

**Clean-Label Approaches to Modify Starch Functionality and
Digestibility in Bread: Role of Germination and Plant-Based Phenolics**

By

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Dedication

To all the women who dare to choose their own path and pursue their dreams.

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List of Abbreviations

Adj- Adjusted

ANOVA- Analysis of variance

CWRS- Canadian Western Red Spring

Cy 3-gluE- cyanidin-3-glucoside equivalents

DC- Diluted control

DHT- Dry heating treatment

DSC- Differential scanning calorimetry

FN- Falling number

G- Grape

G 6- 6-hour germination

G 12- 12-hour germination

G 18- 18-hour germination

G 24- 24-hour germination

GAE- Gallic acid equivalents

GOPOD- Glucose oxidase–peroxidase

GSI- GlutoPeak strength index

HHP- High hydrostatic pressure

HMT- Heat moisture treatment

MAE- Microwave-assisted extraction

MTI- Mixing tolerance index

MW- Molecular weight

OSA- Octenyl succinate anhydride

P- Pomegranate

PEF- Pulsed electric field
PSP- Purple sweet potato
QE- Quercetin equivalents
RDS- Rapidly digestible starch
RH- Relative humidity
RS- Resistant starch
RVA- Rapid visco analyzer
SDS- Slowly digestible starch
TAC- Total anthocyanin content
TFC- Total flavonoid content
TPC - Total phenolic content

Thesis Abstract

Starch digestibility of wheat-based bakery foods plays an important role in determining their nutritional quality and potential impact on postprandial glycemic response. Modifying starch hydrolysis in bread systems to meet consumers' demand for clean label has therefore become an important research focus in cereal science. This thesis investigated alternative strategies to control starch functionality and digestibility in bread systems through sustainable, process- and formulation-based approaches.

First, the role of clean-label starch in bakery and confectionery applications is critically reviewed, highlighting emerging physical, enzymatic, and genetic modification techniques that improve functionality while aligning with sustainability and consumer expectations. This establishes the framework for exploring non-chemical strategies to modulate starch behaviour.

Building on the previous chapter, the effect of wheat germination on flour functionality and bread performance was investigated. Germination increased α -amylase activity, leading to significant changes in starch properties and dough behaviour. Peak viscosity was identified as a practical quantitative marker to control germination level, showing strong correlations with α -amylase activity ($r=-0.84$, $p<0.0001$) and FN ($r=0.98$, $p<0.0001$). Viscosity-adjusted germination allowed better control of enzymatic activity, improving dough handling and producing more consistent bread texture while reducing starch hydrolysis during digestion.

The impact of grain composition was explored using purple wheat, where the germination interacts with the inherent phenolic compounds. Increasing germination time enhanced enzymatic activity and progressively reduced starch pasting ability and gluten aggregation strength. Germinated purple wheat exhibited stronger viscosity breakdown compared with common wheat and produced bread with a distinctive reddish–yellow crumb due to anthocyanin transformations during processing. Phenolic compounds, particularly anthocyanins, were strongly associated with starch

digestion behaviour, showing positive correlations with RS ($r=0.861$, $p=0.006$) and negative correlations with starch hydrolysis rate ($r=-0.918$, $p=0.001$). These findings demonstrate that pigmented wheat exhibits a distinct germination response compared with common wheat.

Finally, a formulation-based approach was examined through the incorporation of phenolic-rich plant powders, including 5 g/100 g of PSP, G, and P, into wheat bread formulations, respectively. Phenolic enrichment modified gluten aggregation, starch pasting properties, and dough mixing behaviour while maintaining acceptable bread quality. DSC results indicated the formation of starch–phenolic complexes, evidenced by characteristic thermal transitions ($\sim 100^{\circ}\text{C}$ and $\sim 130^{\circ}\text{C}$), suggesting the presence of stable complexes that likely limited enzymatic hydrolysis of starch. Among the tested ingredients, P powder showed the strongest inhibitory effect on starch digestion, significantly ($p<0.05$) reducing RDS and increasing RS. Correlation analyses confirmed strong relationships between phenolic content and starch digestion kinetics, corresponding to the phenolic-starch complex formation.

Overall, this research demonstrates that starch digestibility in wheat bread can be modulated through both grain-level biochemical modification and formulation strategies using phenolic-rich ingredients. The findings highlight the interactions among enzymatic activity, starch structure, phenolic compounds, and dough matrix properties, providing new insights for the development of cereal-based foods with improved nutritional functionality and potential benefits for glycemic control.

Chapter 1 Overview

1.1 General introduction

Wheat is one of the most widely cultivated cereal crops in the world and serves as a primary staple food for a large proportion of the global population. Wheat-based products provide an important source of carbohydrates, proteins, and essential nutrients in human diets (Garg et al., 2021). Among these products, bread remains one of the most widely consumed wheat-derived foods due to its accessibility, versatility, and cultural significance across many regions (Khalid et al., 2023). The quality of bread is largely determined by the functional properties of wheat flour and the interactions among its major components during dough development and baking (Akram et al., 2017).

Starch is the most abundant component of wheat flour, typically accounting for approximately 70–75 g/100 g of its dry weight (Tinceva, 2019). As the primary carbohydrate in wheat-based foods, starch plays a critical role in determining both the technological and nutritional properties of bread. During breadmaking, starch granules absorb water and undergo gelatinization during baking, disrupting their crystalline structure and allowing amylose and amylopectin molecules to interact with surrounding components such as gluten proteins and lipids (Akram et al., 2017). These transformations contribute to the formation of bread crumb structure and greatly influence product texture, shelf life, and overall quality.

Structurally, starch is a semi-crystalline polysaccharide composed of two main glucose polymers: amylose and amylopectin (Amagliani et al., 2016). Amylose is a predominantly linear molecule consisting of α -(1→4)-linked glucose units, whereas amylopectin is a highly branched polymer containing both α -(1→4) and α -(1→6) glycosidic linkages. These polymers are organized into complex granule structures within the wheat endosperm, forming alternating crystalline and

amorphous regions that determine the physicochemical behaviour of starch during food processing and digestion (Mua & Jackson, 1997).

Following consumption, starch is hydrolyzed by digestive enzymes into glucose, which is subsequently absorbed and utilized as an energy source in the human body. The rate and extent of starch digestion can vary depending on starch structure and its interactions with other food components (Magallanes-Cruz et al., 2017). Based on digestion characteristics, starch is generally classified into RDS, SDS, and RS. Rapid digestion of starch may lead to elevated postprandial blood glucose levels, which have been associated with metabolic disorders such as obesity and type 2 diabetes (Chai et al., 2013). Consequently, increasing attention has been directed toward strategies that can modulate starch digestibility in cereal-based foods in order to improve their nutritional quality and potential health benefits.

To modify starch functionality and digestibility in food systems, both native starch and modified starch have been widely used in the food industry. Native starch refers to starch that retains its natural structure without chemical or physical modification. However, native starch often exhibits limitations in terms of stability, processing tolerance, and functional performance under industrial conditions (Gunaratne, 2023). Therefore, various modification techniques, including chemical, enzymatic, and physical treatments, have been developed to produce modified starch with improved functional properties. Despite their technological advantages, chemically modified starches have raised concerns among consumers who increasingly prefer foods made with minimally processed ingredients (Maniglia et al., 2021).

This consumer demand has led to growing interest in the development of clean-label starch, which refers to starch ingredients that maintain natural or minimally modified characteristics while still providing desirable functional properties in food products. Approaches such as enzymatic

treatment, physical modification, and the use of natural ingredients have been explored as alternatives to traditional chemical modification methods. In cereal-based foods such as bread, natural strategies that can modulate starch functionality and digestibility while maintaining clean label characteristics are therefore of considerable interest (Amante et al., 2021).

Among these strategies, enzymatic activity plays an important role in modifying starch functionality. In wheat flour systems, α -amylase is a key enzyme capable of hydrolyzing α -(1 $\rightarrow$ 4) glycosidic linkages in starch molecules, producing smaller dextrins and fermentable sugars that contribute to yeast fermentation and dough development (Caballero et al., 2007). The level of amylase activity is commonly evaluated using the Falling Number test, which indirectly reflects the extent of starch degradation caused by α -amylase (Kaur et al., 2024). Maintaining appropriate enzymatic activity is essential for achieving desirable flour functionality and bread quality.

One natural process that significantly influences enzymatic activity in wheat is germination. During germination, metabolic processes are activated within the grain, leading to increased synthesis of hydrolytic enzymes such as α -amylase (Chinma et al., 2022). These enzymatic changes can modify starch structure, alter starch gelatinization behaviour, and affect dough rheology and breadmaking performance (Kaur et al., 2024). In addition to enzymatic changes, germination may also influence the availability of bioactive compounds, including phenolic compounds, which may further affect starch functionality and digestibility (Cornejo et al., 2015). Phenolic compounds represent a large group of plant secondary metabolites characterized by aromatic rings bearing hydroxyl groups (Czajkowska–González et al., 2021). These compounds are widely distributed in plant-derived foods and are well known for their antioxidant activity and potential health benefits (Lu et al., 2021). In recent years, phenolic compounds have also attracted considerable attention due to their potential to modulate carbohydrate digestion (Kwasny et al.,

2022). The type and concentration of polyphenolic compounds also significantly influenced wheat starch digestibility, such as 5 mg (+)-catechin, showing the most pronounced inhibitory effects compared to other phenolic acids, flavanones, and anthocyanins on starch digestion (Kwasny et al., 2022).

Pigmented cereals and plant-based foods are particularly rich sources of phenolic compounds. For example, pigmented wheat varieties such as purple wheat contain higher concentrations of phenolic compounds compared with conventional wheat due to the presence of anthocyanins and other phenolic constituents in the outer layers of the grain (Abdel-Aal et al., 2016). Similarly, several fruits and vegetables, including PSP, G, and P, are recognized as important sources of phenolic compounds (Li et al., 2019; Milincic et al., 2025; Oliveira et al., 2020). These plant materials have increasingly been explored as functional ingredients in cereal-based foods to enhance nutritional value and introduce bioactive compounds into widely consumed products (Agirbas et al., 2021).

Beyond their antioxidant properties, phenolic compounds may influence starch digestion through several mechanisms. One mechanism involves inhibiting digestive enzymes, particularly α -amylase, which hydrolyze starch during digestion. Certain phenolic compounds can interact with these enzymes and reduce their catalytic activity, thereby slowing starch hydrolysis and glucose release (Santamaria et al., 2025). In addition, phenolic compounds may interact directly with starch molecules through hydrogen bonding or hydrophobic interactions, leading to the formation of starch-polyphenol complexes that alter starch structure and reduce its susceptibility to enzymatic digestion (Moreno-Zaragoza et al., 2025). Phenolic compounds may also influence starch functionality during food processing. Interactions between polyphenols and starch can modify

starch gelatinization, pasting behaviour, and retrogradation properties, which may ultimately affect the texture and structural characteristics of cereal-based foods (Paixão e Silva et al., 2021).

Despite increasing interest in developing cereal-based foods with improved functionality, several important knowledge gaps remain regarding the mechanisms regulating starch digestibility in wheat-based products. Germination has been widely applied to modify wheat composition and enzymatic activity; however, the extent to which germination-induced changes in α -amylase activity influence starch digestibility in final baked products remains poorly understood. In addition, there is no study on the purple wheat germination and its effect on starch digestibility. Furthermore, plant-derived ingredients from fruits and vegetables are increasingly incorporated into bakery products as sources of phenolic compounds and dietary fibre. However, most previous studies have examined individual ingredients or focused primarily on product quality, while the relationships between phenolic composition, dough functionality, starch physicochemical behaviour, and starch digestion kinetics remain insufficiently explored.

Therefore, a comprehensive approach integrating grain biochemical modification (germination), phenolic-rich ingredients, dough functionality, and starch digestion behaviour is needed to better understand how these factors collectively modulate starch digestibility in wheat bread.

1.2 Hypothesis

The hypotheses proposed in this series of studies are as follows:

1. Peak viscosity can serve as a reliable indicator of the degree of germination and its impact on flour functionality and starch digestibility.
2. Germination of purple wheat will modify flour functionality and bread quality by enhancing enzymatic activity, which may subsequently influence starch digestibility.

3. Incorporating phenolic-rich plant powders at a 5 g/100 g substitution level will modify starch properties, weaken gluten development, and reduce starch digestibility through interactions between polyphenols and starch.

1.3 Objectives

1.3.1 General Objective

The overall objective of this research was to investigate how to control starch functionality and digestibility in bread systems through process and formulation strategies aligned with clean-label trends.

1.3.2 Specific Objectives

This body of work aims to address the following objectives:

1. To critically review and synthesize current advancements in clean-label starch sources and modification technologies, and to assess their implications for functionality, sustainability, and innovation in bakery and confectionery applications.
2. Evaluate the effects of germination on wheat flour functionality and starch digestibility by using peak viscosity as a reliable marker of the degree of germination.
3. Compare how controlled germination influences the functional properties of purple wheat flour and the quality of the bread made from it with common wheat.
4. Investigate the effects of incorporating three phenolic-rich plant powders (PSP, G, and P) at a 5 g/100 g substitution level on starch pasting and thermal behaviour, dough development, bread quality, and *in vitro* starch digestibility.

1.4 Structure of the Thesis

This study is organized into the following chapters:

Chapter 2: The literature review explores clean-label starch in bakery and confectionery products, detailing its functional roles and the limitations of chemically modified starches. It emphasizes the rising demand for natural alternatives and discusses strategies to enhance starch functionality through eco-friendly methods like physical treatments, enzymatic activity, germination, and the addition of novel starch from natural ingredients, which can also affect starch digestibility and the nutritional value of wheat-based foods.

Chapter 3: Examines apparent viscosity as a marker of wheat germination and its ability to predict bread quality and starch digestibility. The chapter investigates how germination-induced changes in α -amylase activity affect wheat flour functionality and starch pasting properties. It further explores the relationship between viscosity parameters, bread quality, and *in vitro* starch digestibility, highlighting the potential of viscosity as a practical indicator of germination effects.

Chapter 4: Investigates the effects of germination on purple wheat flour functionality, bread quality, and starch digestibility, with particular emphasis on comparisons with germinated common wheat. The chapter evaluates how germination influences flour properties and bread characteristics while considering the naturally higher phenolic content of purple wheat. Special attention is given to the role of phenolic compounds as potential α -amylase inhibitors and their contribution to modulating starch digestion.

Chapter 5: Evaluates the effects of incorporating phenolic-rich plant powders, including PSP, G, and P, into refined wheat flour on dough functionality, bread quality, and starch digestibility. The chapter examines how a 5 g/100 g substitution level influences starch pasting and thermal properties, dough development, and bread characteristics. Particular attention is given to the interactions between phenolic compounds and starch, and their potential role in modulating starch digestion in wheat-based products.

Chapter 6: The concluding chapter presents an overall discussion and conclusion of all three studies, summarizing the main findings and emphasizing the possible future directions.

Chapter 2_Literature Review

DISCLAIMER: This chapter is based on the following published book chapter: Xiaohang Zou, Nicola Gasparre, and Cristina M. Rosell (2026). In PS Panesar & Vikas Kumar (Eds.), Clean Label Starch Processes, Properties, and Functionality (chapter 9). Elsevier Inc. ISBN: 9780443334795. Publication Date: 01-Mar-26.

Clean-label starch in bakery and confectionery

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Abstract

Starch plays a crucial role in the bakery and confectionery industries, where it is extensively used as either a primary ingredient or a multifunctional additive that significantly enhances the quality of the final products. While native starch exhibits certain limitations that can impact the desired characteristics of these products, modified starch has been developed through physical, chemical, enzymatic, and genetic methods to augment their desirable qualities. However, concerns regarding the environmental harm and potential health implications of traditionally chemically modified starch have led to a growing consumer demand for natural and minimally processed foods, driving the incorporation of clean-label starches in bakery and confectionery products.

Clean-label starch can be derived from natural sources or modified through environmentally friendly methods. The sources of native starches include fruits, vegetables, grains, and legumes. Research into eco-friendly alternatives focuses on options that are both effective and sustainable. Promising approaches include physical treatments such as heat treatments, microwave treatment,

ultrasound, HHP, PEF, and cold plasma; enzymatic treatments involving amylase and glycosyltransferases; and genetic treatments for breeding, such as CRISPR-Cas9 and RNA silencing. These modified starches not only improve the texture, shelf life, and structural characteristics of products but also contribute to the development of innovative formulations with enhanced nutritional profiles and functional attributes.

This chapter explores the diverse functional roles of starch in bakery and confectionery applications, discusses the latest advancements in clean-label starch modification technology and utilization, addresses the challenges involved, and highlights future trends regarding the use of clean-label starch in the bakery and confectionery sectors.

Keywords: bakery products, clean-label, confectionery, natural ingredients, starch modification.

2.1 Introduction

Starch, a prevalent polysaccharide found in essential food items such as rice, wheat, corn, and potatoes, functions as a crucial caloric source in human nutrition. Rice and corn are the top two important sources of calories worldwide, and cassava is the third most significant caloric source in tropical regions, following rice and maize (Dini et al., 2023). Composed of long chains of glucose molecules, starch is broken down during digestion, releasing glucose, utilized by the body for energy production. This energy fuels essential bodily functions, from muscle contractions to brain activity (Amagliani et al., 2016).

Starch is an adaptable component utilized across multiple sectors; in particular, it holds appeal as a food component for modifying texture due to its dual characteristics of being natural and safe. In bakery applications, starches enhance texture, improve shelf life, and provide fat-replacement properties, while in confectionery they contribute to desirable texture, flavour, gelling, and

stabilizing properties. They are also particularly valuable in gluten-free formulations given that they can create a pseudo-gluten network (Gasparre & Rosell, 2023).

However, starch is often characterized by poor viscosity and water retention capacity, low stability in acidic environments, limited solubility in cold aqueous systems, a pronounced tendency toward retrogradation, and high sensitivity to shear forces. Native starch application is limited by these inherent constraints. Thus, starch is often modified to improve functionality and expand applications (Gunaratne, 2023). For this purpose, various methods (chemical, physical, enzymatic, and genetic) have been employed to produce starches with specific properties for industrial use. Starch modification entails the incorporation of novel functional groups into starch molecules or the modification of starch molecule size and granule properties. Acetylated and hydroxypropylated starches, for example, are frequently employed in bakery and pastry products because of their improved functional characteristics (Cisse et al., 2023). Concerns about the environmental and health risks of traditional starch modification methods are rising (Maniglia et al., 2021). This has spurred research into eco-friendly alternatives that offer both effectiveness and sustainability, while meeting consumer preferences for natural and sustainable products, often referred to as "clean label" ingredients (Maruyama et al., 2021). Such methods can improve starch properties, including viscosity, freeze-thaw stability, and water-binding capacity, offering a versatile solution for enhancing the quality of bakery and confectionery products (Bensaad et al., 2022).

Consumers have shown a strong interest in two key aspects of bakery and confectionery products. First, they prioritize functional ingredients that align with health and wellness trends. Second, there is a significant demand for confectionery that offers an indulgent, sensory-rich experience (McDonagh, 2012). Sustainability has risen to the forefront of the baking industry's agenda, driven by consumers' growing concerns about environmental impact, food sourcing, and production

practices. This often involves avoiding chemical modifications in favour of natural colours, flavours, starches, and sweeteners derived from sources like fruits, vegetables, and grains. Although regulatory standards for "clean label" vary across countries, the principal trend emphasizes ingredient transparency, safety, and a shift towards simpler, more natural options (Galanakis, 2022; Asioli et al., 2017). From an economic standpoint, the worldwide clean-label starch market achieved sales of approximately US \$1,272 million in 2018 and exhibited a growth rate of 3.6% from 2018 to 2022. With the rising demand for plant-derived functional ingredients and convenient ready-to-eat products, the global market is projected to attain a value of around US \$2,700 million with a growth rate of 5.4% from 2023 to 2033 (Persistence Market Research Company, 2023). Hence, it is clear that despite the regulatory and economic challenges surrounding the "clean label" trend, its market continues to grow significantly, driven by increasing consumer demand for plant-based, natural ingredients. This growth underscores the importance of developing innovative, sustainable methods to meet these expectations. In this context, the following chapter will focus on environmentally sustainable approaches for modifying starch and their applications in bakery and confectionery.

2.2 Starch in bakery and confectionery

Conventional starches such as corn, potato, wheat, tapioca, and rice are widely used in bakery and confectionery products, playing a key role in shaping the texture, structure, and overall quality of the final product (Table 2.1). In particular, these starches are particularly effective as gelling agents, working synergistically with sugars, fat replacers, and texturizers to achieve the desired structural integrity and sensory experience (Arik Kibar, 2023; Gupta et al., 2023; Serinyel and Öztürk, 2017).

Table 2.1 The characteristics and utilization of conventional native starch in bakery and confectionery

Starch type	Source	Characteristics	Utilization in bakery	Utilization in confectionery	Reference
Corn starch	Fruits of the Gramineae family	White powder, neutral flavour. High water retention capacity, high viscosity, and stability to acid and heating.	Thickening pie fillings, custards. Gelling agent, thickener, anti-caking agent.	Chewy candies; marshmallows; gumdrops.	(Mishra and Rai, 2006; Tincheva, 2019)
Wheat starch	Fruits of Triticum species	Soft wheat starches exhibit low paste viscosity due to high amounts of surface lipids and proteins. Hard cultivars exhibit elevated levels of small granules and amylose, alongside a reduced gelatinization temperature.	Bread and cake structure; thickening custards	Licorice; gummy candies; chewy sweets	(Tincheva, 2019)
Rice starch	Seeds of the grass species <i>Oryza sativa</i>	Fine and granule size like fat globules, hypoallergenic, smooth texture. Waxy and low-amylose rice starches had high swelling capacity compared to high-amylose rice starches.	Gluten-free baking; improving crumb texture, fat replacer, and extending shelf-life.	Producing maltodextrins; Clear gels in jellies; candy coatings; stabilizers, fillers, flavour carriers, sweetness reducers, texture modifiers.	(Aleman et al., 2023; Amagliani et al., 2016)
Potato starch	Tubers of Solanaceae family	High viscosity, swelling capacity, and solubility. Low tendency to retrograde. Low gelatinization temperature.	Thickener, gluten-free baked goods; moisture retention in cakes.	Jelly candies; gummy bears; thickening fruit fillings.	(Aleman et al., 2023; Tincheva, 2019)

Tapioca starch	Cassava roots of Euphorbiaceae family	Amylose has high molecular weight, high viscosity, high solubility, smooth texture, and strong gas bubble-holding ability due to the strong gelation properties.	Gluten-free baked goods; binding agent in doughs	Sweeteners; puddings; chewy candies; bubble tea pearls	(Aleman et al., 2023; Dini et al., 2023)
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2.2.1 Starch in bakery

Starch derived from different botanical sources exhibits diverse morphologies, including variations in granule size, structure, and composition, as well as functional properties such as solubility, viscosity, gelling and flow characteristics, and water-holding capacity. The origin of starch is a critical factor to consider when selecting the appropriate type for specific applications (Amagliani et al., 2016; Mishra and Rai, 2006; Tincheva, 2019). Starches derived from various sources are extensively utilized in the food sector for texture modification and stabilization. For instance, native potato and tapioca starches produce clear, low-viscosity gels, making them ideal for applications like cake-making or dry soup production. The incorporation of a specific amount of medium-gelatinized potato starch into wheat flour has been shown to make the texture softer with more uniform gas cells to improve the overall performance of starch-based products and increase consumer acceptance (Cheng et al., 2022).

Modified starches, such as OSA modified starch, provide superior benefits as fat replacers in bakery applications compared to native starch. Fat plays a vital role in bakery products, contributing to taste, appearance, and texture. However, due to its high caloric content and potential health risks, including cardiovascular diseases and obesity, there is a growing demand for reducing or replacing fat with lower-fat alternatives. 4% OSA-modified wheat and tapioca starches were utilized as fat replacers in bread making, demonstrating comparable dough and bread characteristics to the control samples (Balic et al., 2017). In addition, OSA-modified starch enhanced SDS levels, offering advantages for low-calorie bread formulation utilizing OSA starches as fat substitutes (Balic et al., 2017). Punia et al. (2019) also demonstrated that incorporating 30% OSA-modified mung bean starch into cake formulations effectively reduced fat content without compromising sensory qualities. This modification also enhanced the cake's

texture, colour, and water retention properties, making it a recommended alternative for healthier baked goods. The incorporation of starches along with bakery enzymes, such as α -amylase and hemicellulase, during the bread-making process significantly enhances specific loaf volume, improves crumb microstructure, and extends freshness (Matsushita et al., 2020). Furthermore, combining starches with hazelnut skin, cross-linked starch, or oxidized starch can enhance the loaf volume and softer crumb structure. It addressed textural complications related to fiber enrichment while preserving sensory qualities (Durmus et al., 2024). The properties of starches, such as retrogradation and resistance to digestion, are crucial factors in determining the characteristics of bakery products (Aleman et al., 2023; Juárez-García et al., 2006). Starches can interact with lipases in baked goods, affecting the shelf life and quality of the products (Carpen et al., 2019).

2.2.2 Starch in confectionery

Confectionery refers to a diverse array of products, including boiled sweets (fondant, caramels, hard candy, fudge, and toffee), aerated confections (nougats and marshmallows), gelled candies (licorice, jellies, and gums), coatings, and chocolate confections. The primary ingredients in confectionery products are various types of sugars, such as sucrose, inverted sugar, fructose, and glucose syrup (Hahnadev et al., 2018).

Starch serves as a gelling agent in confectionery, utilized alone or with gelatin (J. Singh et al., 2007). It helps to create the desired texture and consistency in sweet treats such as gummies, jellies, and marshmallows. Starch-based gelling agents can form clear, stable gels across a wide range of temperatures and conditions. They also contribute to a smooth mouthfeel and can be used to control the setting time and firmness of the final product (Arik Kibar, 2023). In some confectionery products, starch is also utilized to prevent moisture loss and extend shelf life. In starch-based fudge, starch is combined with sugar to form a syrup of a specific consistency, acting as the main gelling

agent. Since starch becomes a thick paste with high viscosity when heated in water, affecting the boiling of fudge, starch must meet certain criteria: (1) strong gelation ability; (2) low viscosity to maintain fluidity at high temperatures; (3) good water solubility; (4) good transparency; (5) normal odour and colour. Native starches typically fall short of meeting the specific requirements for soft candy production, leading to the widespread use of modified starches. Modified corn starch and gelatinized starch offer the texture and consistency needed for soft candies. Modified corn starch is increasingly popular in the confectionery industry due to its high gel strength, while potato-modified starch offers low pasting viscosity, mild odour, and high transparency (Autio et al., 1992). Acid-modified starch and oxidized starch are commonly used in soft candies (Karim & Bhat, 2008). In gelatin-based candies, modified starch acts as a filler, giving body structure. As starch is not the main gelling agent, the requirements for gel strength can be slightly reduced; namely, lower viscosity and higher transparency are required to complement gelatin, which has higher viscosity and transparency. Furthermore, including starch in gelatin soft candy formulation helps to reduce production costs (Arik Kibar, 2023; Marfil et al., 2012).

The use of starch in confectionery products serves several important functions, without significantly altering its flavour, offering an economical means of increasing the size of the product. Starch contributes to the desired chewy or firm texture in candies, gummies, and other confections. Moderate addition of starch can also reduce the ductility of gelatin, improve an overly hard texture, and make gelatin-based fondant easier to bite while maintaining high elasticity and chewiness (Marfil et al., 2012). Moreover, starch helps bind the ingredients together, ensuring a uniform distribution of flavours and other components throughout the confectionery item. As a thermally irreversible colloid, starch enhances the thermal stability of the reversible gel formed by gelatin in fudge, stabilizing the overall structure of the confectionery, preventing ingredient separation, and

improving the product's shelf life (Firoozmand et al., 2009). Additionally, the moisture-absorbing properties of starch maintain the right consistency, preventing the confectionery from becoming excessively sticky or dry (İlhan et al., 2022).

2.2.3 Starch application in gluten-free products

The increasing demand for gluten-free products necessitates the use of various starches and additives to achieve desirable dough characteristics, particularly for individuals with celiac disease. Gluten-free bakery items typically utilize diverse starches to replicate gluten's viscoelastic characteristics and enhance product quality (Gasparre and Rosell, 2023; Houben et al., 2012). Starches from tapioca, potato, and corn are utilized in gluten-free formulations to improve attributes like volume, colour, crumb structure, and texture (Horstmann et al., 2017). Very often, starches are combined with gums to achieve the desired textures and properties, ensuring the final product meets both structural and sensory expectations (Boz, 2021; Ike, 2024; Oliveira Teotônio et al., 2021). The gelatinization of starch is essential for dough expansion and crumb texture during baking. Properties such as transition temperatures and pasting viscosities are essential for entrapping gas bubbles within the product, thereby enhancing its texture and determining its appearance (Abdel-Aal, 2009). Starch serves to bind water and form a gas-permeable structure, which is vital for achieving the desired characteristics in the final baked product. Additionally, RS content contributes to increasing the specific volume of the cakes, decreasing surface porosity, and improving nutritional value (Tsatsaragkou et al., 2015). Overall, the diverse applications of starches in gluten-free bakery products highlight their significance in achieving desirable textures, structures, and qualities in the absence of gluten, catering to individuals with gluten allergies, intolerance, or sensitivity. (Tauferová & Jirsová, 2021; Gularte et al., 2012).

2.3 Clean label and clean-label starch

2.3.1 Definition and movement of clean label

Clean-label ingredients comprise natural colors, preservatives, emulsifiers, flavors, sweeteners, and antioxidants (Asioli et al., 2017). The rise of clean labeling is not a random occurrence. Today's society values natural products and advocates for sustainable development. In the 1960s, the European Union introduced the E-code system to standardize the labeling of additives in the market. This system allows consumers to easily understand how many additives are used in a product without needing to know their specific names. An E-number is a code assigned to substances that are approved for use as food additives in the EU and the United Kingdom. These substances include preservatives, colours, emulsifiers, stabilizers, thickeners, and other agents used to enhance the quality, shelf life, or appearance of food. Each E-number represents a specific additive and its role in food processing, providing a standardized way to identify and regulate these substances across different languages and countries (Saltmarsh, 2015). E-numbers help enforce safety standards by ensuring that only approved additives are used in food products and offer consumers a way to recognize additives in ingredient lists, allowing them to make informed choices.

The term 'clean label' has emerged to describe food products with ingredient lists that avoid E-numbers or chemically sounding names, opting instead for more natural and consumer-friendly descriptions. This concept has also expanded to include foods with reduced fat, sugar, and salt content, offering a healthier nutritional profile. However, it is important to note that many items marketed as 'clean label' may still contain added ingredients, which are labeled using their common names rather than their E-number equivalents. For example, 'ascorbic acid' or 'vitamin C' might be listed instead of E300 (Baines, 2012). With living standards improving worldwide, consumers

increasingly seek products labeled as natural, green, healthy, safe, and nutritious. Clean labeling meets these demands by minimizing the appearance of E-codes and emphasizing the natural attributes of food products on their labels. This approach has led to the popularity of additive-free products and has significantly increased manufacturers' profit margins, impacting both food manufacturers and ingredient suppliers. Clean-label products typically adhere to three principles: no added ingredients, a simple list of recognizable natural ingredients, and minimal processing to avoid them being perceived as artificial or heavily processed (Figure 2.1) (Aschemann-Witzel et al., 2019).



Figure 2.1 Key elements of clean label

The clean label movement began in the late 20th century and gained significant momentum in the 21st century as consumers became more conscious of what they were eating. In the 1990s, consumer demand for transparency in food labeling increased due to concerns about food safety and synthetic additives (Baines, 2012). This led to the introduction of the Nutrition Labeling and Education Act by the FDA in 1994, which mandated nutritional labeling on most packaged foods. The early 2000s saw a rise in the popularity of organic and natural foods, leading to the formal

adoption of the term "clean label". In 2006, the Clean Label Project, a nonprofit organization, was established to advocate for clean labeling in food products. Throughout the 2020s, clean-label products continued to grow, with a focus on innovation in food technology and increased consumer interest in health and transparency. Efforts were made to standardize the definition of "clean label," with some organizations advocating for clearer guidelines and certification standards (Weinrich & Spiller, 2016).

2.3.2 Clean Label in the food industry

Clean Label has rapidly developed in the global food industry, bringing new life to the sector. Over the past decade, more products have claimed to feature clean labels (Aschemann-Witzel et al., 2019). Various industry research reports, forums, conferences, food exhibitions, and expert lectures include content related to clean labeling. Several major global market research and consulting firms have ranked clean labeling as the top food industry trend since 2014 and have also evaluated clean labeling as one of the top 10 consumer health and nutrition trends worldwide (Maruyama et al., 2021). The market for clean-label ingredients was valued at approximately USD 47 billion in 2022, with a projected compound annual growth rate exceeding 6.6% from 2023 to 2032 (Global Market Insights, 2023). The market is segmented by form into powder and liquid categories. In 2022, powdered forms comprised about 55% of the market and are expected to grow profitably until 2032. The market share of powdered clean-label ingredients is notable due to their practicality and adaptability. Such ingredients, including powdered natural flavours, sweeteners, and colours, find prevalent application in baking, confectionery, dry mixes, and beverages. They ensure uniform dosing and frequently exhibit prolonged shelf life, making them advantageous for producers. Furthermore, bakery and confectionery items constitute a vital sector within the clean-label ingredients market. (Global Market Insights, 2023). Consumers prioritize natural ingredients

in baked goods, leading to significant market share for clean-label components like natural flavors and sweeteners.

