) showed that gliadins improve incorporation of air as bubbles into the dough matrix during mixing by reducing water's surface tension. Moreover, the distribution of those bubbles in the wheat flour dough depends on the gluten components (Koksel, Strybulevych, Page, et al., 2017). Glutenins are known for their ability to retain gas within the dough structure during mixing and fermentation, while gliadins are responsible for the extensibility required for the expansion of gas cells during proofing and baking (Marchetti et al., 2012).

Because of its effect on the rheological properties of the dough, development of the dough is of paramount importance in wheat flour dough formation (Cauvain, 2015b). Optimal dough development (the formation of a soft viscoelastic material with maximal resistance to mixing) is the primary character of wheat flour doughs destined for various wheat-based products (Cauvain, 2015b). The glutenin fraction plays a major role in the formation of entanglements and disulfide bonds between protein molecules in order to form a continuous network of gluten proteins at the point of optimal dough development (Delcour & Hoseney, 2010a). Since dough development is governed by gluten network development, nitrogen fertilization can impact this process. A greater wheat protein content, thus more gluten proteins, brought about by increased nitrogen fertilization, has been reported to prolong the development time for the dough (Kharel et al., 2011; Park et al., 2014).

In summary, the specific viscoelastic character of the dough is governed to a great extent by dough formation processes, especially dough development (Cauvain, 2015b). Accordingly, the rheological properties of an optimally developed dough will be governed by the nitrogen functionality delivered from the developing wheat plant. The dough rheological properties discussed throughout this chapter refer to the rheological properties of a fully developed wheat flour dough, unless otherwise stated. In industrial bread production, dough development occurs mainly during the mixing step, where shear and extensional forces promote interactions between proteins and the formation of the gluten network (Sluimer, 2005b). The rheological properties of dough are assessed by shear and extensional rheological tests in order to understand dough's behavior under the forces present in the production process (Dobraszczyk et al., 2001).

2.5 Evaluation of dough mechanical properties

The tests described in this section have been employed in numerous research studies to understand dough deformation and flow when subject to extensional forces (Anderssen et al., 2004). Shear tests are also frequently employed to quantify wheat flour dough rheology, a deformation mode relevant to dough processing because of the shear forces required for dough development (Sluimer, 2005b). Besides extensional and shear tests, tests are also necessary to provide information on dough properties during mixing. Breadmaking industries often rely on mixing tests to obtain, for example, information regarding the time necessary in the mixer to produce an optimally developed dough as well as the optimum water amount that the flour is capable of absorbing (Dobraszczyk & Morgenstern, 2003). Finally, some newly developed

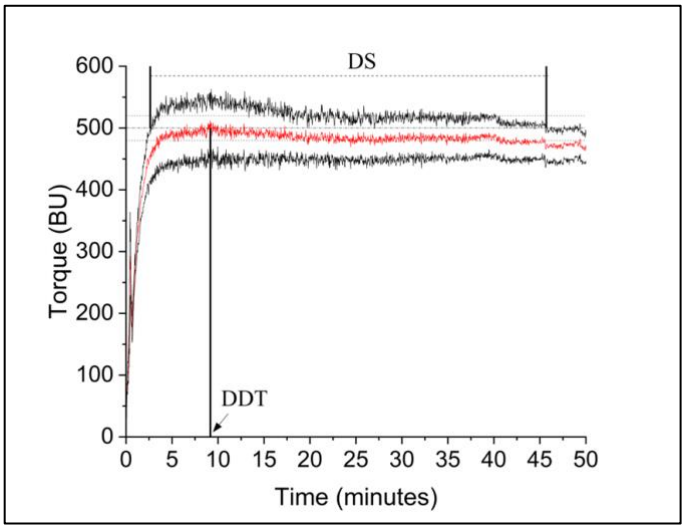
techniques, e.g., low-intensity ultrasound tests, are also available for rheological characterization of wheat flour dough (Koksel et al., 2016). In the next sections, each one of these rheological measurements will be discussed in detail.

2.5.1 Mixing tests

Examples of mixing tests are the farinograph and the mixograph, which are empirical methods that are utilized to evaluate gluten strength during dough mixing process (T. D. Hadnadev et al., 2011)

The farinograph is a popular piece of equipment that is used to measure the torque required for mixing and kneading of the dough. This torque is recorded and analyzed to determine various parameters related to gluten strength (T. D. Hadnadev et al., 2011; Spies, 1990). The parameters commonly obtained are the dough development time (*DDT*, min), the dough stability time (*DS*, min), and the optimum water absorption (*FAB*, % at 14% moisture content). The *DDT* is a measure of the time the dough needs to be mixed until the point of maximum torque is achieved, as shown in Figure 2.4. The glutens from wheat flour doughs with longer *DDT* are classified as stronger when compared to doughs with shorter *DDT* (1 to 3 minutes) (AACC International, 2011; NDSU, 2018). At the dough development time, the dough is considered to have optimum consistency, i.e., maximum resistance to mixing, and to be fully hydrated (Spies, 1990).

Figure 2.4. Example of a farinogram obtained in this research study, showing dough stability time (*DS*, min) and dough development time (*DDT*, min). Red curve indicates the center of the farinograph curve and black curves indicate the top and bottom of the farinograph curve.



The *DS* is the time difference between the point where the top of the torque curve (top black curve) achieves the optimum consistency line (500 BU) and the point where the top of the curve (top black curve) leaves the optimum consistency line (500 BU), also shown in Figure 2.4. This parameter defines how resistant the dough is to the deformations of the mixing process (T. D. Hadnadev et al., 2011). Wheat flour doughs with strong gluten structure show more resistance to deformations that take place during the mixing process, thus having a longer *DS* (AACC International, 2011; T. D. Hadnadev et al., 2011; NDSU, 2018). Finally, according to the approved methodology (AACC International, 2011), *FAB* is the flour water absorption (*WA*) measured by the farinograph, at 14% moisture basis, and translates how much water is necessary to hydrate the wheat flour in order to form an optimally developed dough. The *FAB* varies mostly with protein content and level of damaged starch in the flour (T. D. Hadnadev et al., 2011).

A large and growing body of literature has investigated the gluten strength by performing farinograph measurements on doughs (Järvan et al., 2017; Malunga et al., 2019; Miś et al., 2012; Sobczyk et al., 2017; Struck et al., 2018; Wilson et al., 2020). By using the parameters *DDT* and *DS* from the farinograph, Sobczyk et al. (2017) compared the rheological properties of old, modern spelt varieties, and a common wheat variety (control for comparison), and used these parameters to select the spelt variety with the best suitability for bread baking. Farinograph tests can also be employed to study changes in gluten strength when new ingredients, such as dietary fiber, are added into the dough formulation (Miś et al., 2012; Struck et al., 2018). More recently, this empirical rheology tool was utilized to study the effect of genotype and sulfur fertilization treatments on the gluten strength of different wheat varieties (Wilson et al., 2020). Wilson et al. (2020) performed rheological tests utilizing the farinograph on flours from five wheat varieties receiving two levels of sulfur (0 and 22 kg ha^{-1}). The authors obtained valuable information on the effect of sulfur fertilization on gluten strength by observing, for example, significantly longer DS and *DDT* from wheat that received sulfur when compared to samples receiving no sulfur fertilizer (Wilson et al., 2020).

Farinograph tests are popular and well accepted in the baking industry because this piece of equipment allows one to obtain valuable information on dough performance, e.g., water absorption (*FAB*), time to achieve maximum torque (*DDT*) and resistance to deformation during mixing (T. D. Hadnadev et al., 2011). However, the dough's ability to satisfactorily perform during

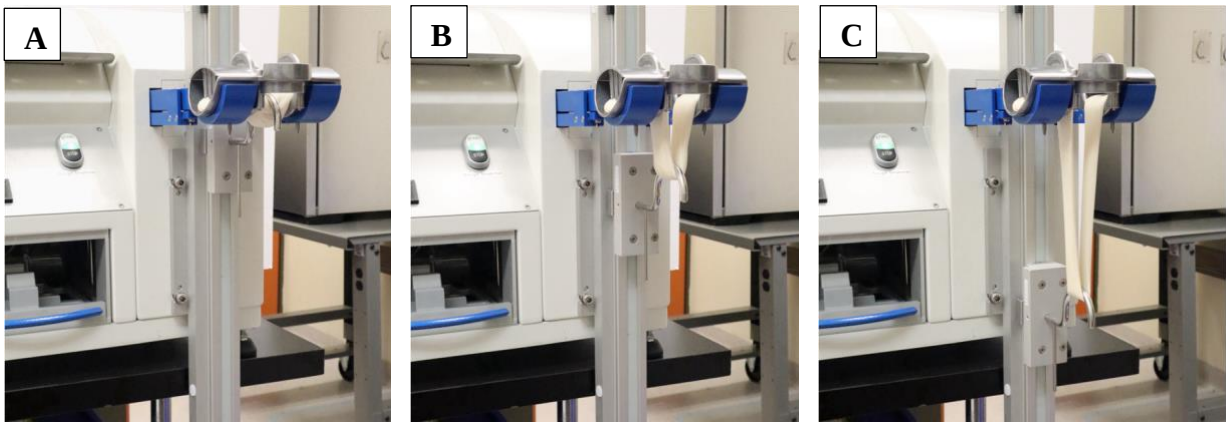
fermentation, i.e., allow bubble expansion and hold the produced gas, depends on the balance between extensibility and elasticity. An example of a well-recognized piece of equipment that measures dough extensibility and resistance to extension, i.e., elasticity, is the Brabender Extensograph (Bloksma, 1972; T. D. Hadnadev et al., 2011).

2.5.2 Extensional tests

The Brabender Extensograph is a piece of equipment that provides load-extension data for evaluation of the extensibility of the dough and its resistance to extension (Bloksma, 1972). A hook, traveling down at constant speed, stretches a cylindrical piece of dough (150 g) into a V-shaped conformation until the dough ruptures (Figure 2.5). As the hook stretches the sample, the force is recorded as a function of the extension. The main parameters obtained from this test are the maximum resistance to extension (R_{max}), in "Brabender Units" (BU), the extensibility (E) measured in millimeters (mm) at the point where the force drops to zero, and their ratio (R_{max}/E) (Bloksma, 1972). A dough suitable for good breadmaking shows greater resistance to extension, and thus higher R_{max}/E, compared to a dough which is more extensible with a lower R_{max}; in contrast, higher extensibility is a primary consideration for products such as cookies (S. Wang et al., 2013).

The SMS/Kieffer dough and gluten extensibility rig is a small version of the Brabender Extensograph that utilizes much less dough (0.4 g) and operates at somewhat lower strain rates (maximum of 0.25-0.30 s^{-1} at a speed of 300 mm min^{-1} (Dunnewind et al., 2004)) compared to the Brabender Extensograph (maximum of 0.35-0.40 s^{-1} at a speed of 840 mm min^{-1} (Dunnewind et al., 2004)). The test can be performed in any materials testing machine. As the dough is stretched upward by the hook, the force (N) and the distance traveled by the hook (mm) are recorded until the dough piece ruptures (Dunnewind et al., 2004). From the force-distance curve, the main parameters derived from this test are the maximum resistance to extension (R_{max}), now acquired in non-empirical units (N), and the dough extensibility (E) in mm (Anderssen et al., 2004).

Figure 2.5. Brabender Extensograph-E, situated in the Ellis Building, University of Manitoba, Winnipeg, MB, in operation. Hook touching the wheat flour dough (A), hook extending the dough (B), and dough extended just prior to rupture (C).



Dough properties subject to extensional forces can also be acquired from uniaxial elongational tests (Charalambides et al., 2006; McCann et al., 2016). A dough piece is clamped between two fixtures and the upper fixture moves upward (usually at a constant speed, but also at defined strain rates) (Uthayakumaran et al., 2000; Uthayakumaran, Newberry, et al., 2002), stretching the dough piece until it ruptures (Uthayakumaran et al., 2000). Some researchers have utilized a digital camera in order to obtain images of the dough piece while it is being stretched (Charalambides et al., 2006; Uthayakumaran et al., 2014). The elongational stress (*ES*, Pa) is calculated by dividing the recorded force (N) by the area of the dough piece (m^2), and the Hencky strain (ε_H) is calculated by Eq. 2.1:

$$\varepsilon_H = ln\left(\frac{L}{L_0}\right) \qquad \text{Eq. 2.1}$$

where L_0 is the initial length of the dough piece and L is the length at a given point in the test. Therefore, a piece of dough with a high elongational stress (*ES*) indicates a strong dough and a large Hencky strain at maximum stress represents an extensible dough (Uthayakumaran et al., 2014). From elongational tests, the rupture strain has also been reported as a means of characterizing the dough's extensional properties (Uthayakumaran, Beasley, et al., 2002). Moreover, the apparent extensional viscosity (*AEV*, Pa.s) can also be obtained from the elongational stress (Pa) and the strain rate (s^{-1}). Since length of specimens is substantially greater

than width, measurements of stress and strain in the initial stretching of the specimen permit a Young's modulus to be obtained (McCann et al., 2016).

Dai & Tanner (2012) showed that dough preparation for extensional testing has a large effect on the rheological results. Sample preparation methods that induced high levels of preorientation had higher *ES* values compared to less preoriented samples. Therefore, in order to obtain sound rheological data from uniaxial extensional tests for modelling dough extensional behavior, one must minimize deformations during dough preparation ($\varepsilon_H < 0.5$) (S. C. Dai & Tanner, 2012).

Uniaxial extensional tests can impose uncontrolled extensional deformations on highly extensible materials such as dough (Meerts et al., 2017b). To address this, an extensional method, where the sample is stretched between two rotational drums and sample deformation is measured through video imaging, was introduced by Meerts et al. (2017b). This extensional viscosity fixture (EVR) has recently been employed to perform extensional measurements on dough (Meerts et al., 2017b).

Extensional tests are able to provide necessary information on the ability of the dough to extend and to resist the extension process. Conducting extensograph tests, for example, allows one to understand how the wheat flour dough would perform during breadmaking steps where extensional forces are present (Cauvain, 2018; Dobraszczyk & Morgenstern, 2003). Moreover, as per Dobraszczyk & Morgenstern (2003), empirical tests, e.g., farinograph and extensograph, are preferred by industries due to the robustness of the equipment, the non-requirement of highly skilled personnel to perform the tests, and the acceptability of the data for quality control purposes. Moreover, empirical tests provide dough rheological properties in arbitrary units, such as Brabender Units (BU) (Dobraszczyk & Morgenstern, 2003). However, because empirical tests are performed by applying large stress or strains, which are not controlled nor uniform, these tests do not allow testing to be performed under small or controlled strains and stresses (Dobraszczyk & Morgenstern, 2003; Weipert, 1990). Fundamental shear tests, performed in controlled stress or strain rheometers, are a good alternative to evaluate dough rheological properties over a wide range of controlled stresses or strains and to provide dough mechanical properties in well-known physical units (Dobraszczyk & Morgenstern, 2003; Weipert, 1990). Therefore, a more comprehensive understanding of the relationship between wheat flour dough rheological

properties and free asparagine concentration can be obtained because it will be possible to evaluate this relationship at various magnitudes of strains and stresses.

2.5.3 Shear tests

Shear deformation tests are frequently employed to study wheat flour dough rheological properties. Although simple shear tests have been used, the vast majority of tests deform the dough specimen in pure shear mode. The shear properties of wheat flour dough can be evaluated within its linear viscoelastic region (LVR), i.e., where the shear moduli are independent of the applied stress or strain (Barnes, 2000). For wheat flour doughs, the LVR occurs where the maximum strain is usually 1% or less (Uthayakumaran, Beasley, et al., 2002; Uthayakumaran, Newberry, et al., 2002), and this test can be applied in linear oscillatory mode, simple shear flow, creep-recovery and stress relaxation tests. Some researchers have evaluated dough's rheological properties in shear oscillatory mode outside of the LVR (Yazar et al., 2016), but data are more complex to interpret compared to those acquired within the LVR since the viscoelastic modulus varies as a function of strain amplitude. Creep-recovery and stress relaxation tests can be employed both within and outside of the LVR. Properties derived from large strain measurements are likely more relevant because of the large strains induced in production processes (Dobraszczyk & Morgenstern, 2003).

In oscillatory shear tests within the LVR, a sinusoidal shear strain or stress at a specific angular frequency (ω) permits the shear modulus of the dough, $G(\omega)$, to be acquired. Because the stress and strain are out of phase in the oscillatory motion of the viscoelastic dough piece, the shear modulus is defined in elastic and viscous terms (Eq. 2.2):

$$G(\omega) = \mathrm{G}'(\omega) + i\mathrm{G}''(\omega) \qquad \text{Eq. 2.2}$$

The magnitude of the shear modulus $G^*(\omega)$ can be calculated by $\sqrt{(G')^2 + (G'')^2}$. The elastic response is represented by the storage modulus *G*' (Pa), which is a measure of the energy stored in the sample per oscillatory cycle. The viscous response is represented by the loss modulus *G″* (Pa) which is an indication of the viscous dissipation, the energy lost per cycle. Another parameter frequently discussed in the literature is the extent of the phase lag, usually expressed as the loss tangent ($\tan \delta = G''/G'$), which represents the balance between energy lost (viscously) and energy recovered (elastically).

The creep-recovery test is performed by "instantaneously" applying a constant shear stress (σ, Pa) and measuring the resulting shear strain ($\gamma(t)$) over a period of time. In the recovery step, the shear stress is removed and any recovery of the strain is measured, again as a function of time (Van Vliet, 2014). The shear strain in the creep-recovery test is often utilized to calculate the compliance $J(t)$ (Pa^{-1}):

$$J(t) = \frac{\gamma(t)}{\sigma} \quad \text{Eq. 2.3}$$

The stress relaxation test is performed by "instantaneously" inducing a specific shear strain (γ) in the sample and measuring the shear stress ($\sigma(t)$, Pa) as a function of time (Van Vliet, 2014). For viscoelastic materials, the stress decreases gradually until a final equilibrium stress is attained (Uthayakumaran, Beasley, et al., 2002). Most stress relaxation tests on breadmaking dough indicate that dough is a viscoelastic solid, since its equilibrium stress is larger than zero (Yang et al., 2019). In the case of a highly hydrated "dough" (such as a batter), the equilibrium stress is expected to approach zero (Letang et al., 2001). The measured shear stress as a function of time can be utilized to derive the stress relaxation modulus ($G(t)$, Pa) using Eq. 2.4 (Van Vliet, 2014).

$$G(t) = \frac{\sigma(t)}{\gamma} \quad \text{Eq. 2.4}$$

The stress relaxation modulus translates into how elastic-like the wheat flour dough is, i.e., doughs suitable for breadmaking have a larger relaxation modulus compared to dough used for other purposes (W. Li et al., 2003).

Although less common among dough rheological studies reported in the literature, oscillatory shear tests within the LVR can also be applied over time, at a fixed angular frequency, and $G^*(t)$ can be acquired. Linear oscillatory time sweep tests have been applied to study dough rheological properties over time (Bonilla et al., 2020; X. Zhao et al., 2019). Although most of the rheological studies that compose current literature use non-yeasted doughs, yeast is an essential ingredient in breadmaking and its activity changes the rheological properties of dough (Campbell & Martin, 2012; Meerts et al., 2018). When measured within the LVR and at a constant angular frequency, dough rheological properties are not expected to suffer great changes over time.

Therefore, linear oscillatory time sweep tests have been shown to be a successful tool to evaluate changes in dough rheological properties due to yeast activity, as measured by changes in shear moduli and tan δ as a function of time (Meerts et al., 2018; Verheyen et al., 2014).

Fundamental shear rheometry includes well-defined tests that allows the researcher to apply a wide range of defined and controlled stress or strains, obtaining accurate and reproducible results (Dobraszczyk & Morgenstern, 2003; Weipert, 1990, 2006). Because the applied stresses or strains are defined, fundamental rheometry allows one to obtain dough rheological properties in absolute and well-known physical units (Weipert, 2006). The possession of dough rheological properties measured in physical units allows the researcher to compare results obtained on rheometers from different manufacturers (Weipert, 2006). Additionally, because empirical tests only apply high deformation forces, it is not possible to obtain a comprehensive understanding of dough properties under small strains, e.g., strain imposed during fermentation (Weipert, 2006). Thus, the measurement of dough rheological properties over a wide range of strains will allow for a more comprehensive study on the relationship between gluten strength and free asparagine concentration. Moreover, as an alternative means of obtaining dough rheological properties at low strains and at high frequencies, low-intensity ultrasound, a low-cost and non-destructive technique, can be employed.

2.5.4 Low-intensity ultrasound

Ultrasound testing can be characterized as high-intensity (usually at frequencies up to 100 kHz), which is not used for materials' testing, and low-intensity ultrasound which usually involve the application of low power levels (<100 mW) and which can be conducted over a range of frequencies (usually 0.05-20 MHz). The latter is classified as a non-destructive test and can be applied to study the properties of materials, such as food (Povey & McClements, 1989). Low-intensity ultrasound tests are performed by applying an external excitation to a material so that a periodic displacement of its microstructural components propagates through it. This periodic displacement depends on the properties of the material, the frequency of the excitation, and how the excitation is applied to the material (Scanlon & Page, 2015). The mode of propagation of the ultrasonic waves can be classified as longitudinal or shear (Raj et al., 2004a). The latter displaces the material's structure perpendicularly to the wave's direction, being successfully realized only in solids, which can support shear wave propagation (Raj et al., 2004a). In fluids, shear waves are highly attenuated and cannot be easily detected after propagating into the sample (Raj et al.,

2004a). Longitudinal waves, on the other hand, cause displacement of the material's microregions in the same direction as the wave's propagation (Raj et al., 2004a). This type of wave propagation can successfully occur in solids, liquids, and gases (Raj et al., 2004a).

Regarding the techniques utilized for ultrasound tests, one can apply transmission or reflection modes. In the reflection technique, data is collected from ultrasound waves that are reflected from the material's surface, thus being a technique that is suitable for measuring the properties of highly attenuating materials (Kulmyrzaev et al., 2000). The transmission technique is characterized by wave propagation through the sample, thus being able to interact with the material's interior and not only its surface (Coupland, 2004; Scanlon & Page, 2015).

Parameters that one can obtain from wave propagation through a material are the wave's attenuation coefficient (α) and phase velocity (v_p) (Povey & McClements, 1989; Scanlon & Page, 2015). The wave attenuation occurs due to the decrease of amplitude as the wave travels through a material, caused by losses occurring in the material's matrix as well as scattering from any obstacles embedded inside it. Other effects that can cause a reduction in amplitude include losses at interfaces between the transducers and sample, as well as wave diffraction due to obstacles and interference phenomena; these effects need to be taken into account in order to determine the material's attenuation coefficient (Povey & McClements, 1989). In homogeneous materials, the wave attenuation occurs mainly due to the sample's viscosity or by absorption of the acoustic energy by the sample (Cheeke, 2002). In heterogeneous materials, such as wheat flour dough that contains a dough matrix phase and bubbles, the wave attenuation occurs mainly due to scattering. The wave attenuation due to scattering happens, for example, when the propagating signal encounters a large difference in density, i.e., between air bubbles and the dough phase, when traveling into the dough. The phase velocity is a measurement of how fast the phase of the wave, at a certain frequency (ω), travels in the dough's structure (Koksel et al., 2016; Povey, 1997). The attenuation coefficient (α), phase velocity (v_p) and the sample's density (ρ) are then utilized to calculate the longitudinal moduli $M(\omega)$. Similar to the shear modulus, the longitudinal modulus is defined in elastic and viscous terms, as per Eq. 2.5 (Khorshidi, 2016).

$$M(\omega) = M'(\omega) + iM''(\omega) \qquad \text{Eq. 2.5}$$

The magnitude of the longitudinal modulus $M^*(\omega)$ can be calculated by $\sqrt{(M')^2 + (M'')^2}$. The elastic response is represented by the longitudinal storage modulus M' (Pa), calculated using Eq. 2.6, and the elongational viscous response is represented by the longitudinal loss modulus M'' (Pa), calculated using Eq. 2.7 (Elmehdi, 2001; Khorshidi, 2016). Analogous to shear oscillatory tests, the longitudinal loss tangent ($\tan\delta = M''/M'$) can also be calculated, representing the balance between viscous and elastic responses.

$$M'(\omega) = \frac{\rho {v_p}^2 \left[1-(\alpha v_p/2\omega)^2\right]}{\left[1+(\alpha v_p/2\omega)^2\right]^2} \quad \text{Eq. 2.6}$$

$$M''(\omega) = \frac{2\rho {v_p}^2 (\alpha v_p/2\omega)}{\left[1+(\alpha v_p/2\omega)^2\right]^2} \quad \text{Eq. 2.7}$$

A considerable amount of literature has been published applying low-intensity ultrasound tests to evaluate bubbles and mechanical properties in non-yeasted (Koksel et al., 2016; Koksel & Scanlon, 2018; Peressini et al., 2016, Elmehdi, 2001) and yeasted doughs (Lee et al., 2004a; Skaf et al., 2009; Strybulevych et al., 2012). Lee et al. (2004) performed low-intensity ultrasound tests at a frequency of 5 MHz to evaluate the fermentation process in wheat flour doughs. The authors encountered difficulties in obtaining accurate results after 30 minutes of fermentation due to signal scattering and high attenuation during bubble growth. However, they concluded that ultrasound is a powerful tool to monitor the fermentation process as well as to determine dough rheological properties during fermentation (Lee et al., 2004a). Later, Strybulevych et al. (2012) proposed an alternate method of evaluating bubble growth during fermentation by a reflectance technique. This technique was effective for observing changes during fermentation at high volume fractions of CO_2 in the dough, which is more challenging when using transmission due to high attenuation during fermentation (Strybulevych et al., 2012). Moreover, low-intensity ultrasound was also successfully applied on non-yeasted dough to study the effect of bakery enzymes (Koksel & Scanlon, 2018) and other ingredients (Peressini et al., 2016) on dough properties.

2.6 Current knowledge on wheat protein and free asparagine and their relationship to dough rheological properties

As previously discussed, an increase in nitrogen fertilizer delivered to wheat crops causes an increase in wheat grain protein content. Therefore, understanding the effect of the grain's protein content on dough rheology is desirable in order to evaluate the direct impact of nitrogen and sulfur fertilization, as well as environment and genotype, on dough properties.

However, disentangling the effects of protein content on dough rheology from those of protein quality is not straightforward. As remarked by Edwards et al. (1999), "generally, studies that compared doughs of variable protein content used wheats of diverse genetic origin that varied also in gluten strength, making it difficult to isolate effects solely due to protein content" (p. 642). Further complicating attempts to resolve the effects of protein content per se on the rheology of dough is the fact that higher protein content flours generally have higher optimal water absorptions for dough make-up. Not only does additional water depress the steady state shear (Lefebvre & Mahmoudi, 2007) and elongational (Uthayakumaran, Newberry, et al., 2002) viscosities, and the modulus of the dough (Meerts et al., 2017a; Uthayakumaran, Newberry, et al., 2002), even to the point of making dough's solid character disappear (Letang et al., 2001), it also amplifies rheological differences between wheats of different protein content/quality (Navickis et al., 1982). Therefore, although I have attempted to delineate studies examining protein content effects on dough rheology from studies examining protein quality, a clean separation is not possible.

2.6.1 Effects from protein content

Linear oscillatory shear tests on bread wheat flour doughs made at or close to optimal absorption reveal that G' is greater than G'' within the usual analyzed frequency range, indicating that breadmaking doughs behave predominantly elastically (Kaur et al., 2013; Magana-Barajas et al., 2012; Marchetti et al., 2012; Meerts et al., 2017b; Yovchev et al., 2017). Power law relationships between G' and G'' versus frequency are usually reported with fractional exponents for the relationship (Leroy et al., 2010; Peressini et al., 2000).

The relationship between the protein content of the flour and the rheological properties of their resulting doughs (formulated at optimal water absorption) was studied for seventeen European wheat cultivars with flour protein contents ranging from 11.6 to 16.9% (Van Bockstaele et al., 2008a, 2008b). Although the authors' experimental choice does not allow the effect of protein content alone to be studied, strong negative correlations to protein content for both G' and

G" were observed. It is worth noting that almost identical correlations between shear moduli and water absorption were also observed (Van Bockstaele et al., 2008b), so that the sole effect of protein content cannot be teased out from the study.

Although conducted with durum wheat milled to semolina granulation, Edwards et al. (1999) conducted an analysis of protein content on shear modulus values at a constant water absorption (50% on flour weight basis). They composited commercially grown wheat samples from multiple Canadian locations so as to prepare four protein contents treatments (ranging from 8.9 to 14.7%) for three crop years. By employing a range of genotypes, the influence of genotype on differences in protein content was diminished. No relationship was observed between *G*' and protein content when averaged over the three crop years (Edwards et al., 1999), but $\tan \delta$ was strongly positively related to protein content ($r = 0.93$). This was ascribed to the relative proportion of gliadins increasing with increase in wheat protein content when grown under field conditions. For two varieties with different protein contents, Tronsmo et al. (2003) also observed a higher $\tan \delta$ in the optimally hydrated doughs of higher protein content (and presumably higher water absorption). It is worth remarking that Van Bockstaele et al. (2008b) also observed a strong positive relationship between $\tan \delta$ and protein content. Small strain shear measurements seem to indicate that extra protein dissipates shear strain energy, and in many experiments, this effect is exacerbated by the added water content associated with preparation of the dough to its optimal water absorption. Therefore, designing experiments to separate water absorption effects from protein content effects is an important consideration for interpreting rheology outcomes.

When evaluating the stress relaxation properties of doughs made from three wheat varieties grown in different locations, Uthayakumaran et al. (2012) observed that the flour protein content for all three wheat varieties was higher (15.0-16.0%) for locations high in soil nitrogen concentration and low rainfall than for more rainfed locations (protein content about 10.0%). Doughs were formulated at optimum absorption, but differences between water absorption values from the three sites were less in relative terms compared to protein content differences. Measurements at a small strain value showed that the shear relaxation modulus, $G(t)$, was lower as protein content increased (Uthayakumaran et al., 2012).

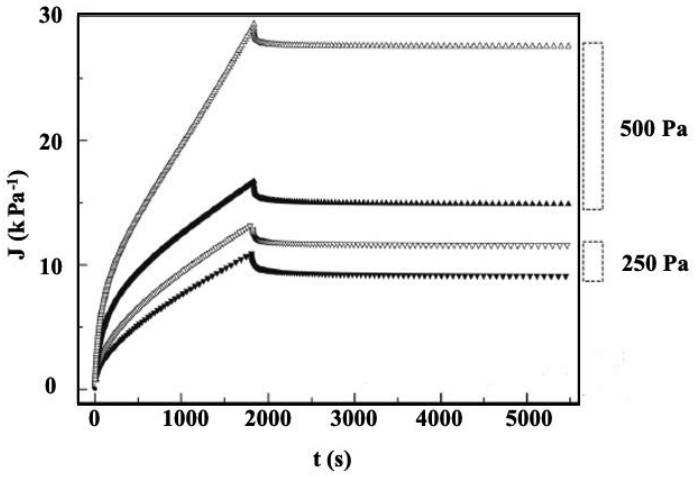
Magana-Barajas et al. (2012) studied the stress relaxation process (shear strain of 15%) of wheat flour doughs made with different wheat varieties. They reported higher initial $G(t)$ and slightly lower relaxation times for doughs made from higher protein wheat varieties (Magana-

Barajas et al., 2012). In a stress relaxation study by W. Li et al. (2003), where 5% shear strain was applied to doughs from different wheat varieties, higher protein content doughs had larger $G(t)$ values, as well as longer relaxation times when compared to lower protein content doughs (W. Li et al., 2003).

Van Bockstaele et al. (2008a) conducted creep and recovery testing of wheat flour doughs made from the same seventeen European wheat cultivars as in their oscillatory study. The maximum creep strain (%) and maximum recovery strain (%) were observed to be positively correlated with the wheat flour protein content both within and outside the linear viscoelastic region (Van Bockstaele et al., 2008a). Interestingly (because of different experimental designs), the same outcome was observed by Edwards et al. (1999) for maximum creep strain (%) measured outside the linear viscoelastic region. Van Bockstaele et al. (2008b) also showed that outside of the linear viscoelastic region, doughs made from higher protein content flours showed lower relative recovery. This might be due to greater viscous losses during the creep step for the higher protein content doughs, consistent with the conclusion of Edwards et al. (1999) on the effect of gliadins, even though genotype effects were still evident for the European study.

The importance of the magnitude of the shear stress in measurements of the shear properties of doughs was emphasized in a study by Meerts et al. (2017b). Creep measurements in the linear viscoelastic zone showed that wheat flour doughs with higher protein content (15.1%) had a larger maximum creep strain (%) compared to those with lower protein content (12.4%), i.e., results were compatible with those of Van Bockstaele et al. (2008a, 2008b) and Edwards et al. (1999). However, with the application of shear stresses well outside the LVR, the lower protein content dough deformed to a greater extent, and even more so as the shear stress was increased further (Figure 2.6) (Meerts et al., 2017b). Moreover, the wheat flour dough with higher protein content demonstrated a greater recovery capability, thus a more elastic-like behavior, compared to the lower protein content dough (Meerts et al., 2017b).

Figure 2.6. Creep and recovery compliance over time for high protein content (▲) and low protein content (▽) wheat flour doughs subject to shear stresses of 250 Pa and 500 Pa. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature. Food and Bioprocess Technology. The interplay between the main flour constituents in the rheological behaviour of wheat flour dough. Meerts, M., Cardinaels, R., Oosterlinck, F., Courtin, C. M., & Moldenaers P., (2017).



Elongational tests using an extensional viscosity fixture (so that the dough was stretched in a true extensional mode) were conducted on wheat flour doughs from a high protein (15.1%) and a low protein content (12.4%) wheat variety at the same strain rate (Meerts et al., 2017b). A higher extensional viscosity was observed for the higher protein content dough (Meerts et al., 2017b), indicating that the high protein variety dough had a greater resistance to extension.

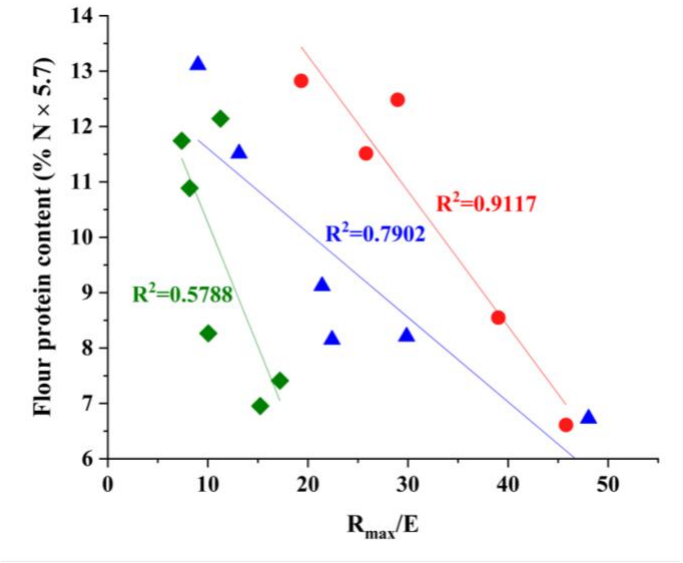
Elongational tests in a tensile mode have also been used to evaluate the effects of protein on dough properties. The elongational stress (*ES*, Pa) for doughs made from three wheat varieties was significantly higher in locations that yielded flours with high protein content (Uthayakumaran et al., 2012). Pursuing a similar experimental strategy as Uthayakumaran et al. (2012), McCann et al. (2016) used two high and two low protein content flours acquired from commercial mills to formulate doughs to their optimal water absorption. They reported higher elongational stress and maximum Hencky strain (ε_H) for wheat flour doughs made from higher protein content flours. The maximum elongational viscosity (Pa.s) and rupture strain were also reported to be greater for doughs made from higher protein content wheats (Uthayakumaran, Newberry, et al., 2002).

Wheat flour protein content was shown by Uthayakumaran et al. (2012) to positively correlate with dough rheological parameters such as the maximum force sustained by the dough under extension (R_{max}, N) and extensibility (E, mm) with the Kieffer test. Also, doughs made from two of the wheat varieties (Janz and Gregory) showed greater extensibility (E) for the locations that yielded high flour protein content. On the other hand, the dough extensibility (E, mm) of a third wheat variety, Guardian, was not greatly affected by location. A higher R_{max}/E was observed for wheat flour doughs made from higher protein wheat compared to lower protein wheat (Uthayakumaran et al., 2012).

In a study by Godfrey et al. (2010), it was demonstrated that for the same wheat variety grown under different nitrogen fertilization treatments, rheological parameters such as R_{max} and R_{max}/E decreased and E increased with increasing protein content. An increase in grain protein content and dough E with increasing nitrogen fertilization demonstrates that there are differential responses of gluten fractions to nitrogen fertilization, e.g., the gliadins increased to a greater extent than the glutenins when nitrogen applications increased from 0 to 288 kg ha^{-1} (Godfrey et al., 2010). Similarly, Janković et al. (2015) found an inverse relationship between wheat flour dough R_{max} and wheat flour protein content for five wheat genotypes with a narrow dispersion of wheat flour protein content (8.9% to 10.5%). The variety with the lowest flour protein content had an R_{max} of 235.0 BU and E of 91.0 mm (R_{max}/E = 2.58), while the variety with the highest protein content had an R_{max} of 102.5 BU and E of 80.0 mm (R_{max}/E = 1.28). As a result of following the standard extensograph method, Janković et al. (2015) formulated doughs to their optimal water absorptions. The values for R_{max} and E in relation to protein content are therefore likely conflated with water effects as well as those attributable to protein content.

In the extensograph study of Godfrey et al. (2010), as wheat flour protein content, and concomitantly dough water content, increased, R_{max} decreased and E increased, causing a decrease in R_{max}/E (Figure 2.7). The reproducibility of these results over 3 crop years indicates that wheat flour dough made from this single breadmaking wheat variety showed greater elastic-like behavior at lower applications of nitrogen to the wheat plant and corresponding lower flour protein contents (Godfrey et al., 2010). One difficulty is in evaluating whether this is due to protein content per se or due to the combined effects of protein and water (since the doughs had been prepared to optimal consistency using different water contents).

Figure 2.7. Relationship between R_{max}/E and flour protein content for the crop years of 2005 (▲), 2006 (●) and 2007 (◆). Graph was created using the data provided in Tables S2 and S3 of the Supporting Information from Godfrey et al. (2010).



Contrasting results to those of Godfrey et al. (2010) were obtained from a glasshouse study by Tyler et al. (2015). After genetically modifying a wheat line to knock down a gene responsible for accumulation of storage proteins, wheat flour protein content decreased from 15.0% to 13.6%. The extensograph parameter R_{max}/E was larger for the control wheat compared to the transgenic one, indicating that wheat with higher protein content resulted in more elastic doughs compared to wheat with lower protein content (Tyler et al., 2015); their study was presumably carried out at optimal water absorption.

Another study evaluated protein content variability for three wheat genotypes grown at the same locations in 2008 and two wheat genotypes in 2009 (Hasniza, Wilkes, et al., 2014). The results of a Kieffer test on whole meal flour dough made with the grains grown in 2008, prepared at optimum absorption, indicated that R_{max} and E were both positively correlated with the nitrogen content of the soil (Hasniza, Wilkes, et al., 2014). Additionally, positive and strong correlation coefficients ($P<0.01$) were observed between grain protein content and both R_{max} and E for all three wheat varieties (Hasniza, Wilkes, et al., 2014). Higher soil nitrogen concentration in two of the locations supported the authors' conclusions of the significant role of nitrogen supply in wheat grain protein content and its effects on dough rheology (Hasniza, Wilkes, et al., 2014).

Fewer studies have included information on growing conditions for their wheat samples, but effects of protein content on dough rheological properties have been reported (M. Hadnadev et al., 2014; Horvat et al., 2015; Kaur et al., 2013; Magana-Barajas et al., 2012; McCann et al., 2016; Meerts et al., 2017b). As an example, bread wheat varieties with protein contents of 12.0% and 13.6% were demonstrated to yield doughs with greater R_{max} compared to lower protein wheat varieties suitable for noodles (protein content of 11.7%) and cakes (9.8%) (Edwards et al., 2001). The R_{max}/E values for wheat flour doughs varying in protein content were reported to range from values below 1.0 up to values as high as 3.9 (Edwards et al., 2001). In a study of four Norwegian wheat cultivars providing 6 levels of flour protein content, the R_{max} and E of wheat flour doughs acquired from both extensograph and Kieffer tests increased as the protein content of the same wheat variety increased (Tronsmo et al., 2003).

Rheological tests that evaluate dough mechanical properties during mixing, such as the farinograph, were also performed to investigate the effects of protein content on dough properties (Godfrey et al., 2010; Katyal et al., 2017; Kaur et al., 2013; Zhu et al., 2001). For example, although no information on growing environment and fertilization treatments were provided, Katyal et al. (2017) utilized 12 Indian wheat varieties, which were classified as hard, medium-hard, and extraordinarily soft, to perform farinograph tests (Katyal et al., 2017). Overall, the authors obtained higher protein content for wheat flours that were classified as hard wheat when compared to extraordinarily soft wheat varieties (Katyal et al., 2017). Moreover, the authors reported that wheat flour protein content was correlated ($p < 0.05$) to *FAB* ($r = 0.606$) and to *DDT* ($r = 0.644$), with no correlation between protein content and *DS* (Katyal et al., 2017). Previously, a study published by Kaur et al. (2013) aimed to evaluate the properties of wheat flour doughs prepared from 18 Indian wheat varieties harvested in the year 2012, with no information on the growing environment and fertilization treatments (Kaur et al., 2013). In this study, the authors also reported that protein content was positively correlated ($p < 0.05$) to *FAB* ($r = 0.583$) and negatively correlated to *DS* ($r = -0.677$), with no significant correlation between protein content and *DDT* (Kaur et al., 2013).

From farinograph tests, Godfrey et al. (2010) observed that flour protein content was positively correlated ($p < 0.05$) to *FAB* ($r = 0.757$), *DDT* ($r = 0.668$), and *DS* ($r = 0.830$) (Godfrey et al., 2010). An increase in *DDT* and *DS*, as flour protein content increased, was also observed by Zhu et al. (2001) when studying six Chinese wheat varieties and 11 Australian wheat varieties

grown under two levels of nitrogen (0 and 200 kg ha^{-1}). The authors observed that wheat flour protein content significantly ($p < 0.05$) increased with nitrogen fertilization (Zhu et al., 2001). The application of nitrogen also caused a significant ($p < 0.05$) increase in *DDT* and *DS*, with Australian wheat varieties showing overall stronger glutens (higher *DDT* and *DS*) when compared to Chinese wheat varieties (Zhu et al., 2001). Therefore, according to the literature, farinograph parameters have shown to be significantly correlated to wheat protein content. Farinograph water absorption (*FAB*) is shown to be positively correlated to protein content. Factors affecting *FAB* have been studied by H. Sapirstein et al. (2018), who evaluated wheat varieties from Canada Western Red Winter (CWRW), Canada Western Special Purpose (CWSP) and breeder lines. Among the studied samples, 26 wheat varieties were grown in 2011 and 52 wheat varieties were grown in 2012. In this study, H. Sapirstein et al. (2018) observed that, for both years, wheat and flour protein content were positively correlated ($p < 0.05$) to *FAB*, with damaged starch, water extractable arabinoxylan content, and flour particle size being other factors affecting *FAB* (Sapirstein et al., 2018).

Although the positive correlation between *FAB* and protein content was unanimous among the previously cited studies, the correlation of protein content to *DDT*, and *DS* was not always significant and positive. Parameters such as *DDT* and *DS* are commonly utilized to evaluate the gluten strength of a flour (Zhu et al., 2001), therefore, protein composition is an important factor that can explain the opposite relationships between protein content and *DDT* or *DS* (Kaur et al., 2013). For example, Diep et al. (2014) performed farinograph tests using nine wheat varieties grown in Western Canada representing two wheat classes; Canada Western Red Spring (CWRS, five varieties) and Canada Prairie Spring Red (CPSR, four varieties) (Diep et al., 2014). The authors reported flour protein content varying from 11.3% to 15.4%, with CWRS generally having higher protein content (13.9% – 15.4%) than CPSR (11.3% – 12.3%) (Diep et al., 2014). According to the authors, although it would be commonly expected that samples with higher protein content would result in stronger wheat flour doughs, farinograph tests showed the opposite result (Diep et al., 2014). CWRS varieties, containing high protein content, resulted in weaker wheat flour doughs when evaluated by *DDT*, whereas *DS* was not an adequate parameter to discriminate between both wheat classes (Diep et al., 2014), thus suggesting that wheat protein composition is also a determinant factor to produce strong doughs.

In order to thoroughly investigate the dough rheological properties of the nine varieties, Diep et al. (2014) performed low-intensity ultrasound measurements on noodle doughs prepared

from the flours. Low-intensity ultrasound was conducted using transducers with a central frequency of 40 kHz, obtaining the attenuation coefficient and the phase velocity, which were utilized to calculate longitudinal moduli and their respective loss tangent (Diep et al., 2014). This study reported that CWRS wheat varieties resulted in the highest phase velocities when compared to CPRS varieties (Diep et al., 2014). The authors hypothesized that a higher number of interactions between proteins could increase the phase velocity, which was also shown by the strong positive correlation between protein content and phase velocity ($r = 0.92$, $p<0.001$) (Diep et al., 2014). However, the authors also pointed out that protein content might not be the only factor affecting phase velocity because, for example, some samples with different protein content showed similar phase velocities (Diep et al., 2014). Regarding the attenuation coefficient, the authors observed that noodle doughs prepared with varieties in the CWRS wheat class showed overall higher attenuation coefficients than CPSR varieties (Diep et al., 2014). However, because not all CWRS wheat varieties resulted in higher attenuation coefficients when compared to CPSR varieties, the authors suggested that other factors, besides protein content, might be influencing the wave attenuation capacity of the samples (Diep et al., 2014). Finally, when the authors calculated the longitudinal moduli, they observed overall higher M' for samples prepared from CPRS varieties when compared to CWRS varieties, indicating a more elastic-like behavior in samples with less protein content (Diep et al., 2014). Moreover, CWRS varieties presented more viscous-like behavior than CPRS varieties as shown by the higher M'' and $\tan\delta$ values (Diep et al., 2014).

A previous study also used low-intensity ultrasound to evaluate the rheological properties of Spanish wheat flour doughs (García-Álvarez et al., 2006). García-Álvarez et al. (2006) performed low-intensity ultrasound at a frequency of 100 kHz on doughs prepared from 36 different wheat flours. Although the authors did not provide the protein content of their wheat flours, they observed that samples that were classified as “strong” produced doughs resulting in lower attenuation coefficients and higher phase velocities when compared to samples classified as “weak” (García-Álvarez et al., 2006). The authors also evaluated doughs prepared from wheat flours classified as “strong bread flour”, “bread flour”, and “biscuit flour” according to their gel-protein shear storage moduli, as reported by Alava et al. (2001). The protein content among the studied wheat flours varied from 8.7% to 12.0% (Alava et al., 2001). For this group of samples, the authors could not obtain a clear discrimination of different wheat flours based on attenuation coefficient and phase

velocity, as samples with different classification had similar outcomes for these two parameters (García-Álvarez et al., 2006). It could be speculated that, because wheat flours were classified according to their gel-protein shear storage moduli, the authors could have obtained a better discrimination among samples if the longitudinal moduli of the wheat flour doughs were calculated.

More recently, Khorshidi et al. (2019) studied the low-intensity ultrasound capability of discriminating between wheat varieties using 11 wheat samples that were selected in order to compose a group with a wide variation in grain and flour characteristics (Khorshidi et al., 2019). The authors conducted low-intensity ultrasound tests at a frequency of 10 MHz, obtaining the attenuation coefficients and phase velocities for the wheat flour dough samples (Khorshidi et al., 2019). From the attenuation coefficient and phase velocity, the authors also calculated the longitudinal storage (M') and loss moduli (M''), and their longitudinal loss tangent ($\tan\delta$) (Khorshidi et al., 2019). Differently than Diep et al. (2014), Khorshidi et al. (2019) did not find significant correlations at $p < 0.05$ for protein content against phase velocity, but significant ($p < 0.05$) correlations were found for grain protein content ($r = 0.702$) and flour protein content ($r = 0.741$) against attenuation coefficient. Moreover, correlations ($p < 0.05$) were found for grain protein content ($r = 0.677$) and flour protein content ($r = 0.621$) against M'' and for grain protein content ($r = 0.760$) and flour protein content ($r = 0.766$) against $\tan\delta$. Therefore, samples with higher protein content resulted in wheat flour doughs with higher $\tan\delta$ values, thus more fluid-like characteristics (Khorshidi et al., 2019). Finally, although no correlation was found between protein content and M', farinograph parameters *DDT* and *DS* were positively ($p < 0.05$) correlated to M' and phase velocity, suggesting that stronger dough samples, i.e., higher *DDT* and *DS* from farinograph, resulted in higher phase velocities and M' (Khorshidi et al., 2019).

As dough rheological properties are affected by wheat protein content, environment, genotype, and nitrogen fertilization treatments affect dough rheological properties. However, as shown throughout this section, wheat protein content is not the only factor involved in the rheological characterization of the dough as a viscoelastic material. Therefore, the role of protein composition, particularly the balance of nitrogen partitioned into gliadins or glutenins, needs to be investigated to achieve a clear understanding of the relationship between nitrogen functionality and wheat flour dough rheology.

2.6.2 Effects from protein composition

Attempts have been made to distinguish the effects of protein quality from those of protein content and the confounding effects of enhanced water absorption. Edwards et al. (1996) reported little difference in the *G*' values of doughs from flours milled from wheats of different common wheat classes (ranging in protein content from 9 to 12%). Given the result of Navickis et al. (1982) that there is better discrimination of dough properties of contrasting classes at higher moisture content, this outcome is not unexpected in a study where doughs were made up to noodle formulation moisture content (Edwards et al., 1996). Nevertheless, a similar outcome was reported by Kim et al. (2008) when they blended a set of 9 hard winter wheat flours of diverse origin to produce two flours with approximately the same protein content and water absorption, but with considerable differences in protein quality based on dough mixing performance. They reported no big differences in *G** for doughs made from these two contrasting quality flours. However, these results differ from the outcome reported by Lindahl & Eliasson (1992). In their study, the shear modulus in the linear viscoelastic regime was compared in doughs that had been made from common wheat or durum wheat at two fixed moisture contents. In both cases, the *G*' of the durum wheat dough was larger than *G*' of the dough made from bread wheat. However, ascribing differences in shear modulus to the effect of wheat protein quality alone is difficult due to the large difference in protein content between the two wheats of their study (10 versus 15%).

The distinctive nature of durum wheat protein quality on the shear properties of dough was confirmed by Peressini et al. (2000). In a study using semolina-sized particles, a range of dough qualities were formulated at a constant moisture content by adding increasing amounts of durum wheat to a bread wheat. The shear modulus in the linear viscoelastic region increased as the amount of durum wheat particles in the blend increased. In addition, Peressini et al. (2000) reported that the exponent for the relationship of shear modulus against frequency decreased as the durum wheat content increased, which the authors ascribed to a greater degree of coordination in the protein network that conferred greater elastic character to the dough.

Substitution studies that have examined the influence of specific protein fractions on the shear properties of dough have pointed to the importance of gliadins and glutenins in changing the shear stiffness of dough, with quality differences evident for glutenins but not for gliadins. For example, in a study on durum wheat where gliadins from three donor cultivars were added, *G*' and *G*'' values decreased relative to those of the base semolina dough (Edwards et al., 2003), and the

effects did not depend on the donor of the gliadins. In contrast, G' and G'' increased with glutenin enrichment, and the stiffening was enhanced with increasing mixing strength of the donor cultivar. The deleterious effect of gliadins on shear stiffness has also been reported by Khatkar et al. (2002), for glutens made from the breadmaking wheat variety Hereward.

Protein quality, as defined by the Gli/Glu ratio (generated intrinsically in the field rather than via substitution in the lab), plays an important role in dough rheological properties. Marchetti et al. (2012) studied the properties of dough made from three wheat varieties within the LVR and reported an inverse relationship between their Gli/Glu ratios and their dynamic shear moduli G' and G'' as well as a direct relationship between wheat Gli/Glu and tan δ values of the dough (Marchetti et al., 2012). Amongst the wheat varieties studied by Kaur et al. (2013), CBW38 and C306 possessed G' values of 17.1 kPa and 5.1 kPa, respectively. The Gli/Glu ratio values of these two varieties determined by Barak et al. (2013) were 0.86 for CBW38 and 1.17 for C306. Therefore, the results from Kaur et al. (2013) confirm that an inverse relationship exists between G' and Gli/Glu. These linear oscillatory shear results concur with previous findings where samples with lower Gli/Glu ratios, from their original or glutenin-enriched flours, possessed higher G' and lower tan δ (Edwards et al., 2003).

Shear stress relaxation measurements on three Australian wheat varieties grown at four locations revealed a significant negative correlation coefficient between Gli/Glu and the relaxation modulus ($G(t)$) within the LVR of the doughs (Uthayakumaran et al., 2014). Thus, even at small strains, i.e., γ = 0.1%, doughs whose protein has a lower Gli/Glu ratio are stiffer compared to doughs with a higher Gli/Glu composition (Uthayakumaran et al., 2014).

In a stress relaxation study of durum doughs made from six cultivars of different glutenin sub-unit composition, but with flour protein contents of very similar values, Rao et al. (2001) showed that the shear stress relaxation modulus decreased as protein quality (defined by mixing strength) decreased. In breadmaking wheats of contrasting breadmaking properties (and protein content varying from 10 to 13%), the stress relaxation modulus did not show differences (Safari-Ardi & Phan-Thien, 1998). Nevertheless, as the shear strain used to induce the stress relaxation test was increased, discrimination did occur in the stress relaxation spectra with a larger relaxation modulus observed for doughs made from the stronger varieties.

In a creep test study by Edwards et al. (2001), where all doughs were tested at a common moisture content, the steady flow viscosity was not substantially different for doughs prepared

from wheats from a very wide range of performance attributes (extra strong bread to cookie). Nevertheless, all viscosities were less than the viscosities of doughs made from durum wheat varieties. Steady flow viscosity was observed to be 19.8 MPa.s for a dough made from a durum wheat cultivar with a Gli/Glu ratio of 0.6 compared to 5.1 MPa.s for a cultivar whose Gli/Glu ratio was 0.86. In a study on the effect of short-term post-harvest storage of wheat (up to 50 days) on the dough strength of three hard white winter varieties, Hadnadev et al. (2014) observed that one variety had a significant glutenin content increase over the storage period. Under constant shear stress (50 Pa), the variety with greater amounts of the glutenin fraction had lower maximum creep compliance (0.8 kPa^{-1}) (M. Hadnadev et al., 2014).

The importance of specific glutenin subunits on the creep response of dough was highlighted in a study by Lefebvre & Mahmoudi (2007). They evaluated the steady-state linear viscosity of doughs made from four near-isogenic lines (NIL) with differing (5+10 and 2+12) glutenin sub-units that had been grown in three different European locations (Sweden, England and France). All doughs were formulated to a constant 46.1% water content on a dough weight basis. With the exception of one of the NILs, the steady state viscosity of the doughs made from the good breadmaking lines (those with the 5+10 subunits) was on average 40 to 90% greater (varying according to country where the wheat had been grown).

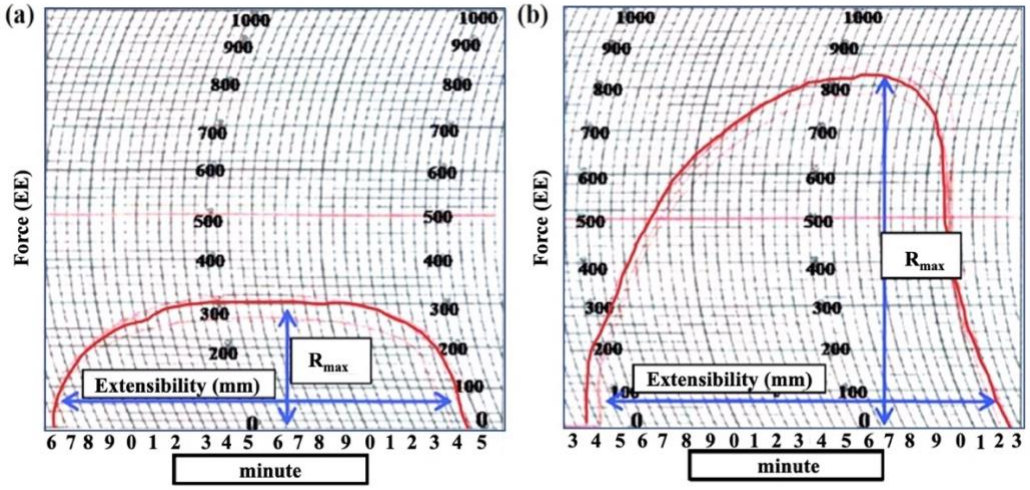
Meerts et al. (2017b) conducted extensional rheology tests on doughs made from commercially milled flours of contrasting strength (but also with a substantial difference in protein content: 15.1 and 12.4% for strong and weak, respectively), but using an extensional viscosity fixture (which better controls the extensional deformation induced in the dough). Despite using optimal absorptions (so that the strong dough had a higher moisture content), the extensional viscosity of the dough made from the strong flour was greater than that made from the weaker flour (Meerts et al., 2017b). Similar findings were obtained for commercially milled Australian flours (but with a protein content difference of only 13.9 to 12%) that were used to create dough specimens that were subject to constant strain-rate tensile tests (Uthayakumaran, Newberry, et al., 2002). The maximum elongational viscosity was greater for the strong flour dough at all strain rates. The authors also showed that moisture content depressed elongational viscosity values regardless of flour type or strain rate (Uthayakumaran, Newberry, et al., 2002).

The Gli/Glu ratio as a protein quality parameter also plays an important role in the results of extension tests on dough, just as it does in shear tests. In a study on the effect of nitrogen

fertilization on wheat grain composition and end-use quality, Godfrey et al. (2010) observed that the Gli/Glu ratio was positively correlated with nitrogen content in the flour, wheat flour dough *E* (mm) and negatively correlated with R_{max} (Godfrey et al., 2010). Hasniza, Wilkes, et al. (2014) evaluated the Gli/Glu ratio for various wheat varieties grown in different locations. The Gli/Glu ratio was negatively correlated ($p<0.05$) with the average daily maximum temperature during grain filling, and positively, but not significantly, correlated with nitrogen content in the soil (Hasniza, Wilkes, et al., 2014). A highly significant ($p<0.001$) positive correlation coefficient was found between the Gli/Glu ratio and dough *E*, but not between Gli/Glu ratio and R_{max} ($p<0.05$) (Hasniza, Wilkes, et al., 2014).

S. Wang et al. (2013) studied the properties of doughs made from the Chinese Spring wheat variety and a genetically modified line (Gli/Glu ratios of 0.61 and 0.47, respectively). The dough made from the wheat with a significantly higher Gli/Glu ratio had a significantly lower R_{max} and R_{max}/E compared to the other dough (Figure 2.8). Employing a Kieffer rig for their extensional test, P. Zhang et al. (2014) also reported a lower R_{max} for a wheat variety with a higher Gli/Glu ratio (1.92) as opposed to those with a lower Gli/Glu ratio (1.33). The P. Zhang et al. (2014) study also revealed positive and negative relationships between Gli/Glu and the extensional parameters *E* and R_{max}/E, respectively. However, in a study of the extensional dough rheology of four commercial wheat varieties grouped into low and high protein categories which both contained a low Gli/Glu wheat variety and a high Gli/Glu one, McCann et al. (2016) found that although the high protein content samples showed higher maximum *ES* and maximum Hencky strain (ε_H) values, there was no significant effect of Gli/Glu on the maximum ε_H and *ES* of low protein and high protein samples, respectively. In this study, for their high protein wheat flour doughs, lower Gli/Glu corresponded to a significantly higher *ES* (McCann et al., 2016), which agrees with the findings of Uthayakumaran et al. (2000), i.e., lower Gli/Glu in wheat produced doughs with higher *ES* compared to higher Gli/Glu ratios in the wheat.

Figure 2.8. Extensograph curves for wheat flour doughs with high (a) and low (b) Gli/Glu, where R_{max} was measured in *EE* (Extensogram Einheit; the force unit used in German) and extensibility measured in mm. Copyright © 2013 Wang et al, Figure 1 from S. Wang et al. (2013). Molecular Mechanisms of HMW Glutenin Subunits from 1Sl Genome of Aegilops longissima Positively Affecting Wheat Breadmaking Quality. PLoS ONE. Licensed (adapted) under CC BY 4.0.



Uthayakumaran et al. (2014) also evaluated the Gli/Glu ratio for three wheat varieties grown at four locations and reported positive and negative correlations between Gli/Glu ratio and the Kieffer extension test parameters *E* (mm) and R_{max} (N), respectively. However, the same authors reported positive correlations between the Gli/Glu ratio and the elongational test parameters *ES* and Hencky strain (ε_H) (Uthayakumaran et al., 2014). An increase in *ES* as a result of an increase in the Gli/Glu ratio is not expected since a higher proportion of gliadins in the protein composition would be expected to diminish the dough's resistance to stretching.

Tyler et al. (2015) evaluated the composition and properties of a control wheat variety (Cadenza) and a genetically modified wheat line, both grown under the same glasshouse conditions. Even though the transgenic wheat had a lower protein content, it had a higher Gli/Glu ratio, which can explain the reported lower R_{max} and R_{max}/E for the transgenic line compared to the control line (Tyler et al., 2015). However, in two research studies carried out in the same laboratory by Gao et al. (2016) and X. Li et al. (2016), where the wheat variety Xinong 1330 and its near isogenic lines (NILs) were compared, it was shown that the doughs made with wheat grains with comparable Gli/Glu possessed significantly different R_{max} and *E* values. The authors attributed

differences in rheological properties of the doughs to differences in subunit composition of the glutenins in the wheat lines (X. Gao et al., 2016; X. Li et al., 2016).

The effect of differences in glutenin subunit composition on R_{max} (determined on a Kieffer rig) was noted in an Australian study (Anderssen et al., 2004). Although no information on protein content was provided, for 8 crosses grown in one location, R_{max} clearly differentiated varieties according to mixing properties that were associated with breadmaking performance. Song et al. (2015) produced two near isogenic lines (NILs) from a wheat variety, which were grown in the same location over two years. The flour protein content was not very different between the NILs and the original wheat variety, varying from 13.8% to 14.6% over the two years studied. Extensograph parameters R_{max} and E were significantly different among three wheat flour doughs, while showing no correlation with the protein content (W. F. Song et al., 2015).

Besides the changes in Gli/Glu ratio due to variations in genetic and environmental conditions, the effect of artificially altered Gli/Glu ratios on dough rheological parameters has been reported for natural and artificial *Fusarium* infection of wheat samples (Horvat et al., 2015) in reconstitution experiments (Uthayakumaran et al., 2000), or by enzymatic manipulation of protein properties (X. Wang et al., 2014). Artificial *Fusarium* infection in different wheat varieties resulted in wheat samples with higher Gli/Glu ratio compared to the same wheat varieties grown under natural Fusarium infection. The higher Gli/Glu ratio samples yielded doughs with much lower R_{max} than the samples with lower Gli/Glu ratios (Horvat et al., 2015). By adding fractionated gliadins and glutenins to a base wheat flour, Uthayakumaran et al. (2000) evaluated the effect of Gli/Glu ratio on dough rheological properties. Doughs with lower Gli/Glu ratio had their maximum Hencky strain (ε_H), which is a corresponding measurement of dough extensibility, decrease, and their elongational viscosity at rupture (corresponding to a dough′s resistance to extension) increase (Uthayakumaran et al., 2000). Using recombinant lipoxygenase (ana-rLOX) which was added to a base wheat flour, X. Wang et al. (2014) showed that by increasing the ana-rLOX concentration during dough preparation, the Gli/Glu ratio decreased, resulting in a dough with higher R_{max} and R_{max}/E and lower E (X. Wang et al., 2014).

Changes in protein composition can also affect dough rheological properties during mixing. Godfrey et al. (2010), as mentioned before, conducted farinograph tests using a single wheat variety grown under different fertilization treatments with nitrogen (0 to 288 kg ha^{-1}) and sulfur (0 and 53 kg ha^{-1}). In this study, when samples received different levels of nitrogen at

constant sulfur of 53 kg ha^{-1}, a high correlation between *FAB* and Gli/Glu ($r = 0.864$, $p < 0.001$) was found. Additionally, Gli/Glu was moderately correlated ($p < 0.05$) to *DDT* ($r = 0.507$) and *DS* ($r = 0.570$). However, Marchetti et al. (2012) obtained a different outcome when evaluating three wheat flours with different Gli/Glu ratios. In this study, the authors reported that the sample with the lowest Gli/Glu ratio resulted in the highest *DDT* and *DS*, whereas the wheat flour with the highest Gli/Glu showed the lowest *DDT* and *DS* values (Marchetti et al., 2012).

In a study from Kaur et al. (2013), farinograph tests also have shown the effect of Gli/Glu on dough properties during mixing. For the same wheat varieties aforementioned from this study, the *DDT* were fairly similar, with CBW38 and C306 having *DDT* values of 2.0 and 2.4 minutes, respectively (Kaur et al., 2013). However, these two samples presented a large difference in *DS* values, with CBW38 (lower Gli/Glu) having a longer stability to mixing (13.8 min) than C306 (1.9 min), which had a higher Gli/Glu (Kaur et al., 2013). A negative relationship between Gli/Glu and farinograph parameters could also be observed in the study from Tyler et al. (2015) who compared their control variety with a transgenic line. In this study, the authors observed that the transgenic line, which had a higher Gli/Glu, presented about a 40% reduction in *DDT* and *DS* when compared to the control wheat variety (Tyler et al., 2015). Gao et al. (2016) and X. Li et al. (2016) have shown that not only the Gli/Glu ratio can affect dough rheological properties, but also the subunits composition of the glutenins. After performing farinograph tests on the wheat variety Xinong 1330 and its near-isogenic lines (NILs), samples with similar Gli/Glu ratio possessed significantly different *DS* and *DDT*, which was the same outcome for R_{max} and *E* mentioned before (X. Gao et al., 2016; X. Li et al., 2016). Therefore, the different outcome for *DS* and *DDT* were attributed to the difference between the glutenin subunits among the samples (X. Gao et al., 2016; X. Li et al., 2016).

The farinograph parameter *DDT* also changed when changes in Gli/Glu occurred due to natural and artificial *Fusarium* infection as shown by Horvat et al. (2015). The authors showed that the artificially infected sample had an increase in Gli content and a decrease in Glu content, mainly HMW-glutenin subunits (Horvat et al., 2015). Besides affecting extensograph parameters, as shown before, *DDT* was significantly lower for the artificially infected sample (higher Gli/Glu) when compared to the sample that was naturally infected by *Fusarium* (Horvat et al., 2015). Finally, the study published by X. Wang et al. (2014) showed that *DS* is more affected by changes in Gli/Glu than *DDT*, agreeing with outcomes from Kaur et al. (2013). After adding the

recombinant lipoxygenase (ana-rLOX) to a base flour, the Gli/Glu decreased as ana-rLOX concentration increased (X. Wang et al., 2014). When comparing the concentration of 30 U g^{-1} to 0 U g^{-1}, i.e., same concentrations used to report extensograph parameters, the resulting *DDT* was slightly lower, but a significant increase in *DS* was observed as the Gli/Glu ratio was reduced (X. Wang et al., 2014).

Currently, there are not enough studies in the literature that correlate changes in dough properties measured by low-intensity ultrasound parameters to changes in wheat protein composition. However, in the study performed by Khorshidi et al. (2019), measurements of wet gluten (GWGlu) and gluten index (GIndex) were performed for all samples. Although no Gli/Glu ratios for the samples were reported, the authors mentioned that GIndex is an indication of polymeric/monomeric proteins (Khorshidi et al., 2019). Additionally, it was reported that there was a negative correlation between GIndex and GWGlu ($r = -0.581$, $p < 0.05$), which led the authors to explain that as wet gluten increased, the ratio of monomeric/polymeric gluten proteins also increased (Khorshidi et al., 2019). When evaluating a group of samples with large variation in grain and flour properties, low-intensity ultrasound results showed that the attenuation coefficient for ultrasound propagation in the dough was positively correlated to GWGlu ($r = 0.606$, $p < 0.05$) (Khorshidi et al., 2019). This outcome suggested that samples with potentially higher Gli/Glu resulted in samples that cause more wave attenuation. Moreover, GWGlu was also positively correlated to the longitudinal modulus tan δ ($r = 0.619$, $r < 0.05$) (Khorshidi et al., 2019), suggesting that samples with higher GWGlu, thus higher ratios of monomeric/polymeric gluten proteins, had weaker glutens than samples with lower GWGlu.

In summary, an increase in wheat Gli/Glu as a result of increased nitrogen fertilization affects dough rheology through decreasing one or more of the dough rheological properties associated with breadmaking dough strength, such as dough *G'*, *G*(*t*), R_{max}, R_{max}/E, *ES*, *AEV*, *DDT*, and *DS*, as well as increasing rheological parameters associated with extensibility, such as Hencky strain (ε_H), strain at rupture, *E*, *G"*, maximum creep strain (%) and tan δ. It is important to mention that nitrogen and sulfur fertilization may impact dough rheological properties to different extents depending on the level of applied nitrogen and sulfur, the timing of the application, and the amount of nitrogen and sulfur uptake by the wheat plant, as discussed in Section 2.3.

2.6.3 Effect of wheat free asparagine concentration

The relationship between free asparagine concentration in wheat and dough rheological properties has not yet been broadly studied and reported in the literature, as recently confirmed by Oddy et al. (2022), with only a few published studies reporting both free asparagine and dough rheological properties (Y. Liu et al., 2011; Malunga et al., 2019; Wilson et al., 2020). Oddy et al. (2022) reviewed published studies evaluating relationships between free asparagine concentration and wheat quality traits, such as baking quality. In their review, Oddy et al. (2022) only discussed the findings from Malunga et al. (2019) when reviewing the relationship between free asparagine concentration and dough rheological properties. Moreover, the majority of the studies discussed by Oddy et al. (2022) involved the evaluation of other quality aspects of wheat, such as protein and gluten content, hardness index, sprouting, and water absorption. Additionally, Oddy et al. (2022) critically evaluated the outcomes from different studies, finding a lack of consistency across studies on the relationship between free asparagine concentration and wheat quality aspects. Therefore, more studies on the relationship between wheat free asparagine concentration and dough rheological properties would shed light on the potential effects of reducing wheat free asparagine levels on bread quality. Malunga et al. (2019) evaluated the free asparagine concentration and the mechanical properties of wheat flour doughs prepared from 30 Canadian hard red spring wheat varieties grown in three provinces in Canada (Alberta, Manitoba, and Saskatchewan). The mechanical properties of doughs prepared from straight grade wheat flour were evaluated using farinograph and extensograph (Malunga et al., 2019). Additionally, the free asparagine concentration was measured on both wholemeal and straight grade wheat flours (Malunga et al., 2019). The authors performed correlation studies and reported negative Pearson correlation coefficients between rheological parameters, e.g., *DDT*, *DS*, and R_{max}, and free asparagine concentration in both wholemeal and straight grade flours (Malunga et al., 2019). However, most of these correlation coefficients were not statistically significant at $p < 0.05$, with only the correlation of R_{max} at 45 minutes against free asparagine concentration in straight grade flour having a *p*-value of 0.0444 (Malunga et al., 2019). Although most of the correlations were not statistically significant, the study from Malunga et al. (2019) showed that samples with low free asparagine concentration generally resulted in stronger glutens.

Liu et al. (2011) conducted rheological studies and free amino acid quantification using a single wheat variety grown at two different locations. The authors milled the wheat grains in order

to produce six mill streams and a patent flour by mixing all the mill streams (Y. Liu et al., 2011). In this study, free amino acid quantification, farinograph, and extensograph were conducted using the mill streams and the patent flour (Y. Liu et al., 2011). After performing correlation studies, the authors found a positive ($p < 0.05$) correlation only between *FAB* and free asparagine ($r = 0.94$) (Y. Liu et al., 2011). No significant relationship was found between free asparagine concentration and dough mechanical properties measured using either the farinograph or extensograph (Y. Liu et al., 2011). However, it is important to note that the authors only reported correlations performed for the parameters *DDT* (farinograph) and *E* (extensograph). The *DS* was not reported in the study because some samples had *DS* values longer than 20 minutes, which was the time limit for the test set by the authors. Although the authors obtained R_{max} data, no correlation studies for R_{max} against free asparagine were mentioned.

