EFFECT OF YELLOW WHOLE AND SPLIT PEA FLOURS WITH DIFFERENT PARTICLE SIZES ON BREAD QUALITY PARAMETERS AND ANTIOXIDANT ACTIVITY IN PAN BREADS

By

Lisa D. Davies-Hoes

A thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Food Science

University of Manitoba

Winnipeg, Manitoba

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Lisa D. Davies-Hoes

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Lisa D. Davies-Hoes

The University of Manitoba requires the signature of all persons using or photocopying this thesis. Please sign below and give address and date.

ACKNOWLEDGEMENTS

I would like to extend a big thank you to my primary advisor, Dr. Martin G. Scanlon. During the completion of my MSc project, Dr. Scanlon was an invaluable resource. He provided direction, guidance and most importantly pushed me to be better. He encouraged critical thinking, leveraging on statistical analysis to identify relationships with my results and to be more clear and concise with my writing. I was also fortunate to have an advisor that was open minded to consider alternate thoughts and ideas. I gained a great deal of knowledge and experience from the completion of my program with Dr. Scanlon.

Thank you to the members of my examining committee, which included Dr. Harry D. Sapirstein and Dr. Rotimi E. Aluko. Dr. Sapirstein provided expertise regarding methodologies and general direction related to the breadmaking and quality aspects of my project. Dr. Aluko was a tremendous resource in helping me select the antioxidant assays and providing tools to facilitate the phenolic and antioxidant testing. I would also like to thank members of Dr. Aluko's laboratory, which included Dennis Labossiere, Dr. Abraham Girgih and other graduate students. Dr. Aluko's laboratory group was extremely helpful in sharing equipment, answering questions and accommodating my time in their lab.

The baking and quality investigation portion of my project was conducted at the Cereal Research Centre. Lab members David Niziol, Jerry Suchy, Kim Kuzminski, and Tatiana Kowalchuk provided exceptional support in explaining best practices and standard operating procedures for the lab equipment.

I would also like to thank the many staff members within the Department of Agricultural and Food Sciences at the University of Manitoba, which included Jennifer McLaren, Carola Lange, Pat Heritage, Denise Aminot-Gilchrist, Babak Sobhi and Heather Weibe. These individuals played a significant role in explaining protocols and policies, general administration as well as lending support with equipment. There were also numerous graduate students that lent support with various projects and coursework during my studies and they included Filiz Koksel, Kun Wang, Yao Wu, Victoria Ndolo, Nasibeh Younes Sinaki, Ikechukwu Agomoh and Xinyang Sun. My thanks also to Dr. Susan Arntfield who always took the time to entertain my questions that pertained to coursework, project work and the AACC product development competition.

I am grateful to the Natural Sciences & Engineering Research Council of Canada Discovery grants program and Manitoba Agri-Food Research and Development Initiative for funding and to Best Cooking Pulses (Portage-la-Prairie, MB, Canada) for supplying samples. Also, I am very thankful that I was approved for the student travel award provided by the University of Manitoba, which allowed me to present a poster at the American Association of Cereal Chemists Conference in Rhode Island, U.S.A in October 2014 and to participate in a product development competition in Minneapolis, U.S.A. in October 2015. Participation in these conferences provided me with an excellent opportunity to discuss my project work and gain valuable industry experience.

I would like to express a special thanks to my husband Frank, who motivated and encouraged me during the completion of my project and who took care of our son when I was extremely busy with my studies. Finally, I would like to send a big thank you to my

son Jackson for his understanding and cooperation during the pursuit of my academic goals.

GENERAL ABSTRACT

Studies have examined nutritional improvements with additions of pea fibre and protein in wheat leavened breads. This study examined 10, 15, 20 and 25% additions of yellow whole and split pea flour with various particle sizes to a Canadian Wheat Red Spring (CWRS) and a commercial wheat flour pan bread using the Canadian Short Process bake method. The objective was to evaluate both yellow pea flour additions and determine optimal substitution levels. A dilution of wheat protein occurred which lowered the amount of gluten available to form a network, thus, causing poor gas retention. Crumb firmness and cell density increased while the whiteness of the crumb grain decreased with increased levels of yellow whole and split pea flour. An evaluation of the phenolic content and antioxidant activity of the flour materials and composite bread samples was also conducted, and bread baking altered the antioxidant power to different extents in the bread samples.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Functional foods are foods that have a positive effect on human health beyond basic nutrition. Some researchers would argue that functional foods promote optimal health while reducing the risk of disease. This research project evaluates the properties of yellow whole and split pea flours in wheat pan bread. The addition of yellow pea flours in a bread formulation is designed to increase both the protein content and the antioxidants of the composite wheat-pea bread samples (Gritton et al., 1975). An increase in yellow pea protein content has been shown to play a role in achieving satiation in human subjects (Smith et al., 2012). In addition to improved satiation, yellow pea flours have been reported to have lower glycemic index values compared to wheat flours, which has been attributed to an abundance of resistant and slowly digestible starch (Dahl et al., 2012). These starches also provide a substrate for the growth of probiotic organisms, and are linked with health benefits such as the reduction of colon cancer and diabetes (Bjorck & Elmstahl, 2003).

Pulse flours have been reported to have higher antioxidant activity of their phenolic compounds compared to wheat flours. Total phenolic content (TPC) for wheat has been reported to be between 22 and 40 mg/100 g; and chickpeas between 78 and 230 mg/100 g (Shahidi & Naczk, 1995). Pulses contain several phenolic compounds, which are considered to be natural antioxidants (Ghanachorloo et al., 2013). Antioxidants in raw materials and food products have been widely investigated as they have been proposed to reduce oxidative damage associated with many health disorders, including cardiovascular disease and cancer.

Pulses have also been recognized as good sources of protein, dietary fibre, minerals such as iron, phosphorous, zinc, manganese and vitamins C, E and B_6. They are also low in fat and a rich source of monounsaturated and polyunsaturated fatty acids. Pulse flours do have superior nutritional properties to wheat flours; however, they have been noted to have a deleterious effect on bread quality, as these flours do not contain gluten proteins. Addition of pulse flours into wheat pan bread results in a dilution of the wheat flour and ultimately a relative decrease of the gluten proteins, which negatively influences the gluten matrix and gas retention of the composite dough sample during bread baking (Sosulski & Wu, 1988). Quantification and analysis of these quality effects, such as changes in loaf volume, will be conducted as a comparison with wheat control pan bread. Quality investigations can serve as a good predictor of consumer acceptability when a comparison is made to a wheat control bread sample. Instruments were selected and tests conducted to evaluate three stages of the bread making process: the investigation evaluated mixing properties, dough handling and quality attributes of the finished bread product. Dough mixing will be evaluated using both a 10g mixograph and farinograph instrument to determine the influence of yellow pea flour additions. Dough samples will be evaluated for extension parameters using a TA XT2 texture analyzer instrument using a Kieffer dough and gluten extensibility rig. Bread quality will be evaluated using loaf volume, specific volume, color, firmness and cell density measurements. Finally, an ASE instrument, using various measurements such as crumb color, will be used to calculate a quality score for the bread samples.

In a study evaluating pea fibre additions into a wheat bread product it was noted that higher viscosity occurred in the wheat-pea fibre composite bread samples (Shum,

2011). Therefore, an RVA instrument will be used to evaluate the viscosity of the wheat and yellow pea flour paste samples. The RVA can be used to evaluate the pasting and gelling behavior that occurs during cooking and cooling cycles that are inherent with dough baking and bread cooling (Angioloni & Collar, 2012). More specifically, the RVA parameters peak viscosity, pasting temperature, and setback during cooling have been highly correlated with bread staling kinetics parameters (Collar, 2003). These parameters have also been valuable predictors of bread firming behavior during storage as well as the sensory scores for fresh baked goods (Collar, 2003; Collar et al., 2006).

In addition to the RVA thermal study, a staling study will also be conducted using a DSC instrument. The DSC instrument will be used to bake the bread dough samples and then evaluate a staling effect based on changes to the amylopectin melting enthalpy, which will be used as a measure of amylopectin retrogradation. Other researchers have utilized the DSC instrument to evaluate the measurement of thermal changes in bread during aging and its association with structure alterations in the starch fraction (Fearn & Russell, 1982). The advantage for this DSC experiment was that the retrogradation enthalpy change can be measured using a small but homogenous baked dough sample (Defloor & Delcour, 1999). Preservation of freshness in bread products plays an important role in consumer acceptability. Therefore, a staleness study can provide invaluable insights regarding bread product freshness that can be used by formulation and product development food scientists.

Both antioxidants and total phenolic content was evaluated for both the raw flour materials and bread samples. To evaluate the samples antioxidant activity, four antioxidant assays will be used and they include: 1,1-diphenyl-2-picrylhydrazyl, Metal

Chelation, Ferric Reducing Power and Superoxide Scavenging. Two or more radical scavenging capacity assays are required to investigate a selected antioxidant preparation since each assay involves different chemical mechanism(s) and may reflect different aspect(s) of the antioxidant properties (Zhu et al., 2010). It has also been postulated that some assays are more sensitive to some compounds and not others. The use of one method has proven not to be effective in evaluating the antioxidant activity content in cereal grains and thus, multiple methods are recommended (Zhu et al., 2010). Pure experimental wheat flour will be used during the antioxidant evaluation to objectively evaluate the activity of the pea flour additions and not additives that are present in commercial bread wheat flours.

The overall objectives of the research are to:

- Investigate substitutions of yellow whole and split pea flours at loadings of 10, 15, 20 and 25% into wheat pan bread. The investigation also evaluated a reduction in particle size of the yellow pea flours to determine if this would have an effect on quality parameters. The quality investigation considered mixing properties, dough handling and bread attributes.
- Investigate thermal properties of the raw flour materials using an RVA, and bread dough samples using a DSC instrument. Bread staling was evaluated with a DSC instrument; this was accomplished by simulating bread baking in the DSC instrument and evaluating staling by measuring the enthalpy change in the bread dough samples.
- Quantify the total phenolic content and antioxidant activity of the raw flour materials and bread samples to evaluate the effect of thermal processing involved

in bread baking and to identify the functionality of the antioxidant compounds based on assay performance. Using the raw flour material in the best manufacturing and processing environment will maximize product development from a functional point of view.

CHAPTER 2: LITERATURE REVIEW

2.1. Wheat background

As a cereal grain, wheat has a global presence and can be found throughout the world including the European Union, China, the United States of America, Canada and Australia. The 2013 crop production was 713 million metric tons (FAOSTAT, 2015), making wheat the third largest crop grown worldwide. Wheat continues to play a pivotal role in meeting the world's food demands. Milling quality wheat grains are milled into flours which can be used in food products such as baked goods, biscuits, crackers and pasta. The milling process can be used to produce both whole and refined wheat flours. There are substantially more vitamins, minerals, antioxidants, and other nutrients in whole-wheat flour than refined flour, as these compounds are concentrated in the outer portions of the grain that are removed during milling (Hemdane et al., 2015). Both whole and refined wheat flours are valuable sources of protein, though they are not considered a complete protein source. The typical value of wheat protein would be 13% (USDA National Nutrient Database). As a protein source, wheat lacks the essential amino acid, lysine (Fenn et al., 2010). Nevertheless, wheat flours are a good source of several micronutrients including thiamin (B1), riboflavin (B2), niacin (B3), iron, insoluble and soluble fibres as well as antioxidants (USDA National Nutrient Database).

2.2. Legumes background

Yellow peas, lentils, chickpeas and dry beans are pulses, and are defined as leguminous crops yielding up to 12 grains or seeds enclosed in a pod (Ma et al., 2015). Crops such as these are harvested solely as a dry grain, which would be why the term pulses does not include harvested crops such as green beans, green peas, and soybeans

(Ma et al., 2015). Pulses are Canada's fifth largest crop and account for 10% of the market in regards to total crop production after wheat, barley, canola, and maize (Ma et al., 2015). The use of pulses as a food ingredient has been emerging in North America and Europe (Roberfroid, 2000).

Pulses are recognized as an inexpensive source of protein, complex carbohydrates, fibre, and minerals (Mohammed et al., 2012). Furthermore, they are rich in essential amino acids lysine and leucine (Mohammed et al., 2012). The protein in legume seeds ranges in content from 17 % to 40 %, contrasting with 7% to 15 % for cereals, and would be equal to the protein content of meat (18% to 25 %) (Bojnanska et al., 2012). Fibre content in cereal products also becomes higher with the addition of legumes. In addition to legumes being excellent sources of protein and fibre, they are also good sources of minerals and vitamins as well as resistant starch (Bojnanska et al., 2012). Pulses have a low glycemic index because of an abundance of resistant starch and slowly digestible starch (Bjorck & Elmstahl, 2003). These starches also provide a substrate for the growth of probiotic organisms, and are linked with health benefits such as the reduction of colon cancer and diabetes (Bjorck & Elmstahl, 2003).

Legume flours have also been reported to have higher antioxidant activity of their phenolic compounds compared to wheat flours. Total phenolic content (TPC) for wheat has been reported to be between 22 and 40 mg/100 g; and chickpeas between 78 and 230 mg/100 g (Shahidi & Naczk, 1995). Pulses contain several phenolic compounds, which are considered to be natural antioxidants (Ghanachorloo et al., 2013). Diets rich in natural antioxidants have the ability to increase the level of reactive antioxidants in the body and

therefore reduce the risk of disease states that are of free radical origin (Diziki et al., 2014).

2.3. Milling background

2.3.1. Flour quality

Protein content, moisture content and ash content should all be considered in the evaluation of a flour sample. For wheat and flour purchasers, protein content has been a key specification because of its relation to many processing properties such as water absorption (Jagannadham et al., 2014). Flour with higher protein content usually requires more water and a longer mixing time to achieve optimum dough consistency. This would be why bakers use protein content results to anticipate water absorption and dough development time for processes and products (Jagannadham et al., 2014).

Gluten plays an important role in cereal crops, accounting for 75 to 85% of the total protein content in wheat flour (Canadian Grain Commission, 2016). Gluten proteins consist mainly of gliadin and glutenin; these proteins play an important role in wheat bread baking (Gomez et al., 2011). Glutenin contributes to the elasticity while the gliadin contributes to the extensibility of a wheat dough sample. A balance between elasticity and extensibility is a requirement for producing a quality wheat bread product (Canadian Grain Commission, 2016). Understanding the factors that influence the gluten's performance becomes important for both the wheat and flour purchasers. Varietal differences exist in wheat samples including gluten strength. Varietal differences in gluten strength can be attributed to compositional differences in both the glutenin and gliadin proteins (Canadian Grain Commission, 2016). Environmental conditions, such as excessive rainfall or high temperatures, can negatively affect the compositional make-up

of the wheat grain and thus, impact gluten strength. Bread made using a good quality variety may have a higher loaf volume than bread made with a lesser variety, even when both varieties have the same protein content (Canadian Grain Commission, 2016). A number of instruments have been developed to determine the quality of flour, and their results are strongly influenced by protein quantity and quality (Gomez et al., 2011).

2.3.2. Flour analysis

2.3.2.1. Farinograph

The most popular of the empirical rheological instruments used to evaluate dough quality is the farinograph. During mixing of the dough sample, using two Z-shaped blades, the farinograph instrument operates to maintain a constant speed and temperature during the test. A farinograph curve provides two important physical properties of a flour: the water absorption, as well as a profile of the mixing characteristics of the dough sample. An optimum amount of water is a requirement for a cohesive viscoelastic dough with optimum gluten strength (Bakare et al., 2016). The optimum water absorption level will differ from flour to flour depending on the quantity of protein present in the flour sample (Bakare et al., 2016). The centre of the curve will be centred on the 500 B.U. line for a dough sample that has achieved optimum absorption. With too much water present in the dough sample, the center of the curve will not reach the 500 B.U. line. A dough sample with too little water will go above the 500 B.U. line.

On the basis of farinograph parameters, wheat flours can be classified as weak, medium, strong, and very strong. Dough strength classifications have been defined as: a weak flour having a water absorption below 55%, a medium flour, 54 to 60%, a strong flour, above 58% and a very strong flour above 64% (Serna-Saldivar, 2012). The key

factors contributing to farinograph absorption include protein and starch content, more specifically the damaged starch present in the dough sample (Bakare et al., 2016). The proportion of soluble and insoluble fibre also influences water absorption in a flour mixture (Autio & Laurikainen, 1997).

The process of dough development begins with the addition of water and mixing until the dough achieves optimum consistency. Initially all ingredients are hydrated and appear like a sticky mass. After further mixing, the viscosity increases, the sticky characteristics of the dough disappear and a consistent dough mass is formed (Khatkar, 1995). This formed dough behaves like a viscoelastic mass and exhibits both elastic and extensible characteristics. Examples of the parameters obtained from the farinograph are the arrival time (time required for the top of the curve to cross the 500 Brabender Unit (BU) line), peak time (time required for the dough to reach maximum consistency without breakdown), departure time or time to breakdown (time lapsed until the top of the curve permanently drops below the 500 BU line), stability (time difference between arrival time and departure time), mixing tolerance index (MTI, the difference in BU's between the peak time and peak time plus 5 minutes), and water absorption (Mohammed et al., 2012).

2.3.2.2. Mixograph

The mixograph uses pins to rapidly develop dough. The peak refers to the dough reaching an optimum consistency. At optimum consistency, the dough behaves like a viscoelastic mass with both elastic and extensible characteristics.

There are a number of flour quality parameters that can be evaluated using a mixograph: peak time, width of curve at peak (wider curve for stronger, elastic doughs),

rate of breakdown after the peak (steeper angles represent doughs with less mixing tolerance), width of the curve after overmixing (% of mixing time) (North Dakota State University, 2015). The dough begins to break down when mixing continues beyond the mixing peak and its ability to resist breakdown is termed the mixing tolerance. Dough with good mixing tolerance will have a large window of time to allow the baker to stop dough mixing while at optimum development. The shape of the curve determines the optimum water absorption of the dough sample while the height of the curve relates to the flour's protein content (Mohammed et al., 2012). A curve with many wild swings before the peak would indicate the dough is too dry and requires more water. A curve that wavers during the development stage and moves side to side indicates too much water has been added to the sample (Mohammed et al., 2012). For a good bread wheat flour, a mixogram should possess the following attributes: high water absorption, moderate mixing times, strong gluten strength and good dough mixing tolerance (North Dakota State University, 2015).

2.3.2.3. Extensograph

Extensional properties of dough are assessed by rheological techniques and are used regularly in the cereal industry. It has been postulated that good gas cell expansion during proofing and baking depends on good dough extensibility (Ktenioudaki & Gallagher, 2012). The balance of elastic and viscous properties of a dough is measured by extension. Resistance to extension and extensibility of the dough are measured by a load/extension curve during dough stretching (Ribotta et al., 2005). The force with which the dough opposes the stretching force is shown in the extensogram. In bread making, it is desirable to achieve a balance between the resistance to extension and extensibility

parameters (Ribotta et al., 2005). This balance in extensibility parameters plays an important role in gas retention during fermentation and oven rise (Ribotta et al., 2005).

2.3.3. Commercial bread flours

Flour milling is a mechanical manufacturing process which produces flour from wheat through comprehensive stages of grinding and separation. Canadian mills produce flour and other products for use in baked goods, biscuits, crackers, pasta, and a wide range of other food products. Wheat of high protein content produces flour suited to bread making. Although protein content alone does not ensure a quality loaf of bread, it has been suggested that a combination of both quality and quantity of protein is needed in the wheat flour to produce one. The protein content for bread flour falls in the 12.5% to 13.5% range (Canadian National Millers Association, 2015). Canadian mills export flour to over thirty countries worldwide (Lyddon, 2014). Canada's principal flour export market is the United States of America (Lyddon, 2014).

Research studies have illustrated that fibrous materials present in wheat flour negatively influence bread quality (Cai et al., 2014). Therefore, investigations have explored new milling techniques that can provide flours with smaller particle sizes (Angelidis et al., 2016). Unfortunately, the literature is not conclusive in supporting the idea that a particle size reduction in fibrous materials can improve bread quality (Cai et al., 2014). That being said, flours with reduced particle size may offer improved health promoting effects and improved functionality compared to flours in their original form (Angelidis et al., 2016).

2.3.4. Yellow pea flours

Unlike wheat, no universal method exists for milling pulse crops. Milling wheat grains involves a series of rolls with different corrugations and speed ratios. Impact or compression mills are used for milling yellow peas and other pulses. Impact mills expose the pulse seeds to a set of rotating hammers or pins to fracture the seeds (Maskus et al., 2016). Compression mills compress the seeds between two hardened surfaces, which include stones or rollers (Maskus et al., 2016). There has been little information recorded about the relationships between the microstructure of legume seeds and milling performance, resulting particle size of flour, and flour composition (Maskus et al., 2016).

It has been established that different grinding methods during flour milling influence both particle size and flour properties, including functional and compositional attributes (Kerr et al., 2000). Protein and starch content in the flour are also affected depending on the grinding method selected. The greatest level of protein was found in pin-milled split pea flour samples, while the highest levels of starch was present in roller milled split pea flour samples (Otto et al., 1997). The highest level of fibre was found in hammer and stone milled split pea flour samples (Otto et al., 1997). Whole legume flours do not have the hull of the seeds removed during the milling process. Therefore, these whole flours would possess higher levels of fibre. End-product attributes should be considered when a method of milling is selected for the yellow peas. Yellow pea flour research has largely been conducted on non-leavened end-products such as tortillas and pita breads (Borsuk et al., 2012). In non-leavened products, replacing wheat flour with yellow pea flour increases the fibre and protein content (Borsuk et al., 2012). A limiting factor with using yellow pea flour instead of wheat flour in a leavened product is the non-

existence of gluten (Mohammed et al., 2012). Gluten proteins play an important role in creating a network structure in bread dough that makes it elastic and extensible (Mohammed et al., 2012). Therefore, yellow pea flour substitutions are recommended to be only partial in pan breads to ensure a portion of the gluten is retained and key quality attributes such as loaf volume are preserved in the final bread product.

2.4. Particle size evaluation

Research on particle size and the potential influence that size may have on final product quality in wheat flours has been conducted for many years. Milling conditions determine both the final particle size and the flour properties. Common techniques used to evaluate particle size in a flour sample include sieve analysis and laser diffraction analysis. Sieve analysis is the simplest of the two methods and therefore, one of the most common approaches taken to evaluate particle size in dry materials. A weighed sample is poured into the top sieve which has the largest screen opening. Each lower sieve in the column has smaller openings than the one above it. The column is placed in a mechanical shaker to shake the column for a fixed amount of time. After shaking, the sample material residing on each sieve is weighed (Dishman, 2006). The weight of the sample in each sieve is divided by the total sample weight; this provides a percentage retained on each of the sieves to create a particle size distribution for the sample material (Dishman, 2006).