As the primary trend for future food research and development, clean labelling sparks consumer enthusiasm and trust, enabling food producers and developers to redefine their strategies and concepts to delve into the true essence of food. During the pandemic, consumer behaviour shifted significantly toward healthier eating habits and an emphasis on boosting immunity. This change resulted in a growing need for clean-label ingredients, which are viewed as healthier and more natural alternatives (Cao & Miao, 2022). As consumer dietary consciousness rises, clean-label product purchases boost. Consequently, manufacturers improved their online visibility and distribution, enhancing consumer accessibility.

In the bakery and cake industry, a leading manufacturer has developed a series of clean-label products, such as dough improvers, dough relaxers, bread and cake bases, flavours, shelf-life extenders, and mold inhibitors. These products are free from partially hydrogenated oils, preservatives, animal-sourced L-cysteine, trans fats, sodium stearoyl lactylate/calcium stearoyl lactylate (SSL/CSL), diacetyl tartrate mono-diglycerides (DATEM), artificial flavours or colours, high fructose corn syrup, potassium bromate, potassium iodate, and azodicarbonamide (BreadPartners, 2024). A clean-label powdered emulsifier and enzyme blend, used as a replacement for glyceryl monostearate, provides prolonged shelf life and superior "spring-back" resilience in finished baked products. This blend offers short-term softness and resiliency for fresh-baked, par-baked, frozen dough, and baked-then-frozen bread (Cox et al., 2021). Stevia solution, a zero-calorie sweetening option for bakery applications, delivers clean-tasting sweetness for various products, including sports nutrition, bakery, and confectionery (Singh et al., 2019). Cargill introduced a soluble corn fibre containing a minimum of 80% fibre content on a dry basis. This

soluble corn fibre serves as a bulking agent with reduced caloric content compared to sugar, exerting minimal effects on colour and texture. The ingredient is plant-based, non-GMO, and gluten-free, offering a means to decrease sugar levels in baked goods (World Grain, 2022).

2.3.3 Clean-label starch

The term "clean label" has emerged in marketing, primarily referring to the use of simple, non-technical language on product packaging, the reduction of allergens and additives, and a focus on using fewer, more recognizable ingredients. It generally applies to products made with a minimal number of ingredients, ensuring that consumers can easily identify them as natural, non-artificial, and wholesome (Maruyama et al., 2021). Some companies have taken this trend further by emphasizing their use of ethically sourced, organic, or non-GMO ingredients. It is important to note that clean labels are not about encouraging consumers to eat fewer packaged foods, but rather about meeting their desire for these foods to be made with more "wholesome" ingredients that are closer to their natural state (Asioli et al., 2017). Because there are no clear legal definitions and specific regulations, the interpretation of "clean label", especially the distinction between "artificial" and "natural" ingredients, is subject to consumer and food producers. Some food companies are taking various approaches to meet consumer demands. The two most common methods include using "free-from" statements and shorter ingredient lists. "Free-from" statements can indicate the absence of specific elements such as trans fats, gluten, artificial colours, flavours, or preservatives. Shorter ingredient lists result from reformulating products to give them a more "home-made" quality (Aschemann-Witzel et al., 2019).

Clean-label starch denotes starches considered natural and minimally processed in food applications. These starches are typically derived from recognizable sources and processed using methods identified as more natural compared to chemically modified starches (Park and Kim,

2021). "Clean-label starch" lacks formal regulation yet is widely recognized in the food industry and among consumers and scientists. Nevertheless, there is an absence of a standardized definition for "clean-label starch" internationally among regulatory entities (Jadhav et al., 2024). Neither the U.S. nor the EU has a formal definition for "clean label." However, the term generally implies that the product contains no artificial ingredients, additives, or preservatives. Starches used in food must be "generally recognized as safe" (GRAS). The FDA requires that the source of starch and any modifications (e.g., chemical modifications) be declared on the label (Office of Regulatory Affairs, 2020). In the EU, clean label generally refers to no artificial additives or E-numbers (codes for substances used as food additives). Starches must be approved for use in food, and any modifications must be declared. Clean-label starches often avoid any E-number-associated additives (EU Commission, 2024). Starches must be listed on the label in Australia and New Zealand; they should not contain chemically modified components, and any modifications should be declared (Food Standards Australia New Zealand, 2023). There is an ongoing effort to provide clearer guidelines and certification for clean-label products as consumer demand continues to grow. In the absence of regulations, some companies and organizations have established their own guidelines to ensure these restrictions align with market demands related to sensitive ingredients and evolving trends. This approach also aims to enhance transparency for consumers. For example, Rich's, a global food company, has developed its own clean-label guidelines. These guidelines include a baseline clean-label ingredient restriction that aligns with common and basic ingredient requirements, as well as a superior clean-label ingredient restriction that expands on the baseline to meet the highest level of market expectations (Rich's Canada, 2023).

The traditional sources of starch mostly come from grains and tubers, such as wheat, corn, tapioca, and rice. However, advancements in clean-label starch production have led to the increased

utilization of starch derived from fruits, vegetables, and legumes such as kiwi, mango, green banana, yam, pea, red beetroot, and okara (Cui et al., 2022; Punia Bangar et al., 2021; Tang et al., 2021; Yang et al., 2022; Zhao et al., 2024; F. Zhu, 2015b). These naturally derived starches offer numerous nutritional benefits, distinct flavors, and a high content of RS. They also provide improved properties such as enhanced flavor, viscosity, gel formation, moisture retention, and texture (Figure 2.2).

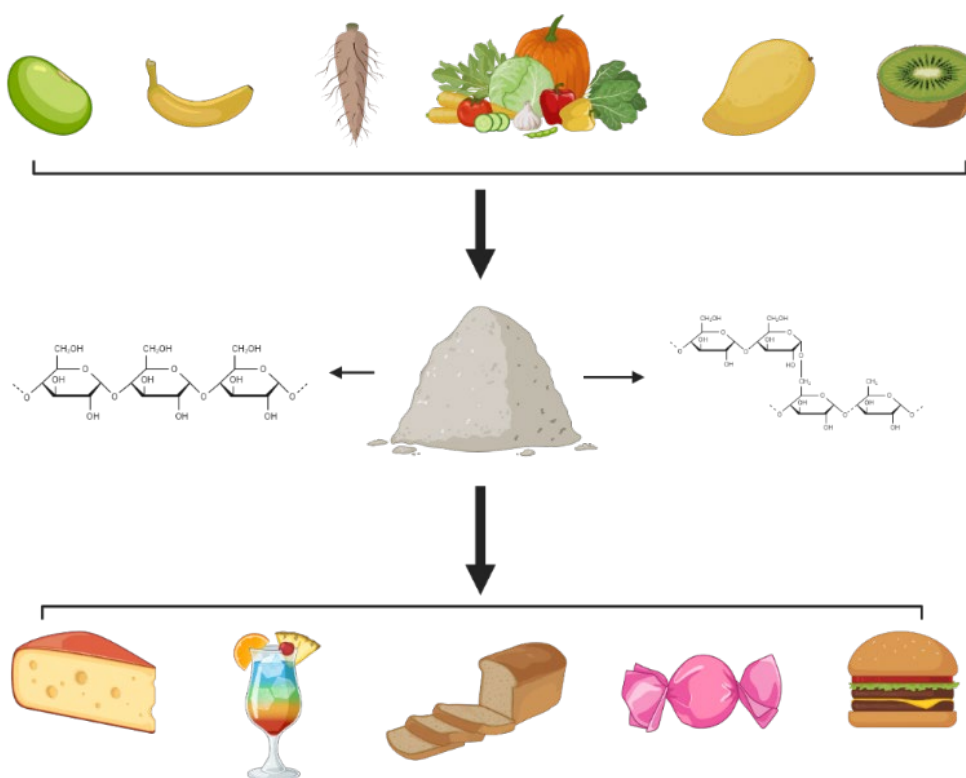


Figure 2.2 Clean-label starches from innovative natural sources and their application in the food industry (Created with BioRender.com, agreement No. *YF27AHSWML*)

The food industry exhibits growing interest in arrowroot starch due to its elevated international market value. Arrowroot starch is recognized for its importance in fine confectionery owing to its unique attributes, imparting lightness and enhanced digestibility. (Amante et al., 2021; Fabiano et al., 2020). Significant alterations transpire in the amorphous area of the starch molecule during baking, attributed to the reaggregation and surface deposition of amylose. This process leads to the formation of a hard gel, which enhances the thermal stability of the starch granule and makes it more resistant to enzymatic degradation. As a result, products made with arrowroot exhibit excellent texture, significant expansion, and notable coloration, contributing to their visual appeal (Amante et al., 2021). Therefore, the formulation of gluten-free bread with arrowroot flour has proven to be a superior alternative to the commonly used rice and potato flour in celiac products, offering enhanced nutritional and sensory quality. Additionally, incorporating up to 30% unripe banana flour enhances RS in baked goods, such as flatbreads and cupcakes, which can improve their nutritional value, while maintaining texture and enhancing sensory properties (Amante et al., 2021).

The mango seed, a byproduct of the processing industry, has significant potential as a source of starch. This starch plays a key role as a sweetening agent in the production of confectionery products, offering a valuable alternative for enhancing sweetness and texture in such applications (Punia Bangar et al., 2021). The amylose content in mango seed starch aligns with that of typical starch sources like maize, barley, and cassava used in food processing. Mango seed starch demonstrated a superior pasting temperature of 83.2°C, in contrast to the lower values of corn starch and potato starch at 76.7°C and 68.6°C, respectively. This phenomenon may be attributable to a more robust crystalline structure that impedes starch granule swelling (Saeaurng & Kuakpetoon, 2018). It is evident that mango seed starch holds significant potential for application

in soups, gravies, and bakeries. Furthermore, its enhanced water retention significantly influences food texture. Mango seed starch is recognized as a valuable ingredient for creating new edible starch-based films and functional foods aimed at managing diabetes due to its excellent thickening properties and high RS levels (Punia et al., 2019).

Kiwi starch, abundant in RS, serves as an effective alternative to wheat flour, improving product structure, baking quality, and storage. Its application in Chinese steamed bread enhances water retention in mixed flour, augments dough gas retention, decreases starch hydrolysis rate, and elevates aroma and colour. Substituting wheat flour with 10-20% kiwi starch enhanced dough viscoelasticity, reduced the bread's specific volume, and improved its chewiness and hardness, thereby extending its shelf life. A 10% substitution was particularly well-received by consumers. This trend aligns with the growing interest in using fruit- and vegetable-derived starches in the food industry to enhance product quality (Zhao et al., 2024). The use of kiwi starch can reduce cooking energy consumption, modify dough rheology, and lead to a more compact and uniform dough microstructure, contributing to improved product quality and consumer acceptance.

Natural colourants, inspired by nature, are extracted from sources such as algae, vegetables, and fruit. One example is a stable and uniform white colour solution derived from natural sources, like native corn starch (Hadnadev et al., 2018). This solution provides a bright white intensity and smooth texture, making it suitable for use in bakery icings and fillings, confectionery products, and a variety of other applications.

2.4 Environmentally friendly starch modification in bakery and confectionery

Chemically modified starches enhance the quality of bakery and confectionery items. Modifications such as acid hydrolysis, oxidation, esterification, and acetylation, lead to specific changes in the physicochemical properties of starch, resulting in delayed starch retrogradation,

reduced staling rates, and stabilized crumb structures in bakery products (Ghalambor et al., 2023). For instance, hydroxypropylated starch can make bread less firm and maintain its softness during storage, while acetylated starch improves bread crumb firmness over time (Moin et al., 2017). On the other hand, cross-linked starches can result in increased specific volume, and low- and medium-cross-linked starches can delay the staling of bread crumb (Wang, 2024). The use of modified starches in bakery products like gluten-free cheese bread has demonstrated positive effects on hardness, number of crumb pores, and overall appearance (Mesa et al., 2019). Furthermore, the inclusion of modified starches in frozen dough has been found to improve the overall quality characteristics of bread, including texture and staling rates (Wang, 2024). Innovative uses of modified starches, such as oxidized starches, in sourdough bread formulations have been investigated to enhance dough quality and functionality (Durmus et al., 2024). Although chemically modified starches provide improved functionalities and properties for food applications, there are growing health concerns associated with certain types of modifications because of the chemical residue in the products. As a result, there is a strong need to replace chemical modifications with sustainable methods to meet customers' clean and purity requirements.

The harmful environmental impact of chemical treatments has prompted the development of alternative, eco-friendly approaches capable of achieving the desired modifications without causing harm (Maniglia et al., 2021). Physical treatments, including thermal and non-thermal processes, besides enzymatic and genetic treatments, are being explored. These approaches provide a more sustainable way to enhance starch functionalities, addressing the increasing consumer preference for sustainable and natural goods (Fig. 2.3).

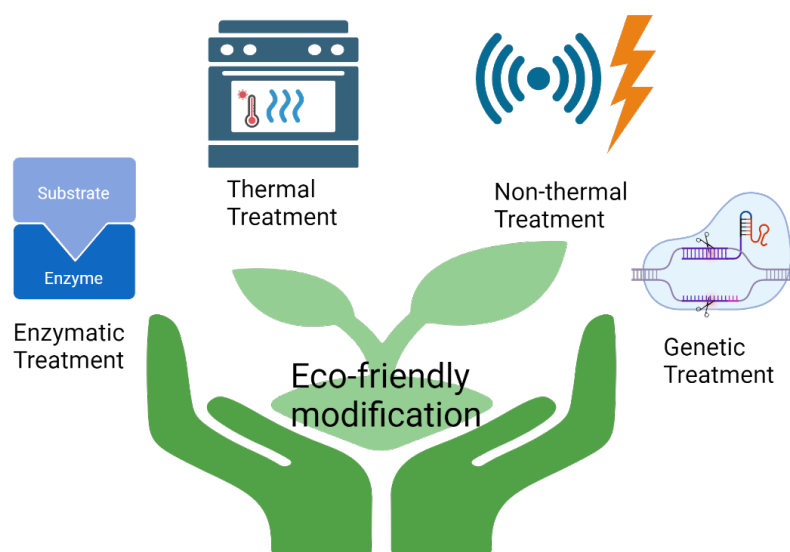


Figure 2.3 Eco-friendly methods for starch modification
(Created with BioRender.com, agreement No. *MT27AC8L4P*)

Table 2.2 summarizes the various treatments of modified clean-label starch, detailing the mechanisms of these methods, their impacts on starch properties, and applications. Physical treatments alter the physical properties of starch without significant chemical changes. These physical modification methods include thermal modification, such as DHT, HMT, and microwaves, as well as non-thermal modification, such as PEF, HHP, ultrasound, and cold plasma. Physically modified starches offer a "clean label" advantage for food companies, besides meeting consumer demand for natural ingredients (Maniglia et al., 2021). Physical treatments of starch primarily affect the arrangement of starch molecules within granules, resulting in significant structural changes. Particularly, the use of physically modified starch in bakery and confectionery products is a growing area of interest due to its ability to improve product flavor, nutrition, texture, and shelf-life. They act as thickeners, emulsifiers, and stabilizers, enhancing the appearance and texture of bakery and confectionery products (Wu et al., 2022). Physically modified starches are less

expensive than other hydrocolloids, providing an economical advantage in food production. However, challenges such as the increase in viscosity with aging and the need for precise control over modification processes remain. These factors can affect the quality and shelf-life of bakery and confectionery products (BeMiller & Huber, 2015). The choice of modification method and starch type is crucial, as different methods and starches yield varying results in terms of stability, digestibility, and functional properties. Treatment selection is contingent upon targeted starch characteristics and application criteria.

Table 2.2 Starch modification technologies and applications

Technology	Mechanism of Action	Impact on starch properties	Applications	Reference
HMT	Alter starch crystalline structure by exposing starch to high temperatures with limited moisture.	Enhanced sweet potato starch, wheat starch thermal stability and retrogradation ability, increase slowly digestible and RS in corn starch.	Short dough biscuits, low glycemic index bakery for diabetes and obesity patients.	(Fonseca et al., 2021); (Y. Zou et al., 2024)
DHT	Alter starch crystalline structure by exposing starch to high temperatures at very low moisture conditions.	Taro starch and rice starch obtained better water absorption and swelling ability and enhanced solubility and viscosity.	Gummy candy, jellybeans; glutinous products.	(H. Gong et al., 2022; Hui et al., 2024) (Burey et al., 2009)
Microwaves	Electromagnetic polarization and dielectric heating break down chemical bonds.	Increases water holding capacity and decreases viscosity in maize starch.	Gluten-free bread; filling regent in confectionery.	(Yi et al., 2024)
PEF	Applies short, high-voltage electrical pulses to create a strong electric field that induces polarization in starch	Increased RDS, enhanced the stability of doughs, and reduced retrogradation and hardness in cassava bread.	Shelf-life extension.	(Conde et al., 2022)

	granules, altering their crystallinity.			
Ultrasound	Exerts of strong mechanical forces on starch granules led to the fracture of the crystalline.	Increase water/oil absorption capacity, swelling index, and RS; decrease pasting viscosity and temperature in non-waxy rice starch.	High-RS bread, functional bakery.	(S. Yu et al., 2013); (Chumsri et al., 2022)
HHP	Alters starch granule structure via pressure-induced gelatinization or disruption	Improves water absorption, solubility, and gel strength in pea starch.	Higher loaf volume; nutrient-rich bakery.	(Gutiérrez et al., 2024)
Cold plasma	Generates reactive species that interact with starch granules, leading to surface erosion and the formation of new functional groups that enhance their reactivity.	Improves solubility, water and oil binding capacity, and swelling power in taro starch.	Gluten-free bakery, confectionery stabilizer,	(Gupta et al., 2024) (Shabbir et al., 2024)
Enzymatic treatment	Uses specific enzymes to alter the molecular structure of starch.	Reduce pasting viscosity, enhance hydrophilicity, inhibit retrogradation, and lower digestibility in corn starch and wheat starch.	Gluten-free bakery, functional bakery for diabetic patients.	(K. Wu et al., 2024) (Miao & BeMiller, 2023)
Genetic treatment	Allows for precise modifications in the starch biosynthetic pathways to achieve desired traits.	Change amylose and amylopectin ratio, increase RS content, low retrogradation, and improve freeze-thaw stability in potato starch and cassava starch.	High-RS bread, filler, and emulsion stabilizer.	(X. Zhao et al., 2018) (Radhika et al., 2014) (Bull et al., 2018)

2.4.1 DHT and HMT

DHT involves subjecting starch to heat at temperatures ranging from 130 to 200°C with a moisture content below 10% (Gong et al., 2022). It involves drying flour to about 4% moisture, then heating up to 120-140°C for up to 30 minutes using methods like rotating drums or fluidized beds. Afterward, the flour is rehydrated to 7-12% moisture content by dispersing water. DHT primarily impacts the proteins on the surface of starch granules, causing them to denature. This weakening of the protein barrier allows for better water absorption and ultimately leads to improved swelling properties of the starch granules (Polachini et al., 2023). DHT induces notable alterations in starch granule morphology. For instance, blue highland barley starch retains its "A"-type crystalline structure but shows decreased amylose content and gelatinization temperature, while solubility and water absorption capacities increase (Liu et al., 2023). Similarly, taro starch granules become agglomerated, and their particle size increases with DHT (Hui et al., 2024). The crystallinity of starch generally decreases with DHT, as observed in corn starch, where the degree of crystallinity is reduced, although the X-ray diffraction pattern remains unchanged (Ji et al., 2016). In maize starch, DHT reduces molecular size and crystallinity while retaining granular structure (Lei et al., 2020). The dry heating process can lead to alterations in the starch molecules, enhancing characteristics such as viscosity, gelatinization, and stability. For instance, DHT improves solubility, water-binding capacity, and transmittance of starch compared to native starch (Rao et al., 2022). DHT affects the thermal properties of starches, such as decreasing the gelatinization enthalpy and increasing the onset and peak temperatures, as seen in glutinous rice starch. This modification enhances the thermal stability of starch, customized for advanced thermal settings, such as glutinous food products (Qiu et al., 2015). DHT can increase the viscosity and gelation properties of starch, which is beneficial for gel products requiring high viscosity, such as gummy

candy and jelly beans (Burey et al., 2009; Hui et al., 2024). DHT enhances the rheological properties of starch, such as increasing the storage modulus and decreasing the $\tan\delta$ values, indicating higher viscoelasticity. This makes DHT-modified starches suitable for applications requiring specific textural properties, such as harder gels and desserts (Qin et al., 2016). Overall, DHT offers a viable approach for modifying starch properties in bakery and confectionery applications. The absence of moisture during the DHT process facilitates the modification of starch properties without the requirement for additional chemicals.

Conversely, HMT is a process that involves exposing starch to controlled heat and moisture levels, which prompts the starch swelling along with the process of starch gelatinization. This swelling contributes to the structure of baked goods by trapping air bubbles and increasing the viscosity of the batter (Polachini et al., 2023). HMT involves heating starch granules beyond gelatinization temperatures of 84°C to 140°C, with moisture levels between 10% and 35%. By changing the temperature, heating time, and moisture content during HMT, starch can be tailored for specific purposes (Fonseca et al., 2021). Modifying starch through HMT alters its structural characteristics, physical properties, and functional attributes, such as crystallinity, morphology, solubility, pasting behavior, gelatinization properties, viscosity, swelling capacity, as well as thermal and freeze-thaw stability. HMT alters the crystalline structure of starch, often changing the X-ray diffraction pattern from B-type to A-type or a combination of A and B-type in various starches such as potato, yam, and breadfruit (Gunaratne, 2023). The relative crystallinity of starch can either diminish or enhance depending on the moisture content used during HMT. For instance, a decrease in crystallinity was detected at lower moisture contents (12% and 15%), while an increase was noted at higher moisture contents (21% and 24%)(Gunaratne, 2023). HMT increases the gelatinization temperature and alters pasting properties, which can enhance the thermal stability and retrogradation ability of

starch. This is beneficial for bakery products that require specific textural properties (Lv et al., 2022; Wang et al., 2024). The addition of HMT-treated cassava flour to wheat flour resulted in improved dough stability, loaf-specific volume, and bread crumb porosity, as well as reduced crumb hardness compared to using only wheat or native cassava flour (Dudu et al., 2020). This indicated that treating flour through HMT can enhance the bread-making qualities and may have potential applications for other gluten-free flours. HMT can modulate the properties of starch in wheat flour, making it suitable for short-dough biscuits. Specifically, HMT diminishes the hydration potential of impaired starch granules through a layer of denatured proteins, which enhances dough deformability, resulting in better biscuit quality (Zou et al., 2024). HMT-modified starches can improve the spread ratio, volume, texture, sensory attributes, and in consequence the consumer acceptance of bakery products (Wang et al., 2021). Overall, HMT is a promising and environmentally friendly technique for modifying starch to create products with desired functionalities.

2.4.2 Microwave treatment

The process is energy-efficient and environmentally friendly, as it requires less time and energy compared to conventional heating methods, while higher heating rates and efficiency can be obtained, which aligns with the sustainability of food production processes (Wu & Xu, 2023). The microwave mechanism consists of two main processes: electromagnetic polarization and dielectric heating. The energy of electromagnetic waves is not high enough to break down hydrogen and chemical bonds, so this stage does not cause any chemical changes. Dielectric heating is the primary factor in microwave heating, producing heat energy through friction and collisions, which increases the temperature of starch granules and alters the starch structure (Yi et al., 2024). Alterations in starch crystallinity and molecular weight may affect product texture and stability

(Zhong et al., 2021). Microwave treatment alters the physicochemical properties of starch, enhancing food stability and texture. The degree of modification is affected by factors like starch type, moisture content, and processing parameters. This treatment typically elevates pasting temperature while reducing peak viscosity, breakdown viscosity, and final viscosity (Yi et al., 2024). These properties help in mimicking fat in confectionery fillings and retarding staling in bread (Oyeyinka et al., 2021). Starch-lipid complexes created through microwave treatment can serve as effective fat replacers in bakery and confectionery products. These complexes provide a creamy texture and good lubricating properties, enhancing the sensory profile of the products without compromising taste or aroma (Agyei-Amponsah et al., 2019).

Modified starches have diverse applications, from providing structure in gluten-free baked goods to acting as gelling agents in confectionery. For example, adding pre-microwaved cassava starch in chickpea flour increased the water-holding capacity, and improved the slice image and shelf-life of gluten-free bread (Garske et al., 2023). Microwave-pretreated gluten-free ingredients may serve as an alternative to clean-label bread formulations. This flexibility facilitates the creation of innovative products catering to varied consumer demands. The advantages of using microwave-clean-label starch in bakery and confectionery applications make it a promising ingredient for the food industry.

2.4.3 PEF

PEF represents a promising technology for the modification of starches in bakery and confectionery applications. By enhancing the functional properties of starch while adhering to clean label standards, PEF not only meets consumer demands for healthier food options but also supports the food industry's shift towards more sustainable processing methods. Pulsed electric field is a form of electroporation that uses a high-intensity electric field (10–80 kV/cm) to create

pores in cell membranes. While PEF treatment operates on the same principle for both microbial cells and starch granules - creating pores in the membrane via electrical pulses - its effect on starch is more complicated. PEF alters the crystallinity of starch granules by disrupting their structure. The specific impact of PEF on starch depends on factors like the granule's shape, crystalline type (A, B, or C), and the plant source of the starch (Achayuthakan, 2023), besides the treatment parameters, including electric field strength and pulse duration (Abbaspourrad & Qiu, 2021; Achayuthakan, 2023). This modification occurs through mechanisms such as charge polarization and the generation of macroscopic space charges, which alter the morphology and crystalline structure of starch granules (Lei, 2023). The versatility of PEF technology also extends to its ability to enhance the extraction of starch from various sources, including grains and tubers, thereby increasing the efficiency of starch production processes (Prabhu et al., 2019). For instance, application of PEF changed the cassava starch's external structure with swelling and disintegration, which disrupted the internal crystalline structure. Studies have shown that PEF can increase the solubility and swelling power of starch, which are critical properties for applications in bakery and confectionery products (Prabhu et al., 2019).

Moreover, the physicochemical changes induced by PEF can lead to improved textural properties in food products. For example, PEF-modified starches have been shown to enhance the stability of doughs, while the reduction in retrogradation and hardness in bread made from PEF-treated starch indicates that such modifications can improve the shelf-life and quality of baked goods (Almeida et al., 2022). PEF efficiency, combined with the clean label appeal, positions PEF-modified starches as a valuable ingredient in the bakery and confectionery sectors, where there is an increasing need for natural and functional food components.

2.4.4 Ultrasound treatment

Ultrasound treatment utilizes high-frequency sound waves to alter products and improve processes. It can be of low intensity ($< 1 \text{ W/cm}^2$) for non-destructive purposes or high intensity ($> 1 \text{ W/cm}^2$) for product modification (Berezina et al., 2018). Starch modification via ultrasound generally occurs in liquid environments, with the primary effect being cavitation. Ultrasound is used in food applications to accelerate mass transfer, enhance properties, improve nutrient accessibility, and modify polysaccharides. Despite its non-thermal nature, ultrasound locally generates heat due to cavitation and free radicals owing to the strong mechanical forces on the granules, which produce and increase the free energy of starch molecules. Acoustic cavitation has the potential to harm starch granules, leading to the fracture of the crystalline structure and a significant decrease in relative crystallinity (Zhang et al., 2019). The efficacy of ultrasonic modification is affected by factors such as sound, frequency, temperature, duration, and characteristics of the starch suspension (Alcázar-Alay, 2015). The damage promoted on the internal structure of starch granules results in lower water binding ability and the remnants of starch granules have a relatively homogeneous crystalline structure which needed high temperature to gain more energy to break intermolecular bonds (Zhang et al., 2019). The ultrasound has significant effect on the thermal and retrogradation properties of starch. In fact, high ultrasound power, strong intensity, and long ultrasonication time were responsible for the raise in the temperature onset and decline in the retrogradation enthalpy of rice starch (Yu et al., 2013). Ultrasonic treatment promotes the formation of amylose-lipid complex formation, enhancing water/oil absorption capacity and swelling index while reducing pasting viscosity and temperature (Chumsri et al., 2022).

2.4.5 HHP

HHP technology can alter the starch's granular, crystalline, and molecular structures, enhancing its functional properties such as pasting, retrogradation, and thermal stability. HHP significantly alters the crystalline structure of starch, transforming A-type or C-type crystals into B-type crystals, which are more stable and desirable for certain food applications. This transformation involves perturbing the orderly configuration of double helices while preserving their integrity, resulting in partial gelatinization (Chen et al., 2023). HHP treatment induces cold gelatinization and protein denaturation, advantageous for nutrient-dense bakery products. This process improves the starch's ability to act as a structuring agent, enhancing the quality of dough and baked products (Gutiérrez et al., 2024).

The pasting properties of high-pressure starches are modified, resulting in higher pasting temperature and altered viscosity profiles, enhancing the gel strength and viscosity. This makes the starch more effective as a thickening and stabilizing agent in food formulations. HHP modification increases swelling power and solubility, particularly at elevated temperatures, leading to improved water and oil absorption capacities of starch, which is beneficial for maintaining moisture and texture in bakery products (Sherin et al., 2024). HHP-modified starch also extends shelf-life by enhancing water retention and reducing starch retrogradation (Chen et al., 2023b; Liu et al., 2023). HHP-modified starch enhances the stability and sensory attributes of confectionery products, serving as a clean-label substitute for conventional thickeners and stabilizers (Sridhar et al., 2024).

While high-pressure clean-label starches offer numerous benefits, it is crucial to evaluate the potential difficulties in their implementation. The high-pressure process can be energy-intensive

and may require specialized equipment, which could increase production costs (Belmiro et al., 2022).

2.4.6 Cold plasma

Cold plasma falls under the category of non-thermal plasma; it is an ionized gas that can be generated under atmospheric conditions or in a vacuum using various types of sources. The process involves exciting gases such as CO₂, Ar, N₂, He, O₂, or air with an intense voltage at room temperature (Shabbir et al., 2024). Cold plasma technology is a viable approach for starch modification to produce clean-label ingredients. Cold plasma can lead to the formation of free radicals, resulting in transglucosidation and the introduction of functional groups (Zhang et al., 2023), besides cross-linking and depolymerization of the starch chains, altering the gelatinization behavior and gel properties (Wang et al., 2024). This non-thermal technology can alter the morphology, crystal structure, and molecular chain structure of starch, and the extent of those changes depends on the type of starch, plasma source, intensity, and duration of treatment (Ma & Jiang, 2024). This treatment alters the surface morphology of starch granules, creating pores and fissures that increase surface roughness, favouring the interaction with other ingredients in food formulations (Gupta et al., 2023).

Cold plasma treatment can cause chain breakage, leading to an increase in short-chain molecules within the starch structure, affecting crystallinity, gelatinization temperature and viscosity (Zhu et al., 2024). Nevertheless, the relative crystallinity of starch can be tailored by adjusting the plasma power and exposure time (Gupta et al., 2023). The rheological behavior of starch is significantly impacted by cold plasma treatment. For example, finger millet starch treated with cold plasma showed a decrease in peak viscosity and an increase in gel strength, attributed to depolymerization

and cross-linking effects (Kaur & Annapure, 2024). These changes can improve the texture and stability of bakery goods, making them more attractive to consumers.

Cold plasma treatment enhances the water and oil absorption capacities of starches, along with their emulsifying and foaming properties. Such modifications may result in superior dough characteristics and stability in baked goods, including bread and pastries (Sarkar et al., 2023). The treatment improves starch's binding capacities, solubility, and swelling power, as noted in proso-millet starch post cold plasma treatment (Chauhan et al., 2023). The treatment also reduces antinutritional factors in starch, such as tannins and phytic acid, while preserving its nutritional components for bakery and confectionery products (Patra et al., 2024).

The impact of cold plasma treatment on hydrophobicity and solubility has been particularly useful for creating starch-based films with better barrier properties for confectionery coating and package applications (Goiana et al., 2024). Cold plasma-treated starch enhances solubility, stability, and texture. This treatment acts as a thickening agent and stabilizer, augmenting food viscosity and texture. It yields a smoother mouthfeel and generates stable gels vital for desserts, fillings, and confectionery (Gupta et al., 2024). Cold plasma-treated starch is essential in gluten-free baking, improving crumb structure and moisture retention in baked goods (Shabbir et al., 2024). Therefore, cold plasma treatment has the potential to transform starch into a versatile ingredient with improved functionalities for various applications in bakery and confectionery.

2.4.7 Enzymatic modification and germination to modify starch

The enzymatic modification utilizes specific enzymes such as glycosyltransferases, amylases, and branching enzymes to alter the molecular structure of starch. Enzymes like amylase, β -amylase, amylo-sucrase, and amylomaltase modify molecular mass, branching distribution, and amylose/amylopectin ratios during reactions with gelatinized starch. (Punia Bangar et al., 2022;

Zhong et al., 2022). The enzymatic modification directly improves freeze-thaw stability and retard retrogradation, critical for starch product quality during storage (Punia Bangar et al., 2022). In bakery products, modified starches are used to improve dough quality, texture, and shelf life. α -Amylase is a key enzyme used in starch modification, playing a crucial role in tailoring starch properties for specific food applications. It works by hydrolyzing the α -1,4-glycosidic bonds in starch molecules randomly, breaking down the long chains into smaller units like dextrins and maltose (Caballero et al., 2007).