A more recent study published by Wilson et al. (2020) reported the free asparagine concentration in four wheat varieties grown in 2017 and five wheat varieties grown in 2018, using three levels of nitrogen (56, 100, and 145 kg ha^{-1}) and two levels of sulfur (0 and 22 kg ha^{-1}) in both years. This study also reported farinograph results for the samples grown in 2018, but no correlation studies were conducted between free asparagine concentration and farinograph parameters by the authors (Wilson et al., 2020). However, when samples received 22 kg ha^{-1} of sulfur, free asparagine concentration significantly decreased and gluten strength significantly increased, as measured by *DDT* and *DS*, when compared to no sulfur fertilization (Wilson et al., 2020). Therefore, as free asparagine concentration in wheat was reduced as a result of sulfur fertilization, the dough samples showed more elastic-like characteristics, which suggests a negative correlation between free asparagine concentration and gluten strength.

As mentioned earlier, the current knowledge on the relationship between wheat free asparagine concentration and dough rheological properties is very limited. Very few studies have been published investigating this relationship (Y. Liu et al., 2011; Malunga et al., 2019). Moreover, the current literature on the relationship between wheat free asparagine concentration and dough rheological properties includes only empirical rheological tests. Therefore, information regarding dough properties, measured under shear rheometry and low-intensity ultrasound, and their relationship to free asparagine concentration is also lacking in the literature.

A more comprehensive understanding of the relationship between free asparagine concentration and dough rheological properties is required by wheat growers, the breadmaking

industry, and the scientific community. Detrimental effects on gluten strength and how the dough performs during processing negatively affects the final product quality, producing bread with unattractive appearance and poor palatability. In order to understand if reducing wheat free asparagine concentration will have detrimental effects on dough rheological properties and, consequently, on the final product quality, the latter can be assessed by evaluating various characteristics, such as the loaf volume, crust formation, and crumb structure (Cauvain, 2015a; Young, 2012).

2.7 Bread quality and safety

After the group of Swedish researchers published their work reporting the presence of acrylamide in carbohydrate-rich foods (Tareke et al., 2002), various researchers investigated the link between wheat free asparagine concentration and acrylamide formation in bread (Claus et al., 2006, 2008; Fredriksson et al., 2004; Surdyk et al., 2004). Surdyk et al. (2004) aimed to study factors influencing the acrylamide content in yeast-leavened breads by using wheat flour and added asparagine and fructose (reducing sugar). In their study, Surdyk et al. (2004) selected a wheat flour with low levels of free asparagine (170 $\mu g\ g^{-1}$) and fructose (50 $\mu g\ g^{-1}$) in order to study the effect of added asparagine and fructose on acrylamide formation in the final bread. The authors added three levels of asparagine (0.1, 0.4, and 0.7g per 100 g of flour) and fructose (0.07, 0.29, and 0.51 g per 100 g of flour) (Surdyk et al., 2004). After baking the breads at 270 ºC for 15 minutes, the authors separated the crust from the crumb and measured the acrylamide content in the freeze-dried samples by LC-MS-MS (Surdyk et al., 2004). In this study, the authors reported no significant effects of fructose, but a significant ($p < 0.005$) effect of asparagine on the acrylamide content (Surdyk et al., 2004). When no precursors were added, the authors quantified 80 $\mu g\ kg^{-1}$ of acrylamide in the dried crust and, at the highest levels of added asparagine, the authors quantified 6000 $\mu g\ kg^{-1}$ of acrylamide in the dried crust (Surdyk et al., 2004). Moreover, this study reported that 99% of the acrylamide in the bread was present in the crust, which was explained by the low internal temperature of the crumb during baking (< 101 ºC) (Surdyk et al., 2004). The authors also studied the effect of time, temperature, and their interactions on acrylamide formation, adding a constant level of asparagine (0.1 g per 100 g of flour) and no fructose, using five temperature levels (150 to 290 ºC) and five different baking times (15 to 35 minutes) (Surdyk et al., 2004). The color of the crust, measured with a Minolta system, was significantly correlated ($p < 0.001$) with the acrylamide content when the recipe was kept constant, with temperature being

the main factor contributing to acrylamide formation (Surdyk et al., 2004). In order to understand if reducing free asparagine would affect the browning/color of the crust, the authors compared the color of the crust of breads prepared with the highest levels of added asparagine (0.7 g per 100 g of flour) with the color of crust of breads with no asparagine added. (Surdyk et al., 2004). The authors observed no significant differences between the color of both breads, whereas the acrylamide content rose from about 700 in the bread with no asparagine added to 6000 μg kg^{-1} in the bread receiving the highest levels of asparagine (Surdyk et al., 2004). Based on the result from the color comparison between the two bread samples, the authors concluded that other amino compounds might be involved in the browning of bread crusts (Surdyk et al., 2004), thus reduction of wheat free asparagine concentration might not be detrimental to bread color.

In the same year, Fredriksson et al. (2004) published a study with eleven milling fractions, including sifted flour and whole-wheat flour, to investigate the effect of fermentation time on the free asparagine concentration of the dough. Wheat flour doughs were prepared by mixing sifted wheat flour (base flour) with eight different milling fractions (50% base flour and 50% milling fraction), which were divided into six pieces whose free asparagine was measured after specific fermentation times (varying from 0 to 6 hours) (Fredriksson et al., 2004). For bread preparation, the sifted wheat flour was mixed with whole-wheat flour and fermented at two temperatures and relative humidity conditions for short (15 min each) and long times (180 min each), and baked at 270 ºC for 15 minutes (Fredriksson et al., 2004). The authors reported a significant decrease in free asparagine concentration after 6 hours of fermentation, compared to values before fermentation (0 hours), with the germ fraction resulting in the lowest decrease (55%) and sifted wheat flour resulting in the highest decrease (96%) (Fredriksson et al., 2004). The authors also reported a significant difference between acrylamide content in the bread crust, quantified using LC-MS-MS, when bread dough was fermented for 0.5 hours (180 μg kg^{-1}) versus bread dough fermented for 6 hours (about 24 μg kg^{-1}) (Fredriksson et al., 2004). Finally, by knowing the crumb and crust weights (values not reported) and considering that the majority of acrylamide is found in the crust (>94%), the authors calculated the acrylamide content in the fresh bread (Fredriksson et al., 2004). For the whole-wheat bread, the authors obtained about 33 μg kg^{-1} of acrylamide for bread after a short fermentation time and 4 μg kg^{-1} of acrylamide after longer fermentation times (Fredriksson et al., 2004).

Later, Claus et al. (2008) also published a research study evaluating the effect of fermentation on free asparagine concentration and acrylamide content in bread roll crusts. Bread rolls were prepared using flour from a single wheat variety, with samples being taken from the dough after various fermentation times (0 to 5.5 hours). As opposed to Fredriksson et al. (2004), Claus et al. (2008) observed a significant decrease in dough free asparagine concentration and acrylamide content in the crusts during the first hour of fermentation. At longer fermentation times, the values for both free asparagine in the dough and acrylamide in the crust remained constant.

The research studies from Fredriksson et al. (2004) and Claus et al. (2008) shows that free asparagine is highly correlated to acrylamide content in bread crusts. Fermentation time can have positive impacts on the safety of the final product by decreasing free asparagine concentration in the dough and, consequently, the acrylamide content in the bread. However, as pointed out by Claus et al. (2008), longer fermentation times should not be encouraged because it can lead to the weakening of the dough's structure, causing negative impacts on the quality of the final bread. Therefore, assuring that wheat flour has low free asparagine concentration is the recommended path to mitigate acrylamide formation.

In another research study, Claus et al. (2006) evaluated the acrylamide content of bread crusts as affected by changes in wheat free asparagine concentration due to agronomic practices and flour extraction rate. The authors studied nine wheat varieties (Ellvis, Enorm, Magnus, Manhattan, Monopol, Terrier, Tommi, Transit, and Wasmo) to determine the effect of genotype (Claus et al., 2006). Two selected wheat varieties (Terrier and Thasos) were used to study the effect of sprouting and another two wheat varieties (Enorm and Tommi) were used to study the effect of nitrogen and sulfur fertilization treatments (Claus et al., 2006). Finally, three wheat treatments (Terrier, Enorm with nitrogen fertilizer, and Enorm control) where used to evaluate the effect of environment, and a single wheat variety (Thasos) was used to study the effect of extraction rate (Claus et al., 2006). From this study, it was reported that there was a great variation due to genotype on the free asparagine content of the wheat flours (from 49 to 249 $\mu g\ g^{-1}$) and doughs (from 19 to 96 $\mu g\ g^{-1}$) and, consequently, a great variation in the acrylamide content of the bread crusts (from 14 to 74 $\mu g\ kg^{-1}$) (Claus et al., 2006). Environmental effects, mainly higher growing temperatures, also caused an increase in free asparagine concentration in the wheat, which led to an increase in the acrylamide content of the bread crust (Claus et al., 2006). Moreover, the authors also observed that the sprouting of two wheat varieties, which can lead to starch and

protein degradation due to enzyme activities, caused an increase mainly in the free asparagine concentration, leading to more acrylamide formation in the bread crust (Claus et al., 2006). One particular wheat variety had a great increase in the free asparagine content after sprouting, which led the acrylamide content to rise from 54.5 μg kg^{-1} (non-sprouted) to 273.4 μg kg^{-1} (sprouted) in the bread crust (Claus et al., 2006). Free asparagine was also increased by nitrogen fertilization (levels added from 0 to 220 kg ha^{-1}), being not significantly affected by sulfur (20 kg ha^{-1}) (Claus et al., 2006). The increase in free asparagine due to nitrogen fertilization was also directly correlated to an increase in the acrylamide contents of the bread crust (Claus et al., 2006). Finally, the authors pointed out that increasing the extraction rate also increased the levels of free asparagine in the flour and, consequently, the acrylamide content in the final bread crust (Claus et al., 2006). More recently, the effect of extraction rate on free asparagine content was also shown in a study published by Malunga et al. (2019) where free asparagine concentrations decreased by at least 40% in white flours compared to their corresponding wholemeal samples.

Therefore, based on the aforementioned research studies, free asparagine concentration in wheat and wheat flour is directly correlated to acrylamide content in bread, which is mostly concentrated in the crust. Therefore, strategies to produce wheat with low free asparagine concentration can tackle the current safety problem regarding the presence of acrylamide in the bread. According to a recent review article aiming to investigate the concentration of acrylamide in various foodstuffs around the word, it was found that much work still needs to be done to decrease acrylamide in bread (Khaneghah et al., 2020). After conducting a meta-analysis using published studies from various countries, the authors reported the acrylamide concentration found in bread, with Canada leading the rank (1145.96 μg kg^{-1}) and Turkey having the least amount (72.00 μg kg^{-1}) (Khaneghah et al., 2020). Bread was ranked the 8^{th} product in the pooled acrylamide concentration (133 μg kg^{-1}) list, with potato-based food leading the rank (740 μg kg^{-1}) (Khaneghah et al., 2020). However, the benchmark levels set by the European Commission (2017) for bread is 50 μg kg^{-1}. Therefore, the data reported by Khaneghah et al. (2020) indicate the necessity of mitigation strategies to decrease acrylamide formation in bread. This necessity urges for investigations on the impacts of wheat free asparagine mitigation strategies on bread quality.

As mentioned before, changes in protein content and composition can affect dough rheological properties and may be detrimental to final bread quality, such as loaf volume (Ktenioudaki et al., 2010; Van Bockstaele et al., 2008a, 2008b; Zghal et al., 2001; F. J. Zhao,

McGrath, et al., 1999). For example, F. J. Zhao, McGrath, et al. (1999) evaluated three wheat varieties (Hereward, Rialto, and Spark) grown at three locations (Bridgets, Woburn, and Ellingham), under three sulfur (0, 20 and 100 kg ha^{-1}) and two nitrogen (180 and 230 or 230 and 280 kg ha^{-1}) fertilization treatments. In order to evaluate wheat flour dough properties, the authors performed extensograph tests and measured the bread quality by performing loaf volume tests and crumb structure evaluation (F. J. Zhao, McGrath, et al., 1999). In this study, the authors observed that wheat variety was the main factor affecting most of the rheological and quality parameters, followed by sulfur fertilization (F. J. Zhao, McGrath, et al., 1999). Moreover, the increase in the sulfur concentration in the grain was negatively correlated to R_{max} (r = -0.267) and positively correlated to E (r = 0.496) and loaf volume (r = 0.529), although no significance for the correlation coefficients was reported (F. J. Zhao, McGrath, et al., 1999). Finally, the authors also observed overall low differences in loaf volume among the three varieties, but higher crumb score for both Hereward and Spark (F. J. Zhao, McGrath, et al., 1999). In this study, the negative correlation between R_{max} and loaf volume could be explained by higher proportions of LMW-glutenin subunits when sulfur was supplied, which can increase dough extensibility and lead to greater loaf volumes (F. J. Zhao, McGrath, et al., 1999).

Later, Zghal et al. (2001) showed how gluten strength is important to obtain satisfactory loaf volumes and crumb structure. The authors performed dough extensograph tests using flour samples representing three wheat classes, i.e., Canada Western Extra Strong (CWES), Canada Western Red Spring (CWRS), and Canada Prairie Spring (CPS), and a fourth sample composed of 50% CWES and 50% CPS (Zghal et al., 2001). In this study, the wheat flour from CWES resulted in the higher R_{max}/E with the CPS flour having the lowest ratio, thus resulting in the weakest dough (Zghal et al., 2001). Moreover, when the three flour types were compared, loaf volume was the highest for the strongest sample (CWES) (Zghal et al., 2001). The authors also found that crumb density and uniformity were also affected by flour type, with CWES showing the lowest density and highest uniformity for the crumb (Zghal et al., 2001).

Van Bockstaele et al., (2008a) performed fundamental rheometry to investigate dough rheological properties of 17 European wheat varieties and correlate the results with the final loaf volume. The authors performed creep-recovery tests, finding that samples with higher protein content resulted in doughs with less ability to recover after the shear stress of 250 Pa was removed, thus presenting a less elastic-like structure (Van Bockstaele et al., 2008a). Additionally, bread loaf

volume measurements were shown to be positively correlated to protein content ($r = 0.750$, $p < 0.01$) and negatively correlated to the ability of the dough to recover ($r = -0.548$, $p<0.05$), indicating that less elastic-like doughs resulted in higher loaf volumes (Van Bockstaele et al., 2008a). The authors also correlated the rheological properties of the same wheat varieties, measured under linear oscillatory shear tests, to bread loaf volume in another study (Van Bockstaele et al., 2008b). In this study, a shear oscillatory time sweep test was performed within the dough's linear viscoelastic region at a frequency of 1 Hz (Van Bockstaele et al., 2008b). The authors reported a fairly strong negative correlation between G^* and loaf volume ($r = -0.824$, $p < 0.01$) and a positive correlation between $\tan\delta$ and loaf volume ($r = 0.833$, $p < 0.01$) (Van Bockstaele et al., 2008b). The results from linear oscillatory tests also showed that samples that resulted in more elastic-like doughs produced breads with lower loaf volume. This could indicate an imbalance in the wheat protein composition, e.g., excessive HMW-glutenin subunits, which could result in very strong doughs with a low ability to expand during fermentation, thus resulting in loaves with low volume.

The high correlation between *E* and loaf volume was reported later by Ktenioudaki et al. (2010) who performed farinograph and extensograph tests using flour of seven wheat varieties from different countries, a Canadian blend, and a control flour containing various Irish varieties. The protein content of the samples varied from 8.2 % (Greek variety) to 13.4 % (Canadian blend), with the Canadian blend resulting in the highest *DDT*, *DS*, R_{max}, and *E* (Ktenioudaki et al., 2010). The authors also divided the samples in three groups, depending on their loaf volume and best crumb structure, with the Canadian blend and two English varieties forming the group with the best breadmaking properties (Ktenioudaki et al., 2010). Moreover, it was reported that dough *E* was highly correlated to loaf volume ($r = 0.8$) while no correlation was found between R_{max} and breadmaking quality (Ktenioudaki et al., 2010). Finally, samples with the highest loaf volumes resulted in bread crumb with a more uniform distribution of cells with smaller volumes, whereas poor breadmaking quality samples resulted in a crumb that had a more open structure and with fewer numbers of cells (Ktenioudaki et al., 2010). This phenomenon was explained by Zghal et al. (2001), who suggested that stronger doughs, i.e., possessing higher strain hardening, suffer less cell coalescence due to a limited cell growth during fermentation, thus resulting in a uniform distribution of small cells.

There is a limited number of studies correlating dough rheological measurements, mainly from shear rheometry and low-intensity ultrasound, with loaf volume and crumb structure. However, from the aforementioned studies, one can expect that samples with an appropriate balance between strength and extensibility will result in high quality bread, i.e., high loaf volume and uniform crumb structure. Therefore, it is of utmost importance to investigate if strategies to reduce wheat free asparagine concentration will be detrimental to dough rheological properties, and thus to the final bread quality.

3 Relationship between free asparagine concentration and gluten strength in Canadian hard spring wheat

3.1 Abstract

Wheat is a major cereal for humankind and its demand for production and exportation looks to grow even more in the coming years. Therefore, safety aspects regarding free asparagine concentration in wheat due to its potential acrylamide formation in the final product must be addressed by wheat growers worldwide. Free asparagine concentration in wheat is affected by three main factors: environment, wheat genotype, and agronomic practices, e.g., nitrogen and sulfur fertilization. However, these three factors also affect wheat protein content and composition. Wheat protein is extremely important in wheat marketing and in delivering satisfactory quality to the final product. The majority of the wheat proteins are gluten proteins, i.e., gliadins and glutenins, which are responsible for producing a viscoelastic dough that results in good quality bread. Therefore, changing protein content and composition can affect dough performance during processing that might result in final products with undesirable qualities. Thus, the proposed research study aimed to investigate if environment, genotype and fertilization treatments would negatively affect the gluten strength and its relationship with free asparagine concentration in wheat. To do so, eight Canadian wheat varieties, grown under four environments and under four fertilization treatments, were studied. To evaluate the dough viscoelastic properties, empirical rheological tests, i.e., farinograph and extensograph, were performed. For final product quality evaluation, specific loaf volume (*SLV*) was also determined. The results showed that environment, genotype and their interaction (G×E) were the main factors affecting gluten strength. Fertilization treatments had little or no effect on gluten strength. Additionally, rheological parameters from the farinograph and extensograph translating into gluten strength were negatively correlated to free asparagine concentration. Overall, no negative effect was observed on *SLV*. In conclusion, environment, genotype and fertilization treatments producing wheat with low free asparagine concentration were not negatively affecting gluten strength . Therefore, selecting wheat varieties and their production for low free asparagine concentration will not impact dough performance during processing nor the desired final product quality.

3.2 Introduction

Acrylamide is a chemical compound that can be present in thermally processed food products, such as bread, as initially detected by Tareke et al. (2002). Acrylamide, when found as a monomer, is a hazardous substance (Raffan & Halford, 2019). Since 1994, acrylamide has been classified as a probable carcinogen to humans (group 2A) (IARC, 1994). As per Friedman (2003), acrylamide is a neurotoxic and carcinogenic compound for laboratory animals, a scientific fact that was also emphasized by the EFSA Panel on Contaminants in the Food Chain (CONTAM) (2015). As per Raffan and Halford (2019) and the EFSA Panel on Contaminants in the Food Chain (CONTAM) (2015), soft bread is one of the wheat-based products with the lowest average acrylamide content (42 µg kg^{-1}) when compared to biscuits, crackers and crispbreads (about 200 µg kg^{-1}). However, due to its high consumption, bread accounted for a significant exposure of humans to acrylamide. Considering the contribution to dietary acrylamide intake, bread is the second major source of acrylamide to humans (about 11%), following potato fries that lead the list with about 24% (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015; Raffan & Halford, 2019). Acknowledging the potential risk that some food products bring to humans in terms of acrylamide exposure, the European Regulation (EU) 2017/2158 set benchmark levels for acrylamide content in food products, such as bread (50 µg kg^{-1}) (European Commission, 2017).

In the food industry, acrylamide is formed through the Maillard reaction between reducing sugars and free amino acids at high temperatures (T > 120ºC) (Tareke et al., 2002). Among the free amino acids, free asparagine was found to be the major precursor to acrylamide (Mottram et al., 2002; Stadler et al., 2002; Zyzak et al., 2003). In 2015, the European Food Safety Authority issued a document titled "Scientific Opinion on Acrylamide in Food", based on a review of scientific studies on acrylamide presence in food products, in which free asparagine was acknowledged as the main precursor to acrylamide (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015). This document also pointed out the importance of the acrylamide toolbox (Food Drink Europe, 2019), developed as a guidance on acrylamide mitigation strategies (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015). Capuano and Fogliano (2011) published a review article analyzing several acrylamide mitigation strategies published in the literature. Examples of these strategies are changing the time-temperature profile during baking, addition of the enzyme asparaginase, and the use of flours extracted at lower extraction rates (e.g., 70% as per Capuano et al. (2010)). However, changing the time-temperature profile

can impact the browning reaction during baking, while addition of asparaginase and the use of refined flours are extremely costly (Capuano & Fogliano, 2011). Therefore, the best strategy that could impose the least effect on the quality and price of the final product should be sought. The Acrylamide Toolbox 2019 introduces strategies that seek acrylamide mitigation through recommending field level actions for delivery of wheat with low free asparagine concentrations (Food Drink Europe, 2019).

Free asparagine in wheat has been shown to be affected by three main factors including environment (Corol et al., 2016; Xie et al., 2021), genotype (Curtis et al., 2009; Xie et al., 2021), and the level of nutrients, i.e., sulfur and nitrogen, in the soil (Claus et al., 2006; Corol et al., 2016; Lea et al., 2007; Muttucumaru et al., 2006; Weber et al., 2008). Selection of wheat varieties with low free asparagine accumulation potential is a strategy to mitigate acrylamide formation in wheat-based products (European Commission, 2014; Food Drink Europe, 2019). It is also important to evaluate how each wheat genotype (G) performs under different environmental conditions (E), as the environment and G×E interactions also play an important role in free asparagine accumulation in wheat (Curtis et al., 2014). In addition, farmers need to maintain a satisfactory level of sulfur in the soil and avoid excessive nitrogen applications through fertilization (Food Drink Europe, 2019).

Nevertheless, one major question that needs to be addressed is how different environmental conditions, and the selection of wheat varieties and fertilization treatments for low free asparagine concentration will affect the quality of the end products. For wheat-based products such as bread, the final quality is highly affected by wheat protein content and composition, i.e., its gluten proteins (Sluimer, 2005a). With a variation of 7 to 20% in wheat grain (Dobraszczyk, 2001), protein content is an important factor considered for wheat classification, application (e.g., bread, biscuits, and cakes) and price (Carson & Edwards, 2009; PDQ, 2020). Moreover, gluten proteins form a viscoelastic material that possesses extensibility due to gliadins, and elasticity, i.e. strength, due to glutenins (Delcour & Hoseney, 2010a; Veraverbeke & Delcour, 2002). This singular property of wheat confers expansion and gas-holding capability to bread doughs during fermentation (Delcour & Hoseney, 2010a), resulting in a bread with a high loaf volume and a satisfactory palatability. Therefore, achieving an optimal balance between gliadins and glutenins (Gli/Glu ratio) is an important requirement for the baking industries to produce high-quality bread (Delcour & Hoseney, 2010a).

Environmental conditions have been reported to influence both protein content and composition (Johansson et al., 2013). For example, drought conditions lead to an increase in protein accumulation in wheat grains (Uthayakumaran et al., 2012; Zhou et al., 2018). Soil texture is another factor that affects protein concentration in wheat, e.g., loamy soils benefit nitrogen uptake by the plants through the roots resulting in higher protein accumulation when compared to sandy soils (Nyiraneza et al., 2012). Regarding growth temperature, it was shown that a wheat variety grown under high temperatures (day 37 ºC/ night 28 ºC) had greater accumulation of sulfur-poor proteins in the grain compared to that grown under low temperatures (24 ºC/17 ºC) (DuPont et al., 2006). Finally, two studies involving a single wheat variety (Soissons) and similar fertilization treatments, performed in Spain by Fuertes-Mendizábal et al. (2010) and in the United Kingdom by Rossmann et al. (2019), led to different Gli/Glu ratios in the wheat samples between these two studies. Fuertes-Mendizábal et al. (2010) obtained Gli/Glu ratios of up to 0.76, while ratios obtained by Rossmann et al. (2019) were all higher than 1.00, confirming that environment could be the cause for this significant difference in the Gli/Glu ratio between the two studies. In addition to the environment, wheat protein content and composition depend on wheat genotype (Mastilović et al., 2018; Panozzo & Eagles, 2000). Variations in the genes coding for the gluten proteins have been shown to affect gluten strength (Payne, 1987; Vancini et al., 2019; X. Wang et al., 2018).

Fertilization treatments also affect protein content and composition. Nitrogen fertilization is widely applied to wheat fields to increase productivity (Ladha et al., 2016), yield (Y. Zhang et al., 2016), and protein content (Abedi et al., 2011). With future challenges regarding wheat production and climate change, e.g., decreased productivity (Gutiérrez-Moya et al., 2021), worldwide demand for nitrogen fertilizer might also increase, i.e., anticipated to reach 111.6 Mt in 2022 (FAO, 2019) compared to 105.7 Mt in 2020/2021 (Statista, 2021c). Increasing nitrogen fertilizer levels to increase productivity and protein content level has been shown to not only increase free asparagine concentration in wheat (Claus et al., 2006; Lea et al., 2007), but also increase the Gli/Glu ratio (Fuertes-Mendizábal et al., 2013; Godfrey et al., 2010). Therefore, nitrogen fertilization can also affect wheat protein composition, particularly gluten proteins, i.e., gliadins and glutenins (Godfrey et al., 2010; Rossmann et al., 2019), which can affect the dough extensibility/elasticity balance. Moreover, sulfur dioxide (SO_2) decrease in the atmosphere has been reported to cause a decrease in sulfur availability, supplied through acid rain, in soils (F. J. Zhao, Hawkesford, et al., 1999). In

addition to positively affecting free asparagine accumulation in wheat (Granvogl et al., 2007; Muttucumaru et al., 2006), sulfur deficiency in the soil has been shown to promote formation of sulfur-poor gluten proteins (Wieser et al., 2004) and to produce wheat with low resistance or elasticity, i.e., gluten strength (Wilson et al., 2020). This is due to a reduction in the formation of sulfur rich amino acids, such as cysteine, that are responsible for the formation of inter- and intra-chain disulfide bonds between and within gluten protein fractions, with the inter-chain bonds being important for gluten stabilization and functionality (P. Shewry, 2019).Therefore, it is necessary to investigate if factors affecting free asparagine in wheat will negatively affect gluten strength.

Two well stablished empirical rheological tests, the farinograph and the extensograph, are usually utilized to evaluate wheat gluten strength through assessment of wheat flour dough resistance to mixing (Järvan et al., 2017; Malalgoda et al., 2018; Malunga et al., 2019; Wilson et al., 2020) and stretching (Malunga et al., 2019; Mastilović et al., 2018; Sehn & Steel, 2017; Sobczyk et al., 2017). For example, Wilson et al. (2020) studied the effect of sulfur fertilization treatments on the gluten strength of four wheat genotypes in 2017 and five wheat genotypes in 2018. The authors reported a significant increase in gluten strength, as measured by the time taken to develop the dough (*DDT*) and the dough resistance to the mixing process (dough stability), when sulfur was provided to the wheat plant (Wilson et al., 2020). In addition, Järvan et al. (2017) studied two wheat cultivars grown in two different locations to evaluate the effect of sulfur fertilization (0 to 39 kg ha^{-1}) on gluten strength, and obtained a positive significant ($p<0.05$) effect of sulfur fertilization on dough stability. Sobczyk et al. (2017) performed extensograph tests to evaluate the difference between old and modern spelt wheat varieties. Rheological data showed a wide range of gluten strength among the samples, with weaker samples presenting a low maximum resistance-to-extensibility ratio (*R/E*), e.g., 0.5, while stronger ones had a much higher *R/E,* e.g., 1.9 (Sobczyk et al., 2017). Sehn and Steel (2017) also performed extensograph tests on eleven commercial whole-wheat samples, one refined wheat flour and two wheat bran samples (fine and coarse). Refined wheat flour and wheat bran fractions were used to prepare eight pre-mixes at various proportions to study the rheological properties of whole-wheat flours and pre-mixes (Sehn & Steel, 2017). Extensograph results were capable of differentiating the samples and, together with the farinograph, were successfully applied to calculate the Sehn-Steel number, utilized to identify wheat samples suitable for breadmaking (Sehn & Steel, 2017). Therefore, wheat flour dough

rheological studies are necessary to evaluate the suitability of wheat flour samples to a desired final product, such as bread.

It is important to understand if strategies to reduce free asparagine accumulation in wheat will have any undesired impact on wheat gluten strength, so that the Canadian wheat growers can take the best path to handle the wheat safety and functionality aspects simultaneously. This study aims to provide an understanding of the effects of genotype, environment and fertilization treatments on gluten strength and its relationship with wheat free asparagine concentration. This study will provide Canadian wheat growers with a valuable understanding on whether strategies implemented to deliver wheat grains with low free asparagine concentration will affect the gluten strength.

3.3 Materials and methods

3.3.1 Wheat and white flour samples

Eight commercial hard spring wheat varieties, six from Canada Western Red Spring class (AAC Brandon, AAC Cameron, AAC Elie, CDC Plentiful, BW5011, and Glenn), one from Canada Prairie Spring Red class (SY Rowyn), and one from Canada Northern Hard Red class (Prosper) were selected for this study. The wheat varieties were grown at four location-years, i.e., three locations in Manitoba, Canada, over 2018 (Carberry and Lilyfield) and 2019 (Grosse-Isle and Lilyfield). Four fertilization treatments, including combinations of two nitrogen levels (100 or 135 kg ha^{-1}) and two sulfur levels (0 or 17 kg ha^{-1}), were applied prior to seeding. At each location, 32 plots were planned in three replicates (blocks), resulting in a total of 96 plots. Within each replicate, a split-plot design was applied. Four plots (main plots) within each block randomly received one of the four fertilization treatments. Within each main plot, the eight wheat varieties were randomly assigned to sub-plots. At harvest, 32 composite samples representing the 32 treatments (4 fertilizations and 8 genotypes) were prepared by combining the wheat grains over the three field replicates. In total, across the four location-years, 128 samples were obtained.

The Manitoba Ag-Weather Program growing season reports from Manitoba Agriculture, Food and Rural Development were utilized for data collection on the weather conditions in 2018 and 2019. The collected weather conditions were crop heat units (CHU), which is calculated using daily maximum and minimum temperatures over the growing period (Government of Canada, 2021), and the total precipitation over the growing period . Soil samples collected at depths of 0-15 cm and 15-60 cm were used for quantification of nitrate-nitrogen (cadmium reduction method)

(Gelderman & Beegle, 2015) and sulfur (barium turbidimetric method) (Franzen, 2015) in the soil. Soil nutrients, before fertilization and wheat seeding, and meteorological information of the four growing environments (location-years) over the growing period are presented in Table 3.1.

Table 3.1. Soil and meteorological data of the four studied environments (location-years).

Site	Year	N/S Ratio (0-15 cm)	N/S Ratio (15-60 cm)	Weather Station	Growing period (days)	Crop Heat Units	Total Precipitation (mm)
Carberry	2018	1.47	3.10	Carberry	115	2467	235
Lilyfield		0.82	0.32	Winnipeg Airport	92	2044	230
Grosse Isle	2019	1.33	1.27	Woodlands	113	2174	204
Lilyfield		1.10	0.59	Winnipeg Airport	120	2323	223

The N/S ratios were calculated using the nutrients available in the soil before application of fertilizers and wheat seeding. Crop Heat Units were calculated using the daily minimum and maximum temperatures over the growing period.

Wheat grain samples were cleaned, tempered to achieve a target moisture of 16.5% (24 hours), and milled to obtain white flour (at 75% extraction rate) and whole-wheat flour. White flours were obtained by milling wheat using a Bühler laboratory flour mill (MLU-202) and a Bühler impact finisher (MLU-302). The Bühler laboratory flour mill produced the flour streams, which were combined to reach the extraction rate at 75%, and the by-products (bran and shorts). To recover a small amount of white flour (about 2-2.5%) remained in the bran and shorts, these by-products were milled in the Bühler impact finisher (MLU-302). The recovered white flour passed through a sieve of 150 µm and the by-products, bran and shorts, were retained on the sieve. To prepare whole-wheat flours, the short and bran fractions, retained on the Bühler impact finisher sieve, were ground in a Jacobson 120-B hammer mill to pass through a 1,190 µm sieve. White flour and the ground short and bran portions were blended to produce the whole-wheat flour for free asparagine quantification and bread preparation.

3.3.2 Free asparagine quantification

Quantification of free asparagine in the whole-wheat flour was carried out as per Xie et al. (2021). The free asparagine was extracted from the whole-wheat flour using 0.01M hydrochloric

acid solution, derivatized using a AccQ•Tag Ultra pre-column derivatization amino acid kit, and quantified using a Waters H class UPLC as described by Malunga et al. (2019).

3.3.3 Empirical rheology

The AACC International Approved Methods 54.21-02 (AACC International, 2011) and 54-10.01(AACC International, 1999b) were followed for farinograph and extensograph experiments, respectively. The farinograph and extensograph experiments were conducted at Cereals Canada using the Brabender Farinograph-E and the Brabender Extensograph-E, respectively. The moisture contents of the white wheat flour samples were analyzed following the AACC International Approved Method 44.15-02 (AACC International, 1999a).

The main farinograph parameters recorded include the dough development time (*DDT*, min), the optimum water absorption at 14% moisture basis (*FAB*, %), and the dough stability time (*DS*, min). For the extensograph, the main parameters recorded, both at 45 and 135 minutes of resting time after dough mixing, were the extensibility (E_{45} and E_{135}, mm) and the maximum resistance to extension (R_{45} and R_{135}, BU).

3.3.4 Bread preparation and specific loaf volume analysis

Bread samples were prepared at Cereals Canada using whole-wheat flour samples (weight adjusted to 14.0% moisture basis). Distilled water based on flour water absorption (Foss calibration, Perten DA7200 NIR), salt (1.65 %), fresh compressed yeast (6.50 %), canola oil (1.75%), dough conditioner (1.00 %), and sugar (0.5%) were added based on whole-wheat flour weight. These ingredients were mixed with the whole-wheat flour in a 200 g pin mixer (National Mfg. Co., Lincoln NE) at the speed of 118 rpm until peak was achieved (determined by P2M software). Each mixed dough was divided into two pieces of 165 g, rounded manually and rested (covered) for 3 minutes. After the resting time, each dough piece was sheeted in 3 passes: 7.9 mm (first gap), 4.8 mm (second gap) and 3.2 mm (third gap), and then moulded. Moulded dough samples were placed in a fermentation cabinet (37 ºC/ RH 85%) for 45 to 60 minutes, and were removed once they reached 90 mm in height. After fermentation, doughs were baked for 20 minutes at 204 ºC. After baking, the bread samples were placed on a baking rack for one hour for cooling before loaf volume measurements.

Bread loaf volume was measured at Cereals Canada based on the AACC International Approved Method 10-14.01 (AACC International, 2014) using laser topography in a Perten TexVol BVM - L370 equipment. After the bread loaf volume was measured, it was divided by the

bread weight (recorded by the mass equivalent in g) in order to report the specific loaf volume (*SLV*, $cm^3\ g^{-1}$).

3.3.5 Statistical analysis

Statistical analysis was performed using JMP 14.0.0 statistical software (SAS Institute Inc., Cary, NC) in order to evaluate the effect of genotype (G), environment (E, location-year), fertilization treatments (F), and the interactions between these factors (G×E, G×F, E×F, and G×E×F). A three-way analysis of variance (ANOVA) was conducted with genotype, environment, fertilization treatments and their interactions as fixed factors. Pearson correlation coefficients were calculated to understand the relationship between field data (grain protein content, grain yield), free asparagine content in whole wheat flour, *SLV* and rheological parameters. Sample means for each factor were compared using Tukey's Honest Significant Difference (HSD) method at $p<0.05$. Principal Component Analysis (PCA) biplots and graphs were depicted using OriginPro 2021b SR1 9.8.5.204 (Student Version). Bread baking as well as all rheological and specific loaf volume measurements were conducted at least in duplicate.

3.4 Results and discussion

3.4.1 Gluten strength

Farinograph and extensograph results were utilized to evaluate the effects of environment, genotype and fertilization on the gluten strength of wheat samples. In Figure 3.1 and Figure 3.2, the extensograph curves (extensograms) and farinograph curves (farinograms), respectively, of representative replicates are shown to demonstrate the breadth of rheological parameters for all samples. The overall ranges for wheat grain data (protein content and yield) and rheological parameters from farinograph and extensograph measurements are presented in Table 3.2.

Figure 3.1. Extensograms of four representative samples showing maximum and minimum values of E_{135} (mm) and R_{135} (BU) obtained among the analyzed samples. The representative samples were SY Rowyn, Lilyfiled-2018, 100 kg N ha^{-1} + 17 kg S ha^{-1} (R_{max}); AAC Brandon, Carberry-2018, 100 kg N ha^{-1} + 0 kg S ha^{-1} (R_{min}); BW5011, Grosse Isle-2019, 135 kg N ha^{-1} + 0 kg S ha^{-1} (E_{max}); and AAC Elie, Lilyfield-2018, 135 kg N ha^{-1} + 0 kg S ha^{-1} (E_{min}).

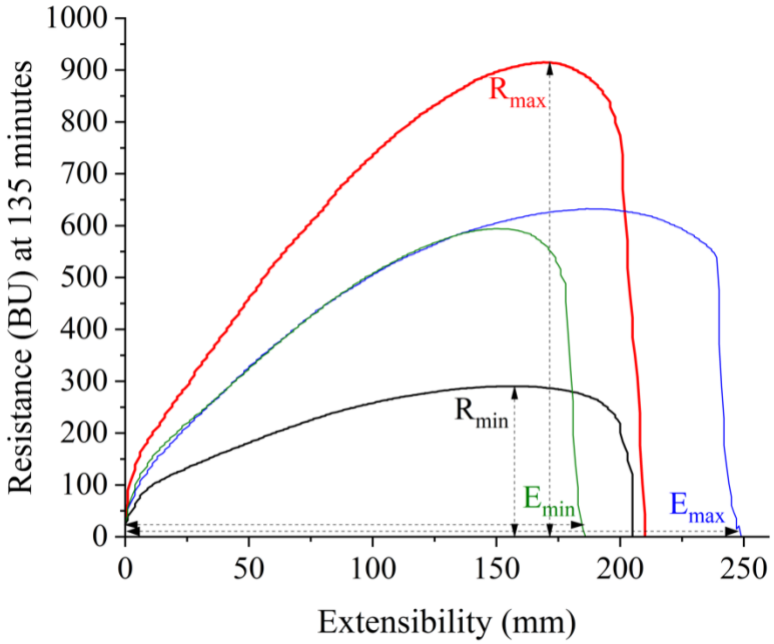


Figure 3.2. Farinograms of four representative samples showing maximum and minimum values of *DS* (top) and *DDT* (bottom), both in minutes, obtained among the analyzed samples. Farinograms are shown as three main curves (mean in red and both maximum and minimum in black), where the optimum consistency line at 500±20 BU is shown by the dashed lines. The duration of the farinograph tests varied from 30 to 50 minutes depending on the *DS* of the sample. The representative samples were SY Rowyn, Lilyfield-2018, 135 kg N ha^{-1} + 0 kg S ha^{-1} (DS_{max}); Prosper, Lilyfield-2019, 135 kg N ha^{-1} + 0 kg S ha^{-1} (DS_{min}); BW5011, Grosse Isle-2019, 100 kg N ha^{-1} + 17 kg S ha^{-1} (DDT_{max}); and AAC Elie, Lilyfield-2018, 100 kg N ha^{-1} + 0 kg S ha^{-1} (DDT_{min}).

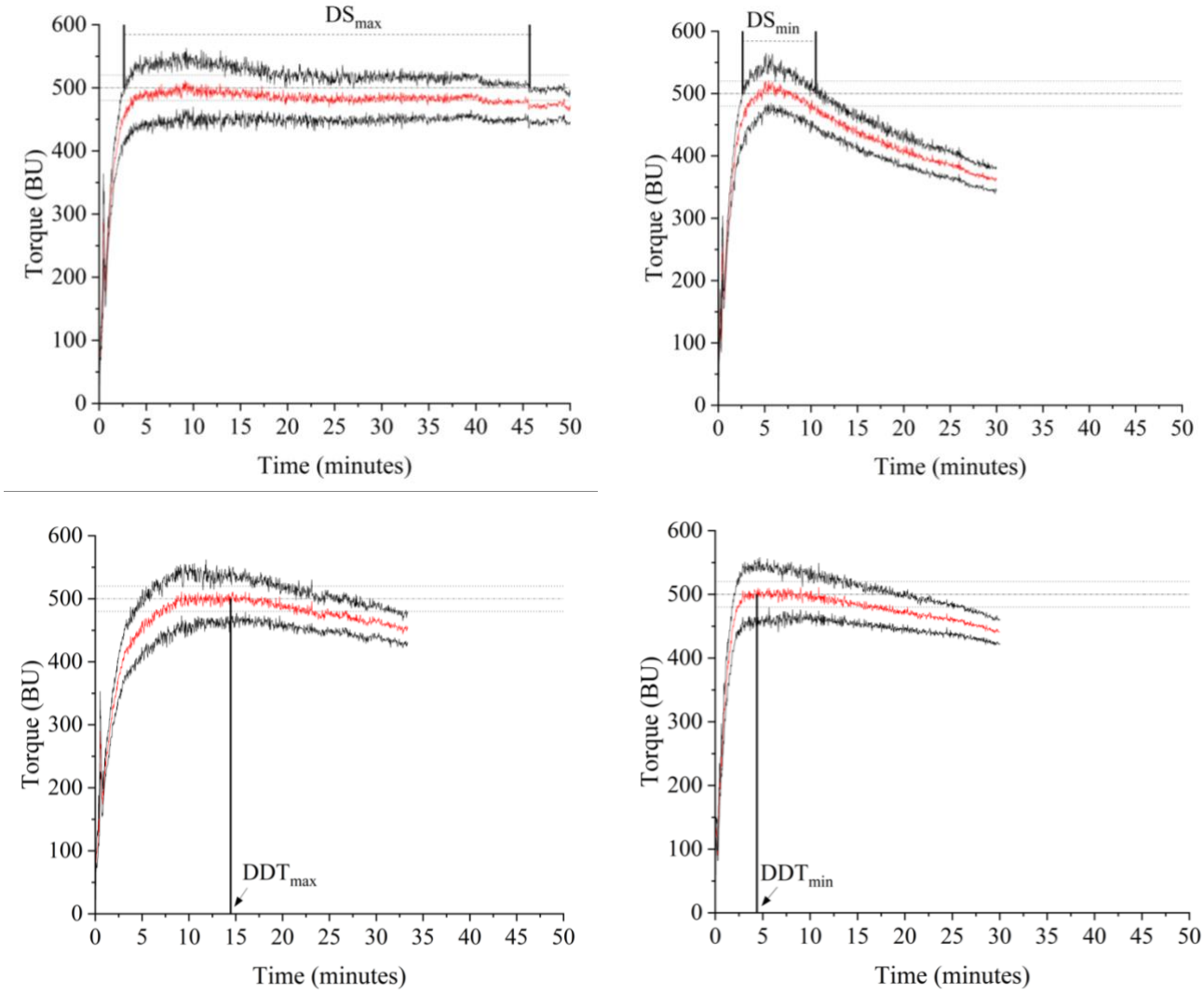


Table 3.2. Minimum and maximum values for field data (wheat protein content and yield) and empirical rheological tests (farinograph and extensograph).

Parameters	Minimum	Maximum
Wheat grain		
Protein (%)	12.3	16.8
Yield (kg/ha)	2259	7168
Farinograph		
Water absorption (%, *FAB*)	59.9	68.2
Dough development time (min, *DDT*)	4.2	14.0
Dough stability (min, *DS*)	6.9	42.4
Extensograph		
Maximum resistance to extension at 45 min (BU, R_{45})	271	785
Extensibility at 45 min (mm, E_{45})	201	252
Resistance to extensibility ratio at 45 min (R_{45}/E_{45})	1.3	3.3
Maximum resistance to extension at 135 min (BU, R_{135})	298	934
Extensibility at 135 min (mm, E_{135})	189	247
Resistance to extensibility ratio at 135 min (R_{135}/E_{135})	1.5	4.5

Canada Western Red Spring wheat varieties, which comprise the majority of the wheat genotypes used in this study, are known to possess large values of *FAB*, *DDT*, and *DS* (Preston et al., 2001). Malunga et al. (2019) investigated the rheological properties of 30 Canada hard red spring wheat genotypes grown across different locations in Canada. The FAB values reported by Malunga et al. (2019), 61.8-67.6%, where comparable to values found in the present study. Values for *DDT* (3.9-7.1 min) and *DS* (6.6-13.7 min) reported by Malunga et al. (2019), were slightly lower than the *DDT* and *DS* values obtained in this research. The extensograph results reported by Malunga et al. (2019), i.e., R_{45} (183-507 BU), E_{45} (229-294 mm) and R_{45}/E_{45} (0.8-1.8) and R_{135} (200-581 BU), E_{135} (224-295 mm), and R_{135}/E_{135} (0.8-2.4), were also lower than values found in this study. The differences in these results could be attributed to the use of different wheat genotypes, growing locations, and fertilization treatments supplied to the wheat plants in the two studies. While the study conducted by Malunga et al. (2019) involved only CWRS wheat genotypes, the present study also included the genotype Prosper (CNHR) and the genotype SY Rowyn (CPSR), which possessed, overall, the highest values for *DS* and extensograph parameters. Among the 30 wheat genotypes studied by Malunga et al. (2019), AAC Brandon, AAC Cameron,

AAC Elie, and CDC Plentiful were in common with the present study. A direct comparison between the results of this study with those of Malunga et al. (2019) to further discuss the role of environment and fertilization treatments on the rheological properties of the common varieties is not possible, as the values of the parameters were not reported by Malunga et al. (2019).

Table 3.3 shows the results of the three-way ANOVA of farinograph and extensograph results. Genotype was the most significant factor affecting *DDT*, R_{45} and R_{135} as well as R_{45}/E_{45} and R_{135}/E_{135}. The second most significant factor was environment, followed by the interaction between genotype and environment (G×E). The *FAB*, *DS*, E_{45} and E_{135} were mainly affected by the environment, followed by the genotype and G×E.

Fertilization treatments had a significant effect on *FAB* and *DDT*, while having no significant effect on *DS* and on extensograph parameters. Remaining interactions between genotype, environment and fertilization treatments (G×F, E×F, and G×E×F) also significantly affected the farinograph parameters *FAB*, *DDT* and *DS*. For the extensograph, the E×F effect was significant only for R_{45} and R_{45}/E_{45}, while the G×F effect was significant for R_{135}, E_{135} and R_{135}/E_{135}. Finally, the G×E×F effect was significant only for extensograph parameters at 135 minutes. The effects of each factor will be discussed in detail in the following sections.

Table 3.3. Three-way analysis of variance for the effect of environment, fertilization, genotype, and their interactions on farinograph and extensograph parameters.

Farinograph		***FAB* (%, 14% m.b.)**		***DDT* (minutes)**		***DS* (minutes)**	
Source	**DF**	**F value**	**Percent of Total Variance (%)**	**F value**	**Percent of Total Variance (%)**	**F value**	**Percent of Total Variance (%)**
Environment (E)	3	8791.26***	50.2	405.22***	19.2	3223.48***	45.5
Genotype (G)	7	2859.79***	38.1	418.81***	46.2	1073.71***	35.4
Fertilization Treatment (F)	3	122.06***	0.7	41.19***	1.9	0.95 n.s.	0.0
G×E	21	156.49***	6.3	41.53***	13.7	143.89***	14.2
G×F	21	37.06***	1.5	9.72***	3.2	7.58***	0.7
E×F	9	36.85***	0.6	24.13***	3.4	19.17***	0.8
G×E×F	63	17.96***	2.2	8.32***	8.3	7.19***	2.1
Error	256	N/A	0.5	N/A	4.0	N/A	1.2
Extensograph		***E_{45}* (mm)**		***R_{45}* (BU)**		***R_{45}/E_{45}***	
Environment (E)	3	178.00***	42.5	815.59***	35.3	357.08***	34.0
Genotype (G)	7	52.91***	29.5	491.94***	49.7	207.23***	46.0
Fertilization Treatment (F)	3	0.35 n.s.	0.1	1.74 n.s.	0.1	0.79 n.s.	0.1
G×E	21	7.92***	13.2	35.16***	10.7	17.40***	11.6
G×F	21	0.68 n.s.	1.1	2.58**	0.8	1.79*	1.2
E×F	9	0.48 n.s.	0.3	2.50*	0.3	2.05*	0.6
G×E×F	63	0.62 n.s.	3.1	1.39 n.s.	1.3	1.25 n.s.	2.5
Error	128	N/A	10.2	N/A	1.8	N/A	4.1
		***E_{135}* (mm)**		***R_{135}* (BU)**		***R_{135}/E_{135}***	
Environment (E)	3	87.79***	31.2	588.61***	26.4	250.90***	23.6
Genotype (G)	7	23.27***	19.3	588.37***	61.6	270.42***	59.5
Fertilization Treatment (F)	3	0.98 n.s.	0.3	1.45 n.s.	0.1	1.28 n.s.	0.1
G×E	21	6.65***	16.5	23.37***	7.3	11.80***	7.8
G×F	21	1.85*	4.6	2.94**	0.9	2.25**	1.5
E×F	9	1.13 n.s.	1.2	1.86 n.s.	0.2	1.39 n.s.	0.4
G×E×F	63	1.57*	11.7	1.65**	1.6	1.56*	3.1
Error	128	N/A	15.2	N/A	1.9	N/A	4.0

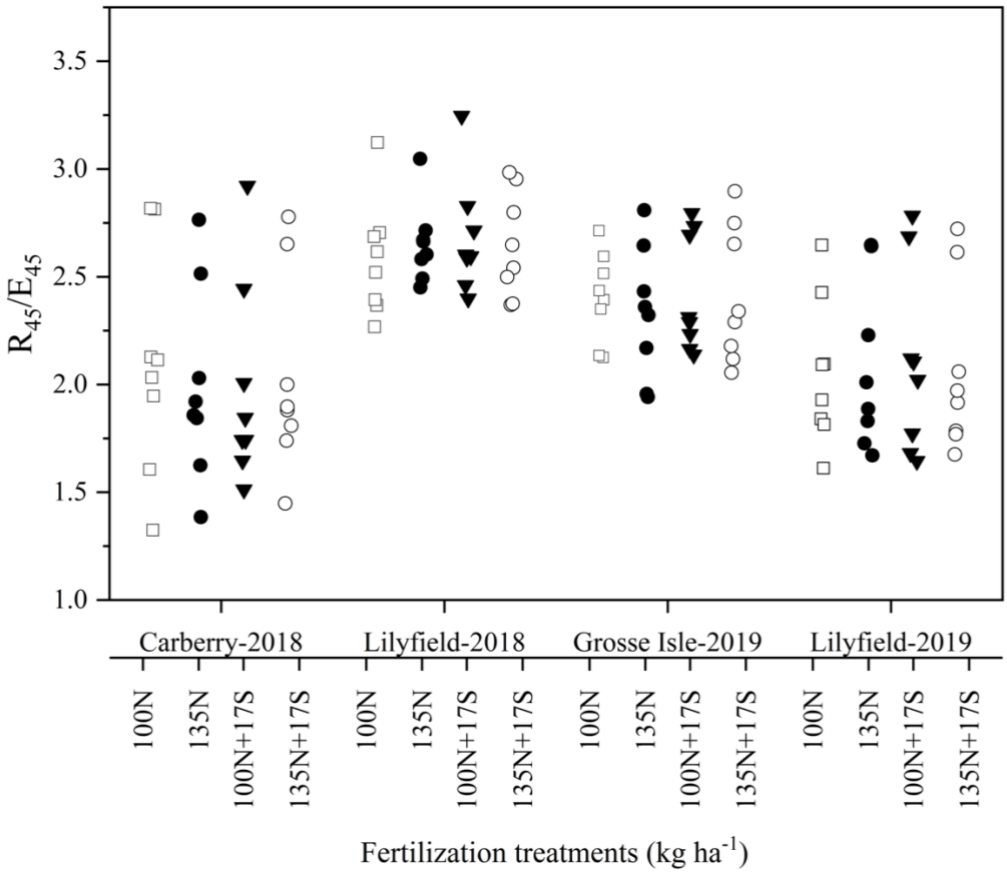
FAB = farinograph water absorption, *DDT* = dough development time, *DS* = dough stability, *E* = dough extensibility, *R*= maximum resistance to extension. Extensograph parameters were recorded after 45 and 135 minutes of dough resting. ***, **, *, and n.s. mean that factors were significant at $p<0.0001$, $p<0.01$, $p<0.05$, and non-significant at $p<0.05$, respectively.

3.4.1.1 Effect of environment

Environment was the most significant factor affecting *FAB*, *DS*, E_{45} and E_{135} (Table 3.3), and grain protein and yield, found in Table 3.4, and the second most significant factor affecting *DDT*, R_{45}, R_{135}, R_{45}/E_{45}, and R_{135}/E_{135}. Very little was found in the literature on the effects of environmental conditions on wheat flour dough rheology. Panozzo & Eagles (2000) studied the dough rheological properties of seven wheat varieties grown in 15 environments (composed of location, sowing time, and irrigation) over the years of 1991 and 1992 in Australia. In that study, Panozzo and Eagles (2000) found a significant ($p<0.01$) effect of environment on *DDT*, *E* and *R*. Additionally, when studying dough rheology of three wheat varieties grown in four different environments (referred to as location in their study) using Kieffer tests, Hasniza et al. (2014) also found a significant ($p<0.001$) effect of environment on dough *R* and *E*.

Results of means comparison by Tukey's HSD tests for field data (grain protein content and grain yield), *FAB*, *DDT*, *DS*, R_{45}, R_{135}, E_{45} , E_{135} , R_{45}/E_{45}, and R_{135}/E_{135} per environment can be found in Table 3.5. In general, samples grown in 2019 had significantly larger *FAB* mean values when compared to samples grown in 2018. However, *DS* mean values of samples from the four environments were all significantly different, with Lilyfield-2019 wheat having the smallest mean value (7.8 min) and Lilyfield-2018 wheat possessing the largest mean value (18.9 min). For *DDT* results, wheat grown at Grosse Isle-2019 had the highest mean value (9.9 min), while wheat from the other environments possessed *DDT* values that were not significantly different (7.8-8.0 min). For extensograph parameters, environment was the main factor affecting dough E_{45} and E_{135}, with Grosse Isle-2019 yielding the most extensible dough (largest *E* values) among all the environments. However, when *R* and *R/E* were evaluated, Lilyfield-2018 yielded the strongest gluten, with the largest mean values for both parameters at 45 (604 BU, 2.6) and 135 (686 BU, 3.3) minutes. For example, in Figure 3.3, which shows the variation in R_{45}/E_{45} among the four site-years (environment) and fertilization treatments, it can be observed that Lilyfield-2018 resulted in samples with overall higher values for R_{45}/E_{45}, followed by Grosse isle-2019. Although genotype was the main factor affecting this rheological parameter (Table 3.3), it is possible to see, by the clear variation among the site-years in Figure 3.3, that environment had great effects on R_{45}/E_{45}. On the other hand, comparison between fertilization treatments within the same environment shows the little effect of this factor on R_{45}/E_{45}, which is expected as per Table 3.3.

Figure 3.3. Distribution of mean values for R_{45}/E_{45} of the eight studied wheat genotypes, among four environments (Carberry-2018, Lilyfield-2018, Grosse Isle-2019, and Lilyfield-2019) and for each fertilization treatment (100 or 135 kg ha^{-1} of nitrogen and 0 or 17 kg ha^{-1} of sulfur).



Regarding field data, the mean protein content (Table 3.5) was significantly higher for the wheat grown in 2019 than for that grown in 2018, with the highest variation taking place at Lilyfield. This significant variation shows that, in addition to the location, weather variations between the two studied years played an important role in the environment effect. A positive correlation between protein content and maximum daily temperatures below 30 ºC, mainly during the grain filling period, has been previously reported (Johansson et al., 2013; Johansson & Svensson, 1998).

Table 3.4. Three-way analysis of variance for the effect of environment, fertilization, genotype, and their interactions on specific loaf volume (*SLV*) and field data (grain protein and yield).

		SLV **($cm^3\ g^{-1}$)**			**Grain protein content (%)**			**Grain yield ($kg\ ha^{-1}$)**
Source	**DF**	**F value**	**Percent of Total Variance (%)**	**DF**	**F value**	**Percent of Total Variance (%)**	**F value**	**Percent of Total Variance (%)**
Environment (E)	3	132.39***	29.1	3	232.95***	53.5	1882.41***	91.2
Genotype (G)	7	59.80***	30.7	7	25.80***	13.8	19.54***	2.2
Fertilization Treatment (F)	3	1.50 $^{n.s.}$	0.3	3	12.25***	2.8	0.66 $^{n.s.}$	0.0
G×E	21	5.64***	8.7	21	1.93**	3.1	4.33***	1.5
G×F	21	3.12***	4.8	21	1.70*	2.7	0.63 $^{n.s.}$	0.2
E×F	9	4.64***	3.1	9	1.09 $^{n.s.}$	0.8	0.87 $^{n.s.}$	0.1
G×E×F	63	3.03***	14.0	63	0.77 $^{n.s.}$	3.7	0.58 $^{n.s.}$	0.6
Error	128	N/A	N/A	256	N/A	N/A	N/A	N/A

***, **, *, and $^{n.s.}$ means that factors were significant at $p<0.0001$, $p<0.01$, $p<0.05$, and non-significant at $p<0.05$, respectively.

Table 3.5. Mean values of empirical rheological parameters, grain protein content, grain yield, and specific loaf volume, averaged over genotypes and fertilization treatments.

	Farinograph			**Extensograph**						**Field Data**		**Bread quality**
Environment	***FAB*** **(%)**	***DDT*** **(min)**	***DS*** **(min)**	E_{45} **(mm)**	R_{45} **(BU)**	R_{45}/E_{45}	E_{135} **(mm)**	R_{135} **(BU)**	R_{135}/E_{135}	**Protein content (%)**	**Yield ($kg\ ha^{-1}$)**	***SLV*** **($cm^3\ g^{-1}$)**
Carberry-2018	63.1±1.5^{c}	8.0±1.9^{b}	12.1±3.9^{c}	218±13^{c}	444±118^{b}	2.0±0.5^{c}	207±10^{c}	519±154^{b}	2.5±0.7^{c}	14.0±0.7^{c}	6034±428^{a}	5.11±0.20^{a}
Lilyfield-2018	62.7±1.4^{c}	8.0±2.0^{b}	18.9±8.2^{a}	228±15^{b}	604±79^{a}	2.6±0.3^{a}	210±12^{c}	686±118^{a}	3.3±0.5^{a}	13.8±0.6^{d}	4990±334^{b}	5.18±0.22^{a}
Lilyfield-2019	66.0±1.2^{a}	7.8±1.3^{b}	9.4±1.8^{d}	224±15^{b}	468±105^{b}	2.1±0.4^{c}	217±14^{b}	540±132^{b}	2.5±0.6^{c}	15.6±0.5^{a}	2759±220^{d}	4.86±0.22^{b}
Grosse Isle-2019	64.0±1.0^{b}	9.9±2.1^{a}	15.6±4.0^{b}	245±6^{a}	587±76^{a}	2.4±0.3^{b}	225±12^{a}	680±111^{a}	3.0±0.6^{b}	15.4±0.4^{b}	3274±107^{c}	4.87±0.23^{b}

Different letters in the same column indicate that values are significantly different at $p<0.05$. Data are presented as mean ± SD.

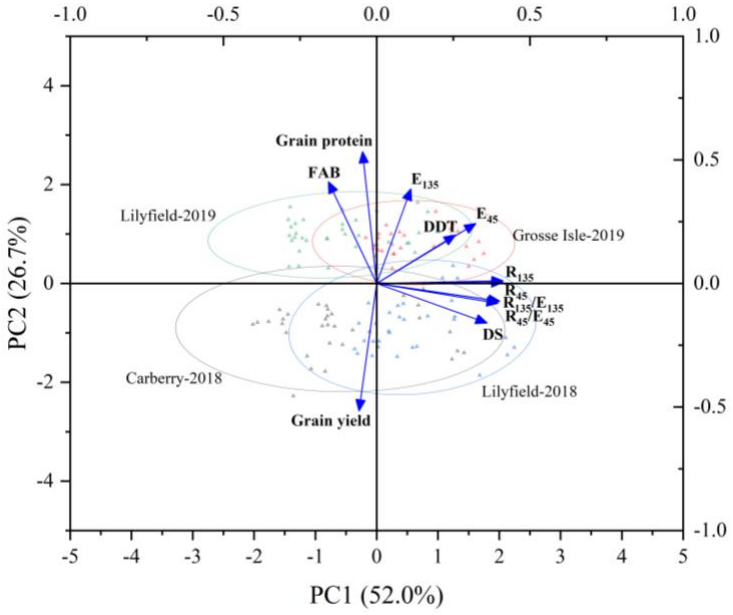
Rainfall and soil moisture can affect grain protein concentration, with lower precipitation contributing to higher protein concentration in wheat grain (Gooding & Davies, 1997a; J. Liu et al., 2021; Ozturk & Aydin, 2004). Although samples grown at Carberry-2018 were exposed to a higher cumulative heat (crop heat units) during the growing period (115 days), compared to those grown at Grosse Isle (113 days) and Lilyfield (120 days) in 2019, they also received more precipitation (Table 3.1). Therefore, the higher average amount of rainfall in 2018 compared to that in 2019 could explain the lower amount of protein content in 2018 wheat compared to the wheat grown in 2019 (Xie et al., 2021).

Soil type can also affect the protein content accumulation in wheat (Nyiraneza et al., 2012). The site Carberry has a Wellwood Orthic Black Chernozem soil, Lilyfield has a Red River Clay soil and Grosse Isle has a Dark Gray Chernozem soil. Wellwood Orthic Black Chernozem soil has a sandy-like characteristic (Fuller, 2010), which allows for a higher moisture loss and nutrient leaching, thus negatively impacting the grain protein content (Gooding & Davies, 1997b). Clay soils, like those at Lilyfield, have high moisture retention capacity (Kandel et al., 2013), but not as much as that of the soil at Grosse Isle, i.e., Dark Gray Chernozem (Fuller, 2010). This could also explain higher mean protein content in wheat grown at Grosse Isle-2019 compared to other wheats, as a result of lesser extent of nutrient leaching at Grosse Isle.

Yield was, overall, negatively correlated with protein content ($r = -0.760$, $p < 0.0001$), which is in accordance with previous studies (Oury & Godin, 2007; Ozturk & Aydin, 2004). Higher yield was achieved in 2018 compared to that in 2019, i.e., highest yield was obtained in Carberry-2018 (6,034 kg ha^{-1}) and lowest yield was observed in Lilyfield-2019 (2,759 kg ha^{-1}). The negative correlation between protein content and grain yield in this study could be explained by the dry matter accumulation in the wheat grain under different precipitation levels. It was hypothesized in the literature that, under low precipitation, carbohydrate accumulation in the grain decreases and the remobilization of nitrogen from the wheat plant to the grain increases (Clarke et al., 1990; J. Liu et al., 2021; Panozzo & Eagles, 1999). This process (higher nitrogen and lower carbohydrate accumulation in wheat grain under low precipitation conditions), which is anticipated to lead to higher concentrations of protein in the grain, could explain the negative relationship between the protein content and the yield. For example, Carberry-2018 had the highest precipitation among the four environments, which could be contributing to the higher yield, while the sandy-like soil of this location could be contributing to less nitrogen available to the plant, due

to nutrient leaching. Moreover, this hypothesis could explain the higher protein content and the lower grain yield in samples grown in 2019 compared to those grown in 2018, as the former were subjected to lower precipitation than the latter (Table 3.1). This negative correlation between the protein content and the yield is visualized in Figure 3.4, which is a PCA biplot for dough rheological parameters and the field data.

Figure 3.4. Biplot from principal component analysis (PCA) of field data (grain protein and yield) and rheological parameters from farinograph and extensograph by environment: Lilyfield-2018 (blue), Carberry-2018 (gray), Lilyfield-2019 (green), and Grosse Isle-2019 (red). Ellipses indicate 95% confidence for each environment.



As mentioned before, in this study, farinograph and extensograph were employed to evaluate the gluten strength. As shown in Figure 3.4, PC1 and PC2 accounted for about 79% of the total variation in the rheological parameters and field data. In this biplot, it is possible to see a positive correlation between *FAB* and grain protein content ($r = 0.692$, $p<0.0001$), which is in accordance with previous studies (Finney, 1945; Koksel & Scanlon, 2012; Sapirstein et al., 2018). Among the four environments, wheat grown at Carberry-2018 and Lilyfield-2019 showed lower gluten strengths, measured by *DS*, R_{45}, R_{135}, R_{45}/E_{45}, and R_{135}/E_{135}, compared to wheat grown at Lilyfield-2018 and Grosse Isle-2019 (Table 3.5). This outcome can also be noted in Figure 3.4,

where the PC1, positively correlated with *DS*, R_{45}, R_{135}, R_{45}/E_{45} and R_{135}/E_{135}, effectively segregated the samples by environment, i.e., Lilyfield-2018 and Grosse Isle-2019 separated from Carberry-2018 and Lilyfield-2019 along PC1, with samples grown at Lilyfield-2018 being more correlated with gluten strength parameters.

In a pot experiment conducted by Zhao et al. (2009), a single wheat variety was treated at post-anthesis with three soil water levels (45, 65 and 85% of the soil water capacity). Zhao et al. (2009) showed that lower water levels (45% of soil water capacity) resulted in a significant decrease in the gliadin-to-glutenin ratio (Gli/Glu) (C. X. Zhao et al., 2009). On the other hand, Kharel et al. (2011) observed a positive correlation between water stress and Gli/Glu, which was then supported by the results from Park et al. (2014). Koga et al. (2016) evaluated the final gluten protein composition of four Norwegian spring wheat varieties grown under three temperature regimens (day/night) of 13/10 ºC (low), 18/15 ºC (medium) and 23/20 ºC (high). The authors showed that wheat grown under the "high" temperature regimen and high N/S (from 16.4 to 17.5) consisted of higher amounts of sulfur-poor gliadins (ω-gliadins) and D-type LMW glutenin subunits (sulfur-poor glutenin subunit) when compared to the other regimens (Koga et al., 2016). Recently, Liu et al. (2021) studied one wheat variety cultivated in China during 2018 and 2019 under irrigated and non-irrigated conditions, as well as high (240 kg ha^{-1}) and normal (120 kg ha^{-1}) nitrogen fertilization. Under normal nitrogen levels, the authors observed a significantly higher amount of ω-gliadins under non-irrigated conditions and a significantly higher amount of glutenins under irrigated conditions (J. Liu et al., 2021). Therefore, these outcomes mean that the accumulation of gliadins, mainly sulfur-poor gliadins, could be highly influenced by environmental conditions, such as drought, which could explain variations in dough rheological properties. Moreover, as the amount of nitrogen in the soil can be influenced by rainfall, e.g., nutrient leaching, drought conditions could provide nitrogen to the plant for longer periods, which could lead to the accumulation of more sulfur-poor gliadins in the wheat, resulting in wheat flour doughs with weaker glutens.

In the present study, as per Table 3.1, samples grown at Carberry-2018 and Lilyfield-2019 were subjected to higher cumulative heat over the growing season. Although the soil in Carberry was sandy-like, thus retaining less moisture, the N/S ratio in the soil was higher than that in other sites, which could also positively impact the Gli/Glu ratio (Fuertes-Mendizábal et al., 2013; Godfrey et al., 2010). Therefore, the lower gluten strength values for wheat grown at Carberry-

2018 and Lilyfield-2019 might be explained by their higher Gli/Glu ratio compared to the wheat grown at the other two environments. Moreover, the positive correlation between protein content and *FAB* could be explained by the higher proportion of gliadins in wheats with higher protein contents. This is supported by previous studies that found a positive correlation between FAB and Gli/Glu (Godfrey et al., 2010) and between *FAB* and ω-gliadins (Malalgoda et al., 2018).

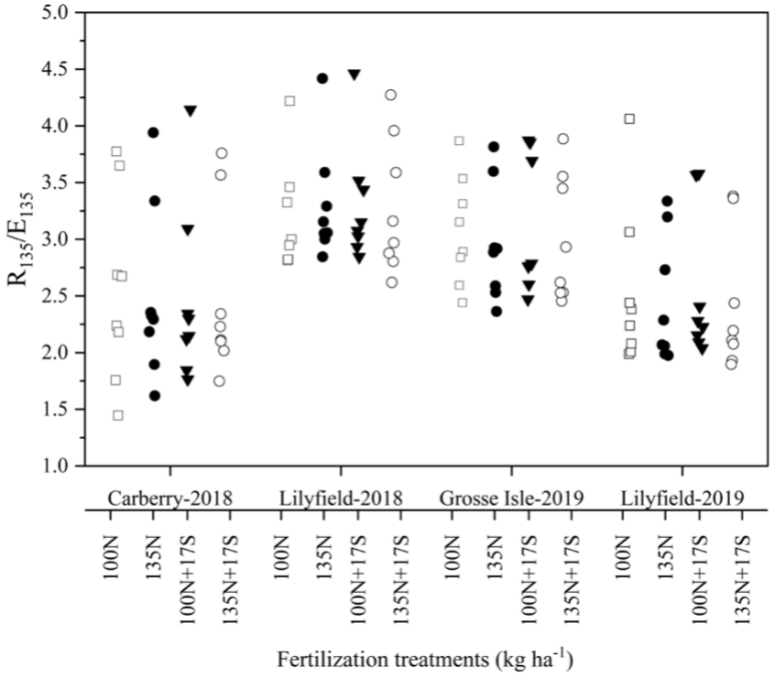
In summary, wheat grown at Lilyfield-2018 and Grosse Isle-2019 possessed stronger glutens. High cumulative heat over the growing season (CHU) and less moisture at Carberry-2018 and Lilyfield-2019 (Table 3.1) might have caused a higher Gli/Glu ratio of wheats grown at these environments compared to those of other environments, which could explain the weaker gluten strength values, as measured by *DS*, R_{45}, R_{135}, R_{45}/E_{45}, and R_{135}/E_{135}.

3.4.1.2 Effect of genotype

In this study, as per Table 3.3, genotype was the most significant factor affecting *DDT*, *R* and *R/E*, and the second most significant factor affecting *FAB*, *DS*, *E* and field parameters (protein content and yield, Table 3.4). These results conform with past studies reporting a significant effect of genotype on *DDT*, *E*, *R* (Panozzo & Eagles, 2000; Wilson et al., 2020), *FAB* and *DS* (Wilson et al., 2020). Also, using Kieffer tests, Hasniza et al. (2014) who studied three Australian wheat genotypes, and Islas-Rubio et al. (2011), who studied five bread wheat lines, reported significant effects of genotype on dough rheological properties (*R* and *E*). In Figure 3.5, a visual representation of how genotype affected the rheological parameter R_{135}/E_{135} is shown, for each environment and fertilization treatment. Although it is possible to observe in Figure 3.5 differences among environments, e.g., Lilyfield-2018 resulting in overall higher values for R_{135}/E_{135} when compared to Lilyfield-2019, genotype was the main factor affecting R_{135} and R_{135}/E_{135}. This effect from genotype can be observed in Figure 3.5 by the large variation of the mean R_{135}/E_{135} values within a given environment and fertilization treatment. Moreover, when compared to Figure 3.3, which shows the same graph but for R_{45}/E_{45}, it is possible to observe an overall increase in R_{135}/E_{135} values, with genotype playing a more important role in R_{135} and R_{135}/E_{135} when compared to R_{45} and R_{45}/E_{45} (Table 3.3). Sobczyk et al., (2017) performed extensograph tests using flour from ten spelt cultivars and lines and a single bread wheat variety for comparison (control). The authors observed that, for spelt cultivars, the *R/E* decreased with resting time, whereas for the bread wheat variety, *R/E* increased when doughs were rested for 135 minutes when compared to 45 minutes (Sobczyk et al., 2017). Sehn and Steel (2017) performed extensograph tests on eleven commercial

whole-wheat flours, on eight pre-mixes made from white flour, coarse and fine bran, and on white flour as control sample. The authors also reported an increase in *R* when resting time increased from 45 to 135 min (Sehn & Steel, 2017), agreeing with results found in this study. Sobczyk et al., (2017) referred to the resting time as fermentation time, even though no yeast was added in the dough prepared for extensograph tests. Therefore, the authors explained that dough performance during extension, at different resting times, could be used to determine if a specific wheat flour is suitable for longer fermentation times or not (Sobczyk et al., 2017). Sobczyk et al., (2017) concluded that spelt varieties that resulted in doughs with lower R_{135}/E_{135}, when compared to R_{45}/E_{45}, should not be subjected to longer fermentation times, which could result in doughs with excessive extensibility and poor bread quality. Based on this discussion, the observed increase in R_{135}/E_{135}, mainly due to overall higher R_{135} when compared to R_{45}, shows that the wheat flour doughs of the present study are suitable for breadmaking with long fermentation times. Moreover, the substantial increase of the effect of genotype for R_{135} and R_{135}/E_{135} (Table 3.3) could confirm the suitability of all wheat varieties involved in this study for breadmaking.