The principle behind laser diffraction analysis involves the assessment of angle sizes as they relate to the degree of light scattered as a laser beam passes through a sample (Malvern, 2007). It has been shown that large particles scatter light at small angles relative to the laser beam, while small particles show the opposite effect by scattering light at larger angles relative to the laser beam (Malvern, 2007).

The angular scattering intensity data would then be analyzed to calculate the size of the particles responsible for creating the scattering pattern, using the Mie theory of light scattering (Malvern, 2007). The particle size is typically recorded as a volume equivalent sphere diameter (Malvern, 2007).

Small particles have high surface-to-volume ratios, which increase the access of enzymes to the interior of the particle (Angelidis et al., 2015). It has been suggested that an increase in the food material's surface area could also increase the rate of water absorption, improve solubility of wheat flour, and increase the site accessibility for chemical reactions (Angelidis et al., 2015). A change in the particle size has been investigated to evaluate its effect on antioxidant properties of a wheat flour sample (Hemdane et al., 2015). Current literature does not show a conclusive relationship between a reduction in particle size and improved phenolic content as well as antioxidant activity in wheat and wheat bran samples.

Research studies have also investigated particle size effects and their influence on the nutritional properties of a sample. It was shown that different particle size treatments of insoluble fibres of carrot and fruits resulted in the re-distribution of fibres from insoluble to soluble, improved their physicochemical properties, and exerted a positive effect on improving intestinal function and health in hamsters (Chau et al., 2007; Wu et al., 2007). Particle size has been an important factor that affects the characteristics and physiological functions of insoluble fibres. Faster starch digestion has also been reported for smaller particle size flours (Angelidis et al., 2015). It has also been suggested that a reduction in particle size may result in greater starch damage and more sugars, due to enzymatic hydrolysis, resulting in softer and stickier dough (Angelidis et al., 2015).

Therefore, effects of particle size on bread quality have been investigated. The influence of particle size on bread quality has had contradictory outcomes in the literature. The replacement of coarse bran with finely ground bran has been shown to both increase and decrease bread loaf volume (Hemdane et al., 2015). If all breads contain the same non-endosperm compounds, it has been postulated that smaller bran particles are more detrimental to bread volume than larger ones. Hemdane and colleagues (2015) postulated that an abrasive effect occurs when fine bran particles are added to achieve the same substitution levels as a coarse bran particle. A larger percentage of small bran particles have an abrasive effect on the gluten network during mixing.

2.5. Influence of wheat and non-wheat flour components on bread making and bread quality

Refined white wheat flour in the Canadian market has been enriched in accordance with regulations outlined in the Food and Drugs Act. The enrichment process required is due to the removal of the bran layer during milling; bran removal negatively impacts the flour's nutritional profile by eliminating certain vitamins and minerals (Hemdane et al., 2015). Removal of the outer layer of the cereal grain during milling has been reported to reduce as many as twenty nutrients such as thiamin, riboflavin, niacin and iron. Cereal grains have also been reported to have low levels of the essential amino acid, lysine (Fenn et al., 2010). One way to address the lysine deficit and maximize the nutritional profile of a final product could be to blend a pulse flour and wheat flour (Bojnanska et al., 2012). A blend of both wheat and legume flours in a pan bread would be a good approach to improving the nutritional profile of a refined wheat flour baked product. A great deal of attention has been given to peas for the following reasons: 1)

they are a good substitute for soya protein (listed as Health Canada's number 1 of 11 allergens) 2) they possess an excellent nutritional profile. Legume flour additions would also encourage higher levels of phenolic content and antioxidant activity in a refined wheat flour baked product (Angioloni & Collar, 2012).

2.5.1. Effect on mixing properties

Using 10-30% flour additions, a study showed a decrease in farinograph mixing resistance of all supplemented dough samples that contained both raw and germinated chickpea flour (Sadowska et al., 2002). However, dough with added raw chickpea flour and dough with the germinated flour both showed a decrease in water absorption. Hsu and colleagues (1980) also showed a decrease in water absorption with the additions of lentil, faba bean and yellow peas. The dough development time (DDT) showed an increase with legume flour additions and was attributed to interactions between the non-wheat proteins and gluten, leading to a delay in the hydration and development of gluten in the presence of the other bread ingredients (Kohajdova et al., 2013). Dough stability (DS) is an index of dough strength, with higher values indicating a stronger dough with a greater tolerance to mixing (Mohammed et al., 2012). The addition of legume flours to wheat flour dough significantly decreased DS, in particular when higher levels of flour were added (Kohajdova et al., 2013). Other studies have reported a decrease in DS for wheat flour dough with increasing levels of various legume flours. The reduction of DS in wheat-legume flour blends results in a general weakening of the gluten network configuration during kneading of the dough sample (Mohammed et al., 2012). Again, this would be attributed to a marked incompatibility between the protein of legume flours and the gluten of the wheat flour (Mohammed et al., 2012).

2.5.2. Effect on dough properties

Although dough rheology has long been investigated, there still remains a significant lack of understanding. This lack of progress is due to the complexity of this biological system (Angioloni & Collar, 2008). Dough represents a typical viscoelastic biopolymer system. It is the intermediate product between flour and baked goods and its rheological behavior is of considerable importance to bread makers. There are direct relationships found between dough rheological properties and the structure and quality of the finished product. Dough that has been defined as too firm or too soft does not process satisfactorily on the dough processing equipment, nor does it produce a satisfactory baked good. Therefore, optimal dough development is important for machinability of the dough as well as the quality of the final product (Angioloni & Collar, 2008).

Uniaxial extension parameters were significantly modified with the substitution of soy protein isolate (SPI) into wheat flour. SPI increased the maximum resistance (Rm) and decreased the extensibility (E) of the dough samples (Roccia et al., 2009). When the soy level increased, these effects were more pronounced. The reduction of the area under the extension curve, which is a measure of the energy required for extension, and has also been taken as a measure of the flour's strength, has indicated a weakening effect of gluten by addition of soy protein. Soy protein produced more resistant, less extensible and weaker dough mixtures. The substitution of soy protein in a wheat flour dough sample resulted in a weakened gluten network that exhibited decreased elasticity but increased viscosity (Roccia et al., 2009). It has been well established in the literature that a weakened gluten network compromises bread quality (Kohajdova et al., 2013).

2.5.3. Effect on bread quality

The dough in the bread making process undergoes a number of physical and chemical changes, such as evaporation of water, formation of a porous structure, volume expansion, protein denaturation, starch gelatinization and crust formation (Mohammed et al., 2012). Quality bread products rely on air being incorporated during mixing of the dough. Hayman and colleagues (1998) established that the stability of the gas cell in bread dough undergoes critical changes during the early stages of baking. However, the mechanism by which the gas cell walls fail still remains unclear. As bread dough would be considered a viscoelastic foam, established theories for foam systems can be applied to the bread dough system, providing some insight into those mechanisms. The two theories for gas cell destabilization that can be applied to bread dough systems are disproportionation (Ostwald ripening) and coalescing of the gas cells (Hayman et al., 1998).

Mixing and extension tests are often used reliably to predict aspects of baking studies (Pollard et al., 2002). However, there has been a lack of correlation between loaf height and mixing parameters for composite flours (Pollard et al., 2002). Pollard and colleagues (2002) reported that it was encouraging that there was a lack of statistical correlation between loaf height and mixing parameters for composite flour blends. These researchers implied that blending the right flour materials could reduce mixing times without adversely affecting loaf volume (Pollard et al., 2002). The benchmark for producing quality bread products has been to deliver a good yield and a high specific loaf volume. The utilization of whole-wheat flour versus refined wheat flour has quality implications in a bread product. These quality implications can be attributed to the

characterization of the flour. Refined wheat flour consists mainly of the endosperm and the whole-wheat flour contains the endosperm, bran, and germ. As the name would imply the entire grain kernel is incorporated into the whole-wheat flour. The introduction of all of the grain influences the raw materials, flour functionality, rheological behavior of the dough, processing and ultimately, the quality of the final baked product (Kohajdova et al., 2013). Pomeranz and colleagues (1977) reported that fibre lowered loaf volume while increasing mixing time, and proposed this was due to lowered gas retention rather than poor gas production. Research evaluating lupin and soybean flours as well as field pea and faba bean concentrates has indicated that increased substitutions result in decreased loaf volumes (Pollard et al., 2002). In another study, researchers evaluated additions of lentil and chickpea flours and decreased loaf volumes were also reported (Bojnanska et al., 2012). Pollard and colleagues (2002) suggest that slightly reduced loaf volumes would still be acceptable to consumers. Ohimain (2015) conveyed the same findings and indicated that 10-20% substitutions in wheat flour produced a bread of acceptable quality with minimal detrimental effects on bread color, crumb structure, and texture as well as shelf life.

2.5.4. Effect on bread staling

Bread staling refers to all changes that occur in bread after baking (D'Appolonia & Morad, 1981). The mechanism(s) involved in staling have been investigated for over 150 years. However, at a molecular level, neither the process of bread baking nor the process of staling has been fully elucidated (Gray & Bemiller, 2003). Changes occur in both the crumb and the crust of the bread. It has been generally accepted that during gelatinization of starch a transition occurs from an ordered state to a relatively disordered

structure, and that during aging of bread the reverse process occurs (Fearn & Russell, 1982).

The RVA can be used to evaluate the pasting and gelling behavior that occurs during cooking and cooling cycles that are inherent with dough baking and bread cooling (Angioloni & Collar, 2012). More specifically, the RVA parameters peak viscosity, pasting temperature, and setback during cooling have been highly correlated with bread staling kinetics parameters (Collar, 2003). The parameters have also been valuable predictors of bread firming behavior during storage as well as the sensory scores for fresh baked goods (Collar, 2003; Collar et al., 2006). The addition of chickpea flour increased the pasting temperature, but decreased the peak viscosity and peak viscosity temperature of the wheat flour suspension (Mohammed et al., 2014). No peak of viscosity curve was found for pure chickpea flour and it has a higher pasting temperature than pure wheat flour. The pasting temperature of wheat and chickpea flour blends ranged between 62°C to 66.5 °C, more than the wheat flour, 60.8 °C, and lower than the chickpea flour, 72 °C. The lowering of the peak viscosity would account for the lowering of gas retention as well as expansion in the final product (Jagannadham et al., 2014). The increase in pasting temperature would suggest that a higher temperature would be required to cook a legume flour sample (Jagannadham et al., 2014).

It has been postulated that staling occurs in the solid phase of the bread crumb and is not influenced by the air cells or specific volume of the bread (Fearn & Russell, 1982). Bread of low specific volume contains a lower volume of air as well as more crumb per unit volume, within which firming can occur, compared to bread with high specific volume (Fearn & Russell, 1982). It follows that any technique, which measures changes

in the solid phase of the crumb directly, should produce results that are independent of specific loaf volume (Fearn & Russell, 1982). Therefore, the Differential Scanning Calorimetry (DSC) instrument is a good technique to evaluate changes in the solid phase of the bread crumb. The DSC has been a fundamental tool in thermal analysis. It looks at how a material's heat capacity has changed with temperature adjustments. The sample and reference(s) pan sit on a thermoelectric disk surrounded by a furnace. As the temperature of the furnace changes, heat is transferred to the sample and reference through the thermoelectric disk. The differential temperature (ΔT) between the sample and reference is converted to a differential heat flow. A change in enthalpy can be used to evaluate staling activity in bread samples.

Fearn and Russell (1982) utilized the DSC instrument to evaluate the measurement of thermal changes in bread during aging and its association with structure alterations in the starch fraction. When stale bread was heated in the DSC instrument, an endotherm curve was reported at 60ºC. This endotherm curve was not present in fresh bread but became visible with increased storage time. Fearn and Russell (1982) used the amylopectin melting enthalpy as a measure of amylopectin retrogradation. Defloor and Delcour (1999) evaluated the impact of reduced farinograph water absorption to determine if water was responsible for the change in amylopectin retrogradation. Adjusted water amounts did influence the retrogradation endotherm but a reduction in the amylopectin retrogradation enthalpy was still observed. The advantage for this DSC experiment was that the retrogradation enthalpy change can be measured using a small but homogenous baked dough sample (Defloor & Delcour, 1999). In a DSC test where a dough sample is baked, a small piece of dough is placed in a hermetically sealed DSC

pan and the sample undergoes calorimeter scan conditions to bake it. There are slightly higher water levels in both control and experimental samples prepared in a DSC instrument compared to a traditional bread making method, since some water evaporation occurs which results in higher retrogradation enthalpy values. This increases the sensitivity of this method to evaluate bread staling properties in baked dough samples (Defloor & Delcour, 1999). Other researchers such as Czuchajowska and Pomeranz (1989) have utilized the DSC technique to evaluate bread staling. However, these researchers used bread crumb instead of a baked dough sample to evaluate bread staling.

2.6. Influence of high fibre non-wheat flour components on bread making and bread quality

Dietary fibre was identified in the 1980s as an important component of a healthy diet (National Research Council, 1989). In the Western world, insufficient fibre in the diet has been identified as a possible cause of chronic disease (National Research Council, 1989). Therefore, the food industry has looked for palatable ways to increase the fibre content of food products. Dietary fibre, from a chemical point of view, consists of cellulose, hemicelluloses, lignin, pectin, β-glucans and gums (Dhinda et al, 2012). It has also been positioned that high-legume breads contain about two to three times the dietary fibre of white wheat bread, and most breads can be labelled as high-fibre breads (6 g DF/100 g food) (Angioloni & Collar, 2012). The practice of manufacturing high-fibre bread products requires very close attention to formulation and processing. Addition of too much fibre produces bread of poor quality in terms of texture, loaf volume, and appearance (Dubois, 1978). High levels of fibre dilute gluten, thus lowering gas retention and causing a decrease in loaf volume. High levels of fibre also result in modification of mixing times and water absorption of doughs (Pomeranz, 1977). The replacement of

portions of wheat flour with legume fibre in bread making has typically utilized ground legume hulls (Dubois, 1978; Shogren et al., 1981; Sosulski & Wu, 1988; Sievert et al., 1990). Results indicate that breads fortified with pea hulls decrease loaf volume, increase water absorption, and decrease the overall bread quality with increased levels of substitution (Sosulski & Wu, 1988).

2.6.1. Effect on mixing properties

The effects of dietary fibre on mixing parameters have been evaluated in several studies. Gomez and colleagues (2003) evaluated several dietary fibre sources and concluded that fibre-supplemented dough samples allowed higher water absorption during the mixing process which implied longer mixing times and a greater tolerance of excess mixing than the fibre-free dough sample. Mohammed and colleagues (2012) evaluated mixing parameters with the addition of dietary fibre from chickpea flour. A dough sample containing 10% chickpea had better stability and resistance to breakdown than the control (Mohammed et al., 2012). The stability value can be used as an index of dough strength, with higher stability values representing stronger dough. This study showed that stability time was related to the substitution level in the dough sample. A 10% chickpea substitution increased stability, while 20% and 30% substitutions decreased the stability of the dough sample (Mohammed et al., 2012). This reduction in dough stability has been attributed to an intense incompatibility between the chickpea protein and wheat gluten (Mohammed et al., 2012). The 30:70 chickpea wheat flour composite blend showed an increase in dough development time (DDT) from 2.1 minutes for the wheat sample to 6 minutes suggesting a weakening of the gluten network during mixing due to the intense incompatibility between the chickpea protein and wheat gluten.

Dalgetty and Baik (2006) showed that the type of dietary fibre addition as well as the quantity of substitution influenced water absorption. Both insoluble fibre and soluble fibre increased water absorption in the pea, chickpea and lentil fibres. However, a decrease in water absorption was recorded for 3% and 5% pea, chickpea or lentil soluble fibre substitutions compared to a 1% substitution, which had resulted in an increase in water absorption compared to the wheat flour control (Dalgetty & Baik, 2006). The quantity of water additions would be considered as important for the distribution of the dough materials, their hydration and the gluten protein network development (Kohajdova et al., 2013). An increase in mixing time was also recorded as a result of an increase in water absorption; the increase recorded was 22-147 seconds for insoluble fibres compared to 27-95 seconds for soluble fibres. Longer mixing times compared to the control sample were observed in the 5% pea or lentil soluble fibres samples, despite a decrease in water absorption. The soluble fibres showed similar resistance to mixing and stability as the control dough. Lentil, pea and chickpea hulls showed less resistance to mixing and stability compared to the control dough (Dalgetty & Baik, 2006). These findings suggest that hydration and fibre types play a role in the mixing properties of dough samples and ultimately, end-product quality.

2.6.2. Effect on dough properties

Dietary fibre when present in dough makes significant changes to the mixing properties, handling ability, extensional behavior and pasting profile of the hydrated flour-fibre blends (Collar et al., 2007). The addition of fibre from a range of sources has been reported to decrease the extensibility of dough, whereas it has been reported to have a variable effect on the resistance to extension (Ktenioudaki & Gallagher, 2012). Other

studies have shown reduced extensibility but increased resistance to extension. It is evident that the type of fibre and quantity of substitution play a role in the dough extension parameters results.

Angioloni and Collar (2008) evaluated the processing effects of chickpea flour additions with an extensograph instrument. The extensibility of the dough after 45, 90 and 135 minutes was reduced by chickpea flour additions, with its value dropping by 7% to 38% of that of the control with the addition of 10% to 30% chickpea flour (Angioloni & Collar, 2008). The initial resistance to deformation (R50) decreased with increased additions of chickpea flour (10% to 30%). The control wheat dough exhibited the highest resistance to deformation after a 90-minute resting period (Angioloni & Collar, 2008). In contrast, the resistance to deformation of dough samples containing chickpea flour continuously increased with time, showing the highest resistance after 135 minutes (Angioloni & Collar, 2008). The parameter R50 predicts the dough handling properties and the fermentation tolerance (Angioloni & Collar, 2008). As a result, the increase in the value of this parameter promoted by chickpea flour additions suggests bad handling behavior and little dough tolerance during the fermentation stage (Angioloni & Collar, 2008). The R50 values appear to approach those of the control when only 10% chickpea flour is added (Angioloni & Collar, 2008). For the blends of 30% chickpea flour, data showed that the resulting dough became very weak, stability was decreased and the degree of softening increased (Angioloni & Collar, 2008). Additionally, the resistance to extension decreased for the blends containing 30% chickpea flour (Angioloni & Collar, 2008). This effect may be heightened by the presence in the chickpea flour of undesirable

enzymes or constituents that interact strongly with wheat gluten proteins and thereby inhibit development of desirable rheological properties (Angioloni & Collar, 2008).

Kteniodaki and Gallagher (2012) reported that resistance to extension increased following the addition of three different sources of fibres (carob, inulin, and pea), with inulin resulting in the highest increase of all three fibres. In this study, the extensibility of dough decreased for all fibre additions except inulin (Kteniodaki & Gallagher, 2012). Interactions between fibre and gluten proteins could explain these results. However, the chemical composition as well as the plant components of each dietary fibre source should be considered in this explanation (Kteniodaki & Gallagher, 2012). The Alveograph results from this study predicted the dough height response during fermentation and showed that the addition of fibres reduced loaf quality. The addition of inulin and carob fibre led to more stable doughs that could withstand longer proofing times (Kteniodaki & Gallagher, 2012).

2.6.3. Effect on bread quality

The effects on loaf volume of fibre fractions were evaluated in a study conducted by Pollard and colleagues (2002). The major effect of lupin flour on decreasing mixing time and increasing peak resistance was attributed to the fibre fraction. Lower gas retention rather than unsatisfactory gas production was attributed to increased mixing times and lower loaf volumes containing oat hull and cellulose fibre (Pomeranz et al., 1977).

It has also been postulated that gluten re-aggregation affected by fibre particles provided a more substantial explanation for the instability of gas cells. However, the principle that there is physical interruption of the gluten matrix by fibre particles cannot

be valid as even fine fibre particle sizes can worsen dough and end-product properties (Kteniodaki & Gallagher, 2012). The authors also found that reducing the particle size can intensify the negative effects of fibre. These authors suggested that the increase in surface area increases the interaction of gluten with active components such as ferulic acid, which impacts gluten's functionality. It should be noted that dough viscoelasticity in highly legume-replaced wheat matrices can be restored using structuring agents (Jagannadham et al., 2014). Structuring agents are used to prevent a disruption in the protein matrix that occurs in highly substituted flour baked products (Collar & Angioloni, 2009). The baking industry utilizes structuring agents such as hydrocolloids to ensure production of consistent baked products as well as to reduce staling and moisture loss during storage (Ho & Noor Azia, 2013).

2.6.4. Effect on bread staling

Many studies have evaluated the effects of dietary fibre additions on bread staling. However, the literature does not show conclusive results on how the dietary fibre impacts bread staling. Dalgetty and Baik (2006) evaluated breads fortified with hulls and insoluble cotyledon legume fibre and showed that these dietary fibre additions retained more moisture during storage. After 7 days of storage, the legume fibre fortified breads showed a decrease in moisture content of 7.7% while the wheat control bread showed a decrease of 9% in moisture content (Dalgetty & Baik, 2006). Sievert and colleagues (1990) evaluated partial additions of soy polysaccharide fibre in steamed bread. All soy polysaccharide fibre steamed bread samples showed an increase in crumb firmness compared to the control wheat bread, while the degree of firmness increased with an increased level of substitution (Sievert et al., 1990). This might suggest that the type of

fibre being substituted with the wheat flour may have different bread staling outcomes. Overall, the results in the literature are inconclusive as to whether a legume addition positively influences bread staling in a wheat composite bread product.

2.7. Nutritional advantages of bread fortification

2.7.1. Protein enrichment

Bread and bakery products have an important role in human nutrition. Generally, wheat bread has been considered to be a good source of energy and irreplaceable nutrients for the human body (Wronkowska et al., 2013). White bread is preferred by most consumers, while many bioactive compounds found in the grain, especially phenolic compounds, are mostly concentrated in the bran and aleurone layer. Legume bread fortification provides many nutritional advantages, which includes protein enrichment. Fortification delivers on providing both quality and quantity of proteins in the final bread product. A great deal of interest has been given to peas as a protein source.