Enzymatically modified starches are also incorporated into gluten-free baked goods to substitute gluten, thereby providing structure and texture to the final product (Hadradev et al., 2018). The incorporation of cyclodextrin glycosyl transferase (CGTase) enhanced the specific volume and crumb firmness of rice bread, while simultaneously diminishing amylopectin retrogradation during storage. CGTase exhibited superior antistaling properties over α -amylase due to its starch-hydrolyzing and cyclizing activities (Gujral et al., 2003). Glycosyltransferases, including 4,6- α -glucanotransferases and 1,4- α -glucan branching enzymes, increase the α -1,6 glycosidic bond ratio and cyclic glucan content while decreasing starch's heavy average molecular weight, resulting in reduced pasting viscosity and retrogradation (Wu et al., 2024). Particularly, highly branched modified starch enhanced hydrophilicity which is effective in retarding staling in gluten-free bread. In confectionery, these starches mimic fat in fillings and act as gelling agents, enhancing product quality and processing efficiency (Hadradev et al., 2018). Additionally, enzymatic treatments can create porous starches with increased specific surface areas, which improve enzyme binding and catalytic efficiency, further enhancing the starch's functional properties (Zhong et al., 2022). For instance sweet potato starch modified by 4- α -glucanotransferase has increased paste viscosity,

which makes an effective thickening agent useful in confectionery applications (Dereje et al., 2020; Zhu & Wang, 2014).

Nature also offers enzyme treatments that alter starch properties; in this context, germination has special relevance. Germination in cereals involves the organisation of stored carbohydrates and proteins in the starchy endosperm cells to provide energy and nutrition for seedling growth (Shahpiri et al., 2014). Amylase enzymes, including α -amylase, β -amylase, and γ -amylase, play a significant role in starch hydrolysis and are the enzyme types most commonly activated during this process. All varieties of amylases have the ability to break down natural starch granules and transform starch into mono- and oligosaccharides that serve as energy sources during cereal germination to facilitate the growth of plumules (Chinma et al., 2022). Germination initiates under proper temperature and humidity conditions, starch breakdown is primarily triggered by alpha-amylase, which is secreted by the aleurone layer into the endosperm (Perata et al., 1992) (Figure 2.4). The expression of α -amylase genes in the aleurone layer is tightly regulated by various signals, including gibberellins and abscisic acid (Ritchie et al., 2000). Additionally, the production of α -amylase during germination is essential for the successful degradation of starch in the endosperm (Loreti et al., 2003). Research indicates that amylase activity peaks on the second day of cereal germination, indicating a peak in enzymatic activity during this period (Bedel et al., 2022).

Germination enhances baking properties and sensory attributes of products (Cardone et al., 2020; Marti et al., 2018). In fact, α -amylases catalyze the hydrolysis of damaged starch present in wheat flour, resulting in the formation of smaller dextrins. This process facilitates the continuous activity of yeast throughout dough fermentation, proofing, and the preliminary stage of baking. As a result, bread volume and crumb texture improve. Additionally, small oligosaccharides and sugars such as glucose and maltose, produced by these enzymes, significantly contribute to enhancing Maillard

reactions that are accountable for the browning of the crust and the creation of a desirable baked aroma (Tiwari et al., 2015). It can also improve the flavor acceptance of ingredients and products, and reduce or eliminate toxic, harmful, or anti-nutritive substances (e.g., tannins, cyanogenic glycosides, phytic acid, trypsin inhibitors, oxalic acid, etc.) in certain grains and legumes (Ojha et al., 2018).

Overall, the enzymatic modification of starch, either by adding enzymes or by using germination to trigger them, is a powerful tool to modify starch functional properties, providing a clean alternative ingredient to the food industry.

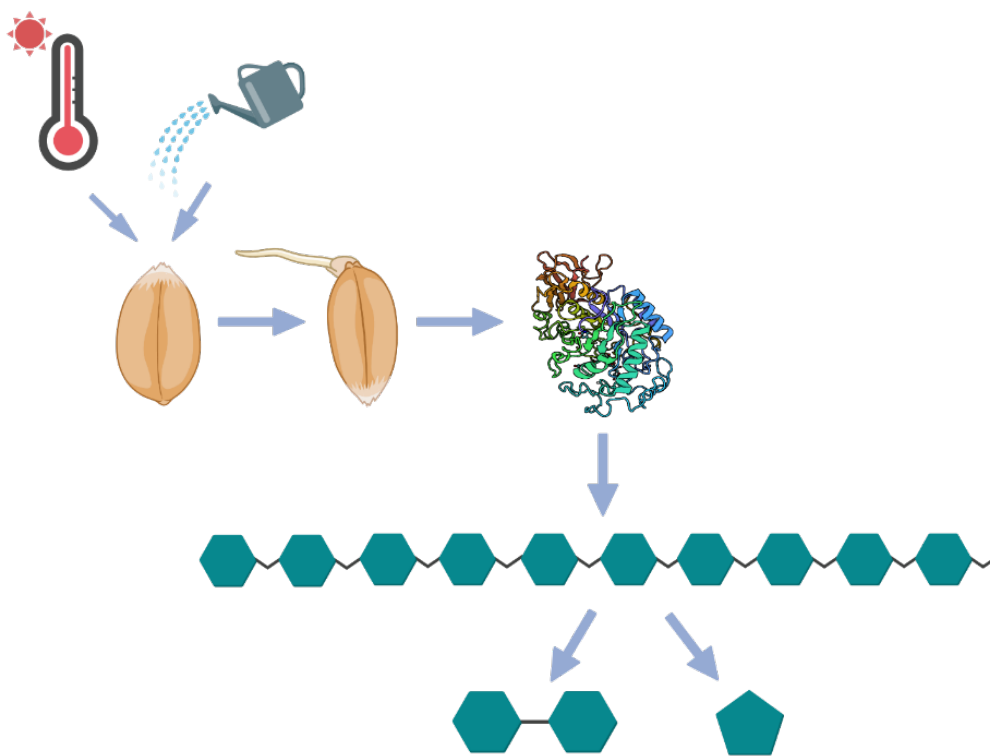


Figure 2.4. Amylase is produced during seed germination, and it hydrolyzes starch into simple sugars. (Created with BioRender.com, Agreement No. *WS27AHRCTS*)

2.4.8 Genetically modified starch

Genetically modified starch has emerged as a significant innovation in food applications due to its enhanced properties and functionalities. Advances in biotechnology have also enabled the production of modified starches with specific particle size distributions and reduced viscosity, which are advantageous for food additives, binders, and other industrial uses (Regina et al., 2014). The modification of starch, particularly through genetic means, allows for the tailoring of its molecular structure and, in consequence, its physicochemical properties to meet specific needs. Advancements in genetic engineering have facilitated the modification of starch in plants by targeting enzymes in the starch biosynthetic pathway. The primary mechanism by which genetically modified starches impact their properties is through the alteration of amylose and amylopectin ratios. This process can lead to starches with improved functionalities such as increased viscosity, better freeze-thaw stability, and enhanced textural attributes, making them suitable for various food applications (Bensaad et al., 2022).

The use of novel breeding technologies and an increased understanding of starch biosynthesis regulation have further expanded the genetic diversity of crops, allowing for the fine-tuning of gene expression to produce starches with value-added consumer benefits. Selective crop breeding has led to the launch of high amylopectin waxy potato and wheat starches. By altering the ratio of amylose to amylopectin, differing functional performance can be achieved. For instance, natural maize starch has about 28% amylose content, while amylomaize starch is a starch from selectively bred maize and may contain up to 80% amylose. High amylose maize starches are being used in various food products because they are highly resistant to processing and also undergo rapid retrogradation if gelatinization occurs. These starches originate from maize via the amylose extender mutation, yielding a starch comprising 40–50% amylose. Selective breeding has enabled

the attainment of amylose concentrations reaching 90% (Klucinec & Keeling, 2005). This kind of genetic modification enabled the application of edible films, coatings, and biodegradable packaging (McDonagh, 2012).

The cassava crop presents a unique challenge for traditional breeding due to its high heterozygosity, allopolyploid composition, and low yield. Therefore, most of the genetic modification research focuses on cassava breeding (Koehorst-van Putten et al., 2012). The amylose content of starch significantly influences its pasting and gelatinization properties. Starch with reduced amylose typically exhibits a lower pasting temperature and higher viscosity compared to normal starch. The application of CRISPR-Cas9 for modifying two genes linked to amylose biosynthesis has effectively diminished or eradicated amylose levels in cassava root starch, resulting in genetically enhanced varieties suitable for food utilization (Bull et al., 2018). Altering amylose content did not affect amylopectin but increased starch granule size. Genetic modifications in cassava to vary amylose levels offer significant potential for diverse food applications and commercial processing (Bull et al., 2018). Additionally, the genetically modified waxy cassava starch through silencing the RNA, which has better paste clarity, low retrogradation, and improved freeze-thaw stability, has shown significant improvements in solubility and stability, making it suitable for various applications, including as a filler, emulsion stabilizer, and consistency modifier (Radhika et al., 2014). Genetically modified cassava yields low-amylose or amylose-free starch has been proven to enhance texture and moisture retention in baked goods. This demonstrates that genetic modification is a viable method for enhancing starch functionality for commercial use (Koehorst-van Putten et al., 2012). Similarly, genetically modified high-amylose potatoes have been obtained, which have high RS content due to modifications in amylose content, starch granule structure, and amylopectin structure (Zhao et al., 2018).

The genetic composition of starch profoundly impacts its structural and functional characteristics, with variations in amylose and amylopectin content dictating its thermal properties, swelling power, and rheological behaviour (Bensaad et al., 2022). Overall, genetically modified starch represents a versatile and sustainable solution for meeting the growing demands of various end-use products, from bakery to confectionery, by leveraging advanced genetic techniques to optimize its properties and performance. However, in many countries, GMOs, including genetically modified starch, are regulated under strict biosafety and food safety protocols. These regulations are designed to assess and manage potential risks to human health and the environment. For instance, the EU employs a science-based regulatory system that focuses on risk assessment rather than benefits, with the European Food Safety Authority (EFSA) playing a central role in evaluating GMOs before they can be marketed (Bartsch, 2014). These regulations aim to balance the potential benefits of GM technologies with the need to protect human health and the environment. However, the stringent nature of these regulations can also pose challenges to innovation and international trade, highlighting the need for ongoing dialogue and harmonization of regulatory standards.

2.5 Health implications of starch in bakery and confectionery products

Starch is an essential dietary element, providing the main source of digestible carbohydrates and greatly influencing total energy consumption. As the main energy-yielding nutrient in daily diets, starch plays a crucial role in fueling human activities (Yin et al., 2010). It is a widespread nutrient globally, providing essential energy to a growing population (Svihus and Hervik, 2016). Because of that, the importance of the starch molecule goes beyond its functional properties during processing. Starch-rich foods exhibit differential digestion rates, affecting their nutritional significance and health implications (Wang et al., 2022).

The digestion of starch is a complicated process that involves mechanical constraints plus the action of enzymes like α -amylase and α -glucosidase, leading to the production of glucose for energy (Dhital et al., 2013). Starch digestion significantly influences postprandial glucose and insulin levels in humans (Šárka et al., 2023). Several factors, such as the plant's characteristics, microstructure (Dini et al., 2024; Santamaria et al., 2021) antinutrients, amylose levels, and dietary fiber components can influence the digestibility of starch in grains (Aleixandre et al., 2021; Toutounji et al., 2019).

Starch can be classified into three groups based on its *in vitro* digestive properties: RDS, SDS, and RS. RDS is quickly broken down into glucose by α -amylase within 20 minutes of entering the body. It is found in amorphous and dispersed starch, which is common in foods cooked with moist heat. RDS is promptly metabolized in the oral cavity and small intestine, resulting in a swift elevation of blood glucose and insulin concentrations (Bello-Perez et al., 2020). SDS, on the other hand, is digested and absorbed more slowly in the small intestine, gradually releasing glucose. The digestion and absorption of SDS occur over a longer period, helping to maintain stable blood glucose levels. The *in vitro* digestion assay for SDS refers to hydrolyzed nutrient fragments in 20-120 minutes. SDS and RS have become significant areas of research in the field of food nutrition. The rate of starch digestion can significantly affect the postprandial glycemic response, with RDS potentially contributing to conditions like type 2 diabetes (Huang et al., 2022). Conversely, SDS can lead to a stable postprandial glycemic response and provide sustained energy (Li et al., 2022). RS, unlike RDS and SDS, is not hydrolyzed by enzymes within 120 minutes in the *in vitro* digestion assay. RS is indigestible and non-absorbable in the human small intestine. Instead, it is utilized by bacterial colonies in the colon through fermentation. RS is broken down by microorganisms in the large intestine, producing short-chain fatty acids, particularly butyric acid,

which acts as a dietary fibre and supports intestinal health (Dura et al., 2017; Patel et al., 2012). RS can be classified into five distinct categories. RS1 is characterized by starch that remains undigestible due to its encapsulation within physical barriers, such as cell walls or the food matrix, which prevent access by digestive enzymes like amylase. This structural protection inhibits starch digestion, making RS1 resistant to breakdown in the digestive system. A notable feature of RS1 is its thermal stability during cooking, meaning it retains its resistance to digestion even after being subjected to heat. RS1 is commonly found in traditional food items, such as whole grains of rice (Nguyen et al., 2023). RS2, known as RS granules, comprises starch granules sourced from specific plants. The structure of the starch in this category can either be long-branched or straight-chained, densely packed in a radial configuration on the granule's surface, thereby resisting enzymatic breakdown. Examples of RS2 sources include raw potato starch, potato starch, and starch from bananas (Dupuis et al., 2014). RS3, called aged, retrograded starch, forms compact crystalline formations at lower temperatures, posing challenges for digestion. This type of RS is prevalent in chilled rice and bread products (Nguyen et al., 2023). RS4 involves chemically modified starch, wherein the starch structure is altered using techniques like pre-gelatinization, esterification, acid and alkali treatments, and cross-linking to modify digestibility. Lastly, RS5 refers to starch-lipid complexes, which impede starch swelling and digestion by creating a helical structure around lipid molecules (Dupuis et al., 2014).

It has been found that the digestibility of starch is closely related to the blood glucose level of the human body. Animal experiments have shown that if the diet contains RS and SDS, it can act as a stable source of glucose and have a stabilizing effect on blood glucose levels (Lehmann & Robin, 2007). The postprandial glycemic response mainly depends on the content level of RDS, whereas SDS releases energy continuously and slowly, keeping the postprandial blood glucose level slowly

rising and reducing the body's resistance to insulin. This can effectively improve the postprandial glycemic load and regulate blood glucose in patients with type 2 diabetes mellitus (Nguyen et al., 2023). Foods with high content of RS also provide a feeling of satiety, and they can be used as slimming food for obese people and as carbohydrate supplements for fitness individuals, providing a range of health benefits for healthy people. Starchy foods rich in RS and SDS can mitigate postprandial glucose and insulin responses, promoting normal blood glucose regulation and health. This dietary starch, which evades small intestinal digestion and reaches the large intestine, enhances fiber intake and provides multiple health advantages (Belobrajdic et al., 2012). Unlike fully digested starch, which provides more energy, RS yields less energy since it is not fully broken down in the small intestine (Englyst et al., 1996).

It is important to stress the impact of starch modification treatments beyond their technological functionality, assessing a potential impact on the digestibility after consumption. DHT modifies starch digestibility, enhancing RDS while diminishing slowly digestible and RS (Liu et al., 2023). HMT can increase the content of SDS and RS, which are beneficial for health, as they lower the glycemic index of foods (Fonseca et al., 2021). Microwave treatment elevates RS levels, functioning as dietary fiber. This alteration improves the nutritional quality of baked goods, catering to health-oriented consumers (Zhong et al., 2021). PEF raised the RDS fraction and led to increased digestibility (Conde et al., 2022). High RS was identified in the ultrasound-treated starch-lipid complex, exhibiting enhanced in vitro bioactivities suitable for functional food applications (Chumsri et al., 2022). The use of HHP in starch processing enhances RS levels and reduces the glycemic index of bakery items, thus promoting digestive health (Chen et al., 2023). A key area of interest is the impact of enzymatic modifications on starch digestibility, which has significant implications for the nutritional profile and various physiological responses, including

glycemic control. Research has demonstrated that enzymatic modifications can enhance the slow digestion properties of starch, affecting its digestibility and glycemic response. For example, corn starch hydrolyzed by α -amylase and treated with branching enzymes can improve the slow digestion performance (Li et al., 2022). Enzymatic approaches utilizing enzymes like α -amylase, pullulanase, and branching enzymes have been identified as effective means to modify starch digestibility. The modification alters molecular structures, enhancing starch granule resistance to enzymatic digestion (Zhang & Bao, 2023). Moreover, enzymatic interventions during or post-gelatinization can modify structural determinants of starch digestion and glycemic responses to lower starch digestibility (Korompokis et al., 2021). This suggests that enzymatic modifications can be customized to achieve specific outcomes in terms of starch digestibility and subsequent glycemic responses. Furthermore, the rates of starch digestion critically affect postprandial glucose regulation, associated with obesity, diabetes, and cardiovascular disease. 1,4- α -glucan branching enzymes-modified starch displays a reduced digestion rate, offering a dietary alternative to decrease external glucose production, thus advised for patients with particular requirements (Wu et al., 2024).

Germination is a biologically driven process that is cost-effective, environmentally friendly, and free from solvents, which can break down starch into simple sugar and change the starch fraction and digestibility. Additionally, germination can improve nutrient bioavailability, such as the digestibility of protein and starch, and enhance the antioxidant properties (Chinma et al., 2022). Studies have demonstrated that germination significantly enhances starch digestibility in legumes (Preet & Punia, 2000; Urooj & Puttaraj, 1994). This improvement occurs through structural and metabolic changes during germination, which promote the degradation of starch granules by amylolytic enzymes. These changes increase the accessibility of digestive enzymes, thereby

enhancing starch digestibility (Ma et al., 2020). In addition, the enhanced digestibility of starch has been associated with a decrease in amylose levels, a reduction in granule size, and the removal of anti-nutritional factors (Carré, 2004). However, adding germinated flours to the baking products sometimes can decrease starch digestibility due to the α -amylase inhibition and RS content. For example, oat sprouting led to elevated polyphenol content and inhibited amylase activity, suggesting potential mechanisms for the observed reduction in starch digestibility. These findings highlight the potential of controlled sprouting to develop nutritionally enhanced bread with reduced starch digestibility (Cao et al., 2022). Bread made of 100% sprouted wheat flour had a significantly increased SDS fraction and decreased RDS fraction compared to commercial wheat flour. The outcome was linked to elevated levels of RS and/or high phenolic compounds in sprouted wheat (Marti et al., 2018). Research demonstrated that incorporating 20% sprouted wheat in bread effectively reduced starch digestibility. This reduction was linked to changes in α -amylase inhibitor activity and RS content within the treated sprouts (Świeca et al., 2013). These findings highlight the significance of sprouting conditions in altering starch content, bioavailability, and glycemic index estimates.

In conclusion, starch is a crucial source of calories for humans, with its digestion and properties impacting energy release, glycemic response, and overall health outcomes. The digestibility of starch plays an important role in the nutritional aspect of creating cereal-based diets that promote low and consistent postprandial blood glucose levels, characterized by a high presence of slowly digestible and indigestible carbohydrates. Understanding the complexities of starch digestion is essential for optimizing dietary choices and promoting human well-being.

2.6 Conclusions and future perspectives

Starch is a fundamental ingredient in bakery and confectionery products, offering versatile functionalities that contribute to texture, structure, stability, flavor, color, and overall quality. The selection and utilization of specific starches, whether native or modified, play a significant role in determining the functional properties and characteristics of a wide range of baked goods and confectionery items.

Clean-label starches represent a significant innovation in response to consumer demand for natural, minimally processed ingredients. Their development reflects broader trends toward transparency and simplicity in food labeling, and ongoing research is likely to enhance their functionality and range of applications further. Clean-label starches can replace traditional additives in baked goods, meeting consumer demand for transparency and natural ingredients. This shift can also lead to the development of new product lines catering to health-conscious consumers. Manufacturers can leverage clean-label starches to differentiate their products in a competitive market, appealing to the growing segment of health-conscious consumers. This transition may also lead to cost savings by reducing the need for synthetic additives.

Clean-label processing techniques, including physical treatments such as heat treatment, microwave processing, ultrasound, PEF, HHP, and cold plasma, as well as enzymatic treatments involving amylase and glycosyltransferases, are employed to produce starches that adhere to clean-label standards. Genetic engineering and breeding techniques, such as CRISPR-Cas9 and RNA silencing, also contribute to this effort. Technological advances have continued to improve the functionality of clean-label starches, making them suitable for a broader range of applications, including processed foods, baked goods, and convenience foods. The reliance on the latest clean-modified technology, natural flavors and sweeteners, and label-friendly texture improvers has

become increasingly prevalent in the food industry. Consequently, clean-label starches have become mainstream in food manufacturing, particularly in products marketed as natural or organic. The increased preference for foods with transparent and simple ingredient lists has further accelerated the adoption of clean-label starches.

Future research could explore new formulations using natural materials through eco-friendly starch modification methods to meet the specific requirements of various industries, including food, packaging, and pharmaceuticals. Research efforts could also focus on evaluating the economic feasibility and cost-effectiveness of incorporating these clean-label ingredients into starch modification processes, ensuring their practicality and affordability for manufacturers. Long-term assessments of the environmental impact and sustainability of innovative starch modification treatments are crucial for understanding their benefits and drawbacks over time.

Due to the lack of legislative regulation regarding clean labels and clean label labelling, merchants often seize the opportunity to manipulate and inflate the significance of these products, leading to higher prices. Many aspects remain unclear, highlighting the need for laws and regulations to improve oversight and promote the use of transparent labelling in the market.

Collaboration can play a critical role in enabling the food industry to have a greater long-term impact on the environment. Collective efforts among researchers, industry stakeholders, and regulatory bodies could be beneficial in establishing guidelines and standards for the adoption of clean-label starch modification technologies, promoting their widespread acceptance and integration into manufacturing processes.

CRedit Author contributions statement

Xiaohang Zou: Writing – original draft

Nicola Gasparre: Writing – review & editing

Cristina M. Rosell: Writing – review & editing; Conceptualization; Supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

Chapter 3_Experimental Section 1

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Apparent viscosity as a marker of wheat germination and predictor of bread quality and starch digestibility

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Abstract

Background and objectives: Wheat germination enhances its α -amylase activity, affecting the functional properties of flour and bread. Studies reported a variety of germination conditions, which do not allow for comparison studies. This study evaluated the impact of germination on wheat flour functionality, breadmaking performance, and nutritional quality using peak viscosity after heating as a marker to control the degree of germination.

Findings: Wheat kernels were germinated until peak viscosity was reduced by 50% compared to the control. A strong negative correlation was found between α -amylase activity and peak viscosity ($r=-0.84$, $p<0.0001$), and a strong positive correlation between peak viscosity and FN ($r=0.98$, $p<0.0001$), validating the use of peak viscosity as a predictor of enzymatic starch degradation. Bread from fixed-time germinated flour showed varying reductions in hardness and chewiness, whereas bread from adjusted germinated flour demonstrated more consistent levels.

Conclusions: Viscosity-adjusted germination effectively controls enzymatic activity and influences both dough handling and bread texture. It also leads to slower starch hydrolysis.

Significance and novelty: Peak viscosity is proposed as a practical and quantitative marker of wheat germination level. By controlling enzymatic activity through viscosity adjustment, it is possible to optimize bread quality and nutritional outcomes.

Keywords: α -Amylase; breadmaking; crumb texture; FN; mixing performance.

3.1 Introduction

Significant changes in the composition and functionality of wheat grains have been described as direct consequences of the endogenous enzymes activated during germination. It has been described that this natural process promotes the breakdown of complex molecules, enhancing nutritional value, reducing anti-nutritional factors, and improving *in vitro* digestibility (Chinma et al., 2022; Cornejo et al., 2015). Among the key enzymes activated during germination, α -amylases play a central role by hydrolyzing starch and releasing fermentable sugars, which are vital for bread fermentation and quality. Because of that, wheat germination and the changes occurring during germination have been the focus of different research. For instance, apparent viscosity measurements have shown a marked decline during 6 to 36 h of germination at 21°C, becoming nearly undetectable after 36 h (Kaur et al., 2024). Similarly, wheat germinated for 48 h at 20°C showed substantial starch degradation and accumulation of simple sugars, attributed to elevated α -amylase activity (Cardone et al., 2020). Those studies investigated the effects of germination, but the wide range of experimental conditions used makes it challenging to compare results or identify an optimal germination condition for desired flour properties.

Germinated wheat has attracted attention for its potential to enhance bread quality (Dhillon et al., 2020). It has been reported that flour from germinated wheat (48 h at 20°C and 90% RH) presents decreased water absorption, short development time, and reduced mixing stability (Cardone et al., 2020). Despite these changes, bread produced with 100% germinated flour shows increased loaf volume and softer crumb (Cardone et al., 2020). However, extending the germination period to 72-90 hours under the same conditions and incorporating only 50% germinated flour further enhances crumb softness but also results in a darker crust coloration (Marti et al., 2018). In another study, Tian et al. (2019) applied varying germination times (7, 8, and 10 h at 30°C and 95% RH) to three hard red winter wheat varieties resulting in different degrees of germination, as determined by FN: approximately 550 s for the control, 350 ± 40 s (low), 250 ± 40 s (medium), and 120 ± 40 s (high). Despite these differences in germination level, no significant changes in loaf volume were observed among breads produced with 100% germinated flours across treatments. On the other hand, adding 10% germinated flour (96 h at 20°C and 80% RH) reduced the FN and peak viscosity by more than 70% and 80%, respectively, while shortening dough stability time and affecting final bread texture and volume (Sun et al., 2023).

Beyond its effect on wheat functionality and baking quality, amylase is also instrumental in starch digestibility, which influences postprandial glucose levels and has implications for managing diabetes (Korompokis et al., 2021a). Increasing germination time and temperature increased hydrolytic enzyme activity, making the wheat starch more digestible. It has been described that the *in vitro* starch digestibility rate of germinated wheat flours increased from 47.5% to 61.6% when germinating from 12 h to 48 h (Singh et al., 2021). Furthermore, germination alters starch fraction distribution, leading to increased RDS and decreased SDS and RS. After 72 h of germination, the RDS of wheat flour increased from 26.5% to 36.2%, making the starch more susceptible to

enzymatic breakdown (Kaur & Gill, 2021). Conversely, Marti et al. (2018) found that sprouted wheat flour contained lower RDS and higher SDS compared to bread made from non-germinated wheat flour.

Previous research has primarily focused on how variations in germination time and temperature influence flour functionality and associated enzyme activity (Chen et al., 2017; Singh et al., 2021). However, the reported effects vary widely due to differences in protocols and the intrinsic properties of wheat grains. Despite the growing interest in germinated wheat, limited attention has been given to the consistent evaluation of viscosity or the influence of elevated α -amylase activity on starch digestibility in bread. To address these gaps, the present study aimed to evaluate the effects of germination on wheat flour functionality and starch digestibility by using peak viscosity as a reliable marker of the degree of germination. The specific objectives were: (i) to examine the properties of germinated wheat by comparing different commercial wheat blends germinated for the same duration, (ii) to evaluate the effects of controlled germination on flour functionality and bread starch digestibility, using peak viscosity as a reliable marker of the degree of germination.

3.2 Materials and Methods

Three commercial blends of CWRS wheat samples were generously provided by Cereals Canada (Winnipeg, Canada). Instant dry yeast was donated by Lallemand Inc. (Montreal, QC, Canada). Salt was acquired in the local market. All chemicals were of analytical grade.

3.2.1 Germination process under controlled conditions

The samples used in this study were CWRS wheat, which, unlike winter wheat, does not require vernalization to transition to reproductive growth. Germination was performed following the methodology described by Kaur et al. (2024). Briefly, wheat kernels were initially soaked in a 1% (v/v) sodium hypochlorite solution for 15 minutes. After soaking, the kernels were thoroughly

washed with distilled water until a neutral pH was achieved. The germination process was conducted at 21°C with 80% RH, followed by drying the kernels at 50°C for 9 h until their moisture content dropped below 15%. The dried kernels were then milled using a laboratory hammer mill (Perten Lab Mill 3100, Perten Instruments AB, Huddinge, Sweden) to produce flour with a particle size < 0.5 mm. Three commercial wheat blends were used for the germination, named CWRS 1, CWRS 2, and CWRS 3. A preliminary germination test was conducted with CWRS 1, recording the apparent viscosity of the resulting germinated flour during the germination period, using an RVA (RVA-4, Newport Scientific, Warriewood, Australia). The standard method, AACCI Method 76-21.01 (AACC International, 2009), was used to assess the pasting profile of the flours. The peak viscosity was taken as a key parameter to evaluate the level of starch hydrolysis by comparing the maximum peak viscosity during heating of ungerminated and germinated kernels. Germination of CWRS 1 for 18 h resulted in a 50% reduction in peak viscosity for the germinated flour. Two different germination conditions were applied to other wheat blends: first, an 18-hour germination to compare with CWRS 1, and second, adjusted germination times to achieve a 50% reduction in their peak viscosity. Germination durations of 15 and 16 h were required for CWRS 2 and CWRS, respectively, to reach a 50% reduction in their initial peak viscosity for ungerminated kernels.

3.2.2 Flour characterization

Flours were characterized by assessing the proximate composition, FN, and α -amylase activity. Proximate composition was determined following standard methods (AACC International, 2009), including ash (AACCI 08-01.01), moisture (AACCI 44-40.01), protein (AACCI 46-11.01), and fat (AACCI 30-25.01) contents. D-glucose was analyzed using the D-glucose assay kit (GOPOD Format) (Neogen-Megazyme International Ireland Ltd, Bray, Ireland).

The FN was measured according to the AACCI-approved method 56-81.03 (AACC International, 2009) using the Perten FN system 1310 (Perten Instruments AB, Stockholm, Sweden). A-Amylase activity was assessed using the Ceralpha analysis kit (Neogen-Megazyme International Ireland Ltd, Bray, Ireland) with the modification reported by (Kaur et al., 2024).

3.2.3 Mixing properties

The mixing characteristics were determined using a Micro-doughLAB mixer (Perten Instruments, Springfield, IL, USA). Four grams of the flour from each sample were weighed based on a 14% moisture basis and mixed at a speed of 63 rpm at 30°C for 20 minutes. The dough consistency was recorded; water absorption, dough development time, stability, and MTI were identified following AACCI Method 54-70.01 (AACC International, 2009).

3.2.4 Fermentation kinetics and bread making

The formulation for bread making consisted of 100 g of wheat flour (14% moisture basis), 1.5 g of salt, 1.0 g of instant dry yeast, and the optimum amount of water determined from the Micro-doughLAB results. All the ingredients were mixed using an Artisan mini tilt-head stand mixer (KitchenAid, St. Joseph, Michigan, USA). The optimal mixing time determined from Micro-doughLAB was applied. The dough was then divided into 50 g portions, manually shaped and placed into a silicone baking tray to proof at 85% RH and 30°C. To record the dough volume increase during proofing, 3 g of dough was put in a graduated cylinder (Martins et al., 2022). Loaves were baked when the volume during proofing reached 75% of the maximum dough volume. Baking was performed at 180°C for 15 minutes. Bread was left to cool down at room temperature for one hour and then packed in polyethylene pouches till further analysis.

3.2.5 Bread quality measurement

The texture properties of bread were evaluated using a Texture Analyzer TA. XT2i (Stable Micro Systems, Surrey, UK) by measuring a crumb disk of 1 cm thickness x 2 cm diameter collected from the central region. Compression strain of 50% was applied at a speed of 1 mm s⁻¹ utilizing a 36 mm aluminum cylindrical probe. The following parameters were measured: hardness, springiness, chewiness, cohesiveness, and resilience.

Colour measurement was performed using a Minolta Colour Spectrophotometer (Konica-Minolta Sensing Americas, New Jersey, USA) to assess L^* , a^* , and b^* values. Where L^* represented lightness, a^* indicated the range from greenness to redness, and b^* measured the spectrum from blueness to yellowness, from negative to positive values, respectively.

3.2.6 *In vitro* starch digestibility

The hydrolysis kinetics of bread were evaluated using the method described by Santamaria et al. (2021) using porcine pancreatic α -amylase (0.9 U/mL) and amyloglucosidase (214.5 U/mL). Dried bread (200 mg) was suspended in 4 mL of a 0.1 M sodium maleate buffer (pH 6.9) that contained porcine pancreatic α -amylase (0.9 U/mL) utilizing an Ultra Turrax T18 basic homogenizer (IKA-Werke GmbH and Co. KG, Staufen, Germany). The slurry was incubated at 37°C for three hours while maintaining constant agitation at a rate of 200 rpm. Aliquots were extracted periodically to quantify glucose release. The residual starch after 16 h of hydrolysis was solubilized using 2 mL of cold 1.7 M NaOH and subsequently hydrolyzed with amyloglucosidase (143 U/mL) at 50°C for 30 minutes in a shaking water bath to achieve complete hydrolysis. The concentration of glucose was determined using a GOPOD assay and measuring absorbance at 510 nm. The starch content was calculated using glucose (mg) multiplied by 0.9. For the starch enzymatic digestion, three starch fractions were identified based on their hydrolysis rates: RDS, hydrolyzed within the first

20 minutes; SDS, hydrolyzed between 20 and 120 minutes; and RS, representing the starch fraction remaining after 16 hours of digestion. The kinetics of *in vitro* digestion were determined by applying the Box-Lucas model following a first-order equation:

$$C = C_{\infty} (1 - e^{-kt}) \quad (\text{Eq.1})$$

where C indicated the percentage of starch hydrolyzed at a specific time t , C_{∞} represented the equilibrium concentration or the maximum hydrolysis extent, k indicated the kinetic constant, and t referred to the selected time.