Figure 3.5. Distribution of mean values for R_{135}/E_{135} of the eight studied wheat genotypes, among four environments (Carberry-2018, Lilyfield-2018, Grosse Isle-2019, and Lilyfield-2019) and for each fertilization treatment (100 or 135 kg ha^{-1} of nitrogen and 0 or 17 kg ha^{-1} of sulfur).



Mean values of field and rheological parameters for each variety, averaged over environment and fertilization, as well as the means comparison by Tukey-Kramer HSD are provided in Table 3.6. A visual representation of the rheological data acquired for each variety, averaged for each environment (n=4) and fertilization treatments (n=4) combination, through empirical tests, i.e., farinograph and extensograph, is provided in Figure 3.6. Therefore, Figure 3.6 allows one to focus the observation on the variation of the rheological parameters for each variety, regardless of environment or fertilization treatment. For farinograph results, the varieties Glenn and AAC Elie showed the highest *FAB* (65.3 %) while SY Rowyn had the lowest *FAB* (62.5 %). Glenn also showed the highest *DDT* (10.8 min), followed by SY Rowyn (9.9 min), while SY Rowyn showed the highest *DS* (23.2 min), followed by Glenn (16.1 min). Liu et al. (2011) also performed farinograph tests using flour prepared from the wheat variety Glenn, grown at two locations, and reported a *FAB* of 65.7% and *DDT* of 9.0 minutes, values which are comparable to the results from the present study.

Prosper, on the other hand, was the genotype that possessed the weakest gluten, as measured by farinograph tests, with the lowest values for *DDT* (6.1 min) and *DS* (10.6 min). The *DS* for Prosper (10.6 min) was not significantly different from *DS* for AAC Cameron (11.5 min), BW5011 (12.2 min), AAC Brandon (12.3 min), AAC Elie (12.8 min) and CDC Plentiful (13.2 min). This shows that *DS* was not as sensitive to differences in wheat genotype in this study. For extensograph results, the genotype SY Rowyn had the highest R_{45} (674 BU), R_{45}/E_{45} (2.8), R_{135} (821 BU) and R_{135}/E_{135} (3.9), thus producing the strongest gluten among all genotypes. The most extensible, thus weakest gluten, belonged to AAC Brandon with an E_{45} of 216 mm and R_{45}/E_{45} of 1.9 as well as an E_{135} of 201 mm and R_{135}/E_{135} of 2.3. These results are in line with recent findings from Tozatti et al. (2020), who reported data from mixing (micro-doughLAB and mixograph) and shear rheometry tests. These authors showed that Glenn and AAC Brandon yielded one of the strongest and weakest glutens, respectively, among 25 Canadian wheat genotypes from Canada Western Red Spring (CWRS), Canada Northern Hard Red (CNHR), Canada Western Hard White Spring (CWHWS), Canada Western Special Purpose (CWSP), Canada Prairie Spring Red (CPSR) and Canada Western Extra Strong (CWES) wheat classes (Tozatti et al., 2020).

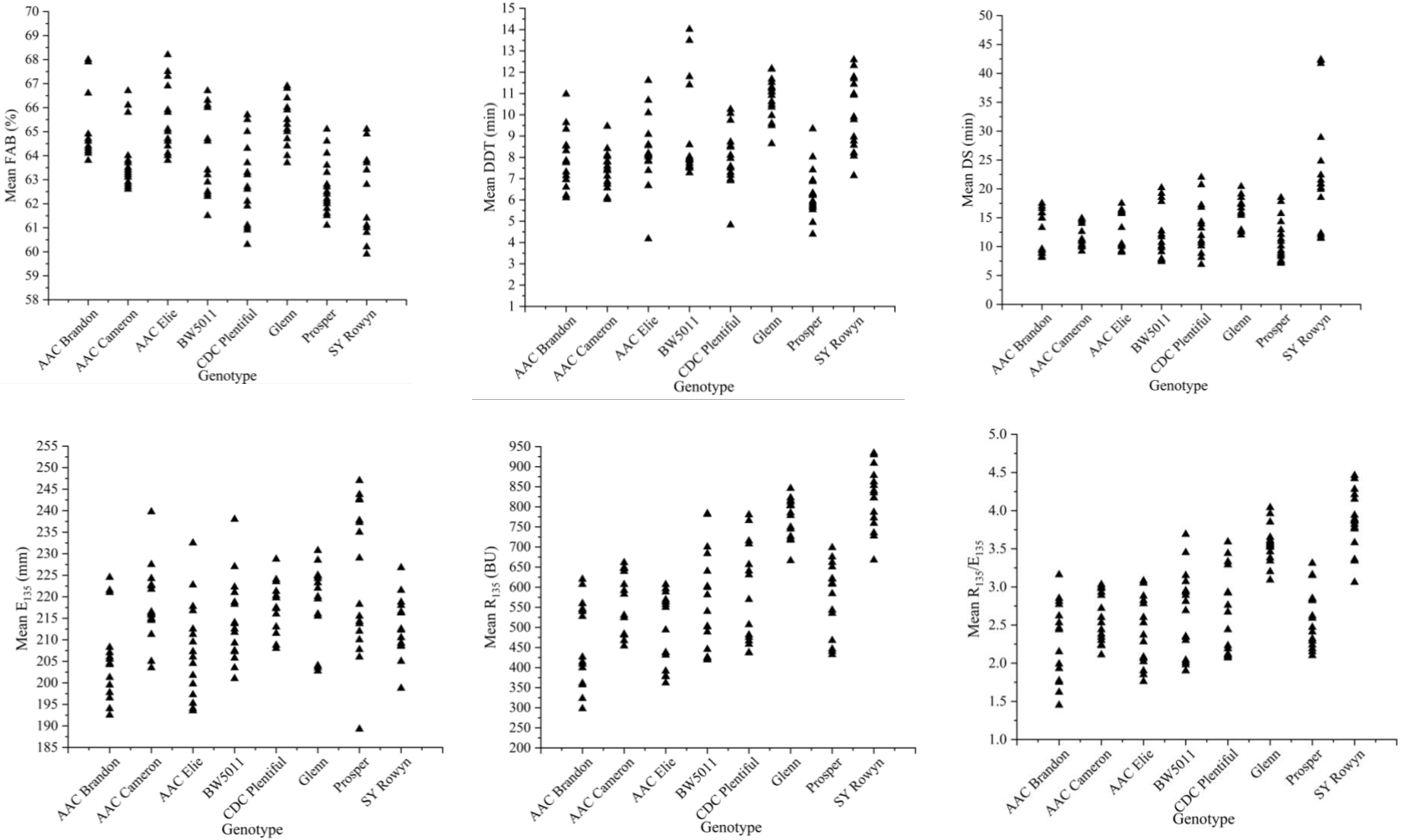
Regarding field data, protein content was significantly ($p<0.05$) correlated with *DDT* ($r = 0.343$), E_{45} ($r = 0.204$) and E_{135} ($r = 0.436$) and significantly ($p<0.05$) correlated with *DS* ($r = -0.293$), R_{45}/E_{45} ($r = -0.222$), and R_{135}/E_{135} ($r = -0.185$).

Table 3.6. Mean values of quality parameters averaged over environment and fertilization treatments.

	Farinograph						Extensograph			Field Data		Bread
Genotype	***FAB*** **(%)**	***DDT*** **(min)**	***DS*** **(min)**	E_{45} **(mm)**	R_{45} **(BU)**	R_{45}/E_{45}	E_{135} **(mm)**	R_{135} **(BU)**	R_{135}/E_{135}	**Protein content (%)**	**Yield (kg ha^{-1})**	***SLV*** **(cm^3 g^{-1})**
AAC Brandon	65.1±1.3^{a}	7.9±1.4^{c}	12.3±3.4^{c}	216±16^{e}	418±94^{d}	1.9±0.4^{e}	207±13^{d}	468±104^{c}	2.3±0.5^{e}	14.6±0.8^{b}	4315±1295^{a}	4.78±0.28^{d}
AAC Cameron	64.0±1.3^{b}	7.4±1.0^{c}	11.5±2.1^{c}	231±13^{bcd}	524±69^{b}	2.3±0.3^{c}	218±12^{ab}	568±78^{b}	2.6±0.3^{cd}	14.7±0.8^{ab}	4271±1303^{a}	4.98±0.25^{bc}
AAC Elie	65.3±1.4^{a}	8.3±1.7^{bc}	12.8±3.3^{c}	217±16^{e}	445±79^{cd}	2.1±0.3^{de}	208±13^{cd}	494±89^{c}	2.4±0.4^{de}	14.9±0.8^{ab}	4502±1370^{a}	4.81±0.17^{cd}
CDC Plentiful	62.8±1.7^{c}	8.1±1.4^{c}	13.2±4.8^{bc}	227±15^{cd}	504±119^{b}	2.2±0.4^{cd}	217±9^{b}	589±122^{b}	2.7±0.6^{c}	14.9±0.8^{ab}	3973±1100^{a}	5.21±0.24^{a}
BW5011	63.8±1.6^{b}	9.0±2.3^{b}	12.2±4.4^{c}	225±16^{d}	489±115^{bc}	2.1±0.4^{cd}	214±13^{bc}	570±122^{b}	2.7±0.6^{c}	15.3±1.0^{a}	4387±1390^{a}	4.94±0.29^{bcd}
Glenn	65.3±1.4^{a}	10.8±1.1^{a}	16.1±2.5^{b}	241±11^{a}	642±40^{a}	2.7±0.2^{b}	218±13^{ab}	774±58^{a}	3.6±0.4^{b}	14.9±0.8^{ab}	4023±1281^{a}	5.08±0.16^{ab}
Prosper	62.7±1.1^{c}	6.1±1.0^{d}	10.6±3.5^{c}	234±15^{abc}	509±77^{b}	2.2±0.3^{cd}	224±18^{a}	565±84^{b}	2.5±0.4^{cd}	13.8±1.1^{c}	4557±1598^{a}	5.18±0.19^{a}
SY Rowyn	62.5±1.9^{c}	9.9±1.8^{a}	23.2±10.4^{a}	237±13^{ab}	674±61^{a}	2.8±0.3^{a}	213±10^{bcd}	821±82^{a}	3.9±0.5^{a}	14.4±0.9^{bc}	4087±1431^{a}	5.05±0.17^{ab}

Different letters in the same column means that values are significantly different at $p<0.05$. Data are presented as mean ± SD.

Figure 3.6. Mean farinograph and extensograph (at 135 min) parameters for eight wheat genotypes, averaged over each environment (n=4) and fertilization treatment (n=4) combination, of this study.



The negative relationship between protein content and gluten strength in this study is in agreement with results found by Janković et al. (2015). These authors studied extensional dough properties for five bread wheat genotypes (coded from BW1 to BW5), grown at the same location. Janković et al. (2015) observed that the genotypes with low protein content, e.g. BW5 with 9.1% of protein content, had stronger glutens (*R/E* = 3.15) compared to genotypes with high protein content, e.g. BW4 with 10.5% of protein content which had an *R/E* of 1.28 (Janković et al., 2015).

Although genotype significantly affected the gluten strength as measured by empirical rheology, Figure 3.6 shows a variation in the range of the mean values (representing each environment-fertilization treatment combination) for each genotype. For example, mean values of E_{135} for Prosper varied from 189 to 247 mm, while SY Rowyn had mean *DS* values varying from 6.9 up to 42 min. On the other hand, AAC Cameron showed a tighter variation for some mean values, e.g., *DS* varying from 9.2 to 15 minutes. This could indicate that each genotype performs differently depending on interactions with other factors, such as environment (Table 3.3). Additionally, the smaller effect of wheat genotype on gluten strength, compared to that of environment, could be explained by the wheat classes involved in this study. Although SY Rowyn and AAC Brandon presented the highest and lowest gluten strengths, respectively, their rheological properties still translated into an elastic-like dough (e.g., *R/E* values of all genotypes in this study were larger than 1). A greater role of genotype on gluten strength could have been observed if wheat genotypes from soft wheat classes were also included in this study.

3.4.1.3 Effect of fertilization

Nitrogen and sulfur fertilization treatments have been shown to impact gluten strength as evaluated by empirical rheology tests (Godfrey et al., 2010; Järvan et al., 2017; Wilson et al., 2020). Järvan et al. (2017) studied two winter wheat varieties, grown at two different locations in northern and southern Estonia under nitrogen fertilization (100 kg ha^{-1}) without and with sulfur fertilization (varying from 6.8 to 39 kg ha^{-1}). The authors reported an increase in DS when the wheat grains received sulfur compared to the treatments without sulfur (Järvan et al., 2017). Wilson et al. (2020) reported farinograph results of five wheat genotypes grown in 2018 in the USA, averaged over three nitrogen fertilization treatments (56, 100 and 145 k ha^{-1}) to compare the effect of 0 and 22 kg ha^{-1} sulfur fertilization treatments. The authors reported a significant increase in *FAB*, *DDT* and *DS* when 22 kg ha^{-1} of sulfur was provided to the wheat plant compared to no sulfur fertilization (Wilson et al., 2020). The effect of nitrogen fertilization was studied by Godfrey

et al. (2010) utilizing a single wheat variety grown at Rothamsted, in England, over three years. The authors applied nitrogen fertilizer from 0 to 288 kg ha^{-1}, keeping sulfur fertilization level at 53 kg ha^{-1}. As a result, a positive correlation ($p<0.05$) between nitrogen rate and dough *FAB, DDT*, *DS*, and *E* was observed (Godfrey et al., 2010). However, the fertilization treatments used in the present study did not have the same effect on dough rheological properties as reported in the literature.

According to Table 3.3, fertilization treatments had a small (percent of variation < 1.9%) or even non-significant ($p > 0.05$) effect on the rheological parameters of wheat. Table 3.7 provides the dough rheological properties, averaged over environment and genotype, for each fertilization treatment. With the exception of *DDT*, the dough rheological properties from farinograph and extensograph were all statistically equal ($p > 0.05$) among the four fertilization treatments. The *DDT* was significantly higher for the treatment with 100 kg N ha^{-1} and 17 kg S ha^{-1} (8.8 min) when compared with the fertilization treatment providing only 100 kg N ha^{-1} and no sulfur (8.0 min). Moreover, while no significant differences were found between the mean grain yield values for the different fertilization treatments, averaged over environment and genotype, the mean grain protein content, also averaged over environment and genotype, was significantly lower when wheat received 100 kg N ha^{-1} and no sulfur (14.4 %) compared to fertilization treatments containing sulfur (Table 3.7).

Godfrey et al. (2010), in the previously mentioned study, observed a positive correlation between nitrogen fertilization (0 - 288 kg ha^{-1}), under an optimum sulfur fertilization (53 kg ha^{-1}), and the nitrogen content in the flour. The significant ($p<0.0001$) positive effect of nitrogen fertilization (0, 150 and 300 kg ha^{-1}) on protein content of wheat flour from five bread wheat genotypes was also confirmed by Islas-Rubio et al. (2011). Moreover, Godfrey et al. (2010) reported a positive correlation between wheat flour nitrogen content and Gli/Glu ratio, which itself had a positive correlation with *DDT*, *DS* and *E*, and a negative correlation with R. Islas-Rubio et al. (2011) also observed an increase in Gli/Glu (0.9 to 1.1) with increasing nitrogen application (0 to 300 kg ha^{-1}). The authors also observed a significant effect of nitrogen fertilization on dough E and R from Kieffer tests (Islas-Rubio et al., 2011). Under drought conditions, Liu et al. (2021) studied a single wheat variety under 120 kg ha^{-1} (normal) and 240 kg ha^{-1} (high) nitrogen fertilization treatments and observed a significant increase in the sulfur rich (γ– and α/β–) gliadins

Table 3.7. Means comparison, averaged over genotype and environment, for empirical rheology, grain protein content, grain yield, and specific loaf volume.

	Farinograph			Extensograph						Field Data		Bread quality
Fertilization (kg ha^{-1})	***FAB* (%)**	***DDT* (min)**	***DS* (min)**	***E*$_{45}$ (mm)**	***R*$_{45}$ (BU)**	***R*$_{45}$/*E*$_{45}$**	***E*$_{135}$ (mm)**	***R*$_{135}$ (BU)**	***R*$_{135}$/*E*$_{135}$**	**Protein content (%)**	**Yield (kg ha^{-1})**	***SLV* (cm^3 g^{-1})**
100 N	63.7±1.9 ^{a}	8.0±2.0^{b}	13.8±4.8^{a}	228±16^{a}	525±112^{a}	2.3±0.4^{a}	214±12^{a}	605±146^{a}	2.8±0.7^{a}	14.4±1.0^{b}	4299±1389^{a}	5.01±0.27^{a}
135 N	64.0±1.8^{a}	8.5±2.1^{b}	14.0±6.5^{a}	229±18^{a}	521±121^{a}	2.3±0.4^{a}	215±15^{a}	603±152^{a}	2.8±0.7^{a}	14.7±1.0^{ab}	4248±1370^{a}	4.99±0.29^{a}
100N + 17S	64.0±1.7^{a}	8.8±2.0^{a}	14.1±6.6^{a}	229±16^{a}	530±124^{a}	2.3±0.5^{a}	215±14^{a}	613±154^{a}	2.8±0.7^{a}	14.8±0.8^{a}	4274±1341^{a}	4.99±0.25^{a}
135N + 17S	64.1±1.8^{a}	8.5±1.8^{ab}	14.2±6.8^{a}	229±16^{a}	527±120^{a}	2.3±0.4^{a}	216±14^{a}	603±153^{a}	2.8±0.7^{a}	14.9±1.0^{a}	4236±1313^{a}	5.03±0.24^{a}

Different letters in the same column means that values are significantly different at $p<0.05$. Data are presented as mean ± SD.

mainly at the “high” nitrogen level. The glutenin subunits levels, on the other hand, were significantly higher under “high” nitrogen levels with normal irrigation regimen (J. Liu et al., 2021). In the same study by Liu et al. (2021), the “high” nitrogen level in the soil, with and without drought condition, improved gluten strength, although the maximum *DDT* (4.5 min) and *DS* (4.1 min) values obtained were significantly smaller than the values found in this study (14.0 min for *DDT* and 42.4 min for *DS*). Moreover, Liu et al. (2021) did not mention the soil sulfur content, which allows one to speculate that the soil sulfur content of their study was optimum allowing for sulfur-rich protein formation under “high” nitrogen levels.

In a study by Godfrey et al. (2010), farinograph parameters (*DDT* and *DS*) as well as extensograph E were shown to be in a significantly ($p < 0.05$) positive correlation with gliadin levels. On the other hand, *R* was positively correlated with glutenins (Godfrey et al., 2010). The positive correlation between gliadins (ω−, and γ/ α− gliadins) and gluten strength (*DDT* and *DS*) from Godfrey et al. (2010) is surprising, as the sulfur rich glutenin fractions are the main proteins responsible for an increase in wheat gluten strength (Steinfurth et al., 2011). Malalgoda et al. (2018) studied 30 hard red spring wheat cultivars from North Dakota, released between 1910 and 2013, and observed a positive correlation ($p<0.001$) between ω-gliadins and gluten strength (*DDT* and DS). Additionally, Godfrey et al. (2010) did not find any significant correlation between glutenins, *DDT* and *DS*. These outcomes suggest that not only glutenins, but gliadins could also have an impact on gluten strength. Therefore, a more extensible dough could be a result of increasing nitrogen levels under optimum sulfur fertilization (Godfrey et al., 2010).

When sulfur is supplied to the wheat plant, sulfur-rich proteins are synthesized to a greater extent compared to the sulfur-poor proteins (Steinfurth et al., 2011). As mentioned before, in this study, a positive correlation ($p<0.05$) between *DDT* and protein content was found. Based on the results from Godfrey et al. (2010) and Malalgoda et al. (2018), this positive correlation between *DDT* and protein content, might suggest that the nitrogen supply, mainly without sulfur, could result in an increase of ω-gliadins that might positively affect *DDT*. A possible explanation to the positive effect of ω-gliadins on *DDT* could be due to the lack of cysteine residues in its molecule. The lack of the ability to form disulfide bonds with other gluten fractions, mainly glutenins, could delay the process of dough development, thus increasing *DDT*. This possible explanation is based on the conclusions from Sapirstein and Fu (2000) regarding the dough development process, which, in summary, occurs due to disaggregation of glutenins and further interactions with

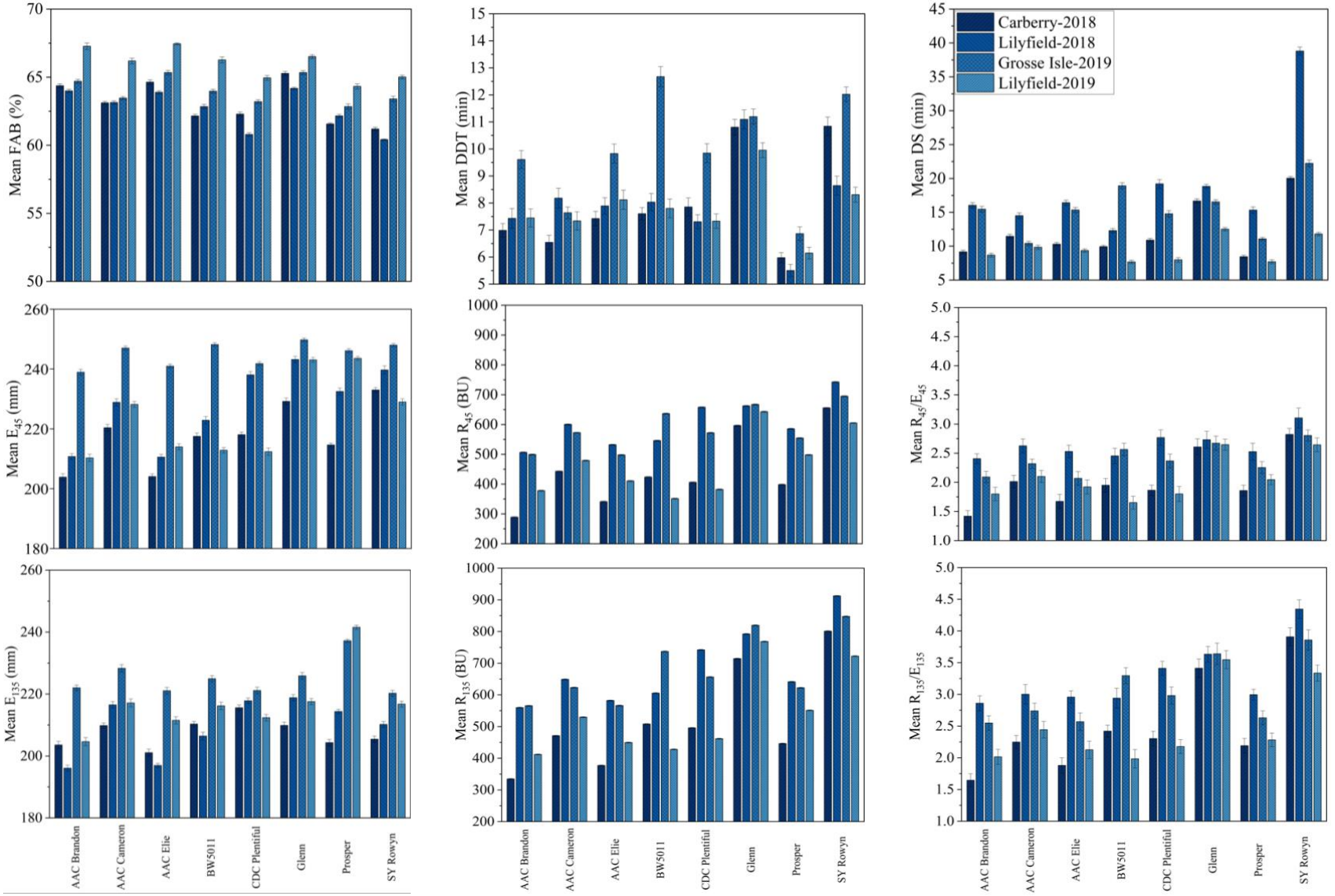
gliadins. Finally, Malalgoda et al. (2018) provided another explanation for the positive correlation between ω-gliadins and *DDT*, based on the close link between genes encoding low molecular weight glutenin subunits and gliadins. Malalgoda et al. (2018) explained that the accumulation of ω-gliadins that possess a close genetic link with low molecular weight glutenin subunits might have a positive effect on *DDT*. At the same time, the positive correlation between E_{45}, E_{135} and the protein content might be an indication that more gliadins are present in the wheat samples with higher protein contents. This increase in gliadins led to a weaker gluten which is reflected in an increase in dough extensibility.

In summary, in this study, fertilization treatments did not play a major role in the dough rheological properties. This contradictory result, when compared to previous publications, might be explained by the nutrient content in the soil of this study. As reported in the literature, an N/S ratio higher than 17 could be an indicator of sulfur deficiency in wheat grains (Randall et al., 1981; Stockmann et al., 2018). In the present study, the N/S ratio was lower than 17 for all four environments (Table 3.1), indicating no sulfur deficiency in the soils, as also concluded by Xie et al. (2021) for all four environments and by Malunga et al. (2020) for both locations in 2018. Previous studies that showed significant effects of sulfur fertilization on gluten strength were performed on sulfur deficient soils (Järvan et al., 2017; Wilson et al., 2020), which could explain their observation of the significant effect of sulfur fertilization on rheological properties.

3.4.1.4 Effect of interactions between genotype and environment

In this study, some of the interactions between the three factors (E, G and F) also significantly affected the dough rheological properties. As shown in Table 3.3, G×E was the third most important factor affecting gluten strength (after E and G). This outcome indicates that wheat genotypes had different rheological behavior depending on the growth environment. The remaining interactions (G×F, E×F, and G×E×F) had small or non-significant ($p > 0.05$) effects on dough rheological parameters. Therefore, the focus of this section will be on the G×E effect on dough rheological properties. The G×E effect can be visualized in Figure 3.7. As an example of the G×E effect on *DDT*, the genotype BW5011 possessed the highest value when grown at Grosse Isle-2019 (12.7 min) whereas Glenn and SY Rowyn had the highest *DDT* values (10.8 min) when grown in Carberry-2018. Another example would be the significant effect of G×E on *DS* where

Figure 3.7. Mean values for farinograph and extensograph tests for wheat genotypes and environment, averaged over four fertilization treatments.



SY Rowyn had the highest *DS* when grown at Lilyfield-2018 (38.8 min). However, when samples where grown in Lilyfield-2019, Glenn possessed the highest mean value (12.5 min). Finally, for FAB, when samples were grown in Carberry-2018, Glenn had the highest value (65%), while for samples grown in Lilyfield-2019, the highest *FAB* was obtained by AAC Elie (67%).

For extensograph results, the effect of G×E on E_{135} was more pronounced compared to that on E_{45} (Table 3.3). On the other hand, the G×E effect was greater for R_{45} and R_{45}/E_{45} compared to that on R_{135} and R_{135}/E_{135} (Table 3.3). For example, from Figure 3.7, E_{45} from samples grown in Grosse Isle-2019 had the highest value for Glenn (250 mm), whereas among samples grown in Lilyfield-2019, Prosper had the highest E_{45} value (244 mm). For both R_{45} and $R_{45}/E_{45,}$ when samples were grown in Lilyfield-2018, SY Rowyn had the highest values (742 BU and 3.1, respectively). However, when samples were grown in Lilyfield-2018, Glenn had the highest R_{45} and R_{45}/E_{45} values (643 BU and 2.6, respectively). When samples were tested after longer relaxation period (135 min), E_{135} had the highest value for Prosper among samples grown in Lilyfield-2019 (242 mm), whereas CDC Plentiful had the highest E_{135} value (216 mm) among samples grown in Carberry-2018. Finally, for R_{135} and $R_{135}/E_{135,}$ the same trend as that of R_{45} and R_{45}/E_{45} was observed. Rowyn had the highest values for R_{135} and R_{135}/E_{135} (913 BU and 4.3, respectively) among samples grown in Lilyfield-2018, however, when samples were grown in Lilyfield-2019, Glenn had the highest R_{135} and R_{135}/E_{135} values (769 BU and 3.5, respectively).

When studying three wheat genotypes grown at four environments (referred to as location), Hasniza et al. (2014) also reported a significant ($p<0.01$) effect of G×E on dough rheological parameters (*R* and *E*). In an Australian study conducted by Panozzo and Eagles (2000), the dough rheological properties from seven wheat cultivars grown at 15 environments were analyzed using farinograph and extensograph. In addition to the effects of genotype and environment, the authors also reported a significant ($p<0.01$) effect of G×E on *DDT*, *E* and *R* (Panozzo & Eagles, 2000). The results from the present study are in agreement with the results reported in the literature in regards to the fact that among all involved factors, environment, genotype, and G×E were the most significant ones affecting gluten strength. Therefore, it is necessary to account for the interaction between genotype and environment while selecting high gluten strength wheat genotypes.

3.4.2 Relationship between free asparagine concentration, specific loaf volume and gluten strength

To assess the relationship between free asparagine concentration, specific loaf volume (SLV), and gluten strength, Pearson correlation coefficients were determined. As per Xie et al. (2021), free asparagine was significantly ($p<0.0001$) affected by E, G, F, and their interactions (G×E, G×F, E×F, and G×E×F). Among these factors, E, G, and G×E had the largest effects with a percent of total variance of 44, 31, and 18%, respectively (Xie et al., 2021). Moreover, fertilization had little effect on free asparagine concentration, however, the treatment with 100 kg N ha^{-1} and no sulfur resulted in samples with lower free asparagine concentration when compared to the mean values for the other treatments (Xie et al., 2021). As mentioned before, the same trend was observed for rheological parameters determined using farinograph and extensograph, with E, G, and G×E being the most significant factors affecting gluten strength. Overall, free asparagine concentration was significantly ($p<0.05$) negatively correlated with dough rheological properties, i.e., *DDT* (r = -0.319), *DS* (r = -0.617), E_{45} (r = -0.730), R_{45} (r = -0.744), and R_{45}/E_{45} (r = -0.680) as well as E_{135} (r = -0.343), R_{135} (r = -0.698) and R_{135}/E_{135} (r = -0.648), as shown in Figure 3.8 for the extensograph parameters including the 128 samples of this study.

Moreover, in order to analyze how *R* and *E* were correlated, at 45 and 135 minutes of resting, Figure 3.9 shows the correlation of R_{45} to E_{45} and of R_{135} to $E_{135.}$ In Figure 3.9, a weaker correlation between R_{135} against E_{135} is evident when compared to R_{45} against E_{45}. These results could be due to the increase in *R* and the decrease in *E* values when dough is tested after resting for 135 minutes when compared to the results at shorter resting times (45 minutes), as previously discussed. When tested at 135 minutes, wheat flour dough samples showed stronger doughs, characterized by a shift to higher resistance to extension (R_{135}) values and to lower extensibility (E_{135}) values. It was interesting to observe these substantial changes in dough resistance to extension and dough extensibility values as the resting period increased, showing that dough rheology changes substantially over time. The graphs in Figure 3.9 show that wheat flour dough samples might suffer structural changes over time that lead to a higher resistance to uniaxial deformations and less fluid-like characteristics, characterized by lower extensibilities.

Figure 3.8. Correlation between extensograph parameters (E_{45}, R_{45}, E_{135}, and R_{135}) against free asparagine concentration (µg g^{-1}) and Pearson's r correlation analysis. Data points represent means and SE (n = 2).

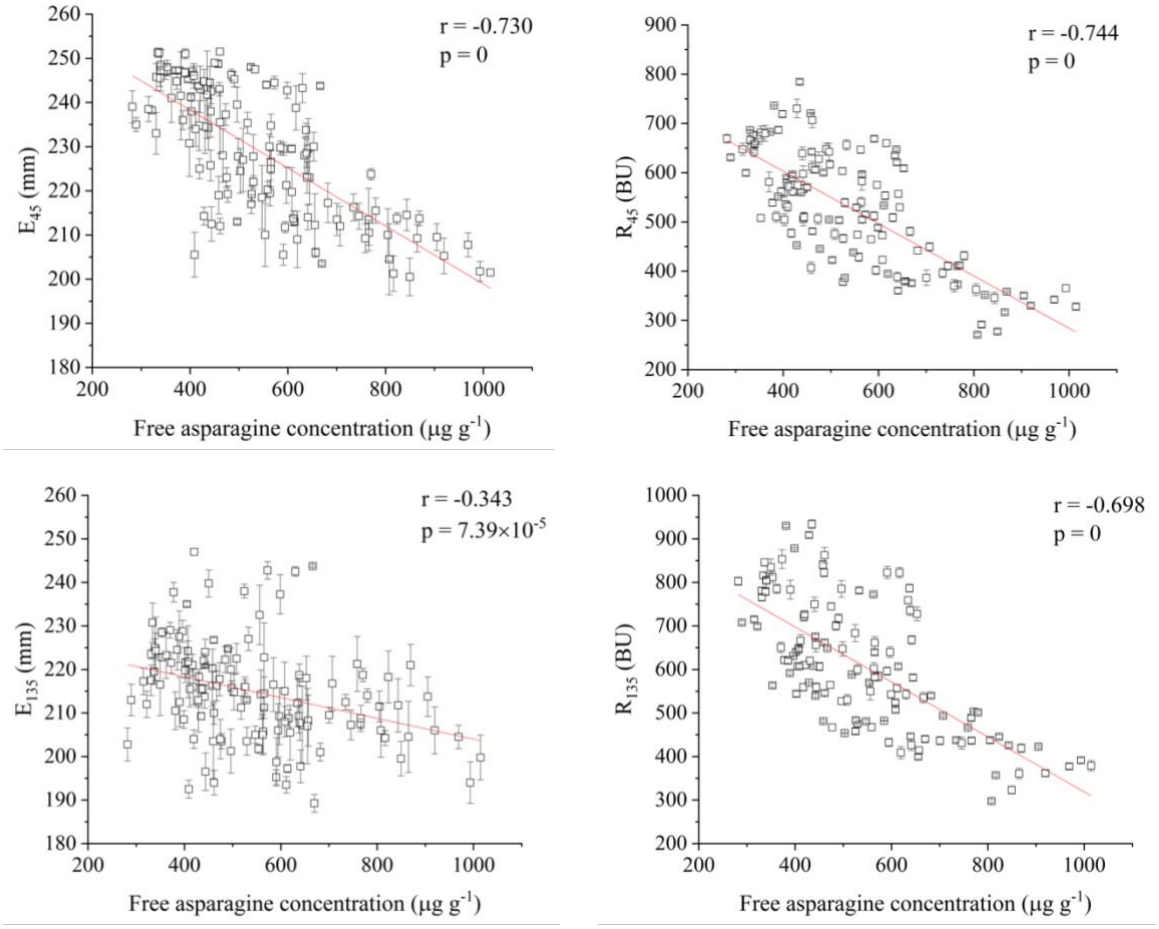
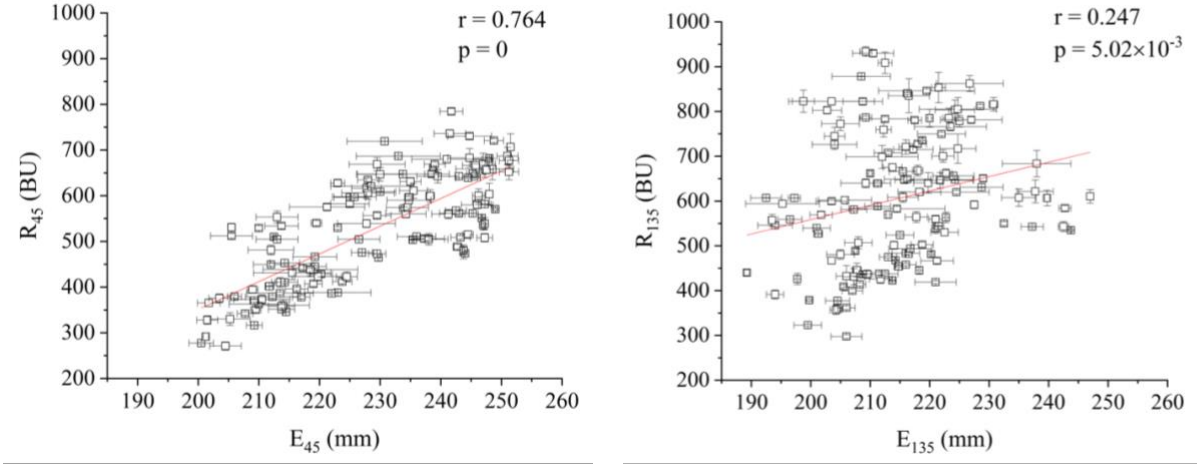


Figure 3.9. Correlation between R_{45} against E_{45} (left) and R_{135} against E_{135} (right), and Pearson's r correlation analysis. Data points represent means and SE (n = 2).



In order to demonstrate how free asparagine concentration was correlated with gluten strength evaluated by empirical tests, a contour plot was built using one rheological parameter translating gluten strength from each test (farinograph and extensograph). Among the rheological parameters from farinograph and extensograph measurements, *DS* and R_{135}/E_{135} were chosen to build the contour plot (Figure 3.10) against free asparagine concentration. *DS* was chosen to represent the farinograph test, as this parameter shows how stable a wheat flour dough is during the mixing process, therefore, translating into a gluten strength characterization. R_{135}/E_{135} was chosen from the extensograph because it indicates the elasticity-to-extensibility ratio of the dough at longer relaxation times, which represents dough behavior during fermentation (Sobczyk et al., 2017). The contour plot clearly shows that samples with low free asparagine concentration had overall high stability to mixing (*DS*) and elasticity-to-extensibility ratio (*R/E*), thus high gluten strengths. In order to evaluate the correlation between free asparagine concentration, *SLV* and dough rheological properties, a second Principal Component Analysis was conducted including free asparagine concentration and *SLV* data, as shown in Figure 3.11. The negative correlation between free asparagine and gluten strength is also displayed in the PCA biplot shown in Figure 3.11. In this biplot, PC1 and PC2 account for about 74% of the total variation.

One can clearly see that the visual representation of the relationship between free asparagine concentration and gluten strength parameters in the contour plot (Figure 3.10) supports the display of the same parameters, i.e., *DS*, R_{135}/E_{135} and free asparagine concentration, in the

Figure 3.10. Contour plot between gluten strength, measured by *DS* (min) and R_{135}/E_{135}, and wheat free asparagine concentration (μg g^{-1}).

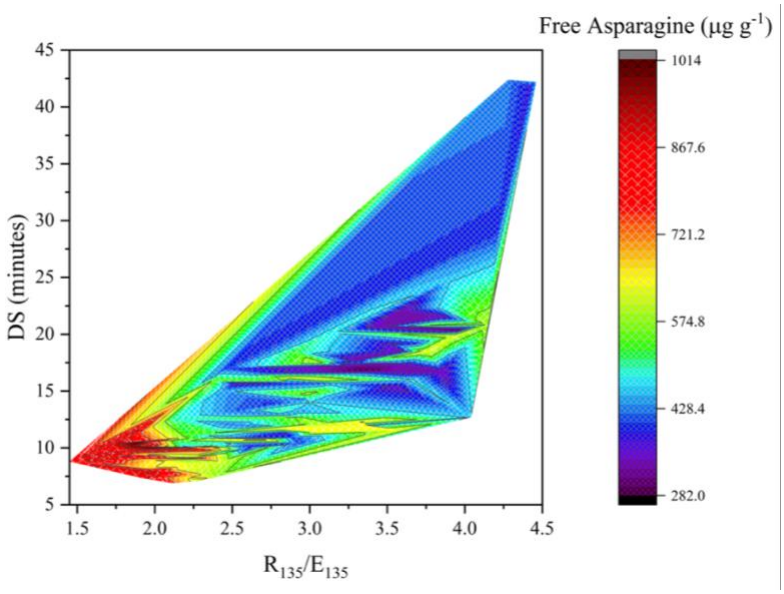
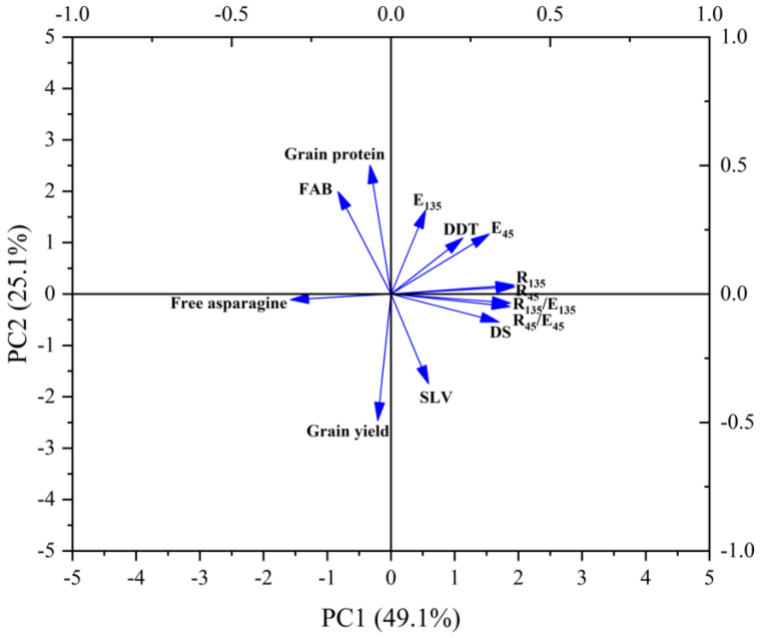


Figure 3.11. Biplot from principal component analysis of field data (grain protein and grain yield), free asparagine concentration, specific loaf volume (*SLV*) and rheological parameters from farinograph and extensograph tests.



PCA biplot (Figure 3.11). Moreover, the PCA biplot shows that if any other parameter representing gluten strength, i.e., R_{45}, R_{135}, and R_{45}/E_{45}, were chosen to build the contour plot against free asparagine concentration, the same negative correlation would have been achieved. In Figure 3.11, PC1 is mostly composed of extensograph parameters (E_{45}, R_{45}, R_{135}, R_{45}/E_{45} and R_{135}/E_{135}), *DS* and free asparagine concentration. The PC2 is mainly composed of grain protein content, grain yield, *FAB*, E_{135} and *SLV*. This can be observed by evaluating the eigenvectors of the PCA in Table 3.8, which shows the eigenvectors of the first five principal components that explain more than 90% of the variances.

Table 3.8. Eigenvectors for the first five principal components analyzed for field data and wheat properties including free asparagine concentration, empirical rheological parameters and specific loaf volume.

	PC1	PC2	PC3	PC4	PC5
Grain protein	-0.0655	**0.5010**	0.0666	0.2579	0.1887
Grain yield	-0.0416	**-0.4925**	0.0852	0.3380	-0.3145
Free asparagine	**-0.3158**	-0.0221	0.2298	0.3120	0.2744
FAB	-0.1666	**0.3981**	0.2791	-0.0668	0.2626
DDT	0.2254	0.2178	0.3919	0.6385	-0.2560
DS	**0.3391**	-0.1083	0.2552	0.0075	-0.2544
R_{45}	**0.3882**	0.0278	0.0186	-0.0962	0.1479
E_{45}	**0.3082**	0.2339	-0.3114	0.0549	-0.2544
R_{45}/E_{45}	**0.3734**	-0.0485	0.1190	-0.1612	0.2678
R_{135}	**0.3862**	0.0351	0.0733	-0.0074	0.1672
E_{135}	0.1086	**0.3258**	-0.5993	0.2285	-0.1386
R_{135}/E_{135}	**0.3752**	-0.0350	0.1991	-0.0643	0.2021
SLV	0.1180	**-0.3491**	-0.3512	0.4637	0.5871

From Table 3.8, the PC1 is highly positively affected by R_{45}, R_{135}, R_{45}/E_{45}, R_{135}/E_{135}, and *DS* (shown in bold) and negatively affected by free asparagine concentration (shown in bold). Therefore, one can notice that free asparagine concentration was negatively correlated to gluten strength, as mentioned before, along PC1. PC2, on the other hand, is positively affected by grain protein, *FAB*, and E_{135}, and negatively affected by grain yield and SLV. As mentioned before, PC1 and PC2 account for the majority of the variations (74%), and so will be the focus of the PCA in this study. Addition of the three following principal components (PC3, PC4, and PC5) helps us

explain about 92% of the variation in the data, with the remaining principal components (PC6 to PC13) having less contribution (remaining 8% in total), and therefore, not shown in Table 3.8.

Very little has been published on the relationship between free asparagine and gluten strength. In a study by Malunga et al. (2019) on 30 Canadian Hard Red Spring (CWRS) wheat varieties, negative correlation coefficients were found between free asparagine concentration and white flour *DDT* (r = –0.225), *DS* (r = –0.289), R_{45} (r = –0.370) and E_{45} (r = –0.142), and R_{135} (r = –0.361), E_{135} (r = –0.077) and R_{135}/E_{135} (r = –0.329) (Malunga et al., 2019). However, only the Pearson correlation coefficient between free asparagine concentration and R_{45} was significant at $p<0.05$ (Malunga et al., 2019). Liu et al. (2011) also reported no significant correlation between free asparagine concentration and gluten strength (*DDT* and E_{45}) of the wheat genotype Glenn. Ohm et al. (2017) evaluated the free asparagine concentration in 75 Hard Red Spring wheat genotypes, including six cultivars and 59 experimental lines, grown at three locations in North Dakota. The authors also performed a gluten strength analysis using mixograph tests, reporting the "mixograph pattern" varying from 1 to 10, with 1 characterized as very weak and 10 as very strong, but found no significant correlation coefficients between free asparagine concentration and mixograph pattern (Ohm et al., 2017).

Wilson et al. (2020) studied the effect of sulfur fertilization treatments on wheat free asparagine and gluten strength, but provided no information on the correlation between the two parameters. However, the authors reported significantly ($p<0.05$) higher mean DS values and lower free asparagine concentrations of the five wheat genotypes of their study as a result of a 22 kg ha^{-1} sulfur fertilization treatment (Wilson et al., 2020).

In this study, the SLV of the final bread was significantly ($p<0.0001$) affected by G (30.7% of total variance), E (29.1 % of total variance), and the interactions G×E, G×F, E×F, and G×E×F (a total of 30.6% of total variance). The fertilization treatment did not affect SLV significantly (Table 3.4). Similar results regarding the significant effects of environment, genotype and their interaction on bread loaf volume have been reported in the literature (Islas-Rubio et al., 2011; Panozzo & Eagles, 2000). Panozzo and Eagles (2000) also showed significant ($p<0.01$) effect of E, G, and G×E loaf volume of breads made with seven wheat varieties grown at 15 environments. Islas-Rubio et al. (2011) also analyzed loaf volume of breads prepared from 5 wheat lines grown under three nitrogen fertilization levels, and reported significant ($p<0.0001$) effects of G, F, and G×F on loaf volume.

Regarding correlations with field data, significantly ($p<0.0001$) negative correlation was found between *SLV* and protein content ($r = -0.516$), while a significantly ($p<0.0001$) positive correlation was found between *SLV* and grain yield ($r = 0.464$). This outcome could also be observed in the PCA biplot (Figure 3.11), where SLV and grain yield possess a negative charge and grain protein content has a positive charge along the PC2 axis. However, a positive correlation between protein content and loaf volume has been repeatedly reported in the literature (Dowell et al., 2008; Finney et al., 1957; Rossmann et al., 2019; Van Bockstaele et al., 2008a). Moreover, Popa et al. (2014) studied 19 flour samples from the Romanian wheat harvest of 2012 and found no significant correlation between bread loaf volume and the flour protein content. The authors introduced a parameter calculated from gluten index and wet gluten measurements called gluten remaining on the sieve (GRS) representing the stronger fraction of gluten, which was strongly correlated with bread loaf volume ($r = 0.79$, $p<0.001$) (Popa et al., 2014). This result could be an indication that the bread volume is dictated by the strong fraction of gluten, likely the glutenins, and not by the total gluten content.

Moreover, a significantly negative correlation between *FAB* and *SLV* (-0.582, $p<0.0001$) was found, which contradicts the results of previous studies reporting a positive correlation between loaf volume and *FAB* (Van Bockstaele et al., 2008a; Wooding et al., 2000). Van Bockstaele et al. (2008) studied 17 wheat cultivars available in the European market, performed farinograph experiments and loaf volume measurements, and reported a strong positive correlation between *FAB* and loaf volume ($r = 0.75$, $p<0.01$). Wooding et al. (2000) also found a positive correlation between bread loaf volume and *FAB* ($r = 0.54$, $p<0.01$) when studying a single wheat variety grown under different nitrogen and sulfur fertilization levels. This contrast could be explained by possible differences in the Gli/Glu ratio among the studies. Van Bockstaele et al. (2008) and Wooding et al. (2000) had a positive correlation between *FAB* and loaf volume, which could be an indication that, as protein content increased, the Gli/Glu ratio was still adequate for breadmaking. In this study, *FAB* was positively correlated with protein content ($r = 0.692$, $p<0.0001$), but protein content was negatively correlated with *SLV* ($r = -0.516$, $p<0.0001$). This outcome could be an indication that as protein content increased, more Gli/Glu was formed that in turn led to a decrease in the *SLV* in the present study.

In addition, no significant correlation was found between *DDT* and *SLV* in this study, while *DS* was significantly correlated with *SLV* ($r = 0.208$, $p < 0.05$). Moreover, Wooding et al. (2000)

found a positive ($p<0.001$) correlation between *DDT* and loaf volume and no significant correlation with *DS*. There were also significant ($p<0.01$) correlations between *SLV* and extensograph parameters R_{45} ($r = 0.255$), R_{45}/E_{45} ($r = 0.299$), R_{135} ($r = 0.249$) and R_{135}/E_{135} ($r = 0.260$), while no significant correlation was found between *SLV* and extensograph E_{45} and E_{135}. Wooding et al. (2000), however, found a positive correlation between E and SLV ($r = 0.49$, $p<0.01$), and no significant correlation between *R* and *SLV*. It is expected that samples yielding high gluten strength produce bread with high loaf volumes (Islas-Rubio et al., 2011). However, the viscous characteristic of the dough, thus its extensibility, is also important to guarantee the growth/expansion of the dough during fermentation. Therefore, a satisfactory balance between *R* and *E* is necessary to achieve optimum baking results (Islas-Rubio et al., 2011).

The results of this study suggest that as protein content increases, gliadins are formed to a greater extent than glutenins. As proposed in section 3.4.1.1, when the synthesis of starch is reduced due to environmental conditions, more nitrogen is mobilized into the grain. This nitrogen could be allocated to gliadin formation, thus increasing protein content and decreasing gluten strength and *SLV*. Moreover, samples with higher grain yield and gluten strength could indicate a more adequate starch accumulation and a Gli/Glu ratio close to an optimum value, resulting in samples with higher *SLV*. This hypothesis is supported by the negative ($p<0.0001$) correlation between grain yield and E_{45} ($r = -0.428$) and E_{135} ($r = -0.534$). Finally, a weak, but significant negative correlation was found between *SLV* and wheat free asparagine concentration ($r = -0.241$, $p<0.01$). This conforms with the results of a study by Malunga et al. (2019) where a negative, though non-significant correlation was found between *SLV* and free asparagine concentration in whole wheat and white flours.

Overall, there was a slight variation in *SLV* (4.33 - 5.60 $cm^3\ g^{-1}$) of the samples of this study. Sehn & Steel (2017) performed farinograph and extensograph tests to study the rheological properties of doughs prepared from eleven whole-wheat commercial flour, eight pre-mixes containing white flour, coarse and fine bran, and a control white flour. Sehn & Steel (2017) also performed *SLV* measurements on the final breads, made from whole-wheat flours, pre-mixes and a white flour control, and correlated it with a dimensionless number (*Sehn-Steel*), calculated from farinograph and extensograph parameters, as per Eq. 3.1.

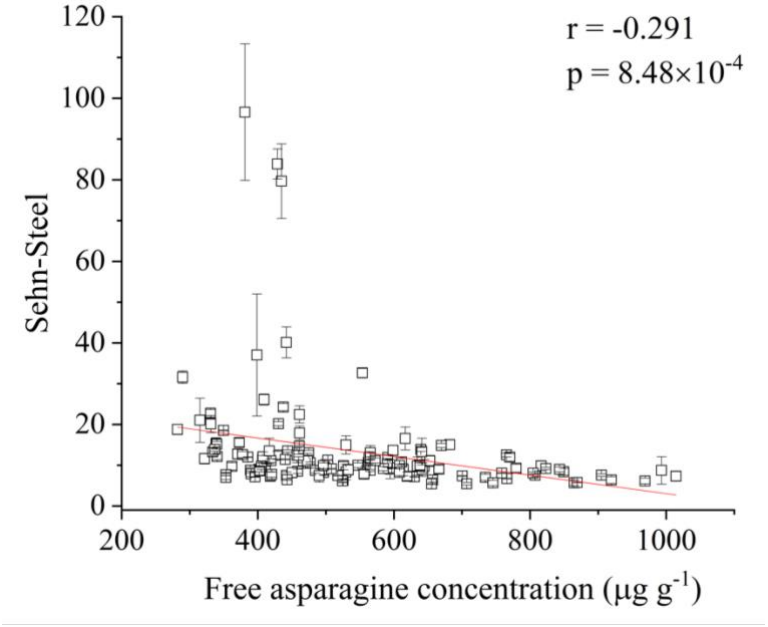
$$Sehn - Steel = (FAB - 14) \times \left(\frac{DS}{DDT}\right)^2 \times \left(\frac{500\,BU}{MTI}\right)^{-1} \times \left(\frac{R_{135}}{R_{45}}\right)^4 \times \left(\frac{E_{135}}{E_{45}}\right)^{-2} \qquad \text{Eq. 3.1}$$

where *FAB* (%), *DS* (min), *DDT* (min), and mixing tolerance index (*MTI*, BU) are obtained from farinograph tests, with 500 BU being the optimum consistency line from farinograph, and R_{45}, R_{135}, E_{45} and E_{135} are the parameters obtained from the extensograph. The *Sehn-Steel* number was developed by the authors with the main goal of using dough rheological data to classify whole-wheat flours as "suitable" or "unsuitable" to breadmaking (Sehn & Steel, 2017). When correlating this number with *SLV*, the *Sehn-Steel* dimensionless number was shown to be a good predictor for *SLV* in their study, with *Sehn-Steel* values of 0-62 identifying unsuitable wheat for breadmaking (*SLV* < 3.8 $cm^3\ g^{-1}$) and *Sehn-Steel* of 62-200 identifying wheat as suitable for breadmaking (*SLV* ≥ 3.8 $cm^3\ g^{-1}$) (Sehn & Steel, 2017). Eq. 3.1 was also applied in the present study to calculate the *Sehn-Steel* dimensionless number from the farinograph and extensograph parameters, resulting in mean *Sehn-Steel* numbers varying from 5.4 to 97. A large variation in this dimensionless number was also found by Sehn & Steel (2017) (0 up to about 180). However, no correlation was found between *Sehn-Steel* values and SLV in the present study. A potential reason for this outcome is that Sehn & Steel (2017) calculated the *Sehn-Steel* dimensionless number using rheological data from the same wheat flours utilized for breadmaking. Conversely, in the present study, farinograph and extensograph were conducted using white flours whereas the bread samples were made from whole-wheat flours, which could explain the lack of correlation between the *Sehn-Steel* number and *SLV* in the present study. Additionally, Sehn & Steel (2017) selected the *SLV* value of 3.8 $cm^3\ g^{-1}$ as the minimum *SLV* for bread to be considered as acceptable which was the lowest *SLV* obtained in a previous study (Ishida & Steel, 2014). Therefore, comparing the *SLV* from the present study with the ones obtained by Sehn & Steel (2017), all the wheat flour samples resulted in bread of acceptable *SLV* (> 4.33 $cm^3\ g^{-1}$), as per the Sehn & Steel (2017) categorization. Moreover, the tight range in SLV values found in the present study, due to all wheat genotypes being suitable for breadmaking, could be a second reason for the lack of correlation between the *Sehn-Steel* dimensionless number and the *SLV* in the present study.

Finally, in order to evaluate if there is a relationship between the *Sehn-Steel* numbers against free asparagine concentration, a correlation study was performed as shown in Figure 3.12. Although the Pearson correlation coefficient did not show strong relationships, the negative correlation was significant at $p<0.05$. Overall, Figure 3.12 shows that samples with higher free asparagine concentrations resulted in lower *Sehn-Steel* dimensionless numbers, thus indicating that samples with high concentration of this free amino acid tend to produce bread with poor final

quality. Therefore, this negative correlation could indicate that, besides analysing the farinograph and extensograph parameters separately, the rheological data could be combined in one single number (*Sehn-Steel*) in order to evaluate if low levels of free asparagine in wheat could be detrimental to the final bread quality.

Figure 3.12. Correlation between *Sehn-Steel* number against free asparagine concentration (µg g^{-1}) and Pearson's r correlation analysis. Data points represent means and SE (n = 2).



These results provide further support to the hypothesis that wheat grains with low levels of free asparagine concentration possess strong glutens. However, this overall relationship needs to be interpreted with caution, as it might vary mostly with genotype, environment and G×E, which were the main factors affecting both free asparagine content and gluten strength. Therefore, a careful selection of factors, such as wheat genotype, is essential in successfully selecting wheat with low free asparagine concentration and acceptable gluten strength.

3.5 Conclusion

Free asparagine concentration in wheat is mostly affected by environment, genotype, and fertilization. These factors also affect protein content and composition, which dictate changes in the dough rheological properties and the quality of the final products. Therefore, a critical question

that needed to be addressed in the present study was whether strategies to reduce free asparagine accumulation in wheat would negatively impact wheat gluten strength and SLV.

The present study showed that, similar to the free asparagine concentration, gluten strength and SLV were mostly affected by environment, genotype, and their interactions. As the soils involved in this study were not sulfur deficient, because all of the calculated N/S ratios before fertilization were lower than 17, fertilization treatments played only a very small role in the studied parameters. More importantly, it was found that factors causing a reduction in wheat free asparagine concentration did not have a negative impact on gluten strength and *SLV*. Therefore, this study concludes that Canadian wheat growers, seeking the delivery of a safe and high-quality wheat to the market, should work towards the selection of wheat varieties that yield lower free asparagine concentration. Additionally, although no significant effects of nitrogen fertilization on the gluten strength parameters were observed, its application did significantly increase free asparagine concentration in whole-wheat flours. Therefore, keeping nitrogen fertilization at commercial levels (100 kg ha^{-1}) to ensure no significant increase in wheat free asparagine content is another final recommendation to Canadian wheat growers.

3.6 Connection between Chapters 3 and 4

The empirical rheological studies conducted in Chapter 3 are widely applied in the breadmaking industry. The farinograph results provided information on the dough's rheological properties during mixing. The extensograph parameters provided critical information on wheat flour dough samples related to their extensibility and resistance to extension at 45 and 135 minutes of resting time. However, the results obtained in Chapter 3 from empirical tests were all reported in arbitrary units, i.e., Brabender Units. Moreover, because it was not possible to control the stresses and strains imposed during rheological measurements, it was not possible to obtain dough rheological properties at controlled small strains. Therefore, in order to obtain comprehensive information on dough rheological properties and their relationship to wheat free asparagine concentration, fundamental shear tests were performed on a set of 10 selected samples in Chapter 4. Shear dynamic and static tests allow one to evaluate dough rheological properties within and outside of the dough's linear viscoelastic region, obtaining dough rheological properties over a wide range of stresses and strains. Finally, because yeast is a critical ingredient in breadmaking, shear tests were also applied to evaluate the changes in dough rheological properties during fermentation and their correlation to wheat free asparagine concentration (Chapter 4).

4 The relationship between wheat free asparagine concentration and dough rheological properties: A dynamic and static shear rheology approach

4.1 Abstract

Acrylamide, a probable carninogen, was found to be present in various foodstuffs, including bread. Producing wheat with low free asparagine levels is one strategy to meet international goals for acrylamide mitigation in bread. However, factors affecting wheat free asparagine concentration also affect wheat protein content and composition, thus potentially impacting the bread quality. Changes in wheat protein content and composition can affect dough viscoelasticity, which can be investigated through rheological measurements. Therefore, this study aimed to investigate the relationship between wheat free asparagine concentration and dough rheological properties using shear dynamic and static rheometry. From a population of 128 wheat flour samples, 10 samples were selected, to provide a uniformly distributed set of samples covering a wide range of free asparagine concentrations. Linear oscillatory time sweeps were performed on yeasted and non-yeasted dough samples, while linear oscillatory frequency sweep, creep-recovery and stress relaxation tests were performed on non-yeasted dough samples. Linear oscillatory time sweeps showed that dough rheological properties have significant variations over time; when yeast was added, dough softening effects were observed due to the fermentation process. Changes in yeasted dough rheological properties were not significantly correlated with free asparagine concentration. Within the linear viscoelastic region, correlation between free asparagine concentration and dough rheological properties were not prominent. However, static shear tests indicated significant correlations between dough rheological parameters and free asparagine. Dough rheological parameters were correlated to grain protein content and water absorption, but no significant correlations were found between dough rheological parameters, other wheat characteristics or specific loaf volume (*SLV*), a key bread quality parameter. In summary, wheat with high levels of free asparagine concentration and protein content resulted in wheat flour doughs with more fluid-like characteristics, i.e., weaker glutens. The main explanation for this outcome is the possible increase in the gliadin-to-glutenin ratio in samples with higher protein content and free asparagine concentration, producing more fluid-like doughs. However, as all wheat varieties involved in this study are suitable for breadmaking, these changes in protein quality were not enough to cause detrimental effects on bread *SLV*, which had little variation in this study.

4.2 Introduction

Bread is one of the oldest processed foods (Cauvain, 2015a) and an extremely important source of energy to humans in various countries around the world (D'Odorico et al., 2014). In 2021 alone, the global average consumption of bread per person was about 27 kg (Statista, 2022). Methods of breadmaking continue to be developed and tailored to improve bread quality, with international wheat trading being one of the most important causes for changes and adaptations in bread production (Cauvain, 2012). Therefore, maintaining quality in the final bread product is a key point in the production process and in bread marketing.

The singular component of wheat that drives good bread volume and crumb appearance is the protein, more specifically, the gluten proteins, i.e., the gliadins and glutenins (Delcour & Hoseney, 2010a; Veraverbeke & Delcour, 2002). Wheat that is suitable for breadmaking usually contains high protein content (12-15%) (Carson & Edwards, 2009). In addition, the wheat protein composition, i.e., the gliadin-to-glutenin ratio (Gli/Glu), must be of such a value that a dough can be formed that is capable of expanding during fermentation yet strong enough to resist the expansion process so that gas cell rupture does not occur. This peculiar characteristic is only obtained because of the gliadins and glutenins that confer fluid and elastic properties, respectively, to the viscoelastic wheat flour dough (Delcour & Hoseney, 2010a). Therefore, viscoelastic doughs that possess more elastic properties, i.e., behave like a viscoelastic solid are known to have stronger glutens, as opposed to doughs that possess more fluid-like properties, thus having weaker glutens. Therefore, changes in wheat protein content and composition can be detrimental to final bread quality.

Free asparagine is an amino acid, naturally present in wheat, that was found to be the main precursor to acrylamide formation in bread (Curtis et al., 2016; Mottram et al., 2002). Since acrylamide has been detected in carbohydrate-rich foods, as first reported in 2002 by a Swedish research group (Tareke et al., 2002), a global battle has been established to reduce or eliminate this compound in food products (Raffan & Halford, 2019). Being a probable carcinogen to humans (IARC, 1994), acrylamide concentration becomes a problem for baking industries due to high consumption of bread by humankind (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015). Therefore, one of the main strategies to reduce acrylamide in bread is through the production of wheat with low free asparagine concentration, firstly proposed by Friedman

(2003) and currently endorsed by the European Commission (2014) and by the Acrylamide Toolbox 2019 (Food Drink Europe, 2019).

Current global efforts to produce wheat with low free asparagine concentration are important because they will improve food safety through acrylamide reduction (Maan et al., 2020). However, because bread quality is affected by protein quality, such efforts are of concern due to their possible effects on wheat protein content and composition. Factors affecting free asparagine concentration in wheat, i.e., growth environment (Navrotskyi et al., 2018; Xie et al., 2021), genotype (Ohm et al., 2017), and nutrient availability, i.e., nitrogen and sulfur (Claus et al., 2006; Lea et al., 2007), also affect wheat protein content and composition, which can pose a threat to wheat protein quality. Therefore, an investigation of how free asparagine concentration in wheat affects dough viscoelasticity shines a light on concerns related to any detrimental effects on bread quality resulting from free asparagine mitigation strategies.

Dough viscoelasticity and its correlation with breadmaking quality is a complex subject that has received considerable attention from various cereal scientists and is still the focus of various research studies (Campbell & Martin, 2012; MacRitchie, 2016). In order to characterize dough viscoelasticity, rheological measurements are commonly applied. Rheology is a subject of great difficulty, explored by scientists and engineers, that studies the flow and deformation of materials. The term “rheology” was coined by Professor Bingham of Lafayette College and his definition was accepted in 1929 upon the foundation of the American Society of Rheology (Barnes et al., 1989a). In breadmaking, bakers rely on their experience and observations to improve their production process. However, with growth in population, and the growth in industrial bread production, new and more standardized methods of dough evaluation were necessary, leading many scientists to study dough properties through rheological measurements (Campbell & Martin, 2012).

Wheat flour dough is a two-phase viscoelastic material composed of a dough matrix and a gas phase (Scanlon & Page, 2015; Scanlon & Zghal, 2001). Because it is viscoelastic, dough, like many other foodstuffs, deviates from being an ideal Hookean solid or Newtonian liquid, and this complicates its mechanical characterization (Menjivar, 1990). Fundamental rheological studies allow scientists to apply controlled stresses or strains on a dough piece, obtaining information on dough rheological properties in well-defined physical units that can be utilized for process development and engineering calculations (Menjivar, 1990). During bread production, from

mixing to fermentation, wheat flour dough is subjected to shear and extensional forces with a wide range of strain rates (Bloksma, 1990). Therefore, fundamental rheological tests can be applied over a wide range of stresses and strains in order to investigate dough physical properties.

Shear rheological tests have been successfully applied to study and characterize wheat flour doughs (Jekle & Becker, 2011; W. Li et al., 2003; Sun et al., 2020; Yovchev et al., 2017). Wheat flour dough possesses linear viscoelastic characteristics when subjected to small shear strains (< 1%) (Uthayakumaran, Newberry, et al., 2002). Thus, in order to evaluate dough rheological properties within the linear viscoelastic region of dough (LVR), dynamic shear rheological tests, i.e., linear shear oscillatory tests, are usually employed (Jekle & Becker, 2011; Sun et al., 2020; Yovchev et al., 2017). Dynamic shear tests are carried out by applying a sinusoidal oscillatory shear stress or strain at a particular frequency, or over a range of frequencies, and measuring the resulting shear strain or stress (Van Vliet, 2014). From these tests, one can obtain information on the material's linear viscoelasticity by evaluating the storage modulus (G') and loss modulus (G''), both used to calculate the magnitude of the complex shear modulus (G^*) and $\tan\delta$ (Barnes et al., 1989b; Van Vliet, 2014). Although most rheological studies on dough have been carried out on unfermented dough systems (Dobraszczyk & Morgenstern, 2003), dynamic tests can also be employed to evaluate dough properties altered by yeast activity (Meerts et al., 2018).

Although dough properties are often assessed within the LVR, static shear tests are more commonly employed outside of the LVR, under large strains or stresses, because they are more closely related to the ones occurring during the breadmaking process (Dobraszczyk & Morgenstern, 2003). Creep-recovery and stress relaxation are static tests that evaluate a material's response under a constant shear stress and strain, respectively, over time (Barnes et al., 1989b; Van Vliet, 2014). From creep-recovery, one can obtain the creep compliance ($J(t)$), which is inversely proportional to dough's elastic-like characteristic, i.e., gluten strength (Jekle & Becker, 2011). From stress relaxation, scientists can obtain parameters such as the relaxation modulus ($G(t)$), which is a direct measure of gluten strength (W. Li et al., 2003). Moreover, although not comparable with literature on empirical rheological tests, some studies have successfully correlated fundamental rheological data with bread quality parameters, such as loaf volume (Van Bockstaele et al., 2008b, 2008a).

Therefore, subjecting wheat flour dough to shear dynamic and static tests can provide the scientific community with a deeper understanding of dough rheological properties in well-known

physical units. To our knowledge, there are no published studies investigating the relationship between dough rheological parameters assessed by fundamental shear measurements and the free asparagine concentration in the wheat used to make the dough. Thus, evaluating dough rheological properties within the LVR, and also at high stresses and strains, will allow us to comprehensively investigate how dough rheological properties are correlated to free asparagine concentration. Finally, studying dough rheological changes during fermentation will allow us to acquire a broader understanding of dough rheological properties in relation to free asparagine concentration in wheat, providing a richer and more complete report to both the scientific community and to the breadmaking industry.

4.3 Materials and methods

4.3.1 White flour sample selection

Wheat grain samples were milled at Cereals Canada to obtain white wheat flour at 75% extraction rate using a Bühler laboratory flour mill (MLU-202) and a Bühler impact finisher (MLU-302). The short and bran fractions, obtained as by-products from the white flour preparation, were ground using a Jacobson 120-B hammer mill to pass through a 1,190 µm sieve. The ground portions of short and bran where blended with the white flour in order to obtain the whole-wheat flour. While white wheat flour samples were used for dough rheological tests, whole-wheat flours were utilized for free asparagine quantification, as per Xie et al. (2020), for bread preparation and for bread quality evaluation through specific loaf volume measurements. For the rheological analyses in this chapter, white wheat flour samples were selected based on the free asparagine concentration of whole-wheat flours, prepared as described by Xie et al. (2020), from the initial population of 128 samples. Free asparagine quantification in the whole-wheat flour was carried out by Xie et al. (2020) by extracting the free asparagine from the whole-wheat flours using 0.01M hydrochloric acid solution. The extracted free asparagine was derivatized using AccQ•Tag Ultra pre-column derivatization amino acid kit and quantified using a Waters H class UPLC as described by Malunga et al. (2019).

Selection of ten samples from the population of 128 samples was carried out using the Kennard-Stone algorithm. This involves a sequential selection of n samples from a population of N samples ($n \leq N$) in a p-dimensional space, where p is the number of factors to be included as a parameter for sample selection. The Kennard-Stone algorithm aims to select a sample set that contains uniformly distributed samples in relation to a factor p, representing the whole population.

This algorithm calculates the Euclidian distance between two samples based on p according to Eq. 4.1:

$$D_{vu}^2 = \sum_{k=1}^{p}(x_{kv} - x_{ku})^2 \quad \text{Eq. 4.1}$$

Where D_{vu}^2 is the Euclidean distance between any two samples (v and u), and k is the number of points considered in the design (Kennard & Stone, 1969). For this experiment, n was equal to 10, N was equal to 128 and p was equal to just one parameter: free asparagine concentration, thus k will remain equal to 1. The algorithm started by selecting two samples with the largest distance in relation to one another: the highest and lowest free asparagine concentration, the remaining number of samples to be selected now being equal to 8 (excluding the first two samples with the highest and lowest free asparagine concentration). Then, a sequential calculation between the remaining samples was done, selecting the samples that contained the highest Euclidian distance from the initial samples, until the set of 10 samples was completed (Table 4.1).

Table 4.1. Mean free asparagine concentration for the ten selected samples through the Kennard-Stone algorithm.