2.7.2. Antioxidants

Over the last four decades a great deal of attention has been paid to antioxidants in the way of research. The literature has been centered on two areas of interest: (1) food preservation (2) potential health benefits. Although the term antioxidant originally referred to molecules that prevent the consumption of oxygen by human tissues, it has evolved to refer to the prevention of oxidative systems as a whole (Finley et al., 2011). An antioxidant would be a molecule that slows or prevents the oxidation of another molecule, and therefore can be considered as a reductant. It would be important to note, however, that not all reductants are necessarily antioxidants.

Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom (Bravo, 1998). The presence of polyphenols in plant food is largely influenced by genetic factors and environmental conditions (Bravo, 1998). Other factors, such as germination, degree of ripeness, type of processing, and storage also influence the content of plant phenolics. Natural polyphenols can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. They occur primarily in conjugated form, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar unit to an aromatic carbon atom also occur (Bravo, 1998). As naturally occurring antioxidants, phenolic compounds have been reported to possess beneficial bioactivities, including anti-allergen, anti-viral, anti-inflammatory, and anti-mutagenic properties (Dziki et al., 2014).

The efficiency of polyphenols as antioxidant compounds greatly depends on their chemical structure. Phenols themselves are inactive as antioxidants, but ortho- and para-di-phenolics have antioxidant capacity, which increases with the substitution of H atoms by ethyl or n-butyl groups. Antioxidants can be classified either as “primary antioxidants” (those which actively inhibit oxidation reactions) or “secondary antioxidants” (those which inhibit oxidation indirectly, by mechanisms such as oxygen-scavenging, binding pro-oxidants, etc.) (Craft et al., 2012). Much of primary antioxidant chemistry reactions can be grouped into the categories of hydrogen-atom transfer (HAT) and single-electron transfer (SET) reactions, both of which are applicable to phenolic antioxidant action (Craft et al., 2012). Phenolics are also considered to operate as secondary oxidants due

to their ability to bind with potentially pro-oxidative metal ions. There are two mechanisms of actions involved with antioxidants:

i. Chain breaking antioxidant activity is where the primary antioxidant donates an electron to the free radical present in a system (Shahidi, 2007).

ii. The secondary antioxidants act to remove reactive oxygen species (ROS) and reactive nitrogen species (RNS) initiators; thereby sequestering the chain-initiating catalysts in a system (Shahidi, 2007).

It would be important to note that it will be difficult to compare results obtained from different studies. In many cases, different assay methodologies, extraction methods, sample concentrations and units of measures are used in these studies. Therefore, finding a head to head comparison amongst various studies within the realm of antioxidants can be challenging.

2.7.2.1. Antioxidants in wheat and legumes

In legumes and cereals, flavonoids, phenolic acids and tannins are the primary antioxidants. Un-bound phytochemicals contribute more to total antioxidants in chickpeas, soybeans, yellow and green peas than bound phytochemicals (Han & Baik, 2008), whereas lentils have shown the opposite effect by contributing more antioxidants in a bound form versus the un-bound form (Han & Baik, 2008). In chickpeas, peas and soybeans there were no increases in bound phytochemicals as a result of hull removal. It appears that the hulls from chickpeas and soybeans are an insignificant source of bound phytochemicals (Han & Baik, 2008). Total phenolic content (TPC) for wheat has been

reported to be between 22 and 40 mg/100 g; and in chickpeas between 78 and 230 mg/100 g (Shahidi & Naczk, 1995). Based on these results, it would appear that legumes offer greater levels of polyphenols compared to wheat.

2.7.2.2. Effect of processing on antioxidant activity

It has also become clear that many health benefits from antioxidants in the diet may occur in response to relatively low exposures (Jensen et al., 2011). It has been postulated that certain levels of reactive oxygen species (ROS) are needed to stimulate many key processes in the body such as cell signaling and homeostasis. Therefore, removal of too many ROS may have negative implications to the human body.

A good portion of data has been collected looking at additions of dietary fibre into bread products including those from legume crops. However, limited research has been conducted that comprehensively evaluates the TPC and antioxidant capacity of these composite blend breads. Some antioxidant research has been conducted on grains or seeds, raw and thermally treated flours and some finished baked products such as bread. Other than compositional aspects of the bread, the effect of an anti-oxidative extract in food products depends on the type of antioxidants present in the extract and their solubility in the specific food matrix (Jensen et al., 2011).

There is ample evidence that the physical state of the food matrix plays a key role in the release, mass transfer, accessibility and biochemical stability of many food components (Palafox-Carlos et al., 2011). Antioxidants are often located in natural cellular compartments or within assemblies produced during processing. While some compounds are destroyed during food processing, many newer compounds might be introduced into the food system (Dziki et al., 2014). Food processing induces complex

physicochemical changes in food systems and has the potential to affect the availability of their antioxidant properties (Moore et al., 2009). Potential mechanisms through which processing may alter the antioxidant availability in food may include chemical, physical, or enzymatic reactions (such as oxidation or isomerization), leaching, or release of antioxidants from or their binding to food matrices (Moore et al., 2009).

The effect of baking on the concentration and antioxidant activities of phenols in bread has been studied (Moore et al., 2009). Baking resulted in an increase in free phenolic acids, while bound phenolic acids decreased in bread (Moore et al., 2009). The results obtained by Antiochia and colleagues (2012) indicated that in general flours show higher antioxidant capacity values than the corresponding breads and that crusts always show an antioxidant capacity larger than the crumb. Though the effect of baking appeared to be dependent on the type of baked product, type of phenolic, recipe and baking conditions, the wholegrain products should be considered as good sources of phenolic antioxidants. For Lakin, a hard white winter wheat variety, baked pizza crusts prepared under three different processing conditions had an increased level of extractable soluble free ferulic acid and a decreased level of soluble conjugated ferulic acid content compared with the unbaked sample (El-Sayed et al., 2013). This indicated that thermal treatments might enhance the release of free ferulic acid. For Trego, a hard white winter wheat variety, baking under all three tested thermal processing conditions had no significant influence on extractable level of soluble free ferulic acid, but decreased soluble conjugated ferulic acid levels were observed. It was noted that increasing the degree of thermal processing may decrease the extractable level of both soluble free and soluble conjugated ferulic acid regardless of wheat variety (Moore et al., 2009). In a

previous study, El-Sayed and colleagues (2012) found that the baking process improved antioxidant properties of the wholegrain bread, cookie and muffin. In their study, free phenolic acids significantly increased in bread, cookie and muffin while bound phenolic acids decreased in bread and were only slightly affected in the cookies and muffins.

Processing clearly plays a role in the release of phenolic compounds that directly impacts the TPC and AOX activity (Nayak et al., 2011). However, the literature is not univocal regarding baking effects on TPC. In fact, while Gelinas and McKinnon (2006) showed an increase of TPC, Leenhardt and colleagues (2006) reported that phenolic compounds are destroyed during the baking process. Moreover, the methods used for extraction and evaluation of antioxidant capacity varies, making it rather difficult to make a comparison amongst the studies and thus, extrapolating to formulate general conclusions.

2.7.2.3. Extraction method

It has been known that the yield of a chemical extraction depends on the type of solvents (with varying polarities and pH), extraction time, and temperature as well as on the chemical compositions and physical characteristics of the sample (Xu & Chang, 2007). Under the same condition of extraction time and temperature, the solvent used and the chemical properties of the food sample are the two most important factors. Oomah and colleagues (2011) found that ethanol was the best antioxidant extract for yellow peas in their research study. However, methanol as a solvent was not considered during their investigation.

It has also been postulated that the type of extraction should be considered when investigating the antioxidants in a food sample. It has been suggested that a chemical

extraction would be more suitable to assess the changes of antioxidant properties caused by bran enrichments (Menga et al., 2010), while an enzymatic extraction would be a suitable procedure to evaluate changes in antioxidant properties caused by processing (Menga et al., 2010).

Two or more radical scavenging capacity assays are required to investigate a selected antioxidant preparation since each assay involves different chemical mechanism(s) and may reflect different aspect(s) of the antioxidant properties. It has also been postulated that some assays are more sensitive to some compounds and not others. The use of one method has proven not to be effective in evaluating the antioxidant activity content in cereal grains and thus, multiple methods are recommended (Zhu et al., 2010).

2.7.2.4. Antioxidant activity assays

2.7.2.4.1. Total phenolic content (TPC)

Different assay protocols have been used to evaluate total antioxidants of bread, but the limiting factor in all of them would be the solubility of the compounds responsible for antioxidant behavior in the reaction media (Delgado-Andrade et al., 2010). For this reason, alcoholic and aqueous reaction media, as well as enzymatic hydrolysis, have been used to extract the active moieties of the food matrix (Delgado-Andrade et al., 2010). But whatever the extraction procedure, there is always an insoluble fraction of antioxidant material present in the food, among which insoluble proteins, tannins and melanoidins must be considered. Therefore, the total antioxidant capacity of systems has been systematically underestimated (Delgado-Andrade et al., 2010). In addition, overestimates of TPC may occur. For example, Folin-Ciocalteu (FC) reagent used in the

TPC method measures total phenolic content (TPC) without distinguishing between phenolic structures. FC reagent may react with sugars and peptides, many of which are soluble in aqueous solutions and therefore, may partly account for the phenolic content in the extracted samples.

2.7.2.4.2. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

DPPH would not be specific to any one particular antioxidant component, thus, applying to the overall antioxidant capacity of the sample. In the presence of an antioxidant, DPPH radicals are reduced and the reduction reaction results in the de-colorization of the solution. The loss of color, which can be monitored spectrophotometrically at 517 nm, reflects the reducing ability of the antioxidant towards the DPPH radical (Frankel, 2007). The DPPH assay does not use a substrate, and is considered an indirect method of antioxidant assessment (Dominguez, 2013). Indirect methods are used to measure the capacity of a molecule to reduce a stable free radical by hydrogen or electron transfer (Dominguez, 2013). As a result, this assay method provides no information on the protective activity of the antioxidant towards a specific food or biological system (Frankel & Meyer, 2000). DPPH has been commonly used as an assay for several reasons: it does not dimerize in either solid state or in solution; it can accept electrons or hydrogen atoms but can be difficult to be oxidized; and it would be both a simple and inexpensive test (Nenadis & Tsimidou, 2010). The DPPH method would be an approved method to evaluate food phenolics.

Nayak and colleagues (2011) evaluated processing effects by examining how extrusion influenced the flavonoids of potato and pea flour samples. At a substitution level of 35% (35:65, purple potato flour: dry pea flour), the extruded samples showed less

flavonoids than non-extruded ones. At a substitution level of 50%, no differences existed amongst the extruded and non-extruded samples. Finally, at a 65% substitution level the extruded samples showed less flavonoids than non-extruded ones. The results show that the impact of processing on the flavonoids was variable and that the sample composition from a substitution perspective may also play a role. In the same study, the DPPH antioxidant activity of legumes was decreased significantly by conventional and pressure boiling (Nayak et al., 2011). In contrast, Stanisavljevi and colleagues (2013) reported that cooking legume flour for 45 minutes at 100°C increased its DPPH activity. Based on these study results, it is evident that the type of processing influences the antioxidant activity of the sample.

El-Sayed and colleagues (2013) investigated the effect of baking temperature and length of baking and their effects on antioxidant activity in whole-wheat bread pizza crusts. For relative DPPH scavenging capacity (RDSC) the results indicated that increasing thermal treatment from 7 to 14 minutes at 204°C or from 204°C to 288°C for 7 minutes significantly increased RDSC between 50% and 82% for pizza crusts made from two wheat varieties (El Sayed et al., 2013).

2.7.2.4.3. Ferric reducing power (FRAP) assay

The ferric reducing power (FRAP) assay is a direct test of total antioxidant power. The reducing capacity of a compound is evaluated by this assay. In the FRAP assay, a single electron would be transferred from the AOX molecule to the oxidant. The assay is also non-specific, and compounds with lower redox potential than Fe+3 will initiate Fe+2 formation.

The FRAP values of legumes was decreased significantly by conventional and pressure boiling (Nayak et al., 2011). As previously noted, limited antioxidant research exists for finished legume products. From a raw material perspective, Durrazo (2013) showed that soft wheat flour (158.10 mg/100g; 2.28 μmol/g) was similar to the chickpea flour (149.87 mg/100g; 3.73 μmol/g) for both the TPC and the FRAP assay. Green and red lentil flours showed greater levels of TPC and FRAP activity (365.80 mg/100g to 737.32 mg/100g; 55.65 μmol/g to 140.32 μmol/g) compared to both the soft wheat and the chickpea flour. The unanswered question would be whether processing will increase or decrease these FRAP values in a finished product such as bread.

2.7.2.4.4. Metal chelation assay

Food, and in general, biological oxidation, are almost exclusively metal-ion promoted reactions and in this respect, iron being the most abundant metal ion, would be commonly involved (Kanner, 2010). Iron is an essential element for aerobic life and is required for the biosynthesis of a variety of iron-containing proteins and for DNA synthesis. The deleterious properties of iron are channeled and controlled within enzymes, whereas within iron binding and storage proteins, it would be sequestered in a poorly or non-reactive form, such as in transferrin, lactoferrin, and ferritins (Kanner, 2010). Ferritins are the main source of 'free' iron in cells of plant or animal origin.

Chelating agents can significantly affect the kinetics of lipid peroxidation induced by metal ions. Chelators, such as ferrozine, which stabilize ferrous ions in a low spin state, prevent electron transfer to oxygen when oxygen is present (Kanner, 2010). In many foods, iron promoted oxidation can be decreased by removal of metals from raw materials, and avoiding metal contamination from sources such as processing equipment,

food ingredients and packaging. When pro-oxidants cannot be removed from foods, their activity can be controlled by chelators or sequestering agents. Simple chelation of transition metals does not guarantee that they will not promote lipid oxidation (Kanner, 2010).

Han and Baik (2008) showed that the metal chelating activity of legumes showed a very different pattern compared with free radical scavenging and lipid peroxidation inhibiting activities. Chickpeas and peas exhibited greater chelating activities than lentils, but were lower in free radical scavenging and lipid peroxidation inhibiting activities.

Torres-Fuentes and colleagues (2012) evaluated the iron-chelating activity of chickpea hydrolysate sub-fractions and compared them to the chickpea protein hydrolysate. The purified sub-fractions (fractions that were further fractionated by gel filtration chromotography) showed the highest iron-chelating activity, between 11 and 17-fold higher than the chelating activity of the chickpea protein hydrolysate. A range of different iron-chelating activities was observed within these sub-fractions. Torres-Fuentes and colleagues (2012) indicated that a positive correlation existed between the histidine content (above 20%) while the peptide size was not a determinant in the metal chelation assay. Pownall and colleagues (2010) suggested that the presence of an imidazole ring in histidine contributes to the metal chelating activity. Pownall and colleagues (2010) examined compositional aspects of pea seed samples to try and target what was responsible for the metal chelating activity. There is limited research in the area of both raw and processed legumes and their respective phenolic content and antioxidant activities. Additional research needs to be conducted to identify what legumes are the

best metal chelators and elucidate what makes them good chelators of metal ions. Yellow pea seeds have been identified as good metal chelators; however, additional studies are required to evaluate yellow pea fortified foods and determine the effects of processing (Pownall et al., 2010).

2.7.2.4.5. Superoxide scavenging (SO) assay

In 1968, superoxide dismutase (SOD), a specific enzyme, was discovered whose function was the detoxification of the superoxide radical (Frankel, 2007). Superoxide is a highly reactive oxygen radical formed by a single electron reduction reaction that occurs during normal cell metabolism (Frankel, 2007). Superoxide radical anion would be produced as a result of the donation of one electron to oxygen. It has been postulated that superoxide radical anions are formed in foods when xanthine oxidase (XOD) acts on xanthine in the presence of molecular oxygen (Craft et al., 2012). Xanthine oxidase is present in bovine milk, while xanthine is found in caffeine, theobromine and theophylline, which can include chocolate, cocoa and tea, as well as foods containing these items.

Li and colleagues (2008) evaluated the superoxide anion scavenging of different chickpea protein hydrolysate (CPH) fractions. All samples in this experiment showed considerable scavenging abilities compared to the control, which did not undergo the pyrogallol autoxidation reaction. Addition of these CPH fractions at 2.0 mg/ml led to a range of superoxide anion scavenging activities between 35% and 69%. Each CPH fraction produced a different superoxide scavenging activity percentage. Therefore, further investigation to identify compositional differences between CPH fractions would be required. The results did show that chickpeas are effective superoxide anion

scavengers, but whether these activity levels increase or decrease due to processing still needs to be determined.

2.7.2.4.6. Comparison of antioxidant assays

There are a few studies that have shown a statistical correlation between TPC and DPPH assay results when investigating legume samples (Xu et al., 2007; Anton et al., 2008; Stanislavlijevic, 2013; Xu & Chang, 2007). This statistical correlation suggests that the phenolic content of the legume sample has a linear relationship to its DPPH scavenging activity. Therefore, it might be reasonable to conclude that the processing effects observed in a TPC assay could also be correlated to the results of a DPPH assay. Currently, the literature has not been able to show a statistical correlation in results between TPC and other antioxidant assays such as FRAP. However, legume research in the area of TPC, and antioxidant activity has been limited.

2.8. Effect of particle size on antioxidant activity

A reduction in flour particle size could positively influence bread quality attributes such as loaf volume but could also increase the surface area of flour particles leading to increased antioxidant activity. Hemery and colleagues (2011) evaluated the biochemical composition of sterols, folates, and alkylresorsinols as well as whole compounds such as phytic acid and ferulic constituents after milling (Hemery et al., 2011). Only the phytic acid and ferulic constituents showed a difference in their biochemical composition after milling (Hemery et al., 2011). A size reduction in the wheat bran led to an increase in chelating activity, reducing power, and TPC (Hemery et al., 2011), while the DPPH activity showed the opposite effect and decreased activity with an increase in the surface area of the wheat bran material (Hemery et al., 2011).

The effect of particle size on antioxidant activities has also studied by Moore and colleagues (2009) in whole-wheat pizza crusts. These researchers showed no statistical differences between two different particle sizes (20 and 80 mesh size) and their antioxidant activity. This result was consistent across the five extract assays used to evaluate both the antioxidant activity and phenolic content in the pizza crust samples (Moore et al., 2009). Regardless of the absence of particle size effects on antioxidant activity, this research identified that process parameters such as dough fermentation time, baking time and temperature have the potential to alter the antioxidant activity in whole-wheat pizza crusts. El-Sayed and colleagues (2013) also evaluated particle size in whole-wheat pizza crusts. The research outcomes were in agreement with the work conducted by Moore and colleagues (2009) since they reported that a reduced wheat bran particle size did not impact its antioxidant properties during processing (El-Sayed et al., 2013).

Zhou and colleagues (2004) evaluated the effect of micronization of aleurone and micronized aleurone in Swiss red wheat and reported an increase in extractable antioxidant properties following micronization; however, it was unclear whether these increases were a result of increased extraction surface area or other processes involved in micronization such as thermal effects. Another study by Cheng and colleagues (2006) found that bran particle size reduction increased its antioxidant properties. Unfortunately, this reduction in particle size also contributed to greater losses in antioxidant activity during storage and thermal processing. Evaluating the effect of particle size on antioxidant activity in finished products is complex and should consider all processing steps as well as flour storage.

2.9. Bioavailability of antioxidants

Nutritionists understand the importance of knowing a person's daily intake of dietary polyphenols, as well as the bioavailability of those ingested polyphenols. Little information has been documented about the absorption of polyphenols in the gastrointestinal tract and whether they are retained in the body after absorption. Chemical structure primarily determines the absorption and metabolism of food phenolics. The role of particle size or the concentration and extractability of phytochemicals in relation to the exposed surface area in digestion has not been well evaluated (Brewer et al., 2014). It has been suggested that after mastication, wheat bran particle size is slightly altered, and the bran is not significantly digested in the large intestine; the wheat bran then travels to the distal colon where it undergoes fermentation (Brewer et al., 2014). Hemery and colleagues (2011) reported that a reduction in wheat bran particle size was correlated with an increase in bioaccessibility of phenolic acids. This would suggest that the initial particle size is important from an availability perspective during digestion (Brewer et al., 2014).

The variability present in phenolic compounds has made the study of their bioavailability as well as their physiologic and nutritional effects challenging. It has been reported that un-bound phenolics are absorbed through the intestinal tract in both in vivo experimental rats and in vitro experiments of isolated rat jejenum (Bravo, 1998). However, in order for absorption to occur, the glycosides must be hydrolyzed to their corresponding aglycones. While bound phenolics are 15 to 30 times more effective at quenching peroxyl radicals than are un-bound phenols, these compounds are not absorbed

(Bravo, 1998). Therefore, it could be argued that an emphasis should be given to un-bound phenolics when evaluating phenolic enriched food products.

Dietary fibre, while increasing the viscosity of the luminal contents, may also reduce rates of antioxidant absorption. This occurs mainly by physically trapping the antioxidants within the fibre matrix in the chyme. Antioxidants within the fibrous particles must first be released into the continuous solution before they can be absorbed through the gut wall (Bravo, 1998). Han and Baik (2008) suggested that fibrous particles and their rate of release of antioxidants into the surrounding intestinal fluid would be inversely proportional to particle size and directly proportional to solute gradient. Additional physical properties, such as the state of the solute as well as the structure and surface properties of the particle, also influence the release of the antioxidants (Bravo, 1998). It is clear that more research is required to fully elucidate the effects of dietary fibre as well as surface area on the rates of absorption for antioxidant compounds.

CHAPTER 3: INCORPORATION OF YELLOW SPLIT AND WHOLE PEA FLOUR OF DIFFERENT PARTICLE SIZES INTO WHEAT PAN BREAD

Abstract

In the Western world, insufficient fibre in the diet has been identified as a possible cause of chronic disease. Therefore, the food industry has looked for palatable ways to increase the fibre content of food products. High-legume content breads contain about two to three times the dietary fibre of white wheat bread, and most bread can be labeled as high-fibre breads (6 g DF/100 g food). The practice of manufacturing high-fibre bread products requires very close attention to formulation and processing. Addition of too much fibre produces bread of poor quality in terms of texture, loaf volume and appearance. This study examined 10, 15, 20 and 25% additions of yellow split and whole pea flour with 0.5 and 0.75 mm particle sizes to a Canadian Wheat Red Spring (CWRS) and commercial wheat flour pan bread using the Canadian Short Process bake method. The objective was to evaluate hulled and hull-less pea flour additions and determine optimal substitution levels.