3.2.7 Statistical analysis

Experimental data were statistically evaluated using the method of one-way ANOVA to assess significant differences among samples using the Tukey test ($p < 0.05$). Pearson correlation analysis was performed to evaluate the correlations between parameters. The correlation coefficients R and associated p -values were calculated to determine the strength and direction of associations between variables. The data were reported as means $\pm$ standard deviations ($n \geq 3$). Data were analyzed with OriginLab 2023 software (Northampton, Massachusetts, USA).

3.3 Results and discussion

3.3.1 Germination of wheat kernels and impact on the apparent viscosity, amylase activity, and proximate composition of the flours

Three CWRs commercial wheat blends were used to reflect the materials most frequently encountered by both the food industry and consumers, germinated using two different germination approaches, including fixed time and adapted time. In the first set, samples were compared by applying the same germination time (18 h). In the second one, kernels were germinated until their apparent peak viscosity was reduced by 50% compared to their initial one of the ungerminated kernels. Table 3.1 shows the apparent peak viscosity of the wheat samples. CWRs 1 required 18

h of germination to have a ~50% peak viscosity reduction as observed for the ungerminated wheat, while in the case of CWRS 2 and CWRS 3, the germination times required for that viscosity reduction were 15 and 16 h, respectively. Therefore, after germination for an adapted time, the apparent viscosity ranged from 577 cP to 694 cP, whereas germination for a fixed time, even with the same class of wheat, gave a much more extended reduction (up to ~40%) in the apparent viscosity. According to our previous results (Kaur et al., 2024), CWRS flour from 18-hour germinated wheat exhibited 50% of the peak viscosity of its ungerminated wheat, as measured using the RVA, which was comparable to the results obtained for CWRS 1. Gao et al. (2022) reported that the peak viscosity of wheat flour increased from 1677 cP (ungerminated) to 2401 cP after 6 h of germination at 33°C, but then began to decline after 12 h, reaching 2176 cP at 18 h. In contrast, Grassi et al. (2018) found that while the control sample had a peak viscosity of 1890 cP, it declined slightly after 24 h of germination (21°C and 90% RH) and dropped sharply after 38 h, reaching just 196 cP by 72 h. Outcomes from the current study highlighted that wheat samples from the same class (CWRS), when germinated for the same duration, can exhibit different functional behaviours, indicating that germination time must be adjusted to allow meaningful comparisons of their properties. This performance-based approach addresses a significant limitation in existing literature, where the sole use of germination durations impedes direct comparisons.

Table 3.1 Impact of different germination conditions on the amylase activity

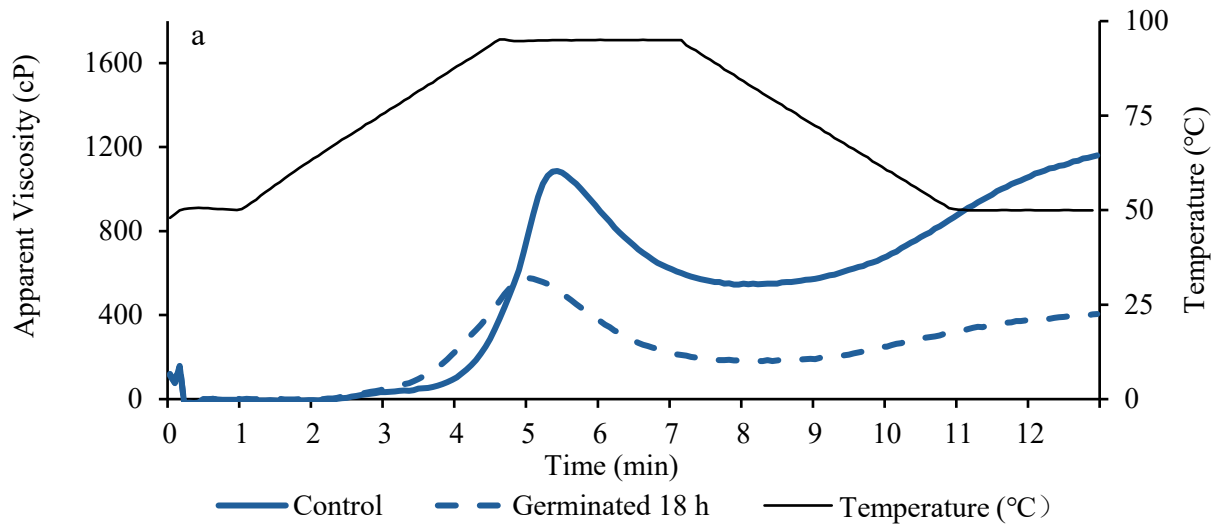
Wheat	Germination	Apparent peak viscosity (cP)	α -amylase activity (CU/g)	FN (s)
CWRS 1	Control	1094 $\pm$ 2 ^c	0.276 $\pm$ 0.015 ^d	365 $\pm$ 2 ^b

	G18	577±8 ^f	0.556±0.028 ^{bc}	202±3 ^e
CWRS 2	Control	1405±21 ^a	0.098±0.010 ^e	457±8 ^a
	G18	422±5 ^g	0.637±0.026 ^b	198±4 ^e
	Adj	694±12 ^d	0.196±0.030 ^d	233±2 ^d
CWRS 3	Control	1246±14 ^b	0.202±0.010 ^d	371±5 ^b
	G18	430±3 ^g	0.842±0.084 ^a	181±4 ^f
	Adj	624±1 ^e	0.476±0.057 ^c	257±7 ^c

G 18: germinated wheat flour from 18hour germination. Adj: germinated wheat flour from germination time 15 and 16 h for CWRS 2 and CWRS 3, respectively. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p<0.05$).

Plots of the pasting behaviour of the native and germinated flours are shown in Fig. 3.1 (a, b, and c). As expected, the native flours exhibited higher apparent viscosity than their germinated counterparts. The germinated sample showed a significantly ($p<0.05$) lower apparent peak viscosity. Germinated samples for fixed or adjusted times displayed flattened viscosity curves, indicating reduced starch swelling and gelatinization due to enzyme degradation. The final viscosities for germinated samples were also significantly ($p<0.05$) lower than their control. Nevertheless, the extent of the impact of germination on the final viscosity varied with the wheat samples, observing a much lower effect with CWRS 2. Considering that this parameter reflects the apparent viscosity of a starch paste upon cooling, which results from the amylose recrystallization, that difference might be connected with the amylose content of the flours. In fact, CWRS 2 had an amylose content of 18.93 ± 1.35 g/100 g in flour, which is significantly higher ($p<0.05$) than the amylose content in CWRS 1 and CWRS 3, which were 13.14 ± 0.65 g/100 g and 12.90 ± 0.81 g/100 g, respectively. This indicates that the amylase triggered by germination was primarily acting

on the amylopectin, and in consequence, affecting more CWRS 1 and CWRS 3. Germination resulted in an earlier and reduced viscosity peak due to the breakdown of starch granules into smaller dextrins that allow faster water penetration into the granules, with consequent lower energy and less time required to start the gelatinization (Frauenlob et al., 2018).



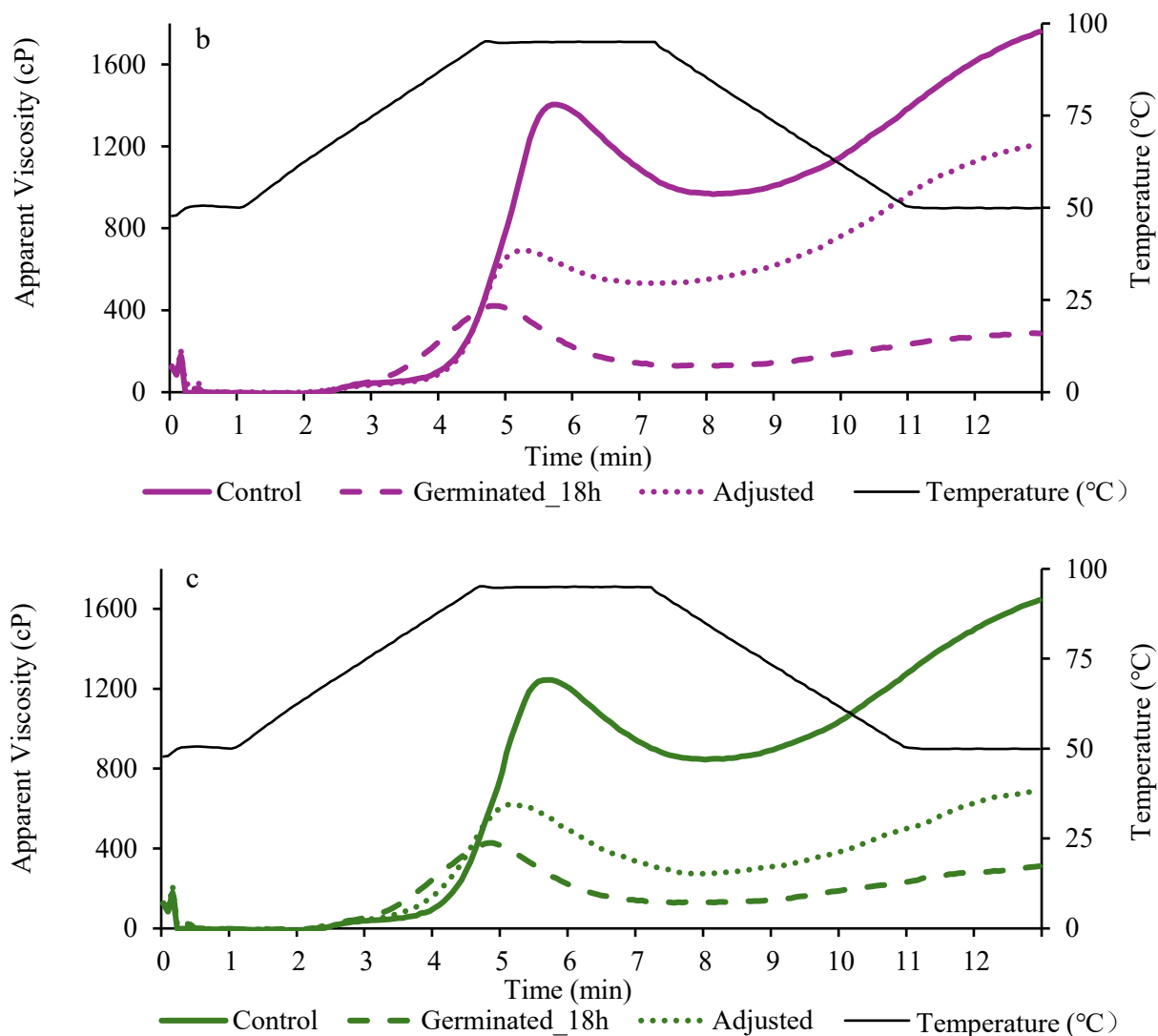


Figure 3.1. RVA curves of three samples under different germination times

a: CWRs 1, b: CWRs 2 and c: CWRs 3. G18: germinated wheat flour from 18-hour germination. Adjusted: germinated wheat flour after 15 h and 16 h for CWRs 2 and CWRs 3, respectively.

Regarding the α -amylase activity, germination significantly ($p < 0.05$) increased it in all wheat samples (CWRs 1, CWRs 2, and CWRs 3) compared to their respective controls (Table 3.1). Kaur et al. (2024) reported α -amylase activity of 0.42 CU/g after germination for 18 h. In the present study, α -amylase activity ranged from 0.556 to 0.842 CU/g, obtaining different levels of α -amylase activity when germinating for a fixed time (18 h). After adjusting the germination time in the

different samples, the α -amylase activity increased by 2-2.5 folds. Conversely, the comparison of the samples at the same germination time brought an increase of α -amylase activity by 2.0, 6.5, and 4.2 times in CWRS 1, CWRS 2, and CWRS 3, respectively. Although peak viscosity is generally associated with α -amylase activity, the measured values did not show a strict one-to-one correspondence. For example, after 18 h of germination, CWRS 3 exhibited the highest α -amylase activity (0.842 CU/g, $p<0.05$), whereas CWRS 2 required a lower activity level (0.196 CU/g) to achieve a comparable 50% reduction in peak viscosity. Thus, each wheat sample reached the 50% apparent viscosity reduction at different times. This discrepancy aligns with studies demonstrating that starch viscosity profiles depend not only on α -amylase levels but also on enzymatic synergies, inhibitor interactions, and molecular structure of starch chains. Zhang et al. (2022) reported that *TaAmy1*-overexpressing wheat lines showed extreme viscosity reductions (80 cP peak viscosity) due to hyperactivation of α -amylase, while adding inhibitors like silver nitrate restored viscosity to >90% of baseline despite unchanged enzyme concentrations. The dynamic nature of enzyme activation during germination further explains why samples reached equivalent adjusted viscosity thresholds at different times. Since the commercial wheat blends used in this study likely consisted of multiple varieties, each sample may have progressed through distinct biochemical maturation stages, altering the functional balance of amylolytic enzymes and their substrates at measurement points.

The FN values decreased significantly ($p<0.05$) in germinated samples, reflecting enhanced α -amylase activity. Adjusted germination times yielded intermediate FN values, inducing moderate enzyme activity. A strong inverse correlation ($p<0.0001$, $r=-0.81$) was observed between α -amylase activity and FN in the range of values obtained after germination. Despite similar reductions in peak viscosity under adjusted germination conditions for CWRS 1 and CWRS 2

(50.6% and 49.9%, respectively), the corresponding increases in α -amylase activity (100% and 135.6%) and reductions in FN (49.0% and 30.7%) were not proportional, suggesting one more time that viscosity did not linearly reflect enzymatic activity or FN across different wheat blends. A negative nonlinear relationship was found between α -amylase activity and FN, as described by Yu et al. (2015), who found that α -amylase activity rose from 0.11 CU/g in ungerminated wheat to 6.96 CU/g after 36 h of germination (20°C), whereas FN decreased from 385 seconds to 63 seconds. Similarly, a strong negative correlation ($p < 0.0001$, $r = -0.84$) was observed between α -amylase activity and peak viscosity for all control, G18, and adjusted samples of CWRS 1, 2, and 3. Higher α -amylase activity led to substantial reductions in peak viscosity due to the enzymatic degradation of starch granules, which diminished their ability to gelatinize and form a viscous paste (Noda et al., 2003). Furthermore, the strong positive correlation ($p < 0.0001$, $r = 0.98$) between peak viscosity and FN suggests that RVA assessments can effectively predict starch enzymatic hydrolysis in flours with varying α -amylase concentrations.

The impact of germination on the proximate composition was assessed (Table 3.2). Protein content ranged from 14.37 g/100 g to 15.55 g/100 g, which was expected since the wheat was from the same CWRS class. Protein content significantly increased during germination, except for CWRS 2. Protein values obtained with the adjusted time germination confirmed that a progressive, steady increase in the protein content is induced. This increase could be attributed to the effective mobilization of high molecular weight glutenin subunits, α -gliadin, and β -gliadin during germination (Świeca et al., 2017). However, this increase may not reflect an actual rise in protein levels but rather a loss of carbohydrates or changes in nitrogenous compounds (Dhillon et al., 2020). Fat content remained unaffected by germination. Except for CWRS 1, the ash content of the germinated samples significantly ($p < 0.05$) decreased, compared to their respective controls.

This observation aligns with Dhillon et al. (2020), who reported approximately a 31% reduction in ash content in wheat germinated for 72 h at 30°C. As anticipated, the carbohydrate content decreased significantly in the germinated samples due to enzyme hydrolysis. However, this decrease did not result in an increase in glucose content, suggesting that α -amylase, as an endoenzyme, hydrolyzed the starch into dextrin or maltose (Grassi et al., 2018). Although the starch content was not quantified in this study, previous research indicated that the total starch content was 62.89 ± 2.82 g/100 g in untreated CWRS wheat and 64.61 ± 3.72 g/100 g in the G18 flour, with no significant changes observed (Kaur et al., 2024).

Table 3.2 Proximate composition of ungerminated and germinated whole wheat kernels expressed as g/100 g (dry basis)

Wheat Samples	Germination	Protein	Fat	Ash	D-glucose	Carbohydrate
CWRS 1	Control	15.55 ± 0.39^b	2.16 ± 0.03^a	1.93 ± 0.01^a	0.04 ± 0.00^b	74.60 ± 0.31^a
	G18	16.76 ± 0.46^a	2.11 ± 0.08^a	1.97 ± 0.02^a	0.04 ± 0.01^b	72.51 ± 0.78^{bc}
CWRS 2	Control	14.71 ± 0.37^{bc}	1.97 ± 0.02^{bc}	1.50 ± 0.01^c	0.05 ± 0.01^b	73.61 ± 0.19^{ab}
	G18	15.48 ± 0.29^b	1.91 ± 0.03^{bc}	1.29 ± 0.03^c	0.05 ± 0.01^b	70.72 ± 0.76^d
	Adj	14.99 ± 0.38^{bc}	1.89 ± 0.02^c	1.40 ± 0.01^d	0.05 ± 0.00^b	71.69 ± 0.25^{cd}
CWRS 3	Control	14.37 ± 0.27^c	1.99 ± 0.04^{bc}	1.63 ± 0.02^b	0.08 ± 0.01^a	72.80 ± 0.48^{bc}
	G18	15.36 ± 0.44^b	2.00 ± 0.04^b	1.53 ± 0.01^c	0.07 ± 0.01^a	70.66 ± 0.41^d
	Adj	14.86 ± 0.24^{bc}	1.97 ± 0.05^{bc}	1.53 ± 0.04^c	0.08 ± 0.01^a	70.88 ± 0.53^d

G18: germinated wheat flour from 18-hour germination. Adj: germinated wheat flour from germination time 15 h and 16 h for CWRS 2 and CWRS 3, respectively. Different letters within each column indicate significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

3.3.2 Mixing properties

The flour mixing performance and dough fermentation parameters are detailed in Table 3.3. Germinated CWRS 1 showed significantly ($p<0.05$) lower water absorption (63.03%) compared to the control (65.36%). This reduction aligns with findings by Ding et al. (2018), who reported a decrease in water absorption from 64% to 61%, 60%, and 59.5% in germinated hard red spring wheat flour after 5, 15, and 24 h of germination, respectively, under conditions of 28°C and 95% RH. Germination shortened the development time, but with the adjusted germination to reduce its apparent viscosity by 50%, no significant impact was observed, except in CWRS 3, where the reduction was around 11%. Similar results were described by Cardone et al. (2020), in which germinated wheat (48 h at 20°C and 90% RH) showed a decrease in dough development time (farinograph) from 5.9 ± 0.2 min to 3.1 ± 0.2 min. Probably, gluten protein hydrolysis, due to germination, led to faster dough formation. Germinated wheat flour exhibited significantly ($p<0.05$) lower stability and quality number compared to the control. Samples demonstrated that closer peak viscosity levels, obtained with the adjusted samples, exhibited similar stability. For instance, the stability of CWRS 1 at G18 decreased by 30.2%, which is comparable to the decline observed in CWRS 2 under the adjusted conditions (26.0%). In contrast, the stability of CWRS 2 and CWRS 3 at G18 showed a more pronounced decrease compared to their respective adjusted samples, reflecting the lower peak viscosity present in G18 relative to the adjusted condition. Conversely, germinated wheat flour showed significantly ($p<0.05$) higher softening and MTI values. For example, G18 of CWRS 1 showed a 29.4% increase in softening, which was comparable to the increase observed in the adjusted sample of CWRS 3 (25.0%), both corresponding to a 50% reduction in peak viscosity. In contrast, the increases in softening were greater in lower peak viscosity samples (G18 of CWRS 2 and CWRS 3) compared to their

respective adjusted samples. These increased softening and MTI values reflected the reduced dough strength because of the enzymatic degradation of high molecular weight proteins (Poudel et al., 2019). A similar trend was observed by Kaur et al. (2024), who confirmed the endoprotease activity during germination by assessing the gluten index and lactic acid retention capacity. In addition to the enzymatic activity triggered by germination, differences in flour properties may also be influenced by changes in starch structure and grain composition. Germination can weaken starch granule integrity, alter the amylose-to-amylopectin ratio, and increase surface porosity, thereby affecting pasting properties (Baranzelli et al., 2019). Likewise, protein mobilization during germination can reduce gluten content and modify the protein–starch matrix, with downstream effects on dough rheology (Shaimerdenova et al., 2025). These compositional changes, together with the enzymatic cocktail present, act in concert to determine the functional behaviour of germinated wheat flours.

Germination reduced the fermentation time, although no significant differences were observed, with the exception of CWRS 3 G18. But that trend was confirmed with the significant increase ($p < 0.05$) observed in the fermentation rate, except for CWRS 1. The elevated levels of α -amylase present in the germinated flour facilitate the hydrolysis of starch into maltose and dextrin, leading to fermentable sugars for the yeast metabolism (Kaur et al., 2024).

Table 3.3 Flour mixing and dough fermentation properties

Wheat Samples	Germination	Water absorption (g/100 g)	Development time (min)	Stability (min)	Softening (mNm)	MTI (mNm)	Quality number	Fermentation time (min)	Fermentation rate (mL/min)
CWRS 1	Control	65.36±0.00 ^a	3.5±0.1 ^c	4.3±0.1 ^c	17±1 ^{bc}	14±1 ^b	54.9±1.3 ^c	42±1 ^{ab}	2.48±0.14 ^a
	G18	63.03±0.00 ^b	3.5±0.1 ^c	3.0±0.1 ^d	22±2 ^a	19±2 ^a	46.8±0.4 ^d	37±2 ^b	2.56±0.24 ^a
CWRS 2	Control	65.80±0.05 ^a	4.7±0.2 ^a	7.7±0.2 ^a	12±1 ^e	9±1 ^c	63.0±2.3 ^a	51±6 ^a	1.81±0.11 ^c
	G18	66.12±0.42 ^a	3.9±0.4 ^{bc}	4.0±0.2 ^c	18±0 ^b	14±1 ^b	54.5±0.8 ^c	42±3 ^{ab}	2.61±0.11 ^a
	Adj	65.80±0.08 ^a	4.4±0.2 ^{ab}	5.7±0.3 ^b	13±0 ^{de}	10±1 ^{de}	60.4±0.6 ^{ab}	43±1 ^{ab}	2.64±0.06 ^a
CWRS 3	Control	63.20±0.04 ^b	4.6±0.2 ^a	7.4±0.2 ^a	12±2 ^c	11±1 ^{cde}	61.2±2.5 ^{ab}	51±6 ^a	1.83±0.08 ^c
	G18	61.50±0.41 ^c	4.0±0.1 ^b	4.2±0.0 ^c	18±1 ^{bc}	13±1 ^{bc}	54.7±1.2 ^c	41±0 ^b	2.44±0 ^{ab}
	Adj	61.97±0.30 ^{bc}	4.1±0.3 ^b	6.0±0.1 ^b	15±1 ^{cd}	12±1 ^{bcd}	58.6±1.3 ^b	44±5 ^{ab}	2.17±0.14 ^b

G18: germinated wheat flour from 18-hour germination. Adj: germinated wheat flour after 15 h and 16 h for CWRS 2 and CWRS 3, respectively. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

3.3.3 Impact of controlled germination on bread properties

Bread was made by adapting the amount of water and dough proofing time according to the values displayed in Table 3.3. The texture profile and colour parameters of the breadcrumbs are summarized in Table 3.4. Breads obtained from the different wheat flours did not show significant differences in the hardness, springiness, cohesiveness, and chewiness. Germination significantly ($p < 0.05$) reduced the crumb hardness by 56%, 49%, and 41%, for CWRS 1, CWRS 2, and CWRS 3, respectively, when applying a fixed germination time. That crumb softening effect was reduced by adjusting the germination time. Likewise, chewiness was significantly ($p < 0.05$) reduced by germination, but the reduction was independent of the extent of the germination. These findings align with those presented by Mendoza Moreno et al. (2025), who observed bread hardness reduction by 55% and 33% when using 100% of wheat germinated for 12 h and 48 h at 25°C, respectively. The increased production of fermentable sugars resulted from elevated amylase activity, improved yeast fermentation, and, in turn, greater gas production and a more open and softer crumb structure (Kaur et al., 2024). Springiness, cohesiveness, and resilience did not show significant differences across treatments, indicating that these parameters are less influenced by changes in α -amylase activity and starch hydrolysis.

Germination had no significant impact on the lightness and redness of the breadcrumbs. However, bread made from germinated wheat flour exhibited significantly higher b^* values, increasing yellowness compared to control bread. This change might be related to the sugar released due to starch hydrolysis, but it has also been reported that an enhancement in the biosynthesis of carotenoids during the germination process, which in turn increases the total yellow pigment content, results in a more yellow breadcrumb (Cankurtaran-Kömürcü & Bilgiçli, 2023).

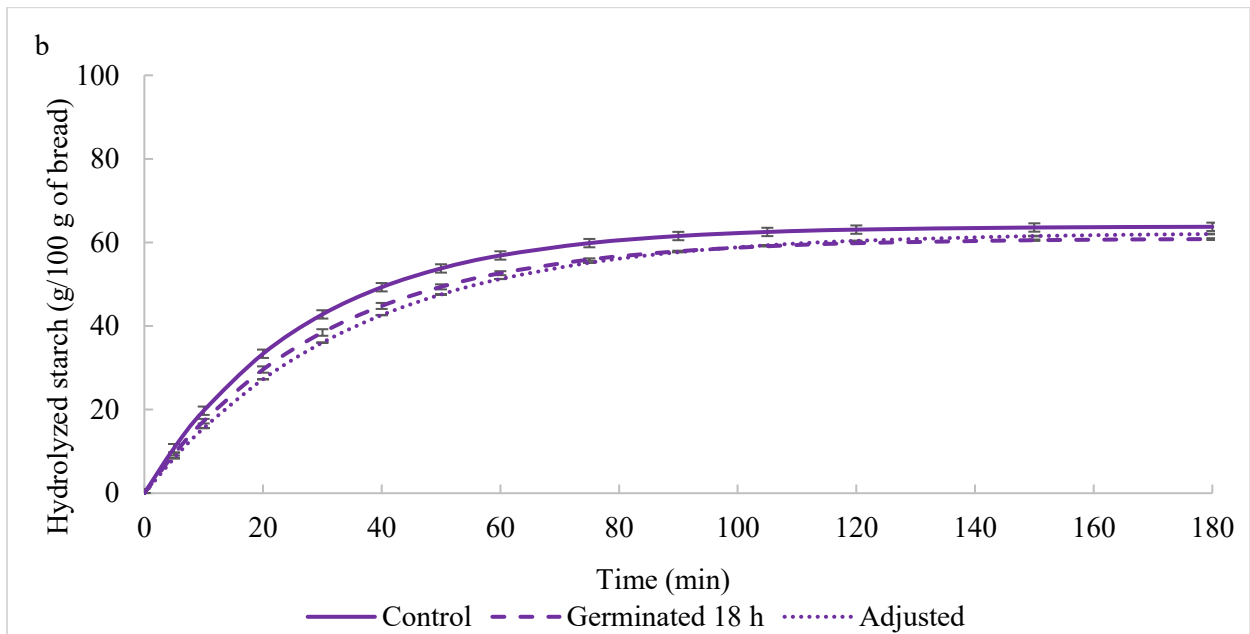
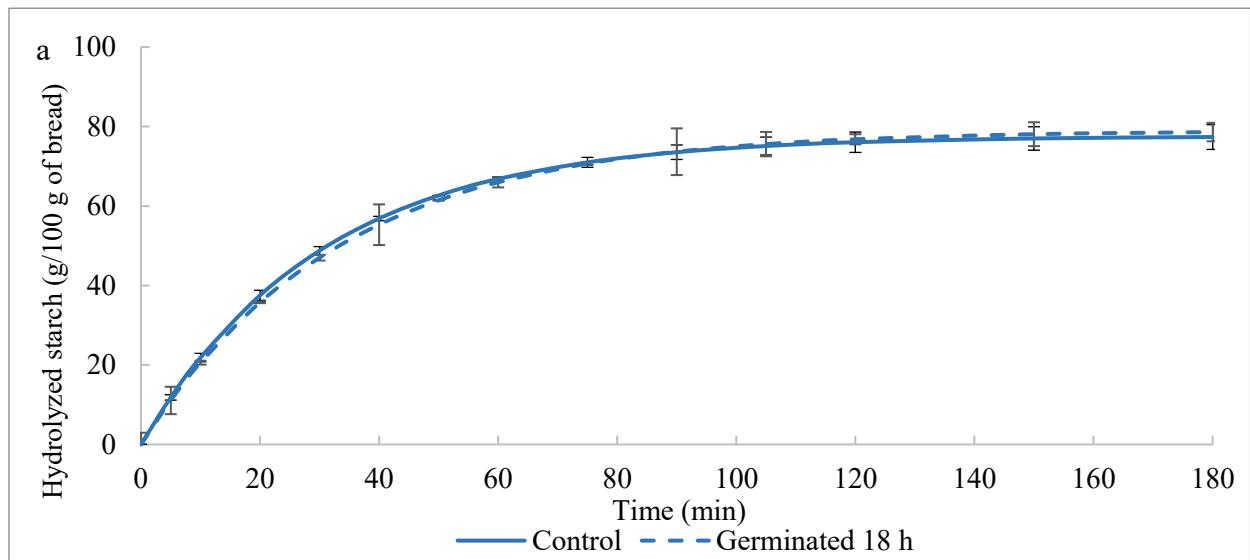
Table 3.4 Impact of germination on bread parameters

Wheat	Germination	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)	Resilience	<i>L</i> *	<i>a</i> *	<i>b</i> *
CWRS 1	Control	1007±74 ^a	0.88±0.01 ^a	0.72±0.02 ^a	700±53 ^a	0.38±0.07 ^c	51.78±2.86 ^a	7.16±0.34 ^a	16.88±0.50 ^{bc}
	G18	567±47 ^c	0.93±0.07 ^a	0.74±0.02 ^a	389±26 ^b	0.40±0.02 ^{bc}	54.10±2.52 ^a	7.28±0.48 ^a	19.83±0.17 ^a
CWRS 2	Control	1019±50 ^a	1.01±0.2 ^a	0.79±0.02 ^a	713±80 ^a	0.46±0.02 ^a	41.02±0.86 ^b	6.69±0.42 ^a	15.50±1.08 ^c
	G18	499±21 ^c	1.09±0.25 ^a	0.79±0.02 ^a	363±41 ^b	0.46±0.01 ^a	43.14±2.74 ^b	6.71±0.55 ^a	19.34±1.51 ^a
	Adj	664±23 ^b	0.88±0.17 ^a	0.77±0.02 ^a	462±41 ^b	0.48±0.06 ^a	41.19±0.62 ^b	6.89±0.66 ^a	18.20±0.32 ^{ab}
CWRS 3	Control	931±8 ^a	1.12±0.07 ^a	0.78±0.01 ^a	704±237 ^a	0.47±0.01 ^a	40.78±2.15 ^b	7.01±0.60 ^a	16.13±0.25 ^c
	G18	386±32 ^d	1.01±0.01 ^a	0.79±0.02 ^a	362±33 ^b	0.46±0.02 ^a	41.11±0.84 ^b	7.05±0.16 ^a	18.96±0.79 ^a
	Adj	582±36 ^{bc}	1.10±0.09 ^a	0.80±0.02 ^a	402±32 ^b	0.44±0.04 ^{ab}	40.83±0.34 ^b	6.97±0.56 ^a	18.18±0.62 ^{ab}

G18: germinated wheat flour from 18 h germination. Adj: germinated wheat flour after 15 h and 16 h for CWRS 2 and CWRS 3, respectively. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

3.3.4 *In vitro* starch digestibility

The potential contribution of germination to the bread healthiness component was assessed through the *in vitro* starch digestibility (Figure 3.2). Resulting plots were fitted to the Box-Lucas model to calculate the kinetic parameters and the starch's vulnerability to digestive enzymes by estimating RDS and SDS fractions, as well as its RS fraction (Table 3.5).



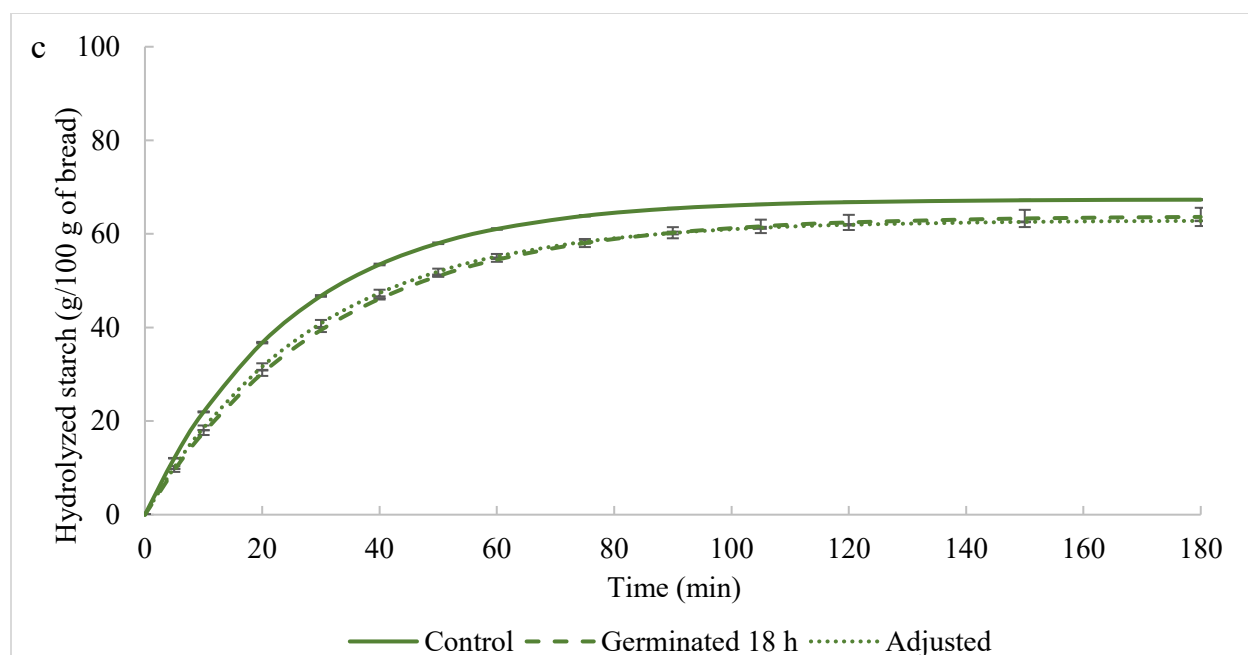


Figure 3.2. *In vitro* starch hydrolysis kinetics of bread made of different whole wheat flours

a: CWRS 1, b: CWRS 2, and c: CWRS 3. G18: germinated wheat flour from germination 18 h. Adj: germinated wheat flour after 15 h and 16 h for CWRS 2 and CWRS 3, respectively.