Sample	Free asparagine concentration (μg g^{-1})
A	1014
B	920
C	869
D	823
E	735
F	645
G	554
H	461
I	373
J	282

4.3.2 Wheat flour dough preparation

Wheat flour dough samples for rheological measurements were prepared from the white flours utilizing a 10 g mixograph (Dynamic Machines Corp, Winnipeg, MB) mixing at 92 rpm and connected to a water bath to maintain a temperature of 30 ºC, according to AACC International

Approved Methods 54-40.02 (AACC International, 1999c). Farinograph experiments were previously conducted to determine the flour water absorption for dough preparation (*FAB*), following AACC International Approved Method 54-21.02 (AACC International, 2011). The preparation of non-yeasted flour doughs was done by mixing wheat flour (weight adjusted for 14.0% m.b.) with the required amount of distilled water determined by the farinograph, and 2% sodium chloride (NaCl, total flour weight basis). For yeasted doughs, an additional 6.5% of fresh compressed yeast and 0.5% of sugar, both on a flour weight basis, were added. The moisture content of the fresh compressed yeast was accounted as water added to adjust added water content for the dough preparation. Prior to dough preparation for shear rheological measurements, the optimum dough development time was determined, for all samples, using the 10 g mixograph and P2M Software (RAR Software Systems, Winnipeg-MB).

4.3.3 Shear rheological measurements

Rheological measurements were made with an AR-2000 rheometer (TA Instruments, New Castle, DE, U.S.A.) connected to a Peltier Plate at 30 ºC. For each rheological measurement, wheat flour dough was mixed until its optimum development time. After mixing, the dough was sheeted in a pasta sheeter machine (Shule® Pasta Machine), passing through three successive gaps until a final gap of 2 mm. A cylindrical piece of dough was cut from the dough sheet using a punch die (diameter of 42 mm) and placed on the center of the Peltier plate, as also performed by Leroy et al. (2010). As per Jekle & Becker (2011), Yovchev et al. (2017) and Sun et al. (2020), a parallel plate with diameter of 40 mm was lowered to clamp the dough until a gap of 2 mm was achieved. Excess dough was trimmed, fine amount of mineral oil was applied on the dough edges to avoid dough surface drying during measurement, and a solvent trap cover was used. For non-yeasted doughs, a relaxation period of 30 minutes was passed prior to starting the rheological measurements (Kim et al., 2008).

4.3.3.1 Linear viscoelastic region

Two wheat flour samples, one with the highest and one with the lowest free asparagine concentration, were selected to prepare yeasted and non-yeasted doughs for determination of a representative linear viscoelastic region (LVR). The LVR was assessed according to Upadhyay et al. (2012) by applying a strain sweep from 0.001 to 10%, keeping the frequency constant at 0.8 Hz. The critical strain was determined when the magnitude of both storage and loss moduli reached 95% of its initial value, as per Uthayakumaran, Newberry, et al. (2002). From this assessment, the

strain of 0.05% was selected, as this strain was less than the critical strain detected in the test, so that all oscillatory tests are performed within the dough's LVR.

4.3.3.2 Yeasted dough

Rheological measurements for yeasted doughs were carried out right after the dough piece was set up between the parallel plates, without a relaxation period. A linear oscillatory time sweep was applied at a constant frequency of 10 Hz and a constant strain of 0.05% for 1200 seconds. Both storage (G') and loss (G'') moduli were recorded over time in order to calculate the magnitude of the complex shear modulus (G^*), as per Eq. 4.2, and the loss tangent ($\tan\delta = G''/G'$).

$$G^* = \sqrt{(G')^2 + (G'')^2} \qquad \text{Eq. 4.2}$$

4.3.3.3 Non-yeasted dough

Rheological measurements for non-yeasted doughs were carried out after the relaxation period. Two sets of measurements were conducted for each replicate using two dough pieces. First, a linear oscillatory frequency sweep was conducted by applying frequencies ranging from 0.1 to 100 Hz, as per Jekle & Becker (2011) and Yovchev et al. (2017), at a constant strain of 0.05%. Both G' and G'' were recorded as a function of frequency in order to calculate G^* (Eq. 4.2) and $\tan\delta$. After the linear oscillatory frequency sweep, a creep-recovery test was conducted on the same dough piece by recording the shear strain. To do so, a constant shear stress of 250 Pa was applied for 180 seconds (creep step) followed by the removal of the shear stress, after which the shear strain was recorded for 360 seconds (recovery step) (Jekle & Becker, 2011; Yovchev et al., 2017). For the creep-recovery test, the final data was reported in terms of compliance ($J(t)$) introduced in Eq. 4.3.

$$J(t) = \frac{\gamma(t)}{\sigma_0} \qquad \text{Eq. 4.3}$$

where $\gamma(t)$ is the recorded shear strain and σ_0 is the constant shear stress. The main parameters obtained from the creep-recovery test were the maximum creep compliance (J_{max}) at t = 180 seconds, the recovery compliance ($J_r = J_{max} - J_{at\ 540\ seconds}$), and the relative elastic part ($J_{el} = J_r/J_{max}$) (Jekle & Becker, 2011; Yovchev et al., 2017).

A linear shear oscillatory time sweep was carried out for 600 seconds on a fresh dough made with the same flour, at a constant frequency of 10 Hz and the shear strain of 0.05%. Both G' and G'' were recorded over time to calculate G^* (Eq. 4.2) and $\tan\delta$. After the linear oscillatory time sweep, a stress relaxation test was performed on the same dough piece by applying a constant shear strain of 5%, with a strain rise time of 0.1 seconds, while recording the shear stress for 1800 seconds as per Li et al. (2003). The main parameter obtained from this test was the relaxation modulus ($G(t)$) (Eq. 4.4) (Van Vliet, 2014).

$$G(t) = \frac{\sigma(t)}{\gamma_o} \qquad \text{Eq. 4.4}$$

where $\sigma(t)$ is the recorded shear stress and γ_0 is the constant shear strain. Another important parameter derived from the stress relaxation test is the relaxation time (τ), which corresponds to the time taken for the relaxation modulus to achieve 36.7% of its initial value, as per the Maxwell model in Eq. 4.5 (Van Vliet, 2014).

$$G(t) = G_\infty + \sum_{i=1}^{n} G_i e^{-t/\tau_i} \qquad \text{Eq. 4.5}$$

where G_∞ is the final relaxation modulus obtained from the fitting, *n* is the number of Maxwell elements, *i* is the *i*th Maxwell element, G_i and τ_i are the relaxation modulus and the relaxation time, respectively, for the *i*th Maxwell element (Van Vliet, 2014). Finally, in order to study the intensity of the relaxation process for the wheat flour dough, the relaxation spectrum ($H(t)$), which defines the continuous relaxation spectrum (Van Vliet, 2014), was calculated using Eq. 4.6. The relaxation spectrum can be approximated to Eq. 4.7 (Ferry, 1970; W. Li et al., 2003).

$$G(t) = G_\infty + \int_{-\infty}^{\infty} H(\tau)\, e^{-t/\tau}\, d\ln\tau_{SR} \qquad \text{Eq. 4.6}$$

$$H(t) = [-(dG(t)/d\ lnt)]_{t=\tau} \qquad \text{Eq. 4.7}$$

The relaxation spectrum is valid within the linear viscoelastic region of the dough (Barnes et al., 1989b), as shown by Rao et al. (2001), who carried out stress relaxation at a constant strain

of 0.05%. Li et al. (2003), on the other hand, performed stress relaxation tests at a constant strain of 5%, the same method as the present study, and also reported relaxation spectrum results. Therefore, a relaxation spectrum was calculated in the present study, but it is emphasized that the equations and methods involved in this calculation are based on the assumption that the rheological parameters have been obtained within the LVR.

4.3.3.4 Fitting equations

To study changes in the time-dependent or frequency-dependent rheological properties of the doughs, equations were fitted for each sample replicate run in the rheometer, i.e., three replicates for each sample, and slopes or exponents were analyzed. For G^* and $\tan\delta$ (non-yeasted) and $\tan\delta$ (yeasted), a linear fit was performed on the linear oscillatory time sweep tests (Eq. 4.8).

$$y(t) = A + Bt \qquad \text{Eq. 4.8}$$

where $y(t)$ corresponds to G^*or $\tan\delta$ over time, *A* is the intercept, *B* is the slope ($dy(t)/dt$), and t is time (seconds). For G^* (yeasted) values resulting from the linear oscillatory time sweep tests, curve fitting was done using an exponential equation (Eq. 4.9).

$$y(t) = A + Be^{-Ct} \qquad \text{Eq. 4.9}$$

where $y(t)$ corresponds to G^* over time, *A* is a constant, *B* is the initial value (G_0^*), *C* is the rate constant, t is time (seconds) and the slope function is $-CBe^{-Ct}$, where $-CB$ is a constant in the slope function that describes the decay in G^* per second (kPa s^{-1}) of fermentation time.

When subjected to a shear oscillatory frequency sweep in the linear viscoelastic region, wheat flour dough follows a power-law behavior as shown in Eq. 4.10 (Ng et al., 2006; Ng & McKinley, 2008).

$$y(t) = S\omega^n \qquad \text{Eq. 4.10}$$

where $y(t)$ corresponds to G^*or $tan\,\delta$ as a function of frequency, *S* corresponds to gel strength, which can also be utilized for gluten strength evaluation (Xinyang, 2019), *n* is an exponent, and ω is angular frequency (rad s^{-1}).

In order to compare and plot the slopes from exponential and linear fitting as well as the power-law exponents, the absolute values, for each sample replicate analyzed in the rheometer and for each rheological parameter, were normalized to a scale ranging from 0 to 1, as per Eq. 4.11.

$$x' = \frac{x - x_{min}}{x_{max} - x_{min}} \quad \text{Eq. 4.11}$$

where x' is the normalized value, x is the original value for each sample replicate analyzed in the rheometer within the same rheological parameter, and x_{max} and x_{min} are the maximum and minimum values, respectively, within the set of samples, for each rheological parameter.

4.3.4 Bread specific loaf volume

Bread samples were prepared at Cereals Canada using whole-wheat flour (weight adjusted to 14.0% moisture basis) as described by Xie et al. (2021). The ingredients added were distilled water, based on water absorption (*WA*, %) determined for whole-wheat flour samples using Near Infrared (NIR) Spectroscopy (Perten DA7200 NIR), NaCl (1.65%), fresh compressed yeast (6.50%), canola oil (1.75%), dough conditioner (1.00 %), and sugar (0.50%), all based on flour weight. The moisture in the fresh compressed yeast was also accounted for in the total water added for dough mixing. These ingredients were mixed with the whole-wheat flour in a 200 g pin mixer (National Mfg. Co., Lincoln NE) at 118 rpm until the peak was achieved (determined by P2M software) (Setia et al., 2020). From the dough mixing process, the mixing time to peak (*MTP*, min) and the power at peak (*Power*, Watt), i.e., maximum power to achieve a dough at optimum consistency, were also recorded.

Each mixed dough was divided into two pieces of 165 g, rounded manually and rested (covered) for 3 minutes. After the resting time, each dough piece was sheeted in 3 passes: 7.9 mm (first gap), 4.8 mm (second gap) and 3.2 mm (third gap), and then moulded. Moulded dough samples were placed in a fermentation cabinet (37 ºC/ RH 85%) for 45 to 60 minutes and were removed once they reached 90 mm in height. After fermentation, doughs were baked for 20 minutes at 204 ºC. After baking, the bread samples were placed on a baking rack for one hour to cool before bread weight (recorded by the mass equivalent in g) and loaf volume measurements were made.

Bread loaf volume was measured at Cereals Canada based on the AACC International Approved Method 10-14.01 (AACC International, 2014) using laser topography with a TexVol bread volume meter (BVM) (Perten- L370). After the bread loaf volume was measured (*LV*, cm^3), it was divided by the bread weight (recorded by the mass equivalent in g), obtained after bread had cooled down from baking, in order to report the specific loaf volume (*SLV*, $cm^3\ g^{-1}$).

4.3.5 Statistical analysis

Fitting of equations and determination of the Pearson correlation coefficients between free asparagine content and dough rheological parameters were conducted using OriginPro software (OriginPro, Version 2021b. OriginLab Corporation, Northampton, MA, USA). The relaxation spectrum was calculated by using an open-source software package "pyReSpect" from Shanbhag (2018), as per Shanbhag (2019). Principal Component Analysis (PCA) was conducted using OriginPro software to investigate the correlations between dough rheological properties, wheat characteristics (free asparagine, protein content, yield), *FAB*, bread dough preparation parameters (*WA*, *MTP*, *Power*) and bread *SLV*. Dough development time measurements on the mixograph and bread specific loaf volume were measured in duplicate. Shear rheological tests were performed in triplicate. The plotted figures throughout this chapter show mean values and error bars, corresponding to standard error (SE) values, unless otherwise stated.

4.4 Results and discussion

4.4.1 Dynamic shear rheology: linear oscillatory time sweep

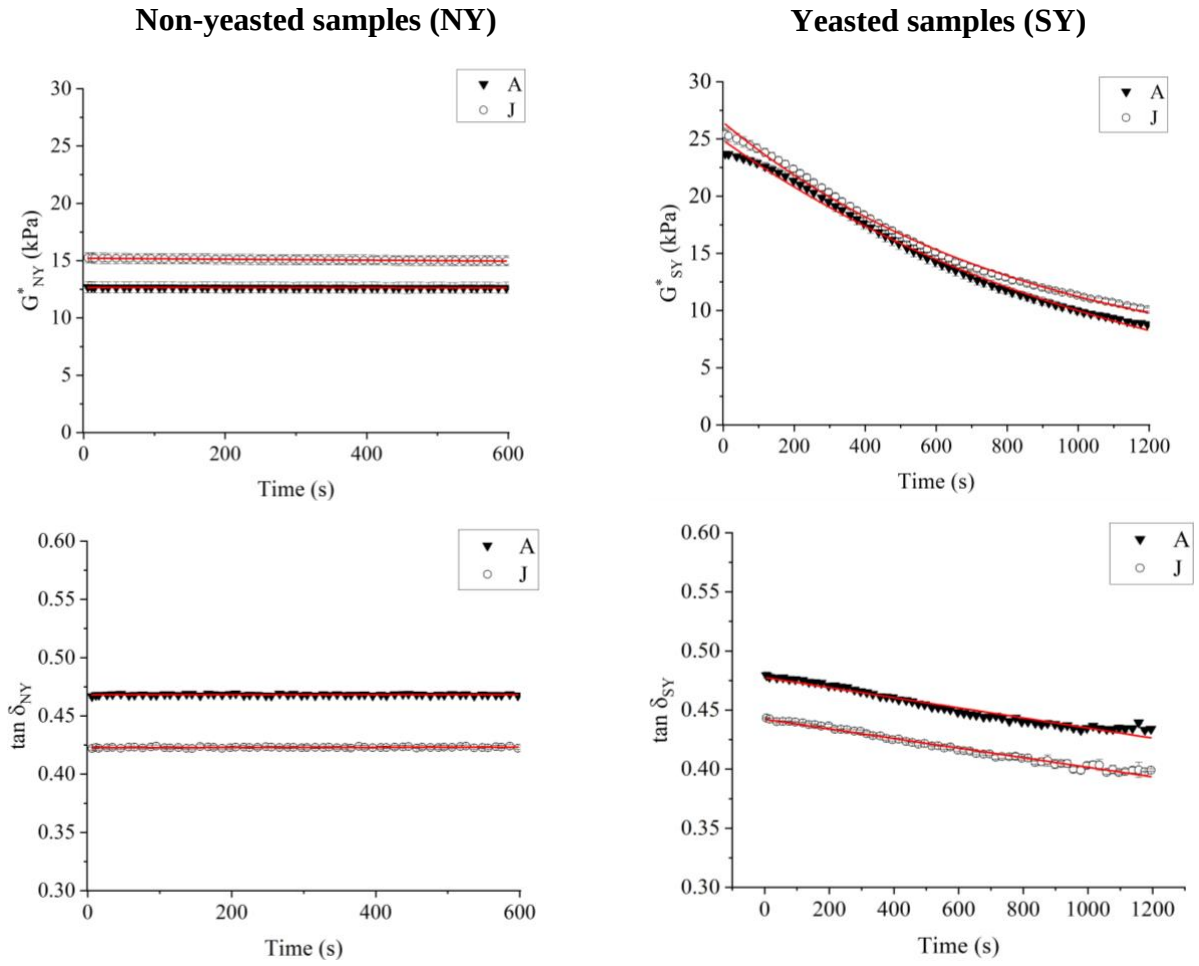
Linear oscillatory time sweep tests were conducted on yeasted and non-yeasted doughs in order to investigate changes in the rheological properties of the doughs over time. In order to evaluate if dough rheological properties change with time, linear fitting was performed for G^* and $\tan\delta$ for the non-yeasted doughs, for all sample replicates analyzed in the rheometer. An example of the evolution of G^* and $\tan\delta$ over time can be observed in Figure 4.1.

For the non-yeasted samples, the linear (Eq. 4.8) fitting had a goodness of fit (R^2) of 0.9999 for G^* and for $\tan\delta$, for all samples. In order to study the changes of these parameters over time, the slopes for each replicate were evaluated, and it was found that all the slopes for G^* were statistically ($p < 0.05$) different from zero, even though from the left hand side of Figure 4.1, there is no evident change. For $\tan\delta$, some replicates had slopes statistically equal to zero ($p < 0.05$), which varied from -5.3×10^{-7} to 1.2×10^{-6} s^{-1}. In addition, the mean slopes varied from -4.2×10^{-4} to -6.3×10^{-5} kPa s^{-1} for $G^*$and, for $\tan\delta$ curves, the mean slopes varied from -1.5×10^{-6} to

2.2×10^{-6} s^{-1} depending on asparagine concentration. When analyzing the rheological properties of dough within the LVR, a slope decay larger than 0.05 has been considered a critical extent of departure which indicates substantial changes in the shear moduli (Uthayakumaran, Newberry, et al., 2002). Therefore, in order to compare the slope values for G^* with the critical slope of 0.05, the absolute slopes for G^* were normalized, including the critical slope value of 0.05 among the values to be normalized. The resulting mean slopes for G^* varied from 3.5×10^{-4} to 7.4×10^{-3} kPa s^{-1} while the normalized slope for 0.05 was 1 (highest decay). For $\tan\delta$, the same normalization for comparison with 0.05 shows a more pronounced difference, with the mean $\tan\delta$ normalized slopes varying from 1.9×10^{-6} to 4.4×10^{-5} s^{-1}, while the critical slope (0.05) was represented by the highest normalized value (1). Therefore, the slopes were all well below 1 in the normalized scale, indicating that the tests all remained within the LVR. However, the statistical analysis for the slopes indicated that significant changes occurred over the period of analysis, as the majority of the replicate slopes were statistically ($p<0.05$) different from zero.

Figure 4.1 shows that there is a clear change in dough rheological properties for the yeasted dough samples. For all replicates, exponential (Eq. 4.9) fitting was done for G^*, and linear (Eq. 4.8) fitting was done for $\tan\delta$, and goodness of fit (R^2) values varying from 0.9986 to 0.9998 for G^* and 0.9999 for $\tan\delta$ were obtained. The exponential rate decay for G^* varied from 4.3×10^{-3} to 1.2×10^{-3} s^{-1}, indicating significant changes in this rheological parameter over time when compared to non-yeasted doughs. For $\tan\delta$, although having less pronounced decays compared to G^* (Figure 4.1), the slopes varied from -8.8×10^{-5} to -2.5×10^{-5} s^{-1}. These slopes were statistically different from zero ($p < 0.05$), and some ten times larger than the slope values found for the non-yeasted samples. From Figure 4.1 and the fitting equation results, it is clear that the rheological properties of yeasted doughs exhibited a more pronounced time dependency than non-yeasted doughs.

Figure 4.1. Magnitude of the complex (G^*) shear modulus and loss tangent (tan δ) as a function of time (seconds) for non-yeasted (NY) and yeasted (SY) wheat flour dough samples with the highest (A, ▼) and lowest (J, ○) free asparagine concentration. Red lines are the linear (non-yeasted and tan δ yeasted) and exponential (G^* yeasted) fits. Error bars (SE, n = 3) are also included in the graphs.



Meerts et al. (2018) performed linear (strain of 0.06%) oscillatory time sweep over two hours to study the rheological changes in a fermenting dough prepared with wheat flour from a single wheat variety. The authors observed a significant decay in G', G'', and tan δ over time, indicating an overall dough softening effect due to the fermentation process (Meerts et al., 2018), which is in agreement with the results of the present study. Moreover, a decay in dough rheological properties translating into a loss of dough strength was also observed in previous studies (Lee et al., 2004b; Lee & Campanella, 2013; Verheyen et al., 2014). In addition to fermenting dough

rheological studies, Verheyen et al. (2014) also evaluated dough pH during fermentation, observing a decay of pH to values as low as 5.3. The dough pH decay has been shown to occur mostly due to succinic acid production by yeast during fermentation (Jayaram et al., 2013). The decay of pH may increase protease activities, which act on gluten proteins and affect the rheological properties of the doughs. The effect of proteases on dough rheological properties was reported by Wikström & Eliasson (1998), who observed dough softening effects upon addition of protease (*Aspergillus oryzae*). Based on the effects of protease on dough properties from Wikström & Eliasson (1998), Verheyen et al. (2014) elucidated that changes in dough rheological properties during fermentation could be due to the decay of the dough pH, which causes an increase in the activity of proteolytic enzymes present in the wheat flour. Besides the pH decay, the increase in the volume fraction of bubbles in the dough has also been hypothesized to cause dough softening (Verheyen et al., 2014). This softening is caused by the increase of bubble sizes that occurs due to the inflation of the dough structure during CO_2 production, which increased the susceptibility of the dough to external forces (Verheyen et al., 2014). Wehrle & Arendt (1998) also evaluated rheological properties of dough during fermentation, and found that dough presented more fluid-like behavior when gas production was at its highest levels. Lee & Campanella (2013) also attributed the effects of fermentation in the rheology of wheat flour doughs to the growth in volume of bubbles, which change the geometry of the dough, leading to changes in dough properties.

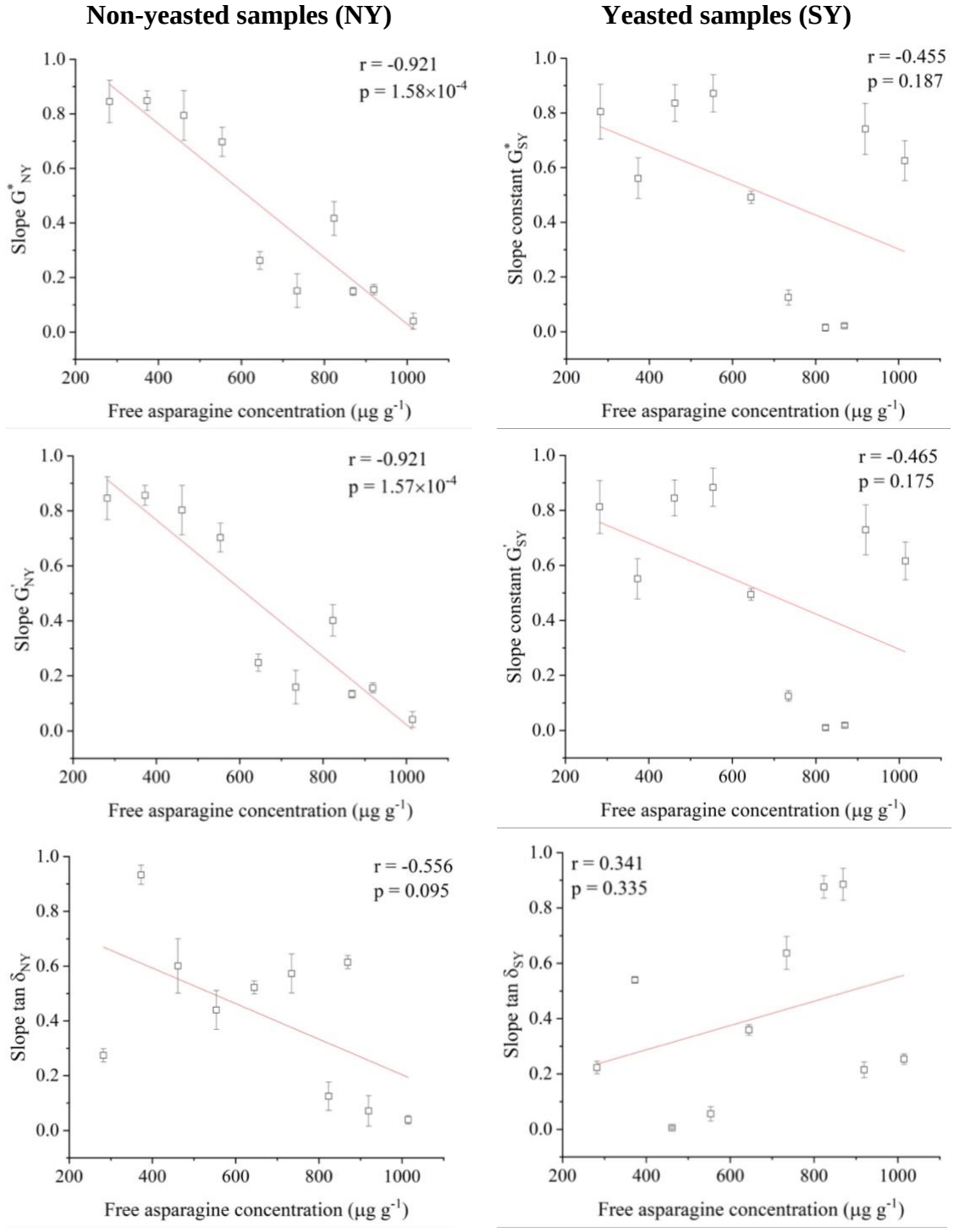
As significant changes in the rheological properties of dough were observed for non-yeasted samples and even more pronounced during fermentation, it was necessary to investigate if these changes were influenced by the free asparagine concentration in the wheat. Therefore, Figure 4.2 shows correlation graphs of the normalized slopes versus time of the rheological parameters (G^* and $\tan\delta$) plotted against free asparagine concentration, for both yeasted and non-yeasted dough samples. In order to confirm the correlation of changes in dough elasticity against free asparagine concentration, the normalized slopes for storage modulus (G') versus time, obtained from linear and exponential fitting for non-yeasted and yeasted doughs, respectively, were also plotted in Figure 4.2.

From Figure 4.2, the slopes for the ${G^*}_{NY}$ and ${G'}_{NY}$ curves over time were negatively ($r = -0.921$, $p<0.05$) correlated with free asparagine concentration, indicating that samples with higher free asparagine concentration had lower slopes, i.e., less changes in G^* and G' over time. Moreover, because of the similar Pearson correlation coefficient between ${G^*}_{NY}$ and ${G'}_{NY}$ against

free asparagine (Figure 4.2), one can notice that G'_{NY} had a prominent contribution to the relationship between slopes of G^*_{NY} and free asparagine concentration. For $\tan\delta_{NY}$, although the correlation was not significant at $p < 0.05$, the Pearson correlation coefficient against free asparagine was significant at $p < 0.10$, also indicating less changes in dough rheological properties over time for samples with higher free asparagine concentration. Therefore, there were evident changes in dough rheological properties over time for non-yeasted samples, and the rate of these changes was significantly correlated to free asparagine concentration. Moreover, no significant correlations, at $p < 0.05$, were found between the slopes for G^*_{NY}, G'_{NY} and $\tan\delta_{NY}$ against protein content and FAB. However, a correlation at $p < 0.10$ was found between protein content and slopes of G^*_{NY} ($r = -0.591$) and G'_{NY} ($r = -0.600$) and between FAB and slopes of G^*_{NY} ($r = -0.577$) and G'_{NY} ($r = -0.575$). No significant correlations were found between slopes of $\tan\delta_{NY}$ and protein content or FAB.

One possible explanation for samples with higher free asparagine concentration showing less changes in moduli, thus maintaining a more elastic-like behavior throughout the analysis, lays in the type of molecular interactions governing the dough rheological properties within the LVR. Amemiya & Menjivar (1992) performed dynamic oscillatory tests within the dough LVR, i.e., at low strains (0.5%), and observed that starch contributions to dough rheological properties were equally important as the protein contributions. The authors explained that, at small strains, short-range interactions, such as van der Waals and hydrogen bonds, are also sensed by the test and have a great impact on the elasticity response of the dough (Amemiya & Menjivar, 1992). The results found in the present study might be explained by findings from Amemiya & Menjivar (1992). Although not statistically significant at $p<0.05$, protein content was positively correlated to free asparagine concentration in this study ($r = 0.530$). Findings from Amemiya & Menjivar (1992) indicated that hydrogen bonds might significantly contribute to the rheological properties of dough. Among gluten proteins, gliadins, mainly ω–gliadins, are rich in the amino acid glutamine, which allows for hydrogen bonding (Delcour et al., 2012). Moreover, M. Li et al. (2021) aimed to study the effect of Gli/Glu ratio on dough rheological properties, i.e., G' and G''measured through frequency sweep tests, and the interactions between the gliadins, the glutenins and starch. The authors utilized a Chinese wheat variety to extract gluten and starch and to prepare gluten at different Gli/Glu ratios. In their study, M. Li et al. (2021) used a mixograph to prepare undeveloped, optimally developed and over-developed dough samples, all at optimum water

Figure 4.2. Absolute normalized slopes of the curves for G^*, G' and tan δ over time against free asparagine concentration (μg g^{-1}) and Pearson's correlation coefficient (r) analysis for both yeasted and non-yeasted dough samples. Data points represent means ± SE (n = 3).



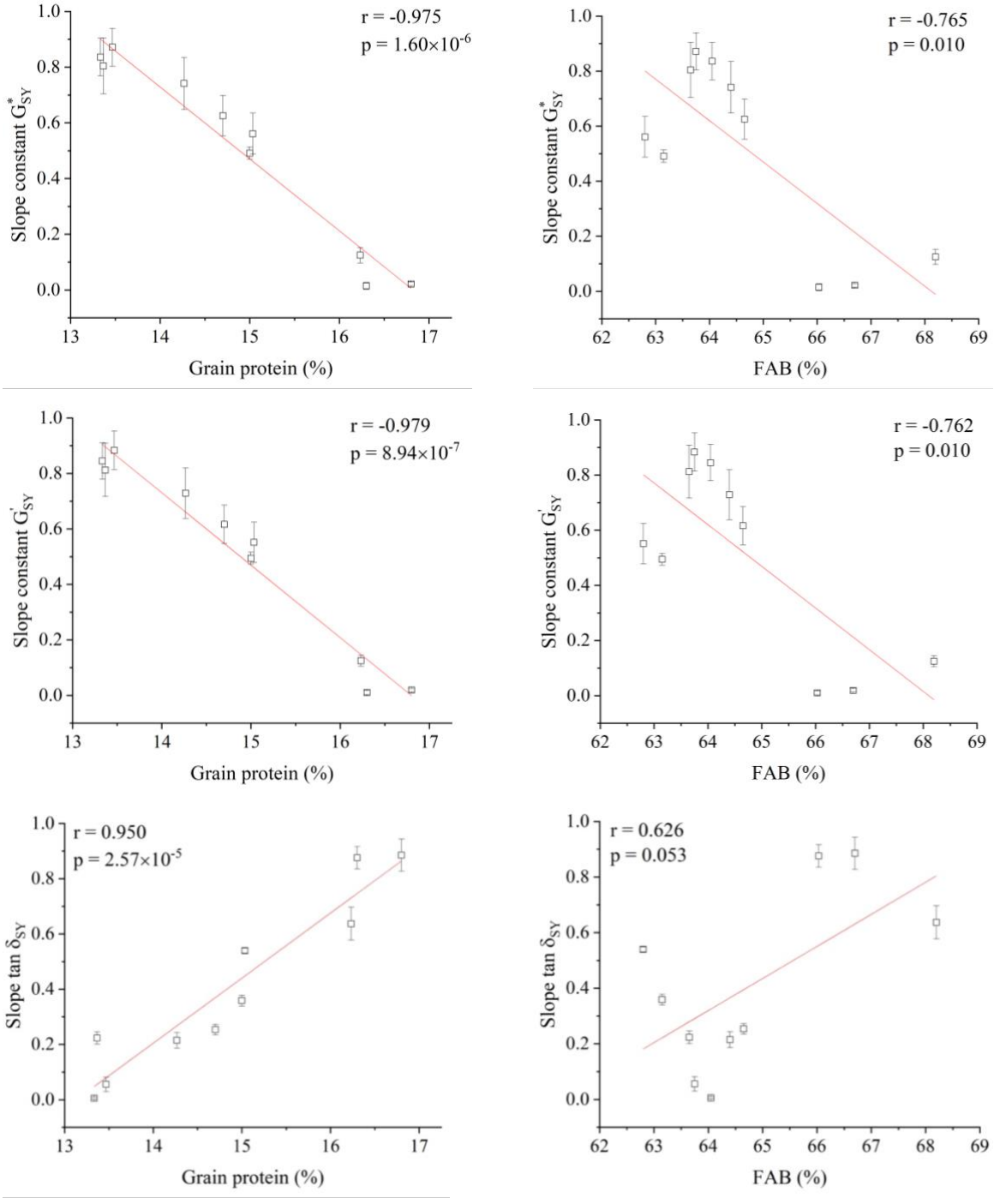
absorption levels but through using different mixing times (M. Li et al., 2021). M. Li et al. (2021)performed linear dynamic oscillatory tests on the dough samples and explained that the dough elasticity, evaluated through G', in optimally developed doughs, at high proportions of Gli/Glu, was explained by the presence of hydrogen bonds and hydrophobic interactions from the gliadins (M. Li et al., 2021). Therefore, a possible explanation for the present findings could be a higher proportion of gliadins relative to glutenins as the protein content increased, thus a higher Gli/Glu ratio in samples with higher free asparagine concentration. The higher proportion of hydrogen bonds from the gliadins, and also gliadins-starch interactions, could be the reason for a more structured material being observed at low strains, with less changes in the moduli over time. Samples with lower protein contents and free asparagine concentrations might have a lower Gli/Glu ratio, thus showing less contribution of short-range interactions on dough rheological properties over time.

For yeasted doughs, on the other hand, a closer look at the correlation studies was necessary. Free amino acids are known to be utilized as a source of nitrogen by yeast during fermentation (De Barber et al., 1989). Fredriksson et al. (2004) evaluated free asparagine concentration in wheat flour doughs during fermentation and observed a decrease in the concentration of this free amino acid in the dough over time. Therefore, it is hypothesized in the present study that samples with higher free asparagine concentration would result in greater changes in dough rheological parameters, i.e., larger slopes, during fermentation. However, as per Figure 4.2, weak and non-significant correlations were found between the slope constant of the curve ${G^*}_{SY}$ and the slope of the curve $tan\ \delta_{SY}$ against free asparagine concentration. This outcome indicates that free asparagine might not play a great role in dough rheological changes during fermentation. However, it is important to note that, in the present study, time sweeps were performed for 20 minutes on yeasted doughs. Fredriksson et al. (2004) observed significant changes in free asparagine concentration after 2 hours of fermentation. This could indicate that results obtained in the present study might be different if the rheological changes had been observed over longer fermentation periods.

Although no correlations were found for the rate of changes in dough rheological properties over time against free asparagine concentration during fermentation, a closer look at the correlations with protein content and water absorption are necessary. For yeasted doughs, Figure 4.3. shows the correlation for the slope constants of the curves for the magnitude of the complex

shear moduli and storage moduli (G^*, G') and the slope of the curve for $\tan\delta$ over time against protein content and FAB. The strong negative correlation between the rate decay of moduli over time and protein content is also consistent with the hypothesis that samples with higher protein content have higher Gli/Glu ratios. The reason for this is because gluten macropolymers, formed by glutenin aggregates linked mainly by disulfide bonds, are highly responsible for dough elasticity (Delcour et al., 2012). However, metabolites that are produced during fermentation might have an impact on dough rheological properties, mainly by decreasing gluten entanglements and aggregation, which leads to a decrease in dough elasticity (Rezaei et al., 2016). Verheyen et al. (2014) observed a steep decrease in G^* during dough fermentation, with dough prepared from a German commercial wheat. The authors concluded that the decrease in dough pH due to metabolites from fermentation increased proteolitic enzyme activities, which affected the gluten network, translating into a decrease in G^*. Moreover, studies involving sourdough fermentation effects in gluten proteins showed that glutenins were degraded during fermentation due to proteolytic activity (Loponen et al., 2004; Wieser et al., 2008), with minimal effects on gliadins, mainly ω−gliadins (Wieser et al., 2008). Therefore, for the present study, the lower decay in the moduli in samples with higher protein content could be explained by higher Gli/Glu ratios in these samples, having short-range interactions less affected by the fermentation process than samples with lower protein content. The strong correlation between slopes of the moduli curves with *FAB*, from Figure 4.3, could also be evidence that samples with higher protein content incorporated more water due to a higher Gli/Glu ratio, leading to an increase in the hydrogen bonding in these samples. Finally, as $\tan\delta$ is indirectly correlated to solid-like properties, the faster decrease in $\tan\delta$ for yeasted samples with higher protein content and *FAB* indicates that, over the 20 minutes of the test, the short-range interactions between gliadins and gliadins-starch were less affected by fermentation, maintaining G' at higher values. In contrast, samples with lower protein content, thus more proportions of glutenin (lower Gli/Glu ratio), were more affected by fermentation, with faster decrease in $G'$and less changes in $\tan\delta$.

Figure 4.3. Absolute normalized slopes of the curves for G^*, G' and tan δ over time against grain protein content (%) and *FAB* (% at 14% m.b.) and Pearson's correlation coefficient (r) analysis for yeasted dough samples. Data points represent means ± SE (n = 3).



Overall, the decay of moduli (G^* and G') for non-yeasted samples were strongly negatively correlated to free asparagine concentration, while no significant correlations were found between decay of moduli and free asparagine concentration for yeasted samples, showing little contribution from this free amino acid on the fermentation rate of the dough samples. On the other hand, the fermentation process was shown to affect samples with lower protein content to a greater extent, causing a faster decrease in moduli for these samples when compared to samples with higher protein content and *FAB*. The possible explanation for the findings lays in the possibility of a higher Gli/Glu ratio in samples with higher free asparagine concentration and protein content.

Therefore, the sensitivity of short-range interactions between gliadins probed by low shear strain tests could explain most of the changes in dough rheological properties when evaluated at low strains. Conversely, samples with lower protein content, thus possibly lower Gli/Glu ratios, could be affected to a greater extent by fermentation due to the effects of low pH and growth of bubbles on the gluten network.

4.4.2 Wheat free asparagine concentration and dough rheological properties: small and large shear strains

Further dough rheological analysis was conducted using small and large shear strains to provide a deeper understanding of the relationship between dough rheological parameters and free asparagine concentration in wheat.

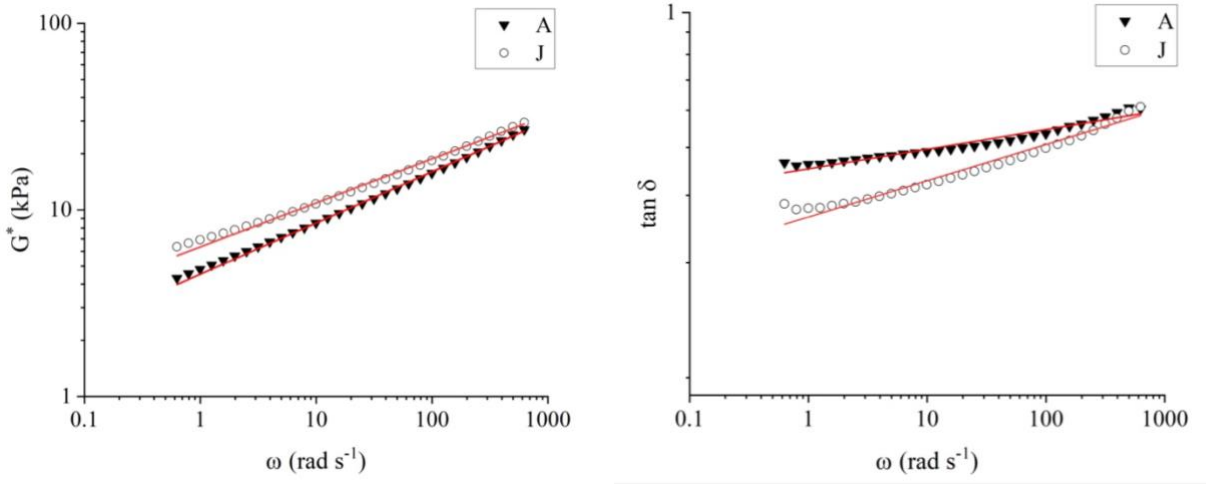
4.4.2.1 Dynamic shear rheology: linear oscillatory frequency sweep

Linear oscillatory frequency sweep tests have been commonly employed to study dough rheological properties over a range of frequencies (Jekle & Becker, 2011; Tozatti et al., 2020; Yovchev et al., 2017). However, to our knowledge, there is no use of this rheological test to evaluate the relationship between the free asparagine concentration in wheat and the rheological properties of the dough made from wheat flour. To study changes in dough rheological properties over a range of frequencies, a power-law equation **(**Eq. 4.10**)** was fitted for G^* and $\tan\delta$ as exemplified in Figure 4.4.

One of the parameters extracted from the best fitted curves, the power-law exponent (*n*), i.e., the indication of the rate of rheological parameter change against frequency, was evaluated. When fitted for each replicate, the power-law curves presented a goodness of fit (R^2) of 0.9999 for G^*, G' and $\tan\delta$ against frequency. For G^*, the mean *n* values varied from 0.243 to 0.307, for G' the mean *n* values varied from 0.225 to 0.295, and for $\tan\delta$ the mean *n* values varied from 0.053

to 0.093. Figure 4.5 shows the correlation between *n*, for G^*, G', and tan δ, against free asparagine concentration.

Figure 4.4. Magnitude of the complex (G^*) shear moduli and loss tangent (tan δ) as a function of angular frequency (ω) for non-yeasted wheat flour dough samples with the highest (A, ▼) and lowest (J, ○) free asparagine concentration. Red lines are the power-law fitting and data points are representatives of the replicates from the frequency sweep results for A and J.

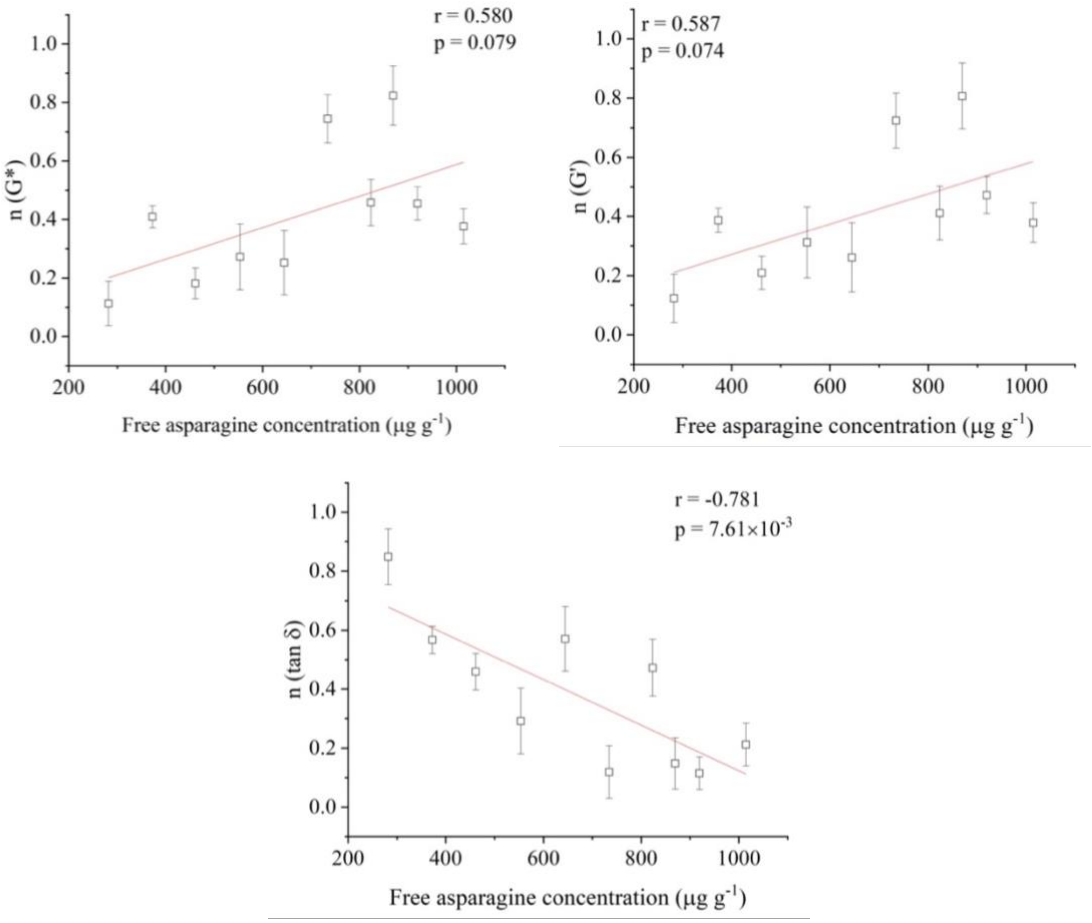


As per Figure 4.4, both G^* and $tan\ \delta$ increased as the frequency increased; however, the rate of increase varies depending on the sample. In Figure 4.5, it is possible to observe that, although not significant, the power-law exponent (*n*) for G^* and G' were positively correlated to free asparagine concentration. However, the *n* for tan δ against frequency was negatively correlated at $p < 0.05$ with free asparagine concentration, meaning that the frequency dependent response of tan δ is lower as free asparagine concentration increases, i.e., the dough samples have more solid tendencies at higher free asparagine concentrations. This outcome could indicate that samples with higher free asparagine concentration levels had a slightly higher increase in G' in relation to G''.

In order to deeply investigate the possible causes for this outcome, Figure 4.6. shows the relationship of power-law exponent (*n*) for G^* and G' against protein content and *FAB*. From Figure 4.6, statistically significant ($p < 0.05$) and positive relationships were found between the exponents against protein content and *FAB*. This outcome could be explained by the possible short-

range interactions present between gliadins and gliadins-starch, such as hydrogen bonding. As mentioned before, samples with high protein content, and overall high free asparagine concentration, could present higher proportions of Gli/Glu ratios compared to samples with lower protein content.

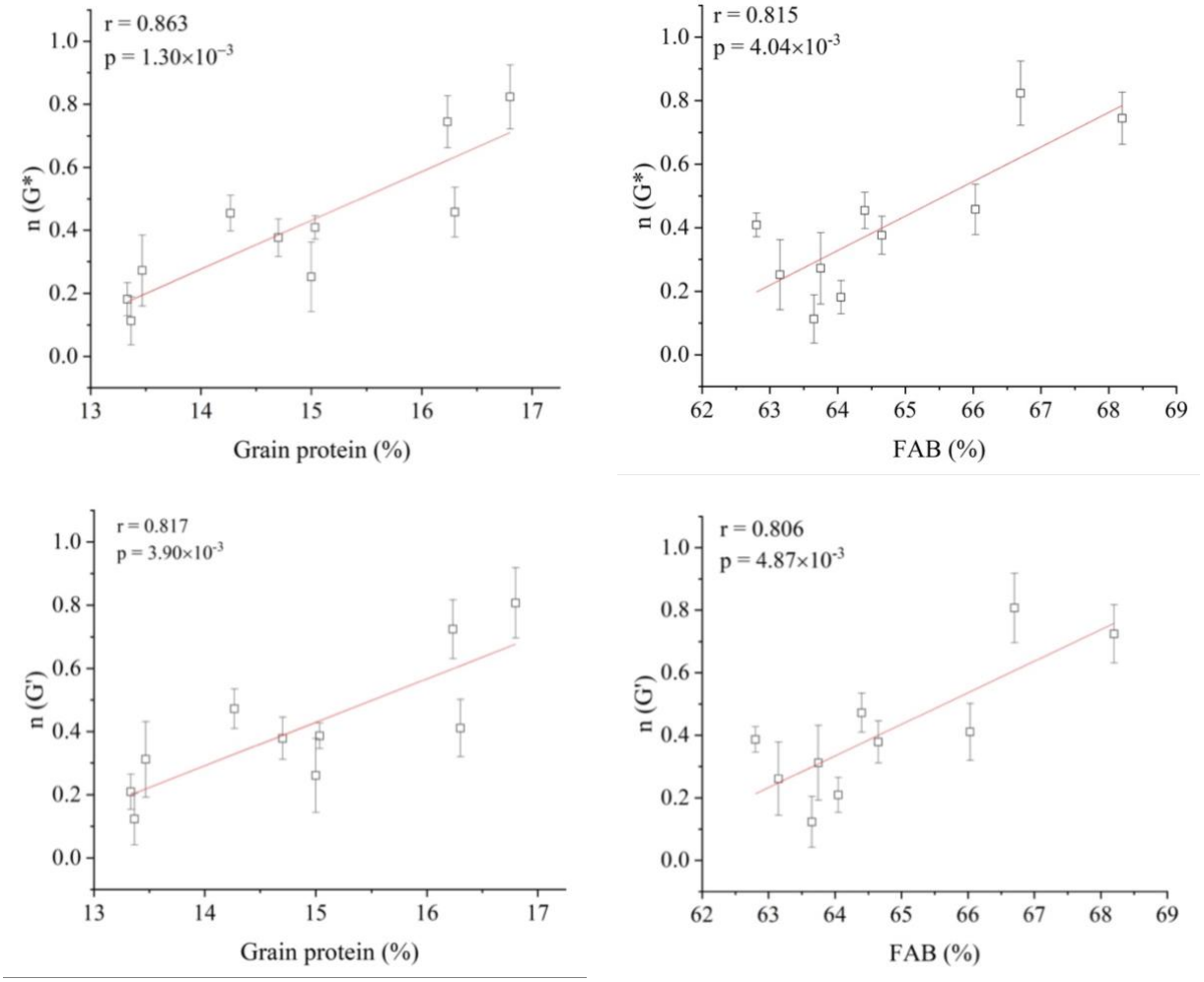
Figure 4.5. Normalized power-law exponent (*n*) for G^*, *G'*, and tan δ, which was obtained from Power-law fitting as a function of frequency, plotted against free asparagine concentration (μg g^{-1}). The Pearson's correlation coefficients (r) are also shown in the graphs. Data points represent means ± SE (n = 3).



Therefore, high concentrations of glutamine in gliadins (Delcour et al., 2012) could also explain higher *FAB* and hydrogen bonding, causing a higher frequency dependence in the moduli at higher protein contents. Although not significant at $p < 0.05$, *n* from tan *δ* against frequency was negatively correlated to protein content (r = -0.404) and *FAB* (r = -605). This negative

correlation could be explained by the short-range interactions effect, at high protein levels, on dough elasticity, showing lesser increase in $\tan\delta$ values of samples with higher free asparagine concentration as frequency increases, thus explaining a more solid character for doughs with higher free asparagine concentration.

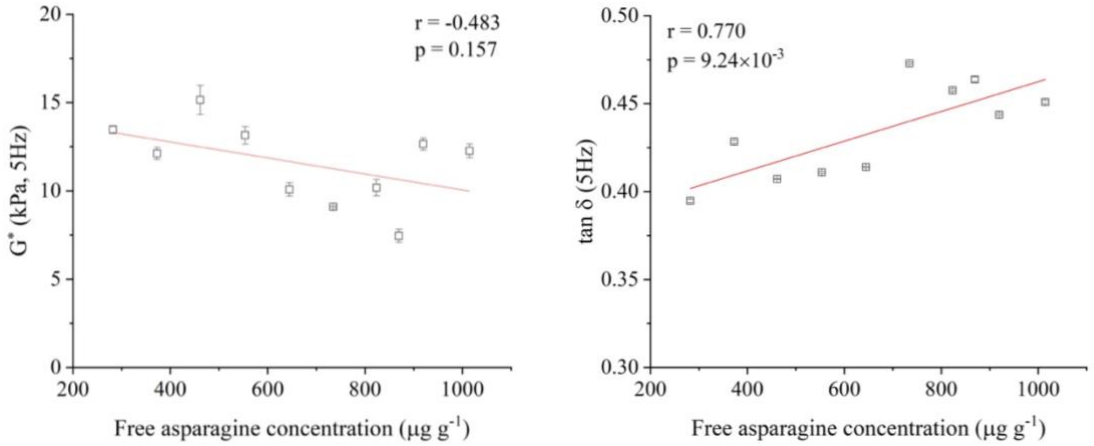
Figure 4.6. Normalized power-law exponent (*n*) for G^* and *G'*, which was obtained from Power-law fitting as a function of frequency, plotted against protein content (%) and *FAB* (%, 14% m.b.). The Pearson's correlation coefficients (r) are also shown in the graphs. Data points represent means ± SE (n = 3).



In addition, in order to observe how rheological parameters were correlated to free asparagine concentration, G^* and $\tan\delta$ were selected at a specific frequency (5 Hz) and plotted

against free asparagine concentration (Figure 4.7). From Figure 4.7, a negative correlation, although not significant, was found between G^* at 5 Hz and free asparagine concentration, meaning that, overall, samples had a lower modulus at high levels of this free amino acid. Research studies performing linear oscillatory frequency sweeps to investigate dough rheological properties have reported loss tangent ($\tan\delta$) values to be inversely proportional to dough solid-like, or gluten strength, characteristics (Jekle & Becker, 2011; Y. Song & Zheng, 2007; Tozatti et al., 2020; Yovchev et al., 2017). Tozatti et al. (2020) evaluated dough rheological properties from 25 Canadian wheat cultivars, prepared at optimum water absorption, reporting G^* values from 9.2 kPa to 18.8 kPa and $\tan\delta$ values ranging from 0.33 to 0.42. The authors reported that, overall, wheat varieties with stronger glutens had higher G^* and lower $tan\ \delta$ (Tozatti et al., 2020). From Figure 4.7, the strong, positive correlation between $\tan\delta$, at 5 Hz, and free asparagine concentration confirms the overall decrease in gluten strength, or dough solid-like properties, when free asparagine concentration increases.

Figure 4.7. Correlation between G^* and tan δ at 5 Hz against free asparagine concentration (μg g^{-1}). The Pearson's correlation coefficients (r) are also shown in the graphs. Data points represent means ± SE (n = 3).



Moreover, at 5 Hz, G^* was negatively correlated to protein content (r = -0.932, $p < 0.05$) and *FAB* (r = -0.662, $p < 0.10$), and $\tan\delta$ was positively correlated, at $p < 0.05$, to protein content (r = 0.854) and *FAB* (r = 0.828). Tozatti et al. (2020) also reported that G^* and $\tan\delta$ were

negatively (r = -0.77, p < 0.01) and positively (r = 0.61, p < 0.01), respectively, correlated to protein content when data was evaluated at an arbitrarily chosen frequency of 1 Hz. Thus, the results from the present study demonstrated that, at a specific frequency, doughs with less solid-like characteristics were observed at high protein content and high free asparagine concentration. However, the frequency dependence analysis showed that, as frequency increased, the doughs with higher protein content and free asparagine concentration shows more solid-like properties. Therefore, data are frequency dependent and, at small strains, short-range interactions could be playing a major role, and imposing difficulties on the evaluation of rheological properties of doughs. As previously mentioned by Amemiya & Menjivar (1992), because the short-range interactions also play an important role in rheological tests within the dough LVR, it is difficult to decouple the effects of long-range interactions between proteins from the short-range interactions. Therefore, rheological tests at larger strain and stresses, outside of the LVR, are also necessary to better understand the relationship between dough rheological properties and free asparagine concentration.

4.4.2.2 Static shear rheology: creep-recovery and stress relaxation

In order to evaluate dough rheological properties outside of the LVR, since dough processing involves large strains and stresses (Dobraszczyk & Morgenstern, 2003), creep-recovery and stress relaxation tests were performed and their standard curves are shown in Figure 4.8. To evaluate how these rheological properties were correlated to free asparagine concentration, Pearson's correlation coefficients (r) were calculated, as shown in Figure 4.9. Contrary to some of the parameters from small strain dynamic tests, static shear tests resulted in dough rheological parameters that were significantly ($p<0.05$) correlated to free asparagine concentration.

When wheat flour dough samples were submitted to a constant shear stress, samples containing higher levels of free asparagine showed higher levels of deformation, thus higher J_{max}, when compared to samples with less free asparagine. The same trend was observed for J_r, which is a parameter that measures the dough capability of recovering after unloading the shear stress. Samples with lower free asparagine concentration had greater recovering of their structure when compared to samples with higher free asparagine concentration, indicating that as free asparagine concentration increased, the gluten strength decreased. This can be observed in Figure 4.9, from the negative correlation between J_{el} and free asparagine concentration, as J_{el} measures the relative elasticity of the samples, i.e., the relative recovery of the creep-compliance (Jekle & Becker, 2011).

Tozatti et al. (2020) also studied dough rheological properties under creep-recovery for 25 Canadian wheat cultivars. These authors also reported high values of J_{max} and low values for J_{el} for samples with lower gluten strength when compared to samples with stronger glutens (Tozatti et al., 2020). Moreover, they found a positive correlation between J_{max} and protein content ($r = 0.65$, $p < 0.01$) and a negative correlation between J_{el} and protein content ($r = -0.36$, $p < 0.01$) (Tozatti et al., 2020). The present study also found significant correlations between creep-recovery parameters, protein content, and also FAB. Parameters that were inversely related to dough solid-like characteristics, i.e., J_{max} and J_r, were positively ($p < 0.05$) correlated to protein content ($r = 0.879$ and $r = 0.934$, respectively) and *FAB* ($r = 0.849$ and $r = 0.830$, respectively). The positive correlations between the parameters J_{max} and J_r against free asparagine concentration (Figure 4.9), protein content, and *FAB* could be explained by the forementioned reason that samples with higher free asparagine content and protein content possess higher Gli/Glu ratios.

This opposite result, when compared to results from dynamic oscillatory shear tests, is expected because as already stated by Amemiya & Menjivar (1992), large strain tests are capable of showing the effects of long-range interactions between proteins. Therefore, the positive correlations for J_{max} and J_r in this study show that samples with high protein content and free asparagine concentration have weaker or less protein-protein interactions, such as disulfide bonds between glutenin subunits. Therefore, this outcome could be explained by a higher proportion of gliadins in these samples, which do not impart dough solid-like characteristics as glutenins do. This explanation is also supported by the negative relationship between J_{el} against protein content ($r = -0.784$, $p < 0.05$) and *FAB* ($r = -0.858$, $p < 0.05$).

For stress relaxation tests, when an "instantaneous" shear strain was applied and the resulting stress was measured over time, dough samples containing lower free asparagine concentration were more resistant to the shear strain and had a longer relaxation process (Figure 4.9). The initial relaxation modulus (G_0) was negatively correlated to free asparagine concentration. The same trend was observed for the relaxation modulus at the end of the test (G_e). A Newtonian-liquid relaxes to $G_e = 0$ right after the strain is imposed and a Hookean-solid will maintain $G_e = G_0$, i.e., it does not relax over time (Barnes et al., 1989b). An optimally developed wheat flour dough is a soft viscoelastic material (Scanlon & Page, 2015) that does not achieve a final relaxation modulus of zero due to its solid-like characteristic. Therefore, G_e is a rheological

Figure 4.8. Plots obtained from creep-recovery and stress relaxation measurements for non-yeasted wheat flour dough samples with the highest (A, ▼) and lowest (J, ○) free asparagine concentration. (A) Compliance over time curves, obtained from creep-recovery tests, where J_{max} is the compliance at 180 seconds and J_r at 540 seconds. (B) Relaxation moduli over time curve, obtained from a stress relaxation test where G_0 is the initial relaxation modulus, G_e is the relaxation modulus at 1800 seconds, G_∞ the final relaxation modulus obtained from the fitting, τ_1 and τ_2 are the relaxation times obtained from fitting a Maxwell model with 2 elements (red line) to the data. (C) Relaxation spectrum over time, obtained from stress relaxation data, where H_{short} is the relaxation spectra at short times (5 seconds) and H_{long} is the relaxation spectra at longer times (300 seconds). Data points in graphs represent means and SE (n = 3).

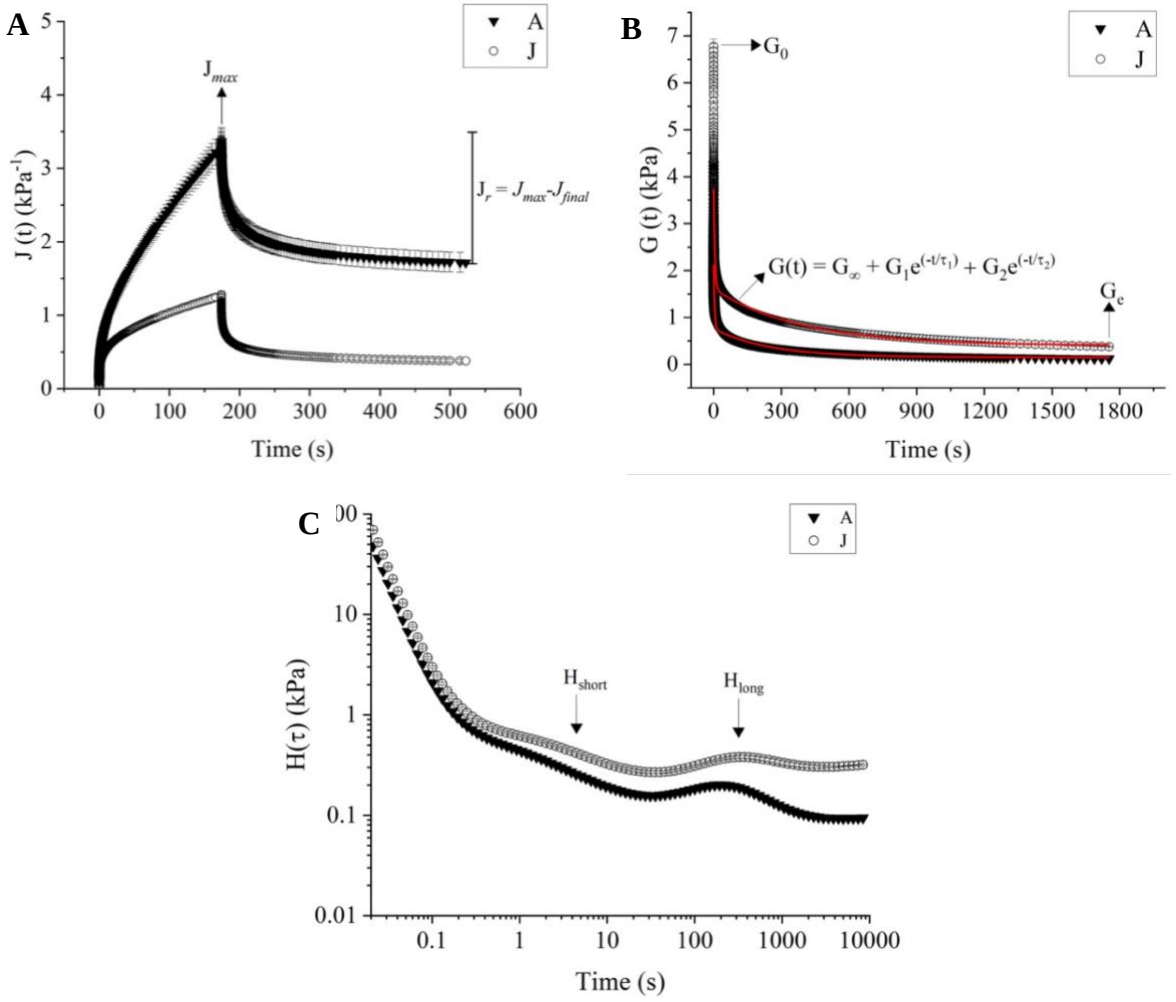
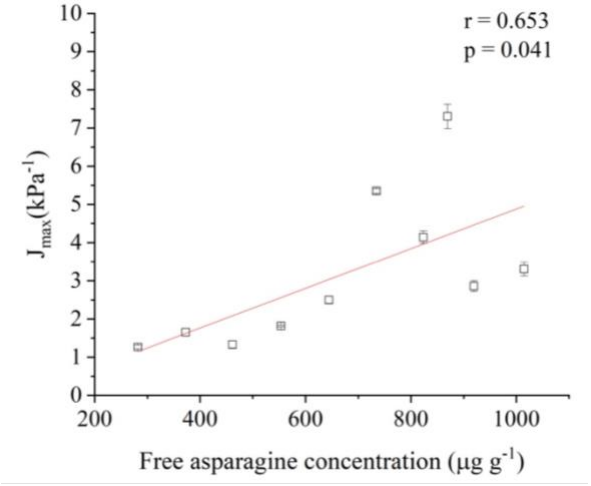
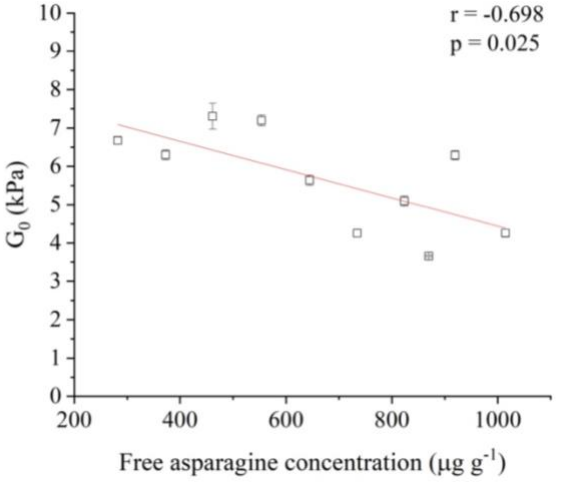
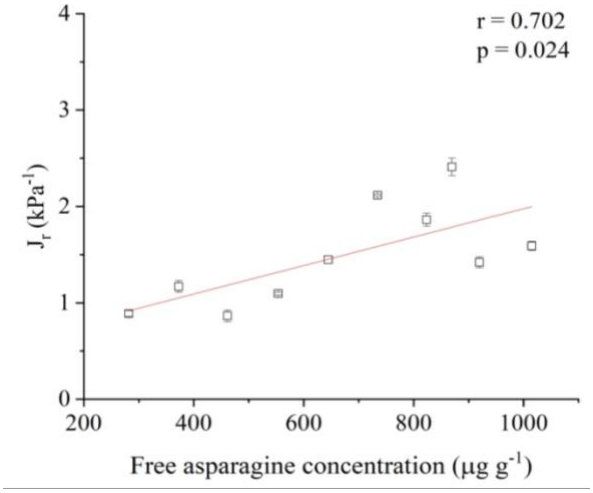
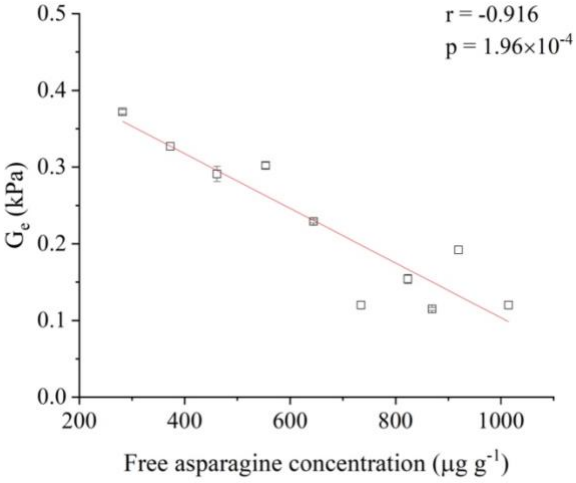


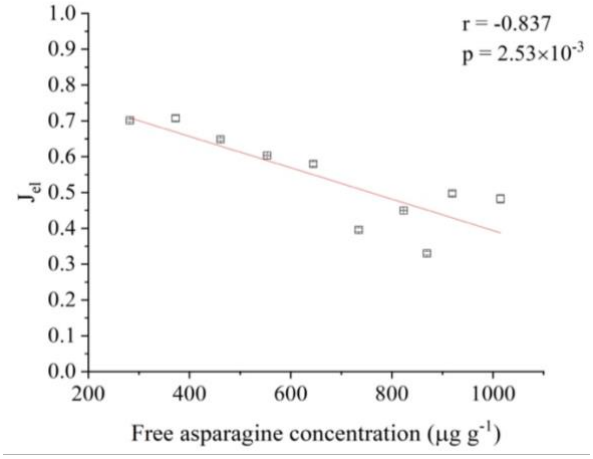
Figure 4.9. Correlation between creep-recovery parameters (J_{max}, J_r, and J_{el}) (left), stress relaxation parameters (G_0 and G_e) (right), against free asparagine concentration (μg g^{-1}) and Pearson's correlation coefficient (r) analysis. Data points represent means ± SE (n = 3).











parameter that translates how solid-like is the dough. As per Figure 4.9, as free asparagine concentration in wheat increases, G_e decreases.

Pearson correlation coefficients were also calculated for G_0 and G_e against protein content and *FAB*. Both G_0 and G_e were negatively ($p < 0.05$) correlated to protein content ($r = -0.861$ and $r = -0.758$, respectively) and FAB ($r = -0.726$ and $r = -0.768$, respectively). This result indicates that samples with higher protein and water content presented lower relaxation moduli, at the beginning and at the end of the stress relaxation test, thus showing less solid-like characteristics. This increased fluidity of the doughs could also be explained by the higher Gli/Glu ratios in these samples, as previously elucidated.

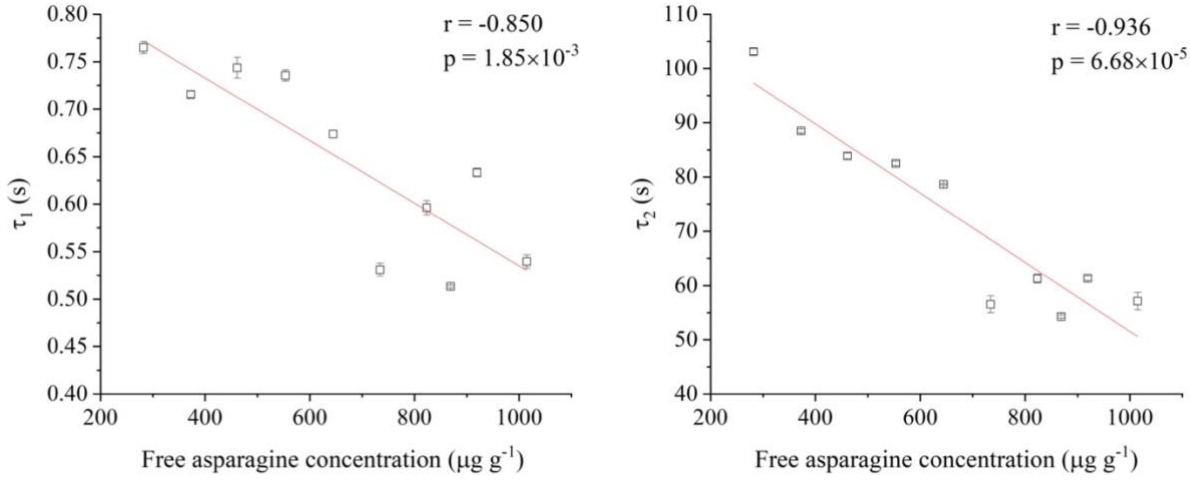
Additionally, the relaxation time (τ), calculated when a Maxwell equation (Eq. 4.5) was fitted to the data for all replicates, with a goodness of fit (R^2) of 0.9999, was also shown to be negatively correlated to free asparagine concentration (Figure 4.10). From Figure 4.8, the relaxation time is the time necessary for $G(t)$ to achieve 36.8% of its Maxwell element's initial value (Ge^{-1}). Dough samples that show longer relaxation times have been characterized as being stronger when compared to samples with shorter relaxation times (W. Li et al., 2003; Rao et al., 2001). Figure 4.10 shows both relaxation times plotted against free asparagine concentration, with the relaxation time for the second Maxwell element (τ_2) exhibiting stronger correlation with free asparagine concentration compared to the first element.

Therefore, the result for the relationship between free asparagine concentration, τ_1 and τ_2 confirms that samples with higher free asparagine concentration levels resulted in doughs with less solid-like characteristics. Correlation studies also showed a negative ($p < 0.05$) relationship between τ_1 against protein content ($r = -0.819$) and *FAB* ($r = -0.795$). For the second Maxwell element, a slightly weaker correlation ($p < 0.05$) was found between τ_2 against protein content ($r = -0.715$) and *FAB* ($r = -0.741$). These results support the previous explanation that samples with higher free asparagine concentration and protein content could possess a higher Gli/Glu ratio. This higher proportion of gliadins could contribute to a more fluid-like dough structure, with faster relaxation times, as opposed to samples with lower free asparagine concentration and protein content.

To our knowledge, no literature was published reporting different relaxation times from Maxwell fit to study dough rheological properties. However, the different relaxation times from both elements could indicate that τ_1 represents the relaxation time of gliadins whereas τ_2

represents the glutenin macropolymer, since it takes a longer time to relax compared to the gliadins. This correlation between Maxwell elements and gluten protein fractions supports the previous speculation that samples with a higher protein content have a higher Gli/Glu ratio, since their correlations were stronger. Godfrey et al. (2010) reported a positive correlation between FAB and Gli/Glu ratio ($r = 0.864$, $p < 0.001$) when studying wheat proteins and dough rheological properties affected by different nitrogen fertilization treatments. Although no Gli/Glu ratio was calculated, Malalgoda et al. (2018) evaluated wheat protein fractions for correlation with dough rheological properties and found that the fraction containing mostly ω-gliadins showed the highest correlation with *FAB* ($r = 0.688$, $p<0.001$). Therefore, the stronger correlation between τ_1 and *FAB* also supports the perspective that the first Maxwell element is a representation of the gliadins fraction.