All percent additions of yellow split and whole pea flour resulted in a decrease in water absorption, dough development time and stability when compared to the CWRS and commercial control flours. The micro-extensibility test showed an increase in resistance to extension compared to the control dough with the CWRS flour while a decrease in resistance to extension compared to the control dough was observed with the PFM P500 commercial flour. A reduction in loaf volume (1%-34%) and impairment of textural properties were observed as the percent addition of yellow split and whole pea flour increased. A dilution of wheat protein occurred which lowered the amount of gluten

available to form a network, thus, causing poor gas retention. Crumb firmness and cell density increased while the whiteness of the crumb grain decreased with increased levels of yellow split and whole pea flour.

3.1. Introduction

Pulses are Canada's fifth largest crop and account for 10% of the market in regards to total crop production after wheat, barley, canola, and maize (Ma et al., 2015). The use of pulses as a food ingredient has been emerging in North America and Europe (Roberfroid, 2000). Incorporation of pulses into a wheat pan bread results in a functional food. Functional foods are foods that have a positive effect on human health beyond basic nutrition. Some researchers argue that functional foods promote optimal health while reducing the risk of disease. Pulses are also recognized as an inexpensive source of protein, complex carbohydrates, fibre, and minerals (Mohammed et al., 2012). Furthermore, they are rich in essential amino acids lysine and leucine and provide a balanced amino acid profile when combined with cereal grains (Mohammed et al., 2012). The protein in legume seeds ranges in content from 17 % to 40 %, contrasting with 7% to 15 % for cereals, and would be equal to the protein content of meat (18% to 25 %) (Bojnanska et al., 2012). Fibre content in cereal products also becomes higher with the addition of pulses.

In bread making, the use of pulse flour produces less favourable breads due to non-wheat proteins and fibre, which deteriorate the rheological properties of dough, and impair the quality of bread. While the addition of pulse flours offers many health benefits, incorporation of them into wheat dough has many quality implications (Sadowska et al., 2003). Therefore, an investigation will be conducted to evaluate the

mixing properties, dough handling and quality attributes of the composite wheat-pea and control bread products. A10g mixograph and a farinograph instrument have been selected to empirically measure the dough mixing properties (Mohammed et al., 2014; Sadowska et al., 2013; Dowell et al., 2008) of the wheat-pea composite dough samples compared to the CWRS and PFM P500 commercial control wheat flours. Extensional parameters of the wheat and wheat-pea composite dough samples will be evaluated using a Kieffer dough and gluten extensibility rig (Suchy et al., 2000). An evaluation of bread quality will include the following test measurements loaf volume, specific volume, crumb color, firmness and cell density. Finally, a bread score or overall quality measurement for the bread samples will be calculated using various imaging measurement parameters captured by an ASE instrument.

An RVA instrument will be used to evaluate the viscosity of the wheat and yellow pea flour samples. The RVA can be used to evaluate the pasting and gelling behavior that occurs during cooking and cooling cycles that are inherent with dough baking and bread cooling (Angioloni & Collar, 2012). More specifically, the RVA parameters peak viscosity, pasting temperature, and setback during cooling have been highly correlated with bread staling kinetics parameters (Collar, 2003). These parameters have been valuable predictors of bread firming behavior during storage as well as the sensory scores for fresh baked goods (Choi et al., 2012; Collar, 2003; Collar et al., 2006).

In addition to the RVA thermal study, a staling study will be conducted using a DSC instrument. The DSC instrument will be used to bake the bread dough samples and then evaluate a staling effect based on changes to the amylopectin melting enthalpy, which will be used as a measure of amylopectin retrogradation. Other researchers have

utilized the DSC instrument to evaluate the measurement of thermal changes in bread during aging and its association with structure alterations in the starch fraction (Fearn & Russell, 1982). Defloor and Delcour (1999) evaluated bread sample aging using a DSC instrument because the instrument proved to be effective in measuring retrogradation enthalpy in small and homogenous dough samples.

3.2. Materials and methods

3.2.1. Materials

Flour samples included a 2013 Buhler milled AC Carberry wheat flour supplied by the Cereal Research Centre (Winnipeg, MB), commercially milled Prairie Flour Mills (PFM) P500 Strong Bakers Flour (Elie, MB), and Best Cooking Pulses (BCP) regular yellow split and whole pea flour (Portage la Prairie, MB). Bread formulation details for the Canadian Short Process (CSP) bake method have been supplied in Table 3.1.

Table 3.1 Bread Formulation (% flour weight basis)

Ingredient	Amount (%)
Flour, adjusted to 14% moisture basis	100.0
Salt (NaCl)	2.4
Sugar	4.0
Fresh yeast (Fleischmann's)	3.0
Malted barley flour (Breiss)	1.0
Shortening (Crisco)	3.0
Ammonium Phosphate (0.1% $NH_4H_2PO_4$ solution)	1.0
Ascorbic acid (150 ppm)	1.0
Whey powder (Health First)	4.0
Dough water	BAB (baker's absorption)

Modified method of Preston (1982).

3.2.2. Methods

3.2.2.1. Preparation of composite flours

The AC Carberry wheat sample was tempered to 16.2% moisture overnight and

an experimental mill was used to produce straight-grade flour (Buhler MLU-202, Uzwil, Switzerland). A rotor beater mill (Retsch SR300, Haan, Germany) was used to re-grind the parent BCP yellow split and whole pea flours to produce the two particle sizes, which included: 0.5mm and 0.75mm . A Malvern Mastersizer 2000 (Malvern, UK) was used to evaluate the particle size distribution for both the parent flour material as well as the re-ground materials. The composite flour sample blends (100 g) were prepared by blending 0, 10, 15, 20 or 25% amounts of the yellow split and whole pea flour for each particle size on an as-is flour basis with the AC Carberry or the PFM P500 control wheat flours.

3.2.2.2. Analytical methods

All samples were measured for moisture using air-oven method (AACC 44-15A) and ash content (AACC 08-01.01). Total nitrogen (N) was determined by method AACC 46-13 using the Leco FP-528 system (Leco Corp., St. Joseph, MI) and percent protein was calculated using a protein factor of 5.7 for wheat and 6.25 for legumes (Fenn et al., 2010).

Farinograph test was performed according to AACC 54-21.01 method using a Farinograph Do-Corder 2200-3 instrument with a 50g bowl (CW Brabender Instruments, Inc., South Hackensack, NJ). Flour weight was based on 14% moisture basis. Analyses were performed in duplicate due to limited raw flour materials.

Mixograph analysis was performed according to the AACC 54-40 method using a water absorption that centered the farinograph curve on the 500- Brabender unit (BU) line and flour weight based on 14%mb using a 10g bowl (National Manufacturing, TMCO, Inc., Lincoln, NE). A circulating water bath set at 30°C was used for the mixograph. Analyses were performed in duplicate due to limited raw flour materials.

The pasting properties of flours were analyzed using AACC method 76-21 'standard 1' profile with a *Rapid Visco Analyzer* (RVA) instrument (Perten Instruments Australia, Macquarie Park, Australia). Analyses were performed in duplicate due to limited raw flour materials.

3.3 Dough quality

3.3.1. Oven spring (mm)

The oven spring of the thermosetting dough can be calculated from two measurements: an initial height measurement of the dough after proofing in a cabinet (37.5°C, 85% RH) for 75 minutes and a final height measurement of the bread after baking (200°C) for 25 minutes. The difference between these recorded measurements is the oven spring of the sample (Equation 1). A commercial electronic caliper was used to measure the heights of the various samples.

Oven Spring (mm)= bread height (mm)-proof dough height (mm) (1)

3.3.2. 2-gram micro-extension test

The 2-gram mixograph and texture analyzer were used to evaluate dough properties in a small-scale extension test using AC Carberry flour or PFM P500 and four composite blends of the AC Carberry or PFM flour with 10, 15, 20 and 25% yellow split and whole pea flour for each particle size. On an initial mixograph run, used to determine the peak dough development time, 2g of flour (14%mb), 2% salt solution and corrected water amount based on the farinograph test were mixed and ran until the dough sample began to break down. The second mixograph was run under the same conditions but mixing was stopped at peak development of the dough based on the initial mixograph run. The dough was removed gently from the pins, rounded into a ball and placed over three channels of a

Teflon coated block. The Teflon block was prepared by placing non-adhesive, mineral-coated Teflon strips in between the channels. The upper half of the block was placed in position and tightly clamped, which distributed the dough equally over the channels to yield dough strips with a uniform geometry. The dough was rested for 45 minutes in the TA-XT2 cabinet (30°C, 95%RH). The individual dough strips were then separated from the Teflon strips, positioned across the Kieffer rig dough holder, and immediately tested on the TA-XT2 (Stable Microsystems, Surrey, UK) with a hook speed of 3.3 mm/sec and a trigger force of 1gf. Resistance to extension (Rmax) was measured as the force (gf) at maximum resistance. Extensibility (E) was measured as the distance (mm) of extension at breaking point of the dough; and area of the curve (EA) was also measured (gf.mm). Reported measurements for this test included: E, EA and Rmax.

3.4. Bread making

The Canadian Short Process (CSP) method with slight modifications was used for bread making. In a glass jar 100 grams of flour adjusted to 14% moisture basis was combined with the following ingredients: 2.4 grams salt, 4.0 grams sugar, 4.0 grams whey powder and 3.0 grams Crisco shortening. Prior to mixing (National Manufacturing, TMCO, Inc., Lincoln, NE), the following ingredients were introduced to the formulation, 1.0 gram of Breiss malted barley flour, 3.0 grams fresh compressed Fleischmann's yeast, 1.0 ml ammonium phosphate monobasic and 1.0 ml of 150-ppm ascorbic acid. Distilled water, based on the 14% corrected value generated by the Brabender Farinograph instrument at 63.0 rpm, was also added.

The dough was mixed at 140rpm to peak development and then carefully removed from the pins and placed into a porcelain bowl, which was placed into a commercial

warming cabinet at 30° C for 15 minutes. The dough was then hand punched seven times and placed back into the warming cabinet for an additional 15 minutes. The dough was then hand rolled twice on a flat and floured surface. This dough piece was put through a sheeter three times on the respective settings: 11/32" gap, 3/16" gap, and finally 1/8" gap (Canadian Grain Commission, Grain Research Laboratory sheeter). The sheeted dough was then put through a molding device for 30 seconds (Canadian Grain Commission, Grain Research Laboratory molder). The molded dough piece was placed into a greased bake pan and put into a proofing cabinet for 75 minutes (37.5° C, 85%RH). After the fixed proofing time, the pan was put into a National Oven at 200°C for 25 minutes. The bread was cooled for 25 minutes and stored in a plastic Ziploc bag overnight. For testing purposes, these day one bread samples were frozen, freeze-dried and then ground using a classic grain mill (NutriMill Inc., St. George, Utah).

3.5. Bread quality

3.5.1. Loaf volume (cm^3)

A rapeseed displacement method was used to establish the loaf volume (AACCI Method 10-05.01, National Pup Loaf Volumeter)**.** Once the volumeter was calibrated with a calibration block of known size (volume of block, cm^3), a cooled loaf of bread was placed into it (25 minutes after baking). The upper portion of the volumeter was placed back into position and locked down. The seed lever was then opened allowing the rapeseeds to fall into the bottom drawer. The level of rapeseed was then measured (volume of rapeseeds with loaf of bread, cm^3). The calculation for loaf volume was:

Loaf Volume (cm^3) = volume of block (cm^3) +volume of rapeseeds with loaf of bread (cm^3) (2)

3.5.2. Specific loaf volume (cm^3 /g)

Specific loaf volume was also determined. The weight of the bread loaf (g) was measured 25 minutes post baking. The following calculation was used for specific volume:

Specific Loaf Volume (cm^3/g) = loaf volume (cm^3) / weight of the loaf (g) (3)

3.5.3. Crumb color

Bread crumb color was measured in CIE color coordinates (L* a* b*) with a Minolta spectrophotometer (Model CM-525i, Minolta, Osaka, Japan) set at 2° observer and "C" illuminant. Color was assessed from coordinate L* measuring brightness, a* measuring yellowness and b* measuring redness in the sample. The color meter was placed on two slices of bread in their center and color determinations were made in duplicate for each baking trial. The following calculation was used to evaluate the whiteness of the samples:

Whiteness Index= $100 - \sqrt{\{(100-L^*)^2+a^{*2}+b^{*2}\}}$ (4)

3.5.4. Bread firmness

Bread crumb texture was determined with a TA-XT2 analyzer (Stable Microsystems, Surrey, UK) fitted with the Texture Expert software. A 36 mm diameter cylindrical aluminum probe was used to conduct a Bread Firmness Test (AACC 74-09). This test was conducted 18 hours after baking on two stacked mechanically sliced bread slices (thickness of 25 mm). The probe compressed the sample until it has indented the slices to 40% of the original product height. Analyses were performed on two bread loaves during each baking trial and a total of 3 baking trials were conducted.

3.5.5. ASE bread scoring system

A single slice of bread was placed into a drawer of the ASE instrument (American Institute of Baking (AIB)). The instrument takes images of the bread slice and completes calculations to yield quality parameters of the bread slice, which included: loaf volume, cell density, cell size, color and a bread score. This instrument was designed to replace the subjective nature of the human evaluation with an objective one.

3.5.6. Differential scanning calorimeter (DSC)

A DSC (DSC Q200, TA Instruments, Waters, LLC, New Castle, DE) was used to evaluate the gelatinization properties of the bread dough samples. Indium was used to calibrate the instrument. Samples of dough were prepared as described in the CSP bread making section (without yeast and shortening). Dough samples of 10-20mg were weighed into stainless steel pans and sealed. The methodology outlined by Leon et al. (1997) with slight modifications (Sanz-Penella et al., 2010) was used to emulate the temperature profiles in the center of the bread crumb during baking under calorimetric conditions. Samples were kept at 30°C for five minutes, then heated from 30 to 130°C at a rate of 11.7 °C/minute, and kept at this temperature for twelve minutes before being cooled back to 30°C.

To evaluate amylopectin retrogradation or aging of the sample, heated-cooled pans were stored at room temperature for up to 72 hours. The stored pans were heated in the calorimeter from 30-130°C, at 10°C/minute. An empty stainless steel pan was used as a reference. Three replicates were conducted for PFM P500 control and 10% yellow pea and wheat composite flour blend dough's. The parameters measured for this test included: onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c)

of the endothermic amylopectin retrogradation curve. Straight lines were drawn between T_o and T_c and the enthalpies were calculated as the area enclosed by the straight line and the endotherm curve. The enthalpies were expressed in Joules per gram of dough sample.

3.6. Statistical analysis

All results were calculated as means from a triplicate analysis**.** Statistical analysis, using a mixed-design ANOVA test in SAS (Statistical Analysis Software 9.3, Cary, NC), was conducted to compare these means. Natural log and square root transformations were conducted on non-Gaussian distributed data sets; determination of the data sets distribution was confirmed by the Shapiro-Wilk test (Buthmann, 2011). A Tukey's post-hoc analysis was carried out to determine which means were significantly different from each other for both the flour and bread samples. Possible interaction effects that may influence the quality of the dough and final bread product(s) were also considered in the analysis: base control wheat flour, type of yellow pea flour (split or whole), particle size (0.5 and 0.75 mm) and level of flour substitution (10, 15, 20 and 25%, respectively). Results were considered significant at a confidence level of $p < 0.05$.

3.7. Results and discussion

3.7.1. Proximate composition

The moisture content, protein and ash content for the wheat and yellow split and whole pea flour samples are presented in Table 3.2. The yellow pea flour protein values were 29.79% for the split and 21.06% for the whole pea flour. The compositions of the pea flour samples were comparable with the results previously reported by Zhou et al. (2005). The AC Carberry wheat flour protein content was reported in this study to be 10.86%, which was 2% lower than what was reported by DePauw et al (2011). The AC

Carberry protein content falls below typical Canadian bread wheat flour. The ash content reported in this study is 0.2% higher than the study conducted by DePauw et al (2011). The commercial flour used, PFM P500 wheat flour, has a protein content of 13.08%, which falls in line with typical bread wheat flour.

Table 3.2 Proximate Compositions for Raw Materials

	Ash Content (%)	Moisture Content (%)	Protein Content (%)
PFM P500 Flour	0.53±0.01	12.10±0.00	13.08±0.03
AC Carberry Flour	0.61±0.02	13.93±0.06	10.86±0.02
Yellow Split Pea Flour	3.23±0.01	7.90±0.00	29.79±0.03
Yellow Whole Pea Flour	2.73±0.03	12.30±0.00	21.06±0.06

14% flour moisture basis

3.7.2. Particle size distributions

A Malvern Mastersizer 2000 instrument used laser diffraction to determine the particle size distribution based on volumetric means for both the yellow split and whole pea flour samples. The particle size distributions for the yellow split and whole pea flours are presented in Table 3.3. The results illustrate that using a larger screen size produces a larger particle size in both the parent yellow split and whole pea flours. In addition, the volumetric mean of the sample is higher than the 0.50 distributions in both parent yellow split and whole pea flour materials. The particle sizes 0.5 and 0.75 mm for the yellow split and whole pea flour were evaluated for the mixing, dough, bread, RVA and DSC parameter testing that was conducted.

Table 3.3 Particle Size Distributions for Raw Flour Materials

Yellow Pea Flour	Volume Weighted Mean (um)	d 0.10 (um)	d 0.50 (um)	d 0.90 (um)
Whole (parent)	390	20	342	843
Split (parent)	45*	4	24	108
Whole (0.5mm)	133*	8	42	377
Whole (0.75mm)	236*	14	188	560
Split (0.5mm)	32	2	16	63
Split (0.75mm)	38	3	21	91

Mastersizer software analysis output was based on an average of three subsamples. Asterisk (*) indicates duplicate analysis for the noted samples.

.

3.7.3. Mixing properties

3.7.3.1. Farinograph test

Four parameters were evaluated from the farinograph test. They included: water absorption (%), dough development time (min), stability (min) and mixing tolerance index (FU). The farinograph test was conducted on both the AC Carberry and PFM P500 control wheat flours and the composite wheat and yellow split and whole pea flour blends, which included 10, 15, 20 and 25% substitution levels. The farinograph parameters provide insights into the quality of the flour and ultimately, suitability for final product usage. The farinograph uses blades for its mixing action. The farinograph results are presented in Tables 3.4 and 3.5 and the statistical analysis results are presented in Table 3.6. The results presented in Tables 3.4 and 3.5 indicated there was a reduction in water absorption when yellow split or whole pea flour was added to either control wheat flours. The reduction in water absorption was in agreement with one study that evaluated a 5% addition of chickpea flour (Mohammed et al., 2012). However, many studies support an increase in water absorption when legume flours are partially

substituted for wheat flour (Mohammed et al., 2014, Bojnanska et al., 2012, Fenn et al., 2008 and Kohajdova et al., 2013). It has been hypothesized that the increase in water absorption is attributed to the increase in protein content (Mohammed et al., 2014).

3.7.3.1.1. Farinograph absorption

The farinograph absorption parameter was reported to be statistically different for base flours AC Carberry and PFM P500, the yellow split and whole pea flour types as well as the substitution level (Table 3.6). The particle sizes 0.5 and 0.75 mm showed no statistical effect for this mixing parameter (Table 3.6). This statistical investigation also reported several significant interactions, which included a two-way interaction between the base control wheat flour and the level of yellow pea flour substitution as well as the type of yellow pea flour and the level of substitution (Table 3.6).

The PFM P500 commercial flour was reported to have a greater water absorption compared to the AC Carberry experimental flour. An increase in either yellow split or whole pea flours in the composite wheat-pea flour blends resulted in a decrease in the farinograph absorption. However, the yellow whole pea flour compared to the yellow split pea flour exhibited less of an effect for this parameter. The decrease in this parameter was in response to the level of substitution in the composite samples. Mohammed and colleagues (2014) showed an increase in farinograph absorption with the addition of chickpea flour. It has been postulated that proteins have functional properties, which include water uptake capacity and mixing strength (Mohammed, 2014). It is clear that the proteins in the pea flour are not exhibiting the same water uptake as the chickpea flour. As with increased levels of protein in a flour sample, increased levels of dietary fibre has also been reported to increase the water absorption of composite samples.

Dalgetty and Baik (2006) validated this finding by reporting the addition of insoluble fibres from pea, lentil and chickpea flours into hard white spring wheat flour increased the water absorption in these composite samples. An increase in water absorption due to an increase in dietary fibre in the composite wheat-whole pea blends did not result.