Starch hydrolysis plots showed the typical rapid increase in hydrolyzed starch during the initial phase (0-30 minutes), followed by a gradual plateau after approximately 120 minutes (Figure 3.2). The plateau indicates the maximum hydrolysis limit under the experimental conditions. Germination promoted a reduction in the starch hydrolysis of the breads, decreasing also the plateau, but that impact was not observed in CWRS 1 bread. For this germinated bread, there was slightly slower starch digestion before 60 minutes compared to their respective control, while faster starch digestion after 90 minutes, and the final hydrolysis extent was slightly higher compared to the control. In contrast, the kinetics of starch digestion in germinated wheat bread made from CWRS 2 and CWRS 3 were slower throughout the entire 180-minute hydrolysis compared to the control group. That trend resulted in slower starch hydrolysis rates, with values around 62%, likely due to the increased content of RS in the sprouted wheat flour. This observation is consistent with previous studies that also reported reduced *in vitro* starch digestibility in bread

formulated with partially sprouted wheat flour. Specifically, bread containing 20% germinated wheat flour (germinated at 20°C for 96 h) and 80% whole wheat flour had a starch digestibility of 55.19%, significantly lower than the 73.05% observed in the control bread. The reduced digestibility may be attributed to interactions of phenolic compounds, digestive enzymes, and starch (Świeca et al., 2017). Minor differences were observed between breads obtained from adjusted and germinated wheats. This result aligns with the natural enzyme modifications occurring during germination. The activation of amylase during germination partially hydrolyzes starch into smaller dextrins that might contribute to the formation of amylose-lipid complexes, which are less susceptible to enzymatic hydrolysis during digestion (Aleixandre et al., 2021).

Table 3.5 Starch fractions of bread prepared from control wheat flour and germinated wheat flour

Wheat Samples	Germination	RDS (g/100 g bread)	SDS (g/100 g bread)	RS (g/100 g bread)
CWRS 1	Control	37.52±1.04 ^a	38.53±3.83 ^{ab}	9.99±0.08 ^b
	G18	35.77±1.38 ^b	41.03±2.88 ^a	11.52±0.01 ^a
CWRS 2	Control	33.37±0.37 ^c	30.04±0.48 ^c	7.61±0.04 ^c
	G18	29.59±0.62 ^e	30.24±0.81 ^c	9.50±0.24 ^c
	Adj	27.26±0.05 ^f	33.13±0.12 ^{bc}	9.47±0.12 ^c
CWRS 3	Control	36.77±0.13 ^{ab}	30.02±0.04 ^c	8.73±0.10 ^d
	G18	30.24±0.47 ^{de}	33.00±1.01 ^{bc}	10.05±0.27 ^b
	Adj	31.65±0.58 ^d	30.31±0.55 ^c	10.12±0.04 ^b

RDS: Rapidly digestible starch. SDS: Slowly digestible starch. RS: Resistant starch. G18: germination 18 h. Adj, germination time adjusted. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

The starch fractions of bread prepared with germinated wheat flour showed significant modifications in starch digestibility, with consistently higher SDS and RS fractions and reduced RDS across all CWRS samples (Table 3.5). For CWRS 1, the germinated groups significantly ($p < 0.05$) increased RS with lower RDS compared to the control, and this trend was consistent in CWRS 2 and CWRS 3. The levels of RS increased by approximately 15%, 12%, and 11% in the germinated samples, respectively, but the germination levels did not significantly affect the RS content. Additionally, the RDS in germinated CWRS 1 decreased by about 5%, while the decrease was more pronounced in germinated CWRS 2 and 3, which showed reductions ranging from 12% to 18%. For SDS, there was a slight increase in germinated wheat bread compared to their controls, but those were not statistically significant. Results agree with those of Marti et al. (2018) reported that had significantly higher SDS and lower RDS in bread made from 100% germinated whole wheat flour (48 h at 20°C) compared to bread made from ungerminated wheat flour. On the other hand, by substituting 20% of whole wheat flour with a germinated one (96 h at 20°C), bread increased ~35% their RS (Świeca et al., 2017). The increased levels of RS in germinated samples can be attributed to structural rearrangements in starch during enzymatic modification. Linear amylose fragments generated by α -amylase interact with endogenous lipids (phospholipids in cereals) to form helical amylose-lipid complexes, which resist enzymatic hydrolysis due to steric hindrance and reduced accessibility of α -1,4/ α -1,6 bonds to amylases (Aleixandre et al., 2021). Similarly, RS resists digestion in the small intestine and undergoes fermentation in the colon, producing short-chain fatty acids that improve gut health and reduce inflammation. The reduction in RDS further supports the bread's potential for improving metabolic responses, as RDS is rapidly hydrolyzed to glucose, leading to spikes in postprandial blood sugar levels (Huang et al., 2022).

3.4 Conclusions

The functionality of germinated wheat flour depends not only on germination time but also on the intrinsic characteristics of wheat, making it difficult to directly compare samples based on the duration of germination alone. Despite applying controlled germination conditions, substantial differences in α -amylase activity and corresponding functional properties were observed among samples germinated for the same length of time. This divergence indicates that germination time alone is an inadequate predictor of flour behaviour.

To address this variability, peak viscosity determined by RVA was proposed in this study as a more robust indicator of the extent of germination and a practical parameter to classify and compare germinated flours. Viscosity more directly reflects α -amylase activity and its functional implications, offering a standardized approach to assessing sprouted flour performance in food applications. Nevertheless, non-linear correlations between α -amylase activity, FN, and peak viscosity were found, with consequent limitation in capturing the complex interplay of enzymatic synergies and endogenous inhibitor interactions in wheat grains.

In terms of dough functionality, germinated wheat flour exhibited reduced viscosity and weakened mixing properties consistent with elevated α -amylase activity. Nonetheless, when used at 100% replacement level in bread formulations, germinated flour produced bread with softer crumb texture and acceptable structural integrity, demonstrating its potential as a functional ingredient for clean-label and nutrient-enhanced baking applications. Critically, the bread made from 100% germinated wheat flour showed a significant reduction in RDS and an increase in RS, reflecting a favourable shift in starch digestibility. These modifications suggest a potential for attenuating postprandial glycemic responses.

Overall, germination, when monitored and categorized through functional indicators like viscosity, emerges as a sustainable, clean-label bioprocess for improving the nutritional and functional quality of wheat-based bakery products.

CRedit Author contributions statement

Xiaohang Zou: Writing – original draft, Investigation; Formal analysis; Data curation.

Nicola Gasparre: Training; Writing – review & editing; Supervision; Conceptualization; Funding acquisition

Cristina M. Rosell: Writing – review & editing; Conceptualization; Supervision; Investigation; Funding acquisition; Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 4_Experimental Section 2

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Effect of germination on purple wheat functionality and starch hydrolysis in bread

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Abstract

Germination is increasingly used to improve the nutritional and functional properties of wheat. However, pigmented wheats may respond differently due to their high phenolic content and distinct kernel structure. This study evaluated the effects of germination (0, 6, 12, and 24 h) on purple wheat flour functionality and on bread texture, colour, and *in vitro* starch digestibility. Germination increased α -amylase activity and reduced FN, leading to lower RVA peak and final viscosity and a progressive weakening of dough aggregation properties. Bread produced from 24 h germinated flour exhibited greater gas cell coalescence and lighter crumb colour due to losses of water-soluble anthocyanins. TPC showed a strong positive correlation with RS ($r=0.861$, $p=0.006$) and a negative correlation with starch hydrolysis rate ($r=-0.918$, $p=0.001$). Additionally, TAC was negatively correlated with RDS ($r=-0.817$, $p=0.013$). These findings demonstrate that phenolic compounds, particularly anthocyanins, modulate starch digestibility and that purple wheat exhibits a distinct germination response compared with common wheat.

Keywords: anthocyanins; bread quality; germinated purple wheat; *in vitro* starch digestibility; TPC.

4.1 Introduction

Pigmented wheat varieties, such as purple, blue, and black wheat, have gained growing attention due to their unique nutritional profiles and bioactive potential (Dhua et al., 2021). Beyond their macronutrient composition, pigmented wheats are rich in phytochemicals with antioxidant and potential health-promoting effects (Shamanin et al., 2022). Among pigmented wheats, purple wheat has drawn particular interest in its high anthocyanin concentration, antioxidant capacity, and potential functional benefits. Specifically, purple pericarp wheat Laval 19 contained 72 µg/g of TAC, while blue aleurone wheat Purendo 38 contained 60 µg/g of TAC (Abdel-Aal et al., 2016). Quantitatively, the TAC in purple wheat has been reported to range from 156.7 to 383.2 mg/kg in bran, 61.3 to 153.3 mg/kg in whole meal, and 3.1 to 14.3 mg/kg in flour, with purple wheat bread containing around 78 mg/kg (Beta et al., 2019). In addition, phenolic compounds represent the largest group of hydrophilic phytochemicals found in purple wheat, with a TPC of about 220-230 mg ferulic acid equivalents /100 g flour, which remain in the final products, increasing their antioxidant potential (Beta et al., 2019). Such findings highlight the strong link between pigmentation and polyphenol accumulation. From a technological perspective, purple wheat flour also displays superior farinograph stability and lower softening degree than conventional wheat, reflecting improved dough strength (Koksel et al., 2023).

Germination has attracted particular attention as a natural bioprocess capable of enhancing the nutritional and functional properties of cereal grains while preserving or even generating bioactive compounds. During germination, endogenous enzymes such as α -amylase and proteases are

activated, hydrolyzing storage macromolecules into simple sugars, amino acids, and peptides (Chinma et al., 2022). This leads to improved nutrient availability, reduced antinutritional factors, and modified flour functionality. Technologically speaking, the hydrolyzing activity of α -amylase partially degrades the starch granules with a consequent decrease in pasting viscosity. Meanwhile, proteolysis leads to a reduction in gluten strength while enhancing the extensibility of the dough (Świeca et al., 2017). Such biochemical changes may influence flour rheology, bread texture, and overall baking performance. For instance, germinated wheat flour showed reduced water absorption and dough development time, suggesting a weakened gluten network (Marti et al., 2018). Likewise, Kaur et al (2024) reported a decrease in pasting properties in 24 h germinated common wheat compared to the control, with a concomitant decrease in the gluten index and gluten performance index. Moreover, germination is increasingly explored as a tool to modulate starch digestibility, which has implications for glycemic response and health-related outcomes. For example, in the 48-hour germinated wheat bread, the level of RDS was lower at 38.18% compared to the reference bread, which had 44.51%. Conversely, the SDS in 48 h germinated wheat bread was higher at 13.15% compared to ungerminated bread, which had 3.32% (Mendoza Moreno et al., 2025). This pattern in the *in vitro* digestibility may be due to the modification of the starch fraction by digestive enzymes (Zou et al., 2025).

While germination of conventional wheat has been widely studied, limited information exists on how this process affects pigmented varieties such as purple wheat. The high anthocyanin content of purple wheat introduces an additional layer of complexity, as these compounds may affect the activity of hydrolytic enzymes, thereby altering starch and protein degradation patterns, dough rheology, and starch digestibility. Therefore, understanding how germination influences the

biochemical and functional characteristics of purple wheat remains an important and unexplored area.

Hence, this study aimed to systematically evaluate how controlled germination affects the functional characteristics of purple wheat flour and the quality of bread derived from it. The analysis of α -amylase activity, dough rheology, bread features, and *in vitro* starch digestibility provides new insights into how germination modulates the functionality and nutritional potential of purple wheat. The findings will contribute to optimizing germination as a clean-label approach to improve the technological and health attributes of anthocyanin-rich cereal products.

4.2 Materials and Methods

Purple pericarp wheat cv. CDC Prime purple was generously provided by Agriculture and Agri-Food Canada, Guelph Research and Development Centre, Guelph, ON, Canada. Although the total starch content of the purple wheat used in the present study was not measured, a previous study reported a value for the purple wheat was 64.5 ± 0.9 g/100 g (Abdel-Aal & Hucl, 1999). Instant dry yeast was donated by Lallemand Inc. (Montreal, QC, Canada). Salt was acquired in the local market. All chemicals were of analytical grade.

4.2.1 Germination process under controlled conditions

Germination was performed following the methodology described by Kaur et al. (2024). Wheat kernels were initially soaked in a 1% (v/v) sodium hypochlorite solution for 15 min. After soaking, the kernels were thoroughly washed with distilled water until they reached a neutral pH. The germination process was conducted at 21°C with an RH of 80%. After germination, the kernels were dried at 50°C for 9 hours until their moisture content dropped below 15%. Germination was carried out for durations of 6, 12, and 24 hours, with the samples designated as G 6, G 12, and G 24, respectively. These controlled germination times were selected according to Kaur et al. (2024),

which could improve wheat flour functionality and bread quality. A non-germinated wheat sample served as the control. The dried kernels were then milled using a laboratory hammer mill (Perten Lab Mill 3100, Perten Instruments AB, Huddinge, Sweden) to produce flour with a particle size of less than 0.5 mm.

4.2.2 Flour characterization

Flours were characterized by assessing the colour, FN, and α -amylase activity. Colour measurement was performed using a Chroma meter CR-410 (Konica Minolta, Tokyo, Japan) to assess L^* , a^* , and b^* values of the flour. Where L^* represented lightness, a^* indicated the range from greenness to redness, and b^* measured the spectrum from blueness to yellowness, from negative to positive values, respectively. The FN was measured according to the AACCI-approved method 56-81.03 (AACC International, 2009) using the Perten FN system 1310 (Perten Instruments AB, Stockholm, Sweden). α -Amylase activity was assessed according to the Ceralpha method (analysis kit Neogen-Megazyme International Ireland Ltd, Bray, Ireland) with the modification reported by Kaur et al. (2024).

4.2.3 Gluten aggregation behavior

The gluten aggregation properties of purple wheat flour were measured using the GlutoPeak instrument (Brabender, Duisburg, Germany). A total of 9 g of whole wheat flour (on a 14% moisture basis) was mixed with 9 g of distilled water in a stainless-steel cup at a speed of 2,750 rpm. The temperature of the sample was maintained at 30°C by circulating water from a water bath. The GlutoPeak parameters analyzed by the corresponding software included: (a) torque maximum, which represented the highest resistance encountered during mixing and was expressed in Brabender units (BU); (b) peak maximum time, defined as the duration before the maximum torque begins to decline, measured in seconds; and (c) GSI, calculated using the formula: $GSI =$

(maximum torque $\times$ peak area) / 1,000, as proposed by Wang et al. (2017). All GlutoPeak tests were conducted at least in duplicate for each batch.

4.2.4 Pasting properties

The standard method 1 from the AACCI Method 76-21.01 (AACC International, 2009) was used to assess the pasting profile of the flours, using an RVA (RVA-4800, PerkinElmer, Shelton, CT, USA).

4.2.5 Mixing properties

The mixing characteristics of the flour samples were determined using a Chopin Mixolab (Chopin Technologies, Villeneuve-la-Garenne, France). This instrument measures the torque (in Nm) produced in real-time as the dough passes between the two kneading arms. Briefly, a 75 g of flour-based dough was prepared based on a 14% moisture content, and the necessary amount of water was added to achieve optimal consistency, measured at 1.1 Nm. The dough was then mixed at a speed of 80 rpm at 30°C for a total analysis time of 30 min. The results recorded included water absorption, dough development time, stability, softening, MTI, bandwidth, and energy.

4.2.6 Breadmaking

The bread-making formulation included 100 g of wheat flour (based on 14% moisture content), 1.5 g of salt, 1.0 g of instant dry yeast, and the optimal amount of water as determined by the Mixolab tests. All ingredients were mixed using an Artisan mini tilt-head stand mixer (KitchenAid, St. Joseph, Michigan, USA) for the optimal mixing time specified by the Mixolab. The dough was then divided into 50 g portions, shaped by hand, and placed into a silicone baking tray to proof at 85% RH and 30°C. To record the dough volume increase during proofing, 3 g of dough was put in a graduated cylinder (Martins et al., 2022). Loaves were baked once the dough volume reached 75% of its maximum during the proofing process. The baking temperature was set at 180°C for 15

min. After baking, the bread was left to cool at room temperature for one hour before being packed in polyethylene pouches for further analysis. Two batches were performed for each germination time.

4.2.7 Bread quality measurement

The 2D area and crumb structure of the slices were assessed using the procedure reported by Espinosa-Ramírez et al. (2018) with modifications. Photos of the bread slices were scanned using a Perfection V39 flatbed scanner (Seiko Epson Corp., Nagano, Japan) at a resolution of 600 dpi. The scanned images were then analyzed with FIJI-ImageJ 2.9.0_172 (National Institutes of Health, Rockville, MD, USA). During the analysis, the images were processed by splitting the channels and enhancing the contrast. Additionally, the predefined Otsu's algorithm was applied for segmentation. In each batch, two slices of bread were examined.

The texture properties of bread were assessed using a Texture Analyzer TA. XT2i (Stable Micro Systems, Surrey, UK). A crumb disk measuring 1 cm in thickness and 2 cm in diameter was taken from the central region of the bread sample. A compression strain of 50% was applied at a speed of 1 mm/s using a 36 mm aluminum cylindrical probe. The following parameters were measured: hardness, springiness, chewiness, cohesiveness, and resilience.

Colour measurement was performed for the crust and crumbs as previously described (2.2 Flour characterization).

4.2.8 *In vitro* starch digestibility

The hydrolysis kinetics of bread were evaluated using the method described by Santamaria et al. (2021) using porcine pancreatic α -amylase (0.9 U/mL) and amyloglucosidase (214.5 U/mL). Dried bread (200 mg) was suspended in 4 mL of a 0.1 M sodium maleate buffer (pH 6.9) containing porcine pancreatic α -amylase (0.9 U/mL). This mixture was processed using an Ultra Turrax T18

basic homogenizer (IKA-Werke GmbH and Co. KG, Staufen, Germany). The resulting slurry was incubated at 37°C for three hours, while being continuously agitated at a rate of 200 rpm. Periodic aliquots were taken at 0, 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, and 180 min to measure glucose release.

After 16 hours of hydrolysis, the residual starch was solubilized with 2 mL of cold 1.7 M NaOH and then hydrolyzed using amyloglucosidase (143 U/mL) at 50°C for 30 min in a shaking water bath to ensure complete hydrolysis. The concentration of glucose was determined using a GOPOD assay, with absorbance measured at 510 nm. The starch content was calculated by multiplying the glucose concentration (in mg) by 0.9.

During the enzymatic digestion of starch, three starch fractions were identified based on their hydrolysis rates: RDS, which is hydrolyzed within the first 20 min; SDS, which is hydrolyzed between 20 and 120 min; and RS, which represents the starch fraction remaining after 16 hours of digestion. The kinetics of *in vitro* digestion were determined by applying the Box-Lucas model following the equation mentioned in section 3.2.6.

4.2.9 TPC in bread

The extraction of phenolic compounds from wheat bread was performed using the MAE method described by Kirusnaruban et al. (2025). Briefly, 0.1 g of freeze-dried bread powder was mixed with 9.9 mL of distilled water. A magnetic stirrer was utilized in each extraction to ensure uniform heat distribution during the extraction. The extraction process involved heating the samples to 170°C for 10 min, using the Monowave 400 system (Anton Paar, Graz, Austria) to extract phenolic compounds from bread. After extraction, the phenolic extracts were collected and subjected to centrifugation at $6,000 \times g$ for 15 min at 4°C using a centrifuge (Corning LSE, New York, USA).

The supernatant was carefully transferred to an aluminum foil-covered Falcon tube using a Pasteur pipette to avoid light and subsequently stored at -20°C for further analysis.

The determination of TPC was measured according to Kirusnaruban et al. (2025) with slight modification. Briefly, an aliquot of sample 20 µL was mixed with 80 µL of 0.2 N Folin-Ciocalteu reagent in a 96-well microplate. The mixture was shaken and left to react for 3 min to develop colour. Then, 100 µL of sodium carbonate (Na_2CO_3) solution (7.5%, w/w) was added. The reaction mixture was kept in the dark for 30 min, and the absorbance of the blue-colored complex was measured at 765 nm in a microplate spectrophotometer (BioTek Epoch 2, Santa Clara, USA). Water was used as the blank, and a standard curve was prepared using gallic acid (1 mg/mL) as the stock solution, which was then diluted with water to concentrations ranging from 0.02 mg/mL to 0.4 mg/mL. The TPC was expressed as mg of GAE per mL of solution.

4.2.10 TAC in bread

Anthocyanin extraction was conducted using a method described by Jiang et al. (2024) with some modifications. In brief, a 100 mg sample of freeze-dried bread was extracted using 800 µL of acidified methanol (methanol: 1N HCl = 85:15, v/v) by vigorously shaking it in an Innova 40 incubator shaker (Eppendorf SE, Hamburg, Germany) for 1 hour at 350 rpm in the dark at room temperature. The supernatant was obtained by centrifugation at 8,000 x g for 15 min. TAC measurement followed the pH differential method according to the AOAC Method 2005.02 (Lee et al., 2005). Two aliquots (50 µL each) of extract were diluted 5 times with 0.025 M potassium chloride buffer (pH = 1.0) and 0.4 M sodium acetate buffer (pH = 4.5), respectively. Let both stand in the dark for 30 min at room temperature. The sample absorbance at 520 nm (anthocyanin peak) and 700 nm (haze correction) for both pH 1.0 and 4.5 solutions was measured by a microplate

spectrophotometer (BioTek Epoch 2, Santa Clara, USA). Results were expressed as mg Cy3-gluE per L of solution. The following equation was used for the calculation:

$$\text{TAC (mg / L)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times I} \quad (\text{Eq.2})$$

where A is corrected absorbance = $(A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$; $\text{MW}_{\text{Cy } 3\text{-glu}} = 449.2 \text{ g/mol}$; ϵ is molar absorptivity of cyanidin-3-glucoside = 26900 L/mol/cm ; DF is the dilution factor = 5; I is the path length = 1 cm.

4.2.11 Statistical analysis

Each analysis was performed in two batches, and the results were expressed as mean $\pm$ standard deviation ($n \geq 3$). Experimental data were statistically evaluated using the method of one-way ANOVA to assess significant differences among samples using the Tukey test ($p < 0.05$). Pearson correlation analysis was performed to evaluate the correlations between parameters. The correlation coefficients (r) and associated p -values were calculated to determine the strength and direction of associations between variables. The data were reported as means $\pm$ standard deviations ($n \geq 3$). Data were analyzed with OriginLab 2023 software (Northampton, Massachusetts, USA).

4.3 Results and discussion

4.3.1 Flour properties

Germination significantly ($p < 0.05$) affected flour colour, α -amylase activity, gluten aggregation, and dough rheological properties of purple wheat flour (Table 4.1). The most noticeable and significant change in the colour parameters was observed in b^* values that rose significantly at 24 h ($p < 0.05$), indicating a shift toward greater yellowness. This change may be attributed to pigment degradation and compositional alterations during sprouting. In this study, noticeable pigment loss was also observed during the soaking and germination process, with residual purple coloration visible on the germination trays, suggesting leaching of anthocyanins from the bran layer into the

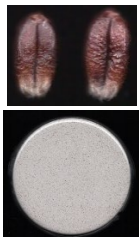
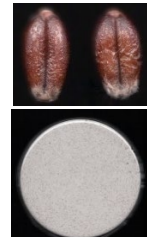
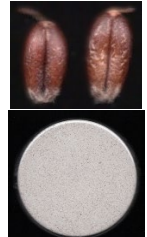
surrounding water. Anthocyanins are water-soluble phenolic pigments, and their molecular structure is vulnerable to nucleophilic attack by water (Francavilla & Joye, 2020). Kernel scanning images further confirmed these visual changes, showing a gradual lightening of kernel colour with increasing germination time. α -Amylase activity increased steadily with germination time, showing a significant ($p<0.05$) rise at 24 hours (0.627 CU/g), which coincided with a sharp decrease in FN from 455 seconds (control) to 75 seconds. The strong inverse relationship ($p<0.0001$, $r=-0.9962$) between α -amylase activity and FN indicates extensive starch hydrolysis, consistent with increased α -amylase production during the later stages of germination, which agrees with previous results observed in CWRS wheat (Kaur et al., 2024).

GlutoPeak analysis revealed that germination significantly ($p<0.05$) impaired gluten aggregation behaviour. Both torque maximum and GSI decreased progressively with germination, showing 32% and 42% reduction at 24 h, respectively (Table 4.1). Therefore, germination substantially weakens the overall aggregation capacity, leading to a looser and less cohesive protein network, which is consistent with the hydrolysis of gluten-forming proteins by the proteases that are triggered during germination (Marti et al., 2018). Grassi et al. (2018) also found that in common wheat, germination led to a decrease in the torque maximum by 14%, showing that germination had a lesser impact on the gluten aggregation in common wheat.

Germination significantly ($p<0.05$) reduced water absorption, development time, and dough stability, with the magnitude of reduction increasing with germination time (Table 4.1). Particularly for 24-hour germinated wheat flour, the water absorption, development time, and stability dropped sharply to $66.4\pm0.6\%$, 3.1 ± 0.3 min, and 2.9 ± 0.2 min compared to the control flour, $69.8\pm0.4\%$, 5.5 ± 0.4 min, and 4.7 ± 0.2 min, respectively. Thus, dough from germinated purple wheat flours developed faster but led to weak gluten with increasing softening after prolonged

mixing. Enhanced α -amylase and protease activities during germination likely degraded starch and gluten proteins, reducing the time needed to reach peak consistency. These results clearly reflected the progressive weakening of the gluten network with increasing germination time, supporting GlutoPeak results. Ding et al. (2018) found that water absorption decreased from 66% and 64% to 61% and 59.5% in germinated hard white wheat and hard red spring wheat, respectively, after 15 h of germination at 28°C and 95% RH. This reduction was due to the enzymatic hydrolysis of large molecules. The partial degradation of glutenin and gliadin likely impaired gluten network formation, resulting in lower dough resistance and reduced stability under mechanical stress (Marti et al., 2015). The MTI was 34.8% higher in the 24 h germinated sample compared to the control, reflecting greater susceptibility to mechanical weakening. Similarly, Kaur et al. (2024) found that MTI increased by 24.3% after 24 h germination of common wheat. Although bandwidth remained unchanged, the overall energy required for dough mixing declined sharply from 8.7 kJ in the control to 4.3 kJ after 24 h germination, confirming a substantial loss in dough strength. These rheological changes may result from proteases disrupting the gluten matrix and from protein depolymerization due to intense protease activity in germinated wheat (Marti et al., 2018). Overall, considering reported values in common wheat, it seems that germination affected the flour functionality of purple wheat to a greater extent compared to common wheat.

Table 4.1 Effect of germination on flour properties and dough rheology

Control	G 6	G 12	G 24
			

Flour colour

<i>L</i> *	72.86±0.01 ^{ab}	72.25±0.23 ^b	73.55±0.86 ^a	73.53±0.42 ^a
<i>a</i> *	3.83±0.01 ^{ab}	3.93±0.07 ^{ab}	4.00±0.08 ^a	3.82±0.11 ^b
<i>b</i> *	5.82±0.01 ^b	5.77±0.06 ^b	5.85±0.11 ^b	6.34±0.26 ^a
α-Amylase activity (CU/g)	0.074±0.005 ^c	0.105±0.008 ^{bc}	0.132±0.011 ^b	0.627±0.026 ^a
FN (s)	455±9 ^a	426±2 ^b	393±14 ^c	75±3 ^d

GlutoPeak parameters

Peak maximum time (s)	32±2 ^a	31±1 ^a	30±1 ^a	30±2 ^a
Torque maximum (BU)	85±1 ^a	80±1 ^{ab}	76±4 ^b	58±3 ^c
GSI (AU)	93±3 ^a	78±6 ^b	73±2 ^b	54±2 ^c

Mixolab parameters

Water absorption (g/100 g)	69.8±0.4 ^a	69.3±0.9 ^a	68.2±0.6 ^a	66.4±0.6 ^b
Development time (min)	5.5±0.4 ^a	4.5±0.2 ^b	3.9±0.3 ^c	3.1±0.3 ^d
Stability (min)	4.7±0.2 ^a	4.0±0.4 ^b	3.7±0.2 ^b	2.9±0.2 ^c
Softening (mNm)	0.25±0.01 ^b	0.24±0.02 ^b	0.27±0.01 ^b	0.42±0.03 ^a
MTI (mNm)	0.23±0.02 ^b	0.20±0.02 ^b	0.22±0.02 ^b	0.31±0.04 ^a
Bandwidth (Nm)	0.21±0.01 ^a	0.21±0.01 ^a	0.21±0.01 ^a	0.21±0.01 ^a
Energy (KJ)	8.7±0.1 ^a	6.5±0.5 ^b	6.1±0.4 ^b	4.3±0.5 ^c

G 6: germinated wheat flour from 6 h germination; G 12: germinated wheat flour from 12 h germination; G 24: germinated wheat flour from 24 h germination. GSI: GlutoPeak strength index; MTI: mixing tolerance index. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

4.3.2 Pasting properties of germinated purple wheat flour

Germination time had a significant ($p < 0.05$) effect on the pasting properties of whole purple wheat flour, as evidenced by the RVA plots (Figure 4.1). The control sample (ungerminated) exhibited the highest apparent peak viscosity (1550 ± 1 cP), final viscosity (2003 ± 2 cP), and setback (961 ± 8 cP), indicating good starch gelatinization and retrogradation potential. In contrast, samples germinated for 6 h and 12 h showed progressively reduced peak viscosities (1411 ± 111 cP and 1204 ± 56 cP, respectively) and final viscosities (1858 ± 124 and 1560 ± 51 cP), suggesting

partial starch hydrolysis due to increased α -amylase activity during early germination. A dramatic shift was observed after 24 h of germination, where the RVA plot showed minimal viscosity development. Peak viscosity plummeted to 150 ± 14 cP, and the final viscosity was only 39 ± 4 cP, indicating extensive enzymatic degradation of starch. Similar trends were observed by Kaur et al. (2024), who reported that the apparent peak viscosity, final viscosity, and setback of whole wheat flour reduced significantly after 12 h of germination. However, compared to common wheat, the reduction in the peak viscosity was more pronounced in the colored wheat, which was 90.32% in 24 h germinated purple wheat, compared to 88.34% in 24 h germinated common wheat. This reduction was attributed to the enzymatic degradation of starch, resulting in the breakdown of starch polymers into smaller fragments, which limits their ability to swell and form a viscous paste during heating (Baranzelli et al., 2019).

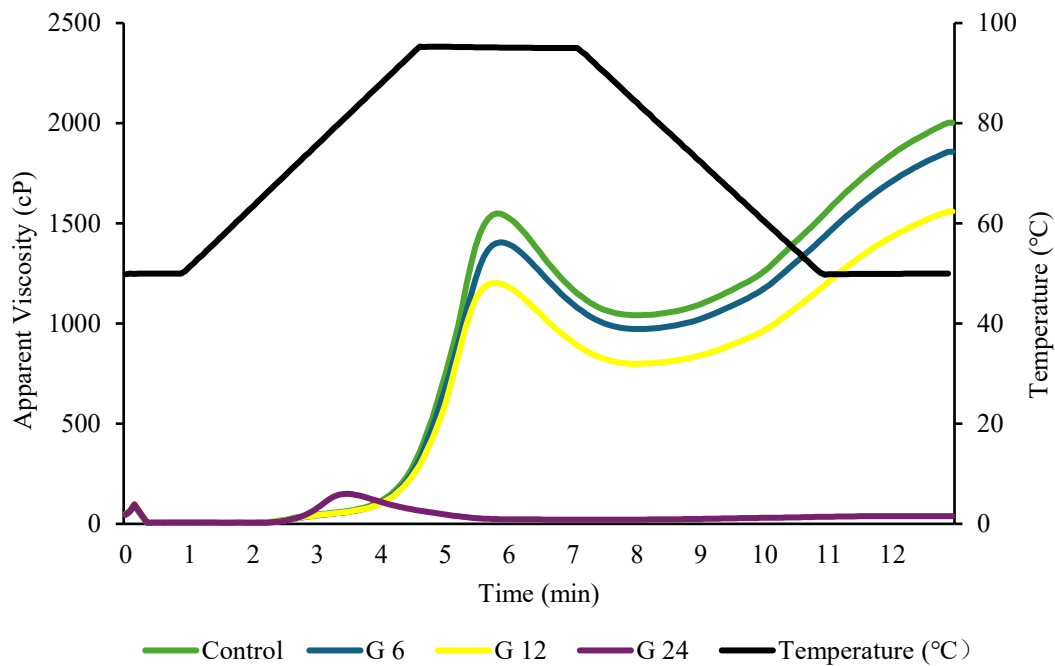


Figure 4.1. RVA plots of purple wheat flour samples under different germination times: 6 hours (G 6), 12 hours (G 12), and 24 hours (G 24).