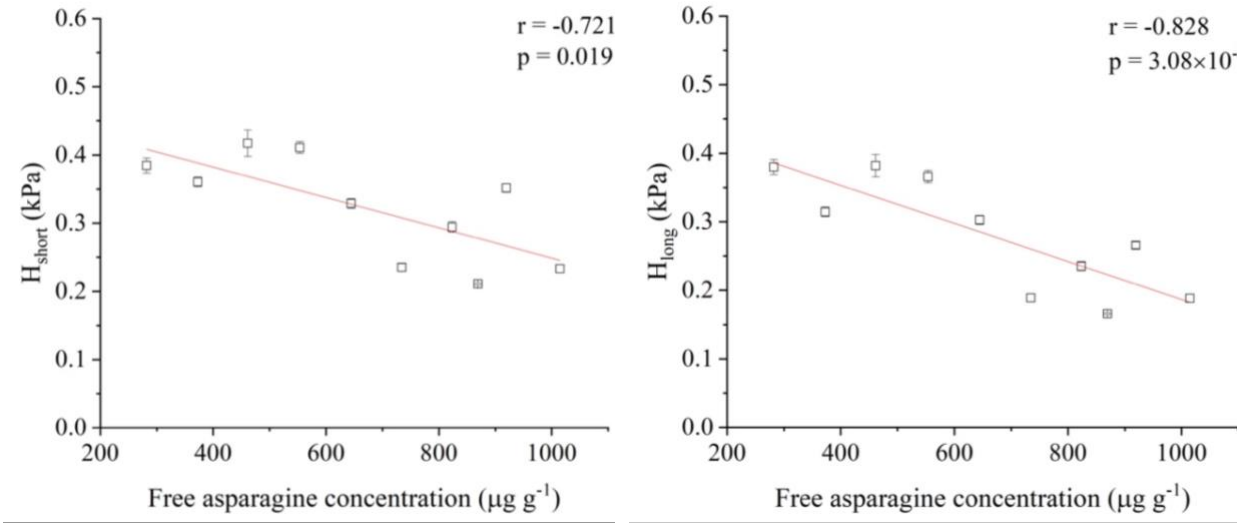
Figure 4.10. Relaxation time τ_1 and τ_2 (seconds) plotted against free asparagine concentration (μg g^{-1}). The Pearson's correlation coefficients (r) are also shown in the graphs. Data points represent means ± SE (n = 3).



From stress relaxation tests, one can also obtain the relaxation spectrum ($H(\tau)$), which represents relaxation processes and moduli densities over time (Van Vliet, 2014). Li et al. (2003) performed stress relaxation tests on wheat flour doughs, glutens, and separated gluten proteins, i.e., gliadins and soluble glutenins, made from two wheat varieties, Hereward, suitable for breadmaking, and Riband, suitable for cookie making. These authors also evaluated the relaxation

spectrum for the studied samples. For wheat flour doughs and glutens, the authors observed two relaxation processes occurring, one at short times (<10 seconds) and another at longer relaxation times (10 to 1000 seconds) (W. Li et al., 2003), which was also observed by Rao et al. (2000, 2001). According to W. Li et al. (2003) and Rao et al. (2000), the first relaxation process is mainly related to gliadins and small molecular weight glutenins, while the second process indicates the presence of a network structure, with entanglements and physical cross-links. When the relaxation spectrum was evaluated for gluten fractions, i.e., gliadin and soluble glutenin, the second relaxation process was not present, indicating the lack of a protein network in these fractions (W. Li et al., 2003). In Figure 4.8, two relaxation processes were observed when $H(\tau)$ was plotted against time, agreeing with the results from Li et al. (2003). Moreover, the resulting two dominant relaxation processes in Figure 4.8 are in agreement with the previous interpretation regarding the relaxation times of the two Maxwell elements in Figure 4.10. Relaxation time τ_1 is in agreement with the first relaxation process occurring at $t < 10$ s while values for τ_2 are in agreement with the second relaxation process in Figure 4.8, occurring after 10 seconds of the test. Figure 4.11 shows $H(\tau)$ chosen at two different times, 5 seconds (H_{short}) and 300 seconds (H_{long}), plotted against free asparagine concentration.

Figure 4.11. Correlation between relaxation spectra at 5 seconds (H_{short}) and at 300 seconds (H_{long}) against free asparagine concentration (μg g^{-1}). The Pearson's correlation coefficients (r) are also shown in the graphs. Data points represent means ± SE (n = 3).



A significant negative correlation was found between the relaxation spectra at short and long relaxation times and free asparagine concentration. As per Li et al. (2003), gliadin fractions were responsible for positively affecting the first relaxation process and negatively affecting the second one, with doughs prepared from Hereward showing overall higher $H(\tau)$ than Riband. In the present study, H_{short} was also negatively correlated to protein content (r = -0.841, $p < 0.05$) and *FAB* (r = - 0.736, $p < 0.05$) and H_{long} was negatively correlated to protein content (r = -0.847, $p < 0.05$) and *FAB* (r = -0.745, $p < 0.05$). Therefore, the negative correlations found in the present study could be an indication that samples with higher free asparagine concentration had weaker protein networks due to possible higher gliadin concentrations in these samples. However, this is a speculation, discussed throughout this chapter, that should be evaluated with caution, since a negative correlation was also found for H_{short}. A plausible explanation for the lack of positive correlation between H_{short}, free asparagine concentration and protein content could be due to the fact that all wheat varieties involved in this study are suitable for breadmaking. Li et al. (2003), on the other hand, compared results from wheat varieties with an enormous difference in rheological properties, showing clear effects from the gliadins on gluten strength.

Overall, within the LVR, although not significant for G^*, samples with higher free asparagine concentration and protein content showed a more solid-like tendency as frequency increased, possibly due to effects of short-range interactions, e.g., hydrogen bonds, on dough rheological properties. On the other hand, for static measurements performed outside of the LVR, strong and negative correlations were found between J_{el}, G_0, G_e and τ_{SR} against free asparagine concentration, protein content and FAB. Therefore, samples with higher free asparagine concentration and protein content resulted in more fluid-like doughs, i.e., weaker, when compared to samples with lower free asparagine concentration. In summary, samples containing higher free asparagine concentrations had greater deformations under creep and less recovery after removing the shear stress. When under constant deformation, these samples showed lower relaxation moduli and faster relaxation processes, indicating a weaker gluten network when compared to samples with lower free asparagine concentration.

4.4.3 Field data and bread specific loaf volume

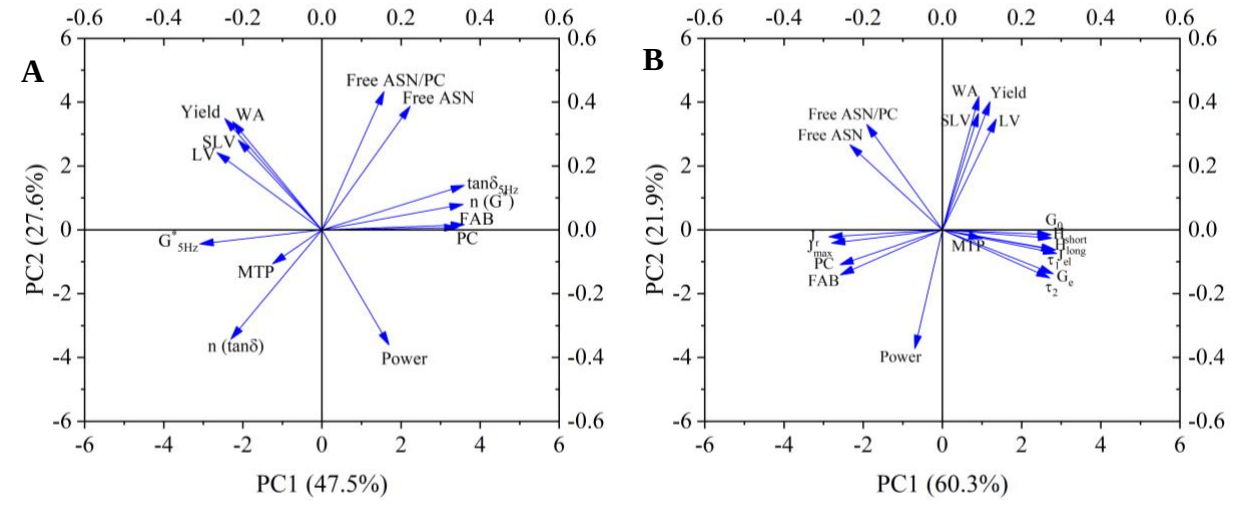
The investigation of the relationship between free asparagine concentration and dough rheological parameters aims to identify if strategies to reduce free asparagine in wheat will negatively affect gluten strength. An adequate gluten strength is necessary to hold the gas produced during fermentation and, at the same time, to allow bubble expansion, thus producing bread with satisfactory loaf volume (Delcour & Hoseney, 2010a). Principal component analysis (PCA) was performed, and Figure 4.12 shows the biplots for rheological parameters, evaluated in white wheat flours, whole-wheat bread specific loaf volume ($cm^3\ g^{-1}$) and loaf volume (cm^3), wheat grain protein content (%), grain yield ($kg\ ha^{-1}$), free asparagine concentration ($\mu g\ g^{-1}$), the proportion of free asparagine to total protein content ($mg\ g^{-1}$), white flour farinograph water absorption (*FAB*, 14% m.b.), and the bread preparation parameters from whole-wheat flours, i.e., water absorption (*WA*, %), mixing time to peak (*MTP*, min), and peak power (*Power*, W).

In Figure 4.12.A, the first two components accounted for about 75% of the total variation in the evaluated parameters, while Figure 4.12.B shows the first two components accounting for 84% of the variation in the data. Overall, when evaluating rheological parameters from frequency sweep tests at small strain (LVR), at a frequency of 5 Hz, G^* showed negative correlation, along PC1, to protein content, FAB, power-law exponent *n* for G^* and $\tan\delta$ at 5Hz. Pearson correlation analysis showed that these negative correlations were all significant at $p < 0.05$. Additionally, no significant correlations were found between G^*, at 5 Hz, and whole-wheat bread quality (*SLV* and

LV) and bread dough preparation parameters (*WA*, *MTP*, and *Power*). While $\tan\delta$, at 5 Hz, which is inversely correlated to dough solid-like properties, was negatively, but not significantly, correlated to *SLV*, *LV* and yield, and positively ($p < 0.05$) correlated to *FAB* and protein content. Moreover, correlation analysis for $\tan\delta$ showed significant relationships ($p<0.05$) against free asparagine, Free ASN/PC, and the power-law exponent *n* for G^*. No significant correlations were found between $\tan\delta$ and bread dough preparation parameters. Finally, the power-law exponent (*n*) for G^* was positively ($p < 0.05$) correlated to protein content and *FAB*, and negatively, but not significantly, correlated to yield, *SLV*, *LV*, *MTP*, and *WA*. Correlation analysis showed statistically significant ($p < 0.05$) and negative relationship between *n* ($\tan\delta$) against free asparagine concentration and Free ASN/PC.

When closely evaluating the correlations in Figure 4.12.B, one can notice that *SLV*, *LV* and yield are on the opposite side (along PC1 and PC2) of J_{max} and J_r, while these two rheological parameters are positively correlated to grain protein content and FAB. Both J_{max} and J_r are inversely proportional to dough solid-like characteristics and, although negatively correlated to *SLV*, *LV* and yield, these correlations were not significant at $p < 0.05$. On the other hand, the correlations between J_{max} and J_r to protein content and *FAB* were strong ($r > 0.8$) and significant at $p < 0.05$. The opposite outcome can be observed for J_{el}, since it is a measure of dough solid-like characteristics. This parameter was negatively correlated to protein content and *FAB* ($p < 0.05$) and positively, but not significantly, correlated to *SLV*, *LV* and yield. From stress relaxation measurements, Figure 4.12.B, all parameters were negatively correlated to protein content and *FAB* ($r > 0.7$, $p < 0.05$), and weakly positively ($p > 0.05$) correlated to *SLV* and yield. Moreover, Figure 4.12 displays the correlation between free asparagine concentration and rheological parameters that was discussed in detail throughout section 4.4.2.

Figure 4.12. PCA biplots for linear oscillatory frequency sweep (A) and static shear tests (B), specific loaf volume (*SLV*, $cm^3\ g^{-1}$), loaf volume (cm^3), grain protein (PC, %), grain yield (Yield, $kg\ ha^{-1}$), water absorption (*FAB*, %), free asparagine concentration (Free ASN, $\mu g\ g^{-1}$), proportion of free asparagine concentration to total protein content (Free ASN/PC), whole-wheat *WA* (%), *MTP* (min) and *Power* (W).



To our knowledge, there are very few studies in the literature that report correlations between shear rheological tests and final loaf volume. When performing frequency sweeps to evaluate rheological properties of dough, made at optimal *FAB*, from 17 European wheat varieties, Van Bockstaele et al. (2008b) reported strong negative correlations between G^* to protein content and to water absorption, and a positive correlation between $\tan\delta$ to protein content and to water absorption. Interestingly, these authors also reported a strong positive and negative correlation, respectively, for $\tan\delta$ and G^* to bread volume (Van Bockstaele et al., 2008b). Moreover, under creep-recovery measurements, the maximum creep and recovery strains (related to J_{max} and J_r) were positively correlated to protein content, water absorption and bread volume, while the percent of recovery (related to J_{el}), was negatively correlated to these parameters (Van Bockstaele et al., 2008a, 2008b). Therefore, the authors observed an overall positive effect of dough fluid-like characteristics on bread volume, indicating that doughs that possessed more solid-like properties yielded lower bread volumes. This negative correlation between G^* and bread volume reported by Van Bockstaele et al. (2008a) might be explained by the high values of G^* obtained by the authors. Data reported by Van Bockstaele et al. (2008a) showed that bread volume only started to increase

when G^* decreased to values lower than 15 kPa. According to Figure 4.7, the majority of G^* values in the present study were lower than 15 kPa. This outcome could be an indication that lack of correlation between G^* and SLV in this study may have occurred due to the fact that this study only involved breadmaking wheat varieties. Van Bockstaele et al. (2008a and 2008b) evaluated a group of 17 wheat varieties that included four baking wheat classes, based on German classification (elite, good quality, lower quality and feed wheat), which was clearly shown by the wide variation in bread volume reported by the authors. Moreover, significant correlations between rheological parameters, protein content and water absorption from Van Bockstaele et al. (2008a and 2008b) might indicate that higher amounts of gliadins are present in doughs with higher protein content, leading to an increased water absorption. The explanation for this hypothesis lies in the study from Godfrey et al. (2010), who observed a positive correlation between *FAB* and Gli/Glu, and from Malalgoda et al. (2018), who observed a positive correlation between sulfur-poor gliadins and FAB. However, for the present study, the possible increase in the Gli/Glu ratio at high protein and free asparagine levels, as speculated throughout this chapter, was not enough to cause detrimental effects to final bread quality. As mentioned before, the lack of significant correlations between *SLV*, dough rheological parameters and wheat properties (free asparagine concentration, protein content, *FAB*, free ASN/PC, and yield) could be explained by narrow variation in the *SLV* and LV values among the samples. For the initial population of 128 samples, the *SLV* varied from 4.3 to 5.6 $cm^3\ g^{-1}$ and *LV* from 597 to 766 cm^3 and, for the 10 selected samples in the present study, there was a slightly tighter range for the *SLV* and *LV* values, with *SLV* varying from 4.6 to 5.5 $cm^3\ g^{-1}$ and *LV* varying from 624 to 745 cm^3.

Even though *SLV* did not have a significant correlation with *FAB*, it was strongly ($p < 0.05$) correlated to *WA* of whole-wheat flours ($r = 0.917$). Moreover, when evaluating the bread *LV*, the values were negatively ($p < 0.05$) correlated to *FAB* ($r = -0.701$) and positively ($p < 0.05$) correlated to *WA* ($r = 0.893$) of whole-wheat flours. The correlation between *LV* and *FAB* could also support the perspective that samples with high *FAB* have a higher Gli/Glu ratio, as per results from Godfrey et al. (2010) and Malalgoda et al. (2018), i.e., samples possessing higher *FAB* having lower gluten strength, thus leading to lower *LV* values. The positive correlation between *SLV*, *LV* and *WA* might be explained by the bran particle size of the whole-wheat flours utilized in this study. Noort et al. (2010) evaluated the effect of bran particle size on dough rheological properties and bread volume. They observed that bran of median particle sizes around 1000 μm had a much higher water binding

capacity and resulted in higher bread volumes when compared to small bran particle sizes, e.g., 100 μm (Noort et al., 2010). The authors proposed two main explanations for this phenomenon, which are the increased interaction between bran particles of small particle size and gluten proteins due to increased surface area, and the liberation of cell components, due to cell rupture, that might negatively affect the gluten proteins (Noort et al., 2010).

Although no correlation studies were performed, results from stress relaxation measurements reported by Li et al. (2003) showed that wheat flour dough made from the Hereward wheat variety showed higher τ_{SR}, $G(t)$, and $H(\tau)$ as well as larger bread volume when compared to dough and bread made from the Riband wheat variety. This outcome shows that gluten strength parameters from stress relaxation are correlated to good breadmaking performance. The results from Li et al. (2003), involving two different wheat varieties in relation to baking performance, could also be an indication that no significant correlation was found between parameters from stress relaxation and SLV, because wheat varieties involved in this study were all suitable for breadmaking.

4.5 Conclusion

Efforts to reduce free asparagine concentration in wheat must take into account possible negative effects on gluten strength, which is essential for final bread volume and crumb appearance. Shear dynamic measurements showed that non-yeasted dough rheological properties within the LVR had small, but significant variations over time. Upon yeast addition, rheological properties showed clear and significant changes over time, with dough becoming softer and more fluid-like. Yet, no significant correlation was found between changes in dough rheological properties and free asparagine concentration during the fermentation period. On the other hand, protein content was a significant factor during fermentation, with samples with higher protein content having a lower decay rate in the rheological properties of their doughs.

The relationships between free asparagine concentration and dough rheological properties were less pronounced within the LVR when tested using dynamic shear tests. When samples were subjected to large strain and stress, significant correlation was found between free asparagine concentration and dough rheological parameters. Overall, samples with higher free asparagine concentration had more fluid-like doughs, thus weaker glutens. Most of the rheological parameters that were inversely correlated to gluten strength showed positive correlation with protein content and FAB, leading to the explanation that higher protein content samples could contain higher

gliadin concentrations. Finally, the fact that all wheat varieties involved in this study were suitable for breadmaking could be a possible explanation for the lack of correlation between rheological parameters, SLV and LV. In summary, shear rheological tests, especially shear static tests at large strain and stress, provided good insights into the relationship between dough rheological properties and free asparagine concentration in wheat. These tests were capable of discriminating wheat samples according to their free asparagine concentration, as shown by the significant correlations with their rheological data, such as compliance (J_{max}, J_r), relaxation moduli (G_0, G_e) and relaxation time (τ_1 and τ_2).

4.6 Connection between Chapters 4 and 5

The understanding of how dough rheological properties change during fermentation and how these changes are correlated to wheat free asparagine concentration were studied in Chapter 4 through linear oscillatory time sweep tests. When changes in non-yeasted dough were studied through linear fitting of G^*, G', and $\tan\delta$, small slopes were observed over time. However, when dough rheological parameters were studied during fermentation, a more pronounced decay in the parameters was observed, indicating dough softening effects. Correlation studies in Chapter 4 showed that changes in dough rheological parameters during fermentation were not correlated, at $p < 0.05$, to free asparagine concentration but were ($p < 0.05$) to wheat protein content and flour water absorption (*FAB*). Linear oscillatory time sweep tests have a great advantage of allowing one to evaluate yeasted and non-yeasted dough rheological properties at small strains and over a range of frequencies. In this study (Chapter 4), dough rheological properties were evaluated at a constant frequency of 10 Hz. In order to further understand changes occurring in dough structure before and after adding yeast, low-intensity ultrasound was performed in Chapter 5, evaluating dough properties at a much higher frequency (10 MHz) than in shear rheometry tests. Low-intensity ultrasound (Chapter 5) is a non-destructive test capable of interrogating the properties of dough samples, thus providing a deeper understanding of the correlation between dough rheological properties against free asparagine concentration, protein content, and *FAB*.

5 Low intensity ultrasound: a novel method to study the relationship between dough rheological properties and free asparagine concentration

5.1 Abstract

Acrylamide presence in bread is a current food safety issue that can be addressed by reducing wheat free asparagine concentration. However, it is crucial to understand if reducing wheat free asparagine concentration will cause detrimental effects on dough rheological properties and the final bread quality. Various methods can be successfully employed to evaluate dough rheological properties, such as empirical and shear rheometry tests. Low-intensity ultrasound (LIU) has been shown to be a promising low-cost alternative for evaluating dough properties. The present study aimed to apply LIU tests to study the relationship between free asparagine concentration in wheat and dough rheological properties, comparing with data from shear tests. Thus, a set of 10 samples, varying in wheat free asparagine concentration, was selected. LIU tests were conducted on non-yeasted and yeasted dough samples and, after selecting the best frequency, i.e., minimal wave attenuation, correlation studies were performed to investigate relationships with free asparagine concentration. Evaluating the attenuation coefficient decay over time of non-yeasted doughs, it was possible to obtain similar correlations between relaxation times and free asparagine concentration from both LIU and shear tests. After the relaxation period, it was observed that the slopes for the elongational loss tangent from LIU showed better correlations to free asparagine concentration compared to the slopes for the magnitude of the complex longitudinal moduli from LIU and the slopes for the loss tangent from shear rheometry. When evaluating dough properties during fermentation, it was observed that both LIU and shear tests showed similar correlations to free asparagine concentration, with the slope for the attenuation coefficient during fermentation showing the strongest correlations. Finally, loaf volume and specific loaf volume were only correlated ($p < 0.05$) to relaxation times obtained from the attenuation coefficient decay. Samples with longer relaxation times resulted in higher loaf volumes, thus indicating that samples with lower free asparagine concentration resulted in good bread quality. Overall, LIU was capable of providing insightful information on dough rheology and show that agronomic strategies to reduce free asparagine concentration in wheat did not negatively affect the final product quality.

5.2 Introduction

Bread is one of the most important foods for humankind, playing an important role in human history (Campbell, 2008), with evidences of its existence since about 2600 B.C.E, when unleavened breads were being made in the Stone Age by cooking a mixture of ground wheat grains with water in hot stones (Wrigley, 2009). Because bread is a staple food, worldwide bread consumption is very high, with an average consumption expected to attain up to 28.5 kg per person in 2021 (Statista, 2022). From a European perspective, bread is evaluated to be attractive and of good quality by some visual aspects, such as loaf volume (Sahi & Little, 2006). Good bread loaf volume, as well as other visual and textural characteristics, is obtained due to the unique ability of wheat to form a viscoelastic dough when wheat flour is mixed with optimum amounts of water (Wrigley et al., 2006). When optimally developed, wheat flour dough is a composite material that contains a dough matrix phase and a gas phase (Scanlon & Page, 2015; Scanlon & Zghal, 2001).

The singular characteristic of wheat dough viscoelasticity is possible due to gluten proteins, which deliver to the dough the ability to expand and to hold the gas produced during fermentation inside the bubbles, thus resulting in a light and aerated loaf of bread (Delcour & Hoseney, 2010a). The fraction of gluten proteins responsible for conferring extensibility to the dough are the gliadins, and the proteins that are responsible for elasticity of the dough, i.e., gluten strength, are the glutenins (Delcour & Hoseney, 2010a). A proper balance between gliadins and glutenins, i.e., adequate Gli/Glu ratio, will result in a bread with high volume, whereas excessive gliadins or glutenins may negatively affect the final bread quality (Delcour & Hoseney, 2010a). Therefore, changes in wheat protein content and composition can affect the dough physical properties and be detrimental to final product quality.

The application of dough rheological measurements, through standardized tests, allows the food industry, as well as scientists, to study dough physical properties (Campbell & Martin, 2012). Dough rheological measurements can be classified as empirical (or descriptive) tests and fundamental tests (Dobraszczyk & Morgenstern, 2003). Empirical tests, such as farinograph and extensograph, are traditional methods, widely employed in the food industry, that provide information on dough properties during mixing (farinograph) and under uniaxial extension (extensograph) (Dobraszczyk & Morgenstern, 2003). The main advantage of empirical tests lies in the low difficulty in carrying them out, which facilitates their application for quality control (Dobraszczyk & Morgenstern, 2003). Conversely, empirical tests that measure dough properties

during mixing, such as farinograph and mixograph, do not allow one to measure the stress imposed on the dough samples as a function of time. This is because, during mixing, only some parts of the dough are in contact with the pins at a certain time, which makes it challenging to define or control the stress during the test (Delcour & Hoseney, 2010b). When a deeper understanding of dough rheological properties is necessary, for example, for measurements under constant stress and to be reported in well-known physical units, shear fundamental tests can be employed (Dobraszczyk & Morgenstern, 2003). Shear fundamental tests are carried out in rheometers, thus allowing the researcher to apply a known and controlled shear stress or strain and obtain the resulting shear strain or stress in well-defined physical units (Menjivar, 1990). Therefore, fundamental tests of this type are commonly used to study the relationship between dough structure and dough properties as well as for processes and engineering calculations (Menjivar, 1990).

Fundamental shear tests can be employed using shear strain or stresses with similar magnitude to those involved in the breadmaking process (Dobraszczyk & Morgenstern, 2003). Examples of these tests are creep-recovery and stress relaxation, which require the application of a constant shear stress (creep-recovery) or strain (stress relaxation) over time (Barnes et al., 1989b; Van Vliet, 2014). However, one of the main advantages of fundamental shear tests is the capability of measuring dough physical properties under very small shear strains (< 1%), i.e., within the linear viscoelastic region (LVR) of the dough (Uthayakumaran, Newberry, et al., 2002). Linear shear oscillatory tests are employed to characterize a viscoelastic material at small strains at a constant frequency over a period of time, i.e., time sweep, or over a range of frequencies, i.e., frequency sweep (Van Vliet, 2014). The main parameters obtained from these tests are the shear storage modulus (G'), which characterizes the energy stored during an oscillation of applied shear strain or stress, and the loss modulus (G''), which defines the energy loss during an oscillation (Van Vliet, 2014). Therefore, G' and G'' translate the dough elastic-like and fluid-like properties, respectively. Additionally, from both of these parameters, other rheological parameters can be calculated, such as the magnitude of the complex shear moduli G^* and the loss tangent ($\tan\delta$) (Barnes et al., 1989b; Van Vliet, 2014). Linear oscillatory shear tests can be successfully employed to measure non-yeasted dough rheological properties (Jekle & Becker, 2011; W. Li et al., 2003; Sun et al., 2020; Yovchev et al., 2017) as well as to investigate dough properties during fermentation (Meerts et al., 2018). However, a main drawback of using shear fundamental tests using rheometers is the narrow frequency range that most rheometers are capable of, usually

allowing one to perform oscillatory tests within a range of 10^{-2} to 10^{2} Hz (Barnes, 2000). An alternative test that allows one to evaluate dough physical properties at low strains and at higher frequency ranges is low-intensity ultrasound (Leroy et al., 2010; Scanlon & Page, 2015).

Low-intensity ultrasound is a technique that has been employed for dough rheological characterization over the last two to three decades (Scanlon & Page, 2015), and has the advantages of requiring less money expenditure and less experimental time than other rheological tests (Khorshidi, Storsley, et al., 2018). Low-intensity ultrasound consists of pressure or vibrational waves that are characterized by having an acoustic intensity ranging from 10^{-14} to 10^{3} W m^{-2} and frequencies ranging from 20 kHz up to 10 GHz (Raj et al., 2004b). Moreover, low-intensity ultrasound is a non-destructive test (Povey, 1997), thus allowing the dough rheological characterization to be performed within the linear viscoelastic region of the dough. Low-intensity ultrasound tests can be carried out by propagating shear or longitudinal waves (Scanlon & Page, 2015). However, because wheat flour dough is composed of a dough matrix phase and a gas phase (Scanlon & Page, 2015), longitudinal waves are better suitable for dough analysis, since shear waves can only be effectively propagated through solids (Raj et al., 2004b).

When low-intensity ultrasound waves propagate through a material, two main parameters are obtained, the phase velocity (v_p) and the attenuation coefficient (α) (Scanlon & Page, 2015). The phase velocity indicates how fast the low-intensity ultrasound waves travel through the dough and the attenuation coefficient defines the decrease in the intensity of the wave with distance due to loss of energy (Raj et al., 2004b). The velocity that waves travel in a low-attenuating material depends on the material's properties, such as density and elastic modulus. For example, low-attenuating materials (homogeneous materials such as oil and water) that are less dense or have a higher resistance to deformation will allow the wave to propagate faster than a dense and less resistive low-attenuating material (McClements & Gunasekaran, 1997). For wave attenuation, the energy loss can happen due to wave scattering and diffraction, which are correlated to the macroscopic structure of the material, or absorption, which is correlated to the microscopic structure of the medium (Raj et al., 2004b). For wheat flour doughs, the air bubbles entrapped during mixing are considered to be a scattering agent depending on the wavelength and the dimensions of the bubble (Koksel et al., 2016; McClements & Gunasekaran, 1997). Therefore, various research studies have been conducted utilizing phase velocity (v_p) and the attenuation coefficient (α) obtained from low-intensity ultrasound to study dough rheological properties

(Elmehdi et al., 2004; Koksel et al., 2016; Peressini et al., 2016; Scanlon, Page, Leroy, Fan, et al., 2011; Scanlon & Page, 2015). Moreover, v_p and α have also been used to calculate the longitudinal storage (M') and loss (M'') moduli in order to provide further information on dough's rheological properties (Elmehdi, 2001; Khorshidi, 2016; Khorshidi et al., 2019; Khorshidi, Thandapilly, et al., 2018).

Peressini et al. (2016), for example, utilized ultrasound tests to evaluate dough properties when prepared from two wheat varieties and using six different treatments. The authors utilized a transducer with central frequency of 3.5 MHz to obtain v_p and α over a range of frequencies. Moreover, the authors observed that depending on the treatment applied for the dough preparation, i.e., variation in the amount of ingredients or mixing time, both v_p and α varied depending on the analyzed frequency (Peressini et al., 2016). Finally, the authors concluded that attenuation coefficients obtained at lower frequencies (0.3 and 0.5 MHz) were the most appropriate to correlate with other dough rheological properties and breadmaking quality (Peressini et al., 2016). More recently, Khorshidi et al. (2019) applied low-intensity ultrasound for discrimination of wheat varieties after conducting a preliminary study to select the best frequency among five frequencies, from 0.5 to 10 MHz (Khorshidi, Thandapilly, et al., 2018). After selecting the frequency of 10 MHz (Khorshidi, Thandapilly, et al., 2018), Khorshidi et al. (2019) studied rheological properties of doughs prepared from 23 Canadian Hard Red Spring (HRS) wheat varieties. When evaluating all the wheat varieties together, the authors did not find significant correlations between empirical rheology and low-intensity ultrasound parameters, finding only a weak ($p<0.10$) positive correlation between v_p, M' and bread mixing time, which was used as an indicator of gluten strength (Khorshidi et al., 2019). The authors attributed the lack of significance between rheological parameters to small differences in quality among the studied varieties. However, when two groups were created to widen the wheat characteristics, the correlations between empirical and low-intensity ultrasound parameters were improved (Khorshidi et al., 2019) showing capability of low-intensity ultrasound for dough rheology evaluation. Finally, although fewer studies have been published in the literature for yeasted doughs compared to non-yeasted doughs, low-intensity ultrasound has also been utilized to study dough rheological properties during fermentation (Elmehdi et al., 2003; Skaf et al., 2009). Therefore, low-intensity ultrasound has proven to be a promising alternative for dough rheology characterization, which could provide data to evaluate the breadmaking quality potential of a wheat variety.

A novel application of low-intensity ultrasound is the investigation of how dough properties are affected when targeting the reduction of free asparagine accumulation in wheat grains, a strategy to mitigate acrylamide formation in wheat-based products. Acrylamide, firstly identified in carbohydrate-rich foods by a Swedish research group (Tareke et al., 2002), is a probable carcinogen to humans (IARC, 1994). Free asparagine, naturally present in wheat, has been shown to be the main precursor to acrylamide formation in bread (Curtis et al., 2016; Mottram et al., 2002). Therefore, efforts to reduce free asparagine concentration in wheat are of outmost importance to abide with food safety aspects when producing bread (Maan et al., 2020). However, current global efforts to reduce acrylamide forming potential of wheat through the reduction of free asparagine concentration might be a threat to dough properties and bread quality. Environment (Navrotskyi et al., 2018), wheat genotype (Ohm et al., 2017), and fertilization treatments with nitrogen and sulfur (Claus et al., 2006; Lea et al., 2007) are the factors that affect free asparagine in wheat, but they also affect wheat protein content and composition. Therefore, aiming for wheat with low free asparagine concentration could affect wheat flour dough rheological properties. Thus, it is of utmost importance to understand the relationship between free asparagine concentration and dough rheological properties in order to understand if producing wheat with low free asparagine will be detrimental to dough properties.

This study aims to apply low-intensity ultrasound tests to evaluate dough rheological properties of yeasted and non-yeasted doughs prepared from samples with a wide range of free asparagine concentrations. In order to investigate the effectiveness of low-intensity ultrasound in evaluating the relationship between dough properties and free asparagine concentration, results will be compared to data obtained from fundamental shear rheology tests.

5.3 Materials and methods

5.3.1 Sample selection

White wheat flours, at 75% extraction rate, were obtained at Cereals Canada by milling wheat grains using a Bühler laboratory flour mill (MLU-202) and a Bühler impact finisher (MLU-302). By-products of white wheat flour preparation, i.e., shorts and bran, were ground using a Jacobson 120-B hammer mill to pass through a 1,190 µm sieve and mixed with the white flour to prepare whole-wheat flour samples. For low-intensity ultrasound tests, white flours were utilized for dough preparation, whereas free asparagine quantification was performed on whole-wheat flours. The free asparagine quantification was performed by Xie et al. (2021) using 0.01M

hydrochloric acid solution to extract the free asparagine from whole-wheat flours. To derivatize and quantify the extracted free asparagine, an AccQ•Tag Ultra pre-column derivatization amino acid kit and a Waters H class UPLC was utilized as per Malunga et al. (2019).

The same selected samples utilized for shear rheological tests (Table 4.1), as described in Chapter 4, were used for low-intensity ultrasound tests (Table 5.1). In summary, a set of 10 white flour samples were selected from an initial population of 128 flour samples. Sample selection was done using the Kennard-Stone algorithm (Kennard & Stone, 1969) based on the free asparagine concentration of the whole-wheat flours. The Kennard-Stone algorithm sequentially selects a uniformly distributed sample set in relation to a factor *p*, according to Eq. 4.1, with the final sample set representing the initial population.

5.3.2 Wheat flour dough preparation

Low-intensity ultrasound measurements were performed on yeasted and non-yeasted wheat flour dough samples. In order to prepare optimally developed doughs for low-intensity ultrasound tests, measurements of dough development time were carried out. The dough development time, for all wheat flour dough samples, was determined utilizing a 10 g mixograph (Dynamic Machines Corp, Winnipeg, MB) mixing at 92 rpm and connected to a water bath to maintain a temperature of 30 ºC, following the AACC International Approved Method 54.40-02 (AACC International, 1999c). The dough development time was determined by the P2M Software (RAR Software Systems, Winnipeg, MB). Farinograph experiments had been previously performed at Cereals Canada in order to determine the flour water absorption used for dough preparation (FAB), as per the AACC International Approved Method 54.21-02 (AACC International, 2011). Non-yeasted dough samples were prepared in the 10 g mixograph through mixing white flour (weight adjusted for 14% m.b.) with distilled water, as determined in the farinograph, and 2% of sodium chloride (NaCl, total flour weight basis). Yeasted dough samples were prepared by adding 6.5% of fresh compressed yeast and 0.5% of sugar, both based on the total flour weight basis. The moisture content of the fresh compressed yeast was accounted as water added to adjust water content for the preparation of the dough samples.

5.3.3 Density measurements

Density measurements were performed using the deionized water displacement method per Diep et al. (2014), Khorshidi et al. (2019) and Koksel & Scanlon (2012), utilizing a 10 mL specific gravity bottle. A sample from the mixed and sheeted dough was separated by cutting with a metal

punch die and placed in a sealed container. After 30 minutes of relaxation, the dough density was measured. An accurately weighed dough piece of ≈0.5-1g was placed in the specific gravity bottle (Wilmad-LabGlass, Vineland, NJ, USA) with the deionized water at 30 ºC. The weight of the water displaced was calculated according to Eq. 5.1.

$$\rho = \frac{W_3 \times 0.9956}{W_2 + W_3 - W_4} \qquad \text{Eq. 5.1}$$

where 0.9956 g cm^{-3} is the water density at 30ºC, W_1 is the weight of empty bottle, W2 is the total weight of bottle filled with deionized water (W_1 + deionized water), W_3 is the weight of the dough piece (≈0.5-1 g), and W_4 is the total weight of bottle with the dough piece (W_2 + W_3). For each mixed dough, density measurements (g cm^{-3}) were made in five repetitions. The mean dough density results and the respective free asparagine concentration in the grain for each sample are shown in Table 5.1 below.

Table 5.1. Mean density values (mean ± SD) for the non-yeasted dough samples prepared from the 10 selected samples, coded from A to J, and their respective free asparagine concentration.

Sample code	Free asparagine concentration (μg g^{-1})	Density (g cm^{-3})
A	1014	1.191 ± 0.009
B	920	1.168 ± 0.009
C	869	1.188 ± 0.009
D	823	1.155 ± 0.010
E	735	1.183 ± 0.009
F	645	1.189 ± 0.009
G	554	1.167 ± 0.011
H	461	1.191 ± 0.012
I	373	1.210 ± 0.015
J	282	1.151 ± 0.008

5.3.4 Low-intensity ultrasound measurements

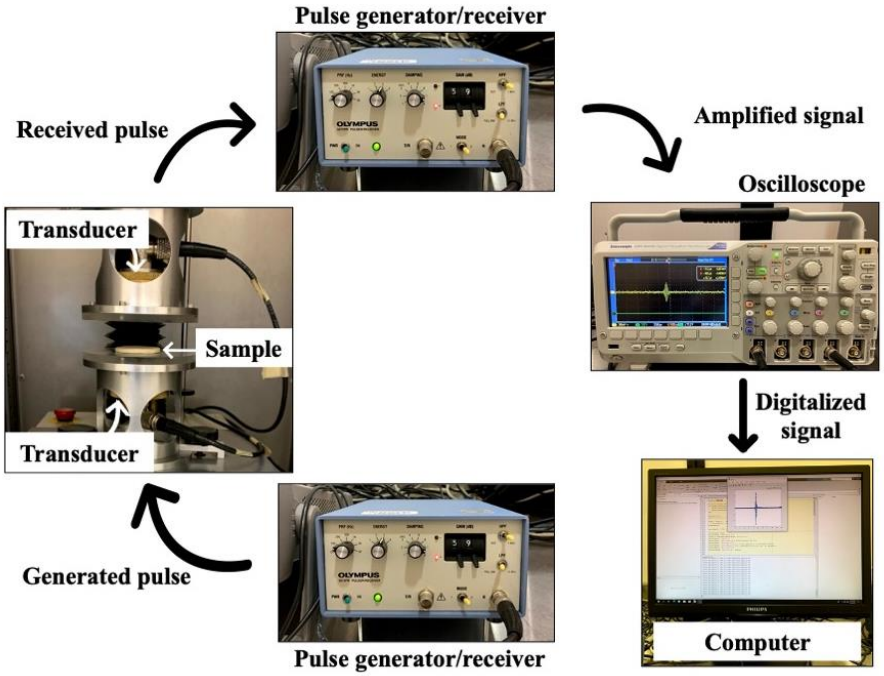
Low-intensity ultrasound experiments were performed in the Agriculture & Agri-Food Canada (AAFC) pilot plant at the University of Manitoba with the set-up illustrated in Figure 5.1. The experiments were conducted as per Khorshidi et al. (2018) using a setup composed of two

transducers (Olympus Canada Inc., Richmond Hill, ON, Canada) with central frequency of 10 MHz, a pulse generator/receiver (5072 PR, Olympus Canada Inc., Richmond Hill, ON, Canada) and an oscilloscope (DPO 2024B, Tektronix Canada Inc., Toronto, ON, Canada) (Figure 5.1). The pulse generator/receiver generates voltage pulses that are converted by the first transducer to acoustic pulses and amplifies the electromagnetic signals from the second transducer after the acoustic pulses have propagated through the dough. The oscilloscope digitizes the pulses received by the pulse generator/receiver and a computer connected to the oscilloscope records the digitized signal. The transducers were connected to a TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK), which was placed inside a temperature-controlled cabinet set at 30ºC. Moreover, two polystyrene disks (Rexolite® 1422, C-Lec Plastics Inc., Philadelphia, PA, USA) of 6.35 mm thickness were bonded to the transducers and attached to the TA-XT machine in order to act as delay plates. Before starting the measurements conducted on a piece of dough, reference signals were acquired by sending ultrasound signals through the delay plates lubricated with Ultrasonix® gel.

Before placing the samples between the transducers, the mixed dough was sheeted in a sheeter machine, continuously reducing the gap until a final gap of 1.3 mm. From the sheeted dough, a circular dough piece was cut using a punch die (diameter of 42 mm). In order to improve the contact between the dough sample and the polystyrene disks, the surface of both disks were lubricated with Ultrasonix® gel. After surface lubrication, the circular piece of dough was placed in the center of the disk and the top transducer was lowered via Exponent Software (Stable Micro Systems, Godalming, UK), which controls the TA-XT machine, until a final gap of 1.1 mm. For non-yeasted doughs, the bottom transducer emitted a sequence of acoustic pulses that propagated though the sample for 2400 seconds, which were received by the top transducer, amplified by the pulse generator/receiver, digitized by the oscilloscope and recorded by a data acquisition program written in MATLAB R2020b (MathWorks, Natick, MA, USA). For the yeasted doughs, the same procedure for signal propagation and acquisition was followed, except that the experimental time was 1200 seconds. The duration of low-intensity ultrasound experiments was set keeping in mind the duration set for shear rheological experiments described in sections 4.3.3.2 and 4.3.3.3.Linear shear oscillatory time sweep tests were conducted for 1200 seconds on yeasted doughs. For non-yeasted doughs, shear rheology was performed for 600 seconds starting after allowing the dough

to rest for 1800 seconds. Therefore, comparison of ultrasound and shear rheology was made only for the last 600 seconds of the ultrasound measurements.

Figure 5.1. Low-intensity ultrasound setup utilized to measure acoustic pulse transmission through yeasted and non-yeasted wheat flour dough samples.



After performing the experiments, the final digitized waveforms for reference and for yeasted and non-yeasted samples were analyzed using Igor Pro 6.3.4.1 (WaveMetrics, Inc., Lake Oswego, Oregon, USA) to determine the attenuation coefficient (α, mm^{-1}) and phase velocity (v_p, km s^{-1}). For non-yeasted doughs, Eq. 5.2 and Eq. 5.3 were utilized to calculate the longitudinal storage (M', GPa) and loss modulus (M'', GPa), respectively, as per Elmehdi et al. (2004), as a function of α and v_p. The magnitude of the complex longitudinal modulus (M^*, GPa) and $\tan\delta$ were also calculated, utilizing pulses acquired during the last 10 minutes of the experiment, using Eq. 5.4 and Eq. 5.5.

$$M' = \frac{\rho v_p\left(1-\alpha^2 {v_p}^2/4\omega^2\right)}{\left(1+\alpha^2 {v_p}^2/4\omega^2\right)^2} \qquad \text{Eq. 5.2}$$

$$M'' = \frac{2\rho {v_p}^3 \alpha / 2\omega}{\left(1 + \alpha^2 {v_p}^2 / 4\omega^2\right)^2} \quad \text{Eq. 5.3}$$

where ρ is the density of the material (g cm^{-3}) and ω (rad s^{-1}) is the angular frequency ($2\pi f$).

$$M^* = \sqrt{M'^2 + M''^2} \quad \text{Eq. 5.4}$$

$$\tan\ \delta = \frac{M''}{M'} \quad \text{Eq. 5.5}$$

5.3.5 Comparison with shear rheology measurements and fitting equations

Interpretation of low-intensity ultrasound results was done by comparing α and v_p from yeasted dough samples with results from linear oscillatory time sweep results. The time sweep tests on yeasted doughs were performed using an AR-2000 rheometer (TA Instruments, New Castle, DE, U.S.A.), set at 30 ºC, by applying a constant shear strain of 0.05% at a constant frequency of 10 Hz for 1200 seconds, as described in Section 4.3.3.2. For non-yeasted doughs, low-intensity ultrasound parameters, i.e., α, v_p, and calculated parameters, i.e., M^* and $\tan\delta$, were compared to results obtained from linear oscillatory time sweep tests. Time sweep tests were performed on non-yeasted dough using the AR-2000 rheometer (TA Instruments, New Castle, DE, U.S.A.), by applying a constant shear strain of 0.05% at a constant frequency of 10 Hz for 600 seconds, as described in Section 4.3.3.3. In addition, for non-yeasted doughs, the initial behavior of α after sample compression was compared to shear stress relaxation results. The results from shear stress relaxation tests were obtained using the same AR-2000 rheometer, by applying a constant shear strain of 5% and recording the resulting shear stress for 1800 seconds, as described in Section 4.3.3.3.

For comparison purposes, fitting of equations was performed as described in Section 4.3.3.3 and 4.3.3.4, for all replicates. For non-yeasted doughs, in order to compare the initial behavior of α after sample compression with shear stress relaxation tests, fitting of α was performed similarly as the fitting for stress relaxation using Eq. 4.5. Fitting of α over time (seconds) was performed using the Maxwell model with 2 elements as per Eq. 5.6.

$$\alpha(t) = \alpha_{\infty} + \sum_{i=1}^{n} \alpha_i e^{-t/\tau_i} \quad \text{Eq. 5.6}$$

where α_{∞} is the final attenuation coefficient obtained from the fitting, *n* is the number of Maxwell elements, i is the i^{th} Maxwell element, α_i and τ_i are the attenuation coefficient and the relaxation time, respectively, for the i^{th} Maxwell element (Van Vliet, 2014).

To study changes in time-dependent rheological properties of non-yeasted doughs, for v_p, M^*, and $\tan\delta$, a linear fit was performed on the low-intensity ultrasound tests using Eq. 5.7, which is similar to Eq.4.8.

$$y(t) = A + Bt \quad \text{Eq. 5.7}$$

where $y(t)$ corresponds to v_p, M^*, or $\tan\delta$ over time, *A* is the intercept, *B* is the slope ($dy(t)/dt$), and t is time (seconds). For yeasted doughs, a linear fit (Eq. 5.7) was performed on the v_p results, but for the attenuation coefficient, two processes were observed to occur over time. First, α exponentially decreased and, as time progressed, a second process characterized by a linear increase was observed. Therefore, using the Piecewise Fit function from Origin Pro (2021b) two equations were separately fitted in each process to study changes in the time-dependent α, first a Maxwell model with two elements was fitted (Eq. 5.8) and second, a linear fit was performed as per Eq. 5.7. The Maxwell model was:

$$\alpha_{SY}(t) = \alpha_{SY,\infty} + \sum_{i=1}^{n} \alpha_{SY,i} e^{-t/\tau_{SY,i}} \quad \text{Eq. 5.8}$$

where $\alpha_{SY}(t)$ corresponds to α_{SY} over time (seconds) for yeasted doughs, α_{∞} is the final attenuation coefficient obtained from the fitting, *n* is the number of Maxwell elements, i is the i^{th} Maxwell element, $\alpha_{SY,i}$ and $\tau_{SY,i}$ are the attenuation coefficient and the relaxation time for yeasted doughs, respectively, for the i^{th} Maxwell element (Van Vliet, 2014).

Finally, in order to compare both low-intensity ultrasound and shear rheology results and to plot the slopes from the equation fitting, the absolute values were normalized to scale in a range between 0 and 1 using Eq. 5.9 (similar to Eq. 4.11).

$$x' = \frac{x - x_{min}}{x_{max} - x_{min}} \quad \text{Eq. 5.9}$$

where x' is the normalized value, x is the original value, and x_{max} and x_{min} are the maximum and minimum values, respectively, within the set of samples, for each rheological parameter.

5.3.6 Bread quality measurement

In this study, bread quality was determined through its loaf volume (LV, cm^3) and specific loaf volume (*SLV*, $cm^3\ g^{-1}$). Bread preparation was carried out at Cereals Canada, using whole-wheat flours, following the complete procedure described in Section 4.3.4. From the dough mixing process, the mixing time to peak (*MTP*, min) and the peak power (*Power*, Watt), i.e., maximum power when the dough was at optimum consistency, were recorded by the P2M software. After bread had cooled down from baking, bread weight (recorded by the mass equivalent in g) was obtained and the loaf volume (*LV*) was measured based on the AACC International Approved Method 10-14.01 (AACC International, 2014) using laser topography with a TexVol bread volume meter (BVM) (Perten- L370). The specific loaf volume (*SLV*) was then obtained by dividing the *LV* by the bread weight.

5.3.7 Statistical analysis

First, for frequency selection, a heat map was built using Igor Pro 6.3.4.1 (WaveMetrics, Inc., Lake Oswego, Oregon, USA) in order to show the *p*-value for Pearson correlation coefficients calculated between α, v_p and free asparagine concentration, grain protein content (%) and water absorption (*FAB*, %), for each time-frequency combination. Moreover, in order to confirm if the Pearson correlation calculation was reliable, a second heat map was built using Python 3.8 to display the *p*-value for the Shapiro-Wilk test performed for α and v_p for all frequency-time combinations. Second, for data obtained from the selected frequency, determination of Pearson correlation coefficients between low-intensity ultrasound parameters, shear rheological parameters and free asparagine concentration, as well as fitting equations, were conducted using OriginPro software (OriginPro, Version 2021b. OriginLab Corporation, Northampton, MA, USA). Finally, Principal Component Analysis (PCA) was conducted using OriginPro software (OriginPro, Version 2021b. OriginLab Corporation, Northampton, MA, USA) to investigate the correlations between dough rheological properties from low-intensity ultrasound and shear rheology tests, wheat characteristics (free asparagine, grain protein content, yield), *FAB*, bread dough preparation

parameters (*WA*, *MTP*, *Power*), and bread quality (*SLV*, *LV*). Dough development time measurements on the mixograph and bread specific loaf volume were measured in duplicate. Low-intensity ultrasound tests were performed at least in duplicate, whereas shear rheological tests were performed in triplicate. The plotted figures throughout this chapter show mean values and error bars, corresponding to standard error (SE) values, unless otherwise stated.

5.4 Results and discussion

5.4.1 Frequency selection

Experimental data have shown that the attenuation coefficient (α, mm^{-1}) and the phase velocity (v_p, km s^{-1}) varied as a function of frequency and time. Figure 5.2 shows the variation of the attenuation coefficient as a function of frequency at two times, i.e., beginning and end of test, for yeasted and non-yeasted doughs prepared from the sample with the median free asparagine concentration value. For the curve at 3 minutes for the non-yeasted doughs in Figure 5.2, the wave attenuation achieve maximum values below 6 MHz, and due to ultrasonic signals being buried in noise at lower frequencies, attenuation coefficient cannot be accurately measured. For the yeasted dough curve at 18 minutes, the same noise interference was observed for frequencies lower than 10 MHz in Figure 5.2. The much higher values of attenuation coefficient at lower frequencies (< 10 MHz) can be explained by the presence of air bubbles (with a particular size distribution) in the dough, which are sensed by the ultrasonic waves, and which act as scattering agents (Scanlon, Page, Leroy, Elmehdi, et al., 2011; Scanlon & Page, 2015). On the other hand, the attenuation coefficient is lower at frequencies around 10 MHz, where ultrasonic wavelengths are small and ultrasound is less scattered by the air bubbles (Scanlon, Page, Leroy, Elmehdi, et al., 2011).

Figure 5.3 shows the variation of the phase velocity as a function of frequency at two times, i.e., beginning and end of test, for yeasted and non-yeasted doughs prepared from the sample with the median free asparagine concentration value. The phase velocities at frequencies below 6 MHz and 8 MHz for non-yeasted and yeasted doughs, respectively, are not reliable as illustrated by their great variation, possibly due to noise interference. However, at frequencies higher than 6 MHz and 8 MHz for non-yeasted and yeasted doughs, respectively, one can note that the phase velocity tends to fall with increasing frequency until it achieves an almost constant value against frequency, regardless of whether the dough contains yeast or not. The phase velocity behavior as a function of frequency can be explained by the presence of air bubbles in the dough, which causes wave attenuation at low frequencies and, consequently, very rapid changes in phase velocity (Scanlon,

Page, Leroy, Elmehdi, et al., 2011). On the other hand, at high frequencies, the short wavelength waves are less scattered by air bubbles and the properties of the dough matrix can be evaluated, resulting in almost no variation in phase velocity as a function of frequency.

Figure 5.2. Curves for the attenuation coefficient (α, mm^{-1}) as a function of frequency (MHz) at 3 and 37 minutes of experiment for non-yeasted (left) and at 2 and 18 minutes for yeasted (right) doughs prepared from sample E (sample with the median free asparagine concentration value, 735 $\mu g\ g^{-1}$). Data points in graphs represent means ± SE ($n \geq 2$).

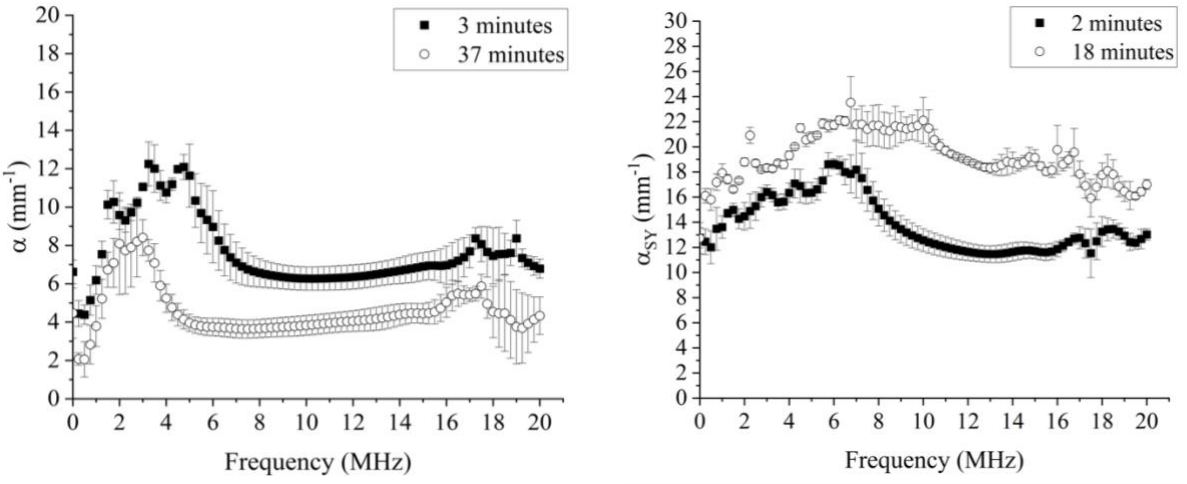
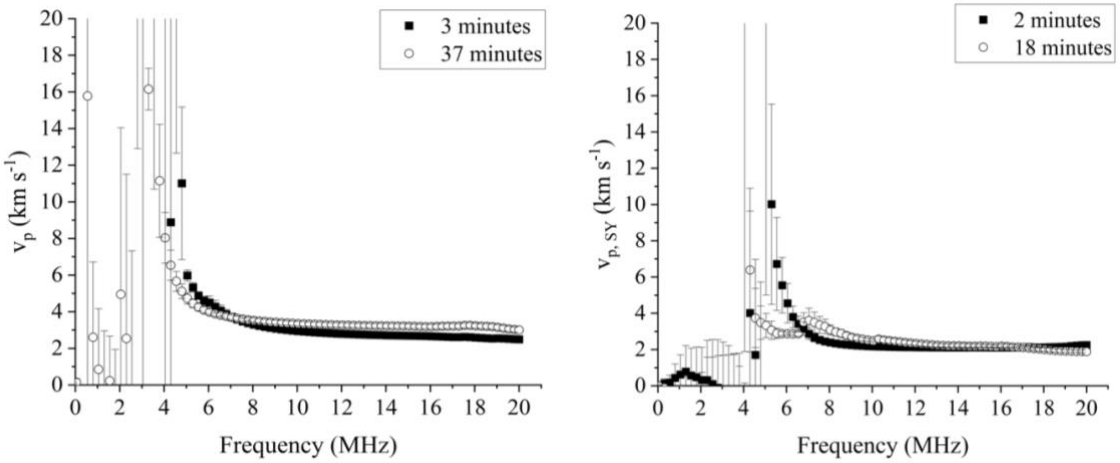


Figure 5.3. Curves for the phase velocity (v_p, km s^{-1}) as a function of frequency (MHz) at 3 and 37 minutes of experiment for non-yeasted (left) and at 2 and 18 minutes for yeasted (right) doughs prepared from sample E (sample with the median free asparagine concentration value, 735 μg g^{-1}). Data points in graphs represent means ± SE (n ≥ 2).



In order to select the frequency to evaluate dough rheology, Figure 5.4 shows examples of heat maps, for non-yeasted dough samples, for the significance of the Pearson correlation coefficients between the low-intensity ultrasound parameter α and free asparagine concentration (μg g^{-1}), grain protein content (%) and water absorption (*FAB*, %). Figure 5.5 shows examples of the same heat maps as Figure 5.4, but for the yeasted dough samples. The same heat maps were built for the correlation between v_p and free asparagine concentration (μg g^{-1}), grain protein content (%) and water absorption (*FAB*, %), for yeasted and non-yeasted samples, in order to analyze the significance of these correlations. For non-yeasted doughs, the heat maps (not shown) are mostly white, indicating no significant correlations between v_p and free asparagine concentration, grain protein content and *FAB* for almost all combinations between time and frequency. A similar outcome was obtained for yeasted doughs, with the majority of the heat maps showing no color, thus indicating no significant correlation for v_p against wheat and flour properties. Figure 5.4 and Figure 5.5 illustrate how the heat maps were built to evaluate the correlation between low-intensity ultrasound parameters (α and v_p) against free asparagine concentration, grain protein content and *FAB*. Also, for illustration purposes, it was chosen to show

on Figure 5.4 and Figure 5.5 only the heat maps for the correlation between α and free asparagine concentration, protein content and water absorption.

Figure 5.4. Heat map figures showing the significance (p-value) of the Pearson correlation coefficients between α and free asparagine concentration (μg g^{-1}), protein content (%) and water absorption (*FAB*, %) for non-yeasted doughs. Values of p higher than 0.05 (non-colored areas) mean that correlations were not significant.

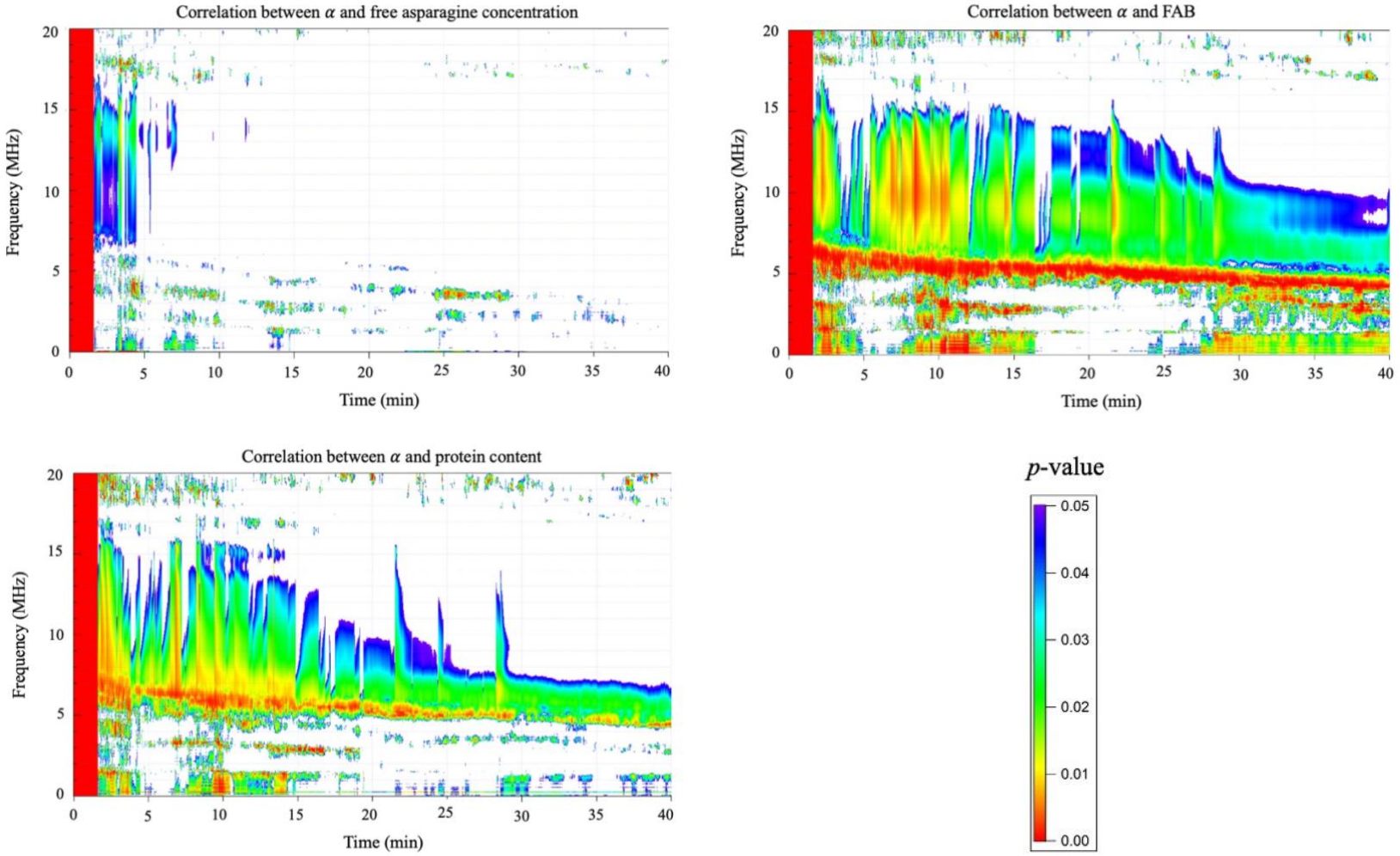
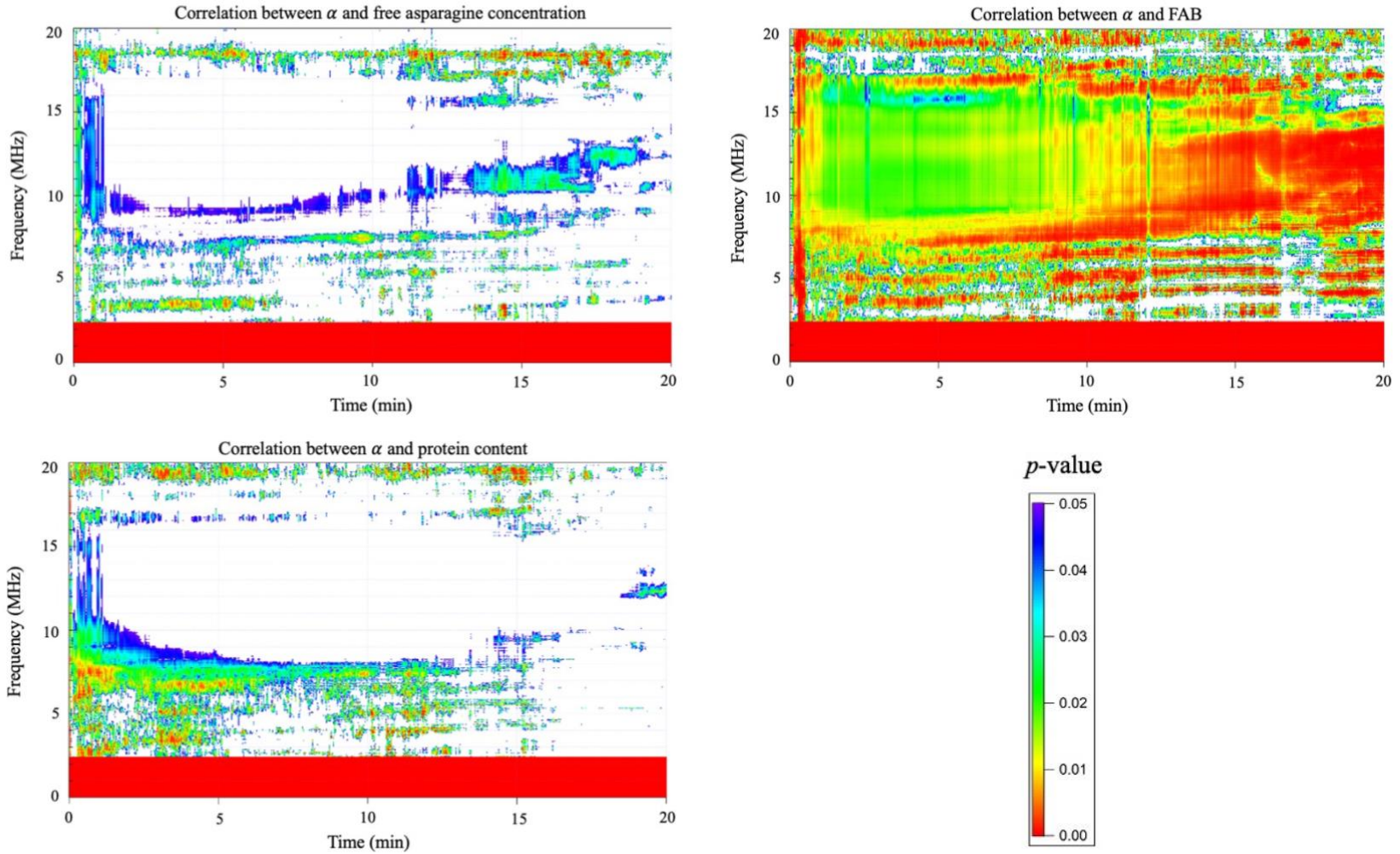


Figure 5.5. Heat map figures showing the significance (p-value) of the Pearson correlation coefficients between α and free asparagine concentration (μg g^{-1}), protein content (%) and water absorption (*FAB*, %) for yeasted doughs. Values of p higher than 0.05 (non-colored areas) mean that correlations were not significant.



Moreover, Figure 5.6 and Figure 5.7 also show the heat map for significance for the Shapiro-Wilk test to evaluate the distribution of α and v_p, respectively, for each time-frequency combination, for both yeasted and non-yeasted dough samples. Moreover, statistical analysis of the free asparagine concentration, protein content, and water absorption shows that data were normally distributed ($p > 0.05$ and $W > 0.90$).

Figure 5.6. Heat map figures showing the W value for a normality test for α data for each time-frequency binomial, for non-yeasted (A) and yeasted (B) doughs. Colored values of W are non-significant ($p > 0.05$) which means that data are close to a normal distribution. Values of W lower than 0.9 (non-colored areas) means that data are not normally distributed.

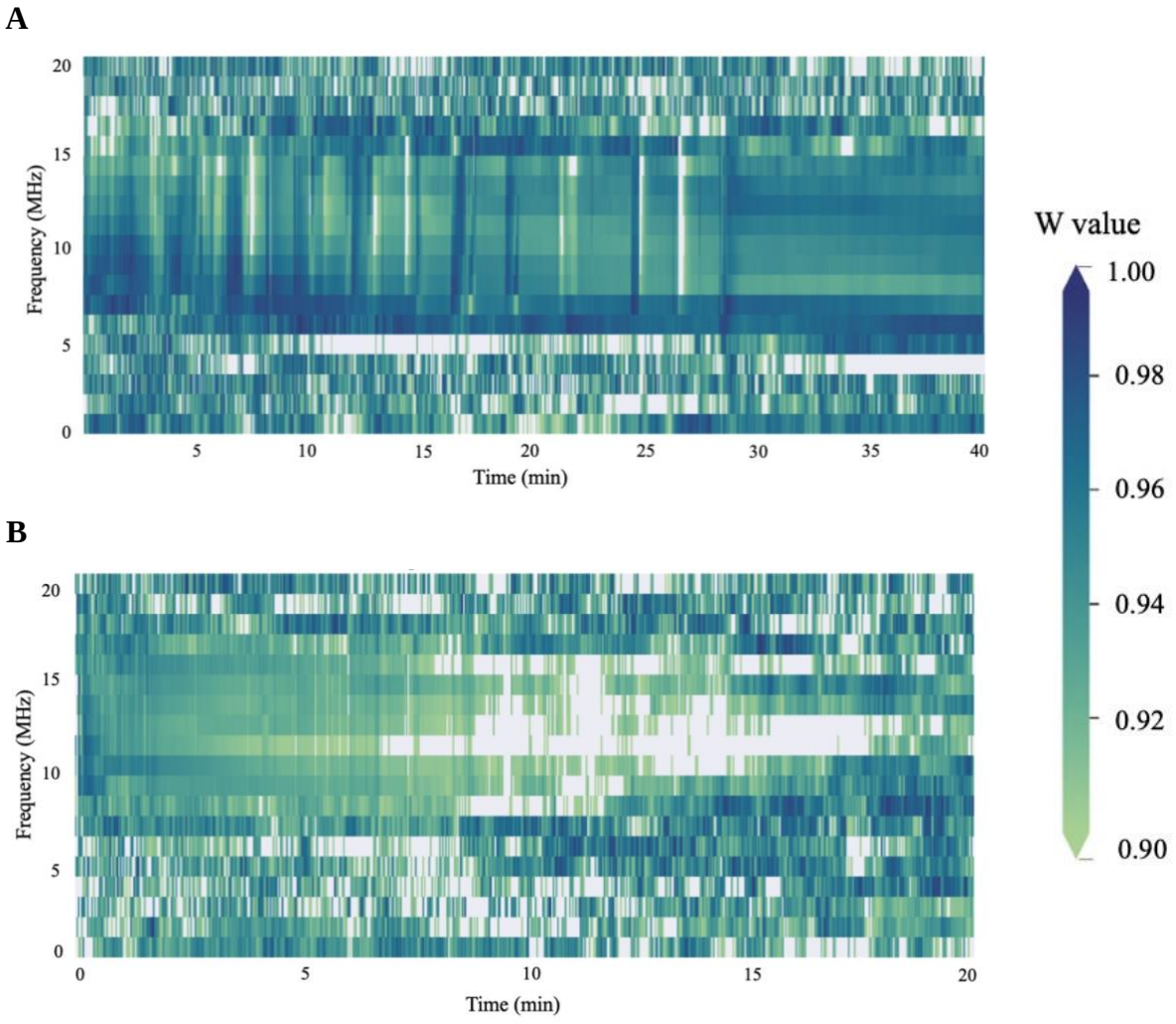
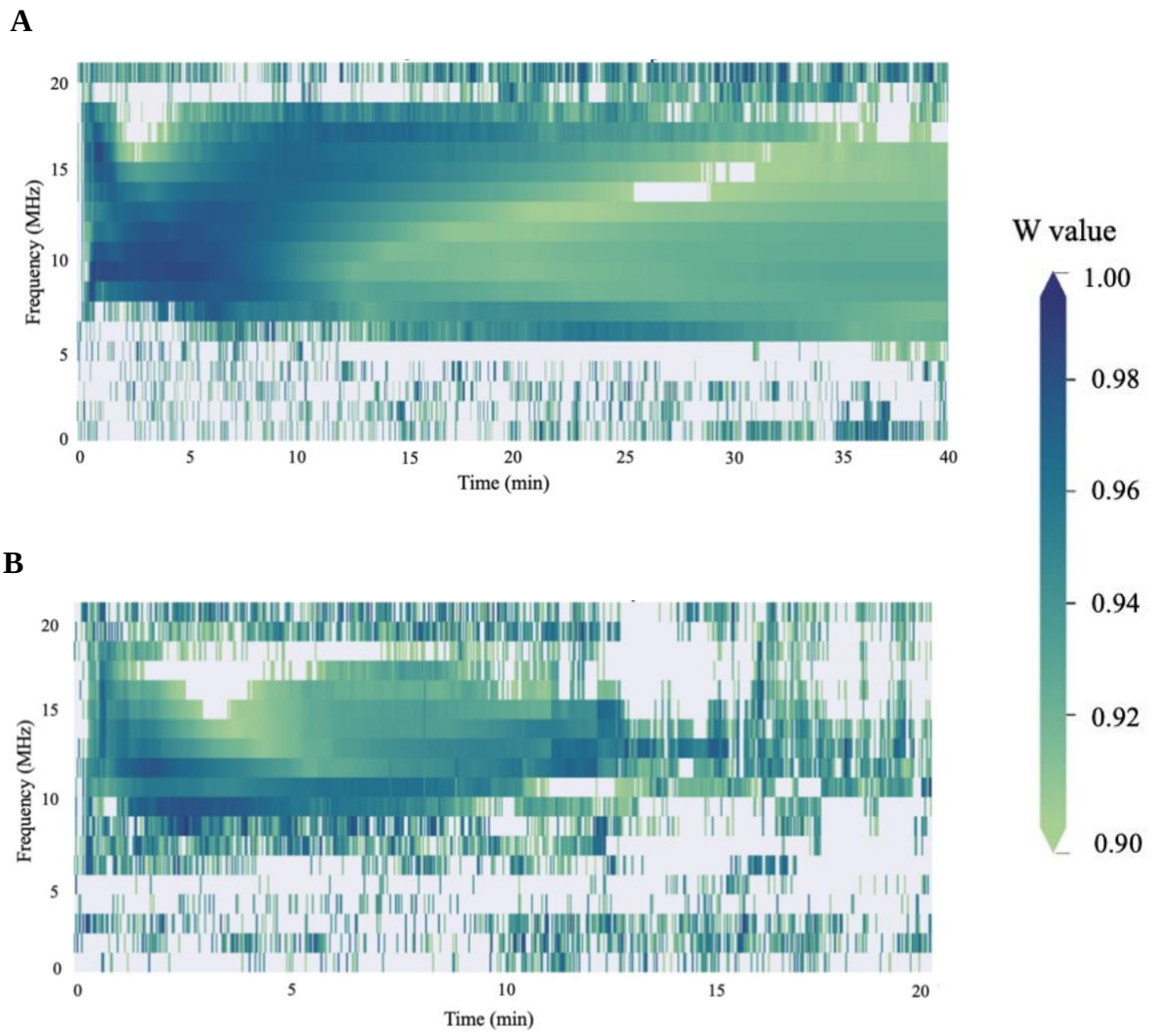


Figure 5.7. Heat map figures showing the W value for a normality test for v_p data for each time-frequency binomial, for non-yeasted (A) and yeasted (B) doughs. Colored values of W are non-significant ($p > 0.05$) which means that data are close to a normal distribution. Values of W lower than 0.90 (non-colored areas) mean that data are not normally distributed.