Table 3.4 Farinograph & Mixograph Results for AC Carberry Control & Composite Yellow Split & Whole Pea Flour Blends for 0.5 & 0.75 mm Particle Sizes

	FAB (%)	DDT (min)	Stability (min)	MTI (FU)	MDT (min)	Peak Dough Resistance (%)
AC Carberry Control	64.8±0.07	5.6±0.92	8.8±0.35	41.0±1.4	4.6±0.42	24.8±0.82
Split 0.5						
10%	58.6±0.07	6.6±0.14	5.6±0.50	59.5±0.7	3.0±0.22	30.0±0.75
15%	58.4±0.64	6.1±0.21	3.4±0.28	85.0±11.3	3.1±0.05	28.3±1.65
20%	56.8±0.14	5.9±0.21	2.3±0.00	107±11.3	3.1±0.29	26.9±1.85
25%	55.2±0.64	5.7±0.21	2.0±0.21	123±29.0	3.5±0.04	23.6±1.29
Split 0.75						
10%	58.2±0.21	6.4±0.21	5.4±0.57	54.0±2.8	2.8±0.03	29.6±1.64
15%	58.1±1.77	6.1±0.57	3.7±0.57	64.5±24.8	3.1±0.08	27.2±1.34
20%	57.4±0.07	5.2±0.28	2.3±0.00	112±12.0	3.0±0.26	26.0±4.75
25%	56.1±0.07	5.6±0.21	1.9±0.14	135±12.0	3.5±0.01	24.2±1.95
Whole 0.5						
10%	57.8±0.35	7.7±0.92	10.7±0.49	23.0±1.4	3.2±0.21	32.1±2.58
15%	58.6±0.71	8.5±0.71	8.1±1.27	34.0±0.7	2.8±0.02	30.1±0.64
20%	58.4±0.14	5.9±0.00	6.2±0.21	43.0±1.4	3.2±0.10	29.5±1.70
25%	57.9±0.21	6.2±0.21	5.1±0.14	46.0±1.4	3.3±0.03	26.9±1.71
Whole 0.75						
10%	57.6±0.00	7.1±1.84	9.9±1.20	23.0±1.4	2.9±0.23	33.9±3.34
15%	59.5±0.00	7.7±0.00	6.7±0.49	29.0±1.4	2.8±0.27	33.1±1.94
20%	58.2±0.07	6.7±0.50	4.4±0.00	64.5±2.1	2.9±0.06	31.3±1.32
25%	57.2±0.28	6.2±0.50	3.2±0.14	82.0±7.1	3.1±0.00	29.3±1.20

FAB (Farinograph absorption), DDT (Farinograph dough development time), Stability (Farinograph stability), MTI (Farinograph mixing tolerance index), MDT (Mixograph development time), Peak Dough Resistance (Mixograph peak dough resistance)

Table 3.5 Farinograph & Mixograph Results for PFM P500 Control & Composite Yellow Split & Whole Pea Flour Blends for 0.5 & 0.75 mm Particle Sizes

		FAB (%)	DDT (min)	Stability (min)	MTI (FU)	MDT (min)	Peak Dough Resistance (%)
PFM P500 Control		62.8±0.28	11.2±0.00	16.3±0.14	16.0±1.4	5.8±0.33	31.6±0.32
Split 0.5							
	10%	62.1±0.14	9.2±0.14	10.4±1.13	16.0±4.2	3.8±0.40	30.8±1.05
	15%	61.8±0.64	6.6±1.34	7.9±0.78	37.0±11.3	3.8±0.06	28.3±2.37
	20%	60.7±0.21	6.9±0.49	5.0±0.14	77.0±2.8	3.6±0.64	25.5±1.15
	25%	59.9±1.27	7.4±0.21	3.9±0.71	72.0±2.1	3.6±0.41	26.0±1.70
Split 0.75							
	10%	60.9±0.57	9.3±2.19	11.2±1.13	33.0±18.4	3.6±0.04	31.0±0.90
	15%	60.3±0.07	8.5±1.13	7.1±0.07	52.0±12.7	3.4±0.20	28.5±0.16
	20%	60.1±0.35	6.5±0.28	5.2±0.14	68.5±10.6	3.6±0.13	25.6±1.07
	25%	59.5±0.35	6.3±0.35	3.5±0.35	78.0±6.4	3.8±0.20	22.9±1.65
Whole 0.5							
	10%	62.3±0.42	9.4±3.54	12.8±0.07	23.5±0.7	3.6±0.57	33.9±2.61
	15%	61.2±1.06	11.2±3.04	11.0±2.69	34.0±7.1	3.3±0.14	33.9±2.01
	20%	61.2±0.14	7.3±0.57	9.9±0.28	50.5±9.2	3.4±0.05	31.9±0.71
	25%	61.3±0.85	7.5±1.13	6.7±2.40	57.0±8.5	3.3±0.29	29.6±1.13
Whole 0.75							
	10%	60.1±1.98	7.0±0.64	12.2±1.48	15.0±12.7	3.6±0.35	35.4±0.62
	15%	61.0±1.34	7.2±1.91	8.1±0.57	27.5±16.3	3.5±0.11	33.8±0.18
	20%	61.0±0.28	7.1±1.27	9.3±0.00	21.5±10.6	2.9±0.12	32.2±2.17
	25%	61.3±0.92	7.2±1.63	6.35±0.07	57.0±9.9	3.3±0.69	29.1±0.44

FAB (Farinograph absorption), DDT (Farinograph dough development time), Stability (Farinograph stability), MTI (Farinograph mixing tolerance index), MDT (Mixograph development time), Peak Dough Resistance (Mixograph peak dough resistance).

Table 3.6 ANOVA Table with Significance of Main Effects & Interactions for Mixing Parameters

Effect	FAB (%)	DDT (min)	Stability (min)	MTI (FU)	MDT (min)	Peak Dough Resistance (%)
Base flour						
AC Carberry	57.7	6.4	5.0	68.0	3.1c	29.1
PFM P500	61.1	7.7	8.1	45.3	3.5b	29.9
Substitution (%)						
0	63.8	8.4	12.5	28.5	5.2a	28.2
10	60.0	7.5	9.3	30.9	3.3b	32.1
15	59.8	7.9	7.4	46.6	3.2b	30.4
20	59.2	6.4	5.6	68.2	3.2b	29.0
25	58.5	6.5	4.1	81.1	3.4b	26.4
Type						
Yellow Split Pea	58.9	6.6	5.0	73.8	3.4b	27.3
Yellow Whole Pea	59.8	7.5	8.1	39.6	3.2c	31.6
Particle size (mm)						
0.5	59.5	7.3	6.8	56.3	3.3b	29.2
0.75	59.3	6.9	6.3	57.1	3.2b	29.8
				P- value		
Base flour (B)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Substitution (S)	<0.001	0.001	<0.001	<0.001	0.19	<0.001
Type (T)	<0.001	0.01	<0.001	<0.001	0.01	<0.001
Particle size (P)	0.28	0.16	0.03	0.73	0.12	0.21
B x S	<0.01	0.91	0.02	0.06	0.06	0.39
B x T	0.98	0.43	0.07	<0.001	0.20	0.05
B x P	0.12	0.47	0.19	0.09	0.80	0.10
S x T	0.01	0.56	0.03	0.002	0.21	0.74
S x P	0.95	0.64	0.79	0.06	0.61	0.74
T x P	0.31	0.06	0.04	0.96	0.67	0.10
B x S x P	0.71	0.70	0.97	0.01	0.84	0.54
B x S x T	0.04	0.93	<0.001	0.19	0.53	0.45
B x P x T	0.08	0.04	0.14	0.01	0.60	0.56
S x P x T	0.16	0.04	0.94	0.49	0.49	0.79
B x S x P x T	0.76	0.29	0.59	0.98	0.69	0.69

Data are means of triplicates. P values <0.05 are statistically significant. Letters have been added to the treatment main effects when interactions were not statistically significant.
FAB (Farinograph absorption), DDT (Farinograph dough development time), Stability (Farinograph stability), MTI (Farinograph mixing tolerance index), MDT (Mixograph development time), Peak Dough Resistance (Mixograph peak dough resistance)

.

3.7.3.1.2. Dough development time

The farinograph dough development time parameter was statistically different for base flours AC Carberry and PFM P500, the yellow split and whole pea flour types as well as the substitution level (Table 3.6). The particle size showed no statistical effect for this mixing parameter (Table 3.6). This statistical analysis did not show interactions amongst these independent variables as well.

The PFM P500 commercial flour was reported to have a greater dough development time compared to the AC Carberry experimental flour. The wheat-pea composite blends had a decrease in dough development time compared to the control wheat flours. It was also reported that this effect was larger with the yellow split pea flour compared to the yellow whole pea flour. Other researchers have reported increases in dough development time as well as non-linear responses to various legume substitutions in composite flour blends (Sadowska et al., 2003, Mohammed et al., 2012 & 2014, Kohajdova et al., 2013 and Bojnanska et al., 2012). Sadowska and colleagues (2003) evaluated raw and germinated pea flour additions milled from Esla pea seeds into wheat dough and reported a similar outcome; the dough development time increased as the amount of pea flour increased in the wheat-pea composite sample. The development of dough is the result of interactions among various flour components during mixing. Water additions play an important role in hydrating the proteins found in flour and an optimum amount of water is needed to develop a cohesive and viscoelastic dough. Optimum water levels differ from flour to flour depending on the quantity of protein and other particles contained in the flour sample. Protein content has been recognized as an important

determinant of how wheat flour will absorb water during dough mixing (Bakare et al., 2016). During mixing of the dough, disulfide bonds are formed, broken and re-formed, until the proteins form a network throughout the dough (Bakare et al., 2016). In composite flour samples, the influence of starches, fibre from non-wheat source, and relatively higher damaged starch in the yellow pea flours on the absorbed water may play a more important role than the effect of protein content in the flour blends as the substitution level of pea flour for wheat flour increases. The inclusion of yellow pea flours in the composite blends increased the starch and fibre contents, while decreasing the quantity and quality of protein needed to sustain the viscoelastic behavior inherent with typical wheat dough.

3.7.3.1.3. Farinograph stability

The farinograph stability parameter was statistically different for base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types as well as the substitution level and particle size (Table 3.6). This statistical investigation also indicated several significant interactions, which included a two-way interaction between the base control wheat flour and the level of yellow pea flour substitution; and a three-way interaction between the base control wheat flour, the level of substitution and the type of yellow pea flour (Table 3.6).

The PFM P500 commercial flour was reported to have greater stability compared to the AC Carberry experimental flour. The wheat-pea composite blends showed a decrease in dough stability compared to the control wheat flours. Fenn and colleagues (2010) also reported a decrease in stability with a yellow pea protein addition into CWRS and CWES wheat flours. A decrease in stability was also shown in a study evaluating raw

and germinated pea flour samples (Sadowska, 2002) as well as other legume flours (Kohajdova et al., 2013). In a study evaluating lentil and chickpea flour, the authors noted an increase in stability at 10% and then a decrease as the substitution level increased in the composite blends (Bojnanska et al., 2012). Mohammed and colleagues (2014) also noted this effect, or trend in the data, when they evaluated chickpea flour additions. Sanz-Penella and colleagues (2010) attributed the increase in both the development time and stability to the high water-binding capacity of the pea product, which results in the gluten phase being compromised during mixing.

3.7.3.1.4. Farinograph mixing tolerance index

The farinograph mixing tolerance index parameter was reported to be statistically different for base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types as well as the substitution level (Table 3.6). The particle size showed no statistical effect for this mixing parameter (Table 3.6). This statistical investigation also indicated several significant interactions for the mixing tolerance index parameter, which included a two-way interaction between the base control wheat flour and the type of yellow pea flour; and the level of yellow pea flour substitution by the type of yellow pea flour. A three-way interaction was also reported between the base control wheat flour, the level of yellow pea flour substitution and the type of yellow pea flour; as well as the base control wheat flour, the type of yellow pea flour and the 0.5 and 0.75 mm particle sizes for the yellow pea flours (Table 3.6).

The PFM P500 commercial flour had a lower mixing tolerance index compared to the AC Carberry experimental flour. The wheat-pea composite blends exhibited an increase in the mixing tolerance index compared to the control wheat flours. The increase

in mixing tolerance index would suggest that the loading of the yellow split and whole pea flour negatively influenced the mixing tolerance of the sample. Deshpande and colleagues (1983) showed an increase in mixing tolerance index when bean flour was substituted at 10, 20 and 30% into wheat flour. It is evident that partially substituting a legume flours into wheat flour influences the mixing tolerance index of the sample. The higher the mixing tolerance index, the lower the resistance of the dough to mechanical damage. The results indicated that fibre decreased the tolerance of wheat flour to mixing (Almeida et al., 2010). It has been postulated that this resistance to damage cannot be explained on the basis of dilution, but would support an interaction between the dietary fibre and wheat proteins in the dough sample (Almeida et al., 2010).

3.7.3.2. Mixograph test

Two parameters were evaluated after the 10g mixograph test was conducted and they included: mixing development time (min) and peak dough resistance (%). The mixograph test was conducted on both the AC Carberry and PFM P500 control wheat flours and the composite wheat and yellow split and whole pea flour blends, which included 10, 15, 20 and 25% substitution levels. While the mixograph also illustrates the mixing parameters of the flour sample like the farinograph, the method of evaluation is slightly different in that pins, rather than blades, are responsible for the mixing action. The mixograph results have been presented with the farinograph results in Tables 3.4 and 3.5.

3.7.3.2.1. Mixograph development time

The mixograph development time parameter was reported to be statistically different for base control wheat flours AC Carberry and PFM P500 as well as the yellow

split and whole pea flour types (Table 3.6). The PFM P500 commercial flour was reported to have a greater mixograph dough development time compared to the AC Carberry experimental flour. The yellow split pea flour had a similar trend and a greater development time was reported compared to the yellow whole pea flour. The substitution levels and particle sizes for both the yellow split and whole pea flours showed no statistical effect for this mixing parameter (Table 3.6). No interactions for the above noted independent variables were identified in this statistical analysis.

3.7.3.2.2. Mixograph peak dough resistance

The mixograph peak dough resistance parameter was reported to be statistically different for base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types as well as the substitution level (Table 3.6), but particle size was not. No interactions for the above noted independent variables were identified in this statistical analysis.

The PFM P500 commercial flour was reported to have greater peak dough resistance compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends showed an increase in peak dough resistance up to a substitution level of 15% followed by a decrease at a substitution level of 20% and greater. A decrease was reported for the yellow split pea flour, while an increase was reported for the yellow whole pea flour compared to the control wheat flours. It has been postulated that the quantity and quality of the protein influences both the mixing time and the peak resistance (Ram, 2009). This implies that the yellow pea flour additions were diluting the gluten in the sample. Gluten plays an important role in

the dough sample strength and how the sample behaves from a development and breakdown perspective (Sanz-Penella et al., 2010).

3.7.4. Dough quality

Two tests were conducted to evaluate the dough quality of the AC Carberry and PFM P500 control wheat flours and the composite control and yellow split and whole pea flour blends; they included oven spring and a 2-gram micro-extension test.

3.7.4.1. Oven spring

The oven spring is the initial volume increase in the bread loaf in the initial stage of baking. Tables 3.7 and 3.8 present the results for oven spring for the AC Carberry control and PFM P500 control and yellow split and whole pea flour composite blends for both particle sizes and the statistical analysis for this data is reported in Table 3.9. The oven spring was statistically different for the substitution level as well as the type of yellow pea flour (Table 3.9). A similar outcome was reported for lentil and navy bean flours with reduced oven spring values in wheat-pulse composite bread samples when compared to a wheat flour control (Borsuk, 2011). No statistical differences were reported between the control wheat flours AC Carberry and PFM P500 and the particle size (Table 3.9). However, a three-way interaction between the substitution level, 0.5 and 0.75 mm particle sizes as well as the type of yellow pea flour was reported for the oven spring (Table 3.9).

In response to the addition of yellow pea flours, the wheat-pea composite blends decreased in oven spring compared to the control wheat flours. The decrease in this parameter was in response to the level of substitution in the composite samples. The yellow split pea flour had a greater effect on reducing oven spring compared to the

yellow whole pea flour. We would expect the loaf volume to follow a similar trend as oven spring as oven spring is considered a predictor of end-product quality.

Table 3.7 Oven Spring and 2-gram Micro-Extension Test Results for AC Carberry Control & Composite Yellow Split & Whole Pea Flour Blends for 0.5 & 0.75 mm Particle Sizes

		Oven Spring (mm)	Rmax (gf)	E (mm)	EA (gf·mm)
AC Carberry Control		16.3±1.8	20.7±0.2	107.5±3	653±170
Split 0.5					
	10%	12.7±5.7	25.2±2.2	103.6±8	1054±78
	15%	11.7±1.3	17.5±0.1	116.3±5	872±75
	20%	6.5±4.6	14.6±0.5	143.7±5	533±12
	25%	5.5±2.0	11.7±0.5	117.1±20	386±68
Split 0.75					
	10%	18.0±8.0	20.2±0.5	110.3±16	1027±150
	15%	15.3±5.2	15.8±0.4	129.2±9	788±151
	20%	6.7±6.8	14.9±0.7	113.8±7	554±35
	25%	7.8±1.7	12.2±0.5	139.4±3	469±27
Whole 0.5					
	10%	15.6±4.7	27.6±1.4	110.2±13	1061±43
	15%	10.3±4.7	19.5±0.9	102.5±1	827±26
	20%	5.2±1.6	18.7±0.2	105.9±5	758±50
	25%	2.2±1.4	14.9±0.4	127.7±5	672±41
Whole 0.75					
	10%	16.3±3.6	23.4±0.6	113.0±4	1183±168
	15%	15.9±5.0	19.2±0.9	108.7±21	816±102
	20%	7.7±3.7	17.3±1.4	135.1±1	705±77
	25%	1.9±1.0	13.6±0.2	138.6±9	493±64

Rmax (Resistance to extension), E (Extensibility), EA (Area under curve)

Table 3.8 Oven Spring and 2-gram Micro-Extension Test Results for PFM P500 Control & Composite Yellow Split & Whole Pea Flour Blends for 0.5 & 0.75 mm Particle Sizes

		Oven Spring (mm)	Rmax (gf)	E (mm)	EA (gf·mm)
PFM P500 Control		22.1±0.2	33.7±0.3	96.4±0	1279±76
Split 0.5					
	10%	14.9±2.0	28.0±0.9	97.2±2	913±206
	15%	11.8±2.6	24.5±0.4	110.3±7	946±102
	20%	8.0±2.5	20.0±0.6	133.2±10	644±55
	25%	2.4±1.6	18.2±0.4	107.8±8	732±37
Split 0.75					
	10%	16.7±2.4	26.0±0.7	109.2±10	1240±43
	15%	12.5±3.7	25.7±0.1	103.8±2	1079±21
	20%	5.5±1.5	18.7±0.4	105.3±4	721±83
	25%	3.8±1.1	16.2±0.2	100.2±3	591±16
Whole 0.5					
	10%	14.8±4.2	27.0±1.3	94.2±10	1084±111
	15%	11.9±3.5	26.1±2.0	85.9±7	920±105
	20%	6.9±3.6	22.1±0.0	103.7±1	840±76
	25%	4.6±0.2	16.8±0.1	94.4±11	524±110
Whole 0.75					
	10%	22.5±4.2	28.1±0.4	94.2±2	1072±18
	15%	17.8±0.1	31.0±0.3	93.2±0	978±116
	20%	5.5±0.5	25.1±0.4	103.4±1	908±67
	25%	0.6±0.1	19.3±2.9	117.8±25	721±155

Rmax (Resistance to extension), E (Extensibility), EA (Area under curve)

Table 3.9 ANOVA Table with Significance of Main Effects & Interactions for Dough Parameters

Effect	Oven Spring (mm)	Rmax (gf)	E (mm)	EA (gf•mm)
Base flour				
AC Carberry	7.8	17.9	119.7	765
PFM P500	7.3	23.3	103.4	870
Substitution (%)				
0	21.1	27.2	101.9	966
10	15.7	25.7	104.0	1079
15	12.8	22.4	106.2	908
20	6.1	18.9	118.0	708
25	2.6	15.4	117.9	573
Type				
Yellow Split pea	8.3	19.3	115.0	784
Yellow Whole pea	6.8	21.9	108.0	850
Particle size (mm)				
0.5	7.5	20.8	109.6	800
0.75	7.6	20.4	113.5	834
		P-value		
Base flour (B)	0.30	<0.001	<0.001	<0.001
Substitution (S)	<0.001	<0.001	<0.001	<0.001
Type (T)	0.03	<0.001	<0.001	0.001
Particle size (P)	0.87	0.05	0.04	0.09
B x S	0.07	<0.001	0.03	0.02
B x T	0.18	0.16	0.10	0.03
B x P	0.11	<0.001	0.04	0.01
S x T	<0.001	<0.001	0.001	0.002
S x P	0.04	<0.001	0.003	0.21
T x P	0.31	<0.001	0.001	0.44
B x S x P	0.23	<0.01	0.52	0.86
B x S x T	0.35	<0.01	0.58	0.36
B x P x T	0.14	<0.001	0.42	0.83
S x P x T	<0.001	0.54	<0.001	0.69
B xS xP xT	0.03	0.08	0.003	<0.001

Data are means of triplicates. P values <0.05 are statistically significant. Letters have been added to the treatment main effects when interactions were not statistically significant.
Rmax (Resistance to extension), E (Extensibility), EA (Area under curve)

3.7.4.2. 2-gram micro-extension test

The 2-gram micro-extension test was conducted and three parameters were evaluated; they included: extensibility (E), area under the curve (EA) and maximum resistance to extension (Rmax). The extension test serves to evaluate the extensibility and strength of the dough sample. The results for these parameters are presented in Tables 3.7 and 3.8 and the results for the statistical analysis are presented in Table 3.9.

3.7.4.2.1. Extensibility

The extensibility parameter was statistically different for base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types, the substitution level and the particle sizes for the yellow pea flours (Table 3.9). This statistical investigation also reported several significant interactions for the extensibility parameter, which included a two-way interaction between the level of yellow pea flour substitution and the 0.5 and 0.75 mm particle sizes for the yellow pea flours; the type of yellow pea flour and the level of yellow pea flour substitution; and the level of yellow pea flour substitution and the 0.5 and 0.75 mm particle sizes for the yellow pea flours. A three-way interaction was also reported between the level of yellow pea flour substitution, the type of yellow pea flour and the particle size for the yellow pea flours. And finally, a highly significant four-way interaction was observed between the base control wheat flour, the level of yellow pea flour substitution, the particle size and the type of yellow pea flours (Table 3.9).

The PFM P500 commercial flour was reported to have lower extensibility compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends increased in extensibility compared to the

control wheat flours. A larger increase was reported for the yellow split flour compared to the yellow whole pea flour. These results are not in agreement with a study evaluating pinto bean flour, which showed a decrease in the extensibility of the wheat-pinto bean composite dough samples (Simons et al., 2014). In that study, the weakening effect began at the initial 5% substitution level in the dough samples (Simons et al., 2014).

3.7.4.2.2. Resistance to extension

The resistance to extension (Rmax) parameter was reported to be statistically different for base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types, the substitution level and the particle size for the yellow pea flours (Table 3.9). This statistical investigation also reported several significant interactions for the Rmax parameter, which included a two-way interaction between the level of yellow pea flour substitution and the particle size; the type of yellow pea flour and the level of yellow pea flour substitution; and the level of yellow pea flour substitution and the particle size; the base control wheat flour and the 0.5 and 0.75 mm particle sizes for the yellow pea flours; as well as the base control wheat flour and the level of yellow pea flour substitution. Multiple three-way interactions were also reported (Table 3.9).

The PFM P500 commercial flour was reported to have greater Rmax compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends had lower Rmax values compared to the control wheat flours. The decrease in Rmax was greater for the yellow split pea flour compared to the yellow whole pea flour. A linear decrease in Rmax was shown in a study where untreated and defatted lupin flour was substituted into wheat flour (Pollard et al., 2002). The results presented by Mohammed and colleagues (2012) for chickpea-wheat flour showed that the

composite flour dough were inferior in strength and exhibited an inextensible texture. Their study clearly demonstrated that the chickpea-wheat flour blend modified the rheological properties of the dough as well as the final product quality attributes. The addition of yellow split pea flour into wheat flour had similar outcomes to those of Mohammed and colleagues (2012).