4.3.3 Dough proofing properties and bread quality of germinated purple wheat

The effect of germination time on dough proofing and bread quality of purple wheat is summarized in Table 4.2. Dough maximum volume decreased significantly ($p < 0.05$) after 6 and 12 hours of germination (95.0 ± 6.4 and 95.0 ± 1.9 mL/100 g, respectively) compared to the control (106.7 ± 0.0 mL/100 g), indicating a reduction in gas-holding capacity, likely due to weakened gluten structure (Boita et al., 2016). However, the volume slightly increased in the 24 h germinated sample (99.2 ± 5.7 mL/100 g), possibly due to higher fermentation activity compensating for weakened dough strength. Fermentation rate increased significantly ($p < 0.05$) with germination time, reaching 1.89 ± 0.13 mL/min in G 24, compared to 1.50 ± 0.00 mL/min in the control. This trend suggests enhanced yeast activity associated with high substrate availability resulting from starch degradation and increased fermentable sugar content during germination. Correspondingly, fermentation time decreased from 45 min in the control to 36 min in G 24, consistent with faster dough expansion.

In the developed breads, porosity and 2D area both significantly ($p < 0.05$) increased in bread made of 24-hour germinated purple wheat flour. The bread crumb area expanded from 8.59 ± 0.07 cm² in the control to 9.93 ± 0.25 cm² in G 24, with a concomitant increase in the crumb porosity. G 24 showed larger and more irregular gas cells, indicating gas bubble coalescence during fermentation or baking, resulting in a looser and more open crumb. The increased α -amylase activity after 24 h, in addition to the activation of the proteases that degrade gluten proteins, weakens the gluten network, diminishing its capacity to maintain the thin walls between gas cells and thus promoting coalescence (Olaerts & Courtin, 2018). Germination is proposed to seek an optimal enzymatic balance point where amylolysis maximizes gas production, while controlled proteolysis reduces the network's stiffness, enhancing its extensibility to accommodate rapid bubble growth.

Nevertheless, beyond this critical threshold, excessive proteolytic activity would lead to dramatic gluten breakdown, causing severe gas loss, reduced loaf volume, and a coarser crumb structure.

Germination significantly ($p<0.05$) affected bread texture (Table 4.2). Hardness remained unchanged from control to G 12 but dropped sharply at G 24, suggesting a much softer crumb due to the thinner walls dividing the gas cells. Springiness, cohesiveness, chewiness, and resilience were stable up to G 12, but all declined markedly in G 24, reflecting reduced elasticity and structural integrity. These textural losses are consistent with progressive hydrolysis of starch and proteins during germination, which alters dough viscoelasticity and ultimately softens the bread texture. These findings agree with Mendoza Moreno et al. (2025), who also reported reduced hardness and springiness in breads made from wheat germinated for 12-48 h; specifically, hardness fell from 24.11 N in the control to 10.80 N after 12 h germination in common wheat, corresponding to a reduction of 55.2%. In contrast, no significant decrease in hardness was observed in 12 h-germinated purple wheat bread.

Colour stands as an immediate determinant of consumer acceptance in bakery products, and in pigmented grains like purple wheat, it is also closely linked to the presence of bioactive compounds such as anthocyanins. In this study, germination significantly ($p<0.05$) affected the colour attributes of both bread crust and crumb (Table 4.2). The crust lightness (L^*) increased progressively from 32.85 ± 0.74 in the control to 34.71 ± 0.52 in G 24. Interestingly, G 6 had a darker crust compared to the control group, likely due to minimal anthocyanin leaching, which may enhance the retention of dark pigments in the pericarp, compared to G 12 and G 24. A similar pattern was observed in the crumb; the L^* value also increased significantly ($p<0.05$) with germination, from 31.70 ± 0.41 in the control to 34.61 ± 0.33 in G 24, indicating a lighter and possibly more uniform internal structure. Crust redness (a^*) increased from 7.30 ± 0.41 in the

control to 10.43 ± 0.28 in G 24, and yellowness (b^*) rose more noticeably, from 4.66 ± 0.40 to 9.63 ± 0.52 . These changes reflect more intense Maillard and caramelization reactions, potentially due to increased reducing sugar levels from starch hydrolysis during germination (F. Wu et al., 2018). Compared to the crust, the crumb showed less pronounced changes in redness but a notable increase in yellowness, which could be influenced by both pigment retention and changes in flour composition during germination. The pronounced increase in all color parameters observed in the G 24 bread, strongly suggests a substantial fading of the purple pigmentation. TAC measurements (Table 4.3) confirm this observation, showing that breads produced from purple wheat germinated for 24 hours retained less than 60% of their original anthocyanins. This phenomenon is largely attributable to the leaching of water-soluble anthocyanins into the soaking water during extended germination, as well as enzymatic degradation during dough mixing, when air incorporation activates polyphenol oxidase and peroxidase (Eliasova et al., 2020). Furthermore, while enzymatic activation in G 24 may release certain bound anthocyanins into more active free forms, these forms are more sensitive to thermal and oxidative degradation during baking (Cavalcanti et al., 2011; L. Yu & Beta, 2015). These trends are consistent with the findings of Koksel et al. (2023), who also reported that purple wheat breads had the lowest L^* values for crust and crumb (40.38 ± 0.89 and 36.80 ± 0.46 , respectively) compared to white wheat bread, due to their higher anthocyanin content.

Table 4.2 Impact of germination on dough proofing and bread parameters

	Control	G 6	G 12	G 24
				
Proofing parameters				
Dough maximum volume (mL /100 g)	106.7 ± 0.0^a	95.0 ± 6.4^b	95.0 ± 1.9^b	99.2 ± 5.7^{ab}

Fermentation rate (mL /min)		1.50±0.00 ^b	1.49±0.15 ^b	1.55±0.04 ^b	1.89±0.13 ^a
Fermentation time (min)		45±0 ^{ab}	49±2 ^a	41±4 ^b	36±0 ^c
Crumb structure					
2D Area (cm ²)		8.59±0.07 ^b	8.65±0.62 ^b	8.68±0.68 ^b	9.93±0.25 ^a
Porosity (%)		24.79 ±0.25 ^b	24.96 ±1.80 ^b	26.71±0.31 ^{ab}	28.65±0.74 ^a
Crumb texture					
Hardness (g)		1932±110 ^a	1961±107 ^a	1899±196 ^a	1002±106 ^b
Springiness		0.94±0.02 ^a	1.01±0.10 ^a	0.94±0.04 ^a	0.82±0.03 ^b
Cohesiveness		0.77±0.01 ^a	0.80±0.04 ^a	0.77±0.03 ^a	0.65±0.02 ^b
Chewiness (g)		1347±134 ^a	1414±78 ^a	1337±138 ^a	526±46 ^b
Resilience		0.42±0.00 ^{ab}	0.44±0.05 ^a	0.41±0.02 ^b	0.27±0.01 ^c
Bread color					
Crust	<i>L</i> *	32.85±0.74 ^b	31.71±0.31 ^c	34.27±0.48 ^a	34.71±0.52 ^a
	<i>a</i> *	7.30±0.41 ^c	8.53±0.21 ^b	10.01±0.56 ^a	10.43±0.28 ^a
	<i>b</i> *	4.66±0.40 ^d	6.30±0.23 ^c	8.24±0.84 ^b	9.63±0.52 ^a
Crumb	<i>L</i> *	31.70±0.41 ^c	33.12±0.20 ^b	34.36±0.17 ^a	34.61±0.33 ^a
	<i>a</i> *	9.37±0.26 ^b	9.33±0.19 ^b	9.54±0.16 ^{ab}	9.91±0.03 ^a
	<i>b</i> *	5.89±0.27 ^d	7.00±0.20 ^c	7.83±0.22 ^b	8.54±0.17 ^a

G 6: germinated wheat flour from 6 h germination; G 12: germinated wheat flour from 12 h germination; G 24: germinated wheat flour from 24 h germination. Different letters within each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

4.3.4 *In vitro* starch digestibility in purple wheat bread

Figure 4. 2 illustrates the *in vitro* starch hydrolysis profiles of breads prepared from purple wheat flour after 0 h (control) or 6, 12, and 24 h of germination. The control and G 6 showed similar rates throughout the digestion and reached the highest equilibrium starch hydrolysis at 180 min. In contrast, G 12 and G 24 breads exhibited faster initial hydrolysis but attained significantly lower final hydrolysis values (~55 g /100 g bread). The kinetic analysis showed that short germination (6 h) does not appreciably alter the starch digestibility of purple wheat bread compared with ungerminated flour, whereas longer germination periods accelerate the initial rate of hydrolysis but reduce the extent of digestion. In breads made from common wheat, germination primarily altered the extent of starch hydrolysis but did not fundamentally change the digestion kinetics. Germination reduced the final hydrolysis levels of common wheat bread, which was attributed to

a reduction in RDS and an increase in RS fractions (Zou et al., 2025). This difference in kinetic behaviour with common wheat suggests that, in purple wheat, germination either induces additional structural or compositional changes rather than a simple reduction in hydrolysable starch or there is restricted enzyme accessibility during digestion. Previous studies have indicated that phenolics and anthocyanins can promote inhibitory effects on α -amylase activity, thereby slowing starch hydrolysis and altering digestion kinetics (Aleixandre et al., 2022; Zhang et al., 2024).

Consistent with this behaviour, breads made with G 12 and G 24 flour contained significantly ($p < 0.05$) higher amounts of RDS and lower values of SDS (Table 4.3). Differences in α -amylase activity encountered in the germinated flours do not fully explain these partners. Although FN values indicated changes in enzyme activity, direct α -amylase assays showed no significant differences between G 6 and G 12. Therefore, the *in vitro* starch hydrolysis behaviour of the purple wheat germinated flours cannot be attributed solely to amylase activity. A more plausible explanation relates to changes in bioactive compounds during germination. The TPC increased markedly with germination, from 4.77 mg /g in the control to 5.95 mg/g in G 12 and 6.34 mg/g in G 24 (Table 4.3), in agreement with previous findings that germination releases phenolics bound to the cell wall matrix, leading to a higher concentration of free forms in common wheat (Baranzelli et al., 2023) and Abyssinian purple wheat (Kassegn et al., 2024). Polyphenols are known to modulate starch digestibility through two primary mechanisms: (i) formation of non-digestible starch–polyphenol V-type inclusion complexes (Type I), and (ii) inhibition of α -amylase activity by polyphenols encapsulated within such complexes (Type II) (Romero Hernández et al. 2022). Literature further supports this interaction, as phenolic-rich sprouted bread (containing 20% germinated common wheat) has been shown to substantially reduce starch digestion in bread

(Świeca et al., 2017), and phenolic acids such as vanillic and syringic acids are likely to interact with α -amylase, exhibiting low half-maximum inhibitory concentration (IC₅₀) values (Aleixandre et al., 2022).

Conversely, TAC decreased during germination, from 101.44 $\mu\text{g/g}$ in the control to 68.24 $\mu\text{g/g}$ and 59.03 $\mu\text{g/g}$ after 12 and 24 h germination, respectively (Table 4.3), but no significant differences were observed between G 12 and G 24. The greatest decrease occurred at the washing and soaking stages, likely due to the transfer of water-soluble anthocyanins to the soaking medium (Kassegn et al. 2024).

Lastly, the RS content (7.03-7.90 g /100 g) did not differ significantly among treatments. However, the RS proportion relative to total starch increased in G 12 and G 24 breads ($11.99 \pm 0.23\%$ and $12.47 \pm 0.56\%$ of the total starch, respectively), compared with the control and G 6 breads ($\sim 10\%$). The significant ($p < 0.05$) increase in RS proportion further indicated that germination may promote the formation of starch–polyphenol complexes, contributing to the reduced digestibility observed in 12 h and 24 h germinated purple wheat samples.

These findings align with previous reports showing that germinated common wheat bread generally exhibits a slower starch hydrolysis and a higher RS proportion (Zou et al., 2025). In contrast, germinated purple wheat bread in the present study showed an increase in RDS, while the overall extent of starch hydrolysis was still reduced. This discrepancy may be attributed to the presence of higher levels of phenolic compounds in purple wheat, which can interact with starch and/or inhibit amylolytic enzymes, thereby limiting starch hydrolysis during digestion.

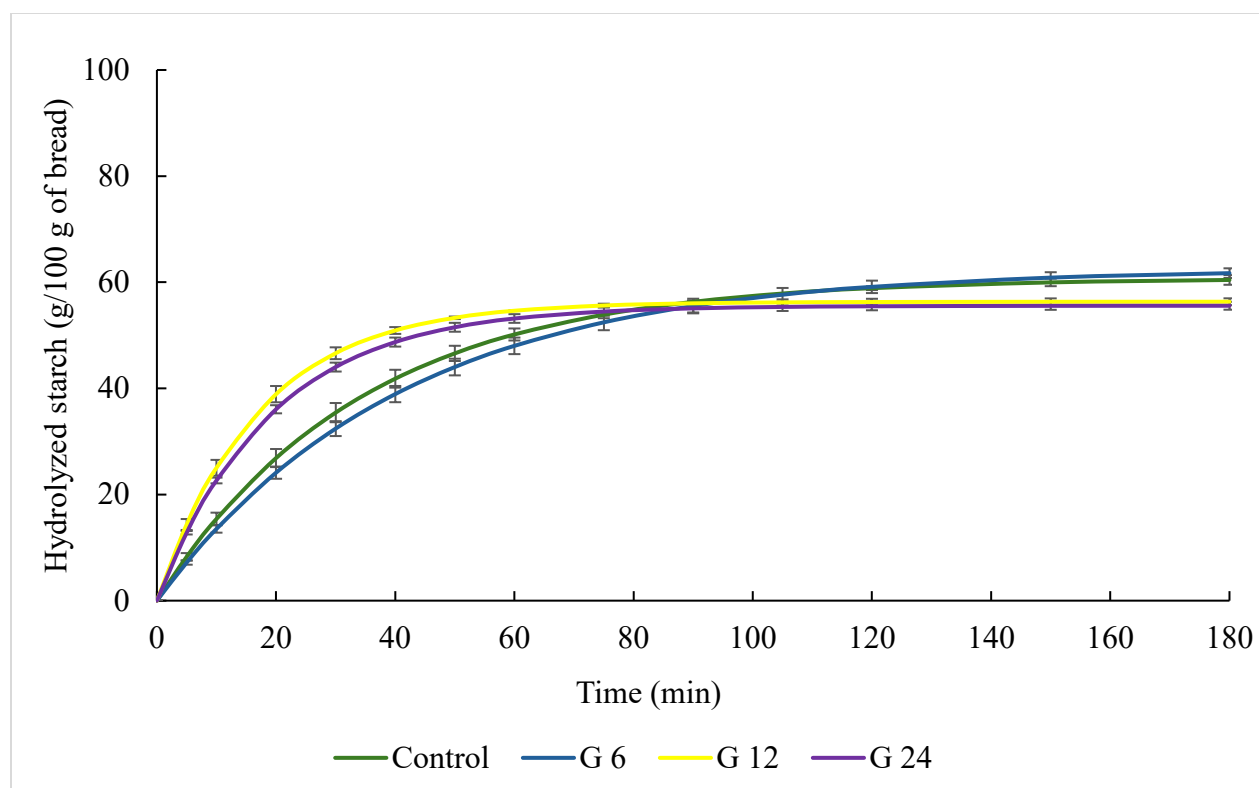


Figure 4.2. *In vitro* starch digestibility in germinated purple wheat bread

G 6: germinated wheat flour from 6 h germination; G 12: germinated wheat flour from 12 h germination; G 24: germinated wheat flour from 24 h germination.

The Pearson correlation analysis showed a strong positive association between TPC and RS proportion respect to total starch ($r=0.861$, $p=0.006$) and a strong negative association between TPC and starch hydrolysis rate at 180 min ($r=-0.918$, $p=0.001$). TAC showed a significant negative correlation to RDS ($r=-0.817$, $p=0.013$) while correlating positively with SDS ($r=0.853$, $p=0.007$). The diverse roles of TPC and TAC in our correlation analysis highlight that the type of polyphenol matters in starch digestion. Polyphenols are a diverse group of compounds, from simple molecules like gallic acid to complex polymers like condensed tannins. Germination enhances both free and bound phenolic acids, along with bound flavonoids, which strengthen the inhibition of α -glucosidase significantly and α -amylase to a lesser extent, particularly regarding the free phenolics

(Gong et al., 2018). This enhanced enzyme inhibition slows starch hydrolysis, contributing to reduced starch digestion during *in vitro* digestion. Conversely, the strong negative correlation between TAC and RDS and the positive correlation with SDS imply that anthocyanins slow starch digestion, enhancing the slowly digestible fraction. These findings align with evidence that anthocyanins reduce bread digestibility via both enzyme inhibition and physical interactions with starch granules (Zhang et al., 2024).

Table 4.3 Starch fractions and polyphenols in germinated purple wheat bread

	Control	G 6	G 12	G 24
RDS (g /100 g bread)	25.94±1.11 ^b	24.12±1.15 ^b	38.91±1.53 ^a	36.06±0.77 ^a
SDS (g /100 g bread)	33.11±1.33 ^a	35.01±0.02 ^a	17.36±2.14 ^b	19.41±0.01 ^b
RS (g /100 g bread)	7.03±0.28 ^a	7.13±0.41 ^a	7.66±0.25 ^a	7.90±0.30 ^a
TPC (mg/g)	4.77±0.04 ^b	4.95±0.27 ^b	5.95±0.04 ^a	6.34±0.06 ^a
TAC (µg/g)	101.44±0.01 ^a	87.74±0.65 ^b	68.24±3.44 ^c	59.03±3.60 ^c

G 6: germinated wheat flour from 6 h germination; G 12: germinated wheat flour from 12 h germination; G 24: germinated wheat flour from 24 h germination. RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. TPC: total phenolic content; TAC: total anthocyanins content. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

4.4 Conclusions

The findings indicate that increasing germination time leads to a gradual loss of pasting ability in purple wheat flour. Short-term germination (6–12 hours) results in moderate changes that still allow for some functionality, whereas extended germination (24 hours) significantly compromises starch integrity, limiting the flour's use in products that depend on viscosity. Gluten aggregation levels gradually decrease from 6 to 24 hours of germination, resulting in diminished dough mixing properties. Additionally, the crust and crumb of the bread exhibited increased lightness and a more reddish-yellow hue with extended germination time, attributable to the loss of water-soluble

anthocyanins. These colour changes were more evident in the crust, likely as a result of surface browning reactions during baking. Moderate germination (6–12 hours) enhances gas production and porosity without severely affecting dough strength, while prolonged germination (24 hours) leads to a softer, more open crumb with reduced mechanical integrity. Furthermore, the findings indicate that adjusting germination time allows for the manipulation of starch digestibility in purple wheat bread. Short germination (6 hours) led to starch hydrolysis similar to that of the control at the initial time point, whereas longer germination (12–24 hours) increased the rapidly digestible fraction but reduced the overall extent of starch hydrolysis. In summary, germinated purple wheat shows strong potential as a functional ingredient when an optimal germination time is applied. This balance not only supports favourable dough handling and bread texture but also results in reduced starch hydrolysis, which may contribute to improved postprandial blood glucose regulation.

Author Contributions

Xiaohang Zou: writing – original draft, investigation, formal analysis, data curation.

Nicola Gasparre: training, writing – review and editing, supervision, and conceptualization.

Cristina M. Rosell: writing – review and editing, conceptualization, supervision, and funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Chapter 5_Experimental Section 3

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From dough development to starch digestibility: effects of phenolic-rich plant powders in wheat bread

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Abstract

The inclusion of phenolic-rich plant powders in wheat bread offers a promising method to modify starch digestibility while preserving technological performance. This study examined how substituting 5 g/100 g of white flour with purple sweet potato (PSP), grape (G), or pomegranate (P) powders affects dough functionality, starch pasting and thermal behaviour, bread quality, and *in vitro* starch digestibility.

Distinct phenolic profiles led to formulation-dependent changes in gluten aggregation and starch properties. While PSP and P accelerated gluten aggregation, they also reduced overall gluten strength. PSP and P systems exhibited greater reductions in peak and final viscosities, along with lower setback values, indicating restricted starch swelling and reduced retrogradation. DSC identified two main thermal transitions (~60°C and ~100°C) in all samples, with PSP displaying

an additional transition at $\sim 130^{\circ}\text{C}$, suggesting the formation of thermally stable starch–phenolic structures. Bread crumb hardness decreased by 13–18% in the substituted samples without compromising crumb structure. *In vitro* starch digestion analysis showed significant ($p < 0.05$) changes in starch hydrolysis. The bread made with P powder exhibited the most substantial inhibitory effect, showing a 19% reduction in C_{∞} , a 43% decrease in k , a 45% reduction in RDS, and a noticeable increase in RS. Pearson correlation analysis confirmed strong associations between total phenolics and the redistribution of starch fractions, as well as hydrolysis kinetics. Overall, phenolic enrichment altered starch digestibility by affecting substrate accessibility and enzymatic efficiency, while maintaining comparable product quality. This study provides valuable insights into the development of functional bakery products.

Keywords: bread quality; phenolic compounds; pomegranate; purple sweet potato; *in vitro* starch digestibility

5.1 Introduction

White bread is a widely consumed staple and a major source of carbohydrates. It has a high starch content, with a significant portion classified as rapidly digestible starch (RDS), which can lead to increased blood sugar levels after consumption. Studies showed that the total starch in fresh white bread crust ranges from 76.8 g/100 g to 80.1 g/100 g, while dietary fibre and resistant starch (RS) comprise only 6.8 g/100 g to 7.2 g/100 g and about 2 g/100 g, respectively (Agama-Acevedo et al., 2019). Likewise, white bread made from common white flour of various cultivars contained approximately 59.0 g/100 g of RDS, 9.5 g/100 g of slowly digestible starch (SDS), and 3.5 g/100 g of RS (Sterbova et al., 2016). This means that most of the starch in white bread is quickly converted to glucose during digestion.

Recently, there has been an increasing emphasis on developing functional bakery goods, particularly by incorporating plant-based ingredients abundant in phenolic compounds, to effectively inhibit starch digestibility and control glucose release (Aleixandre et al., 2022). Feng et al. (2022) found that the addition of 5 g/100 g papaya seeds significantly reduced glucose release during *in vitro* starch digestion compared to control wheat bread, due to insoluble polyphenol and phytochemicals. Incorporating 5 g/100 g-15 g/100 g mushroom powder into breadmaking can slow the hydrolysis rate of starch, likely due to polyphenols inhibiting α -amylase activity and the high dietary fibre content reducing water availability to digestive enzymes through its strong water-binding capacity (Liu et al., 2022).

Phenolic compounds are known to exhibit antioxidant activity and potential health benefits, and accumulating evidence suggests that they may also modulate starch digestibility (Kwasny et al., 2022; Świeca et al., 2017). Several mechanisms have been proposed, including direct inhibition of digestive enzymes and the formation of starch-phenolic complexes that limit enzymatic accessibility (Romero Hernández et al., 2022). Amylose, because of its linear structure and capability to form single helices, is particularly susceptible to this complexation. This can lead to the formation of structures that functionally resemble RS. For instance, a complex made from corn starch and a medicinal plant (*Amphipterygium adstringent*) exhibited a 14.4% increase in RS content compared to corn starch alone (Moreno-Zaragoza et al., 2025). Additional insights were provided when vegetable and fruit powders were added to corn flour for flatbread production. Authors reported that flatbreads with varying concentrations (2, 5, 10 g/100 g) of artichoke and 10 g/100 g lemon powder reduced *in vitro* enzymatic hydrolysis of starch due to phenolic interactions with digestive α -amylase compared to the control (Santamaria et al., 2025).

Plant powders such as purple sweet potato (PSP), grape (G), and pomegranate (P) are characterized by a distinct phenolic profile. PSPs have a vibrant purple-red colour, high levels of anthocyanins, and strong antioxidant properties, with anthocyanin content significantly higher than that of orange-fleshed sweet potatoes (Li et al., 2019). G serves as an important source of phenolic compounds, which are predominantly located in the seeds (60-70%), skins (28-35%), and stems, while the pulp has less than 10%. Seeds are abundant in flavan-3-ols and phenolic acids, skins primarily hold flavanols and anthocyanins, whereas stems contain a high concentration of stilbenes and procyanidins (Milincic et al., 2025). The biological function of P is partially related to the presence of phenolic compounds, particularly gallic acid, ellagic acid, hydrolysable and condensed tannins, along with anthocyanins, which are powerful antioxidants (Oliveira et al., 2020).

While these materials have been individually studied as functional ingredients, most previous research has either focused on flour properties (Gul & Sen, 2017; Paixao e Silva et al., 2021) or product quality (Agirbas et al., 2021; Liu et al., 2020), with very little emphasis on their effect on bread starch digestibility (Rocchetti et al., 2021). Therefore, the objective of this study was to systematically investigate the effects of incorporating three phenolic-rich plant powders (PSP, G, and P) at a 5 g/100 g substitution level on starch pasting and thermal behaviour, dough development, bread quality, and *in vitro* starch digestibility. Special emphasis was placed on understanding how phenolics modulate starch accessibility and enzymatic hydrolysis in wheat bread, thereby providing mechanistic insights that can help in the design of phenolic-enriched bakery products while maintaining the product functionality.

5.2 Materials and Methods

5.2.1 Materials

Organic white flour was kindly provided by Prairie Flour Mills Ltd. (Elie, Manitoba, Canada). The crude protein content was 15.91 g/100 g, and the ash content was 0.70 g/100 g (Tomassini et al., 2026). Although the total starch content was not quantified, based on a previous study, the total starch content was 65-75% of white flour (Shewry, 2009).

PSP powder was purchased from Jiangsu Cuigu Food Co., Ltd. (Taizhou, Jiangsu, China) and characterized by a carbohydrate content of 84 g/100 g and a protein content of 5.9 g/100 g, as shown in the nutrition facts label by the manufacturer. G powder was purchased from Guangzhou Zhiyu Trading Company Ltd. (Guangzhou, Guangdong, China), which contained 95 g/100 g carbohydrates and 0.5 g/100 g protein, according to the manufacturer's label. P powder was obtained from SURAJR, Loblaw's Inc. (Toronto, ON, Canada), containing approximately 90 g/100 g carbohydrates and 2 g/100 g protein. Instant dry yeast was donated by Lallemand Inc. (Montreal, QC, Canada). Salt was acquired in the local market. Porcine pancreatic α -amylase (9.9 U/mg) and amyloglucosidase from *Aspergillus niger* (~120 U/mg) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade.

5.2.2 Flour and powder characterization

White flour and powders were characterized by measuring moisture content, total dietary fibre, and colour. Moisture and total dietary fibre were measured according to the AACCI-approved methods 44-40.01 and 32-07.01, respectively (AACC International, 2009). Colour measurements were conducted using a Chroma Meter CR-410 (Konica Minolta, Tokyo, Japan) to determine the L^* , a^* , and b^* values of the flour and powders. The L^* value indicates lightness, while a^* and b^* represent colour coordinates ranging from green to red and from blue to yellow, respectively.

5.2.3 TPC

Phenolic compounds from white flour and powders were extracted using an MAE method following Kirusnaruban et al. (2025). TPC was determined according to Kirusnaruban et al. (2025) with slight modifications. The detailed procedure was described in 4.2.9.

5.2.4 TFC

TFC was determined following Sari et al. (2023) with slight modifications. Briefly, 20 mg of white flour or powder samples were extracted with 1 mL of cold 80% (v/v) ethanol and incubated for 10 min at 25°C with constant agitation at 250 rpm. The extracts were centrifuged at $3,000 \times g$ for 10 min at 4°C, and the supernatants were collected for analysis.

For TFC quantification, 50 μ L of sample extract was mixed with 100 μ L methanol in a 96-well microplate. After the addition of 30 μ L of 10% (w/v) aluminum chloride solution, the plate was gently mixed and incubated for 3 min. Subsequently, 30 μ L of 1 M sodium acetate solution and 60 μ L methanol were added. The reaction mixtures were incubated in the dark at room temperature for 40 min, and absorbance was measured at 430 nm using a microplate spectrophotometer (BioTek Epoch 2, Santa Clara, USA). A calibration curve was prepared using quercetin standards (0-200 mg/L) under the same conditions. TFC was expressed as mg QE per L of solution.

5.2.5 TAC

Anthocyanins were extracted according to Jiang et al. (2024) with slight modifications. TAC was determined using the pH differential method following the AOAC Method 2005.02 (Lee et al., 2005). The detailed procedure and calculation equation were described in 4.2.10.

5.2.6 Gluten aggregation behaviour

The gluten aggregation properties of the mixture of white flour and powders were measured using the GlutoPeak instrument (Brabender, Duisburg, Germany). For each test, 9 g of sample was mixed

with 9 g of distilled water in a stainless-steel cup at a constant mixing speed of 1,900 rpm (Marti et al., 2015). Five types of samples were prepared. Pure white flour (9 g) served as the control. To account for the dilution effect caused by powder substitution, a diluted white flour sample consisting of 95 g/100 g white flour (8.55 g), marked as DC, was also tested, while maintaining the same water addition. In addition, three composite samples were prepared by replacing 5 g/100 g of white flour with different powders, including PSP powder, G powder, and P powder, resulting in mixtures containing 95 g/100 g white flour and 5 g/100 g powder (w/w). All samples were standardized to a 14% moisture basis.

The test temperature was kept at 30°C using water from an external bath. GlutoPeak parameters calculated included: (a) maximum torque, the highest resistance during gluten aggregation (in Brabender units, BU); and (b) peak maximum time, the time to reach maximum torque (s); and (c) GSI, calculated using the formula: $GSI = (\text{maximum torque} \times \text{peak area}) / 1,000$, according to Wang et al. (2017). All GlutoPeak tests were conducted at least in duplicate for each batch.

5.2.7 Pasting properties

The standard method from the AACCI Method 76-21.01 (AACC International, 2009) was used to assess the pasting profile of the five groups of samples mentioned in section 2.6, using an RVA (RVA-4800, PerkinElmer, Shelton, CT, USA). Gels obtained after RVA analysis were cooled to room temperature. Two aliquots of each gel were vacuum-dried overnight at room temperature using a Vacufuge Plus (Eppendorf, Hamburg, Germany) operated at 250 rpm in V-AQ (vacuum-aqueous) mode. The dried gels were subsequently milled into powders for further analysis.

5.2.8 Thermal properties

Thermal properties were determined using DSC according to Kong et al. (2018) with modifications. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy

(ΔH) were measured using a DSC Q200 (TA Instruments, New Castle, DE, USA) equipped with high-volume aluminum pans. Approximately 7 mg of gel powder was mixed with 21 mg of distilled water (1:3, w/w), hermetically sealed, and equilibrated overnight at room temperature. An empty sealed pan was used as the reference. Samples were equilibrated at 20°C and heated to 130°C at a rate of 5°C/min. Data were analyzed using TA Universal Analysis software (Universal Analysis 2000 v4.2E, TA Instruments).

One point that should be emphasized is that the DC used in the DSC analysis was prepared by weighing 6.65 mg (95% of the standard 7.00 mg sample mass) of dried RVA gel powder obtained from the 100% white flour sample and mixing it with 21.0 mg of distilled water. This approach was adopted to enable comparison of the thermal performance of diluted starch with that of the other sample groups under standardized hydration conditions. It should be noted that this preparation differs from the DC used in RVA measurements, in which dilution was applied at the flour formulation level prior to pasting.

5.2.9 Mixing properties

The mixing properties of flour–powder blends were analyzed using a Mixolab (Chopin Technologies, Villeneuve-la-Garenne, France), which records dough torque (Nm) in real time during mixing and heating. The samples analyzed included 100% white flour and white flour partially substituted (5 g/100 g, w/w) with PSP, G, or P powders. Dough samples (75 g, 14% moisture basis) were prepared, and water was added to reach an optimal consistency of 1.1 Nm. Mixing was performed at 80 rpm and 30°C for 30 min. The parameters recorded included water absorption, dough development time, stability, softening, MTI, bandwidth, and energy.

5.2.10 Breadmaking

Bread was prepared using the same flour formulations as those used in the Mixolab analysis. The breadmaking formula consisted of 100 g of flour or flour-powder mixture (14% moisture basis), 1.5 g salt, 1.0 g instant dry yeast, and the optimal amount of water determined by Mixolab. Ingredients were mixed using an Artisan mini tilt-head stand mixer (KitchenAid, St. Joseph, Michigan, USA) for the optimal mixing time indicated by Mixolab.

The dough was divided into 50 g portions, hand-shaped, and placed in silicone baking trays for proofing at 30°C and 85% relative humidity. Dough volume development during proofing was monitored by placing 3 g of dough in a graduated cylinder, as described by Martins et al. (2022). Baking was initiated when the dough reached 75% of its maximum proofing volume, and loaves were baked at 180°C for 15 min. After baking, bread was cooled at room temperature for 1 h and stored in polyethylene pouches prior to analysis. Two independent batches were prepared for each formulation.

5.2.11 Bread quality measurement

The 2D area and crumb structure of bread slices were analyzed following the method of Espinosa-Ramírez et al. (2018) with modifications. The detailed procedure was described in 4.2.7. Crust and crumb colour were measured as described in Section 5.2.2.