From Figure 5.4, one can observe that only the correlation between α and FAB showed significant ($p < 0.05$) Pearson coefficients throughout the 40 minutes of the test duration, for frequencies from 5 up to 10 MHz. The correlation between α and protein content showed significance in a broad range of frequencies only until 15 minutes of the test, with the Pearson coefficient becoming non-significant at frequencies higher than 7 MHz at longer experimental

times. When evaluating the significance of the Pearson coefficient between α and free asparagine concentration, Figure 5.4 shows that no significant correlation was found for almost all combinations of time and frequency, with some significance appearing only at the beginning of the test, i.e., until 5 minutes of pulse propagation.

When looking at the significance of the correlations for yeasted samples in Figure 5.5, the correlation between α and *FAB* was significant at almost all time-frequency combinations. However, the same outcome was not observed when evaluating the correlations between α and protein content and free asparagine concentration, with only a few regions of the map showing statistical significance. The test of significance for Pearson correlation coefficients can be affected, e.g., increased error or reduced statistical power, when evaluating the bivariate relationships of data that are not normally distributed (Bishara & Hittner, 2012, 2015). Therefore, from the Shapiro-Wilk tests, Figure 5.6 and Figure 5.7 show the distribution of W-values that are non-significant ($p>0.05$) and higher than 0.90, i.e., normally distributed, for each time-frequency combination. Non-colored areas shown in the heat maps in Figure 5.6 and Figure 5.7 indicate that data are not normally distributed and these respective areas, or time-frequency binomials, must be disregarded in Figure 5.4 and Figure 5.5, as they might not present reliable results. The ideal situation expected from the figures above (Figure 5.4 to Figure 5.7) was to obtain the best frequency at which significant and reliable information could be retrieved from all correlation studies. As the ideal situation was not achieved, the frequency selection for dough rheological properties evaluation was carried out by selecting the frequency that was mostly correlated to normally distributed data of α and v_p during the pulse propagation. Considering the data presented in Figure 5.6 and Figure 5.7, the best frequency to be selected was 10 MHz, which was also the central frequency of the transducers used in the present study. The frequency selection was in agreements with Khorshidi, Thandapilly, et al. (2018) who performed low-intensity ultrasound with five frequencies (0.5-10 MHz) on five wheat varieties. The authors observed that signals suffered less attenuation at 10 MHz when compared to lower frequencies. Furthermore, better correlations between dough rheological parameters from ultrasound and parameters from empirical tests were found at 10 MHz (Khorshidi, Thandapilly, et al., 2018).

5.4.2 Non-yeasted dough rheological properties and free asparagine concentration

5.4.2.1 Low-intensity ultrasound and shear stress relaxation

Non-yeasted flour dough is a material composed of two phases: a continuous solid and essentially incompressible phase and a compressible gas phase, made of the air bubbles entrapped during mixing (Scanlon & Page, 2015; Scanlon & Zghal, 2001). Although air bubbles are not the focus of this study, they are indeed an essential component in wheat flour doughs and they have the power to affect dough rheological properties (Campbell, 2008; Campbell & Mougeot, 1999). Low-intensity ultrasound is a technique that has the power of measuring the dough properties as a composite material, sensitive to bubbles and dough matrix when performed at low frequencies (~ 50 kHz) (Scanlon, Page, Leroy, Fan, et al., 2011). When higher frequencies are used, e.g., up to 5 MHz (Scanlon, Page, Leroy, Elmehdi, et al., 2011) or even up to 10 MHz (Scanlon, Page, Leroy, Fan, et al., 2011), the bubbles can be sensed by the ultrasonic waves, with changes in phase velocity and attenuation coefficient being mainly due to air bubbles present in the dough (Scanlon, Page, Leroy, Fan, et al., 2011). At higher frequencies (≥10 MHz), wavelengths are small and ultrasound is generally not affected by bubbles. In this frequency region, the continuous solid phase properties of the dough matrix can be evaluated through phase velocity and attenuation coefficient changes (Scanlon, Page, Leroy, Fan, et al., 2011). Therefore, the selection of the frequency of 10 MHz, in this study, aims to obtain signals that are less affected by bubbles in order to also be able to evaluate viscoelastic dough rheological properties and their relationship with free asparagine concentration. In order to understand the changes in low-intensity ultrasound parameters over time, Figure 5.6 was plotted to show the attenuation coefficient behavior over time for the non-yeasted wheat flour dough samples made from the highest and lowest free asparagine concentration wheat samples. Additionally, in order to compare the behavior of the dough right after sample compression between the two transducers, Figure 5.8 also displays the shear relaxation modulus over time, and, for both curves, a Maxwell model with two elements was fitted, for all replicates, in order to calculate the dough relaxation times (τ_1 and τ_2).

During pulse propagation, ultrasonic waves suffer energy losses, e.g., due to scattering and absorption. Wave attenuation is the rate of energy loss while the wave is being propagated through a material or medium (Raj et al., 2004b). Wave attenuation occurs via absorption of acoustic energy, for example, due to viscous losses and molecular relaxations. In the case of heterogeneous media, wave attenuation is also due to scattering because of the difference between the acoustic

impedance of components in the material (Raj et al., 2004b). For wheat flour doughs, for example, the air bubbles and the continuous phase have a large difference in compressibility (Koksel et al., 2016), which causes a resonant scattering (McClements, 1991).The presence of particles, such as protein aggregates, can also act as scattering agents (McClements & Gunasekaran, 1997). In Figure 5.8, a similar behavior between the decay of the attenuation coefficient and the relaxation modulus from shear rheometric tests was observed. When fitting a Maxwell model with two elements, the goodness of fit (R^2) was of the order of 0.9999 for both α and $G(t)$. From the fitted equations, the relaxation times (τ) were calculated for the decay of the attenuation coefficient ($\tau_{\alpha,1}$ and $\tau_{\alpha,2}$) and for the shear relaxation modulus (τ_1 and τ_2). The relaxation times are the time necessary for the parameter (α_i or G_i) to achieve 36.8% of its initial value. Figure 5.9 shows the relaxation times from both parameters plotted against free asparagine concentration in order to compare data obtained from low-intensity ultrasound with the data obtained from shear rheology tests.

Figure 5.8. Plots obtained from low-intensity ultrasound at 10 MHz and shear stress relaxation measurements for non-yeasted wheat flour dough samples with the highest (A, ▼) and lowest (J, ○) free asparagine concentration. (A) Curves of attenuation coefficient (α) over time, obtained from low-intensity ultrasound tests, where red lines indicate a Maxwell model fitting with two elements, α_∞ is the final attenuation coefficient obtained from the fitting, α_1 and α_2 are the attenuation coefficient for the first and the second Maxwell element, respectively, and τ_1 and τ_2 are the relaxation times for the first and the second Maxwell elements. (B) Curves of relaxation moduli over time, obtained from a shear stress relaxation test, where G_∞ is the final relaxation modulus obtained from the fitting, G_1 and G_2 are the relaxation moduli for the first and second Maxwell element, and τ_1 and τ_2 are the relaxation times obtained from fitting a Maxwell model with 2 elements (red line) to the data. Data points in graphs represent means and SE ($n \geq 2$).

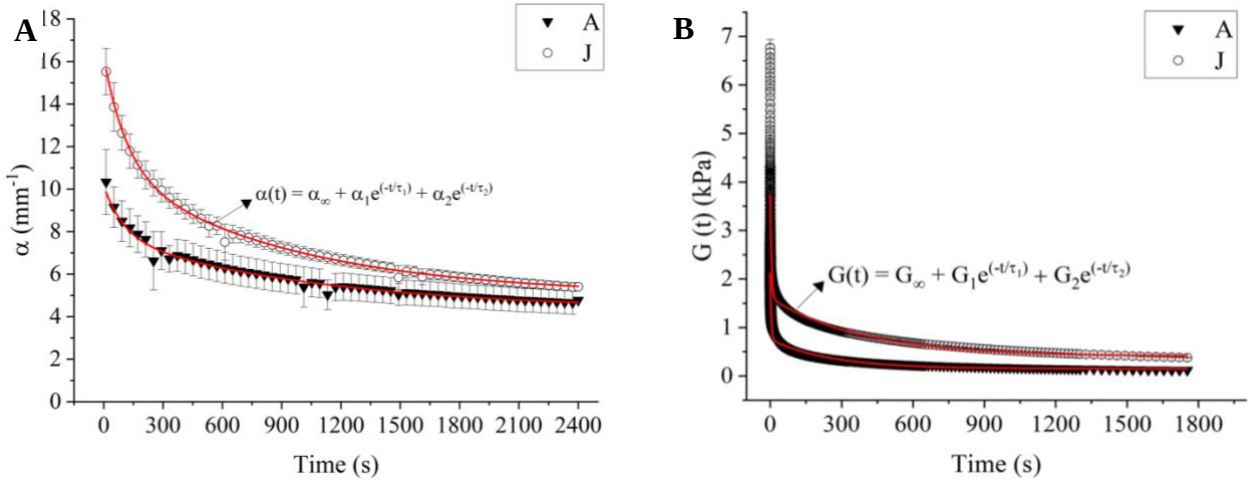
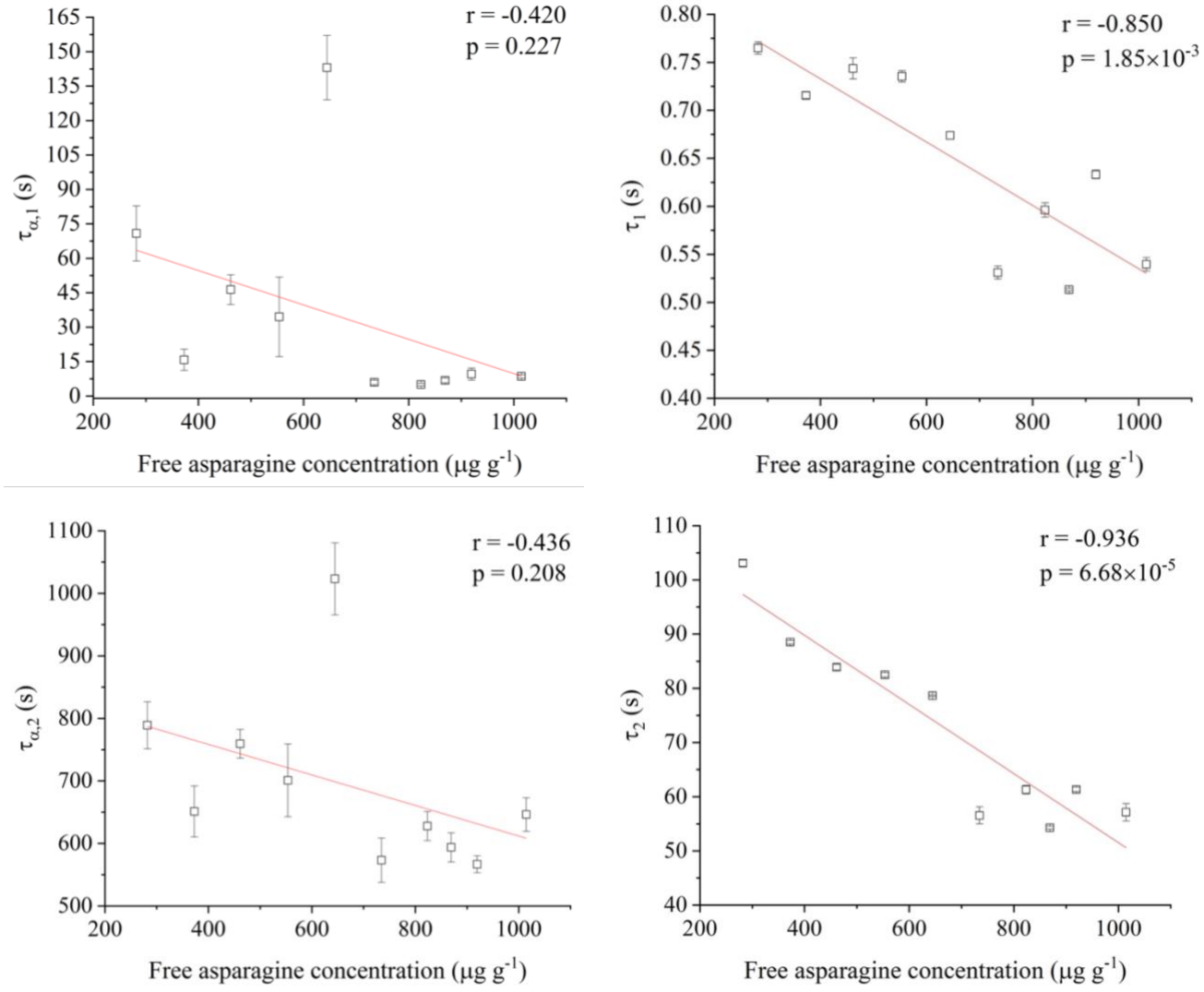


Figure 5.9. Relationship between relaxation times from attenuation coefficient decay (left), and from the shear relaxation modulus (right) against free asparagine concentration (μg g^{-1}). Data points represent means ± SE (n ≥ 2).



When a dough piece is subjected to stress relaxation tests and the respective relaxation spectra is calculated, two relaxation processes can occur over time. The shorter relaxation process, occurring at t < 10 seconds, is generally correlated to gliadins, and the longer process, occurring at t > 10 seconds, is correlated to glutenin macropolymer (W. Li et al., 2003; Rao et al., 2001). In this study, the Maxwell model fitting for the relaxation moduli showed that, based on the magnitude of the relaxation times in Figure 5.9, τ_1 and τ_2 could represent the relaxation time of the gliadins and glutenins, respectively, of the dough. For the low-intensity ultrasound results,

although no relaxation moduli were obtained, it was possible to obtain two relaxation times from the fitting of the attenuation decay curves. In Figure 5.9, when the relationship between relaxation time against free asparagine concentration was evaluated, a negative correlation was observed. Although the correlations against free asparagine concentration were not significant at $p < 0.05$ for low-intensity ultrasound results, a similar trend was observed between both tests. Correlation studies also found that, for shear stress relaxation results, τ_1 was negatively ($p < 0.05$) correlated to grain protein content ($r = -0.819$) and *FAB* ($r = -0.795$) and $\tau_2$was also negatively ($p < 0.05$) to protein content ($r = -0.715$) and *FAB* ($r = -0.741$). From the attenuation decay fit, correlation studies did not find a significant correlation at $p < 0.05$ between relaxation times, protein content and *FAB*. However, a negative trend was observed between $\tau_{\alpha,1}$ and protein content ($r = -0.351$) and *FAB* ($r = -0.537$) and between $\tau_{\alpha,2}$ and protein content ($r = -0.370$) and *FAB* ($r = -0.586$). Wheat flour dough samples with relaxation processes that take longer to occur are known to possess a more elastic-like structure, thus stronger glutens (W. Li et al., 2003). The strong negative correlation between τ_1 and protein content could be an indication that samples with higher protein content possessed higher levels of gliadins when compared to glutenins. This hypothesis could also be confirmed by the stronger negative correlation of τ_1 with *FAB*. This rationale is supported by previous studies that reported Gli/Glu ratio (Godfrey et al., 2010) and sulfur-poor gliadins (Malalgoda et al., 2018) to be positively correlated to *FAB*.

For the low-intensity ultrasound test, when the pulses propagate through the sample, the waves interact with dough components and the dough rheological properties, affected by different ingredients and presence of bubbles, can be retrieved (Koksel et al., 2016; Peressini et al., 2016; Scanlon & Page, 2015). Although not many studies have been published from the high frequency domain (>10 MHz) (Scanlon & Page, 2015), Fan et al. (2013) evaluated wheat flour doughs, mixed at atmospheric pressure and under vacuum, using transducers of central frequency of 20 MHz. By calculating the relaxation time, the authors observed the effects of dough protein molecular relaxation on α and v_p, when subjected to the strains generated by the stress inputs at the surface of the sample (Fan et al., 2013). Moreover, samples mixed under vacuum showed faster molecular relaxation times when compared to doughs mixed at atmospheric pressure, indicating that, in the latter, gluten proteins fastened to air bubble interfaces took longer to relax from the imposed strain (Fan et al., 2013). Additionally, samples mixed at atmospheric pressure showed greater variation in v_p as frequency increased, achieving values between 2 and 2.5 km s^{-1} at frequencies of between

20 and 30 MHz (Fan et al., 2013). On the other hand, vacuum mixed doughs resulted in v_p between 1.80 and 1.85 km s^{-1} for all frequencies higher than 10 MHz (Fan et al., 2013). The attenuation coefficient also was significantly different between atmospheric and vacuum mixed doughs (Fan et al., 2013). For dough mixed in the presence of air, α increased from about 5 mm^{-1} up to 20 mm^{-1} as frequency increased, whereas for vacuum mixed doughs, α was lower, increasing from 1 to about 6 mm^{-1} (Fan et al., 2013).

Differently from Fan et al. (2013), the present study evaluated α and v_p over a period of time (2400 seconds), at constant frequency (10 MHz), after sample compression between the transducers. Therefore, the α and $v_p$variations over time were related to dough relaxation post compression rather than relaxation due to strain imposed by pulse propagation, as reported by Fan et al. (2013). In the present study, the decrease in attenuation coefficient over time (Figure 5.8) could be speculated to be caused by some mechanisms affecting bubble stability when the sample was compressed between the two transducers, such as bubble disproportionation and coalescence. Bubble disproportionation happens when air from smaller bubbles are transferred to bigger ones, which is caused by the difference of the Laplace pressure between the inside and the outside of the bubble (Mills et al., 2003). The difference in Laplace pressure is directly proportional to the surface tension of the bubble and indirectly proportional to the radius of the bubble. Therefore, smaller bubbles will have greater differences in Laplace pressure and, when close to larger bubbles, the gas will be transferred to these larger bubbles, causing the smaller bubbles to disappear (Mills et al., 2003). Bubbles and bubble size distribution are not the focus of the present study, therefore; no direct analysis on bubbles was done in the studied samples. However, it is an almost impossible task to tackle dough rheological behavior, measured under ultrasound tests, without considering the presence and the effects of bubbles on dough structure. Therefore, through a brief interpretation of the literature, regarding dough analysis using low-intensity ultrasound, we might elucidate possible mechanisms occurring in the samples that led to the attenuation decay over time, as observed in Figure 5.6. For example, Leroy et al. (2008) presented a model to calculate the bubble size distribution in non-yeasted doughs compressed to 0.3 mm thickness. Leroy et al. (2008) obtained a distribution of bubbles with a median diameter of 28 μm when dough samples were evaluated after 53 minutes post mixing. When dough samples were evaluated after 96 minutes post mixing, the median diameter increased to 36 μm, showing that the size of the bubbles was

increasing over time due to bubble disproportionation (Leroy et al., 2008). Bellido et al. (2006) performed X-ray microtomography analysis on non-yeasted doughs with a thickness of 2.17 mm, obtaining a mean bubble diameter of about 100 μm after 90 minutes post mixing. In the present study, the mean phase velocity, for all non-yeasted samples up to 10 minutes of test, was about 2.97 km s^{-1}. Therefore, at a frequency (f) of 10 MHz, the ultrasound wavelength ($\lambda = v_p/f$) would be about 297 μm. The region where most of the resonant scattering occurs is called the intermediate region, i.e., when the ultrasound wavelength and the dimensions of the scatterer are similar (Koksel et al., 2016). This intermediate region was defined by McClements & Gunasekaran (1997) as where the bubbles have median diameter values between λ/10 and 50λ. Therefore, for the samples involved in this study, the possible presence of scattering bubbles, i.e., bubbles with diameter values of 30 μm or lower (Koksel, Strybulevych, Aritan, et al., 2017), could be causing some wave attenuation at the beginning of the test, even though bubble effects are expected to be more prominent when yeasted doughs are evaluated. Considering the median size of the bubbles found in wheat flour dough by Bellido et al. (2006) and Leroy et al. (2008), disproportionation events could be happening if the samples from this study have bubbles with similar size as the ones reported by Bellido et al. (2006) and Leroy et al. (2008) at the beginning of the test. Therefore, these small bubbles would disappear over time, with only the bigger ones remaining in the sample. Because the bigger bubbles (d > 50λ (McClements & Gunasekaran, 1997)) are "invisible" to short wavelength waves (high frequencies), waves are then less scattered and the attenuation coefficient decreases (Koksel et al., 2016).

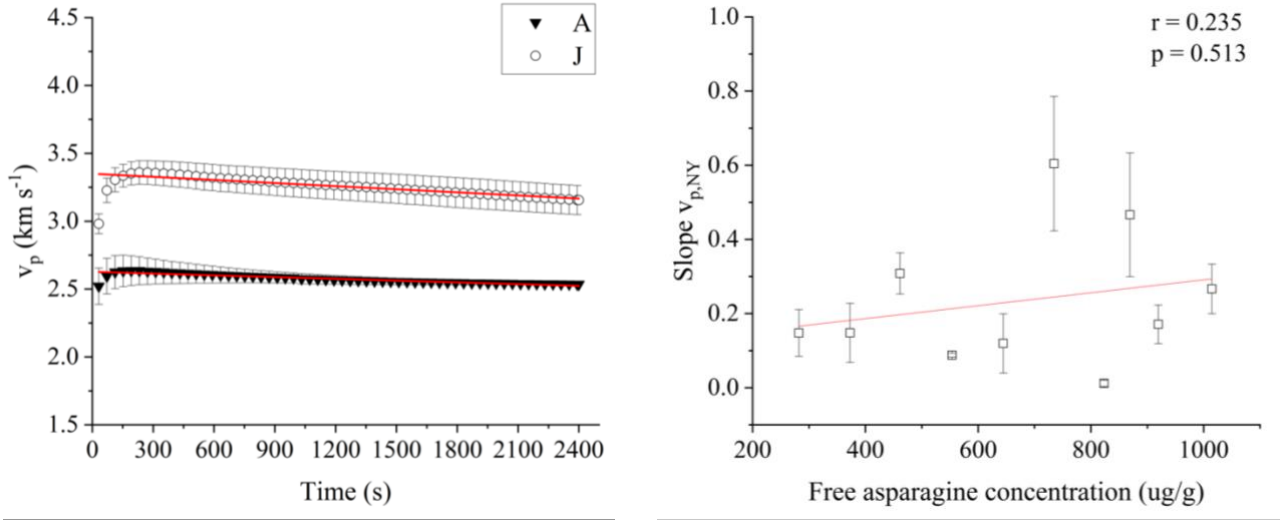
In Figure 5.9, both relaxation times obtained from the fitting of the attenuation coefficient decay over time were negatively ($p > 0.05$) correlated to free asparagine concentration in wheat. This outcome means that samples with higher free asparagine concentration, shown to possess weaker glutens in stress relaxation tests, presented faster attenuation coefficient decays. Additionally, as mentioned before, it was proposed that samples with higher protein content and higher free asparagine concentration could have higher Gli/Glu ratios. Results from low-intensity ultrasound could support this hypothesis, with samples having higher free asparagine having an overall faster attenuation decay.

Besides the attenuation coefficient decay over time, the phase velocity was also obtained for non-yeasted samples over the course of 2400 seconds, as shown in Figure 5.10. The changes in phase velocity were evaluated by performing a linear fitting for each replicate and plotting the

mean slope of the curves against free asparagine concentration (Figure 5.10). The goodness of fit (R^2) for the linear fitting was 0.9999 for all replicates, with the mean slopes varying from -1.09×10^{-4} to 1.98×10^{-4} km s^{-2}, which are the slopes of the fits of the curves represented in the left plot in Figure 5.10.

In Figure 5.10, changes in phase velocity, i.e., slope of the curve, were positively, but not significantly (at $p < 0.05$), correlated to free asparagine concentration. Correlation studies also found that the slopes for the v_p curves over time were positively ($p > 0.05$) correlated to protein content ($r = 0.410$) and positively ($p < 0.05$) correlated to *FAB* ($r = 0.710$). Kidmose et al. (2001) studied doughs prepared from flours of seven wheat varieties using a constant water amount and optimum water absorption. The authors evaluated dough rheological properties under shear oscillatory tests at four different frequencies and using low-intensity ultrasound at 37 kHz (Kidmose et al., 2001). When evaluating the ultrasound velocity of the doughs with the water absorption of the wheat flours, the authors observed a strong negative correlation between ultrasound velocity and *FAB* ($r = -0.94$, $p < 0.001$) (Kidmose et al., 2001).

Figure 5.10. Phase velocity (v_p) as a function of time (seconds), measured at 10 MHz, for non-yeasted dough samples with the highest (A, ▼) and lowest (J, ◯) free asparagine concentration, red lines are the linear fits (left). Relationship between absolute normalized slopes of the curves for v_p over time against free asparagine concentration (μg g^{-1}) and Pearson's r correlation analysis (right). All data points represent means ± SE ($n \geq 2$).



In the present study, the changes in phase velocity over time were positively correlated to the water added to prepare the wheat flour dough samples. Therefore, although low-intensity ultrasound velocity data was not affected by free asparagine concentration, it was affected by *FAB*. Samples with higher water absorption have been shown to have slightly higher slopes, e.g., sample with 68.2% having a slope of 1.98×10^{-4} km s^{-2} as opposed to samples with the lowest *FAB*, 62.8%, having a slope of 3.4×10^{-5} km s^{-2}. Therefore, samples with higher water absorption have been shown to have slightly more changes in v_p over time than samples with less water absorption. Moreover, Kidmose et al. (2001) also pointed out that the development of softer doughs caused a decrease in ultrasound velocity and storage moduli from shear tests. In the present study, the majority of the mean slopes were negative, indicating overall decay in v_p over time, thus overall dough softening over time.

In a more recent study, phase velocity has been shown to have higher values when doughs were mixed at atmospheric pressure when compared to doughs mixed under vacuum conditions (Fan et al., 2013). Additionally, in a study from Leroy et al. (2008), performed to propose a model to investigate bubble size distribution in dough, a v_p value of 1.6 km s^{-1} for a wheat flour dough without air bubbles was reported. In the present study, changes in v_p could also be occurring due to bubble disproportionation during dough relaxation after sample compression. Thus, the overall decay in v_p over time could be occurring due to small bubbles disappearing over time, thus agreeing with the results found for attenuation decay behavior over time. Therefore, while shear stress relaxation tests provide information mostly on dough protein relaxation, low-intensity ultrasound, in addition to dough matrix properties, provides rich information on other components present in the dough, such as air bubbles.

5.4.2.2 Low-intensity ultrasound and linear shear oscillatory time sweep

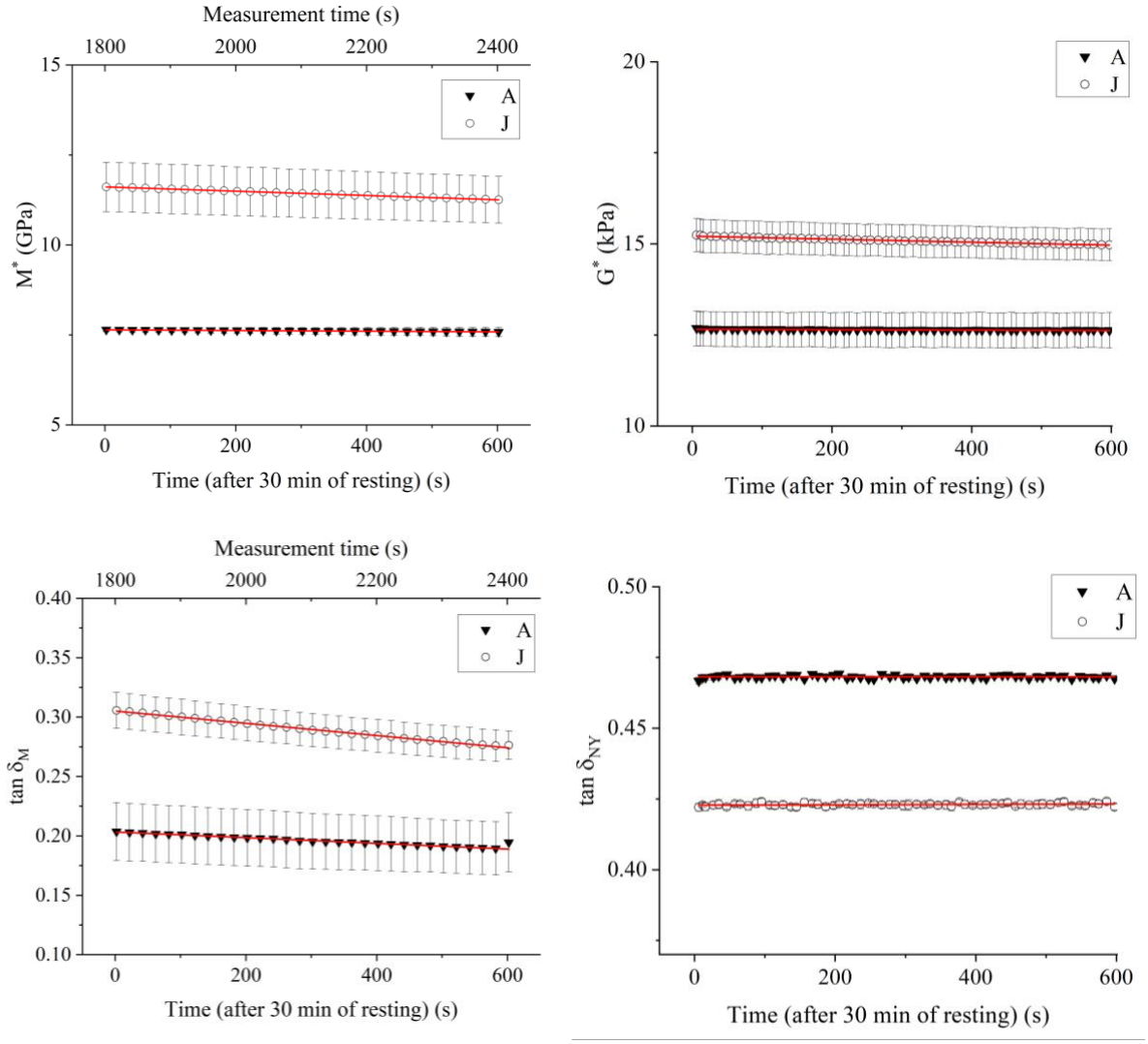
From low-intensity ultrasound parameters, i.e., α and v_p, one can also calculate the magnitude of the complex longitudinal moduli (M^*) and the longitudinal loss tangent ($\tan\delta_M$) using Eq. 5.5 and Eq. 5.6, respectively. Data obtained from linear shear oscillatory time sweep tests (G^* and $\tan\delta$) were utilized in the comparison with dough rheological properties from low-intensity ultrasound (M^* and $\tan\delta_M$). To compare dough rheological properties from low-intensity ultrasound with shear oscillatory time sweep, the analysis of the propagated pulses was performed 1800 seconds after sample compression between the transducers. Moreover, to conduct

longitudinal moduli calculation, dough density measurements were also performed on non-yeasted dough using the dough piece that was separated after sheeting from the tested dough sheet after 1800 seconds of resting in a sealed container. The curves for the magnitude of the complex moduli and loss tangent over time, from both shear oscillatory time sweep (10 Hz) and low-intensity ultrasound (10 MHz), are shown in Figure 5.11. A goodness of fit (R^2) of 0.9999 was obtained after performing a linear fit on the curves of G^*, $\tan\delta$, M^* and $\tan\delta_M$ over time, as represented in Figure 5.11. Additionally, statistical analysis of the curves' slopes showed that, for all replicates, slopes for M^*, G^*, and $\tan\delta_M$ over time were significantly different from zero ($p<0.05$). For G^*, the mean slopes varied from -4.2×10^{-4} to -6.3×10^{-5} kPa s^{-1}, while for M^* the mean slopes varied from -5.9×10^{-4} to 6.2×10^{-4} GPa s^{-1}, with the majority of samples showing negative slopes. For $\tan\delta$, some of the replicates had very small slopes (-5.3×10^{-7} to 1.2×10^{-6} s^{-1}). For $\tan\delta$ the mean slopes varied from -1.5×10^{-6} to 2.2×10^{-6} s^{-1} with some not significantly different from zero, whereas for $\tan\delta_M$, the mean slopes varied from -5.2×10^{-5} to -1.4×10^{-5} s^{-1}, depending on the asparagine concentration. Although the slopes were small for all parameters, it was observed that both parameters from low-intensity ultrasound tests had changes over time about ten times faster than parameters from shear linear oscillatory tests.

To understand how these changes in dough rheological parameters over time were correlated to free asparagine concentration, Figure 5.12 shows the correlation studies that were performed for the absolute normalized slopes against free asparagine concentration. In Figure 5.12, the slopes of the G^* curves over time were negatively ($p<0.05$) correlated to free asparagine concentration. Conversely, the same strong correlation was not found for the slopes of the M^* curves over time, which showed a very weak negative correlation with free asparagine concentration. It was observed, however, a more similar trend for the slopes of $\tan\delta$ and $\tan\delta_M$ curves against time. Although the correlation was not significant at $p < 0.05$ for $\tan\delta$, both correlation studies showed a clear negative relationship against free asparagine concentration.

When evaluating the results for linear shear oscillatory tests, correlation studies also found that slopes of G^* were negatively correlated ($p < 0.10$) to protein content ($r = -0.591$) and to *FAB* ($r= -0.577$) while slopes for $\tan\delta$ showed no significant correlations to either protein content or *FAB*. For low-intensity ultrasound results, correlation studies found that slopes of M^* were positively correlated to grain protein content ($r = 0.429$) and to *FAB* ($r = 0.600$), with only the correlation to *FAB* being significant ($p < 0.10$).

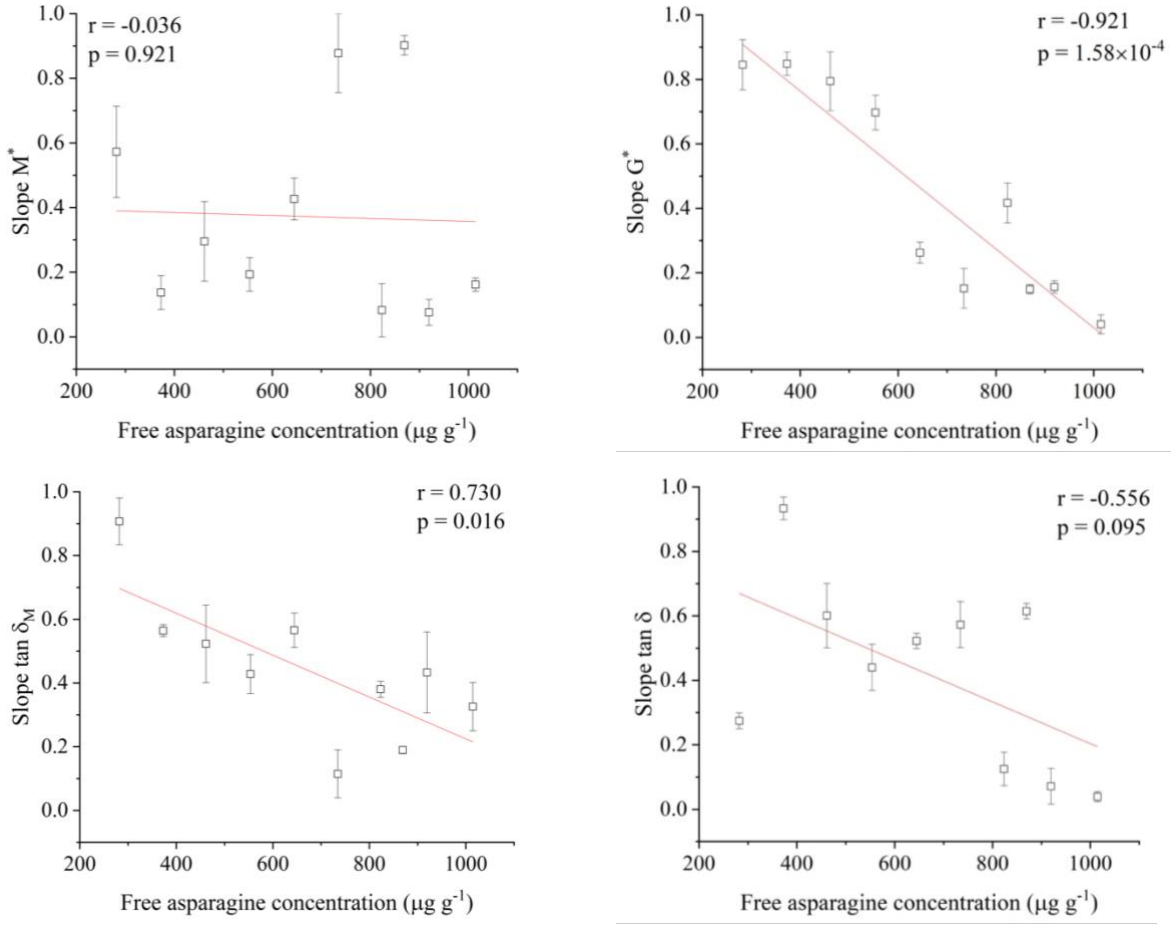
Figure 5.11. Magnitude of the longitudinal shear moduli (M^*) its longitudinal loss tangent (tan δ_M), and dough density (left) and magnitude of the complex shear moduli (G^*) and shear loss tangent (tan δ) (right) as a function of time after 30 minutes of resting for non-yeasted wheat flour dough samples with the highest (A, ▼) and lowest (J, ○) free asparagine concentration. Red lines are linear fits. Data points represent means and SE (n ≥ 2).



Finally, the slopes of the curves for tan δ_M were negatively correlated ($p < 0.05$) to protein content ($r = -0.782$) and to *FAB* ($r = -0.694$). Therefore, when comparing outcomes from both tests, one can notice that, while G^* was highly affected by free asparagine concentration, the same result was not observed for M^*. Moreover, while slopes for G^* were negatively correlated to protein content and FAB, the contrary was observed for the slopes of the M^* curves. Finally, while the

changes in tan δ over time were not greatly affected by free asparagine concentration, protein content and *FAB*, changes in tan δ_M over time were negatively correlated to these parameters.

Figure 5.12. Absolute normalized slopes of the curves for M^* and tan δ_M (left) and G^* and tan δ (right) over time against free asparagine concentration (μg g^{-1}) and Pearson's r correlation analysis for non-yeasted dough samples. Data points represent means ± SE ($n \geq 2$).

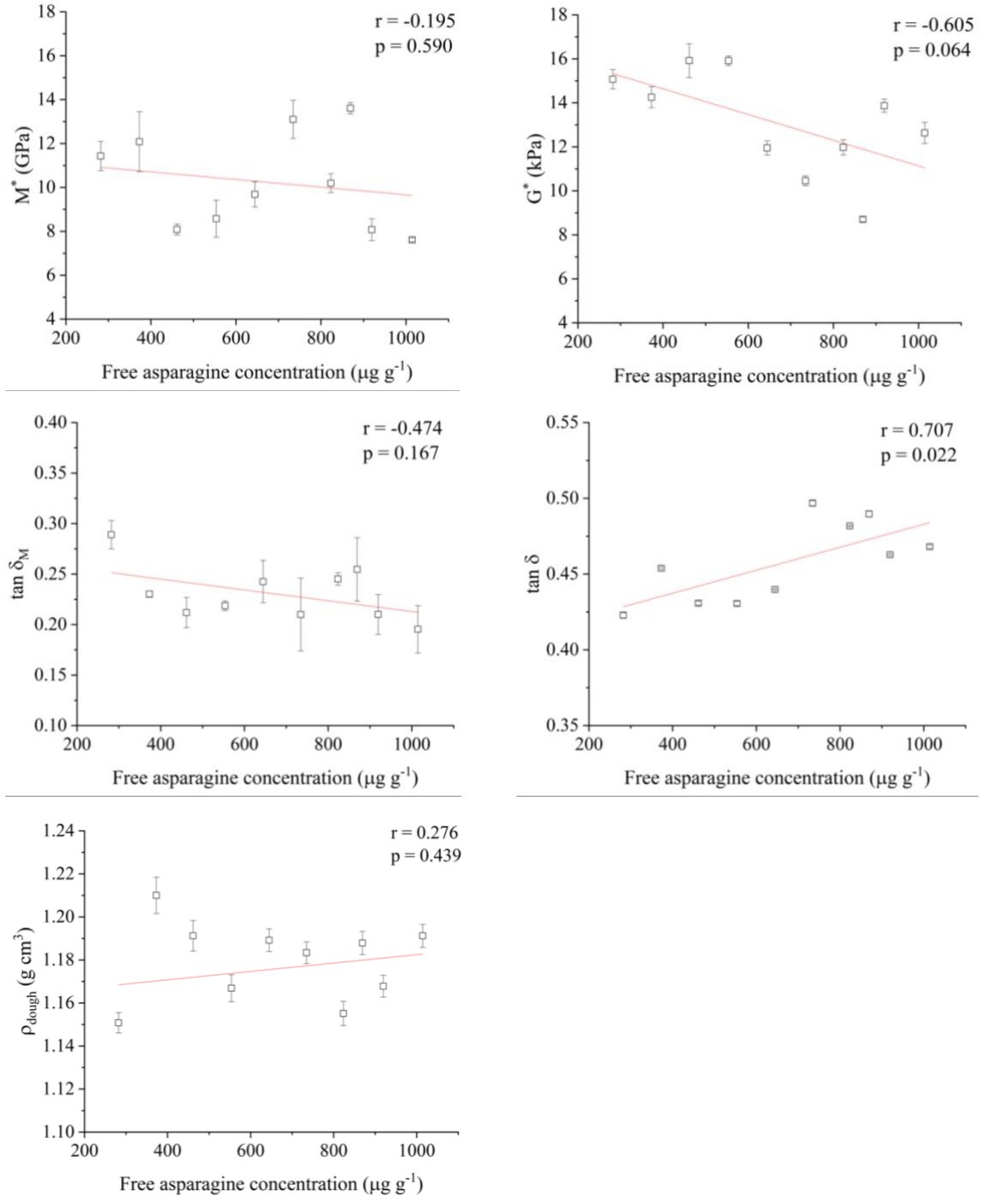


A possible explanation for the shear results lies in the type of molecular interactions between gluten proteins and starch that are sensed by linear shear oscillatory tests. In a study conducted by Amemiya & Menjivar (1992), the authors observed great contributions of short-range interactions between proteins, as well as proteins-starch granule interactions, on the rheological results when dough was evaluated under linear shear oscillatory tests. When analyzing their outcomes, the authors cited the influence of short-range interactions (van der Waals and hydrogen bonds) on the increased elasticity of wheat flour doughs (Amemiya & Menjivar, 1992). Therefore, hydrogen

bonds, mainly occurring due to the presence of gliadins, which contain high glutamine content (Delcour et al., 2012), might be affecting G^*, leading to more elastic-like doughs structures that suffer less changes over time. The negative correlation between slopes for G^* against protein content and FAB could support this hypothesis due to a possibly higher Gli/Glu ratio in samples with higher protein content, as mentioned before. Low-intensity ultrasound tests did not provide the same outcome. In Figure 5.12, changes in the slopes of magnitudes of the complex longitudinal moduli were not correlated to free asparagine concentration, whereas slopes for M^* over time were positively ($p > 0.05$) correlated to protein content and *FAB*. Finally, for both low-intensity ultrasound and shear oscillatory time sweep tests, samples with higher free asparagine concentration had less changes over time in their respective loss tangent (Figure 5.12). However, only changes in $\tan \delta_M$ were negatively ($p < 0.05$) correlated to free asparagine concentration, protein content and *FAB*. This outcome could support the previous hypothesis that samples with higher Gli/Glu ratios showed less changes over time in their rheological parameters due to a greater proportion of hydrogen bonds between gliadins and gliadins-starch. Finally, a moderately strong positive correlation was found between the slopes of the curves for $\tan \delta_M$ against stress relaxation parameters that are directly correlated to more elastic-like doughs, i.e., initial relaxation moduli G_0 ($r = 0.702$) and final relaxation moduli G_e ($r = 0.862$). This positive correlation indicates that samples with lower free asparagine concentration, that showed higher changes in $\tan \delta_M$, also have higher values for their shear relaxation moduli, positively correlated to more structured wheat flour doughs. Overall, although slopes of M^* over time did not show strong correlations with free asparagine concentration or the same outcome as linear shear oscillatory tests, changes in $\tan \delta_M$ over time were fairly strongly correlated to free asparagine concentration in the samples. Additionally, Figure 5.13 shows the correlation studies performed to evaluate if dough rheological parameters from low-intensity ultrasound and linear shear oscillatory tests, at a specific time, were correlated to free asparagine concentration.

In Figure 5.13, one can note a significant negative correlation between magnitude of the complex shear moduli (G^*) and free asparagine concentration. The magnitude of the complex longitudinal moduli (M^*) showed a much weaker and non-significant, correlation against free asparagine concentration, but a negative trend was also observed. Therefore, both outcomes indicate that samples with lower free asparagine concentration have overall larger magnitude for the complex moduli. When looking at the loss tangent from both tests, an opposite outcome was

Figure 5.13. Comparison between low-intensity ultrasound parameters, M^* and tan δ_M, measured at 10 MHz, and dough density (left), and linear shear oscillatory time sweep (at 10 Hz) parameters, M^* and tan δ (right) at 300 seconds plotted against free asparagine concentration (μg g^{-1}) and with Pearson's r correlation analysis. Data points represent means ± SE (n ≥ 2).



observed, with shear tests resulting in overall higher values for $\tan\delta$, but for $\tan\delta_M$ a negative correlation to free asparagine concentration. Finally, dough density (ρ) showed a positive, but non-significant, weak correlation to free asparagine content.

Correlation studies also found that G^* was negatively correlated ($p < 0.05$) to protein content ($r = -0.927$) and to *FAB* ($r = -0.728$). On the other hand, M^* showed positive ($p < 0.05$) correlation to protein content ($r = 0.640$) and positive ($p > 0.05$) correlation to *FAB* ($r = 0.495$). In addition, $\tan\delta$ was found positively correlated ($p < 0.05$) to protein content ($r = 0.902$) and to *FAB* ($r = 0.847$) while $\tan\delta_M$ and dough density (ρ) showed no correlations against protein content and *FAB*. These contradictory outcomes between the different tests indicate that while wheat flour doughs with lower free asparagine concentration, protein content, and *FAB* behaved more elastic-like, the same behavior was not observed under low-intensity ultrasound. A possible explanation for this result could be the magnitude of the frequencies utilized in both tests. While linear shear oscillatory time sweep tests utilized frequencies of 10 Hz, low-intensity ultrasound applied frequencies of 10 MHz. Additionally, longitudinal ultrasound and shear rheology measure different types of mechanical response in the dough, which also explains the different results obtained from the two tests. The capability of the low-intensity ultrasound tests of applying high frequencies and small strains could have resulted in a higher sensibility to short-range interactions present in the dough. Therefore, samples with higher protein content, and possibly higher Gli/Glu ratios, showed a more elastic-like dough, as measured by M^*, when measured at higher frequencies. This outcome was also supported by positive correlation of M' to protein content ($r = 0.648$, $p < 0.05$) and to *FAB* ($r = 0.495$, $p > 0.05$).

To our knowledge, there is a lack of studies in the literature that directly compares the magnitude of the complex shear moduli to magnitude of the complex longitudinal moduli obtained at a constant frequency over a period of time. However, Kidmose et al. (2001) evaluated doughs prepared from seven wheat varieties, over 40 minutes using low-intensity ultrasound and shear tests, and found a strong correlation between ultrasound velocity and G' ($r = 0.93$, $p < 0.01$) for samples prepared at optimum water absorption. For the present study, an opposite correlation was found for v_p against G' ($r = -0.601$, $p < 0.10$) and against G^* ($r = -0.614$, $p < 0.10$). However, v_p was strongly correlated to M^* ($r = 0.994$, $p < 0.05$) and to M' ($r = 0.992$, $p < 0.05$). Khorshidi et al. (2019) performed low-intensity ultrasound measurements at 10 MHz on doughs prepared from 23 hard red spring wheat cultivars. The authors reported the low-intensity ultrasound results in a

PCA biplot, showing a strong positive correlation between v_p and M' (Khorshidi et al., 2019), thus agreeing with the results from the present study. When comparing the coefficient of attenuation (α_{300s}), i.e., selected at 300 seconds of the test, with parameters obtained from shear oscillatory tests, the only significant ($p < 0.05$) correlation that was found was for α_{300s} against $\tan\delta$ ($r = -0.732$). This outcome indicates that samples showing a more elastic-like structure under shear tests, thus lower $\tan\delta$, had higher attenuation coefficients when measured under low-intensity ultrasound. Correlation studies found negative correlations between α against protein content ($r = -0.574$, $p < 0.10$) and *FAB* ($r = -0.664$, $p < 0.05$). Therefore, these results could support the hypothesis in section 5.4.2.1 that samples showing faster attenuation coefficient decay and lower attenuation coefficient could be suffering bubble disproportionation to a higher extent. This would occur due to the possible higher Gli/Glu ratio, as proposed before, which would produce more fluid-like doughs. Fan et al. (2013) utilized a breadmaking wheat flour dough to study the relaxation time after imposing ultrasonic strains to a dough piece. The authors observed that longer relaxation times for air-mixed doughs could be explained by the presence of bubbles (Fan et al., 2013). Glutenin polymers, which are responsible for storing elastic energy, undergo rearrangements while participating on the bubbles' interfacial area, which delays protein's structure rearrangements, thus the relaxation time (Fan et al., 2013). Therefore, samples with higher amount of gliadin could have less glutenin polymer to act on bubble interfacial area, thus producing a higher number of small bubbles which could lead to more bubble disproportionation over time. This conclusion could be supported by Fan (2006) who observed an increase in bubble size and narrow bubble distribution as the time after mixing passed by, an indication of bubble disproportionation. The gas present in the small bubbles would be transferred to bigger bubbles or diffused within the dough structure until lost to the atmosphere, which could have caused the decrease in wave attenuation in the dough. Therefore, samples with more elastic-like structure, thus overall lower free asparagine concentration, protein content and FAB, would have more bubble disproportionation and thus, higher attenuation coefficients.

Overall, although low-intensity ultrasound results were not equivalent to shear tests for non-yeasted doughs, the results showed that low-intensity ultrasound provides insight into dough rheological properties. The results show that low-intensity ultrasound should not be treated as a substitute for shear tests, but a complementary non-destructive test that is capable of examining dough properties at much higher frequencies than shear tests. However, the results reported in this

subsection are for non-yeasted doughs only. Therefore, the next subsection aims to provide an understanding of how parameters from low-intensity ultrasound change over time when yeast, an essential ingredient, is added to the dough, and how they compare with shear oscillatory time sweep tests also performed on yeasted doughs.

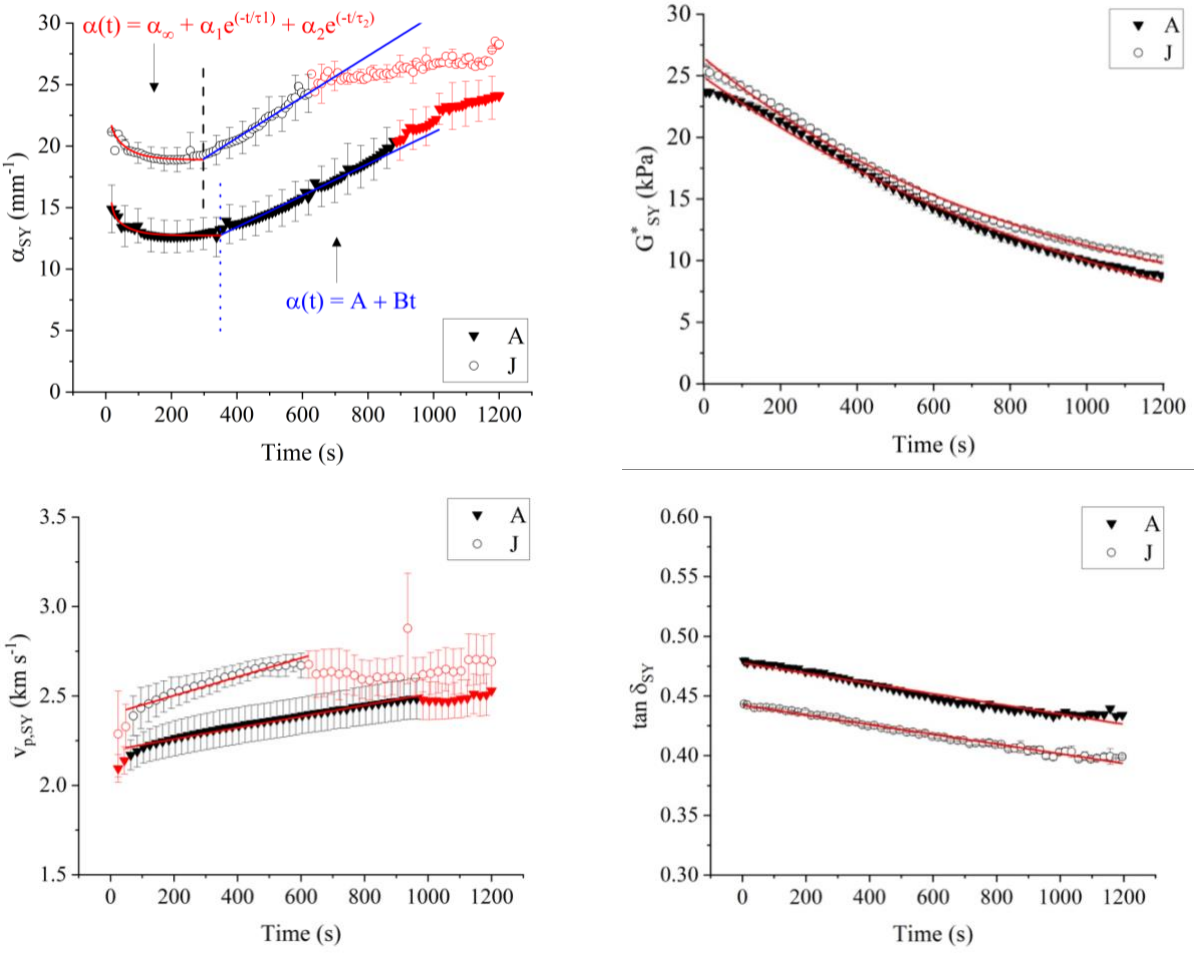
5.4.3 Yeasted dough rheological properties and free asparagine concentration

Yeasted wheat flour doughs were analyzed using low-intensity ultrasound tests for 1200 seconds, following the same procedure as the shear oscillatory time sweep tests. Because the fermentation process changes dough density over time, no density measurements and, therefore, no calculations of M^* and $\tan\delta_M$ were conducted for yeasted doughs. However, data obtained from transmitted pulses were used to obtain α and v_p over time, which are shown in Figure 5.14. Rheological parameters from yeasted doughs when evaluated under oscillatory time sweep tests (G^*_{SY} and $\tan\delta_{SY}$) are also shown in Figure 5.14. Moreover, fitting was performed, for each replicate, in order to evaluate changes in the parameters over time due to the activity of yeast.

From Figure 5.14, when yeasted dough samples were evaluated using shear oscillatory time sweep tests, G^*_{SY} exponentially decreases over time, while $\tan\delta_{SY}$ decreases linearly over time, showing an effect of yeast activity on dough rheological properties. After performing an exponential fitting on the magnitude of the complex shear moduli curve, i.e., G^*_{SY} as a function of time, using Eq. 4.9, the obtained exponential decay rate varied from 4.3×10^{-3} to 1.2×10^{-3} s^{-1} and the mean slope constants varied from -2.7×10^{-2} to -9.9×10^{-3} kPa s^{-1}. The goodness of fit (R^2) obtained by fitting Eq. 4.9 on G^*_{SY} as a function of time varied from 0.9986 to 0.9998, and the linear fitting performed for $\tan\delta_{SY}$ as a function of time using Eq. 5.7 had a goodness of fit of 0.9999 for all replicates. Changes in $\tan\delta_{SY}$ were less pronounced than those for the magnitude of the complex shear moduli, with mean slopes varying from -8.8×10^{-5} to -2.5×10^{-5} s^{-1}. For low-intensity ultrasound tests, the curves for α_{SY} over time, for all replicates, showed an initial decrease, similar to the relaxation process for non-yeasted doughs (Figure 5.8). However, as the yeast starts producing CO_2 in the dough samples, α_{SY} shows a linear increase over time, showing that fermentation significantly affects this parameter. Although less pronounced when compared to α_{SY}, changes in $v_{p,SY}$ were also observed over time, with an overall increase in $v_{p,SY}$ over time, i.e., positive slopes. In order to fit two different equations to evaluate changes in α_{SY}, a Piecewise Fit function was applied using Origin Pro (2021b). A Maxwell equation with two elements (Eq.

5.8) was fitted at the beginning of the test and a linear equation (Eq. 5.7) was fitted after the inflection point detected by the software. The goodness of fit (R^2) for α_{SY} varied from 0.9544 to 0.9980. Moreover, for the first half of the α_{SY} curve, the obtained parameters were the relaxation times ($\tau_{\alpha SY,1}$ and $\tau_{\alpha SY,2}$) utilized to evaluate the decay of α_{SY} after sample compression.

Figure 5.14. Attenuation coefficient (α_{SY}) and phase velocity ($v_{p,SY}$) over time (left), obtained at 10 MHz, and magnitude of the complex shear moduli (G^*_{SY}) and loss tangent (tan δ_{SY}) over time (right), obtained at 10 Hz, for yeasted wheat flour dough samples with the highest (A, ▼) and lowest (J, ◯) free asparagine concentration. Red lines are the Maxwell fitting with two elements (first part α_{SY}), linear fitting (second part α_{SY}, $v_{p,SY}$, tan δ_{SY}) and exponential fitting (G^*_{SY}). For low-intensity ultrasound, red data points show data that were masked and not considered in the fitting, due to noise in the acquired signal, mostly towards the end of the test, due to yeast growth. Data points represent means and error bars ($n \geq 2$).



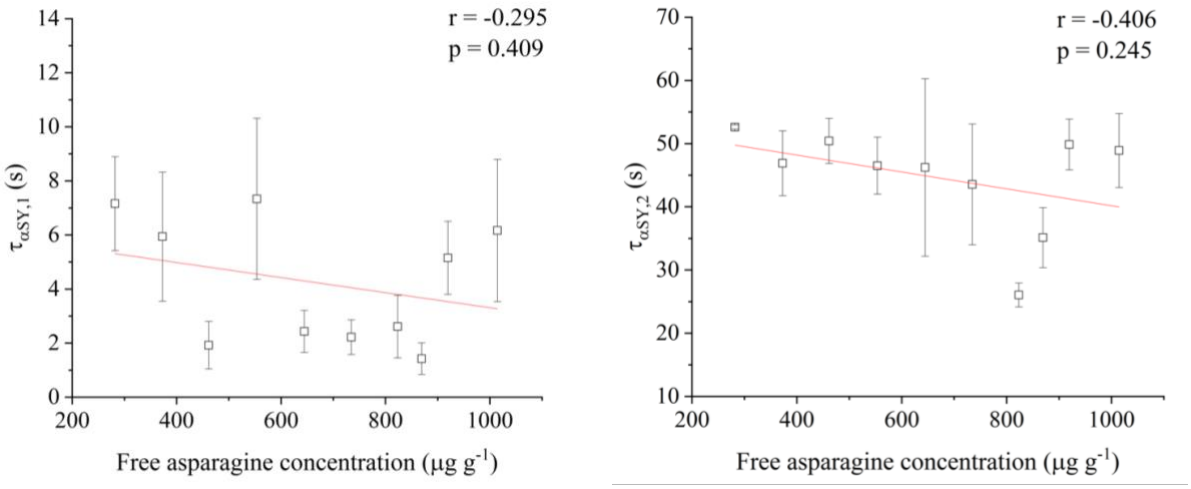
Meanwhile, for the second half of the α_{SY} curve, changes in the attenuation coefficient were evaluated through the slope of the linear fit, with mean slopes varying from 1.1×10^{-2} to 1.7×10^{-2} $mm^{-1}s^{-1}$. When performing linear fitting on $v_{p,SY}$ over time, for all replicates, a goodness of fit (R^2) of about 0.9999 was obtained, with mean slopes varying from 2.5×10^{-4} to 6.6×10^{-4} km s^{-2}. First, in order to investigate if initial changes in α_{SY} had a similar relationship to free asparagine as non-yeasted doughs, correlation studies for the relaxation times from the Maxwell model are shown in Figure 5.15. Although Figure 5.14 shows curves of α_{SY} over time as averages ($n \geq 2$), the fitting parameters obtained to analyze changes in the rheological parameters during fermentation were obtained for each replicate. Therefore, Figure 5.15 contains fitting parameters from individual replicates, which were averaged and used to assess uncertainties.

First, it is important to note that initial values (at *t*=0) of α and G^* from non-yeasted doughs were different than the initial values for α_{SY} and G^*_{SY} (yeasted doughs), with yeasted samples resulting in overall higher values at *t*=0 compared to non-yeasted samples (Figure 5.8.A, Figure 5.11, and Figure 5.14). Because low-intensity ultrasound and shear rheology tests were conducted 15 minutes post-mixing, these differences could be attributed to the yeast presence in the dough. In addition, from Figure 5.15 it was possible to observe that both relaxation times obtained for yeasted dough samples were about ten times smaller than the respective relaxation times obtained from non-yeasted doughs (Figure 5.9). However, the relationships for $\tau_{\alpha SY,1}$ and $\tau_{\alpha SY,2}$ against free asparagine concentration were similar to the same relationships for $\tau_{\alpha,1}$ and $\tau_{\alpha,2}$, i.e., negative but not significant at $p<0.05$. Correlation studies also found that $\tau_{\alpha SY,1}$ was negatively ($p < 0.10$) correlated to protein content ($r = -0.618$) and *FAB* ($r = -0.560$) and $\tau_{\alpha SY,2}$ was negatively correlated to protein content ($r = -0.787$, $p<0.05$) and to *FAB* ($r = -0.559$, $p<0.10$). Although not significant for non-yeasted doughs, a negative correlation was also found between relaxation times, protein content and *FAB*, as mentioned in section 5.4.2.1. Therefore, the similar correlation found for relaxation times against free asparagine concentration, protein content and *FAB*, for both yeasted and non-yeasted doughs, supports the previous hypothesis that samples with higher protein content and *FAB* could have higher Gli/Glu ratios. The higher proportion of gliadins would produce more fluid-like samples, which would be more prone to bubble rearrangements, thus causing a decrease of the wave's attenuation due to the presence of small bubbles in the dough.

As fermentation proceeds, the CO_2 gas, initially dissolved into the dough matrix, migrates to the bubbles and causes them to grow and this also affects the measured rheological properties. Correlation studies are shown in Figure 5.16 in order to understand how changes in dough parameters from low-intensity ultrasound and shear tests, due to this stage of the fermentation process, were correlated to wheat free asparagine concentration.

In Figure 5.16 it is possible to observe that during fermentation, samples that had higher free asparagine concentration showed lower slopes for the α_{SY} and $v_{p,SY}$ curves when fitting the linear portion of the curves (during fermentation), with a significant ($p<0.05$) negative correlation for the α_{SY} slopes. From shear tests, although not significant at $p<0.05$, a negative correlation was observed for the slope constants of G^*_{SY} against free asparagine concentration and a positive negative correlation for the slopes of $\tan \delta_{SY}$ against this free amino acid. When evaluating the results for yeasted doughs analyzed under shear tests, it was hypothesized that samples with lower protein content, thus lower Gli/Glu ratios, would be affected to a greater extent by the fermentation process, explaining the dough softening effect observed in Figure 5.14.

Figure 5.15. Relationship between relaxation times from the attenuation coefficient decay against free asparagine concentration (μg g^{-1}) for yeasted dough samples. Data points represent means and SE ($n \geq 2$).

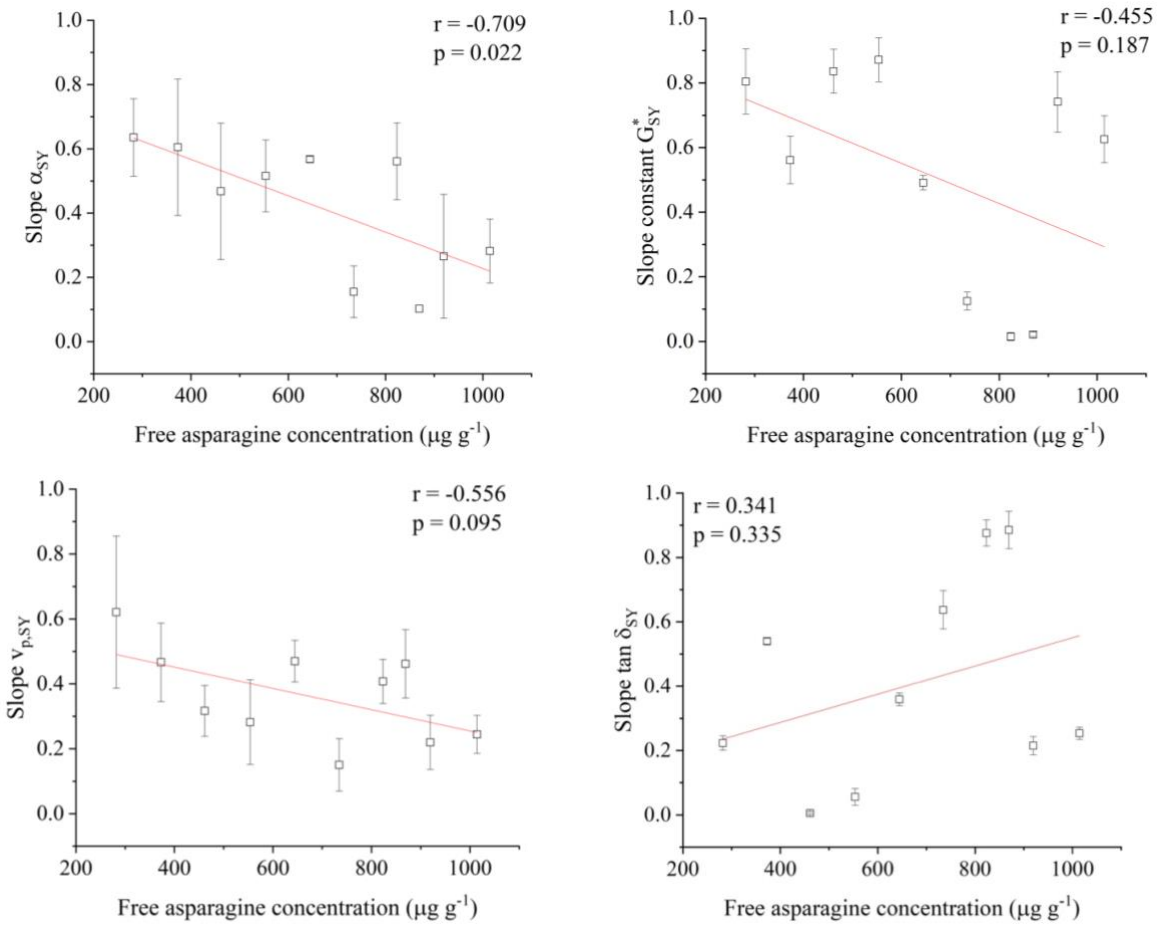


During yeast growth, fermentation metabolites can affect dough rheological properties via changes in gluten entanglements and aggregation, which affect the dough's elastic-like

characteristics, with the structure becoming softer (Rezaei et al., 2016). For example, studies involving sourdough fermentation have shown that glutenins were affected to a greater extent by proteolytic activities that took place in the dough after the pH was decreased by fermentation metabolites (Loponen et al., 2004; Wieser et al., 2008).

Correlation studies also found that slope constants of G^*_{SY} were negatively correlated ($p<0.05$) to protein content ($r = -0.975$) and to *FAB* ($r = -0.765$) while slopes of $\tan \delta_{SY}$ were positively correlated to protein content ($r = 0.950$, $p < 0.05$) and to *FAB* ($r = 0.626$, $p < 0.10$). These correlation results support the hypothesis that samples with higher protein content, thus higher Gli/Glu ratio had a less structured gluten network, thus were less affected by the fermentation process, i.e., they exhibited lower slope constants for G^*_{SY}.

Figure 5.16. Absolute normalized slopes of the curves for α_{SY} and $v_{p,SY}$ from low-intensity ultrasound (left) and for G^*_{SY} and tan δ_{SY} over time (right) against free asparagine concentration (μg g^{-1}) and Pearson's r correlation analysis for yeasted dough samples. Data points represent means ± SE (n ≥ 2).



When evaluating low-intensity ultrasound results in Figure 5.16, one can observe that samples with higher free asparagine also had less change in α_{SY} and $v_{p,SY}$ over time. Correlation studies also found that slopes for α_{SY} were negatively correlated to protein content (r = -0.505, p > 0.05) and to *FAB* (r = -0.740, p < 0.05), while slopes for $v_{p,SY}$ were not correlated to protein content and weakly correlated to *FAB* (r = -0.431, p > 0.05). Elmehdi et al. (2003) studied the effects of fermentation on dough properties, prepared from a single wheat source and at two conditions (atmospheric pressure and under vacuum), using low-intensity ultrasound at frequency of 50 kHz. Contrarily from the results of the present study, Elmehdi et al. (2003) observed a

decrease in ultrasonic velocity as a function of fermentation time. Additionally, the authors observed that samples mixed under vacuum, thus with a smaller amount of air bubbles, had a greater ultrasonic velocity at the beginning of the test (Elmehdi et al., 2003). After 20 minutes of fermentation, the authors observed that, due to CO_2 generation from yeast growth, the velocity had a steep decay. The authors hypothesized that dough acidification during fermentation increased the positive charge on the gluten proteins causing them to repulse each other, thus weakening the dough structure (Elmehdi et al., 2003). Another effect of acidification on dough mixed at atmospheric pressure, i.e., containing air bubbles, was the shrinkage of the air bubbles (Elmehdi et al., 2003). This occurred because as proteins repulsed each other, a compression force was applied on the air bubbles' surface, thus causing them to shrink (Elmehdi et al., 2003). Although Elmehdi et al. (2003) utilized a much lower frequency than the present study, Leroy et al. (2008) reported phase velocities of around 2 km s^{-1} when wheat flour doughs were analyzed at frequencies around 10 MHz, which is in accordance with values found in the present study (Figure 5.14). Although phase velocity did not correlate significantly to free asparagine concentration, protein content and *FAB*, it was possible to observe in Figure 5.14 an increase in velocity in the dough during fermentation, i.e., positive slopes. The increase in dough velocity could be caused by dough softening effects due to fermentation metabolites, as hypothesized by Elmehdi et al. (2003), which would shift the bubble resonance to lower frequencies. This would reduce the impact of the tail of the resonance peak on $v_{p,SY}$ in the high frequency region (10 MHz).