3.7.4.2.3. Area under the curve

The area under the curve (EA) parameter was reported to be statistically different for the base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types and the substitution level (Table 3.9). Particle size for the yellow pea flours did not have a statistical effect on the EA parameter (Table 3.9). This statistical investigation also reported several significant interactions for the area under the curve parameter, which included a two-way interaction between the base control wheat flours and the level of yellow pea flour substitution; the base control wheat flours and the 0.5 and 0.75 mm particle sizes for the yellow pea flours; the level of yellow pea flour substitution and the type of yellow pea flour. A three-way interaction was also reported between the level of yellow pea flour substitution, the type of yellow pea flour and the 0.5 and 0.75 mm particle sizes for the yellow pea flours. And finally, a four-way interaction was observed between the base control wheat flour, the level of yellow pea flour substitution, the 0.5 and 0.75 mm particle sizes for the yellow pea flours and the type of yellow pea flours (Table 3.9).

The PFM P500 commercial flour had a greater EA compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends increased at the 10% substitution level followed by a decrease in EA

compared to the control wheat flours. The yellow split pea flour exhibited a greater decrease in EA compared to the yellow whole pea flour. Mohammed and colleagues (2012) had previously shown a reduction in the area under the curve with all substitution levels for wheat-chickpea flour composite blends.

3.7.5. Bread quality

Several measurements and tests were conducted to evaluate the quality of the final bread product. Measurements for specific loaf volume (cm^3/g), loaf volume (cm^3), whiteness of the crumb, firmness of the bread crumb (gf), and finally the cell density (ASE unit) and bread score (ASE unit) which were calculated by the ASE instrument. These results are presented in Tables 3.10 and 3.11 and the results for the statistical analysis are presented in Table 3.12; the results reported do not support the idea that particle size has an effect on bread quality parameters.

3.7.5.1. Loaf volume

Loaf volume is a key parameter to measure when evaluating bread quality. Gluten and the formation of a gluten network play a pivotal role in the viscoelasticity of the dough and ultimately, gas retention in the dough product. These two factors play a significant role in delivering an acceptable loaf volume in the final bread product. The loaf volume was reported to be statistically different for base control wheat flours AC Carberry and PFM P500 and the substitution level, while the particle size and yellow split and whole pea flour types did not show a statistical difference (Table 3.12). No interactions amongst the above noted independent variables were identified in the statistical analysis.

The PFM P500 commercial flour was reported to have greater loaf volume compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends showed a decrease in loaf volumes compared to the control wheat flours. These results are similar to those of a study conducted to evaluate the addition of chickpea flour into commercial wheat flour (Mohammed et al., 2012). In that study, the loaf volume decreased and was significantly different than the commercial wheat flour at 30% substitution (Mohammed et al., 2012). Hsu and colleagues (1980) also reported a deleterious effect on loaf volume with a 15% addition of legume flours including yellow pea, faba bean and lentil. A reduction in the bread's loaf volume can be attributed to a combination of gluten dilution and disruption of the gluten network structure by the particles present in the yellow split and whole pea flour (Mohammed et al., 2014). Gluten or more precisely glutenin, is the main structure-forming protein in wheat flour that is responsible for the extensible properties needed to produce good bread quality (Bakare et al., 2016). It has been shown that the difference between weak and strong flours can be explained by differences in the molecular mass distribution of their proteins (Bakare et al., 2016). An abundance of glutenin molecules with long chains in wheat flour has been observed to generate highly extensible doughs that yield good bread loaf volumes (Bakare et al., 2016). The influence of particle size on bread quality has had contradictory outcomes in the literature. The replacement of coarse bran with finely ground bran has shown both an increase and a decrease in bread loaf volume (Hemdane et al., 2015). If all breads contain the same non-endosperm components, it has been postulated that smaller bran particles are more detrimental to bread volume than larger ones. The detrimental effects of the smaller bran particles could

be the result of an abrasive effect of the particles on the gluten network during mixing. The abrasion could be caused by more fine bran particles being added than coarse bran particles to achieve the same substitution levels of bran in the bread product (Hemdane et al., 2015).

The composition of the flours would also play an important role in bread quality attributes such as loaf volume. A comparison of chemical composition indicated that legume flours have less starch (50% in legume flours and 75-83% in wheat flour), starch damage (3% in legume flours and 5.5-7.8% in wheat flour) and pentosan content but more protein, fat, ash and fibre than white wheat flours (Naivikul & D'Appolonia, 1978). The total sugar content is higher in all legume flours (4.99-7.22%) than in wheat flour (1.2-1.6%) (Naivikul & D'Appolonia, 1978). Sucrose is the major free sugar in wheat flour but is about 10 times less than the content in the legume flours (Naivikul & D'Appolonia, 1978). The carbohydrates are the major component of most flours and also play an important role in determining the quality of the end-product including loaf volume.

Table 3.10 Bread Quality Parameter Measurements for AC Carberry Control & Composite Yellow Split & Whole Pea Flour Blends for 0.5 & 0.75 mm Particle Sizes

		Loaf Volume (cm^3)	Specific Volume (cm^3/g)	Whiteness Index	Firmness (gf)	Cell Density (ASE Unit)	Bread score (ASE Unit)
AC Carberry Control		883±25	6.0±0.02	74.0±0.4	27±1	76.5±3.2	45.7±0.6
Split 0.5							
	10%	808±81	5.8±0.17	74.2±0.3	53±5	83.6±2.9	50.3±12.6
	15%	758±25	5.3±0.03	72.7±1.0	63±7	86.9±2.2	30.7±2.1
	20%	677±80	5.0±0.50	68.8±0.3	81±8	88.8±0.9	25.7±4.0
	25%	627±28	4.5±0.09	68.3±0.8	109±15	89.9±0.2	6.0±6.1
Split 0.75							
	10%	872±116	5.3±0.21	72.8±0.7	74±6	85.7±0.8	41.3±15.0
	15%	817±95	5.2±0.07	70.9±0.6	90±14	84.8±4.3	26.0±7.9
	20%	702±8	4.8±0.07	69.8±0.5	89±7	88.7±1.5	22.3±9.6
	25%	658±63	4.5±0.03	68.2±0.9	122±12	90.7±1.1	18.3±5.7
Whole 0.5							
	10%	875±59	5.9±0.59	72.3±0.1	34±5	83.3±5.0	56.3±5.5
	15%	783±19	5.3±0.06	72.2±0.8	49±5	85.3±3.1	30.3±0.6
	20%	715±48	4.9±0.47	68.0±0.3	52±5	88.6±3.0	20.3±4.6
	25%	705±0	4.9±0.07	67.2±0.6	60±7	88.0±0.8	11.0±4.0
Whole 0.75							
	10%	855±23	5.6±0.17	73.4±0.3	41±7	83.3±0.9	39.7±6.4
	15%	827±53	5.5±0.49	72.5±0.3	46±4	85.4±1.2	42.7±3.8
	20%	743±39	5.0±0.35	71.1±0.4	65±5	89.1±1.2	16.7±10.3
	25%	647±57	4.4±0.15	68.1±0.3	113±12	90.1±0.8	11.3±0.6

Table 3.11 Bread Quality Parameter Measurements for PFM P500 Control & Composite Yellow Split & Whole Pea Flour Blends for 0.5 & 0.75 mm Particle Sizes

		Loaf Volume (cm^3)	Specific Volume (cm^3/g)	Whiteness Index	Firmness (gf)	Cell Density (ASE Unit)	Bread score (ASE Unit)
PFM P500 Control		1000±66	NA	75.0±1.3	34±2	81.1±3.3	71.0±6.3
Split 0.5							
	10%	940±73	NA	73.1±0.2	58±6	86.6±1.7	50.3±16.6
	15%	850±73	NA	71.6±0.6	54±2	88.7±1.5	27.3±1.5
	20%	733±60	NA	69.8±0.2	72±7	89.6±0.2	23.3±4.5
	25%	720±58	NA	68.1±0.4	100±17	88.2±2.2	15.0±11.5
Split 0.75							
	10%	915±84	NA	72.6±0.1	47±15	84.3±3.7	46.0±2.0
	15%	847±71	NA	70.7±0.2	57±10	88.6±1.2	27.7±4.9
	20%	728±4	NA	68.5±0.3	101±15	89.8±1.8	27.0±6.6
	25%	718±12	NA	66.2±1.0	124±16	91.7±0.2	2.50±0.7
Whole 0.5							
	10%	840±35	NA	72.3±0.7	66.2±8	88.7±0.2	40.5±14.9
	15%	805±17	NA	72.2±0.4	50.9±5	88.4±3.3	23.3±6.5
	20%	733±28	NA	68.0±0.1	77.6±6	90.1±2.2	19.0±5.7
	25%	683±47	NA	67.2±0.7	126.6±8	88.8±1.0	11.0±2.1
Whole 0.75							
	10%	952±25	NA	73.8±1.1	62.5±13	88.8±0.4	71.0±6.2
	15%	870±0	NA	72.5±0.4	52.5±11	86.7±2.3	41.0±2.7
	20%	750±42	NA	71.1±1.0	50.4±13	88.2±0.8	26.0±2.8
	25%	677±4	NA	68.1±0.2	105.5±12	91.3±0.5	4.3±7.5

Table 3.12 ANOVA Table with Significance of Main Effects & Interactions for Bread Quality Parameters

Effect	Loaf Volume (cm^3)	Specific Volume (cm^3•g)	Whiteness Index	Firmness (gf)	Cell Density (ASE Unit)	Bread score (ASE Unit)
Base flour						
AC Carberry	754c	5.1	70.9	71	87.0b	28.1
PFM P500	798b	-	70.3	75	88.7a	28.5
Substitution (%)						
0	956a	6.0	74.5	31	78.8c	58.3
10	882b	5.7	72.9	55	85.5a	49.4
15	820c	5.3	72.1	58	86.8a	31.1
20	723d	4.9	70.0	74	89.1b	22.5
25	679d	4.6	67.9	108	89.8b	9.9
Type						
Yellow Split pea	773b	5.0	70.2	81	87.9a	27.5
Yellow Whole pea	779b	5.2	71.0	66	87.8a	29.0
Particle size (mm)						
0.5	766b	5.2	70.5	69	87.7a	27.5
0.75	779b	5.0	70.7	78	87.9a	29.0
			P- value			
Base flour (B)	<0.001	-	0.01	0.05	0.001	<0.001
Substitution (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Type (T)	0.66	0.17	<0.001	<0.001	0.73	0.31
Particle size (P)	0.13	0.09	0.33	<0.001	0.63	0.34
B x S	0.86	-	0.31	0.001	0.10	0.15
B x T	0.09	-	0.54	<0.001	0.20	0.70
B x P	0.93	-	0.54	<0.001	0.67	0.05
S x T	0.86	0.98	0.02	0.02	0.58	0.05
S x P	0.57	0.36	0.002	0.06	0.10	0.28
T x P	0.86	0.03	<0.001	0.002	0.96	0.02
B x S x P	0.92	-	0.14	0.13	0.56	<0.001
B x S x T	0.92	-	0.01	0.02	0.16	0.01
B x P x T	0.06	-	0.008	0.002	0.57	0.01
T x P x S	0.73	0.39	0.17	0.13	0.97	0.06
B x S x P x T	0.59	-	0.18	<0.001	0.37	0.06

Data are means of triplicates. P values <0.05 are statistically significant. Letters have been added to the treatment main effects when interactions were not statistically significant.

3.7.5.2. Specific loaf volume

The specific loaf volume was reported to be statistically different for substitution level, while the yellow split and whole pea flour types and particle size did not show a statistical difference for the bread samples (Table 3.12). No interactions amongst the above noted independent variables were identified in the statistical analysis (Table 3.12).

Only the one base flour, PFM P500, was evaluated for specific loaf volume. In response to the addition of yellow pea flours, the wheat-pea composite blends had lower specific loaf volumes compared to the control wheat flour. These results are similar to those of a study conducted to evaluate the addition of chickpea flour into commercial wheat flour at 10, 20 and 30% substitution levels (Mohammed et al., 2012). The specific loaf volume decreased and was significantly different than the commercial wheat flour at 20% while the 10% substitution level was not significantly different (Mohammed et al., 2012). The differences in specific loaf volume of composite blends have been attributed to factors such as composition of the flour as well as rheological and pasting properties.

3.7.5.3. Whiteness

Another important quality measurement for bread is the degree of whiteness. The whiteness was statistically different for base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types and the substitution level, while the particle size for the yellow pea flours did not show a statistical difference for this quality parameter (Table 3.12). This statistical investigation also reported several significant interactions for the whiteness index parameter, which included a two-way interaction between the level of yellow pea flour substitution and type of yellow pea flour; the level of yellow pea flour substitution and the particle size for the yellow pea flours; and the

type of yellow pea flour and the particle size for the yellow pea flours. A three-way interaction was also reported between the base control wheat flours, the level of yellow pea flour substitution, and the type of yellow pea flour (Table 3.12).

The AC Carberry experimental flour was reported to have a greater degree of whiteness compared to the PFM P500 commercial flour. A linear reduction in whiteness would be expected as yellow pea flour was being partially substituted into the white pan bread. In this study, the yellow split pea flour showed a greater reduction in whiteness compared to the yellow whole pea flour. Angioloni and Collar (2012) evaluated chickpea, green split pea and defatted soya flour additions in a refined wheat flour bread to determine their effect on whiteness. Their study reported that chickpea negatively impacted whiteness, green pea positively impacted whiteness and soya flour had no statistically significant effect on whiteness. Interestingly, their study also evaluated two structuring agents and their influence on whiteness. The 1% addition of gluten led to a slightly creamy-white crumb color as well as a reduction in crust darkness. Utilization of gluten in wheat-legume composite bread would positively impact quality aspects of the final bread product including crumb and crust color and loaf volume. Fenn and colleagues (2010) reported that additions of 5% and 8% yellow pea protein and 8% chickpea protein reduced the brightness of the crumb color. However, for the cultivar AC Barrie, there was no impact on the brightness of an addition of 5% yellow pea protein. Dalgetty and Baik (2006) went as far as to differentiate the quality of the crumb color by the type of fibre addition. These researchers suggested that soluble fibres produced a more attractive bread product when considering both crumb color and uniformity.

Current literature supports the view that the type of dietary fibre, legume source and wheat cultivar all play a role in the crumb and crust color in the bread product.

3.7.5.4. Firmness

Texture evaluation of the final bread product is an important quality measurement. The parameter bread firmness was reported to be statistically different for the base control wheat flours AC Carberry and PFM P500, the yellow split and whole pea flour types, the substitution level and particle size (Table 3.12). This statistical investigation also reported several significant interactions for the bread firmness parameter, which included a two-way interaction between the base control wheat flours and the level of yellow pea flour substitution; the base control wheat flours and the 0.5 and 0.75 mm particle sizes for the yellow pea flours; the base control wheat flours and type of yellow pea flour; the level of yellow pea flour substitution and the type of yellow pea flour; and the type of yellow pea flour and the 0.5 and 0.75 mm particle sizes for the yellow pea flours. A three-way interaction was also reported between the base control wheat flours, level of yellow pea flour substitution, and the type of yellow pea flour; and the base control wheat flours, the 0.5 and 0.75 mm particle sizes for the yellow pea flours and the type of yellow pea flour. And finally, a four-way interaction was reported between the base control wheat flours, the level of yellow pea flour substitution, the 0.5 and 0.75 mm particle sizes for the yellow pea flours and the type of yellow pea flours (Table 3.12).

The PFM P500 commercial flour produced a firmer bread crumb compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends increased in bread firmness compared to the control wheat

flours. The increases in firmness results are compatible with a study that evaluated yellow pea protein fractions and CWES and CWRS wheat flours. The yellow pea protein fractions resulted in an increase in bread firmness at all substitution levels (Fenn et al., 2010). Angioloni and Collar (2012) also showed an increase in crumb firmness with all substitution levels of chickpea, green pea and soybean flour. The yellow split pea flour produced crumb with a greater a degree of firmness compared to the yellow whole pea flour and the 0.75 mm particle size was firmer than the 0.50 mm particle size. A study conducted by Sakhare and colleagues (2014) investigated particle size effects on bread firmness using wheat flour and reported that an increase in particle size resulted in an increase in bread firmness. A similar outcome was reported in an oat fibre study conducted by Marcin and colleagues (2016).

3.7.5.5. Cell density

Cell density has been associated with the density of the bread sample and thus, the texture. This bread quality parameter was statistically different for the base control wheat flours AC Carberry and PFM P500 and the substitution level, while the yellow pea flour types and particle size did not show a statistical difference (Table 3.12). This statistical analysis did not show interactions amongst the independent variables noted above (Table 3.12).

The PFM P500 commercial flour had a larger value for the bread's cell density compared to the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends had increased cell density compared to the control wheat flours. It has been postulated that the addition of mixed legumes into a

bread formula dramatically affects loaf quality, causing a significant decrease in bread volume and an increase in cell density (Angioloni & Collar, 2011).

3.7.5.6. Bread score

The ASE instrument uses several machine generated measurements such as loaf volume, color and cell density to provide an overall quality score for the bread sample (Lukow et al., 2011). The bread score was reported to be statistically different for the base control wheat flours AC Carberry and PFM P500, and the substitution level, while the yellow split and whole pea flour types and the 0.5 and 0.75 mm particle sizes for the yellow pea flours were not statistically different (Table 3.12). This statistical investigation reported several significant interactions for the bread score, which included a two-way interaction between the base control wheat flours and the level of yellow pea flour substitution; the type of pea flour and the 0.5 and 0.75 mm particle sizes for the yellow pea flours. A three-way interaction was also reported between the base control wheat flours, the level of yellow pea flour substitution, and the type of yellow pea flour; the type of yellow pea flour, the base control wheat flours, and the 0.5 and 0.75 mm particle sizes for the yellow pea flours; and the base control wheat flours, level of yellow pea flour substitution and the 0.5 and 0.75 mm particle sizes for the yellow pea flours (Table 3.12).

The PFM P500 commercial flour had a higher bread score than the AC Carberry experimental flour. In response to the addition of yellow pea flours, the wheat-pea composite blends had decreased bread score compared to the control wheat flours. Angioloni and Collar (2011) showed that substitution of both chickpea and green pea

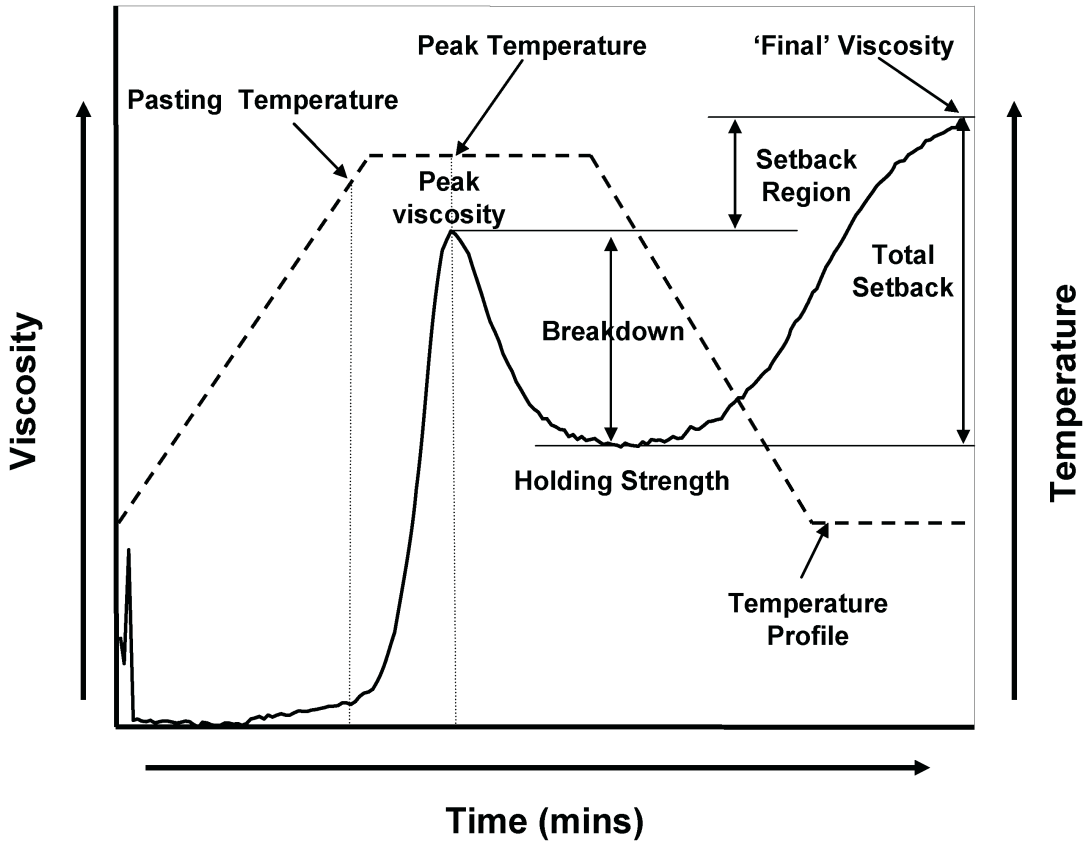
flours maintained acceptable bread quality scores for substitutions of 8% to 20% in refined wheat flour.

3.7.5.7. Thermal properties

3.7.5.7.1. RVA standard pasting test

An RVA standard pasting test was conducted to evaluate the following parameters: peak viscosity, set-back value, trough, breakdown, final viscosity, peak time and pasting temperature. This test creates a product in between the flour and final baked product and evaluates the rheological and pasting characteristics that are relevant to the manufacture of bread as they relate to the machinability of dough and the quality of the final product (Angioloni & Collar, 2008). The parameters for a standard RVA curve are presented in Figure 3.1. This standard test was conducted on the AC Carberry and PFM P500 control wheat flours as well as the yellow split and whole pea flour samples. The standard pasting test parameter results are presented in Table 3.13. Figure 3.2 presents an RVA curve for the control flours, parent yellow split pea flour and the 0.5mm and 0.75 mm yellow split pea flour samples. Figure 3.3 also uses an RVA curve to present the control and yellow whole pea flour sample results.

Figure 3.1 RVA Parameters Identified in a Standard RVA Curve



No statistical differences existed between the AC Carberry and PFM P500 control wheat flours, while a large significant difference was shown between the control flours and the yellow split pea flours for the pasting parameters: peak viscosity and trough. A significant difference was recorded between the 0.75 mm yellow whole pea and all remaining yellow pea flour samples for both of these pasting parameters. No significant difference was recorded between the 0.5 and 0.75 mm particle sizes for the yellow split pea flour. Jagannadham and colleagues (2014) reported a reduction of the peak viscosity and trough when evaluating chickpea and wheat flour samples. Mohammed and colleagues (2014) also reported an effect on thermal or pasting properties with the addition of chickpea flour in commercial wheat flour. It has been postulated that high levels of other components, such as fat and protein, influence swelling power and the

pasting properties of starches (Mohammed et al., 2012). Gomez and colleagues (2003) attributed the decrease in peak viscosity to a lower carbohydrate and high protein content. High peak viscosity values imply that the starch would be suitable for end-products requiring high gel strength and elasticity (Ikegwu et al., 2010). The lowering of the peak viscosity would account for the lowering of gas retention and expansion in the final product (Jagannadham et al., 2014).