5.2.12 *In vitro* starch digestibility

The starch hydrolysis kinetics of bread were determined following the method of Santamaria et al. (2021) using porcine pancreatic α -amylase (0.9 U/mL) and amyloglucosidase (214.5 U/mL). The detailed procedure is described in 4.2.8.

5.2.13 Phenolic compounds in bread

TPC, TFC, and TAC measurements were performed for the freeze-dried bread as previously described in sections 5.2.3-5.2.5.

5.2.14 Statistical analysis

All analyses were conducted in two independent batches, and results are presented as means $\pm$ standard deviations ($n \geq 3$). Statistical differences among samples were evaluated using one-way ANOVA, followed by Tukey's HSD post hoc test at a significance level of $p < 0.05$. Pearson correlation analysis was applied to examine relationships between variables, with correlation coefficients (r) and corresponding p -values calculated to assess the strength and direction of associations. All statistical analyses were performed using OriginLab 2023 software (Northampton, Massachusetts, USA).

5.3 Results and discussion

5.3.1 Characteristics of raw flour and phenolic-rich powders

Compared with white flour, the three phenolic-rich powders exhibited markedly different physicochemical and phenolic-related characteristics (Table 5.1). White flour showed the highest moisture content and lightness, whereas PSP powder, G powder, and P powder displayed significantly ($p < 0.05$) lower lightness and distinct chromatic profiles, reflecting their different pigment compositions. In particular, PSP showed high a^* and negative b^* values, which indicated a pronounced red-blue colour, while G and P powders exhibited comparatively lighter red hues.

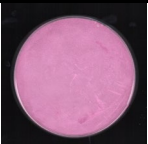
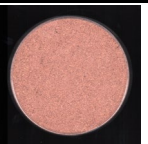
The phenolic-related results highlighted the compositional diversity among the powders. White flour contained negligible amounts of phenolic compounds. In contrast, all three powders showed significantly higher ($p < 0.05$) TPC, although the distribution of TFC and TAC varied substantially. PSP was noted for having a significantly higher ($p < 0.05$) TAC compared to G and P powders,

which aligns with the presence of anthocyanins contributing to its colour. Similarly, Wang et al. (2022) found a high amount of TAC, which was 2.27 mg/g in dried PSP powder. While Surek and Nilufer-Erdil (2014) demonstrated that the TAC of the whole P was 144.7 ± 28.1 mg / 100 g.

Pearson correlation analysis showed significant relationships between phenolic compounds and powder colour parameters. TPC was negatively correlated with lightness ($r = -0.724$, $p < 0.05$), and TFC showed a strong positive correlation with redness ($r = 0.755$, $p < 0.05$) and a strong negative correlation with L^* ($r = -0.920$, $p < 0.01$). In addition, TAC exhibited an extremely strong positive correlation with a^* ($r = 0.979$, $p < 0.001$). These results indicate that phenolic compounds, particularly anthocyanins, contribute to darker and red-purple colouration in the samples. These differences suggest that the powders cannot be differentiated solely based on the TPC; instead, they represent phenolic systems with distinct compositions and varying proportions of phenolic subclasses. The relation TPC: TFC: TAC for PSP, G, and P, being 2: 1: 400, 9: 1: 47, and 7: 1: 50, respectively, showed a closer profile between G and P than PSP.

Most previous studies found that a 5 g/100 g substitution of various plant powders, such as papaya, mushroom, and strawberry, was more effective in controlling starch digestion than lower amounts and comparable to 10 g/100 g (Feng et al., 2022; Liu et al., 2022; Mu et al., 2021). Therefore, for this study, we decided to use a 5 g/100 g substitution of white flour with the previously described plant-based powders.

Table 5.1 Characteristics of raw flour and powders

	Control	PSP	G	P
				
Moisture (g/100 g)	15.65 ± 0.12^a	8.58 ± 0.18^b	5.63 ± 0.08^c	8.63 ± 0.30^b

Total dietary fibre (g/100 g)		2.05±0.09 ^c	8.70±0.30 ^b	0.99±0.08 ^c	24.45±0.76 ^a
	<i>L</i> *	94.38±1.01 ^a	37.89±0.57 ^d	69.12±0.66 ^b	44.13±0.35 ^c
Flour colour	<i>a</i> *	-1.31±0.11 ^d	30.02±0.14 ^a	20.63±0.03 ^b	18.99±0.23 ^c
	<i>b</i> *	14.33±0.35 ^a	-7.77±0.01 ^c	-0.85±0.01 ^b	14.57±0.18 ^a
TPC (mg/g)		0.19±0.01 ^d	7.40±0.24 ^b	4.06±0.21 ^c	19.98±0.60 ^a
TFC (mg/g)		0.18±0.00 ^c	4.46±0.31 ^a	0.46±0.03 ^c	3.00±0.12 ^b
TAC (µg/g)		nd	1784.05±13.56 ^a	21.68±1.88 ^c	149.17±5.49 ^b

Control: white flour; PSP: purple sweet potato powder; G: grape powder; P: pomegranate powder. nd: not detected. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

5.3.2 Functional and thermal properties of dough

GlutoPeak results, shown in Figure 5.1 and Table 5.2, indicated significant differences in gluten aggregation behaviour among the samples.

The control wheat dough showed the typical aggregation plot, with the aggregation peak maximum time at 70 s. For better assessment of the plant-based powders' impact on the gluten aggregation, a diluted control (DC) sample containing the same amount of wheat as in the blended ones was tested. While the DC sample showed a significantly ($p < 0.05$) delayed aggregation time of 107 s, representing an increase of 53%. In contrast, the PSP, G and P samples exhibited significantly shorter peak maximum time values of 40 s, 89s and 49 s, respectively, indicating faster aggregation in the presence of the phenolic-rich powders. The shorter aggregation times suggest a faster formation of the gluten network, likely due to the antioxidant capacity of the phenolic compounds. Previous studies have suggested that polyphenols may interact with gluten proteins through hydrophobic interactions, hydrogen bonding, and potential thiol–disulfide exchange reactions under mixing conditions. Such interactions may involve the disruption and rearrangement of intra- and intermolecular disulfide bonds, which could contribute to modifications in gluten aggregation behaviour and the faster aggregation observed in some formulations (Girard & Awika, 2020). In

fact, significant correlations were observed between phenolic compounds and peak maximum time. Both TPC and TFC were negatively correlated with peak maximum time ($r=-0.651$, $p=0.0414$; $r=-0.865$, $p=0.0012$). The maximum torque exhibited a different pattern. PSP and P showed the highest torque values at 80 and 83 BU, but all the plant powders led to significantly higher torque than the DC. The increased torques suggest a stiffer, yet less extensible gluten structure. This change may be due to strong hydrophobic and hydrogen bond interactions between gluten and polyphenols. In fact, both TPC and TFC were positively correlated with torque maximum ($r=0.721$, $p=0.0186$; $r=0.789$, $p=0.0067$). These hydrophobic interactions may contribute to modifications in gluten network organization reported in previous studies (Jiang et al., 2023).

The GSI further differentiated the samples. The control sample had the highest GSI at 237, reflecting balanced aggregation and network strength, which was reduced in DC. In contrast, PSP induced the largest decrease of GSI, followed by P. Again, a correlation was established with the phenolic compounds; specifically, TFC and TAC were negatively correlated with GSI ($r=-0.918$, $p<0.001$; $r=-0.834$, $p=.039$). The lower GSI values suggest a weakened overall development of the gluten network, despite the higher instantaneous torque observed in some samples. This may result from plant powder-induced hydrophobic and hydrogen bond interactions with gluten, which increased dough rigidity. As an arithmetic indicator of gluten strength, GSI showed a stronger correlation with dough strength than peak time or peak area, suggesting a better reflection of the intrinsic strength of flour, which is less affected by hydration capacity (Wang et al., 2017).

Researchers also reported that the incorporation of 10% P peel powder resulted in a 78% reduction in flour strength. This decrease was attributed to the disruption of the gluten network by dietary fibres in P peel. The added fibres diluted the gluten-forming proteins and interfered with their

proper hydration and alignment, thereby slowing gluten network development and ultimately weakening overall gluten strength (Garcia et al., 2023).

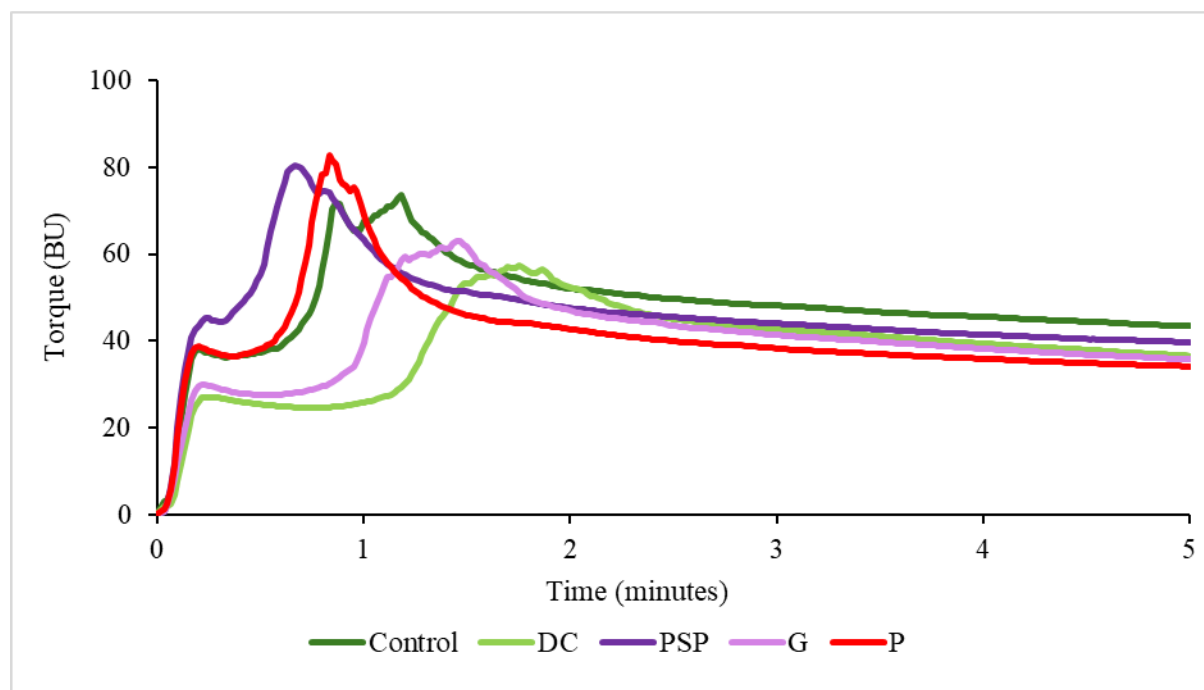


Figure 5.1. GlutoPeak plots of flour samples

Control: white flour; DC: diluted control; PSP: 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: 95 g/100 g of white flour and 5 g/100 g of grape powder; P: 95 g/100 g of white flour and 5 g/100 g of pomegranate powder.

Table 5.2 Gluten aggregation behaviour, pasting, and thermal properties

	Control	DC	PSP	G	P
<i>GlutoPeak parameters</i>					
Peak maximum time (s)	70±1 ^c	107±7 ^a	40±0 ^d	89±2 ^b	49±2 ^d
Torque maximum (BU)	74±1 ^b	59±1 ^d	80±0 ^a	64±1 ^c	83±1 ^a
GSI (AU)	237±0 ^a	202±5 ^b	150±0 ^d	207±5 ^b	173±1 ^c
<i>Pasting properties</i>					
Apparent peak viscosity (cP)	2533±38 ^a	2388±52 ^{ab}	2139±16 ^c	2185±88 ^{bc}	2018±65 ^c
Trough (cP)	1300±95 ^a	1239±62 ^{ab}	1115±65 ^{ab}	1133±51 ^{ab}	1010±67 ^b

Breakdown (cP)		1333±57 ^a	1199±61 ^{ab}	1024±81 ^b	1052±33 ^b	1009±2 ^b
Final viscosity (cP)		2505±92 ^a	2259±66 ^{ab}	2051±11 ^{bc}	2053±16 ^{bc}	1890±91 ^c
Setback (cP)		1205±3 ^a	1021±5 ^b	936±54 ^{bc}	970±35 ^{bc}	881±23 ^c
Peak time (min)		5.7±0.2 ^a	6.0±0.1 ^a	6.0±0.2 ^a	6.1±0.0 ^a	6.0±0.1 ^a
Pasting temperature (°C)		86.4±0.0 ^b	86.8±0.6 ^{ab}	88.9±1.2 ^a	88.0±0.1 ^{ab}	88.8±0.0 ^a
<i>Thermal properties</i>						
Peak 1	T _o (°C)	62.2±0.1 ^a	62.4±0.2 ^a	62.3±0.1 ^a	62.6±0.1 ^a	62.2±0.0 ^a
	T _p (°C)	65.3±0.0 ^a	65.4±0.1 ^a	65.4±0.1 ^a	65.3±0.0 ^a	65.4±0.1 ^a
	T _c (°C)	71.0±0.1 ^a	70.9±0.4 ^a	71.4±0.1 ^a	70.9±1.0 ^a	71.3±0.5 ^a
	ΔH (J/g)	0.21±0.02 ^a	0.19±0.01 ^b	0.16±0.01 ^c	0.18±0.01 ^b	0.16±0.01 ^c
Peak2	T _o (°C)	92.2±1.0 ^{ab}	91.2±0.6 ^b	94.8±0.8 ^a	92.9±0.8 ^{ab}	94.9±0.1 ^a
	T _p (°C)	98.7±0.2 ^a	98.6±0.5 ^a	99.8±1.0 ^a	99.1±0.7 ^a	99.7±0.3 ^a
	T _c (°C)	103.8±0.2 ^{ab}	98.6±0.5 ^b	105.6±0.5 ^a	103.84±0.2 ^{ab}	105.5±0.3 ^a
	ΔH (J/g)	0.19±0.01 ^b	0.19±0.00 ^b	0.19±0.01 ^b	0.18±0.01 ^b	0.23±0.01 ^a
Peak 3	T _o (°C)	nd	nd	130.2±1.2	nd	nd
	T _p (°C)	nd	nd	130.5±0.9	nd	nd
	T _c (°C)	nd	nd	134.6±0.1	nd	nd
	ΔH (J/g)	nd	nd	0.26±0.02	nd	nd

Control: white flour; PSP: 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: 95 g/100 g of white flour and 5 g/100 g of grape powder; P: 95 g/100 g of white flour and 5 g/100 g of pomegranate powder. GSI: GlutoPeak strength index; To: onset temperature; Tp: peak temperature; Tc: conclusion temperature; ΔH: enthalpy; nd: not detected. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

The RVA profiles of the flour samples are presented in Figure 5.2 and summarized in Table 5.2.

The RVA results indicated a consistent reduction, beyond the expected one, due to the flour dilution.

The diluted control was tested to assess the dilution impact when 5 g/100 g of wheat flour was replaced by plant powders. The wheat flour dilution resulted in a reduction of 5.7%, compared to the control. Among the tested blends, the P showed the lowest peak viscosity at 2,018 cP, followed by PSP and G. The reduction in peak viscosity exceeded that anticipated from starch dilution alone, indicating additional contributions from phenolic compounds present in the added powders. As the degree of swelling increases, more phenolics permeate the granules, enhancing the formation

of non-inclusion complexes. Once gelatinization is finished, the majority of phenolic compounds can form complexes with amylose or branched amylopectin, resulting in either inclusion or non-inclusion complexes (Wang et al., 2023). Furthermore, dietary fibre influences starch gelatinization by competing for water, forming a coating around starch granules that restricts swelling and amylose leaching, and interacting with starch to alter viscosity (Zhuang et al., 2024). Trough viscosity and breakdown barely decreased due to the dilution of the wheat flour, but no further reduction was observed due to the blends' inclusion. The final viscosity decreased from 2505 cP in the control sample to 2259 cP due to the dilution of the starch. Further reductions to 2051, 2053, and 1890 cP in the PSP, G, and P samples suggested the impact of the phenolic compounds. Similarly, Mu et al. (2021) also observed that 15 g/100 g strawberry powder significantly reduced the final viscosity, which was explained based on a competition of the phenolic compounds with starch for water. However, the present results suggest the interaction of phenolic compounds with starch chains. Moreover, dietary fibre may influence starch retrogradation by limiting water mobility and interacting with starch molecules to slow retrogradation. Thermodynamic incompatibility between starch and dietary fibre induces phase separation, promoting starch self-association during retrogradation (Zhuang et al., 2024). Compared to DC, setback viscosity is significantly reduced with the addition of the P sample, which indicates reduced short-term retrogradation tendency. The interaction between starch and polyphenols disrupts starch chains and inhibits the aggregation of starch molecules, decreasing relative crystallinity and increasing water-holding capacity, thereby suppressing retrogradation (Zeng et al., 2022).

The relationship between pasting behaviour and phenolics, showing that TPC was significantly negatively correlated with apparent peak viscosity ($r=-0.8337$, $p=0.0027$), trough ($r=-0.8258$,

$p=0.0033$), breakdown ($r=-0.6853$, $p=0.0288$), final viscosity ($r=-0.7942$, $p=0.0061$), and setback ($r=-0.7318$, $p=0.0161$), while positively correlated with pasting temperature ($r=0.7353$, $p=0.0154$). Similarly, TFC was significantly moderately correlated with apparent peak viscosity ($r=-0.7084$, $p=0.0218$), breakdown ($r=-0.6805$, $p=0.0303$), and setback ($r=-0.6501$, $p=0.0419$), and strongly correlated with pasting temperature ($r=0.8167$, $p=0.0039$). No significant correlations were observed between TAC and any RVA parameters. These correlations suggest that TPC- and TFC-rich systems may play key roles in reducing starch swelling and viscosity development, whereas TAC has a limited impact on pasting behaviour.

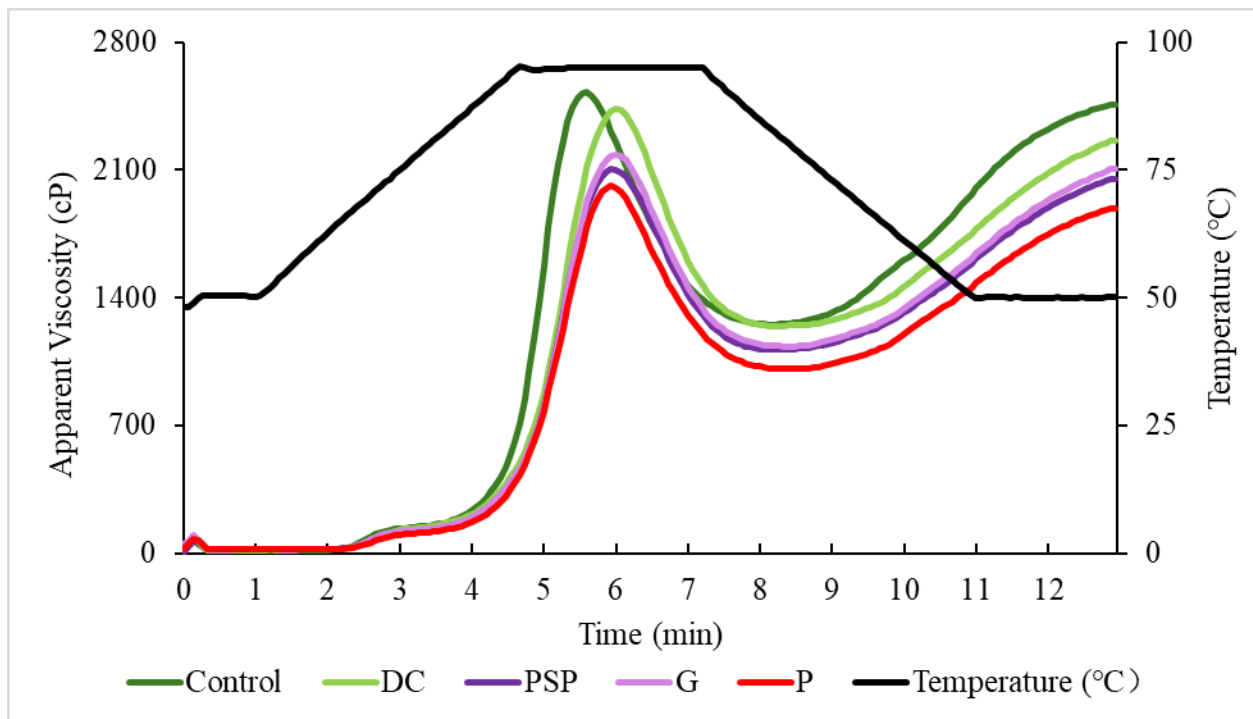


Figure 5.2. RVA plots of flour samples

Control: white flour; DC: diluted control; PSP: 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: 95 g/100 g of white flour and 5 g/100 g of grape powder; P: 95 g/100 g of white flour and 5 g/100 g of pomegranate powder.

To determine the possible formation of amylose-phenolic compound complexes, the thermal transitions of the dried RVA gels were summarized in Table 5.2. Two main endothermic transitions were observed in all samples at approximately 60°C (Peak 1) and 100°C (Peak 2).

For Peak 1, no significant differences were found in T_o , T_p , or T_c . The enthalpy varied significantly among the treatments ($p < 0.05$). DC exhibited lower enthalpy than the control, which was expected due to the starch dilution. Compared to the DC sample, PSP and P gels exhibited the lowest enthalpy values. This may be attributed to the interference of polyphenols with the starch-water matrix during residual gelatinization. Due to their high solubility, polyphenols readily interact with starch, partially disrupting the native crystalline structure during heating, which reduces the energy required to melt the remaining ordered regions (Han et al., 2020).

In fact, both TPC and TFC were significantly negatively correlated with Peak 1 ΔH ($r=-0.7821$ and -0.8213 , respectively; $p=0.0075$ and 0.0036), indicating disruption of native starch crystalline structures.

For Peak 2, the P sample displayed a significantly ($p<0.05$) higher ΔH compared to all other samples, while the other treatments did not show any differences. Compared to DC, PSP and P displayed significantly higher ($p<0.05$) T_o and T_c , whereas T_p remained unchanged. This implies a broader melting temperature range and the presence of more thermally resistant components in the PSP and P systems. It must be stressed that TPC positively correlated with Peak 2 ΔH ($r=0.8446$, $p=0.0021$).

Moreno-Zaragoza et al. (2025) also found that the incorporation of gallic acid or *Amphipterygium adstringens* with high-amylose maize starch increased enthalpy values compared with the control, suggesting that greater energy was needed to disrupt the structure due to the formation of polyphenol-amylose complexes stabilized by hydrophobic interactions.

Additionally, a peak observed at $\sim 130^{\circ}\text{C}$ was detected only in the PSP sample, indicating the formation of highly stable structures. PSP showed the highest content in anthocyanins, which could be responsible for that peak. Nevertheless, anthocyanins interact with amylose through weak, non-covalent associations rather than forming true inclusion complexes (Zhang et al., 2024). Two types of starch-polyphenol complexes have been reported. The V_{I} -type complex is characterized by a single-helical structure, whereas the V_{II} -type complex exhibits a lamellar structure. DSC analysis shows that V_{I} -type complexes typically have melting temperatures between 95 and 105°C , while V_{II} -type complexes display higher melting temperatures ranging from 115 to 130°C (Romero Hernández et al., 2022). Similarly, Kan et al. (2022) studied wheat starch mixed with $5\text{ g}/100\text{ g}$ tannic acid, which identified three endothermic transitions: one at approximately 60°C with ΔH of 12 J/g , associated with the melting of crystalline amylopectin structures; another at 100°C with ΔH of 0.61 J/g , linked to the melting of the amylose-lipid complex; and a third at 150°C with ΔH of 0.89 J/g , associated with the formation of an amylose-tannin complex. In our study, the first two transitions occurred at similar temperature ranges ($\sim 60^{\circ}\text{C}$ and $\sim 100^{\circ}\text{C}$) but exhibited significantly lower enthalpy values. This is consistent with the DSC analysis conducted on dried gels obtained after RVA treatment, as most crystalline regions had already undergone gelatinization during the RVA processing. Additionally, we observed an extra transition at approximately 130°C in the PSP sample, which differs from the earlier report of 150°C . This discrepancy may be due to the different types of phenolic compounds present in plant powders compared to pure tannic acid, resulting in the formation of starch-phenolic structures with varying thermal stability. Furthermore, TAC exhibited a strongly positive correlation with Peak 3 ΔH ($r=0.9979$, $p<0.0001$), suggesting anthocyanins' dominant contribution to the formation of these thermally stable starch-phenolic complexes.

5.3.3 Dough mixing and proofing properties

The incorporation of different plant powders at a 5 g/100 g substitution level significantly ($p<0.05$) affected dough mixing and proofing behaviours, as reflected in Table 5.3. Compared with the control, water absorption increased significantly ($p<0.05$) in the PSP sample and P sample, whereas it decreased markedly ($p<0.05$) in the G formulation.

Development time was significantly prolonged in P (10.1 min), representing a 28% increase relative to the control (7.9 min), indicating delayed gluten network formation. In contrast, PSP showed a substantially shorter development time (5.1 min), suggesting rapid dough development. These results were consistent with the low GlutoPeak peak maximum time and GSI, although the correspondence was not observed with P. In general, a shorter peak maximum time indicates faster gluten aggregation and network formation, which reduces the time required to reach optimum dough consistency in the Mixolab test. However, sample P exhibited a longer dough development time than PSP despite its rapid initial aggregation. This apparent discrepancy may be partially related to the lower pH of the P dough (4.95 after proofing), which could influence gluten hydration and dough stabilization during prolonged mixing. In contrast, the GlutoPeak test mainly reflects early aggregation behaviour under high shear conditions. Therefore, the two measurements may capture different stages of gluten development within the dough system. These differences also imply distinct interactions between flour proteins and powder constituents, particularly polyphenols and organic acids. Regarding dough stability, softening, and MTI, G exhibited comparable stability to the control, while PSP and P showed lower stability and higher softening and MTI. These results indicate that gluten weakening caused by PSP and P may be due to strong polyphenol-protein interactions. Meral and Doğan (2013) found that adding 5 g/100 g G seed to wheat flour did not affect water absorption, but it increased the dough development time and

stability. Discrepancies with the current results might be explained based on the G powder composition. It can be assumed that the G powder used here contains less seed but mostly pulp. The Pearson correlation analysis demonstrated strong associations between gluten aggregation behavior (GlutoPeak) and dough mixing properties (Mixolab). Peak maximum time was positively correlated with dough stability ($r=0.847$, $p=0.0079$), while negatively correlated with softening ($r=-0.868$, $p=0.0052$) and mixing tolerance index (MTI) ($r=-0.914$, $p=0.0015$), suggesting that longer aggregation time reflects enhanced dough strength and resistance to breakdown. In addition, peak maximum time showed a strong negative correlation with water absorption ($r=-0.982$, $p<0.0001$), suggesting that increased water availability accelerates gluten aggregation while reducing the time required to reach maximum aggregation. Similarly, GSI was positively correlated with stability ($r=0.757$, $p=0.0295$) and negatively correlated with softening ($r=-0.889$, $p=0.0032$) and MTI ($r=-0.911$, $p=0.0017$), indicating that stronger gluten aggregation is associated with improved dough resistance to mechanical weakening. GSI was also negatively correlated with water absorption ($r=-0.750$, $p=0.0320$), further suggesting that higher water content weakens gluten network strength, likely due to dilution of protein-protein interactions of the dough matrix. In contrast, torque maximum exhibited an opposite trend compared with peak maximum time and GSI. It was negatively correlated with stability ($r=-0.874$, $p=0.0046$) but positively correlated with water absorption ($r=0.899$, $p=0.0024$) and MTI ($r=0.855$, $p=0.0069$). This increased torque does not reflect a stronger gluten network, but rather a stiffer and more hydrated dough system that is more susceptible to breakdown during continued mixing. Energy values further confirmed the different impact of the powders. P required the highest energy (18.6 kJ) to obtain the dough, which was approximately 30% higher than the control, indicating increased resistance to mixing and stronger dough consistency. In contrast, PSP exhibited the lowest energy, consistent with its

reduced development time and stability. A study revealed that adding 5 g/100 g PSP to white flour reduces the deformation energy of the dough by more than 50% (Ropciuc et al., 2021). Although all composite flour samples resulted in some dilution of gluten due to partial substitution of wheat flour, the more pronounced reduction observed in PSP may also be attributed to the presence of phenolic compounds, which can interfere with gluten network formation and weaken dough structure beyond the effect of gluten dilution alone (Rosell et al., 2009).

Significant differences ($p<0.05$) were observed in the proofing performance of the dough. All substituted samples exhibited a decrease in maximum volume compared to the control, with reductions of 9.0% for PSP, 6.5% for G, and 10.4% for P. These reductions indicate a compromised gas-holding capacity following the addition of the powders. Liu et al. (2020) discovered that adding 5 g/100 g purple potato flour to the dough for steamed bread resulted in a reduction of the maximum dough height compared to dough made with 100% wheat flour. This reduction in height may be attributed to the fact that the purple potato flour disrupted the gluten network structure of the wheat dough, leading to decreased dough stability.

The fermentation rate also reflected a similar trend, with all substituted samples showing lower rates than the control, which resulted in longer fermentation times to get a similar dough volume. The reduced gas retention could also be associated with the inhibitory effects of polyphenolic compounds on yeast metabolism. Previous studies have reported that phenolic compounds can interact with yeast cells and reduce fermentation activity and cell viability, which may lead to lower CO₂ production during dough fermentation (Grinvald et al., 2024).

The dough of substituted samples showed significantly ($p<0.05$) lower pH both before and after proofing compared to the control sample. Dough pH decreased after proofing in all samples, with

P exhibiting the lowest pH of 4.95, which could be due to the presence of organic acids and acidic polyphenols in P powder.

Table 5.3 Dough mixing and proofing properties

	Control	PSP	G	P	
<i>Mixolab parameters</i>					
Water absorption (g/100 g)	65.9±0.0 ^c	68.8±0.1 ^a	64.0±0.6 ^d	67.3±0.3 ^b	
Development time (min)	7.9±0.0 ^b	5.1±0.1 ^c	8.2±0.2 ^b	10.1±0.3 ^a	
Stability (min)	8.8±0.9 ^a	6.2±0.4 ^b	9.3±0.7 ^a	4.5±0.4 ^b	
Softening (Nm)	0.06±0.01 ^c	0.35±0.00 ^a	0.05±0.00 ^c	0.16±0.02 ^b	
MTI (Nm)	0.01±0.00 ^b	0.09±0.01 ^a	0.01±0.00 ^b	0.08±0.01 ^a	
Bandwidth (Nm)	0.06±0.01 ^a	0.06±0.01 ^a	0.09±0.00 ^a	0.05±0.01 ^a	
Energy (KJ)	14.3±0.2 ^b	10.2±0.5 ^c	13.5±0.4 ^b	18.6±0.4 ^a	
<i>Proofing parameters</i>					
Dough maximum volume (mL/100 g)	128.3±2.4 ^a	116.7±0.0 ^b	120.0±10.0 ^b	115.0±2.4 ^b	
Fermentation rate (mL/min)	1.57±0.09 ^a	1.28±0.03 ^b	1.31±0.03 ^b	1.19±0.03 ^b	
Fermentation time (min)	67±1 ^b	76±1 ^a	74±2 ^a	77±0 ^a	
Dough pH	Before proofing	6.02±0.02 ^a	5.98±0.02 ^b	5.89±0.02 ^c	5.32±0.01 ^d
	After proofing	5.74±0.08 ^a	5.57±0.00 ^b	5.48±0.01 ^c	4.95±0.00 ^d

Control: white flour; PSP: 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: 95 g/100 g of white flour and 5 g/100 g of grape powder; P: 95 g/100 g of white flour and 5 g/100 g of pomegranate powder. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

5.3.4 Bread quality

As illustrated in Table 5.4, the addition of fruit and vegetable powders at a 5 g/100 g substitution level had little impact on the slice size. The two-dimensional slice area ranged from 14.15 to 15.25 cm², and crumb porosity varied between 40.34% and 43.46%. The structural integrity and openness of the crumb were largely preserved in all the substituted breads, which was achieved by adapting the fermentation times.

Clear differences in crumb texture were observed among the breads. The control bread had the highest hardness, while the breads with PSP, G, and P substitutions showed significantly lower hardness values ($p<0.05$), with reductions of approximately 13-18%. A similar trend was noted for chewiness, which decreased by about 15-16% in the substituted breads. In contrast, the springiness was unaffected by the incorporation of powder and remained stable across all samples. However, cohesiveness and resilience showed a slight but significant ($p<0.05$) decrease in the G and P breads compared to the control. The results indicate that adding fruit and vegetable powders to the dough primarily leads to a softer crumb and decreased mastication resistance, while preserving the elastic recovery properties of the final bread. Based on the mechanical and pasting analyses discussed earlier, a 5 g/100 g substitution of PSP and P powders resulted in reduced dough strength and increased softening behaviour, as shown by lower stability and higher softening/MTI values. Interestingly, G powder incorporation led to a significant ($p<0.05$) reduction in crumb hardness, while dough stability and softening remained comparable to the control. It seems that crumb softening in G bread was influenced more by starch-protein interactions during baking and cooling, rather than by gluten weakening during mixing. This may be related to the lower setback observed in the pasting properties, which indicates reduced starch retrogradation and helps maintain bread softness for a longer time. Additionally, the lower GSI suggests weaker gluten strength compared to the control.