When evaluating the changes in attenuation coefficient over the course of the fermentation process, those dough samples with lower slopes for α_{SY} over time could indicate that their dough structure was more fluid-like, thus less strong and with an inability to hold gas during fermentation, possibly due to higher Gli/Glu, as previously hypothesized. Although shear tests have shown that samples with lower free asparagine concentration had greater changes in dough softening during fermentation, these samples have shown a possibly greater ability to hold gas bubbles during fermentation than samples with higher free asparagine. Therefore, low-intensity ultrasound tests have been able to provide insightful information about dough changes during fermentation, with results being in accordance with the ones obtained from shear tests.

5.4.4 Field data and bread specific volume

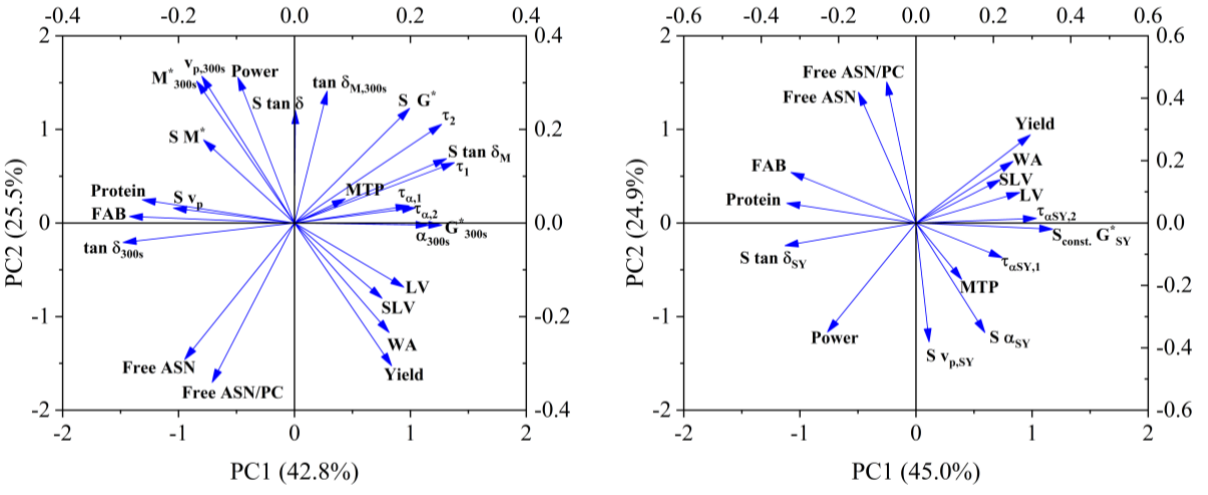
Shear rheology results are utilized, mainly by the scientific community, to evaluate wheat flour dough rheological studies at small and large shear strains and stresses (Dobraszczyk &

Morgenstern, 2003). However, the use of low-intensity ultrasound as a low-cost and non-destructive alternative test has been getting the attention of the scientific community to evaluate wheat flour dough properties (Khorshidi, Storsley, et al., 2018; Scanlon & Page, 2015). Overall, wheat flour dough rheological analyses aim to understand dough properties and determine dough suitability for a specific final product, e.g., dough with high gluten strength to yield bread with acceptable loaf volume (Delcour & Hoseney, 2010a). Therefore, the present study aimed also to understand how dough properties obtained from low-intensity ultrasound tests were correlated to loaf volume in comparison to results from shear tests. For this comparison, a Principal Component Analysis (PCA) was performed including low-intensity ultrasound and shear results from white wheat flours, and loaf volume (*LV*, cm^3) and specific loaf volume (*SLV*, $cm^3\ g^{-1}$) from whole-wheat breads. The PCA analysis also included grain yield ($kg\ ha^{-1}$) and wheat characteristics, i.e., grain protein content (%), free asparagine concentration ($\mu g\ g^{-1}$), and the proportion of free asparagine to total protein content ($mg\ g^{-1}$), as well as white flour farinograph water absorption (*FAB*, 14% m.b.), and the bread preparation parameters from whole-wheat flours, i.e., water absorption (*WA*, %), mixing time to peak (*MTP*, min), and peak power (*Power*, W).

In Figure 5.17, the PCA biplot for non-yeasted doughs shows that the first two principal components account for about 68% of the total variation in the parameters, while in the PCA biplot for yeasted doughs the first two components account for about 70% of the total variation in the data. For non-yeasted doughs, in Figure 5.17, the only two parameters that were positively correlated ($p < 0.05$) to *LV* and *SLV* were the relaxation times obtained from the attenuation decay curve, i.e., $\tau_{\alpha,1}$ and $\tau_{\alpha,2}$. Therefore, samples with longer relaxation times for the attenuation coefficient decay resulted in higher *LV* and *SLV*. Based on the previous discussion, it was hypothesized that samples with shorter relaxation times have higher Gli/Glu which was resulting in weaker glutens. The higher Gli/Glu ratio could be leading to less glutenin polymer available to form air bubbles during mixing. Glutenin polymers are at the interfaces of bubbles, and possibly undergo rearrangements when dough is subjected to ultrasonic strains (Fan et al., 2013). Therefore, samples with higher Gli/Glu could be forming smaller bubbles during mixing. The high number of small air bubbles could accelerate the rate of bubble disproportionation, thus the formation of bigger bubbles with time, which would cause greater wave attenuation. On the other hand, samples with lower Gli/Glu would have stronger glutens which would keep the bubble walls' structure, thus retaining more small bubbles in the dough to act as scattering agents. Moreover, the higher

amount of glutenin polymer participating at the bubbles' interfaces could store more elastic energy from dough compression and delay dough relaxation. Therefore, the slower protein rearrangement could interfere with bubble disproportionation, retaining more small bubbles in the dough over time, thus delaying the attenuation decay. Therefore, the positive correlation between $\tau_{\alpha,1}$, $\tau_{\alpha,2}$, *LV* and *SLV* shows that samples with higher relaxation times, thus lower Gli/Glu and high gluten strength, resulted in high *LV* and *SLV*.

Figure 5.17. PCA biplots for low-intensity ultrasound and shear tests for non-yeasted doughs (left) and for yeasted doughs (right), grain yield (Yield, kg ha^{-1}), specific loaf volume (*SLV*, cm^3 g^{-1}), loaf volume (*LV*, cm^3), grain protein (PC, %), water absorption (*FAB*, %), free asparagine concentration (Free ASN, μg g^{-1}), proportion of free asparagine concentration to total protein content (Free ASN/PC), whole-wheat *WA* (%), *MTP* (min) and *Power* (W). The letter "S" means slope and the 300s means data evaluated at 300 seconds of the test.



When evaluating the PCA for non-yeasted doughs in Figure 5.17, it was possible to observe that the parameters yield, *WA*, *SLV*, *LV*, $\tau_{\alpha,1}$, $\tau_{\alpha,2}$, τ_1, τ_2, slope (S) for tan δ_M, α_{300s}, slope (S) for G^*, G^*_{300s} were on the opposite side of free asparagine, protein content, *FAB*, slope (S) for v_p, slope (S) for M^*, $v_{p,300s}$, M^*_{300s}, and tan δ_{300s} along PC1. Conversely, free asparagine, yield, free asparagine/protein content were on the opposite side as τ_2, slope (S) for G^*, Slope (S) for tan δ, $v_{p,300s}$, M^*_{300s}, and tan $\delta_{M,300s}$ along PC2. Parameters that are on the same side along PC1 or PC2 show a positive, but not necessarily significant at $p<0.05$, correlation. On the other hand, a

negative, but not necessarily significant at $p<0.05$, correlation could be expected between parameters on opposite sides along PC1 or PC2. Unfortunately, there is a lack of an extensive list of published studies correlating low-intensity ultrasound parameters to shear test results and bread quality parameters, such as loaf volume, in the literature. However, when studying wheat flour doughs prepared from six treatments using low-intensity ultrasound, Peressini et al. (2016) reported a high correlation between α and bread volume for frequencies at 0.5 MHz. Although a much higher frequency was utilized in the present study, it was found that α_{300s} was positively, but not significantly at $p < 0.05$, correlated to *SLV* and *LV*. Additionally, Khorshidi et al. (2019) evaluated dough rheological properties of 23 Canadian Hard Red Spring varieties using low-intensity ultrasound at 10 MHz, empirical tests (farinograph and extensograph), as well as the final bread loaf volume. When correlation studies were conducted considering the 23 wheat varieties together, no significant correlations among rheological parameters were found at $p < 0.05$, with only the bread mixing time being correlated at $p < 0.10$ to v_p and M' (Khorshidi et al., 2019). The authors attributed the lack of correlation to the small differences in quality among the samples. To investigate ultrasound's potential to discriminate between wheat varieties, the authors divided the 23 wheat varieties into two groups (Khorshidi et al., 2019). The first group was composed of 11 varieties displaying a wide variation with respect to grain and flour characteristics and the second group was composed of the 12 remaining samples (Khorshidi et al., 2019). When the authors divided the wheat varieties, better correlation coefficients were found, showing the ultrasound capability of discrimination among wheat varieties (Khorshidi et al., 2019). As opposed to the findings of Khorshidi et al. (2019), no significant correlations were found in the present study between MTP from bread preparation and low-intensity ultrasound parameters. However, the *Power* parameter, which is the power measured when the bread dough is mixed to its peak, was positively ($p < 0.05$) correlated to $v_{p,300s}$, M^*, and $\tan \delta_{M,300s}$, indicating that these parameters could also be useful for dough rheological characterization.

For yeasted dough samples, the PCA in Figure 5.17 shows that yield, *SLV*, *LV*, $\tau_{\alpha SY,2}$ and constant slope ($S_{const.}$) for G^*_{SY} were on opposite sides to protein content, *FAB*, *Power*, and slope (S) for $\tan \delta_{SY}$ along PC1. Moreover, *Power*, slope (S) for $v_{p,SY}$ and slope (S) for α_{SY} were on opposite sides to free asparagine, free asparagine/protein content, and yield along PC2. However, there were no significant correlations between *SLV*, *LV* and rheological parameters from low-intensity ultrasound for yeasted doughs. On the other hand, all rheological parameters from low-

intensity ultrasound and shear tests were, to some extent, negatively correlated to free asparagine concentration. Therefore, even though the use of low-intensity ultrasound tests to monitor dough fermentation did not provide significant correlations to final bread quality, it was shown to be more effective than shear tests in studying the relationship between free asparagine concentration and dough properties under fermentation. The main example would be the correlation between the slope for α_{SY} against free asparagine concentration, which was significant ($p<0.05$) and stronger ($r = -0.709$) than the slope constant for G^*_{SY} ($r = -0.454$).

In summary, although no significant correlations were found among most of the dough rheological parameters and bread quality, the PCA biplots could provide a good overview of dough rheological parameters in relation to wheat characteristics and bread quality parameters. The lack of significant correlation between *SLV*, *LV* and bread dough mixing parameters to dough rheological properties from low-intensity ultrasound tests could be attributed to the wheat varieties utilized in this study. The same observation from Khorshidi et al. (2019) could be applied to the present study, i.e., small differences among the studied samples could contribute to the lack of correlation. The wheat varieties involved in this study are all suitable for breadmaking, thus resulting in *SLV* and *LV* with a tight range of variation, i.e., *SLV* varied from 4.6 to 5.5 $cm^3\ g^{-1}$ and *LV* varied from 624 to 745 cm^3. Therefore, although samples varied in free asparagine concentration, decreasing free asparagine concentration in the wheat had no significant detrimental effects on the final product quality.

5.5 Conclusion

Food safety concerns related to acrylamide in bread can be addressed by reducing free asparagine content in wheat. However, it is necessary to investigate if reducing free asparagine in wheat could negatively impact dough rheological properties and final bread quality. Low-intensity ultrasound, a low-cost and a non-destructive test, could be an alternative to investigate the relationship between free asparagine concentration and dough rheological properties. After analysis of the relationship of low-intensity parameters (α and v_p) against free asparagine concentration, protein content and *FAB*, the frequency of 10 MHz was selected to retrieve data for correlation studies. When comparing the coefficient of attenuation decay over time after sample compression with shear stress relaxation data, although not significant for low-intensity ultrasound, relaxation times obtained from both tests were negatively correlated to free asparagine concentration. It was hypothesized, from negative correlations between relaxation times against

protein content and *FAB*, that samples with higher free asparagine and protein content had faster relaxation decays, which could be caused by bubble disproportionation. After the relaxation period of 1800 seconds, it was shown that low-intensity ultrasound resulted in different outcomes, for example for M^*, when compared to G^* from linear shear oscillatory time sweep tests. Although not all parameters from low-intensity ultrasound showed significant correlations to free asparagine concentration, the slopes for $\tan \delta_M$ over time showed a fairly strong negative correlation to free asparagine concentration. Results from low-intensity ultrasound supported the previous hypothesis that samples with higher protein content and *FAB* could have higher Gli/Glu ratios, thus more fluid-like doughs. Additionally, it was hypothesized that samples with higher protein content and *FAB* could be having more bubble disproportionation, causing less wave attenuation over time when compared to samples with lower protein content and FAB. When evaluating changes in dough properties during fermentation, slopes for α_{SY} over time from low-intensity ultrasound showed better correlation to free asparagine concentration than slope constant of G^*_{SY} over time from shear tests.

Additionally, the lack of correlation between dough rheological and bread quality parameters should not be attributed specifically to the rheological tests, but to the fact that samples involved in this study were all suitable for breadmaking. Inclusion of wheat varieties with a wider variation in protein content and composition could result in different outcomes. Finally, it was possible to conclude that low-intensity ultrasound could be successfully used as a complementary test to investigate the relationship between free asparagine concentration and dough rheological properties.

6 General discussion

Awareness regarding the presence of acrylamide, a probable carcinogen, in bakery products has been the subject of attention in the past 15-20 years. Strategies to mitigate acrylamide formation in bakery products have been investigated in the literature (Sarion et al., 2021). One effective strategy is to utilize wheat with low levels of free asparagine, because this amino acid is a precursor to acrylamide formation (Mottram et al., 2002; Stadler et al., 2002). However, factors affecting wheat free asparagine concentration also affect wheat protein content and composition (Godfrey et al., 2010; Johansson et al., 2013; Park et al., 2014; Wilson et al., 2020), thus affecting dough rheological properties. Little is known in the literature on the relationship between wheat free asparagine concentration and dough rheological properties. Thus, this thesis aimed to investigate the relationship between wheat free asparagine concentration and dough properties using different rheological tests, i.e., empirical tests, shear rheometry, and low-intensity ultrasound. This thesis also sought to investigate if changes in dough rheological properties during fermentation were affected by wheat free asparagine concentration.

To fulfill the goal of this thesis, empirical tests were performed and discussed in Chapter 3 in a comprehensive investigation of the effects of environment, genotype, and fertilization treatments on dough rheological properties. The relationship between rheological properties of non-yeasted doughs and wheat free asparagine concentration was also investigated in Chapter 3. This relationship was also studied in Chapter 4 by analyzing the rheological properties of non-yeasted doughs prepared from 10 selected wheat flour samples with a wide variation in free asparagine concentration using shear fundamental rheological tests. In Chapter 5, low-intensity ultrasound was applied to the same set of 10 samples to study the rheological properties of non-yeasted doughs, which were also correlated to wheat free asparagine concentration. Additionally, shear oscillatory tests and low-intensity ultrasound were used to investigate the properties of yeasted doughs in Chapter 4 and 5, respectively. The results from both chapters were compared to wheat free asparagine concentration data to investigate the effect of this free amino acid on dough rheological properties during fermentation.

To obtain bread with a desirable loaf volume, wheat flour dough must have an adequate balance between two main properties, strength and extensibility. Glutenins form intra and inter-polypeptide disulfide bonds which provide strength, or elastic-like characteristics, to the dough structure (Cauvain, 2015b). Gliadins, on the other hand, allow the dough to expand, thus conferring fluid-like characteristics, i.e., extensibility (Cauvain, 2015b). Wheat flour doughs that are

excessively strong or excessively extensible will produce bread with poor loaf volume (Spies, 1990). Thus, an adequate dough strength/extensibility balance is necessary to retain the carbon dioxide inside the bubbles and to allow the bubbles to grow in volume during fermentation (Cauvain, 2015b; Spies, 1990). Dough rheological tests allow one to evaluate dough strength and extensibility in order to investigate if a reduction in wheat free asparagine concentration will be detrimental to bread quality.

Although rheological studies are commonly conducted on non-yeasted doughs (Dobraszczyk & Morgenstern, 2003), yeast is an essential ingredient in breadmaking (Cauvain, 2015a). During fermentation, dough rheological properties change due to yeast metabolites affecting the gluten's structure (Spies, 1990; Verheyen et al., 2014). Therefore, yeast's effect on the relationship between wheat free asparagine concentration and dough rheological properties must also be investigated. Thus, the main goals of this chapter are to investigate if the wheat free asparagine concentration affects dough strength and extensibility and to evaluate if the relationship between wheat free asparagine and dough properties is affected by yeast addition.

6.1 Non-yeasted doughs

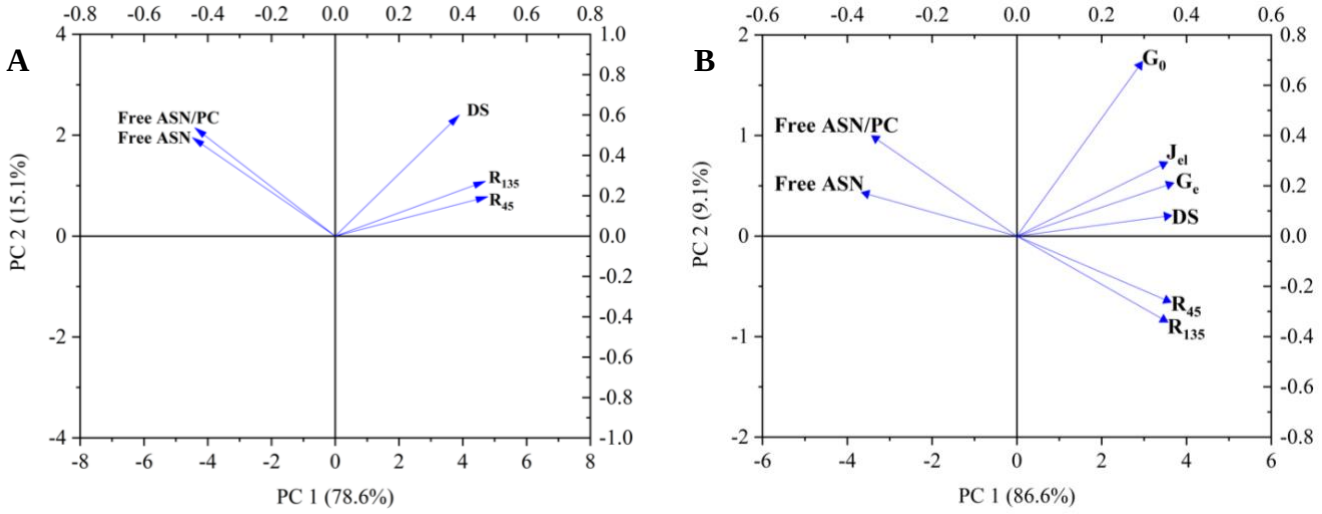
6.1.1 Strength of non-yeasted doughs

As aforementioned, dough strength is an essential property to guarantee gas-holding capacity during fermentation (Cauvain, 2015b; Spies, 1990). Thus, the main goal of this section is to investigate if dough strength, evaluated through empirical, shear rheometry and low-intensity ultrasound tests, is affected by wheat free asparagine concentration. In order to fulfill the goal of this section, some parameters translating into gluten strength were selected and the Pearson's correlation coefficients (r) against wheat free asparagine concentration were calculated.

Additionally, Principal Component Analysis was conducted to illustrate, in one biplot, how the various selected dough strength parameters and the free asparagine concentration were associated, shown in Figure 6.1. In Figure 6.1.A, the first two components account for about 94% of the total variation in the data and includes the parameters from the empirical tests, wheat free asparagine concentration, and the ratio of free asparagine/protein content for the 128 wheat flour samples. The selected parameters were the dough stability time (*DS*) from the farinograph and, from the extensograph, the maximum resistance to extension at 45 and 135 minutes (R_{45} and R_{135}). In Figure 6.1.B, the first two components account for about 96% of the total variation in the data. Figure 6.1.B includes parameters from empirical and shear rheology tests, wheat free asparagine

concentration, and the ratio of free asparagine/protein content for the 10 selected samples as described in Chapter 4. The selected parameters from empirical tests were the same as in Figure 6.1.A. For shear tests, three parameters were selected, i.e., the relative elastic part (J_{el}) from the creep-recovery test and, from the stress relaxation test, the initial (G_0) and final (G_e) relaxation moduli.

Figure 6.1. PCA biplots for the full set of 128 wheat flour samples (A) and for the selected 10 wheat flour samples (B), including parameters from empirical rheological tests (A and B), shear rheometry (B), wheat free asparagine concentration (Free ASN, μg g^{-1}), and proportion of free asparagine concentration relative to total protein content (Free ASN/PC , mg g^{-1}).



Correlation studies for the rheological parameters in Figure 6.1 against free asparagine concentration and the ratio of free asparagine/protein content showed that the correlation coefficients were significant at $p < 0.05$. According to Xie et al. (2021), the wheat free asparagine concentration, quantified in 128 whole-wheat flour samples, was significantly ($p < 0.05$) affected by growing environment, genotype, and fertilization treatments (nitrogen and sulfur). From Chapter 3, the main factors affecting dough rheological parameters were growing environment and genotype, with little or no significant ($p < 0.05$) effect from fertilization treatments. In Figure 6.1.A, dough strength parameters were negatively correlated to wheat free asparagine concentration and to the ratio of free asparagine/protein content along PC1. Correlation coefficients for *DS* against free asparagine concentration ($r = -0.617$) and against the ratio of free

asparagine/protein content (r = -0.562) were significant at $p < 0.05$. The DS parameter from the farinograph indicates how resistant the dough is to the deformations occurring during the mixing process, with longer *DS* indicating stronger doughs (T. D. Hadnadev et al., 2011). From the extensograph tests, stronger negative correlations were found between dough strength (R_{45} and R_{135}) and free asparagine concentration, i.e., higher Pearson's correlation coefficients values as shown below, when compared to *DS* from farinograph. The extensograph provides the maximum resistance to extension until rupture after resting the sample for 45 minutes (R_{45}) or for 135 minutes (R_{135}) (Bloksma, 1972). Dough samples showing high values for R are known to be strong (Bloksma, 1972; S. Wang et al., 2013). Correlation studies showed that R_{45} was negatively correlated to free asparagine concentration (r = -0.744) and to the ratio of free asparagine/protein content (r = -0.729). The dough strength parameter R_{135} was also negatively correlated to free asparagine concentration (r = -0.698) and to the ratio of free asparagine/protein content (r = -0.691). Therefore, the biplot in Figure 6.1.A shows that wheat samples with lower free asparagine concentration produced stronger doughs compared to wheat samples with higher free asparagine concentration.

In the biplot from Figure 6.1.B, most of the rheological parameters from the 10 selected samples were negatively correlated to free asparagine concentration along PC1. In Figure 6.1.B, the parameters from empirical tests were also all negatively correlated ($p < 0.05$) to free asparagine concentration and to the ratio of free asparagine/protein content. Additionally, the Pearson's correlation coefficients were stronger for this set of samples when compared to the full set of grain samples. The dough strength rheological parameter R_{45} was strongly correlated to free asparagine concentration (r = -0.939) and to the ratio of free asparagine/protein content (r = -0.915). The parameter R_{135} was also strongly correlated to free asparagine (r = -0.916) and to the ratio of free asparagine/protein content (r = -0.913). The dough strength parameter from the farinograph, i.e., *DS*, was also fairly strongly correlated ($p < 0.05$) to free asparagine concentration (r = -0.894) and to the ratio of free asparagine/protein content (r = -0.803). This overall negative correlation between dough strength, as measured by *DS*, R_{45}, and R_{135}, and wheat free asparagine concentration is in agreement with results from Malunga et al. (2019), although their correlation coefficients were only significant at $p < 0.05$ for R_{135}.

Agreeing with empirical tests, shear strain tests performed outside of the LVR also showed that dough strength was negatively correlated to free asparagine concentration. From a creep-

recovery test, the J_{el} indicates how elastic-like the dough is, i.e., how much strain energy was stored when the sample was subjected to a constant shear stress (Jekle & Becker, 2011). The parameter J_{el} had a fairly strong correlation coefficient against free asparagine concentration (r = -0.837) and against the ratio of free asparagine/protein content (r = -0.720). From shear stress relaxation tests a similar outcome was observed. Both the initial relaxation moduli (G_0) and the relaxation moduli at the end of the test (G_e) are obtained by dividing the shear stress ($\sigma(t)$) by the constant shear strain (γ), and are an indication of the material's elasticity (Van Vliet, 2014; Yang et al., 2019). A significant ($p < 0.05$) correlation was found between G_0 and free asparagine concentration (r = -0.698). For G_e, a stronger negative correlation to free asparagine concentration (r = -0.916) and to the ratio of free asparagine/protein content (r = -0.821) was found. Overall, PCA analysis and correlation studies showed that dough strength, when evaluated under large strain tests (empirical and fundamental), was negatively ($p < 0.05$) affected by increasing amounts of free asparagine concentration in the wheat.

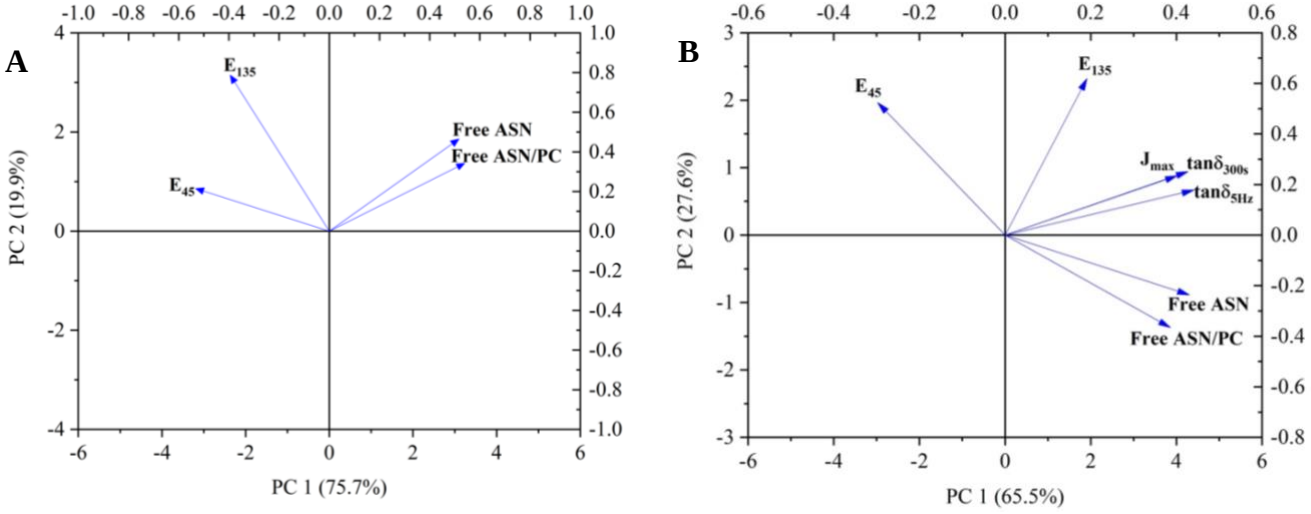
6.1.2 Extensibility of non-yeasted doughs

Dough extensibility is also an essential component in the breadmaking process (Cauvain, 2015b; Spies, 1990). In order to obtain a high-quality product, the wheat flour dough must have an adequate extensibility to allow the expansion of air bubbles during fermentation (Cauvain, 2015b). However, excessive dough extensibility results in a bread with undesirable shape and volume (Spies, 1990). Therefore, the relationship between wheat free asparagine and dough extensibility parameters, obtained from empirical tests and shear rheometry, will be explored in this section. The main goal of this section is to understand if overall dough extensibility is affected by wheat free asparagine concentration. Therefore, some parameters that translate into dough extensibility were selected from the empirical and shear rheology tests, and their Pearson's correlation coefficients (r) against wheat free asparagine concentration were calculated.

Principal Component Analysis was conducted to illustrate, in one biplot, how the selected dough extensibility parameters and the free asparagine concentration were associated, shown in Figure 6.2. In Figure 6.2.A, the first two components account for about 96% of the total variation in the data and include the parameters from the extensograph, wheat free asparagine concentration, and the ratio of free asparagine/protein content for the 128 wheat flour samples. The parameters selected from the empirical tests were the dough extensibility after 45 minutes (E_{45}) and after 135 minutes (E_{135}) of resting time. In Figure 6.2.B, the first two components account for about 93% of

the total variation in the data. Figure 6.2.B includes parameters from both the extensograph and shear rheometry, wheat free asparagine concentration, and the ratio of free asparagine/protein content for the same 10 selected samples described in 6.1.1. The selected parameters from the extensograph were the same as in Figure 6.2.A. For shear rheometry performed within the LVR, two parameters were selected, i.e., the loss tangent at 300 seconds of testing (tan δ_{300s}) from time sweep tests and the loss tangent at a frequency of 5Hz (tan δ_{5Hz}) from frequency sweep tests. For tests performed outside of the LVR, the maximum creep compliance (J_{max}) was selected from creep-recovery tests.

Figure 6.2. PCA biplots for the full set of 128 wheat flour samples (A) and for the selected 10 wheat flour samples (B), including parameters from the extensograph (A and B), shear rheometry (B), wheat free asparagine concentration (free ASN, μg g^{-1}), and proportion of free asparagine concentration relative to total protein content (Free ASN/PC, mg g^{-1}).



The extensogram, i.e., the force-extension curve from the extensograph, allows one to obtain the dough extensibility, which is the length of the dough at the point of rupture (Bloksma, 1972). From Figure 6.2.A, dough extensibility parameters were negatively correlated to free asparagine concentration and to the ratio of free asparagine/protein content along PC1. Additionally, correlation studies showed that the negative correlations between dough extensibility and free asparagine concentration were significant at $p < 0.05$. The parameter E_{45} showed much stronger correlations to free asparagine concentration ($r = -0.730$) and to the ratio of free

asparagine/protein content (r = -0.781) than E_{135} against free asparagine concentration and the ratio of free asparagine/protein content (r = -0.343 and r = -0.449, respectively). Although most of the samples showed decreased extensibility resulting from the resting period, which is a common characteristic for breadmaking wheat (Sobczyk et al., 2017), some high free asparagine concentration samples showed greater extensibility over time. Indeed, only 13 samples out of 128 samples had their E_{135} values larger than E_{45}, but the majority of these (11 samples) contained high levels of free asparagine (equal or higher than 657 μg g^{-1}). In Figure 6.2.B, a fairly strong correlation was still observed between E_{45} and free asparagine concentration (r = -0.793) and the ratio of free asparagine/protein content (r = -0.855). However, a shift in the correlation between E_{135} and free asparagine could be observed along PC1 (Figure 6.2.B). Additionally, the correlation coefficients were weak and non-significant at $p < 0.05$ for E_{135} against free asparagine concentration (r = 0.134) and the ratio of free asparagine/protein content (r = -0.050). On the other hand, the selected parameters from shear tests were positively correlated to free asparagine concentration along PC1 in Figure 6.2.B. Correlation studies showed a positive relationship ($p < 0.05$) between $\tan \delta_{300s}$ and free asparagine concentration (r = 0.707). Also, the parameter $\tan \delta_{5Hz}$ was shown to be correlated to free asparagine concentration (r = 0.770) and to the ratio of free asparagine/protein content (r = 0.640). The parameter loss tangent ($\tan \delta$), obtained from linear oscillatory shear tests, is inversely correlated to the material's elastic-like properties, i.e., high values of $\tan \delta$ indicate a more fluid-like material (Van Vliet, 2014). This parameter is calculated by dividing the material's viscous response (G'') by its elastic response (G') to the applied shear stress (Van Vliet, 2014). When evaluated under creep-recovery tests, dough samples also showed a positive correlation between J_{max} and free asparagine concentration (r = 0.653). The parameter J_{max} is calculated at the end of the creep step (strain ($\gamma(t)$)/stress (σ)) and through its measurement of the maximum deformation experienced by the dough before the shear stress is removed, translates into an assessment of the dough's fluid-like characteristics (Jekle & Becker, 2011). Therefore, shear tests showed that dough extensibility was positively correlated to free asparagine concentration.

From Figure 6.2.A and from the correlation coefficients for E_{45} and E_{135} against free asparagine concentration and the ratio of free asparagine/protein content, one can observe a stronger negative correlation between E_{45} and free asparagine concentration compared to correlations for E_{135}. The different outcomes between the two resting times could be explained by

the wheat protein composition, i.e., the Gli/Glu ratio. According to Cauvain (2015b), during mixing, HMW glutenin subunits are potentially broken into smaller molecules, followed by subsequent polymerization into larger units during resting. For this thesis, a hypothesis that samples with higher protein content and higher free asparagine concentration could have higher Gli/Glu ratios was discussed in Chapters 3, 4, and 5. When farinograph tests were performed, the dough development time (*DDT*), which is the time required for the dough to achieve the optimum consistency (Spies, 1990), was shown to be negatively correlated ($p < 0.05$) to wheat free asparagine concentration ($r = -0.319$). Therefore, samples with lower free asparagine concentration, potentially having higher levels of glutenin, required longer time for dough development compared to samples with higher free asparagine levels. The longer *DDT* that was necessary to develop the dough to optimum consistency could have led to an increased breakdown of HMW glutenin, thus producing larger amounts of smaller molecules in samples with low free asparagine levels. Therefore, samples with lower free asparagine concentration would require longer resting times for full glutenin polymerization, thus showing higher E_{45} than E_{135}. On the other hand, samples with higher free asparagine concentration, potentially having less glutenin, would have less glutenin breakdown due to their shorter *DDT*. At short resting times, samples with higher free asparagine concentration would achieve glutenin polymerization faster than samples with lower free asparagine concentration, thus showing lower E_{45}. Due to the potential differences in amounts of small molecules of glutenin in samples with different free asparagine levels, a strong negative correlation was found between wheat free asparagine concentration and E_{45}. As glutenin polymerizes in samples with low free asparagine levels, the negative correlation between wheat free asparagine concentration and E_{135} becomes weaker, thus explaining the different outcomes observed for E_{45} and E_{135} relationships with free asparagine concentration.

For the set of 10 selected samples, the different outcome between E_{45} and the remaining dough extensibility parameters becomes more evident due to the uniform distribution of free asparagine concentration across the range of samples. From Figure 6.2.B, it is possible to observe a clear difference between the correlation for E_{45} as well as the shear parameters ($\tan \delta_{300s}$, $\tan \delta_{5Hz}$, and J_{max}) against free asparagine concentration. While the correlation between E_{45} and free asparagine concentration continues to be negative, the correlation between E_{135} shifts to a positive value (although non-significant at $p < 0.05$). From shear oscillatory and creep-recovery tests, parameters translating into dough extensibility were positively correlated to free asparagine

concentration. A possible explanation for the different outcome from extensograph and shear rheometry could be found in the type of testing method. For example, the extensograph applies a uniaxial extension to the dough, while a shear deformation, at smaller strains, is applied when using a rheometer with parallel plates. The higher sensibility of extensional tests to entanglements present in the gluten structure, mostly due to HMW glutenin subunits (Dobraszczyk & Morgenstern, 2003), could lead to the negative correlation between wheat free asparagine concentration and E_{45}. On the other hand, shear tests could be more sensitive to gliadins present in the samples, mostly in samples with higher free asparagine concentration (higher Gli/Glu ratio). The higher sensibility of shear tests to gliadins, when compared to extensional tests, could result in the positive correlation between wheat free asparagine concentration and shear parameters.

6.2 Yeasted doughs

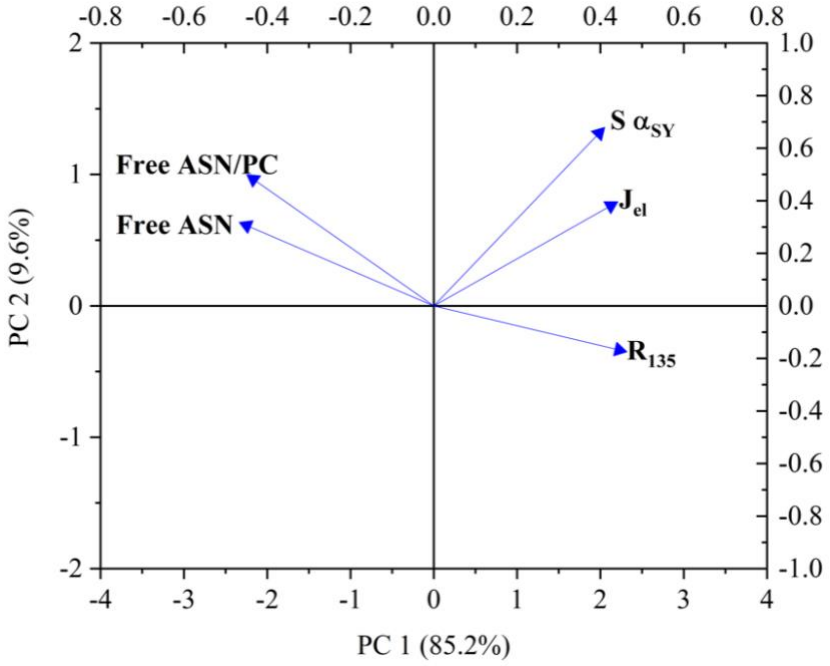
6.2.1 Strength of yeasted doughs

Yeast is an essential ingredient in bread production (Delcour & Hoseney, 2010c). During fermentation, yeast produces carbon dioxide, which provides volume to the bread, and organic acids, responsible for aroma formation (Cauvain, 2015a). Additionally to bread volume and aroma, yeast metabolites also affect dough rheological properties (Delcour & Hoseney, 2010c). Therefore, the main goal of this section is to understand if the addition of yeast affects the relationship between wheat free asparagine concentration and dough strength.

To address the main goal of this section, a rheological parameter was selected from low-intensity ultrasound tests ($S\ \alpha_{SY}$). This parameter was selected based on the hypothesis discussed in Chapter 5 regarding its correlation to dough strength and based on its relationship to other dough strength parameters.

Principal Component Analysis was conducted to illustrate how the rheological parameters from yeasted doughs, translating into dough strength, are correlated to free asparagine concentration, with the biplot shown in Figure 6.3, where the first two components account for about 95% of the total variation in the data. From low-intensity ultrasound tests, the slope for the attenuation coefficient over time for yeasted doughs ($S\ \alpha_{SY}$) was chosen to investigate how yeast affected the relationship between wheat free asparagine concentration and the changes in α_{SY} during fermentation. For comparison purposes, parameters translating into dough strength from non-yeasted doughs (R_{135} and J_{el}) were included in Figure 6.3.

Figure 6.3. PCA biplot for the selected 10 wheat flour samples, including the parameter from low-intensity ultrasound (S α_{SY}) for yeasted doughs, wheat free asparagine concentration (free ASN, μg g^{-1}), and proportion of free asparagine concentration relative to total protein content (Free ASN/PC, mg g^{-1}). For comparison, two selected dough strength parameters from empirical tests (R_{135}) and shear rheometry (J_{el}) conducted on non-yeasted doughs are also shown.



In Figure 6.3, the parameter $S\,\alpha_{SY}$ is negatively correlated to wheat free asparagine concentration and to the ratio of free asparagine/protein content and positively correlated to dough strength parameters measured on non-yeasted doughs (J_{el} and R_{135}) along PC1. From low-intensity ultrasound, the parameter α provides information on the wave's amplitude decay due to losses that occur during wave propagation into the material (Povey & McClements, 1989; Scanlon & Page, 2015). Air bubbles are examples of scattering agents known to cause wave amplitude decay (Koksel et al., 2016). Thus, during fermentation, the increase in the attenuation coefficient over time (positive slopes) was hypothesized to happen due to growth of gas bubbles. Faster growth of bubbles is seen in samples of higher dough strength, and lower amounts of free asparagine. Correlation studies found a positive correlation ($p < 0.05$) for $S\,\alpha_{SY}$, against dough strength parameters from section 6.1.1, such as R_{45} (r = 0.741), R_{135} (r = 0.748), DS (r = 0.694), G_0 (r = 0.691) and G_e (r = 0.791), and J_{el} (r = 0.832).

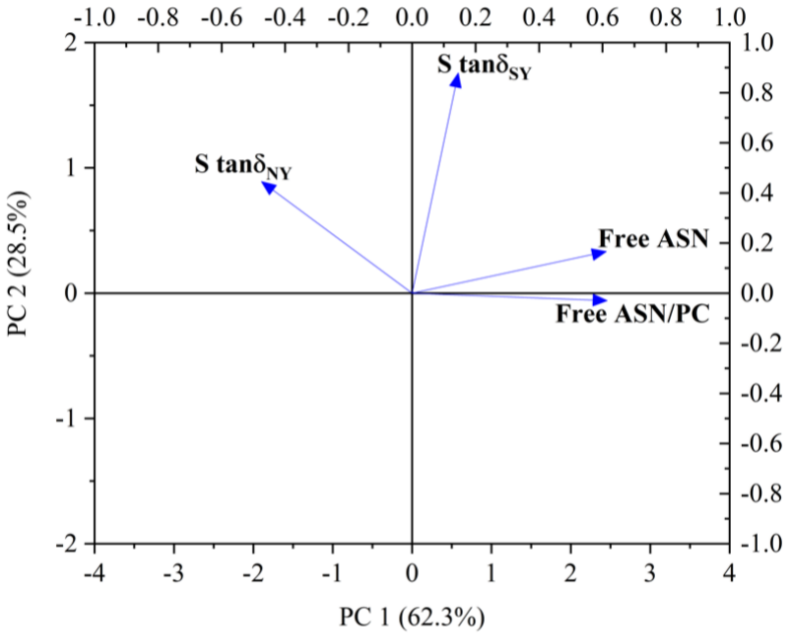
In order to evaluate the effect of yeast addition, correlation studies showed that $S\ \alpha_{SY}$ was negatively correlated ($p < 0.05$) to wheat free asparagine concentration ($r = -0.709$) and to the ratio of free asparagine/protein content ($r = -0.654$). As discussed in Chapter 5, the lower $S\ \alpha_{SY}$ values for samples with higher free asparagine concentration could be explained by higher Gli/Glu ratios of these samples. $S\ \alpha_{SY}$ shows that dough strength indications for non-yeasted doughs are confirmed when yeast is added to the dough.

6.2.2 Extensibility of yeasted doughs

Besides dough strength, fermentation metabolites can also affect dough extensibility due to their effects on gluten structure (Delcour & Hoseney, 2010c). Therefore, the objective of this section is to investigate if the addition of yeast affected the relationship between wheat free asparagine concentration and dough extensibility, which was previously investigated in section 6.1.2 for non-yeasted doughs.

In order to fulfill the objective of this section, a rheological parameter was selected from linear shear oscillatory tests performed using yeasted dough samples. The selected parameter was the slope of the loss tangent over time for yeasted doughs ($S\ \tan\ \delta_{SY}$). During fermentation, it was observed that $S\ \tan\ \delta_{SY}$ decreased over time, thus resulting in curve with negative slopes. For comparison purposes, the same slope obtained for non-yeasted doughs ($S\ \tan\ \delta_{SY}$) was also evaluated. Principal Component Analysis was carried out to illustrate how the rheological parameter from yeasted doughs, translating into dough extensibility, is correlated to free asparagine concentration, with the biplot shown in Figure 6.4. In the biplot below (Figure 6.4), the first two principal components account for about 91% of the total variation in the data. Moreover, the biplot shows $S\ \tan\ \delta_{SY}$ being positively (but weakly) correlated to wheat free asparagine concentration and to the ratio of free asparagine/protein content along PC1 while $S\ \tan\ \delta_{NY}$ is negatively correlated to free asparagine concentration along PC1.

Figure 6.4. PCA biplot for the selected 10 wheat flour samples, including the selected parameter from shear rheometry (S tan δ_{SY}) for yeasted doughs, wheat free asparagine concentration (free ASN, μg g^{-1}), and proportion of free asparagine concentration relative to total protein content (Free ASN/PC, mg g^{-1}). For comparison, a selected dough strength parameter from shear rheometry (S tan δ_{NY}) conducted on non-yeasted doughs is also shown.



As per earlier discussion, the short-range interactions existing between proteins, mainly gliadins due to their higher level of the amino acid glutamine (Delcour et al., 2012), could also affect dough rheological properties within the LVR. Therefore, the slopes for the loss tangent of non-yeasted doughs ($S \tan \delta_{NY}$) had smaller values for samples with higher free asparagine concentration potentially because of the higher Gli/Glu ratios of these samples. The higher proportion of gliadins relative to glutenins would lead to a higher proportion of hydrogen bonds in samples with higher levels of free asparagine, thus resulting in a more structured material showing less changes in $\tan \delta_{NY}$ over time. On the other hand, samples with lower free asparagine concentrations would have a lower proportion of hydrogen bonds, resulting from the potentially lower Gli/Glu ratios, with less contributions of short-range interactions on dough properties over time. However, fermentation metabolites in yeasted doughs affect mainly the glutenin macropolymer (Loponen et al., 2004; Wieser et al., 2008). Thus, these metabolites could be affecting, to a greater extent, the samples with lower free asparagine concentration and protein

content, which would have proportionally more glutenins than gliadins (lower Gli/Glu ratio) when compared to samples with higher free asparagine concentration. In order to compare $S \tan \delta_{SY}$ with other parameters translating into dough extensibility from non-yeasted doughs, the Pearson correlation coefficients were calculated. Correlation studies found a positive relationship ($p < 0.05$) between $S \tan \delta_{SY}$ and parameters translating into dough extensibility such as E_{135} (r = 0.901), J_{max} (r = 0.784), $\tan \delta_{5Hz}$ (r = 0.730), and $\tan \delta_{300s}$ (r = 0.799).

The Pearson's correlation coefficients for $S \tan \delta_{SY}$ against free asparagine concentration (r = 0.341) and against the ratio of free asparagine/protein content (r = 0.132) were weak and non-significant at $p < 0.05$. Nevertheless, Figure 6.4 shows a similar biplot to Figure 6.2.B, indicating that, although weak, a positive correlation between wheat free asparagine concentration and dough extensibility was also observed after yeast addition. Therefore, yeast addition did not significantly affect the previous positive relationship between free asparagine concentration and dough extensibility.

6.3 Conclusion

The reduction of free asparagine concentration in wheat grains is one effective strategy to reduce acrylamide formation in bakery products. However, the factors affecting the concentration of this free amino acid in wheat also affect dough properties, such as strength and extensibility, thus affecting the final product quality. The evaluation of the dough rheological parameters obtained from different rheological tests, varying from classical methods to novel techniques, allows one to study the effect of reducing free asparagine concentration on dough properties. Principal Component Analysis and correlation studies showed that wheat free asparagine concentration and various dough strength parameters (*DS*, R_{45}, R_{135}, J_{el}, G_0, and G_e) were negatively correlated. Moreover, the same statistical analysis showed that dough extensibility parameters ($\tan \delta_{300s}$, $\tan \delta_{5Hz}$, and J_{max}) were positively correlated to wheat free asparagine concentration when analyzed under fundamental rheological tests. However, when extensograph tests were performed for the full set of samples, E_{45} showed a strong negative correlation to wheat free asparagine concentration, while a weaker, but negative correlation was found for E_{135}. The differences between E_{45} and E_{135} was attributed to the potentially higher proportion of glutenins in samples with low free asparagine levels, thus requiring longer mixing times, which would lead to increased content of small molecules of glutenins. The lower amounts of small glutenin molecules in samples with higher free asparagine levels could lead to faster glutenin polymerization at 45

minutes, thus resulting in doughs with lower E_{45} and a strong negative correlation to free asparagine concentration.

Additionally, by evaluating a rheological parameter obtained from fermenting dough ($S\ \alpha_{SY}$) and its correlation to non-yeasted dough strength parameters, it was observed that the addition of yeast did not alter the negative relationship between the free amino acid and dough strength. Finally, for the evaluation of the extensibility of yeasted doughs, a rheological parameter from linear oscillatory shear tests was selected ($S \tan \delta_{SY}$). It was observed that $S \tan \delta_{SY}$ was positively correlated to rheological parameters translating into extensibility from non-yeasted doughs (E_{135}, J_{max}, $\tan \delta_{5Hz}$, and $\tan \delta_{300s}$). Although non-significant at $p < 0.05$, the positive correlations between $S \tan \delta_{SY}$ and free asparagine concentration showed that the addition of yeast did not affect the relationship between wheat free asparagine concentration and dough extensibility.

7 Conclusion and future work

7.1 Conclusion

Because of the current concern regarding the potential for high levels of free asparagine in wheat, this thesis aimed to investigate the relationship between wheat free asparagine concentration and gluten strength of yeasted and non-yeasted doughs. This is necessary because the baking industry needs to ensure that steps taken to reduce free asparagine content do not adversely affect gluten strength, and thus breadmaking quality.

First, Chapter 3 of this thesis showed that environment, genotype and their interactions were the main factors affecting gluten strength and bread quality (SLV), with little or no significant effect from fertilization treatments. Interestingly, for the extensograph parameters, genotype played a greater role on parameters obtained after 135 minutes of resting (R_{135} and R_{135}/E_{135}) compared to parameters obtained after 45 minutes (R_{45} and R_{45}/E_{45}). The improvement in *R* and *R/E* as resting time increased was an indication of the wheat's suitability for breadmaking with long fermentation periods. Additionally, correlation studies in Chapter 3 showed that wheat free asparagine concentration was negatively correlated to various gluten strength parameters, e.g., *DDT*, *DS*, R_{45}, R_{45}/E_{45}, R_{135} and R_{135}/E_{135}. Therefore, reducing free asparagine levels in wheat was not detrimental to gluten strength. In this study, it was also hypothesized in Chapter 3 that samples with higher protein content and *FAB,* and overall higher free asparagine concentration, had higher Gli/Glu ratios, thus producing weaker glutens on a per unit protein basis.

Shear rheometry (Chapter 4) and low-intensity ultrasound (Chapter 5) were used for evaluation of rheological properties within the dough's linear viscoelastic region (LVR). Overall, as wheat free asparagine concentration increased, non-yeasted doughs were shown to possess stronger glutens when evaluated over time (shear oscillatory time sweep tests) or when evaluated over frequency (shear oscillatory frequency sweep tests). It was hypothesized that besides disulfide bonds, short-range interactions, such as hydrogen bonding, also affected the changes in dough rheological properties. When dough rheological properties were evaluated at a specific time or frequency, shear tests showed that wheat free asparagine concentration was negatively correlated to gluten strength.

When tested outside of the dough's LVR (Chapter 4), shear transient tests, i.e., creep-recovery and stress relaxation, also showed that wheat free asparagine concentration was negatively correlated to gluten strength. Additionally, for stress relaxation tests, Maxwell model fitting showed that the first (τ_1) and second (τ_2) relaxation times where negatively correlated to

free asparagine concentration, indicating that samples with lower free asparagine levels presented more elastic-like structures. Finally, relaxation spectra analysis showed two relaxation processes, a short one, which is known to be correlated to gliadins, and a long one, known to be correlated to the glutenins. The relaxation spectra from both processes were also negatively correlated to free asparagine concentration. The expected positive correlation between the short relaxation process and free asparagine concentration was not obtained, possibly due to the good breadmaking suitability of all the wheat varieties selected for this study.

For low-intensity ultrasound, Maxwell model fitting for the attenuation coefficient (α) curve showed a negative, but non-significant, correlation for both relaxation times against free asparagine concentration, thus agreeing with shear stress relaxation results. This negative correlation supported the hypothesis that the decay of the attenuation coefficient over time was caused mainly by bubble disproportionation. Thus, samples with more free asparagine produced weaker doughs, allowing for smaller bubbles to disappear, thus leading to a faster α decay. Moreover, α was negatively correlated to protein content and FAB, indicating that samples with higher Gli/Glu ratios had lower attenuation coefficients. Thus, samples with higher Gli/Glu had proportionately less glutenin polymers to form bubble interfacial areas, resulting in a higher number of small bubbles, which would then lead to more bubble disproportionation.

For yeasted doughs analyzed under shear tests, no significant effects from free asparagine concentration were observed in the changes of dough rheological properties during fermentation. However, a significant effect of protein content was observed, as samples with less protein content showed faster decay in gluten strength parameters over time. It was concluded that fermentation metabolites were potentially affecting the glutenin macropolymer and causing dough softening. From low-intensity ultrasound, changes in α during fermentation were negatively correlated to free asparagine concentration and protein content. It was hypothesized that samples with higher protein content had a weaker structure, with less gas-holding capability, thus causing a slower increase in α during fermentation. Additionally in the yeasted doughs, no significant correlations were found between the slopes of phase velocity against time and the concentration of free asparagine or protein content. However, the overall increase in the phase velocity over time (positive slopes), mainly for samples with low free asparagine concentration, was also hypothesized to be due to dough softening effects caused by the fermentation process.

Finally, from Chapter 6, it was possible to conclude that overall dough strength was negatively correlated to wheat free asparagine concentration. Additionally, dough extensibility was positively correlated to wheat free asparagine concentration, with only E_{45} showing a strong negative correlation against this free amino acid. When the effects of yeast addition were evaluated, no significant variations were observed in the relationship for wheat free asparagine concentration against dough strength or extensibility.

The present study addressed important questions regarding the necessity of maintaining the high quality of Canadian wheat varieties while reducing their free asparagine concentration. In conclusion, this study advises cereal growers to select wheat varieties that accumulate low free asparagine levels and to continue the application of commercial nitrogen fertilization levels (100 kg ha^{-1}), as sulfur deficiency was not an issue in the studied locations. Additionally, this study provides a comprehensive investigation of the relationship between wheat free asparagine concentration and dough rheological properties. Overall, the present work showed that taking steps to reduce free asparagine levels in wheat would not be detrimental to gluten strength and final bread quality.

7.2 Future work

The present study addresses current concerns on potential effects of reducing wheat free asparagine concentration on dough rheological properties and bread quality. The main hypothesis extensively discussed in this study is that samples accumulating higher levels of free asparagine and protein content also had higher Gli/Glu ratios. The hypothesis regarding variation in the Gli/Glu ratio explained the results obtained via various rheological tests and the relationship between wheat free asparagine concentration and gluten strength. Therefore, a potential follow-up study could include the separation and quantification of the wheat gluten protein fractions to confirm this hypothesis. The study of the relationship between wheat free asparagine concentration and gluten protein fractions along with dough rheological measurements would provide a solid link between reducing free asparagine levels in wheat and how gluten strength is controlled for a given protein content.

As discussed in Chapter 4, the free amino acid asparagine was observed to be consumed by yeast during fermentation (Fredriksson et al., 2004). This raised the question of how dough rheological properties would change during fermentation based on their respective wheat free asparagine concentration. As observed in this study, wheat free asparagine concentration was not

significantly correlated to changes in dough rheological properties during fermentation. However, the present study evaluated fermenting dough properties for 20 minutes, whereas the consumption of free asparagine by yeast has been observed over a span of 2 hours (Fredriksson et al., 2004). Therefore, another follow-up research study could include the evaluation of dough rheological properties during 2 hours of fermentation. This would allow one to understand if free asparagine concentration affects changes in dough rheological properties over the whole fermentation period. However, as the volume of the dough changes during fermentation, the measurement apparatus to continuously measure dough properties must account for these changes, thus requiring a more complex set-up to accomplish the task.

Finally, wheat free asparagine concentration is affected by growing conditions, with high temperatures and drought increasing its accumulation (Oddy et al., 2020), which can also affect wheat protein content and composition, as extensively discussed in Chapter 3. Current concerns around climate change and its potential effects on agriculture in the Canadian Prairies, such as moisture deficits, and hotter and drier summers (Chipanshi et al., 2022), could fuel another follow-up research study. Studies on adaptation measures to cope with climate change effects are a necessity nowadays and, due to this high urgency, adaptation measures are becoming the subject of attention worldwide (Berrang-Ford et al., 2021; Biesbroek et al., 2022). Thus, it is paramount to investigate climate change effects in wheat grains in order to select adaptation strategies to maintain the production of high-quality wheat with low levels of free asparagine. Therefore, another follow-up study could evaluate the relationship between wheat free asparagine concentration and dough rheological properties of different wheat varieties grown under widely different climate variation conditions. This follow-up study could be conducted in a glasshouse experiment mimicking expected climate conditions for the next decades, such as higher temperatures, drier conditions, and different soil types from northern regions (in case of agriculture shift to north as an adaptation method). Understanding how climate change can impact wheat free asparagine concentration as well as its protein content and composition can shed light on measures that can be taken now, such as breeding for new wheat varieties.

8 References

AACC International. (1999a). Approved methods of analysis. Method 44-15.02. Moisture-air-oven method. In *AACC International* (11th ed., pp. 1–4). http://dx.doi.org/10.1094/AACCIntMethod-44-15.02

AACC International. (1999b). Approved methods of analysis. Method 54-10.01.Extensigraph method, general. In *AACC International* (11th ed., pp. 1–2). http://dx.doi.org/10.1094/AACCIntMethod-54-10.01

AACC International. (1999c). Approved methods of analysis. Method 54-40.02. Mixograph. In *AACC International* (11th ed., pp. 1–6). AACC International.

AACC International. (2011). Approved methods of analysis. Method 54-21.02. Rheological behavior of flour by farinograph: Constant flour weight procedure. In *AACC International* (11th ed., pp. 1–8).

AACC International. (2014). Approved methods of analysis. Method 10-14.01. Determination of bread volume by laser topography: BVM method. In *AACC International* (11th ed., pp. 1–2).

Abedi, T., Alemzadeh, A., & Kazemeini, S. A. (2011). Wheat yield and grain protein response to nitrogen amount and timing. *Australian Journal of Crop Science, 5*(3), 330–336.

Abt, E., Robin, L. P., McGrath, S., Srinivasan, J., DiNovi, M., Adachi, Y., & Chirtel, S. (2019). Acrylamide levels and dietary exposure from foods in the United States, an update based on 2011-2015 data. *Food Additives & Contaminants: Part A*, *36*(10), 1475–1490. https://doi.org/10.1080/19440049.2019.1637548

Alava, J. M., Millar, S. J., & Salmon, S. E. (2001). The Determination of wheat breadmaking performance and bread dough mixing time by NIR spectroscopy for high speed mixers. *Journal of Cereal Science*, *33*(1), 71–81. https://doi.org/10.1006/jcrs.2000.0341

Amemiya, J. I., & Menjivar, J. A. (1992). Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. *Journal of Food Engineering*, *16*(1–2), 91–108. https://doi.org/10.1016/0260-8774(92)90022-X

Andačić, I. M., Tot, A., Ivešić, M., Krivohlavek, A., Thirumdas, R., Barba, F. J., Sabolović, M. B., Kljusurić, J. G., & Brnčić, S. R. (2020). Exposure of the Croatian adult population to acrylamide through bread and bakery products. *Food Chemistry*, *322*(April). https://doi.org/10.1016/j.foodchem.2020.126771

Anderssen, R. S., Bekes, F., Gras, P. W., Nikolov, A., & Wood, J. T. (2004). Wheat-flour dough extensibility as a discriminator for wheat varieties. *Journal of Cereal Science*, *39*(2), 195–203. https://doi.org/10.1016/j.jcs.2003.10.002

Arvanitoyannis, I. S., & Dionisopoulou, N. (2014). Acrylamide: Formation, occurrence in food products, detection methods, and legislation. *Critical Reviews in Food Science and Nutrition*, *54*(6), 708–733. https://doi.org/10.1080/10408398.2011.606378

Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Effect of composition of gluten proteins and dough rheological properties on the cookie-making quality. *British Food Journal*, *115*(4), 564–574. https://doi.org/10.1108/00070701311317847

Barnes, H. A. (2000). Linear viscoelasticity and time effects. In *A Handbook of Elementary Rheology* (pp. 81–105). The University of Wales Institute of Non-Newtonian Fluid Mechanics.

Barnes, H. A., Hutton, J. F., & Walters, K. (1989a). Introduction. In *An Introduction to Rheology* (First, pp. 1–10). Elsevier Science Publishers B.V.

Barnes, H. A., Hutton, J. F., & Walters, K. (1989b). Linear viscoelasticity. In *An Introduction to Rheology* (First, pp. 37–54). Elsevier Science Publishers B.V.

Bellido, G. G., Scanlon, M. G., Page, J. H., & Hallgrimsson, B. (2006). The bubble size distribution in wheat flour dough. *Food Research International, 39*(10), 1058–1066. https://doi.org/10.1016/j.foodres.2006.07.020

Belton, P. S. (2012). The molecular basis of dough rheology. In S. Cauvain (Ed.), *Breadmaking: Improving quality* (2nd ed., pp. 337–351). Woodhead Publishing Limited. https://doi.org/10.1533/9780857095695.2.337

Berrang-Ford, L., Siders, A. R., Lesnikowski, A., Fischer, A. P., Callaghan, M. W., Haddaway, N. R., Mach, K. J., Araos, M., Shah, M. A. R., Wannewitz, M., Doshi, D., Leiter, T., Matavel, C., Musah-Surugu, J. I., Wong-Parodi, G., Antwi-Agyei, P., Ajibade, I., Chauhan, N., Kakenmaster, W., … Abu, T. Z. (2021). A systematic global stocktake of evidence on human adaptation to climate change. *Nature Climate Change*, *11*(11), 989–1000. https://doi.org/10.1038/s41558-021-01170-y

Biesbroek, R., Wright, S. J., Eguren, S. K., Bonotto, A., & Athanasiadis, I. N. (2022). Policy attention to climate change impacts, adaptation and vulnerability: A global assessment of National Communications (1994–2019). *Climate Policy*, *22*(1), 97–111. https://doi.org/10.1080/14693062.2021.2018986

Bishara, A. J., & Hittner, J. B. (2012). Testing the significance of a correlation with nonnormal data: Comparison of Pearson, Spearman, transformation, and resampling approaches. *Psychological Methods*, *17*(3), 399–417. https://doi.org/10.1037/a0028087

Bishara, A. J., & Hittner, J. B. (2015). Reducing bias and error in the correlation coefficient due to nonnormality. *Educational and Psychological Measurement*, *75*(5), 785–804. https://doi.org/10.1177/0013164414557639

Blake-Kalff, M. M. A., Hawkesford, M. J., Zhao, F. J., & McGrath, S. P. (2000). Diagnosing sulfur deficiency in field-grown oilseed rape (Brassica napus L.) and wheat (Triticum aestivum L.). *Plant and Soil*, *225*, 97–107. https://doi.org/10.1023/A:1026503812267

Bloksma, A. H. (1972). Rheology of wheat flour doughs. *Journal of Texture Studies*, *3*, 3–17.

Bloksma, A. H. (1990). Rheology of the breadmaking process. *Cereal Foods World*, *35*(2), 228–236.

Bonilla, J. C., Erturk, M. Y., & Kokini, J. L. (2020). Understanding the role of gluten subunits (LMW, HMW glutenins and gliadin) in the networking behavior of a weak soft wheat dough and a strong semolina wheat flour dough and the relationship with linear and non-linear rheology. *Food Hydrocolloids*, *108*, 106002. https://doi.org/10.1016/j.foodhyd.2020.106002

Campbell, G. M. (2008). A history of aerated foods. In G. M. Campbell, M. G. Scanlon, & D. L. Pyle (Eds.), *Bubbles in food 2—Novelty, health and luxury* (pp. 1–22). AACC International.

Campbell, G. M., & Martin, P. J. (2012). Bread aeration and dough rheology: An introduction. In *Breadmaking: Improving quality* (pp. 299–336). Woodhead Publishing Limited. https://doi.org/10.1533/9780857095695.2.299

Campbell, G. M., & Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science & Technology*, *10*(9), 283–296. https://doi.org/10.1016/S0924-2244(00)00008-X

Canadian Grain Commission. (2021). *Exports of Canadian grain and wheat flour*. Canadian Grain Comission. https://grainscanada.gc.ca/en/grain-research/statistics/exports-grain-wheat-flour/

Capuano, E., Ferrigno, A., Acampa, I., Serpen, A., Açar, Ö. Ç., Gökmen, V., & Fogliano, V. (2009). Effect of Flour Type on Maillard Reaction and Acrylamide Formation During Toasting of Bread Crisp Model Systems and Mitigation Strategies. *Food Research International*, *42*(9), 1295–1302. https://doi.org/10.1016/j.foodres.2009.03.018

Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *LWT - Food Science and Technology*, *44*(4), 793–810. https://doi.org/10.1016/j.lwt.2010.11.002

Capuano, E., Garofalo, G., Napolitano, A., Zielinski, H., & Fogliano, V. (2010). Rye flour extraction rate affects Maillard reaction development, antioxidant activity, and acrylamide formation in bread crisps. *Cereal Chemistry*, *87*(2), 131–136. https://doi.org/10.1094/CCHEM-87-2-0131

Carson, G. R., & Edwards, N. M. (2009). Criteria of wheat and flour quality. In K. Khan, & P. R. Shewry (Eds.), *Wheat chemistry and technology* (Fourth, pp. 97–118). AACC International.

Cauvain, S. P. (2012). Breadmaking: An overview. In S. P. Cauvain (Ed.), *Breadmaking: Improving quality* (Second, pp. 9–31). Woodhead Publishing Limited.

Cauvain, S. P. (2015a). Bread: The product. In *Technology of Breadmaking* (Third, pp. 1–23). Springer International Publishing.

Cauvain, S. P. (2015b). Principles of dough formation. In *Technology of breadmaking* (3rd ed., pp. 303–338). Springer International Publishing.

Cauvain, S. P. (2018). The relevance of testing to the manufacture of bread and fermented products. In S. P. Cauvain (Ed.), *The ICC handbook of cereals, flour, dough and product testing. Methods and aplications* (Second, pp. 143–175). DEStech Publications.

Charalambides, M. N., Wanigasooriya, L., Williams, G. J., Goh, S. M., & Chakrabarti, S. (2006). Large deformation extensional rheology of bread dough. *Rheologica Acta*, *46*(2), 239–248. https://doi.org/10.1007/s00397-006-0104-4

Cheeke, J. D. N. (2002). Bulk Waves in Fluids. In *Fundamentals and Applications of Ultrasonic Waves* (pp. 15–58). CRC Press.

Chipanshi, A., Berry, M., Zhang, Y., Qian, B., & Steier, G. (2022). Agroclimatic indices across the Canadian Prairies under a changing climate and their implications for agriculture. *International Journal of Climatology*, *42*(4), 2351–2367. https://doi.org/10.1002/joc.7369

Chope, G. A., Wan, Y., Penson, S. P., Bhandari, D. G., Powers, S. J., Shewry, P. R., & Hawkesford, M. J. (2014). Effects of genotype, season, and nitrogen nutrition on gene expression and protein accumulation in wheat grain. *Journal of Agricultural and Food Chemistry*, *62*(19), 4399–4407. https://doi.org/10.1021/jf500625c

Claeys, W. L., De Vleeschouwer, K., & Hendrickx, M. E. (2005). Quantifying the formation of carcinogens during food processing: Acrylamide. *Trends in Food Science and Technology*, *16*(5), 181–193. https://doi.org/10.1016/j.tifs.2005.01.005

Clarke, J. M., Campbell, C. A., Cutforth, H. W., DePauw, R. M., & Winkleman, G. E. (1990). Nitrogen and phosphorus uptake, translocation, and utilization efficiency of wheat in relation to environment and cultivar yield and protein levels. *Canadian Journal of Plant Science*, *70*(4), 965–977. https://doi.org/10.4141/cjps90-119

Claus, A., Mongili, M., Weisz, G., Schieber, A., & Carle, R. (2008). Impact of formulation and technological factors on the acrylamide content of wheat bread and bread rolls. *Journal of Cereal Science*, *47*(3), 546–554. https://doi.org/10.1016/j.jcs.2007.06.011

Claus, A., Schreiter, P., Weber, A., Graeff, S., Herrmann, W., Claupein, W., Schieber, A., & Carle, R. (2006). Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads. *Journal of Agricultural and Food Chemistry*, *54*(23), 8968–8976. https://doi.org/10.1021/jf061936f

Corol, D. I., Ravel, C., Rakszegi, M., Charmet, G., Bedo, Z., Beale, M. H., Shewry, P. R., & Ward, J. L. (2016). 1H-NMR screening for the high-throughput determination of genotype and environmental effects on the content of asparagine in wheat grain. *Plant Biotechnology Journal*, *14*(1), 128–139. https://doi.org/10.1111/pbi.12364

Coupland, J. N. (2004). Low intensity ultrasound. *Food Research International*, *37*(6), 537–543. https://doi.org/10.1016/j.foodres.2004.01.011

Curtis, T. Y., Muttucumaru, N., Shewry, P. R., Parry, M. A. J., Powers, S. J., Elmore, J. S., Mottram, D. S., Hook, S., & Halford, N. G. (2009). Effects of genotype and environment on free amino acid levels in wheat grain: Implications for acrylamide formation during processing. *Journal of Agricultural and Food Chemistry*, *57*(3), 1013–1021. https://doi.org/10.1021/jf8031292

Curtis, T. Y., Postles, J., & Halford, N. G. (2014). Reducing the potential for processing contaminant formation in cereal products. *Journal of Cereal Science*, *59*(3), 382–392. https://doi.org/10.1016/j.jcs.2013.11.002

Curtis, T. Y., Powers, S. J., & Halford, N. G. (2016). Effects of fungicide treatment on free amino acid concentration and acrylamide-forming potential in wheat. *Journal of Agricultural and Food Chemistry*, *64*(51), 9689–9696. https://doi.org/10.1021/acs.jafc.6b04520

Curtis, T. Y., Raffan, S., Wan, Y., King, R., Gonzalez-Uriarte, A., & Halford, N. G. (2019). Contrasting gene expression patterns in grain of high and low asparagine wheat genotypes

in response to sulphur supply. *BMC Genomics*, *20*(1), 1–18. https://doi.org/10.1186/s12864-019-5991-8

Dai, S. C., & Tanner, R. I. (2012). Aspects of elongational testing with bread dough. *Journal of Rheology*, *56*(2), 385–395. https://doi.org/10.1122/1.3690178

Dai, Z., Plessis, A., Vincent, J., Duchateau, N., Besson, A., Dardevet, M., Prodhomme, D., Gibon, Y., Hilbert, G., Pailloux, M., Ravel, C., & Martre, P. (2015). Transcriptional and metabolic alternations rebalance wheat grain storage protein accumulation under variable nitrogen and sulfur supply. *The Plant Journal*, *83*(2), 326–343. https://doi.org/10.1111/tpj.12881

De Barber, B. C., Prieto, J. A., & Collar, C. (1989). Reversed-phase high-performance liquid chromatography analysis of changes in free amino acids during wheat bread dough fermentation. In *Cereal Chemistry* (Vol. 66).

Delcour, J. A., & Hoseney, R. C. (2010a). Proteins of Cereals. In *Principles of cereal science and technology* (3rd ed., pp. 56–69). AACC International.

Delcour, J. A., & Hoseney, R. C. (2010b). Rheology of doughs and batters. In *Principles of cereal science and technology* (3rd ed., pp. 81–88). AACC International.

Delcour, J. A., & Hoseney, R. C. (2010c). Yeast-leavened products. In *Principles of cereal science and technology* (3rd ed., pp. 151–176). AACC International.

Delcour, J. A., Joye, I. J., Pareyt, B., Wilderjans, E., Brijs, K., & Lagrain, B. (2012). Wheat gluten functionality as a quality determinant in cereal-based food products. *Annual Review of Food Science and Technology*, *3*(1), 469–492. https://doi.org/10.1146/annurev-food-022811-101303

Diep, S., Daugelaite, D., Strybulevych, A., Scanlon, M. G., Page, J., & Hatcher, D. (2014). Use of ultrasound to discern differences in Asian noodles prepared across wheat classes and

between varieties. *Canadian Journal of Plant Science*, *94*(3), 525–534. https://doi.org/10.4141/CJPS2013-043

Dobraszczyk, B. J. (2001). Wheat and flour. In D. A. V. Dendy & B. J. Dobraszczyk (Eds.), *Cereals and cereals products. Chemistry and technology* (pp. 100–139). Aspen Publishers, Inc.