For the pasting parameters set-back value, breakdown and final viscosity, the AC Carberry and PFM P500 control wheat flours were significantly different, while a larger significant difference was exhibited between the control flours and the yellow split pea flours. The breakdown parameter had the greatest difference between the control wheat flours and the yellow split pea flours. The yellow split and whole pea flours had a similar trend for both the parent material and 0.5 mm particle sizes for the set-back value. The yellow split 0.75 mm particle size was not significantly different than the 0.5 mm particle size, while the yellow whole pea 0.75 mm particle size was significantly less than its respective parent and 0.5 mm particle size. It has been postulated that higher set-back values are reflective of less retrogradation during the cooling process (Ikegwu et al., 2010). Based on this postulate, the composite wheat-pea breads would be expected to exhibit a higher degree of retrogradation. A reduction of the set-back value, breakdown and final viscosity parameters was also recorded when chickpea flour was compared to the wheat flour samples by Jagannadham and colleagues (2014).

For the pasting parameter peak time, no statistical difference existed between the AC Carberry and PFM P500 control wheat flours, while a significant difference was recorded between the PFM P500 control wheat flour and the yellow split pea parent flour

sample. The yellow split pea parent flour sample had a higher peak time parameter compared to the PFM P500 control wheat flour. A higher peak time parameter was also recorded for chickpea flour samples compared to wheat flour samples by Jagannadham and colleagues (2014). No statistical differences were reported between the control wheat flours and the 0.5 and 0.75 mm particle sizes for both the yellow split and whole pea flour samples. This trend differed from what was noted for the peak viscosity and trough pasting parameters.

The final pasting parameter, pasting temperature, showed no statistical difference between the AC Carberry and PFM P500 control wheat flours. The yellow split pea 0.5 mm particle size was significantly different than both the control flours as well as the balance of the yellow split and whole pea flour samples. A non-significant increase was recorded for the yellow pea flour samples compared to the control flours. An increase in pasting temperature was also recorded when chickpea and wheat flour samples were evaluated by Jagannadham and colleagues (2014). Sanz-Penella and colleagues (2010) showed the same trend (increase in pasting temperature) with the addition of resistant pea starch from a modified pea starch in a wheat dough sample. The increase in pasting temperature suggests that a higher temperature would be required to cook legume flour samples (Jagannadham et al., 2014).

The above reported RVA results are in agreement with Jagannadham and colleagues (2014), in that the peak, breakdown, final, set-back and trough viscosities were lower for the legume pastes compared to the control wheat flour pastes. The pasting temperature was found to increase in the chickpea flour pastes compared to the control wheat flour pastes.

Table 3.13 RVA parameters for AC Carberry, PFM P500 & Yellow Split & Whole Pea Flour Samples

	Peak Viscosity (RVU)	Set-Back Value (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Peak Time (min)	Pasting Temperat-ure (°C)
AC Carberry	3259a	1381b	2109a	1148.1b	3490b	6.3ab	66.1b
PFM P500	3403a	1768a	1941a	1461.6a	3823a	6.1b	67.5b
Split (parent)	1058b	725d	1014b	43.8b	1739c	7.0a	77.3b
Split 0.5 mm	1175b	851cd	1144b	30.1b	2162b	6.9ab	59.1c
Split 0.75 mm	1307b	941c	1280b	26.2b	2221b	6.4ab	76.3b
Whole (parent)	1048b	722d	1021b	26.6b	1743c	6.6ab	75.1b
Whole 0.5 mm	1206b	739cd	1194b	10.4c	1933bc	6.2ab	74.0b
Whole 0.75 mm	668c	400e	640c	28.4b	1039d	6.9ab	78.4b

Data are means of triplicates. Within each column, means with the same letter are not significantly different ($P > 0.05$).

Figure 3.2 RVA curve for AC Carberry, PFM P500 & Yellow Split Pea Flour Samples

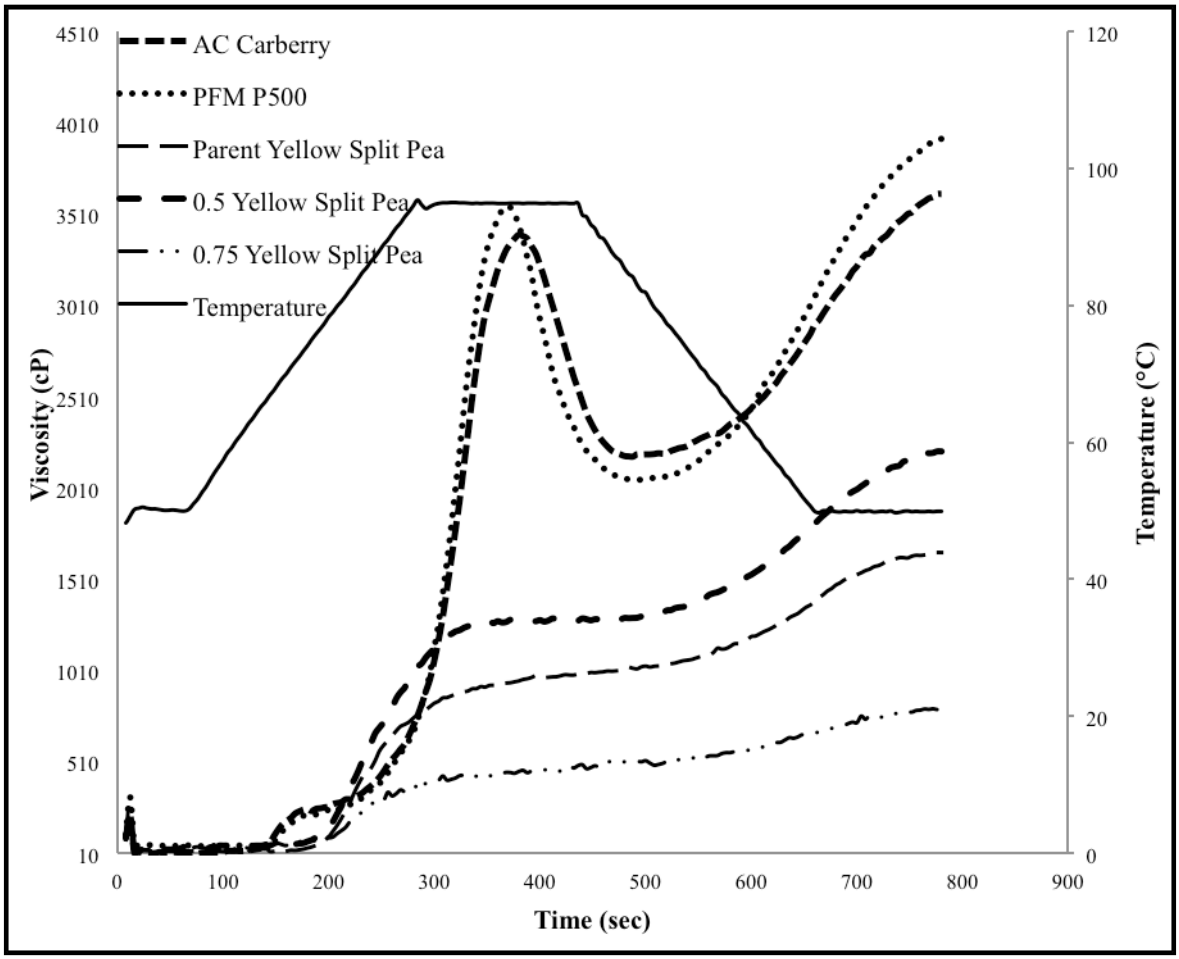
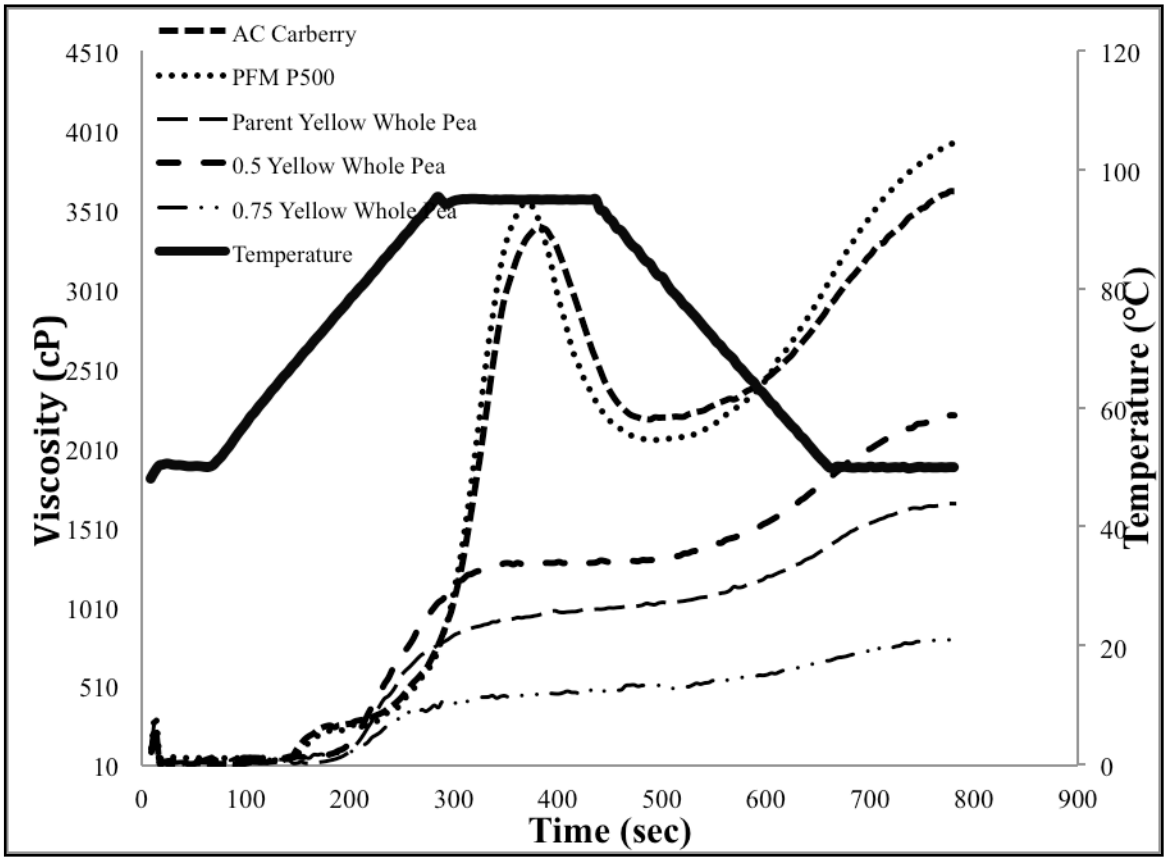


Figure 3.3 RVA curve for AC Carberry, PFM P500 & Yellow Whole Pea Flour Samples



3.7.5.7.2. Storage evaluation of the bread dough samples using a DSC instrument

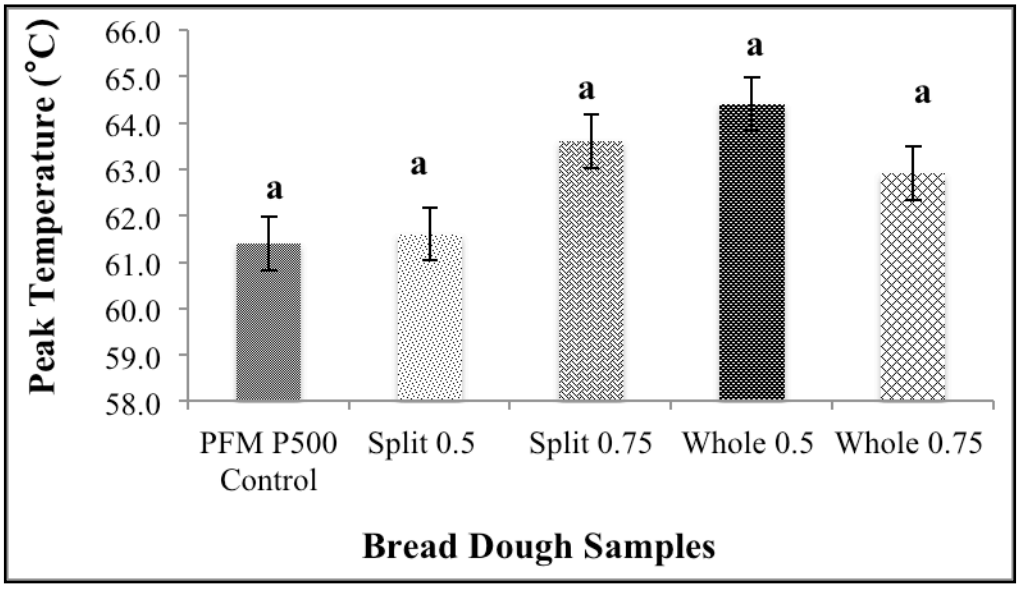
The DSC instrument was used to evaluate the gelatinization enthalpy of a bread dough sample to quantify the amylopectin retrogradation of the samples. Other authors have also used the DSC to evaluate retrogradation in bread dough samples (Defloor & Delcour, 1999 and Wronkowska et al., 2013). In this study, the bread dough samples were evaluated after storage of the bread for days 0, 1, 2 and 3. This test was conducted using PFM P500 as the control and yellow split and whole pea flours for the 10, 15, 20 and 25% substitution levels. For industrial applicability, the commercial grade flour was

selected to evaluate the DSC parameters. Two parameters were evaluated in the DSC: peak temperature and enthalpy change. These test parameter results are presented in Figures 3.4 and 3.5.

The peak temperature parameter was significantly different between Day 0 and Days 1, 2 and 3; an increase in peak temperature was reported. The PFM P500 control wheat flour exhibited the smallest peak temperature compared to the yellow pea flours (Figure 3.4). The parameter enthalpy change showed a similar trend as peak temperature as Day 0 was significantly different than Days 1, 2 and 3 except this parameter decreased as the sample storage time increased. Day 1 was also significantly different than Days 2 and 3, while Days 2 and 3 were not significantly different from each other. The enthalpy change exhibited the following trend in the samples: PFM P500 control, 0.5 and 0.75 mm yellow whole pea-PFM P500 10% composite flour blends < 0.5 and 0.75 mm yellow split pea-PFM P500 10% composite flour blends (Figure 3.5). A linear decrease was shown with the parameter enthalpy change for the yellow split-PFM P500 10% composite flour blends compared to the PFM P500 control and the yellow whole pea composite flour blends. Collar and colleagues (2015) evaluated the addition of tef, green pea and buckwheat flour and the impact on the enthalpy change evaluated in the DSC. The results in this study showed a reduction in the enthalpy change with higher levels of substitution in these composite bread dough samples. Dalgetty and Baik (2006) evaluated pea hull fibre, pea insoluble fibre and pea soluble fibre additions to wheat flour. Their 7-day study indicated that both the pea hull fibre and soluble fibre led to a reduction in enthalpy change while the insoluble fibre showed no difference to the control wheat flour sample. Their results suggest that the addition of pea hull and soluble fibre has the ability

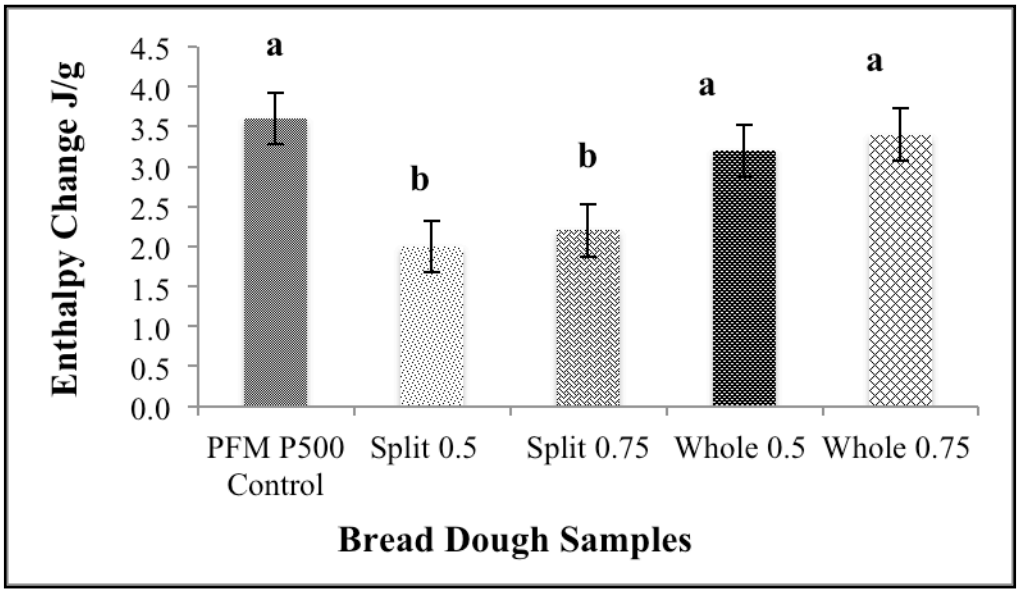
to slow down retrogradation. The composite 10% wheat-split pea blends for both particle sizes showed a decrease in enthalpy change compared to the PFM P500 control flour. Therefore, the results for the 10% wheat-split-pea flour composite blends were the same as Dalgetty and Baik's results with a reduction in the enthalpy change or a slowing of retrogradation in the bread dough samples. The composite 10% wheat-whole pea blends for both particle sizes did not show a significant difference compared to the PFM P500 control flour. These results are justified based on the compositional make up of the dietary fibre as outlined in the yellow pea flour product specification sheets supplied by Best Cooking Pulses: 18.4% insoluble fibre in the yellow whole pea flour compared to 7.2% insoluble fibre in the yellow split pea flour. Further investigations should be conducted to evaluate the effect of increased substitution levels in the bread dough samples as well as a longer storage period, such as 7 days.

Figure 3.4 DSC Peak Temperatures for PFM P500 Control & 10% Wheat-Yellow Split & Whole Pea Flour Bread Dough Samples



Data illustrated as least squares mean ± standard error of the population mean. Within each column, means with the same letter are not significantly different ($P > 0.05$).

Figure 3.5 DSC Enthalpy Change for PFM P500 Control & 10% Wheat-Yellow Split & Whole Pea Bread Dough Samples



Data illustrated as least squares mean ± standard error of the population mean. Within each column, means with the same letter are not significantly different ($P > 0.05$).

3.8. Conclusion

Both the PFM P500 commercial and AC Carberry wheat flours were used as control flours to evaluate the effect of yellow split and whole pea flour additions in the composite wheat-pea bread samples from a quality perspective. Quality deteriorations were expected due to a dilution of the wheat flour with the yellow pea flour additions. Based on the reported results for the mixing parameters conducted on the farinograph and mixograph instruments, a definitive relationship between the mixing and bread quality parameters could not be established. The type of yellow pea flour had a statistical effect on all mixing parameters. The decreases in mixing parameters corresponded with impairments in dough properties, which also included a four-way interaction (base control wheat flour x type of

yellow pea flour x level of yellow pea flour substitution x particle size for yellow pea flour) for the oven spring, extensibility and area under the curve. Consequently, a decrease in loaf volume with increasing levels of yellow split and whole pea flour in the composite wheat-split pea bread samples was reported. The type of yellow pea flour did not have a significant effect on loaf volume. The whiteness index decreased, and the firmness and cell density increased as the load of yellow split and whole pea flour increased in the bread samples. The type of yellow pea flour had a significant effect on both the whiteness index and firmness bread quality parameters. If an improved loaf volume is desired, additional dough improving agents will need to be explored for the composite wheat- pea bread samples. Interestingly, particle size played a minor role in this quality study. Particle size had a significant effect on extensional parameters E and EA as well as bread firmness. A larger particle size resulted in an increase in bread firmness.

A thermal investigation was conducted using RVA and DSC instruments to evaluate viscosity changes and bread staling in the composite wheat-split and wheat-whole pea dough samples. The RVA parameters peak viscosity, set back value, trough, breakdown and final viscosity for the yellow split and whole pea flours were statistically lower than both control wheat flours. A lower set back value at the peak of the curve indicates higher diastatic activity, which has been shown to correlate with lower retrogradation. The yellow split and whole pea flour blend RVA results had a lower set back value compared to the control wheat flours, which would imply a lowering of retrogradation for the yellow pea flour samples. The yellow whole pea flour samples had lower set back values than the yellow split pea flour samples, which would suggest that the yellow whole pea flour would have a greater effect on reducing retrogradation in the yellow pea samples.

Based on the DSC study, the peak temperature parameter was significantly different between Day 0 and Days 1, 2 and 3; an increase in peak temperature was reported. The parameter enthalpy change showed that Day 0 was significantly different than Days 1, 2 and 3 and this parameter decreased as the sample storage time increased. Day 1 was also significantly different than Days 2 and 3, while Days 2 and 3 were not significantly different from each other. No statistical difference was reported for the parameter enthalpy change between the PFM control wheat flour and yellow whole-PFM P500 10% composite flour blends, while a statistical decrease was shown between the yellow split-PFM P500 10% composite flour blends and the yellow whole-PFM P500 10% composite flour blends. The statistical difference between the yellow pea flours would suggest that a retrogradation reduction occurs in the yellow split-PFM P500 10% composite flour blend samples. Unfortunately, the RVA and DSC results for retrogradation or bread staling are not in agreement. To validate these DSC results an alternate evaluation method should be conducted to evaluate bread staling in the composite wheat-pea bread samples.