Similar results were found in previous research, which indicated that adding 4% G seed powder to wheat bread significantly decreases cohesiveness and chewiness compared to the control. Conversely, hardness and springiness increased due to low moisture content and the bread's water-holding capacity, while resilience remained largely unchanged (Koç & Atar Kayabaşı, 2023).

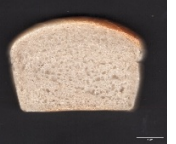
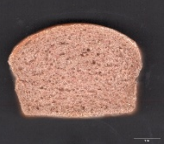
Adverse results were observed by Gül and Şen (2017) when comparing the control bread to the breads containing 5 -10 g/100 g P seed powder, observing increased firmness. Likely, differences in the breadmaking settings could explain the divergent results. Gul and Sen (2017) used two-step fermentation with longer fermentation time and elevated baking temperature to 275°C. These could promote a more compact crumb structure and greater moisture loss, leading to increased hardness.

Significant ($p<0.05$) differences in both crust and crumb colour were observed following powder incorporation (Table 4), which were also evident in the bread images. For the crust, all substituted breads exhibited significantly ($p<0.05$) lower L^* values than the control, indicating darker crusts. The colour changes result from higher concentrations of reducing sugars like glucose, fructose, and maltose in plant powders, enhancing the Maillard reaction and bread browning (Santiago et al., 2015). Crust redness (a^*) increased markedly ($p<0.05$) with powder addition, especially in P bread, followed by PSP and G, reflecting the contribution of naturally pigmented compounds. Correspondingly, b^* values decreased in PSP and P breads, indicating reduced yellowness.

The colour differences were more noticeable in the crumbs of the different breads. The control bread crumb exhibited the highest lightness, while the crumbs from the PSP and P bread showed significantly ($p<0.05$) lower lightness values, at 50.85 and 52.92, respectively. The a^* values of the crumbs increased dramatically ($p<0.05$) in all the substituted breads, particularly in the PSP and P breads, which were 15.33 and 13.05, respectively, compared to the near-zero value of the control bread (0.26). At the same time, the b^* values, which indicate yellowness, decreased sharply in the PSP bread, while the G and P breads maintained relatively higher levels of yellowness. Similarly, previous research found that adding 4 g/100 g PSP to wheat flour resulted in a bread crumb colour with lower L^* and b^* values, but a higher a^* value. The darker colour of the bread

crumbs was attributed to the natural dark purple hue of anthocyanin pigments (Santiago et al., 2015). These results indicate that crumb colour closely reflects the intrinsic pigmentation of the powders, since significant ($p<0.05$) correlations were observed between powder colour and crumb colour parameters. Powder luminosity showed a strong correlation with crumb L^* ($r=0.9642$, $p=0.0001$), powder a^* was positively correlated with crumb a^* ($r=0.7341$, $p=0.0381$), and similarly, powder b^* was positively correlated with crumb b^* ($r=0.7833$, $p=0.0215$). Specifically, powders with higher redness tended to produce darker and less yellow crumbs, while lighter powders resulted in brighter, more yellow crumbs. Regarding the crust's colour, weaker and fewer correlations were found, which suggests that crust appearance is less directly governed by the original powder pigmentation. Instead, it is likely influenced by additional factors during the baking process, including Maillard reactions and caramelization, which can partially mask or override the contribution of powder-derived pigments (Rozanska et al., 2025).

Table 5.4 Texture and colour characteristics of bread

	Control	PSP	G	P
				
Crumb structure				
2D area (cm ²)	15.00±0.39 ^{ab}	15.25±0.38 ^a	14.15±0.51 ^b	14.54±0.57 ^{ab}
Porosity (%)	42.74 ±1.12 ^{ab}	43.46 ±1.09 ^a	40.34±1.45 ^b	41.44±1.64 ^{ab}
Crumb texture				
Hardness (g)	695±46 ^a	593±51 ^b	572±43 ^b	605±40 ^b
Springiness	1.04±0.08 ^a	1.04±0.05 ^a	1.01±0.02 ^a	1.01±0.03 ^a
Cohesiveness	0.87±0.02 ^a	0.86±0.01 ^{ab}	0.85±0.01 ^b	0.85±0.01 ^b
Chewiness (g)	605±41 ^a	507±38 ^b	507±33 ^b	513±38 ^b
Resilience	0.49±0.01 ^{ab}	0.50±0.01 ^a	0.47±0.01 ^c	0.47±0.01 ^c

Bread colour

	<i>L</i> *	68.06±2.97 ^a	56.16±2.91 ^b	58.32±4.25 ^b	55.25±3.60 ^b
Crust	<i>a</i> *	6.15±0.46 ^c	12.44±1.01 ^b	11.36±1.95 ^b	15.20±1.96 ^a
	<i>b</i> *	31.33±2.52 ^a	20.79±1.56 ^b	28.42±2.75 ^a	18.52±1.72 ^b
	<i>L</i> *	81.90±0.49 ^a	50.85±0.95 ^d	75.21±1.60 ^b	52.92±0.76 ^c
Crumb	<i>a</i> *	0.26±0.04 ^d	13.05±0.33 ^b	4.42±0.15 ^c	15.33±0.20 ^a
	<i>b</i> *	22.98±0.34 ^a	1.05±0.13 ^d	18.01±0.15 ^b	16.42±0.18 ^c

Control: bread made of white flour; PSP: bread made of 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: bread made of 95 g/100 g of white flour and 5 g/100 g of grape powder; P: bread made of 95 g/100 g of white flour and 5 g/100 g of pomegranate powder. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

5.3.5 Bread *in vitro* starch digestibility

The *in vitro* starch hydrolysis curves for the different breads suggested the impact of the phenolic-rich powders on the starch polymers (Figure 5.3). Both the G bread and the control bread exhibited a faster hydrolysis rate throughout the digestion process. In contrast, P bread consistently displayed the lowest hydrolysis curve, indicating the slowest rate of starch digestion. This behaviour was consistent with the significant negative correlations observed between TPC and both k ($r = -0.736$, $p = 0.0374$) and C_{∞} ($r = -0.976$, $p < 0.0001$), suggesting an association between phenolic-rich systems and reduced starch hydrolysis behaviour. The PSP formulation showed an intermediate response, with a slightly lower hydrolysis rate than the control during the initial stages but rapidly reaching a plateau. These trends were further confirmed by kinetic modelling presented in Table 5.5. The theoretical maximum hydrolysis extent (C_{∞}) was highest for G bread at 63.66 g/100 g, which is comparable to the control bread at 63.04 g/100 g, but significantly ($p < 0.05$) reduced in PSP and P. Compared to the control, C_{∞} decreased by approximately 11% in PSP and by 19% in P bread.

More importantly, the kinetic constant (k) demonstrated a notable formulation effect. G bread had the highest k value at 0.048 min⁻¹, which was significantly ($p < 0.05$) higher than the control at

0.040 min⁻¹, indicating a faster initial hydrolysis. Conversely, P bread exhibited a markedly ($p<0.05$) reduced k value of 0.023 min⁻¹, reflecting an approximately 43% decrease compared to the control. PSP showed no significant difference in k despite a lower C_{∞} , suggesting reduced total digestible starch without substantial suppression of the initial hydrolysis rate.

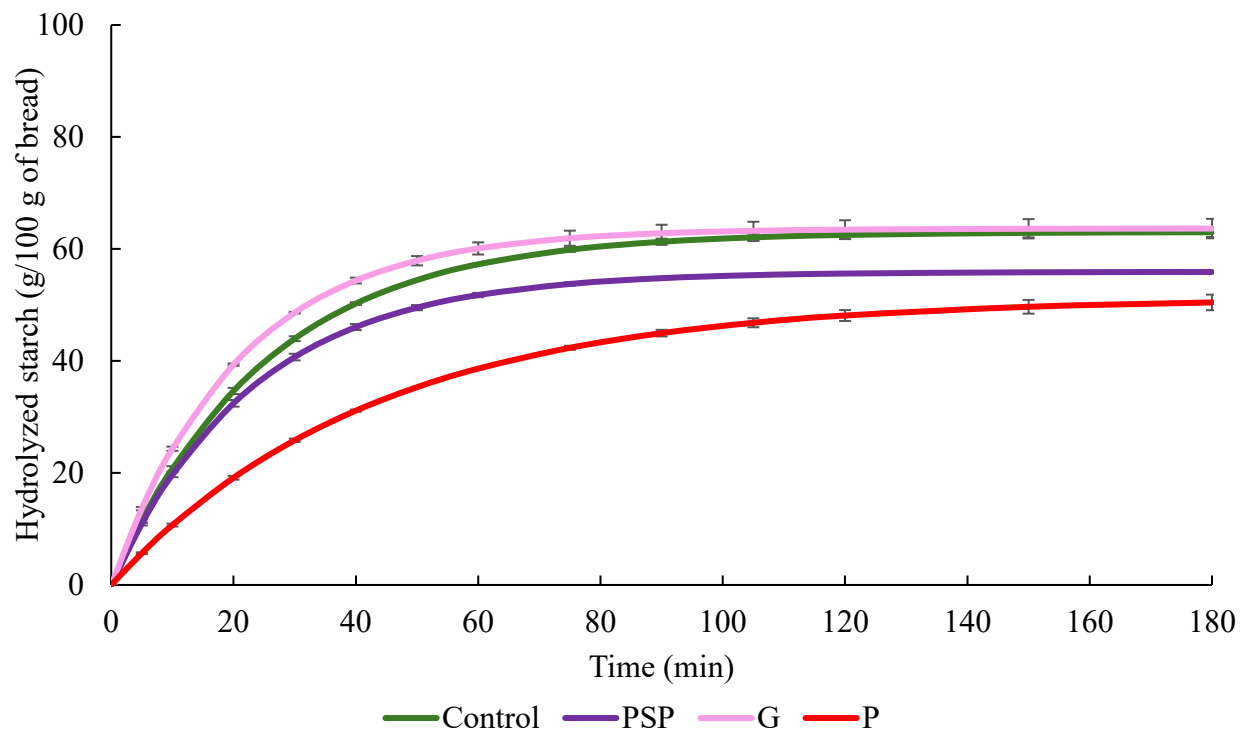


Figure 5.3. *In vitro* starch hydrolysis kinetics of bread

Control: bread made of white flour; PSP: bread made of 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: bread made of 95 g/100 g of white flour and 5 g/100 g of grape powder; P: bread made of 95 g/100 g of white flour and 5 g/100 g of pomegranate powder.

Significant differences in starch fractions were also observed among formulations (Table 5.5). Compared with the control, the P bread exhibited the most significant starch fraction modifications. RDS decreased from 34.65 to 19.15 g/100 g bread, which was approximate 45% reduction, corresponding to a decline in RDS proportion from 52.5% to 32.1% of total starch. In parallel, RS

was 11.50 g/100 g bread, nearly doubled compared to control bread (5.83 g/100 g bread). These compositional shifts may lead to both C_{∞} and k being significantly lower in P bread. In agreement with these results, TPC showed a strong negative correlation with RDS ($r=-0.877$, $p=0.0043$) and a positive correlation with RS ($r=0.993$, $p<0.0001$). Similar relationships were observed for TFC, suggesting that phenolic-rich formulations were associated with redistribution of starch fractions toward lower RDS and higher RS contents. RS was negatively correlated with C_{∞} ($r=-0.958$, $p=0.0002$), reinforcing the consistency between starch fraction redistribution and hydrolysis kinetics. Similarly, Liu et al. (2022) found that there is a negative correlation between AUC and TPC ($r=0.794$, $p<0.01$); thus, the presence of polyphenols may contribute to reduced starch accessibility during digestion, thereby hindering the enzyme-catalyzed breakdown of starch into sugar.

PSP showed an intermediate modification; RDS decreased modestly to 32.43 g/100 g bread, while RS increased to 9.86 g/100 g bread. Correspondingly, PSP showed a significant reduction in C_{∞} but not a significant difference in k , suggesting partial structural modulation of starch without strong suppression of the hydrolysis rate. These results imply that PSP phenolic enrichment induced starch digestion inhibition, but the extent of phenolic-starch interaction was less pronounced than in P bread.

In contrast, the G powder formulation exhibited the highest RDS and k , consistent with a high hydrolysis curve, despite moderate phenolic enrichment. Correspondingly, the positive relationship between RDS and k ($r=0.966$, $p<0.0001$) further reflected the rapid hydrolysis behaviour observed in G bread. This may be partly explained by compositional differences of the G powder that influenced the bread matrix rather than the digestibility of the powder itself. Compared with PSP and P powders, G powder contained substantially lower total dietary fibre

(0.99 g/100 g), which may have provided less physical restriction to water mobility, starch hydration, and enzyme diffusion during digestion. Although sugar composition was not directly determined, the high carbohydrate and low fibre contents of G powder may indicate a more soluble and pulp-derived matrix, which could have contributed to the higher hydrolysis rate observed in G bread. Literature reported that bread fortified with 10% G pomace powder led to the hydrolysis index decreasing from 100 to 86.45, while the predicted glycemic index dropped from 94.20 to 82.72 (Rocchetti et al., 2021). These differences support that the commercial G powder used in this study was primarily extracted from the G pulp itself, while the seeds and skin may be removed for smoothness and silkiness.

A substantial reduction in TPC, TFC, and TAC was observed after baking when compared with the corresponding powders. Although plant powders exhibited markedly elevated TPC, TFC, and TAC values (Table 5.1), their levels decreased considerably in the final bread products. Apart from the dilution due to bread moisture content, this decline can be attributed to multiple processing-related factors, including thermal degradation, oxidative reactions during baking, and, importantly, reduced extractability due to phenolic interactions with starch and lipid matrices. Similar results were also found by researchers who noted that the TPC was 0.5 mg/g in wheat bread and increased to 2.2 mg/g in bread that contained 5 g/100 g P peel powder. (Garcia et al., 2023).

A similar trend was observed for TFC, where P showed the highest level, followed by PSP and G. In contrast, TAC was highest in PSP bread, consistent with the anthocyanin-rich profile of PSP. Interestingly, although PSP powder originally contained the highest TFC (Table 5.1), the highest TFC in bread was observed in the P formulation. This apparent shift may reflect differential stability during baking. Furthermore, the lower pH associated with P bread may have contributed to partial stabilization of certain phenolics. An increase in TPC and a concomitant decrease in the

area under the hydrolysis curve were found in sweet potato starch pastes enriched with blackcurrant and strawberry, which may be due to the interaction of phenolic compounds with starch, inhibiting starch degradation (Mu et al., 2021). A similar correlation has been identified in a previous study. Liu et al. (2022) found that there is a negative correlation between AUC and total phenolic content (TPC) ($r=0.794$, $p<0.01$). This suggests that increasing the phenol content in bread may influence its digestion. The presence of polyphenols could inhibit the activity of digestive enzymes, thereby hindering the enzyme-catalyzed breakdown of starch into sugar.

At the starch molecular level, several interaction pathways may explain the observed redistribution. Phenolic compounds can associate with starch chains via hydrogen bonding and hydrophobic interactions, and amylose is generally more prone to complexation than amylopectin due to its relatively linear structure and ability to form single helices. It is therefore plausible that phenolics promoted the formation of amylose-associated complexes with reduced enzyme susceptibility. Depending on phenolic structure and processing conditions, such interactions may resemble a complexed starch fraction functionally behaving like RS by limiting α -amylase access or slowing chain depolymerization (Bello-Perez et al., 2025; Wang et al., 2023).

Table 5.5 Starch fractions and phenolic compounds in bread

	Control	PSP	G	P
RDS (g/100 g bread)	34.65±0.56 ^b	32.43±0.58 ^c	39.35±0.21 ^a	19.15±0.34 ^d
SDS (g/100 g bread)	27.86±1.28 ^{ab}	23.18±0.42 ^b	24.10±1.88 ^b	28.97±1.31 ^a
RS (g/100 g bread)	5.83±0.04 ^d	9.86±0.01 ^b	7.06±0.04 ^c	11.50±0.05 ^a
C _∞ (g/100 g bread)	63.04±0.85 ^a	55.92±0.13 ^b	63.66±1.74 ^a	51.23±1.61 ^b
<i>k</i> (min ⁻¹)	0.040±0.001 ^b	0.044±0.000 ^{ab}	0.048±0.003 ^a	0.023±0.001 ^c
TPC (mg/g)	0.06±0.00 ^d	1.39±0.04 ^b	0.24±0.00 ^c	2.08±0.02 ^a
TFC (µg/g)	16.29±1.89 ^d	301.41±9.42 ^b	56.83±1.90 ^c	452.92±7.60 ^a
TAC (µg/g)	nd	82.88±4.88 ^a	6.54±0.62 ^c	27.85±2.46 ^b

Control: bread made of white flour; PSP: bread made of 95 g/100 g of white flour and 5 g/100 g of purple sweet potato powder; G: bread made of 95 g/100 g of white flour and 5 g/100 g of grape powder; P: bread made of 95 g/100 g of white flour and 5 g/100 g of pomegranate powder. RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. C_{∞} : maximum hydrolysis extent; k : kinetic constant; TPC: total phenolic content; TFC: total flavonoid content; TAC: total anthocyanins content; nd: not detected. Different letters in each parameter are highlighted by different superscript letters indicating significant differences (one-way ANOVA, Tukey test, $p < 0.05$).

5.4 Conclusions

The incorporation of phenolic-rich plant powders at a 5 g/100 g substitution level induced systematic modifications in dough rheology, starch functionality, and *in vitro* starch enzymatic digestibility while maintaining acceptable bread quality. The magnitude of these effects differed among PSP, G, and P formulations and may be associated with differences in phenolic composition and other matrix-related components, such as dietary fibre. PSP and P powders exhibited a stronger weakening effect on gluten aggregation, as indicated by lower GSI values compared with G powder. Additionally, PSP and P powders reduced starch swelling and retrogradation tendency, as evidenced by decreased RVA peak and setback viscosities. Thermal analysis further suggested the possible formation of phenolic-starch structures in selected systems, particularly in the PSP formulation. Although gluten development was partially weakened, crumb structure remained intact, and bread softness was improved, indicating that technological performance was not compromised. The incorporation of these pigmented fruit and vegetable powders produced visually distinctive bread while maintaining an acceptable crumb structure.

Starch digestibility was substantially modulated by phenolic enrichment. Among the formulations, P bread exhibited the strongest potential inhibitory effect, characterized by a reduced hydrolysis rate, lower RDS content, and increased RS fraction. Pearson correlation analysis revealed significant associations between phenolic content and digestion kinetics, supporting a direct role of phenolics in regulating starch hydrolysis behaviour. These findings offer mechanistic insights

for designing functional wheat bread that modulates digestibility while maintaining technological quality.

Author Contributions

Xiaohang Zou: writing – original draft, investigation, formal analysis, data curation.

Nicola Gasparre: training, writing – review and editing, supervision, and conceptualization.

Cristina M. Rosell: writing – review and editing, conceptualization, supervision, and funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Chapter 6 _General Discussion and Conclusions

6.1 General Discussion

The present thesis investigated how biochemical modification of wheat and the incorporation of phenolic-rich plant ingredients influence dough functionality, bread quality, and starch digestibility in wheat-based systems. Particular attention was given to understanding how germination-induced enzymatic activity, starch physicochemical properties, and phenolic compounds interact within the bread matrix to regulate starch hydrolysis behaviour. By combining grain-level modification strategies with formulation approaches using phenolic-rich ingredients, this work provides new insights into the mechanisms controlling starch digestibility in bread systems.

6.1.1 Germination as a strategy to modify wheat functionality and starch accessibility

Germination is widely recognized as a biochemical process capable of activating endogenous enzymes and modifying cereal grain composition. During germination, hydrolytic enzymes, such as α -amylase, are synthesized or activated to mobilize starch reserves required for seedling growth. In wheat systems, this enzymatic activation significantly affects flour functionality, dough rheology, and final product quality. Previous studies have shown that increased α -amylase activity during germination leads to partial degradation of starch granules, reducing paste viscosity and altering dough properties (Cardone et al., 2020; Marti et al., 2018). However, the results were varied under different germination conditions and varying levels of amylase activity.

The results obtained in this thesis confirm that germination plays a critical role in modifying starch functionality and breadmaking performance. Adjusting germination conditions to obtain the same level of viscosity enabled better control of enzymatic activity, thereby allowing evaluation of flour pasting properties and dough behaviour at the same enzyme level. The results demonstrated that the technological impact of germination depends strongly on the extent of enzymatic activity. While moderate germination levels provided manageable technological changes, excessive

germination resulted in substantial loss of dough strength and reduced breadmaking suitability. Similar observations have been reported in previous studies where high levels of α -amylase led to excessive starch degradation and weakened dough structure (Kaur et al., 2024; Marti et al., 2018). Even though apparent peak viscosity was correlated with α -amylase activity, dough and bread quality did not show a linear relationship with enzyme levels. This may be attributed to proteases produced during germination, which hydrolyze proteins and consequently affect gluten quality (Shaimerdenova et al., 2025). Germination influences starch accessibility during digestion by altering the starch fractions and contributing to the formation of starch-lipid or starch-phenolic complexes, which may increase RS. This highlights the importance of germination in potentially regulating starch hydrolysis in cereal-based foods. Therefore, it is crucial to control germination conditions to achieve a balance between functional improvements and technological performance.

6.1.2 Unique response of pigmented wheat to germination

An important aspect of this thesis is the investigation of pigmented wheat, particularly purple wheat, which differs from conventional wheat varieties due to its high phenolic and anthocyanin content. Pigmented wheat varieties have attracted increasing attention as potential functional ingredients because of their bioactive compounds and associated health benefits (Yu & Beta, 2015). The results of this work demonstrate that purple wheat responds differently to germination compared with common wheat. Germination progressively reduced the pasting ability of purple wheat flour and weakened gluten aggregation, which affected dough mixing properties. Moderate germination levels (6-12 hours) maintained satisfactory dough functionality while enhancing gas production and crumb porosity. In contrast, excessive germination (24 hours) led to a significant loss of starch integrity and markedly reduced dough strength. A notable difference observed between germinated purple wheat and common wheat was the stronger viscosity breakdown

exhibited by purple wheat during pasting. This behaviour indicates a greater susceptibility of starch granules to enzymatic and thermal degradation. The presence of phenolic compounds and anthocyanins may contribute to this response by influencing starch structure and interactions within the flour matrix (Wang et al., 2023). Germination also influenced purple wheat bread appearance, producing loaves with a characteristic reddish–yellow crumb and crust. These colour changes are likely related to the existence of anthocyanins. Similar colour changes have been reported in studies investigating anthocyanin-rich cereals and plant materials (Koksel et al., 2023). In addition to technological changes, germinated purple wheat bread exhibited distinct starch digestibility behaviour compared with bread produced from common wheat. Germination increased the RS in bread made from both common wheat and purple wheat. However, germination affected starch hydrolysis differently in these two types of wheat. In bread made from common wheat, germination decreased RDS to obtain lower starch hydrolysis. In contrast, the situation in purple wheat bread is more complex, with RDS increasing by 40% while SDS decreased by 50%. This difference is likely associated with the phenolic composition of purple wheat. Phenolic compounds have been reported to interact with starch and digestive enzymes, potentially affecting starch hydrolysis kinetics and the distribution of starch fractions during digestion (McDougall & Stewart, 2005). Different phenolic compounds can have varying effects on starch digestion. In this study, TAC showed a strong positive correlation with the SDS fraction and a negative correlation with RDS. In contrast, TPC only correlated with RS. These findings suggest that anthocyanins may have a more significant influence on starch digestibility. This effect may be due to the ability of anthocyanins to interact with starch granules, which promotes the formation of more ordered crystalline structures, particularly B-type crystallinity, during the cooling process (Ou et al., 2022).

The increase in starch crystallinity, associated with higher levels of anthocyanins, could contribute to reduced potential digestibility by modifying the microstructure of the starch.

6.1.3 Phenolic-rich ingredients as modulators of starch digestibility

In addition to grain-level modification through germination, this thesis explored formulation strategies aimed at modifying starch digestibility by incorporating phenolic-rich plant powders into wheat bread. Ingredients such as PSP, G, and P powders are rich in phenolic compounds and dietary fibre, both of which have been reported to influence starch digestion behaviour.

The incorporation of these plant powders modified several technological properties of the dough and bread systems. Changes in gluten aggregation behaviour, dough mixing properties, and starch pasting characteristics were observed, indicating that plant powders interact with both starch and protein components of the dough matrix. Phenolic–protein interactions altered gluten aggregation behaviour, as reflected by changes in GlutoPeak parameters and dough mixing properties. These interactions, involving hydrogen bonding and disruption of disulfide linkages, led to modified gluten network development, which in turn affected gas retention and dough stability. Simultaneously, phenolic–starch interactions reduced pasting viscosities and setback values, indicating restricted starch swelling and reduced retrogradation. These changes contributed to a softer crumb texture and delayed firming; the overall bread quality remained acceptable, suggesting that phenolic enrichment can be achieved without severely compromising technological performance.

One of the most significant findings of this study is the influence of phenolic-rich ingredients on starch digestion kinetics. Among the tested ingredients, P powder showed the strongest inhibitory effect on starch hydrolysis, which exhibited lower C_{∞} , reduced hydrolysis rate (k), decreased RDS, and increased RS. These findings are consistent with previous research showing that phenolic

compounds possibly reduce starch digestibility by inhibiting digestive enzymes or forming complexes with starch molecules (Zhu, 2015a).

Correlation analyses performed in this study further suggested strong relationships between phenolic content and starch digestion parameters. These results support the hypothesis that phenolic compounds may reduce starch accessibility to digestive enzymes. Two main mechanisms have been proposed in the literature to explain this phenomenon. First, phenolic compounds can inhibit digestive enzymes such as α -amylase and α -glucosidase, thereby slowing starch hydrolysis. Second, and more importantly, phenolics interact with starch during thermal processing to form ordered structures, including V-type or higher-order starch–phenolic complexes, as suggested by DSC transitions observed in this study. The appearance of additional thermal transitions (e.g., $\sim 130^{\circ}\text{C}$ in PSP systems) supports the formation of thermally stable complexes, which are associated with increased structural resistance to enzymatic digestion. These structural rearrangements, occurring during gelatinization and cooling, promote the development of more ordered crystalline regions, which are characteristic of RS and contribute to reduced digestibility (Romero Hernández et al., 2022).

Furthermore, this study highlighted that the potential effect of phenolics on starch digestibility was strongly source dependent. P exhibited the strongest inhibitory effect on starch hydrolysis, likely due to its high content of hydrolyzable tannins and phenolic acids (Oliveira et al., 2020). In contrast, PSP, characterized by high anthocyanin content, showed moderate inhibition, suggesting that anthocyanins contribute to structural modification but with lower binding strength or stability during baking. G powder, despite containing phenolics, showed limited inhibitory effects on starch digestion, which may be attributed to differences in phenolic composition as well as the presence of soluble sugars that increase substrate availability and counteract inhibitory effects (San Martín-

Hernandez et al., 2021). These findings emphasize that total phenolic content alone is not sufficient to predict functionality; instead, phenolic profile and chemical structure are critical determinants. In addition to phenolic compounds, the dietary fibre introduced by the plant powders may have contributed to the differences in starch digestibility. This effect was particularly relevant for the pomegranate powder, which contained substantially more fibre than the purple sweet potato and grape powders. Fibre may compete with starch for available water during baking, restrict starch swelling and gelatinization, and create a physical barrier that limits the accessibility and diffusion of digestive enzymes (Zhang et al., 2022). Soluble fibre components may also increase the viscosity of the digestion medium and further slow starch hydrolysis (Zhang et al., 2021). Therefore, the lower hydrolysis rate and extent observed in the pomegranate-enriched bread may have resulted from the combined effects of fibre, phenolic compounds, acidity, and other matrix components, rather than from phenolics alone. Since soluble and insoluble fibre fractions and their physicochemical properties were not determined, their individual contributions could not be distinguished in the present study.

6.2 General Conclusions

This thesis explored how biochemical modifications of wheat and the incorporation of phenolic-rich plant ingredients affect dough functionality, bread quality, and starch digestibility in wheat-based products. A key focus was on understanding the interactions between enzymatic activity, starch physicochemical properties, and phenolic compounds within the bread matrix, particularly as they relate to starch hydrolysis behaviour.

- Wheat germination served as a strategy for controlling enzymatic activity and enhancing bread functionality. By adjusting the germination level based on viscosity-related indicators, we were able to regulate α -amylase activity, which significantly impacted dough

handling, bread texture, and starch digestion behaviour. Viscosity-adjusted germination offers a practical approach for balancing technological performance with nutritional functionality. Controlling enzymatic activity through germination enables the optimization of breadmaking performance while also influencing starch hydrolysis rates.

- Purple wheat responded uniquely to germination, offering both functional and nutritional benefits for developing cereal-based foods with modified starch digestibility. Longer germination times progressively diminished the pasting ability of purple wheat flour and weakened gluten aggregation, thereby affecting dough mixing behaviour. Moderate germination levels (6–12 hours) maintained acceptable dough functionality while enhancing gas production and crumb porosity. Conversely, extended germination (24 hours) resulted in a significant loss of starch integrity and dough strength. Compared to germinated common wheat, germinated purple wheat exhibited a greater breakdown of viscosity during pasting, indicating an increased susceptibility of starch granules to enzymatic and thermal degradation. Additionally, germination led to breads with a distinct reddish-yellow crumb and crust, attributed to transformations of anthocyanins during processing. Germinated purple wheat bread also demonstrated different starch digestibility characteristics compared to common wheat, likely linked to its phenolic composition. The interactions between phenolic compounds, starch, and digestive enzymes influenced starch hydrolysis kinetics and the distribution of starch fractions.
- The addition of powders from PSP, G, and P systematically altered dough rheology, starch functionality, and digestion behaviour while maintaining acceptable bread quality. The enrichment with phenolic compounds affected gluten aggregation, starch pasting properties, and thermal behaviour. Notably, PSP and P powders decreased starch swelling and

retrogradation tendency, likely due to interactions between phenolic compounds and starch molecules. Despite a slight weakening of gluten development, the bread structure remained intact, and crumb softness improved, demonstrating that phenolic enrichment can be achieved without sacrificing technological performance. Most importantly, the inclusion of phenolic compounds significantly modulated starch digestibility. Among the tested ingredients, P powder exhibited the strongest inhibitory effect on starch hydrolysis, resulting in a lower hydrolysis rate, reduced RDS, and increased RS content. Strong correlations between phenolic content and starch digestion kinetics indicated that phenolic compounds play a crucial role in potentially regulating enzymatic starch hydrolysis by inhibiting enzymes and forming complexes with starch.

Overall, this thesis showed that starch digestibility in wheat bread can be potentially modified through both biochemical changes at the grain level and formulation strategies involving phenolic-rich ingredients. The findings emphasized the importance of the interactions between enzymatic activity, starch structure, phenolic compounds, and the dough matrix in determining starch accessibility during digestion. By integrating knowledge of cereal biochemistry, dough functionality, and digestion behaviour, this research provided new insights into the mechanisms that control starch digestibility in bread systems, contributing to the development of cereal-based foods with improved nutritional functionality, particularly through the modulation of starch hydrolysis and its potential impact on postprandial glycemic response, while maintaining desirable technological properties.

6.3 Future Directions

While this thesis provides new insights into the roles of germination and phenolic-rich ingredients in modifying wheat functionality and starch digestibility, several aspects warrant further investigation.

First, sensory evaluation should be incorporated in future studies. Although the present work focused on physicochemical properties, dough behaviour, and starch digestibility, consumer acceptance is critical for practical applications. Phenolic-rich ingredients such as purple wheat, G, P, and PSP powders may introduce bitterness or astringency, which could influence the sensory quality of bread. Therefore, systematic sensory testing would be essential to evaluate consumer perception and determine acceptable incorporation levels of these ingredients.

Second, the effects of phenolic-rich ingredients on starch digestion should be further validated through *in vivo* studies. The current thesis evaluated starch digestibility using *in vitro* models. However, it is still entirely theoretical until the breads undergo testing in humans. Human digestion is more complex and influenced by physiological factors. Future work could involve animal models or clinical trials to better understand how phenolic compounds and germination-derived changes influence glycemic response and carbohydrate digestion *in vivo*.

Third, the influence of pH and different organic acids on polyphenol–starch interactions should be explored. During fermentation and baking, acids such as lactic acid and acetic acid can alter the pH of dough systems, which may affect the stability and activity of phenolic compounds. Investigating how different acids and pH conditions influence polyphenol functionality, their inhibitory effects on α -amylase, and the formation of phenolic starch complexes could provide further insight into strategies for modulating starch digestion in bakery products.

Furthermore, further investigation of the roles of phenolic compounds and dietary fibre in starch digestion is needed. Fractionation and reconstitution experiments could be used to separate their individual and combined effects, for example, by comparing whole plant powders with phenolic- or fibre-depleted fractions and then adding the isolated components back. The physicochemical properties of dietary fibre, such as solubility, water-binding capacity, and viscosity, should also be examined, as they may affect enzyme diffusion and starch accessibility. In addition, enzyme inhibition assays, microscopy, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray diffraction (XRD) could be used to determine whether the reduced starch digestion is related to direct enzyme inhibition, interactions among starch, phenolics, and fibre, physical barriers within the bread matrix, or changes in starch crystalline structure. These studies would provide clearer evidence of the mechanisms involved.

Finally, further characterization of the phenolic composition of plant powders would help clarify the mechanisms underlying their effects on starch digestion. Advanced analytical techniques such as LC–MS could be used to identify and quantify individual phenolic compounds present in various sources, including purple wheat, phenolic-rich vegetables and fruits. Establishing correlations between specific phenolic compounds and starch digestibility may help determine which phenolics are most effective in modulating enzymatic starch hydrolysis.

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