Dobraszczyk, B. J., Campbell, G. M., & Gan, Z. (2001). Bread: A unique food. In D. A. V. Dendy & B. J. Dobraszczyk (Eds.), *Cereals and cereals products. Chemistry and technology* (pp. 182–227). Aspen Publishers, Inc.

Dobraszczyk, B. J., & Morgenstern, M. P. (2003). Rheology and the breadmaking process. *Journal of Cereal Science*, *38*(3), 229–245. https://doi.org/10.1016/S0733-5210(03)00059-6

D'Odorico, P., Carr, J. A., Laio, F., Ridolfi, L., & Vandoni, S. (2014). Feeding humanity through global food trade. *Earth's Future*, *2*(9), 458–469. https://doi.org/10.1002/2014EF000250

Dowell, F. E., Maghirang, E. B., Pierce, R. O., Lookhart, G. L., Bean, S. R., Xie, F., Caley, M. S., Wilson, J. D., Seabourn, B. W., Ram, M. S., Park, S. H., & Chung, O. K. (2008). Relationship of bread quality to kernel, flour, and dough properties. *Cereal Chemistry Journal*, *85*(1), 82–91. https://doi.org/10.1094/CCHEM-85-1-0082

Dunnewind, B., Sliwinski, E. L., Grolle, K., & Van Vliet, T. (2004). The Kieffer dough and gluten extensibility rig—An experimental evaluation. *Journal of Texture Studies*, *34*(5–6), 537–560. https://doi.org/10.1111/j.1745-4603.2003.tb01080.x

DuPont, F. M., Hurkman, W. J., Vensel, W. H., Chan, R., Lopez, R., Tanaka, C. K., & Altenbach, S. B. (2006). Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. *Journal of Cereal Science*, *44*(1), 101–112. https://doi.org/10.1016/j.jcs.2006.04.003

Edwards, N. M., Dexter, J. E., Scanlon, M. G., & Cenkowski, S. (1999). Relationship of creep-recovery and dynamic oscillatory measurements to durum wheat physical dough properties. *Cereal Chemistry*, *76*(5), 638–645. https://doi.org/10.1094/CCHEM.1999.76.5.638

Edwards, N. M., Mulvaney, S. J., Scanlon, M. G., & Dexter, J. E. (2003). Role of gluten and its components in determining durum semolina dough viscoelastic properties. *Cereal Chemistry*, *80*(6), 755–763. https://doi.org/10.1094/CCHEM.2003.80.6.755

Edwards, N. M., Peressini, D., Dexter, J. E., & Mulvaney, S. J. (2001). Viscoelastic properties of durum wheat and common wheat dough of different strengths. *Rheologica Acta*, *40*(2), 142–153. https://doi.org/10.1007/s003970000147

Edwards, N. M., Scanlon, M. G., Kruger, J. E., & Dexter', J. E. (1996). Oriental noodle dough rheology: Relationship to water absorption, formulation, and work input during dough sheeting. *Cereal Chemistry*, *73*(6), 708–711.

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2015). Scientific Opinion on acrylamide in food. *EFSA Journal*, *13*(6). https://doi.org/10.2903/j.efsa.2015.4104

Elmehdi, H. M. (2001). *An ultrasonic investigation of the effect of voids on the mechanical properties of bread dough and the role of gas cells in determining the cellular structure of freeze-dried breadcrumb* [PhD Thesis]. University of Manitoba.

Elmehdi, H. M., Page, J. H., & Scanlon, M. G. (2003). Monitoring dough fermentation using acoustic waves. *Food and Bioproducts Processing: Transactions of the Institution of of Chemical Engineers, Part C*, *81*(3), 217–223. https://doi.org/10.1205/096030803322437983

Elmehdi, H. M., Page, J. H., & Scanlon, M. G. (2004). Ultrasonic investigation of the effect of mixing under reduced pressure on the mechanical properties of bread dough. *Cereal Chemistry*, *81*(4), 504–510. https://doi.org/10.1094/CCHEM.2004.81.4.504

Esposito, F., Velotto, S., Rea, T., Stasi, T., & Cirillo, T. (2020). Occurrence of acrylamide in italian baked products and dietary exposure assessment. *Molecules*, *25*(18), 1–10. https://doi.org/10.3390/molecules25184156

European Commission. (2014). *Summary Report of The Standing Committee on the Food Chain and Animal Health Held in Brussels on 20 February 2014 (Section Toxicological Safety of the Food Chain)*.

European Commission. (2017). *Commission Regulation (EU) 2017/2158 of 20: Establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food*. Off. J. Eur. Union.

European Food Safety Authority. (2012). Update on acrylamide levels in food from monitoring years 2007 to 2010. In *EFSA Journal* (No. 10; Vol. 10, p. 2938). https://doi.org/10.2903/j.efsa.2012.2938

Fan, Y. (2006). *Using ultrasound to investigate relaxation and resonance phenomena in wheat flour dough* [MSc Thesis]. University of Manitoba.

Fan, Y., Scanlon, M. G., & Page, J. H. (2013). Influence of internal interfacial area on nanosecond relaxation of wheat gluten proteins as probed by broadband ultrasonic spectroscopy. *Colloids and Surfaces B: Biointerfaces*, *112*, 466–473. https://doi.org/10.1016/j.colsurfb.2013.05.032

FAO. (2019). World fertilizer trends and outlook to 2022. In *World fertilizer trends and outlook to 2022*. http://www.fao.org/3/ca6746en/CA6746EN.pdf

FAO. (2021). *Food Outlook – Biannual Report on Global Food Markets*. FAO. https://doi.org/10.4060/cb4479en

FAO/WHO. (2011). *Evaluation of certain food additives and contaminants. 72nd Report of the Joint FAO/WHO Expert Committee on Food Additive (JECFA)*. WHO Press.

Fayle, S. E., & Gerrard, J. A. (2002). What is the Maillard reaction? In *The Maillard Reaction* (pp. 1–8). Royal Soc. Chem. https://doi.org/10.1039/9781847552105-00001

Ferry, J. D. (1970). Approximate interrelations among the linear viscoelastic functions. In *Viscoelastic properties of polymers* (2nd ed., pp. 88–108). John Wiley & Sons.

Finney, K. F. (1945). Methods of estimating and the effect of variety and protein level on the baking absorption of flour. *Cereal Chemistry*, *22*(2), 149–158.

Finney, K. F., Meyer, J. W., Smith, F. W., & Fryer, H. C. (1957). Effect of foliar spraying of Pawnee wheat with urea solutions on yield, protein content, and protein quality. *Agronomy Journal*, *49*(7), 341–347. https://doi.org/10.2134/agronj1957.00021962004900070001x

Flæte, N. E. S., Hollung, K., Ruud, L., Sogn, T., Færgestad, E. M., Skarpeid, H. J., Magnus, E. M., & Uhlen, A. K. (2005). Combined nitrogen and sulphur fertilisation and its effect on wheat quality and protein composition measured by SE-FPLC and proteomics. *Journal of Cereal Science*, *41*(3), 357–369. https://doi.org/10.1016/j.jcs.2005.01.003

Food Drink Europe. (2019). *Acrylamide Toolbox 2019* (pp. 1–68). Food Drink Europe.

Franzen, D. W. (2015). Sulfate-Sulfur. In *Recommended Chemical Soil Test Procedures for the North Central Region* (p. 8.1-8.6). Missouri Agricultural Experiment Station SB 1001.

Fredriksson, H., Tallving, J., Rosén, J., & Aman, P. (2004). Fermentation reduces free asparagine in dough and acrylamide content in bread. *Cereal Chemistry*, *81*(5), 650–653. https://doi.org/10.1094/CCHEM.2004.81.5.650

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. A review. *Journal of Agricultural and Food Chemistry*, *51*(16), 4504–4526. https://doi.org/10.1021/jf030204+

Fuertes-Mendizábal, T., Aizpurua, A., González-Moro, M. B., & Estavillo, J. M. (2010). Improving wheat breadmaking quality by splitting the N fertilizer rate. *European Journal of Agronomy*, *33*(1), 52–61. https://doi.org/10.1016/j.eja.2010.03.001

Fuertes-Mendizábal, T., González-Torralba, J., Arregui, L. M., González-Murua, C., González-Moro, M. B., & Estavillo, J. M. (2013). Ammonium as sole N source improves grain quality in wheat. *Journal of the Science of Food and Agriculture*, *93*(9), 2162–2171. https://doi.org/10.1002/jsfa.6022

Fuller, L. (2010). Chernozemic soils of the prairie region of Western Canada. *Prairie Soils & Crops Journal*, *3*, 37–45.

Gaju, O., Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., Hubbart, S., & Foulkes, M. J. (2014). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crops Research*, *155*, 213–223. https://doi.org/10.1016/j.fcr.2013.09.003

Gao, R., Curtis, T. Y., Powers, S. J., Xu, H., Huang, J., & Halford, N. G. (2016). Food safety: Structure and expression of the asparagine synthetase gene family of wheat. *Journal of Cereal Science*, *68*, 122–131. https://doi.org/10.1016/j.jcs.2016.01.010

Gao, X., Liu, T., Yu, J., Li, L., Feng, Y., & Li, X. (2016). Influence of high-molecular-weight glutenin subunit composition at Glu-B1 locus on secondary and micro structures of gluten in wheat (Triticum aestivum L.). *Food Chemistry*, *197*, 1184–1190. https://doi.org/10.1016/j.foodchem.2015.11.085

Gao, X., Lukow, O. M., & Grant, C. A. (2012). Grain concentrations of protein, iron and zinc and bread making quality in spring wheat as affected by seeding date and nitrogen fertilizer management. *Journal of Geochemical Exploration*, *121*, 36–44. https://doi.org/10.1016/j.gexplo.2012.02.005

García-Álvarez, J., Alava, J. M., Chavez, J. A., Turo, A., Garcia, M. J., & Salazar, J. (2006). Ultrasonic characterisation of flour-water systems: A new approach to investigate dough properties. *Ultrasonics*, *44*(SUPPL.), 1051–1055. https://doi.org/10.1016/j.ultras.2006.05.099

Gelderman, R. H., & Beegle, D. (2015). Nitrate-Nitrogen. In *Recommended Chemical Soil Test Procedures for the North Central Region* (p. 5.1-5.4). Missouri Agricultural Experiment Station SB 1001.

Giuliani, M. M., Giuzio, L., de Caro, A., & Flagella, Z. (2011). Relationships between nitrogen utilization and grain technological quality in durum wheat: II. Grain yield and quality. *Agronomy Journal*, *103*(6), 1668–1675. https://doi.org/10.2134/agronj2011.0154

Godfrey, D., Hawkesford, M. J., Powers, S. J., Millar, S., & Shewry, P. R. (2010). Effects of crop nutrition on wheat grain composition and end use quality. *Journal of Agricultural and Food Chemistry*, *58*(5), 3012–3021. https://doi.org/10.1021/jf9040645

González-Torralba, J., Arazuri, S., Jarén, C., & Arregui, L. M. (2011). Stable quality traits of soft winter wheat under nonlimiting nitrogen conditions. *Crop Science*, *51*(6), 2820–2828. https://doi.org/10.2135/cropsci2011.02.0084

Gooding, M. J. (2009). The wheat crop. In K. Khan & P. R. Shewry (Eds.), *Wheat Chemistry and Technology* (4th ed., pp. 19–50). AACC International.

Gooding, M. J., & Davies, W. P. (1997a). Crop environment and grain quality. In *Wheat Production and Utilization* (pp. 108–127). Cab International.

Gooding, M. J., & Davies, W. P. (1997b). Crop nutrition and fertilizer use. In *Wheat Production and Utilization. Systems, Quality and the Environment* (pp. 146–183). Cab International.

Government of Canada. (2021). *Crop (corn) heat units*. https://open.canada.ca/data/en/dataset/07875f11-c342-4450-bdaa-0cdeaf43c0c9

Granvogl, M., Wieser, H., Koehler, P., Von Tucher, S., & Schieberle, P. (2007). Influence of sulfur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking. *Journal of Agricultural and Food Chemistry*, *55*(10), 4271–4277. https://doi.org/10.1021/jf070262l

Grosch, W., & Wieser, H. (1999). Redox reactions in wheat dough as affected by ascorbic acid. *Journal of Cereal Science*, *29*(1), 1–16. https://doi.org/10.1006/jcrs.1998.0218

Guerrini, L., Napoli, M., Mancini, M., Masella, P., Cappelli, A., Parenti, A., & Orlandini, S. (2020). Wheat grain composition, dough rheology and bread quality as affected by nitrogen and sulfur fertilization and seeding density. *Agronomy*, *10*(2), 233. https://doi.org/10.3390/agronomy10020233

Gutiérrez-Moya, E., Adenso-Díaz, B., & Lozano, S. (2021). Analysis and vulnerability of the international wheat trade network. *Food Security*, *13*(1), 113–128. https://doi.org/10.1007/s12571-020-01117-9

Hadnadev, M., Hadnadev, T. D., Pojić, M., Torbica, A., Jelena, T., Rakita, S., & Hajnal, E. J. (2014). Changes in the rheological properties of wheat dough during short-term storage of

wheat. *Journal of the Science of Food and Agriculture*, *95*(3), 569–575. https://doi.org/10.1002/jsfa.6782

Hadnadev, T. D., Pojic, M., Hadnadev, M., & Torbica, A. (2011). The role of empirical rheology in flour quality control. In I. Akyar (Ed.), *Wide Spectra of Quality Control* (pp. 335–360). Intech. https://doi.org/10.5772/24148

Hasniza, N. M. Z., Copeland, L., & Wilkes, M. A. (2014). Globulin expression in grain of Australian hard wheat cultivars is affected by growth environment. *Cereal Chemistry Journal*, *91*(2), 159–168. https://doi.org/10.1094/CCHEM-05-13-0108-R

Hasniza, N. M. Z., Wilkes, M. A., Uthayakumaran, S., & Copeland, L. (2014). Growth environment influences grain protein composition and dough functional properties in three Australian wheat cultivars. *Cereal Chemistry*, *91*(2), 169–175. https://doi.org/10.1094/CCHEM-05-13-0109-R

Hawkesford, M. J. (2014). Reducing the reliance on nitrogen fertilizer for wheat production. *Journal of Cereal Science*, *59*(3), 276–283. https://doi.org/10.1016/j.jcs.2013.12.001

Heffer, P., Gruère, A., & Roberts, T. (2017). Assessment of fertilizer use by crop at the global level. In *Assessment of Fertilizer Use by Crop at the Global Level*. International Fertilizer Association and International Plant Nutrition Institute. https://www.ifastat.org/plant-nutrition

Horvat, D., Spanic, V., Dvojkovic, K., Simic, G., Magdic, D., & Nevistic, A. (2015). The influence of Fusarium infection on wheat (Triticum aestivum L.) proteins distribution and baking quality. *Cereal Research Communications*, *43*(1), 61–71. https://doi.org/10.1556/CRC.2014.0023

IARC. (1994). Acrylamide. In *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans—Some Industrial Chemicals* (pp. 389–433).

Igrejas, G., & Branlard, G. (2020). The importance of wheat. In G. Igrejas, T. M. Ikeda, & C. Guzman (Eds.), *Wheat quality for improving processing and human health* (pp. 1–8). Springer Nature.

Ishida, P. M. G., & Steel, C. J. (2014). Physicochemical and sensory characteristics of pan bread samples available in the Brazilian market. *Food Science and Technology (Campinas)*, *34*(4), 746–754. https://doi.org/10.1590/1678-457X.6453

Islas-Rubio, A. R., Chávez-Quiroz, K., Vásquez-Lara, F., Silva-Espinoza, B., Granados-Nevárez, M. del C., Gonzalez-Ríos, H., & Camacho-Casas, M. (2011). Effect of split applications of urea on protein size distribution, physical dough properties, and baking performance of five experimental bread wheat lines. *Agricultural Sciences*, *02*(03), 181–190. https://doi.org/10.4236/as.2011.23025

Janković, M., Barać, M., Pešić, M., Dodig, D., Kandić, V., & Žilić, S. (2015). The polypeptide composition, structural properties and antioxidant capacity of gluten proteins of diverse bread and durum wheat varieties, and their relationship to the rheological performance of dough. *International Journal of Food Science and Technology*, *50*(10), 2236–2245. https://doi.org/10.1111/ijfs.12894

Järvan, M., Lukme, L., Adamson, A., & Akk, A. (2017). Responses of wheat yield, quality and bread-making properties on the sulphur fertilization. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, *67*(5), 444–452. https://doi.org/10.1080/09064710.2017.1293725

Jayaram, V. B., Cuyvers, S., Lagrain, B., Verstrepen, K. J., Delcour, J. A., & Courtin, C. M. (2013). Mapping of Saccharomyces cerevisiae metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. *Food Chemistry*, *136*(2), 301–308. https://doi.org/10.1016/j.foodchem.2012.08.039

Jekle, M., & Becker, T. (2011). Dough microstructure: Novel analysis by quantification using confocal laser scanning microscopy. *Food Research International*, *44*(4), 984–991. https://doi.org/10.1016/j.foodres.2011.02.036

Johansson, E., Malik, A. H., Hussain, A., Rasheed, F., Newson, W. R., Plivelic, T., Hedenqvist, M. S., Gällstedt, M., & Kuktaite, R. (2013). Wheat gluten polymer structures: The impact of genotype, environment, and processing on their functionality in various applications. *Cereal Chemistry*, *90*(4), 367–376. https://doi.org/10.1094/CCHEM-08-12-0105-FI

Johansson, E., & Svensson, G. (1998). Variation in bread-making quality: Effects of weather parameters on protein concentration and quality in some Swedish wheat cultivars grown during the period 1975–1996. *Journal of the Science of Food and Agriculture*, *78*, 109–118.

Johnson, K. A., Gorzinski, S. J., Bodner, K. M., Campbell, R. A., C.H. Wolf, Friedman, M. A., & Mast, R. W. (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicology and Applied Pharmacology*, *85*(2), 154–168. https://doi.org/10.1016/0041-008X(86)90109-2

Kandel, H. J., Brodshaug, J. A., Steele, D. D., Ransom, J. K., DeSutter, T. M., & Sands, G. R. (2013). Subsurface drainage effects on soil penetration resistance and water table depth on a clay soil in the Red River of the North Valley, USA. *Agricultural Engineering International: CIGR Journal*, *15*(1), 1–10.

Karimi, G., & Rashedinia, M. (2014). Acrylamide. In P. Wexler (Ed.), *Encyclopedia of Toxicology* (Third, pp. 69–73). Academic Press.

Katyal, M., Singh, N., Virdi, A. S., Kaur, A., Chopra, N., Ahlawat, A. K., & Singh, A. M. (2017). Extraordinarily soft, medium-hard and hard Indian wheat varieties: Composition, protein profile, dough and baking properties. *Food Research International*, *100*(August), 306–317. https://doi.org/10.1016/j.foodres.2017.08.050

Kaur, A., Singh, N., Ahlawat, A. K., Kaur, S., Singh, A. M., Chauhan, H., & Singh, G. P. (2013). Diversity in grain, flour, dough and gluten properties amongst Indian wheat cultivars varying in high molecular weight subunits (HMW-GS). *Food Research International*, *53*(1), 63–72. https://doi.org/10.1016/j.foodres.2013.03.009

Kennard, A. R. W., & Stone, L. A. (1969). Computer aided design of experiments. *Technometrics*, *11*(1), 137–148.

Khaneghah, A. M., Fakhri, Y., Nematollahi, A., Seilani, F., & Vasseghian, Y. (2020). The concentration of acrylamide in different food products: A global systematic review, meta-analysis, and meta-regression. *Food Reviews International*, 1–19. https://doi.org/10.1080/87559129.2020.1791175

Kharel, T. P., Clay, D. E., Clay, S. A., Beck, D., Reese, C., Carlson, G., & Park, H. (2011). Nitrogen and water stress affect winter wheat yield and dough quality. *Agronomy Journal*, *103*(5), 1389–1396. https://doi.org/10.2134/agronj2011.0011

Khatkar, B. S., Fido, R. J., Tatham, A. S., & Schofield, J. D. (2002). Functional properties of wheat gliadins. II. Effects on dynamic rheological properties of wheat gluten. *Journal of Cereal Science*, *35*(3), 307–313. https://doi.org/10.1006/jcrs.2001.0430

Khorshidi, A. S. (2016). *Use of Ultrasound to Determine the Effects of Sheeting Work Input and Barley β -glucan Addition on Mechanical Properties of Asian Wheat Noodles* [PhD Thesis]. University of Manitoba.

Khorshidi, A. S., Ames, N., Cuthbert, R., Sopiwnyk, E., & Thandapilly, S. J. (2019). Application of low-intensity ultrasound as a rapid, cost-effective tool to wheat screening: Discrimination of Canadian varieties at 10 MHz. *Journal of Cereal Science*, *88*(May), 9–15. https://doi.org/10.1016/j.jcs.2019.05.001

Khorshidi, A. S., Storsley, J., Malunga, L. N., Thandapilly, S. J., & Ames, N. (2018). Advancing the science of wheat quality evaluation using nuclear magnetic resonance (NMR) and ultrasound-based techniques. *Cereal Chemistry*, *95*(3), 347–364. https://doi.org/10.1002/cche.10040

Khorshidi, A. S., Thandapilly, S. J., & Ames, N. (2018). Application of low-intensity ultrasound as a rapid, cost-effective tool to wheat screening: A systematic frequency selection. *Journal of Cereal Science*, *82*(June), 190–197. https://doi.org/10.1016/j.jcs.2018.06.011

Kidmose, U., Pedersen, L., & Nielsen, M. (2001). Ultrasonics in evaluating rheological properties of dough from different wheat varieties and during ageing. *Journal of Texture Studies*, *32*(5–6), 321–334. https://doi.org/10.1111/j.1745-4603.2001.tb01239.x

Kim, Y. R., Cornillon, P., Campanella, O. H., Stroshine, R. L., Lee, S., & Shim, J. Y. (2008). Small and large deformation rheology for hard wheat flour dough as influenced by mixing and resting. *Journal of Food Science*, *73*(1), 1–8. https://doi.org/10.1111/j.1750-3841.2007.00599.x

Koga, S., Böcker, U., Moldestad, A., Tosi, P., Shewry, P. R., Mosleth, E. F., & Uhlen, A. K. (2016). Influence of temperature during grain filling on gluten viscoelastic properties and

gluten protein composition: Influence of temperature during grain filling on gluten characteristics. *Journal of the Science of Food and Agriculture*, *96*(1), 122–130. https://doi.org/10.1002/jsfa.7068

Koksel, F., & Scanlon, M. G. (2012). Effects of composition on dough development and air entrainment in doughs made from gluten-starch blends. *Journal of Cereal Science*, *56*(2), 445–450. https://doi.org/10.1016/j.jcs.2012.05.013

Koksel, F., & Scanlon, M. G. (2018). Investigation of the influence of bakery enzymes on non-yeasted dough properties during mixing. *Journal of Cereal Science*, *79*, 86–92. https://doi.org/10.1016/j.jcs.2017.10.002

Koksel, F., Scanlon, M. G., & Page, J. H. (2016). Ultrasound as a tool to study bubbles in dough and dough mechanical properties: A review. *Food Research International*, *89*, 74–89. https://doi.org/10.1016/j.foodres.2016.09.015

Koksel, F., Strybulevych, A., Aritan, S., Page, J. H., & Scanlon, M. G. (2017). The use of synchrotron X-rays and ultrasonics for investigating the bubble size distribution and its evolution in bread dough. *Journal of Cereal Science*, *78*, 10–18. https://doi.org/10.1016/j.jcs.2017.06.022

Koksel, F., Strybulevych, A., Page, J. H., & Scanlon, M. G. (2017). Ultrasonic investigation of the effects of composition on the volume fraction of bubbles and changes in their relative sizes in non-yeasted gluten-starch blend doughs. *Journal of Food Engineering*, *204*, 1–7. https://doi.org/10.1016/j.jfoodeng.2017.01.027

Ktenioudaki, A., Butler, F., & Gallagher, E. (2010). Rheological properties and baking quality of wheat varieties from various geographical regions. *Journal of Cereal Science*, *51*(3), 402–408. https://doi.org/10.1016/j.jcs.2010.02.009

Kulmyrzaev, A., Cancelliere, C., & McClements, D. J. (2000). Characterization of aerated foods using ultrasonic reflectance spectroscopy. *Journal of Food Engineering*, 7.

Ladha, J. K., Tirol-Padre, A., Reddy, C. K., Cassman, K. G., Verma, S., Powlson, D. S., van Kessel, C., de B. Richter, D., Chakraborty, D., & Pathak, H. (2016). Global nitrogen budgets in cereals: A 50-year assessment for maize, rice and wheat production systems. *Scientific Reports*, *6*(1), 19355. https://doi.org/10.1038/srep19355

Landolfi, V., D'Auria, G., Nicolai, M. A., Nitride, C., Blandino, M., & Ferranti, P. (2021). The effect of nitrogen fertilization on the expression of protein in wheat and tritordeum varieties using a proteomic approach. *Food Research International*, *148*, 110617. https://doi.org/10.1016/j.foodres.2021.110617

Lea, P. J., Sodek, L., Parry, M. A. J., Shewry, P. R., & Halford, N. G. (2007a). Asparagine in plants. *Annals of Applied Biology*, *150*(1), 1–26. https://doi.org/10.1111/j.1744-7348.2006.00104.x

Lea, P. J., Sodek, L., Parry, M. A. J., Shewry, P. R., & Halford, N. G. (2007b). Asparagine in plants. *Annals of Applied Biology*, *150*(1), 1–26 . https://doi.org/10.1111/j.1744-7348.2006.00104.x

Lee, S., & Campanella, O. (2013). Impulse viscoelastic characterization of wheat flour dough during fermentation. *Journal of Food Engineering*, *118*(3), 266–270. https://doi.org/10.1016/j.jfoodeng.2013.04.024

Lee, S., Pyrak-Nolte, L. J., & Campanella, O. (2004a). Properties of dough during fermentation. *Journal of Texture Studies*, *35*, 33–51.

Lee, S., Pyrak-Nolte, L. J., & Campanella, O. (2004b). Determination of ultrasonic-based rheological properties of dough during fermentation. *Journal of Texture Studies*, *35*(1), 33–52. https://doi.org/10.1111/j.1745-4603.2004.tb00821.x

Lefebvre, J., & Mahmoudi, N. (2007). The pattern of the linear viscoelastic behaviour of wheat flour dough as delineated from the effects of water content and high molecular weight glutenin subunits composition. *Journal of Cereal Science*, *45*(1), 49–58. https://doi.org/10.1016/j.jcs.2006.06.005

Leroy, V., Fan, Y., Strybulevych, A. L., Bellido, G. G., Page, J. H., & Scanlon, M. G. (2008). Investigating the bubble size distribution in dough using ultrasound. In *Bubbles in Food 2* (pp. 51–60). Elsevier. https://doi.org/10.1016/B978-1-891127-59-5.50009-2

Leroy, V., Pitura, K. M., Scanlon, M. G., & Page, J. H. (2010). The complex shear modulus of dough over a wide frequency range. *Journal of Non-Newtonian Fluid Mechanics*, *165*(9–10), 475–478. https://doi.org/10.1016/j.jnnfm.2010.02.001

Letang, C., Piau, M., Verdier, C., & Lefebvre, L. (2001). Characterization of wheat flour-water doughs: A new method using ultrasound. *Journal of Food Engineering*, *39*(2), 133–141.

Li, M., Deng, X., Xu, X., Liu, N., Wang, Z., & Yan, Y. (2019). Effects of water deficit and different nitrogen fertilizer treatments on the quality of wheat for Chinese fresh white noodles and steamed bread and the composition of storage proteins. *Journal of the Science of Food and Agriculture*, *99*(14), 6431–6443. https://doi.org/10.1002/jsfa.9922

Li, M., Yue, Q., Liu, C., Zheng, X., Hong, J., Wang, N., & Bian, K. (2021). Interaction between gliadin/glutenin and starch granules in dough during mixing. *LWT*, *148*, 111624. https://doi.org/10.1016/j.lwt.2021.111624

Li, W., Dobraszczyk, B. J., & Schofield, J. D. (2003). Stress relaxation behavior of wheat dough, gluten, and gluten protein fractions. *Cereal Chemistry*, *80*(3), 333–338. https://doi.org/10.1094/CCHEM.2003.80.3.333

Li, X., Liu, T., Song, L., Zhang, H., Li, L., & Gao, X. (2016). Influence of high-molecular-weight glutenin subunit composition at Glu-A1 and Glu-D1 loci on secondary and micro structures of gluten in wheat (Triticum aestivum L.). *Food Chemistry*, *213*, 728–734. https://doi.org/10.1016/j.foodchem.2016.07.043

Lindahl, L., & Eliasson, A. -C. (1992). A comparison of some rheological properties of durum and wheat flour doughs. *Cereal Chemistry*, *69*(1), 30–34.

Lingnert, H., Grivas, S., Jägerstad, M., Skog, K., Lingnert, H., Grivas, S., Ja, M., & Skog, K. (2016). Acrylamide in food: Mechanisms of formation and influencing factors during heating of foods. *Journal of Nutrition*, *6480*(4), 159–172. https://doi.org/10.1080/110264802762225273

Liu, J., Zhang, J., Zhu, G., Zhu, D., & Yan, Y. (2021). Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality. *The Crop Journal*, S2214514121001057. https://doi.org/10.1016/j.cj.2021.04.006

Liu, Y., Ohm, J.-B., Hareland, G., Wiersma, J., & Kaiser, D. (2011). Sulfur, protein size distribution, and free amino acids in flour mill streams and their relationship to dough rheology and breadmaking traits. *Cereal Chemistry Journal*, *88*(2), 109–116. https://doi.org/10.1094/CCHEM-06-10-0086

Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T., & Salovaara, H. (2004). Degradation of HMW glutenins during wheat sourdough fermentations. *Cereal Chemistry Journal*, *81*(1), 87–93. https://doi.org/10.1094/CCHEM.2004.81.1.87

Maan, A. A., Anjum, M. A., Khan, M. K. I., Nazir, A., Saeed, F., Afzaal, M., & Aadil, R. M. (2020). Acrylamide formation and different mitigation strategies during food processing – a review. *Food Reviews International*, 1–18. https://doi.org/10.1080/87559129.2020.1719505

MacRitchie, F. (2016). Seventy years of research into breadmaking quality. *Journal of Cereal Science*, *70*, 123–131. https://doi.org/10.1016/j.jcs.2016.05.020

Magana-Barajas, E., Ramirez-Wong, B., Torres-Chavez, P. I., & Morales-Rosas, I. (2012). Use of the stress-relaxation and dynamic tests to evaluate the viscoelastic properties of dough from soft wheat cultivars. In J. D. Vicente (Ed.), *Viscoelasticity—From theory to biological applications*. IntechOpen. https://doi.org/10.5772/45993

Malalgoda, M., Ohm, J. B., Meinhardt, S., & Simsek, S. (2018). Association between gluten protein composition and breadmaking quality characteristics in historical and modern spring wheat: *Cereal Chemistry*, *95*(2), 226–238. https://doi.org/10.1002/cche.10014

Malunga, L. N., Ames, N., Khorshidi, A. S., Thandapilly, S. J., Yan, W., Cuthbert, R., Dyck, A., Waterer, J., Sopiwnyk, E., Malcolmson, L., & Scanlon, M. G. (2020). Association of free asparagine concentration in wheat with cultivar, location, fetilizer, and their interaction. *Food Chemistry*, *Submitted for publication*.

Malunga, L. N., Ames, N. P., Masatcioglu, M. T., Khorshidi, A. S., Thandapilly, S. J., Cuthbert, R. D., Sopiwnyk, E., & Scanlon, M. G. (2019). Free asparagine concentrations in Canadian

hard red spring wheat cultivars. *Canadian Journal of Plant Science*, *99*(3), 338–347. https://doi.org/10.1139/cjps-2018-0134

Marchetti, L., Cardós, M., Campaña, L., & Ferrero, C. (2012). Effect of glutens of different quality on dough characteristics and breadmaking performance. *LWT - Food Science and Technology*, *46*(1), 224–231. https://doi.org/10.1016/j.lwt.2011.10.002

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany*, *105*(7), 1141–1157. https://doi.org/10.1093/aob/mcq028

Mastilović, J., Živančev, D., Lončar, E., Malbaša, R., Hristov, N., & Kevrešan, Ž. (2018). Effects of high temperatures and drought during anthesis and grain filling period on wheat processing quality and underlying gluten structural changes. *Journal of the Science of Food and Agriculture*, *98*(8), 2898–2907. https://doi.org/10.1002/jsfa.8784

McCann, T. H., Le Gall, M., & Day, L. (2016). Extensional dough rheology—Impact of flour composition and extension speed. *Journal of Cereal Science*, *69*, 228–237. https://doi.org/10.1016/j.jcs.2016.03.012

McClements, D. J. (1991). Ultrasonic characterisation of emulsions and suspensions. *Advances in Colloid and Interface Science*, *37*(1–2), 33–72. https://doi.org/10.1016/0001-8686(91)80038-L

McClements, D. J., & Gunasekaran, S. (1997). Ultrasonic characterization of foods and drinks: Principles, methods, and applications. *Critical Reviews in Food Science and Nutrition*, *37*(1), 1–46. https://doi.org/10.1080/10408399709527766

Meerts, M., Cardinaels, R., Oosterlinck, F., Courtin, C. M., & Moldenaers, P. (2017a). The impact of water content and mixing time on the linear and non-linear rheology of wheat flour dough. *Food Biophysics*, *12*(2), 151–163. https://doi.org/10.1007/s11483-017-9472-9

Meerts, M., Cardinaels, R., Oosterlinck, F., Courtin, C. M., & Moldenaers, P. (2017b). The interplay between the main flour constituents in the rheological behaviour of wheat flour dough. *Food and Bioprocess Technology*, *10*(2), 249–265. https://doi.org/10.1007/s11947-016-1810-2

Meerts, M., Vaes, D., Botteldoorn, S., Courtin, C. M., Cardinaels, R., & Moldenaers, P. (2018). The time-dependent rheology of fermenting wheat flour dough: Effects of salt and sugar. *Rheologica Acta*, *57*(12), 813–827. https://doi.org/10.1007/s00397-018-1113-9

Mencin, M., Abramovič, H., Vidrih, R., & Schreiner, M. (2020). Acrylamide levels in food products on the Slovenian market. *Food Control*, *114*(January). https://doi.org/10.1016/j.foodcont.2020.107267

Menjivar, J. A. (1990). Fundamental aspects of dough rheology. In H. Faridi & J. M. Faubion (Eds.), *Dough rheology and baked product texture* (pp. 1–28). Van Nostrand Reinhold.

Mills, E. N. C., Wilde, P. J., Salt, L. J., & Skeggs, P. (2003). Bubble formation and stabilization in bread dough. *Food and Bioproducts Processing*, *81*(3), 189–193. https://doi.org/10.1205/096030803322437956

Miś, A., Grundas, S., Dziki, D., & Laskowski, J. (2012). Use of farinograph measurements for predicting extensograph traits of bread dough enriched with carob fibre and oat wholemeal. *Journal of Food Engineering*, *108*(1), 1–12. https://doi.org/10.1016/j.jfoodeng.2011.08.007

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *International Weekly Journal of Science*, *419*(6906), 448–449. https://doi.org/10.1038/419448a

Muttucumaru, N., Halford, N. G., Elmore, J. S., Dodson, A. T., Parry, M., Shewry, P. R., & Mottram, D. S. (2006). Formation of high levels of acrylamide during the processing of flour derived from sulfate-deprived wheat. *Journal of Agricultural and Food Chemistry*, *54*(23), 8951–8955. https://doi.org/10.1021/jf0623081

Navickis, L. L., Anderson, R. A., Bagley, E. B., & Jasberg, B. K. (1982). Viscoelastic properties of wheat flour doughs: Variation of dynamic moduli with water and protein content. *Journal of Texture Studies*, *13*(2), 249–264. https://doi.org/10.1111/j.1745-4603.1982.tb01399.x

Navrotskyi, S., Baenziger, P. S., Regassa, T., Guttieri, M. J., & Rose, D. J. (2018). Variation in asparagine concentration in Nebraska wheat. *Cereal Chemistry*, *95*(2), 264–273. https://doi.org/10.1002/cche.10023

NDSU. (2018). Wheat quality and carbohydrate research—Farinograph. In *North Dakota State University—Department of Plant Sciences*. https://www.ndsu.edu/faculty/simsek/wheat/farinograph.html

Ng, T. S. K., & McKinley, G. H. (2008). Power law gels at finite strains: The nonlinear rheology of gluten gels. *Journal of Rheology*, *52*(2), 417–449. https://doi.org/10.1122/1.2828018

Ng, T. S. K., McKinley, G. H., & Padmanabhan, M. (2006). Linear to non-linear rheology of wheat flour dough. *Applied Rheology*, *16*(5), 265–274. https://doi.org/10.1515/arh-2006-0019

Noort, M. W. J., van Haaster, D., Hemery, Y., Schols, H. A., & Hamer, R. J. (2010). The effect of particle size of wheat bran fractions on bread quality – Evidence for fibre–protein

interactions. *Journal of Cereal Science*, *52*(1), 59–64 .
https://doi.org/10.1016/j.jcs.2010.03.003

Nyiraneza, J., Cambouris, A. N., Ziadi, N., Tremblay, N., & Nolin, M. C. (2012). Spring wheat yield and quality related to soil texture and nitrogen fertilization. *Agronomy Journal*, *104*(3), 589–599. https://doi.org/10.2134/agronj2011.0342

Oddy, J., Raffan, S., Wilkinson, M. D., Elmore, J. S., & Halford, N. G. (2020). Stress, nutrients and genotype: Understanding and managing asparagine accumulation in wheat grain. *CABI Agriculture and Bioscience*, *1*(1), 10. https://doi.org/10.1186/s43170-020-00010-x

Oddy, J., Raffan, S., Wilkinson, M. D., Elmore, J. S., & Halford, N. G. (2022). Understanding the relationships between free asparagine in grain and other traits to breed low-asparagine wheat. *Plants*, *11*(5), 669. https://doi.org/10.3390/plants11050669

OECD/FAO. (2021). *OECD-FAO Agricultural Outlook 2021-2030*. OECD Publishing. https://doi.org/10.1787/19428846-en

Ohm, J.-B., Mergoum, M., & Simsek, S. (2017). Variation of free asparagine concentration and association with quality parameters for Hard Red Spring wheat grown in North Dakota. *Cereal Chemistry Journal*, *94*(4), 712–716. https://doi.org/10.1094/CCHEM-12-16-0290-R

Ooms, N., Jansens, K. J. A., Pareyt, B., Reyniers, S., Brijs, K., & Delcour, J. A. (2018). The impact of disulfide bond dynamics in wheat gluten protein on the development of fermented pastry crumb. *Food Chemistry*, *242*, 68–74. https://doi.org/10.1016/j.foodchem.2017.09.007

Oury, F.-X., & Godin, C. (2007). Yield and grain protein concentration in bread wheat: How to use the negative relationship between the two characters to identify favourable genotypes? *Euphytica*, *157*(1–2), 45–57. https://doi.org/10.1007/s10681-007-9395-5

Ozturk, A., & Aydin, F. (2004). Effect of water stress at various growth stages on some quality characteristics of winter wheat. *Journal of Agronomy and Crop Science*, *190*(2), 93–99. https://doi.org/10.1046/j.1439-037X.2003.00080.x

Panozzo, J. F., & Eagles, H. A. (1999). Rate and duration of grain filling and grain nitrogen accumulation of wheat cultivars grown in different environments. *Australian Journal of Agricultural Research*, *50*(6), 1007. https://doi.org/10.1071/AR98146

Panozzo, J. F., & Eagles, H. A. (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Australian Journal of Agricultural Research*, *51*(5), 629. https://doi.org/10.1071/AR99137

Park, H., Clay, D. E., Hall, R. G., Rohila, J. S., Kharel, T. P., Clay, S. A., & Lee, S. (2014). Winter wheat quality responses to water, environment, and nitrogen fertilization. *Communications in Soil Science and Plant Analysis*, *45*(14), 1894–1905. https://doi.org/10.1080/00103624.2014.909833

Payne, P. I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Ann. Rev. Plant Physiol*, *38*, 141–153.

PDQ. (2020). Average Grade Spreads. In *Price & Data Quotes Dashboard*. https://www.pdqinfo.ca/dashboard

Peressini, D., Braunstein, D., Page, J. H., Strybulevych, A., Lagazio, C., & Scanlon, M. G. (2016). Relation between ultrasonic properties, rheology and baking quality for bread doughs of widely differing formulation. *Journal of the Science of Food and Agriculture*, *97*(8), 2366–2374. https://doi.org/10.1002/jsfa.8048

Peressini, D., Sensidoni, A., Pollini, C. M., & De Cindio, B. (2000). Rheology of wheat doughs for fresh pasta production: Influence of semolina-flour blends and salt content. *Journal of Texture Studies*, *31*(2), 163–182.

Popa, N., Tamba-Berehoiu, R.-M., Hutan, M., & Popescu, S. (2014). The significance of some flour quality parameters as quality predictors of bread. *Scientific Bulletin. Series F. Biotechnologies*, *XVIII*, 135–140.

Povey, M. J. W. (1997). *Ultrasonic techniques for fluids characterization*. Academic Press.

Povey, M. J. W., & McClements, D. J. (1989). Ultrasonics in food engineering. Part I: Introduction and experimental methods. *Journal of Food Engineering*, *8*(4), 217–245. https://doi.org/10.1016/0260-8774(88)90015-5

Preston, K. R., Hucl, P., Townley-Smith, T. F., Dexter, J. E., Williams, P. C., & Stevenson, S. G. (2001). Effects of cultivar and environment on farinograph and Canadian short process mixing properties of Canada Western Red Spring wheat. *Canadian Journal of Plant Science*, *81*(3), 391–398. https://doi.org/10.4141/P00-137

Raffan, S., & Halford, N. G. (2019). Acrylamide in food: Progress in and prospects for genetic and agronomic solutions. *Annals of Applied Biology*, *175*(3), 259–281. https://doi.org/10.1111/aab.12536

Raj, B., Rajendran, V., & Palanichamy, P. (2004a). Applications of ultrasound—General and advanced. In *Science and Technology of Ultrasonics* (pp. 105–134). Alpha Science International Ltd.

Raj, B., Rajendran, V., & Palanichamy, P. (2004b). Fundamentals of ultrasonics. In *Science and Technology of Ultrasonics* (pp. 4–36). Alpha Science International Ltd.

Randall, P. J., Spencer, K., & Freney, J. R. (1981). Sulfur and nitrogen fertilizer effects on wheat. I Concentrations of sulfur and nitrogen and the nitrogen to sulfur ratio in grain, in relation to the yield response. *Australian Journal of Agricultural Research*, *32*, 203–212.

Rao, V. K., Mulvaney, S. J., & Dexter, J. E. (2000). Rheological characterisation of long- and short-mixing flours based on stress-relaxation. *Journal of Cereal Science*, *31*(2), 159–171. https://doi.org/10.1006/jcrs.1999.0295

Rao, V. K., Mulvaney, S. J., Dexter, J. E., Edwards, N. M., & Peressini, D. (2001). Stress-relaxation properties of mixograph semolina-water doughs from durum wheat cultivars of variable strength in relation to mixing characteristics, bread- and pasta-making performance. *Journal of Cereal Science*, *34*(2), 215–232. https://doi.org/10.1006/jcrs.2001.0384

Rezaei, M. N., Jayaram, V. B., Verstrepen, K. J., & Courtin, C. M. (2016). The impact of yeast fermentation on dough matrix properties. *Journal of the Science of Food and Agriculture*, *96*(11), 3741–3748. https://doi.org/10.1002/jsfa.7562

Rossmann, A., Buchner, P., Savill, G. P., Hawkesford, M. J., Scherf, K. A., & Mühling, K. H. (2019). Foliar N application at anthesis alters grain protein composition and enhances baking quality in winter wheat only under a low N fertiliser regimen. *European Journal of Agronomy*, *109*(July 2018), 125909. https://doi.org/10.1016/j.eja.2019.04.004

Safari-Ardi, M., & Phan-Thien, N. (1998). Stress relaxation and oscillatory tests to distinguish between doughs prepared from wheat flours of different varietal origin. *Cereal Chemistry*, *75*(1), 80–84. https://doi.org/10.1094/CCHEM.1998.75.1.80

Sahi, S. S., & Little, K. (2006). Quality control. In Y. H. Hui, H. Corke, I. De Leyn, W.-K. Nip, & N. Cross (Eds.), *Bakery products. Science and Technology* (1st ed., pp. 319–336). Blackwell Publishing.

Sang, S., Xu, D., Ma, Y., Jin, Y., Wu, F., & Xu, X. (2020). Effect of egg yolk on the properties of wheat dough and bread. *Food Bioscience*, *37*, 100689. https://doi.org/10.1016/j.fbio.2020.100689

Sapirstein, H. D., & Fu, B. X. (2000). Evidence for varying interaction of gliadin and glutenin. In P. R. Shewry & A. S. Tatham (Eds.), *Wheat Gluten* (pp. 425–429). Royal Soc. Chem.

Sapirstein, H. D., Wu, Y., Koksel, F., & Graf, R. (2018). A study of factors influencing the water absorption capacity of Canadian hard red winter wheat flour. *Journal of Cereal Science*, *81*, 52–59. https://doi.org/10.1016/j.jcs.2018.01.012

Sarion, C., Codină, G. G., & Dabija, A. (2021). Acrylamide in bakery products: A review on health risks, legal regulations and strategies to reduce its formation. *International Journal of Environmental Research and Public Health*, *18*(8), 4332. https://doi.org/10.3390/ijerph18084332

Scanlon, M. G., & Page, J. H. (2015). Probing the properties of dough with low-intensity ultrasound. *Cereal Chemistry*, *92*(2), 121–133. https://doi.org/10.1094/CCHEM-11-13-0244-IA

Scanlon, M. G., Page, J. H., Leroy, V., Elmehdi, H. M., Fan, Y., & Mehta, K. L. . (2011). Examining the effect of dough matrix and bubbles on the properties of dough using low-intensity ultrasound. In R. N. Chibbar & J. E. Dexter (Eds.), *Proceedings of the 4th International Wheat Quality Conference* (pp. 389–394). AGROBIOS (International).

Scanlon, M. G., Page, J. H., Leroy, V., Fan, Y., Elmehdi, H. M., Kiefte, A., & Mehta, K. L. . (2011). Using low frequency ultrasound to evaluate the properties of wheat flour doughs. In R. N. Chibbar & J. E. Dexter (Eds.), *Proceedings of the 4th International Wheat Quality Conference* (pp. 271–282). AGROBIOS (International).

Scanlon, M. G., & Zghal, M. C. (2001). Bread properties and crumb structure. *Food Research International*, *34*(10), 841–864. https://doi.org/10.1016/S0963-9969(01)00109-0

Scheerer, U., Haensch, R., Mendel, R. R., Kopriva, S., Rennenberg, H., & Herschbach, C. (2010). Sulphur flux through the sulphate assimilation pathway is differently controlled by adenosine 5′-phosphosulphate reductase under stress and in transgenic poplar plants overexpressing γ-ECS, SO, or APR. *Journal of Experimental Botany*, *61*(2), 609–622. https://doi.org/10.1093/jxb/erp327

Sehn, G. A. R., & Steel, C. J. (2017). Classification of whole wheat flour using a dimensionless number. *Journal of Food Science and Technology*, *54*(12), 3827–3836. https://doi.org/10.1007/s13197-017-2811-5

Setia, R., Dai, Z., Nickerson, M. T., Sopiwnyk, E., Malcolmson, L., & Ai, Y. (2020). Properties and bread-baking performance of wheat flour composited with germinated pulse flours. *Cereal Chemistry*, *97*(2), 459–471. https://doi.org/10.1002/cche.10261

Shanbhag, S. (2018). *PyReSpect-time*. https://github.com/shane5ul/pyReSpect-time#pyrespect-time

Shanbhag, S. (2019). pyReSpect: A computer program to extract discrete and continuous spectra from stress relaxation experiments. *Macromolecular Theory and Simulations*, *28*(3), 1–10. https://doi.org/10.1002/mats.201900005

Shewry, P. (2019). What is gluten—Why is it special? *Frontiers in Nutrition*, *6*, 101. https://doi.org/10.3389/fnut.2019.00101

Shewry, P. R., Halford, N. G., Tatham, A. S., Popineau, Y., Lafiandra, D., & Belton, P. S. (2003). The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. In *Advances in Food and Nutrition Research* (Vol. 45). https://doi.org/10.1016/s1043-4526(03)45006-7

Shewry, P. R., & Tatham, A. S. (1990). The prolamin storage proteins of cereal seeds: Structure and evolution. *Biochemical Journal*, *267*(1), 1–12. https://doi.org/10.1042/bj2670001

Shewry, P. R., & Tatham, A. S. (1997). Disulphide bonds in wheat gluten proteins. *Journal of Cereal Science*, *25*(3), 207–227. https://doi.org/10.1006/jcrs.1996.0100

Shewry, P. R., Tatham, A. S., Forde, J., Kreis, M., & Miflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science*, *4*(2), 97–106. https://doi.org/10.1016/S0733-5210(86)80012-1

Skaf, A., Nassar, G., Lefebvre, F., & Nongaillard, B. (2009). A new acoustic technique to monitor bread dough during the fermentation phase. *Journal of Food Engineering*, *93*(3), 365–378. https://doi.org/10.1016/j.jfoodeng.2009.02.005

Sluimer, P. (2005a). Basic Ingredients. In *Principles of Breadmaking. Functionality of Raw Materials and Process Steps* (pp. 17–48). American Association of Cereal Chemists.

Sluimer, P. (2005b). Mixing. In *Principles of breadmaking. Functionality of raw materials and process steps* (pp. 81–111). American Association of Cereal Chemists.

Sobczyk, A., Pycia, K., Stankowski, S., Jaworska, G., & Kuźniar, P. (2017). Evaluation of the rheological properties of dough and quality of bread made with the flour obtained from old

cultivars and modern breeding lines of spelt (Triticum aestivum ssp. Spelta). *Journal of Cereal Science, 77*, 35–41. https://doi.org/10.1016/j.jcs.2017.07.013

Song, W. F., Ren, Z. Y., Zhang, Y. B., Zhao, H. B., Lv, X. B., Li, J. L., Guo, C. H., Song, Q. J., Zhang, C. L., Xin, W. L., & Xiao, Z. M. (2015). Effects of allelic variation in glutenin subunits and gliadins on baking-quality in near-isogenic lines of common wheat cv. Longmai 19. *Cereal Research Communications*, *43*(2), 284–294. https://doi.org/10.1556/CRC.2014.0041

Song, Y., & Zheng, Q. (2007). Dynamic rheological properties of wheat flour dough and proteins. *Trends in Food Science and Technology*, *18*(3), 132–138. https://doi.org/10.1016/j.tifs.2006.11.003

Spies, R. (1990). Application of rheology in the bread industry. In H. Faridi & J. M. Faubion (Eds.), *Dough rheology and baked product texture* (pp. 343–361). Van Nostrand Reinhold.

Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., Robert, M.-C., & Riediker, S. (2002). Acrylamide from Maillard reaction products. *Nature*, *419*(6906), 449–450. https://doi.org/10.1038/419449a

Stadler, R. H., & Scholz, G. (2004). Acrylamide: An update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control. *Nutrition Reviews*, *62*(12), 449–467. https://doi.org/10.1301/nr.2004.janr.449-467

Statista. (2021a). *Bread—Canada*. https://www.statista.com/outlook/cmo/food/bread-cereal-products/bread/canada

Statista. (2021b). *Bread—United Kingdom*. https://www.statista.com/outlook/cmo/food/bread-cereal-products/bread/united-kingdom

Statista. (2021c). *Global demand for agricultural fertilizer by nutrient from 2011/2012 to 2021/2022*. https://www.statista.com/statistics/438930/fertilizer-demand-globally-by-nutrient/

Statista. (2021d). *Volume of the bread market in the United Kingdom from 2012 to 2025*. https://www.statista.com/forecasts/1191977/volume-bread-market-uk

Statista. (2022). *Bread—Worldwide*. https://www.statista.com/outlook/cmo/food/bread-cereal-products/bread/worldwide#revenue

Steinfurth, D., Muhling, K. H., & Zorb, C. (2011). Impact of nitrogen and sulfur fertilization on gluten composition, and baking quality of wheat. In D. S. Fellstone (Ed.), *Gluten: Properties, modifications and dietary intolerance* (pp. 27–47). Nova Science Publishers, Inc.

Stockmann, F., Weber, E. A., Schreiter, P., Merkt, N., Claupein, W., & Graeff-Hönninger, S. (2018). Impact of nitrogen and sulfur supply on the potential of acrylamide formation in organically and conventionally grown winter wheat. *Agronomy*, *8*(12), 1–19. https://doi.org/10.3390/agronomy8120284

Struck, S., Straube, D., Zahn, S., & Rohm, H. (2018). Interaction of wheat macromolecules and berry pomace in model dough: Rheology and microstructure. *Journal of Food Engineering*, *223*, 109–115. https://doi.org/10.1016/j.jfoodeng.2017.12.011

Strybulevych, A., Leroy, V., Shum, A. L., Koksel, H. F., Scanlon, M. G., & Page, J. H. (2012). Use of an ultrasonic reflectance technique to examine bubble size changes in dough. *IOP Conference Series: Materials Science and Engineering*, *42*(1). https://doi.org/10.1088/1757-899X/42/1/012037

Sun, X., Koksel, F., Nickerson, M. T., & Scanlon, M. G. (2020). Modeling the viscoelastic behavior of wheat flour dough prepared from a wide range of formulations. *Food Hydrocolloids*, *98*, 105129. https://doi.org/10.1016/j.foodhyd.2019.05.030

Surdyk, N., Rosén, J., Andersson, R., & Åman, P. (2004). Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. *Journal of Agricultural and Food Chemistry*, *52*(7), 2047–2051. https://doi.org/10.1021/jf034999w

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2000). Acrylamide: A cooking carcinogen? *Chemical Research in Toxicology*, *13*(6), 517–522. https://doi.org/10.1021/tx9901938

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, *50*(17), 4998–5006. https://doi.org/10.1021/jf020302f

Tozatti, P., Güldiken, B., Fleitas, M. C., Chibbar, R. N., Hucl, P., & Nickerson, M. T. (2020). The interrelationships between wheat quality, composition, and dough rheology for a range of Western Canadian wheat cultivars. *Cereal Chemistry*, *97*(5), 1010–1025. https://doi.org/10.1002/cche.10324

Triboi, E., Abad, A., Michelena, A., Lloveras, J., Ollier, J. L., & Daniel, C. (2000). Environmental effects on the quality of two wheat genotypes: 1. Quantitative and qualitative variation of storage proteins. *European Journal of Agronomy*, *13*(1), 47–64. https://doi.org/10.1016/S1161-0301(00)00059-9

Tronsmo, K. M., Magnus, E. M., Baardseth, P., Schofield, J. D., Aamodt, A., & Færgestad, E. M. (2003). Comparison of small and large deformation rheological properties of wheat dough

and gluten. *Cereal Chemistry*, *80*(5), 587–595. https://doi.org/10.1094/CCHEM.2003.80.5.587

Tyler, A. M., Bhandari, D. G., Poole, M., Napier, J. A., Jones, H. D., Lu, C., & Lycett, G. W. (2015). Gluten quality of bread wheat is associated with activity of RabD GTPases. *Plant Biotechnology Journal*, *13*(2), 163–176. https://doi.org/10.1111/pbi.12231

Upadhyay, R., Ghosal, D., & Mehra, A. (2012). Characterization of bread dough: Rheological properties and microstructure. *Journal of Food Engineering*, *109*(1), 104–113. https://doi.org/10.1016/j.jfoodeng.2011.09.028

Uthayakumaran, S., Beasley, H. L., Stoddard, F. L., Keentok, M., Phan-Thien, N., Tanner, R. I., & Békés, F. (2002). Synergistic and additive effects of three high molecular weight glutenin subunit loci. I. Effects on wheat dough rheology. *Cereal Chemistry*, *79*(2), 294–300. https://doi.org/10.1094/CCHEM.2002.79.2.294

Uthayakumaran, S., Newberry, M., Keentok, M., Stoddard, F. L., & Békés, F. (2000). Basic rheology of bread dough with modified protein content and glutenin-to-gliadin ratios. *Cereal Chemistry*, *77*(6), 744–749. https://doi.org/10.1094/CCHEM.2000.77.6.744

Uthayakumaran, S., Newberry, M., Phan-Thien, N., & Tanner, R. (2002). Small and large strain rheology of wheat gluten. *Rheologica Acta*, *41*(1), 162–172. https://doi.org/10.1007/s003970200015

Uthayakumaran, S., Tanner, R. I., Dai, S. C., Qi, F., & Wrigley, C. W. (2014). Relationships between traditional and fundamental dough-testing methods. *Cereal Research Communications*, *42*(2), 282–292. https://doi.org/10.1556/CRC.2013.0055

Uthayakumaran, S., Tanner, R. I., Dai, S., Qi, F., Newberry, M., Wrigley, C., & Copeland, L. (2012). Genotype-based stability of dough quality in wheat from different growth

environments. *Journal of Agricultural Science*, *4*(7), p41. https://doi.org/10.5539/jas.v4n7p41

Uthayakumaran, S., Zhao, F. J., Sivri, D., Roohani, M., Batey, I. L., & Wrigley, C. W. (2007). Defect identification in wheat grain by micro-fluidic electrophoresis: Sulfur deficiency and bug damage. *Cereal Chemistry Journal*, *84*(4), 301–303. https://doi.org/10.1094/CCHEM-84-4-0301

Van Bockstaele, F., De Leyn, I., Eeckhout, M., & Dewettinck, K. (2008a). Rheological properties of wheat flour dough and the relationship with bread volume. I. Creep-recovery measurements. *Cereal Chemistry*, *85*(6), 753–761. https://doi.org/10.1094/CCHEM-85-6-0762

Van Bockstaele, F., De Leyn, I., Eeckhout, M., & Dewettinck, K. (2008b). Rheological properties of wheat flour dough and their relationship with bread volume. II. Dynamic oscillation measurements. *Cereal Chemistry*, *85*(6), 762–768. https://doi.org/10.1094/CCHEM-85-6-0762

Van Vliet, T. (2014). Measuring methods. In *Rheology and fracture mechanics of foods* (pp. 91–108). CRC Press Taylor & Francis Group.

Vancini, C., Torres, G. A. M., Miranda, M. Z. de, Consoli, L., Bonow, S., & Grando, M. F. (2019). Impact of high-molecular-weight glutenin alleles on wheat technological quality. *Pesquisa Agropecuária Brasileira*, *54*, e00639. https://doi.org/10.1590/s1678-3921.pab2019.v54.00639

Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition*, *42*(3), 179–208. https://doi.org/10.1080/10408690290825510

Verheyen, C., Jekle, M., & Becker, T. (2014). Effects of Saccharomyces cerevisiae on the structural kinetics of wheat dough during fermentation. *LWT - Food Science and Technology*, *58*(1), 194–202. https://doi.org/10.1016/j.lwt.2014.02.050

Vestreng, V., Myhre, G., Fagerli, H., Reis, S., & Tarrason, L. (2007). Twenty-five years of continuous sulphur dioxide emission reduction in Europe. *Atmos. Chem. Phys.*, 19.

Wang, K., Ma, J., Islam, S., Yan, Y., Appels, R., Yan, G., & Ma, W. (2015). Detection of cysteine residue numbers in wheat gluten proteins by MALDI-TOF. *International Journal of Mass Spectrometry*, *392*, 91–95. https://doi.org/10.1016/j.ijms.2015.09.014

Wang, S., Yu, Z., Cao, M., Shen, X., Li, N., Li, X., Ma, W., Weißgerber, H., Zeller, F., Hsam, S., & Yan, Y. (2013). Molecular mechanisms of HMW glutenin subunits from 1Sl genome of Aegilops longissima positively affecting wheat breadmaking quality. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0058947

Wang, X., Lu, F., Zhang, C., Lu, Y., Bie, X., Xie, Y., & Lu, Z. (2014). Effects of recombinated Anabaena sp. Lipoxygenase on the protein component and dough property of wheat flour. *Journal of Agricultural and Food Chemistry*, *62*(40), 9885–9892. https://doi.org/10.1021/jf503238h

Wang, X., Zhang, Y., Zhang, B., Florides, C. G., Gao, Z., Wang, Z., Zhang, X., & Wei, Y. (2018). Comparison of quality properties between high-molecular-weight glutenin subunits 5 + 10 and 2 + 12 near-isogenic lines under three common wheat genetic backgrounds. *Cereal Chemistry*, *95*(4), 575–583. https://doi.org/10.1002/cche.10061

Weber, E. A., Graeff, S., Koller, W. D., Hermann, W., Merkt, N., & Claupein, W. (2008). Impact of nitrogen amount and timing on the potential of acrylamide formation in winter wheat

(Triticum aestivum L.). *Field Crops Research*, *106*(1), 44–52. https://doi.org/10.1016/j.fcr.2007.10.011

Wehrle, K., & Arendt, E. K. (1998). Rheological changes in wheat sourdough during controlled and spontaneous fermentation. *Cereal Chemistry Journal*, *75*(6), 882–886. https://doi.org/10.1094/CCHEM.1998.75.6.882

Weipert, D. (1990). The benefits of basic rheometry in studying dough rheology. *Cereal Chemistry*, *67*(4), 311–317.

Weipert, D. (2006). Fundamentals of rheology and spectrometry. In F. W. Popper L., Schäfer W. (Ed.), *Future of Flour – A Compendium of Flour Improvement* (pp. 117–146).

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, *24*(2), 115–119. https://doi.org/10.1016/j.fm.2006.07.004

Wieser, H., Bushuk, W., & MacRitchie, F. (2006). The polymeric glutenins. In C. W. Wrigley, F. Bekes, & W. Bushuk (Eds.), *Gliadin and Glutenin—The Unique Balance of Wheat Quality* (pp. 213–240). AACC International.

Wieser, H., Gutser, R., & Von Tucher, S. (2004). Influence of sulphur fertilisation on quantities and proportions of gluten protein types in wheat flour. *Journal of Cereal Science*, *40*(3), 239–244. https://doi.org/10.1016/j.jcs.2004.05.005

Wieser, H., & Seilmeier, W. (1998). The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour. *Journal of the Science of Food and Agriculture*, *76*(1), 49–55. https://doi.org/10.1002/(sici)1097-0010(199801)76:1<49::aid-jsfa950>3.0.co;2-2

Wieser, H., Vermeulen, N., Gaertner, F., & Vogel, R. F. (2008). Effects of different Lactobacillus and Enterococcus strains and chemical acidification regarding degradation of gluten

proteins during sourdough fermentation. *European Food Research and Technology*, *226*(6), 1495–1502. https://doi.org/10.1007/s00217-007-0681-1

Wikström, K., & Eliasson, A.-C. (1998). Effects of enzymes and oxidizing agents on shear stress relaxation of wheat flour dough: Additions of protease, glucose oxidase, ascorbic acid, and potassium bromate. *Cereal Chemistry Journal*, *75*(3), 331–337. https://doi.org/10.1094/CCHEM.1998.75.3.331

Wilson, T. L., Guttieri, M. J., Nelson, N. O., Fritz, A., & Tilley, M. (2020). Nitrogen and sulfur effects on hard winter wheat quality and asparagine concentration. *Journal of Cereal Science*, *93*, 102969. https://doi.org/10.1016/j.jcs.2020.102969

Wooding, A. R., Kavale, S., MacRitchie, F., Stoddard, F. L., & Wallace, A. (2000). Effects of nitrogen and sulfur fertilizer on protein composition, mixing requirements, and dough strength of four wheat cultivars. *Cereal Chemistry Journal*, *77*(6), 798–807. https://doi.org/10.1094/CCHEM.2000.77.6.798

World Health Organization. (2006). *Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Contaminants*. https://apps.who.int/iris/bitstream/handle/10665/43258/WHO_TRS_930_eng.pdf?sequence=1

Wrigley, C. W. (1996). Giant proteins with flour power. *Nature*, *381*(6585), 738–739. https://doi.org/10.1038/381738a0

Wrigley, C. W. (2009). Wheat: A unique grain for the world. In K. Khan & P. Shewry (Eds.), *Wheat Chemistry and Technology* (Fourth, pp. 1–17). AACC International.

Wrigley, C. W., Békés, F., & Bushuk, W. (2006). Gluten: A balance of gliadin and glutenin. In *Gliadin and glutenin—The unique balance of wheat quality* (pp. 3–32). AACC International.

Wunsch, N.-G. (2020). Global bread and bakery products market size 2017-2025. *Statista*. https://www.statista.com/statistics/947363/bread-and-bakery-products-market-size-global/

Xie, Y., Malunga, N., Ames, N., Waterer, J., Khorshidi, A., & Scanlon, M. (2021). Effects of growing environment, genotype, and commercial fertilization levels on free asparagine concentration in Western Canadian wheat. *Cereal Chemistry*, cche.10364. https://doi.org/10.1002/cche.10364

Xinyang, S. (2019). *The effects of wheat flour, water, salt and mixing on the rheological properties and the gas phase of bread dough* [PhD Thesis]. University of Manitoba.

Xu, H., Curtis, T. Y., Powers, S. J., Raffan, S., Gao, R., Huang, J., Heiner, M., Gilbert, D. R., & Halford, N. G. (2018). Genomic, biochemical, and modeling analyses of asparagine synthetases from wheat. *Frontiers in Plant Science*, *8*(January), 1–12. https://doi.org/10.3389/fpls.2017.02237

Xue, C., Erley, G. S. A., Rossmann, A., Schuster, R., Koehler, P., & Mühling, K. H. (2016). Split nitrogen application improves wheat baking quality by influencing protein composition rather than concentration. *Frontiers in Plant Science*, *7*(JUNE2016), 1–11. https://doi.org/10.3389/fpls.2016.00738

Yang, Y., Guan, E., Zhang, T., Li, M., & Bian, K. (2019). Influence of water addition methods on water mobility characterization and rheological properties of wheat flour dough. *Journal of Cereal Science*, *89*, 102791. https://doi.org/10.1016/j.jcs.2019.102791

Yaylayan, V. A., Wnorowski, A., & Perez Locas, C. (2003). Why asparagine needs carbohydrates to generate acrylamide. *Journal of Agricultural and Food Chemistry*, *51*(6), 1753–1757. https://doi.org/10.1021/jf0261506

Yazar, G., Duvarci, O. C., Tavman, S., & Kokini, J. L. (2016). Effect of mixing on LAOS properties of hard wheat flour dough. *Journal of Food Engineering*, *190*, 195–204. https://doi.org/10.1016/j.jfoodeng.2016.06.011

Yovchev, A., Briggs, C. E., Stone, A. K., Scanlon, M. G., Tyler, R. T., Hucl, P. J., & Nickerson, M. T. (2017). Interrelationships of flour, dough, and bread properties under reduced salt level conditions. *Cereal Chemistry*, *94*, 760–769. https://doi.org/10.1094/CCHEM-12-16-0285-R

Yu, Z., She, M., Zheng, T., Diepeveen, D., Islam, S., Zhao, Y., Zhang, Y., Tang, G., Zhang, Y., Zhang, J., Blanchard, C. L., & Ma, W. (2021). Impact and mechanism of sulphur-deficiency on modern wheat farming nitrogen-related sustainability and gliadin content. *Communications Biology*, *4*(1). https://doi.org/10.1038/s42003-021-02458-7

Zghal, M. C., Scanlon, M. G., & Sapirstein, H. D. (2001). Effects of flour strength, baking absorption, and processing conditions on the structure and mechanical properties of bread crumb. *Cereal Chemistry Journal*, *78*(1), 1–7. https://doi.org/10.1094/CCHEM.2001.78.1.1

Zhang, M., Ma, D., Wang, C., Zhao, H., Zhu, Y., & Guo, T. (2016). Responses of amino acid composition to nitrogen application in high- and low-protein wheat cultivars at two planting environments. *Crop Science*, *56*(3), 1277–1287. https://doi.org/10.2135/cropsci2015.08.0504

Zhang, P., Jondiko, T. O., Tilley, M., & Awika, J. M. (2014). Effect of high molecular weight glutenin subunit composition in common wheat on dough properties and steamed bread quality. *Journal of the Science of Food and Agriculture*, *94*(13), 2801–2806. https://doi.org/10.1002/jsfa.6635

Zhang, Y., Dai, X., Jia, D., Li, H., Wang, Y., Li, C., Xu, H., & He, M. (2016). Effects of plant density on grain yield, protein size distribution, and breadmaking quality of winter wheat grown under two nitrogen fertilisation rates. *European Journal of Agronomy*, *73*, 1–10. https://doi.org/10.1016/j.eja.2015.11.015

Zhao, C. X., He, M. R., Wang, Z. L., Wang, Y. F., & Lin, Q. (2009). Effects of different water availability at post-anthesis stage on grain nutrition and quality in strong-gluten winter wheat. *Comptes Rendus - Biologies*, *332*(8), 759–764. https://doi.org/10.1016/j.crvi.2009.03.003

Zhao, F. J., Hawkesford, M. J., & McGrath, S. P. (1999). Sulphur assimilation and effects on yield and quality of wheat. *Journal of Cereal Science*, *30*(1), 1–17. https://doi.org/10.1006/jcrs.1998.0241

Zhao, F. J., McGrath, S. P., Salmon, S. E., Withers, P. J. A., Shewry, P. R., Evans, E. J., & Monaghan, J. M. (1999). Responses of breadmaking quality to sulphur in three wheat varieties. *Journal of the Science of Food and Agriculture*, *79*(13), 1865–1874. https://doi.org/10.1002/(SICI)1097-0010(199910)79:13<1865::AID-JSFA449>3.0.CO;2-R

Zhao, F. J., Salmon, S. E., Withers, P. J. A., Monaghan, J. M., Evans, E. J., Shewry, P. R., & McGrath, S. P. (1999). Variation in the breadmaking quality and rheological properties of

wheat in relation to sulphur nutrition under field conditions. *Journal of Cereal Science*, *30*(1), 19–31. https://doi.org/10.1006/jcrs.1998.0244

Zhao, X., Li, W., Zhang, H., Liu, X., & Zhang, Z. (2019). Disparate dynamic viscoelastic responses of wheat flour doughs coated with different oils for preventing water evaporation during time sweeps and their mechanisms decoupled. *Journal of Food Science and Technology*, *56*(1), 462–472. https://doi.org/10.1007/s13197-018-3508-0

Zheng, T., Qi, P.-F., Cao, Y.-L., Han, Y.-N., Ma, H.-L., Guo, Z.-R., Wang, Y., Qiao, Y.-Y., Hua, S.-Y., Yu, H.-Y., Wang, J.-P., Zhu, J., Zhou, C.-Y., Zhang, Y.-Z., Chen, Q., Kong, L., Wang, J.-R., Jiang, Q.-T., Yan, Z.-H., … Zheng, Y.-L. (2018). Mechanisms of wheat (Triticum aestivum) grain storage proteins in response to nitrogen application and its impacts on processing quality. *Scientific Reports*, *8*(1), 11928. https://doi.org/10.1038/s41598-018-30451-4

Zhou, J., Liu, D., Deng, X., Zhen, S., Wang, Z., & Yan, Y. (2018). Effects of water deficit on breadmaking quality and storage protein compositions in bread wheat (*Triticum aestivum* L.): Effects of water deficit on storage proteins in bread wheat. *Journal of the Science of Food and Agriculture*, *98*(11), 4357–4368. https://doi.org/10.1002/jsfa.8968

Zhu, J., Huang, S., Khan, K., & O'Brien, L. (2001). Relationship of protein quantity, quality and dough properties with Chinese steamed bread quality. *Journal of Cereal Science*, *33*(2), 205–212. https://doi.org/10.1006/jcrs.2000.0358

Zörb, C., Steinfurth, D., Seling, S., LangenkÄmper, G., Koehler, P., Wieser, H., Lindhauer, M. G., & Mühling, K. H. (2009). Quantitative protein composition and baking quality of winter wheat as affected by late sulfur fertilization. *Journal of Agricultural and Food Chemistry*, *57*(9), 3877–3885. https://doi.org/10.1021/jf8038988

Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P., & Villagran, M. D. (2003). Acrylamide formation mechanism in heated foods. *Journal of Agricultural and Food Chemistry*, *51*(16), 4782–4787. https://doi.org/10.1021/jf034180i