CHAPTER 4: EFFECT OF PEA FLOURS WITH DIFFERENT PARTICLE SIZES ON ANTIOXIDANT ACTIVITY IN PAN BREADS

Lisa D. Davies-Hoes*, Martin G. Scanlon, Abraham T. Girgih, Rotimi E. Aluko

(Reprinted from Cereal Chem. 2017. 94, 866-872)

****I completed the experiments, wrote the paper and coordinated with the other team members to include their intellectual contributions.***

Abstract

Pulses are good sources of vitamins and minerals as well as antioxidants. Current literature supports a role for antioxidants in reducing oxidative damage associated with many health disorders, including cardiovascular disease and cancer. The effects of substitution of 10% (w/w) yellow whole or split pea flour (various particle sizes) in white wheat flour (Canadian Wheat Red Spring (CWRS)) on the phenolic and antioxidant activity of the leavened bread was examined. Antioxidant activity was evaluated using four assays, which included 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), metal chelation (MC), and superoxide (SO) radical scavenging assays. The bread samples had reduced DPPH (5-11% scavenging activity) and MC (5-10% scavenging activity) values compared to their respective raw flours. Decreased activity in both the DPPH and MC assays can be attributed to a reduction in the antioxidant capacity in the bread samples due to dilution with white wheat flour. The MC values for bread samples showed little variability between the yellow whole and split pea flours (8-10% scavenging activity for yellow whole and 9% for split pea flours), which suggests that the antioxidant activity of bread samples is not dramatically affected by the seed coats. Most pea fractions increased the FRAP scavenging activity and decreased the SO scavenging activity values for the bread samples compared to their respective raw flours. We conclude that thermal processing enhanced the antioxidant activity of the bread samples, limiting the dilution effect associated with flour addition during dough make-up.

4.1. Introduction

White wheat flour has been reported to have as much as 2-3 times lower antioxidant activity compared to whole-wheat flour (Delcour et al., 2012). This is because a large variety of bioactive compounds, including phenolic antioxidants, are predominantly located in the seed coat of the whole grain (Stanisavlijvec et al., 2013). The milling of cereal grains results in a reduction of both nutrients and micronutrients; removal of the outer layers of the cereal grain reduces the amount of many nutrients such as thiamin, riboflavin, niacin and iron.

The addition of pulse flours to white wheat flours merits attention since the high level of bioactive compounds present in pulses may complement some nutritional deficiencies of a cereal-based diet (Rizello et al., 2014). For example, pulses increase both the quantity and quality of the protein source by contributing lysine, the limiting amino acid present in cereal grains. The protein content in pulse seeds ranges from 17 % to 40 %, contrasting with 7% to 15 % for cereals, and would be equal to the 18-25% protein content of meat (Bojnanska et al., 2012). A composite wheat-pea flour blend will therefore result in an improved and more complete protein source. The fibre content in cereal products also becomes higher with the addition of pulses. Therefore, a great deal of attention has been paid to the addition of pulse ingredients in processed foods because of their potential nutritional and therapeutic benefits (Diziki et al., 2014).

Pulses also contain several phenolic compounds, which are considered to be natural antioxidants (Ghanachorloo et al., 2013). Diets rich in natural antioxidants have the ability to increase the level of reactive antioxidants in the body and therefore reduce the risk of health disorders that are of free radical origin (Diziki et al., 2014). Free

radicals are produced in the body as by-products of normal metabolism and as a result of exposure to environmental pollutants (Sardesai, 1995).

It has been postulated that the potential antioxidant activities of pulse-enriched breads are mainly attributed to the phenolic acids rather than other compounds present in the bread (Diziki et al., 2014). Evaluation of total phenolic content and antioxidant activity in bread samples is a way of quantifying potential health benefits associated with the enrichment of white wheat flour pan bread with pulse flours.

Processing can affect the antioxidant activity, thereby altering the benefits to consumers of bread made with pulse flours. Thermal processing (baking) of bread, as well as mixing, has been reported to negatively affect antioxidant activity. During dough production, the hydrated environment activates oxidative enzymes, such as polyphenol oxidases, which may degrade the phenolic compounds (Diziki et al., 2014). However, it was also suggested that the process of fermentation as well as baking during bread making increase antioxidant activity (Diziki et al., 2014). This is because novel compounds are formed during thermal processing of food through the Maillard reaction, with a significant portion of the phenolic compounds being recovered as polymerized compounds (Sharma & Gujral, 2014; Han & Koh, 2011, Swieca et al., 2013; Borrelli et al., 2013; Gelinas & McKinnon, 2006). It has been postulated that Maillard reaction products have antioxidant potential similar to that of chemical additives commonly used as standard food antioxidants (Finot, 1990; Sharma & Gujral, 2014). Overall antioxidant properties of the food products may remain the same or be enhanced by the development of Maillard reaction products, even though the concentration of natural antioxidants, such

as phenolic compounds, are significantly reduced as a consequence of thermal processing (Sharma & Gujral, 2014).

Though previous studies have examined the antioxidant activity of pea fibre-wheat flour composite breads, there is scant information on the antioxidant activity of yellow pea-wheat flour composite breads, especially the influence of particle size. The influence of particle size was studied since the surface area of particles can be an important contributing factor to bioavailability of active food components. For example, the rate of antioxidant release from dietary fibre within the intestinal tract is thought to be inversely proportional to particle size (Adiotomre et al., 1990). Moreover, disruption of the natural food matrix could influence the rate of nutrient release and absorption within the gastrointestinal tract (Parada & Aguilera, 2007). Therefore, the objective of this investigation was to determine the extent to which regrinding of pea flour particles to reduce their size affected the free total phenolic content (TPC) and antioxidant activity of white wheat and yellow pea flours and the bread samples made from them.

4.2. Materials and methods

4.2.1. Materials

Flour and bread samples were evaluated for free TPC and antioxidant activity. The flour samples were a white wheat flour, which was Buhler milled from the wheat cultivar AC Carberry (AC), and parent yellow whole and split pea flours (Best Cooking Pulses, Portage-la-Prairie, MB, Canada). Regrinding of these parent yellow pea flours produced the following fractions designated by the screen size used for preparation: yellow whole (2.0, 0.75, and 0.5 mm), and yellow split (0.75 and 0.5 mm). Pan bread samples comprising 10% yellow whole or split pea flour in white wheat flour were

prepared from each of the reground particle sizes. Previous work has also shown incorporation of up to 10% pea flour in white pan bread has minimal effect on bread quality (Repetsky & Klein, 1982). Control breads were prepared from flour prepared from AC wheat.

4.2.1.1. Preparation of yellow whole and split pea flours with different particle size

A Retsch SR300 rotor beater mill (Haan, Germany) was used to regrind the parent whole and yellow split pea flours into reduced size flour fractions using the following screen sizes: 2.0, 0.75, and 0.5 mm for yellow whole pea flour and 0.75 and 0.5 mm for yellow split pea flour. Small quantities of the yellow pea flours were put through the mill during regrinding to avoid choke feeding.

4.2.2. Methods

4.2.2.1. Particle size distribution for yellow whole and split pea flour

The yellow whole and split pea flours, as well as the reground materials, were analyzed with a Malvern Mastersizer 2000 (Malvern, U.K.) for particle size distribution using a dry laser diffraction method. Three tablespoons of each sample were added to the instrument to evaluate particle size for each test run. The test was conducted in triplicate. The average for three tests was calculated and reported for each sample, except when noted for duplicate testing. The principle behind laser diffraction involves the assessment of angle sizes as it relates to the degree of light scattered as a laser beam passes through the sample. Large particles scatter light at small angles relative to the laser beam, whereas small particles show the opposite effect.

4.2.2.2. Bread making

The Canadian short process method (Preston, 1982) with slight modifications was used for bread making. The ingredients shown in Table 4.1 were combined prior to mixing. Distilled water was added, based on the 14% moisture corrected value for the flour that was generated by the Brabender farinograph (operating at 63.0 rpm). Based on the handling properties of the dough, the baking absorption for the AC white wheat flour (control) as well as the composite wheat and yellow pea flour blends was 2–6% less than the respective farinograph absorption. The dough was mixed at 140 rpm to reach peak development, rested for 15min, hand punched seven times, and placed back into the commercial proofing cabinet for an additional 15 min. After sheeting and molding, the dough piece was placed into a greased bake pan and put into a proofing cabinet for 75 min (37.5ºC, 85% relative humidity). The pan was put into a National oven (National Manufacturing, Lincoln, NE, U.S.A.) at 205ºC for 25 min. After baking, the bread was cooled for 25 min and stored in a plastic Ziploc bag overnight. For testing purposes, these day 1 bread samples were frozen, freeze-dried, and then ground with a classic grain mill (NutriMill, St. George, UT, USA). Bread making was conducted as three replicate bakes, and the average was reported for each treatment.

Table 4.1 Bread Formulation (% Flour Weight Basis)

Ingredient	Amount (%)
Flour, adjusted to 14% moisture basis	100.0
Salt (NaCl)	2.4
Sugar	4.0
Fresh yeast (Fleischmann's)	3.0
Malted barley flour (Breiss)	1.0
Shortening (Crisco)	3.0
Ammonium Phosphate (0.1% $NH_4 H_2 PO_4$ solution)	1.0
Ascorbic acid (150 ppm)	1.0
Whey powder (Health First)	4.0
Dough water	Baker's absorption

Modified method of Preston (1982).

4.2.2.3. Extraction of free polyphenols

Each sample was weighed (50 mg) into a centrifuge tube. Methanol (80%) (1 mL) was then added, and the sample was vortexed until evenly dispersed and then shaken at 1,000 rpm for 1 h at 25ºC (Thermomixer R, Hamburg, Germany). The sample was then centrifuged at 16,000 x *g* for 5 min (Agboola et al., 2010).

For each of the four assays (see below), four concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL) of each sample were prepared from the stock sample or supernatant (50 mg/mL) to determine a dose effect, or an optimal concentration level for activity or reducing power in each sample.

4.2.2.4. Determination of free total phenolic content (TPC)

Free TPC was estimated with the Folin–Ciocalteu assay method (Singleton et al., 1999). Preparation of the assay mixture used 50 μL of the extraction sample, 3 mL of distilled water, 250 μL of Folin–Ciocalteu reagent, and 750 μL of 7% (w/w) sodium carbonate. This mixture was vortexed and rested for 8 min at room temperature. An additional 950 μL of distilled water was then added. The final sample mixtures were held

at room temperature for 2 h, and then the absorbances were read on a Synergy H4 microplate reader (Bio-Tek,Winooski, VT, U.S.A.) at 765 nm against the blank control (distilled water) and compared with a known positive control for quantification. Synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyanisole, have been used to create oxidative-stable food products. Therefore, selection of BHT as a positive control in the antioxidant assays was appropriate (Jensen et al., 2011). A gallic acid (GA) standard curve was generated using eight concentrations in the range of 1.15 to 0.10 mg/mL (R^2= 0.97). The TPC of samples was calculated from absorbance values using the GA standard curve (Agboola et al., 2010; Nayak et al., 2011; Stanisavljevic et al., 2013) and was reported as milligrams of GA equivalent per gram of sample.

4.2.2.5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay

DPPH free radical scavenging effects of the phenols in the extracted wheat, yellow pea, and bread samples were evaluated following a modified method of Aluko and Monu (2003) with BHT as the standard (Jensen et al., 2011). Each sample, or BHT solution, was diluted in 0.1M sodium phosphate buffer, pH 7.0, containing 1% (w/v) Triton X-100 to achieve an assay concentration of 1.0 mg/mL. Each cell of the 96-well flat-bottom plate contained 100 µL of sample or BHT solution and 100 µL of the DPPH solution. A blank control was prepared with DPPH and sodium phosphate buffer. The plates were placed into the microplate reader and left for 30 min in the dark, at which point the absorbance (A) at 517 nm was read.

The percent scavenging effect of each sample or BHT solution was calculated as follows:

$$\text{DPPH RS effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where RS is radical scavenging.

4.2.2.6. Chelation of metal ions assay

Metal chelating (MC) activity was measured using a modification of the method reported by Xie et al. (2008). In incubation tubes, the sample or BHT was mixed with 30 μL of FeCl2 solution (2mM) and 1,110 μL of double distilled water to achieve a final concentration of 1.5 mg/mL. Ferrozine (60 μL of a 5mM solution) was added, mixed well, and incubated at room temperature for 10 min. The blank control was prepared as noted earlier with distilled water replacing the sample. The final sample or BHT mixture (200 μL) was transferred into a 96-well flat-bottom plate, and the absorbance was read in the microplate reader at 562 nm. The percent MC activities of each sample or BHT solution were calculated as follows:

$$\text{MC effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

4.2.2.7. Ferric reducing power assay (FRAP)

The reducing powers of the samples were evaluated with the method of Li et al. (2008), which was slightly modified as follows. Each sample or BHT was diluted in 0.2M sodium phosphate buffer (pH 6.6) and then mixed with 125 μL of buffer and 125 μL of 1% potassium ferricyanide to achieve a final concentration of 2.0 mg/mL. The reaction mixture was immersed in a water bath at 50ºC and incubated for 20 min. After the

incubation period, 125 μL of 10% aqueous trichloroacetic acid was added. Then, 125 μL of each mixture was combined with 25 μL of a 1% aqueous ferric chloride solution. An additional100 μL of double-distilled water was added to the reaction mixture. This final reaction mixture stood at room temperature for 10 min and was then centrifuged at 1,500 x *g* for 10 min. The blank control was prepared as noted earlier with sodium phosphate buffer replacing the sample. A 200 μL aliquot of the final reaction mixture was transferred into a 96-well flat-bottom plate, and the absorbance was read in the microplate reader at 700 nm. The percent reducing power activity of each sample or BHT solution was calculated from the following equation:

$$\text{FRAP RP activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (3)$$

where RP is reducing power.

4.2.2.8. Superoxide (SO) radical scavenging assay

The SO scavenging activity was measured following the method of Xie et al. (2008), slightly modified as follows. Each sample or BHT solution (80 μL) in 50mM Tris-HCl buffer (containing 1mM ethylenediaminetetraacetic acid [EDTA], pH 8.3) was mixed with 80 μL of the buffer directly into a clear-bottom 96-well plate to achieve a concentration of 0.5 mg/mL. A 40 μL aliquot of 1.5mM pyrogallol (which was dissolved in 10mM HCl) was then added to each well. The blank control was prepared as noted earlier with Tris-HCl buffer replacing the sample. The reaction rate was measured as a change in absorbance over 4 min (ΔA/min) at room temperature at 420 nm in the microplate reader. Decreased absorbance of the sample mixture indicates increased SO

scavenging activity. The percent scavenging activity of each sample or BHT solution was calculated as follows:

$$\text{SO RS activity (\%)} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100 \quad (4)$$

where RS is radical scavenging.

4.3. Statistical analysis

For each assay, three subsamples were analyzed and the mean value calculated. From the three replicate extractions for each assay, statistical analysis, using a mixed-design ANOVA test in SAS software (version 9.3, SAS Institute, Cary, NC, U.S.A.), was conducted on the data. Log transformations were conducted on non-Gaussian distributed data sets; determination of the data set's distribution was confirmed by the Shapiro–Wilk test (Buthmann, 2011). ATukey's posthoc analysis was carried out to determine which means were significantly different from each other for both the flours and the bread samples. In reporting results, one concentration for the assays was selected, because this was representative of observations at all four concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL). The Pearson correlation coefficient test was used to determine if free TPC was correlated with the antioxidant activity produced by the DPPH, MC, FRAP, or SO assays for both the flours and the bread samples. A 1.0 mg/mL sample concentration was used to evaluate free TPC in the sample set. The following concentrations were utilized for their corresponding antioxidant assays: DPPH, 1.0 mg/mL; MC, 1.5 mg/mL; FRAP, 2.0 mg/mL; and SO, 0.5 mg/mL. These antioxidant assay sample concentrations were utilized to make comparisons for the same base flour and its various particle sizes as evaluated in the free TPC assay.

4.4. Results and discussion

4.4.1. Particle size distribution

The particle size distributions for the two types of parent yellow pea flours and their reground materials are shown in Table 4.2. Not surprisingly, use of a larger screen size produced a larger particle size in both the whole and yellow split pea flours. In addition, for all samples, the mean particle size was larger than the median, an outcome typical of a log-normal distribution that is frequently generated from size reduction processes (Epstein, 1948). The easier-to-break cotyledons generated a smaller particle size for the yellow split pea flours. They also had a relatively narrower distribution of particle sizes (ratio of d0.90 to d0.10), indicative of the difficultly frequently encountered in effectively reducing the size of fibre particles.

Table 4.2 Particle Size Distributions for Raw Flour Materials

Yellow Pea Flour	Volume Weighted Mean (um)	d 0.10 (um)	d 0.50 (um)	d 0.90 (um)
Whole (parent)	390	20	342	843
Split (parent)	45*	4	24	108
Whole (0.5mm)	133*	8	42	377
Whole (0.75mm)	236*	14	188	560
Whole (2.0mm)	321	19	273	706
Split (0.5mm)	32	2	16	63
Split (0.75mm)	38	3	21	91

Mastersizer software analysis output was based on an average of three subsamples. Asterisk (*) indicates duplicate analysis for the noted samples.

4.4.2. Free TPC

Results of the free TPC extraction are presented in Table 4.3. Although the 80% methanol extract contains mainly the free phenolic acids, the data represent estimates of reducing capacity because other Folin reagent-reactive compounds may also be present. The parent yellow split pea flour showed a higher TPC value than the parent yellow whole pea flour. The TPC results for both the yellow whole and split pea flours do not support the concept that the majority of free phenolic compounds are located in the seed coat. The reground yellow whole pea flours showed an increase in free phenolic content when compared with the unground form. The reground yellow whole pea flour samples also showed an increase in free phenolic content with an increase in particle size. Both of these outcomes are unexpected unless additional size reduction is limiting extractability of phenolics. In contrast, because there was not a substantial reduction in the particle size of the split pea flour (Table 4.2), there was not a great degree of variation in free TPC values among the particle sizes. However, the 0.5 mm yellow split flour showed higher levels of phenolic content than the 0.75 mm yellow split pea flour, whereas the 0.75 mm yellow pea flour showed less free phenolic content than its unground form.

Consistent with the research on the yellow pea fractions, the 2.0 mm yellow whole pea bread samples exhibited the highest phenolic content (6.5 mg of GA equivalents/mL), as shown in Figure 4.1. A change in values between the flour raw materials and the composite bread samples owing to dilution by the white wheat flour contributed to the reduced phenolic content in the bread samples. Nevertheless, the TPC values for both the 0.5 and 0.75 mm yellow split pea bread samples showed an increase in activity compared with their respective flour samples. The results suggest that thermal

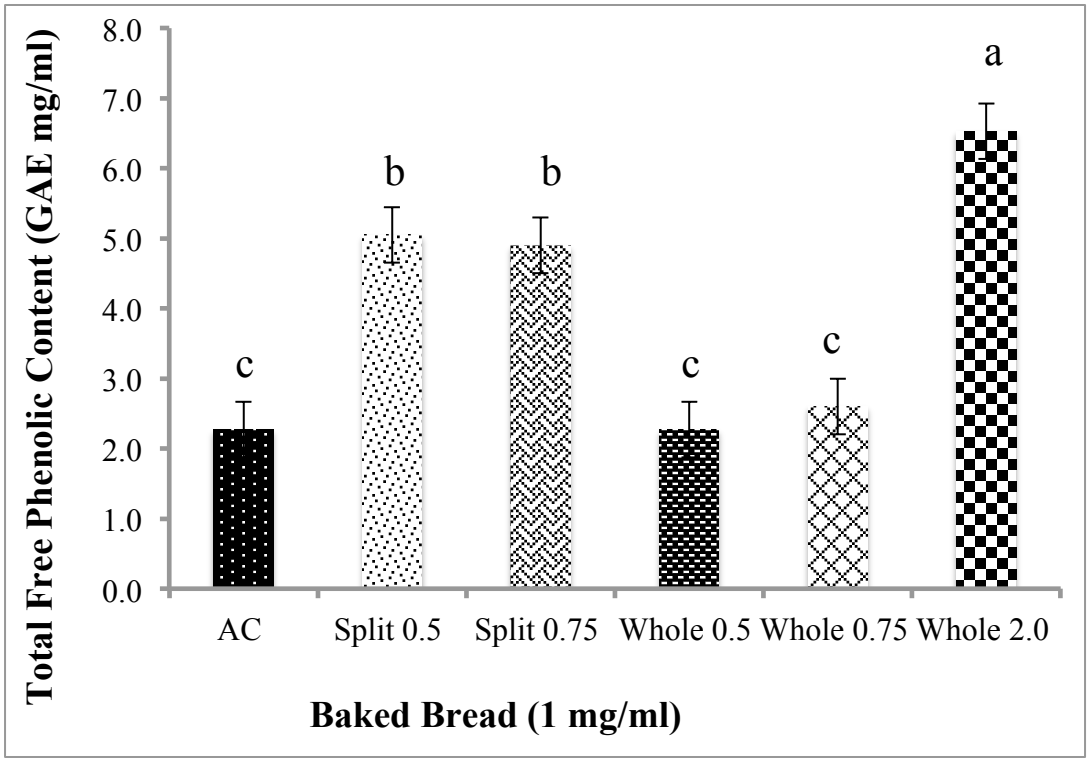
processing during bread baking had a positive influence on the free TPC content of the bread samples, as previously suggested by Lu et al. (2014).

Table 4.3 Free TPC, DPPH, MC, FRAP, and SO for Raw Flours

Sample	Free TPC (GAE mg/ml)	DPPH (%)	MC (%)	FRAP (%)	SO (%)
BHT	…	38a	14c	36a	52bc
AC	4.2c	29bc	25b	29b	45c
Whole	2.3d	25c	52a	31b	64b
Split	4.0c	31b	31b	33a	101a
Split (0.50 mm)	5.2b	26bc	32b	34a	76b
Split (0.75 mm)	2.2d	22c	29b	36a	73b
Whole (0.50 mm)	4.8b	23c	61a	24c	50bc
Whole (0.75 mm)	4.9b	22c	59a	30b	58bc
Whole (2.0 mm)	9.1a	25c	53a	34a	53bc

Data are means of triplicates. Within each column, means with the same letter are not significantly different ($P > 0.05$). TPC = total phenolic content (1.0 mg/mL sample concentration); DPPH = 1,1-diphenyl-2-picrylhydrazyl (1.0 mg/mL sample concentration); MC = metal chelation (1.5 mg/mL sample concentration); FRAP = ferric reducing antioxidant power (2.0 mg/mL sample concentration); SO = superoxide radical scavenging (0.5 mg/mL sample concentration); GAE = gallic acid equivalents; BHT = butylated hydroxyl toluene; AC = AC Carberry wheat white flour; Whole = yellow whole pea flour (parent), and numbers indicate screen size for reground products; and Split = yellow split pea flour (parent), and numbers indicate screen size for reground products.

Figure 4.1. Total Free Phenolic Content for Baked Bread



Total free phenolic content for baked bread. Data illustrated as least squares mean ± standard error of the population mean. Means with same the letter are not significantly different (P > 0.05). Bread sample concentration used in the assay was 1.0 mg/mL. AC = wheat cultivar AC Carberry; and GAE = gallic acid equivalents.

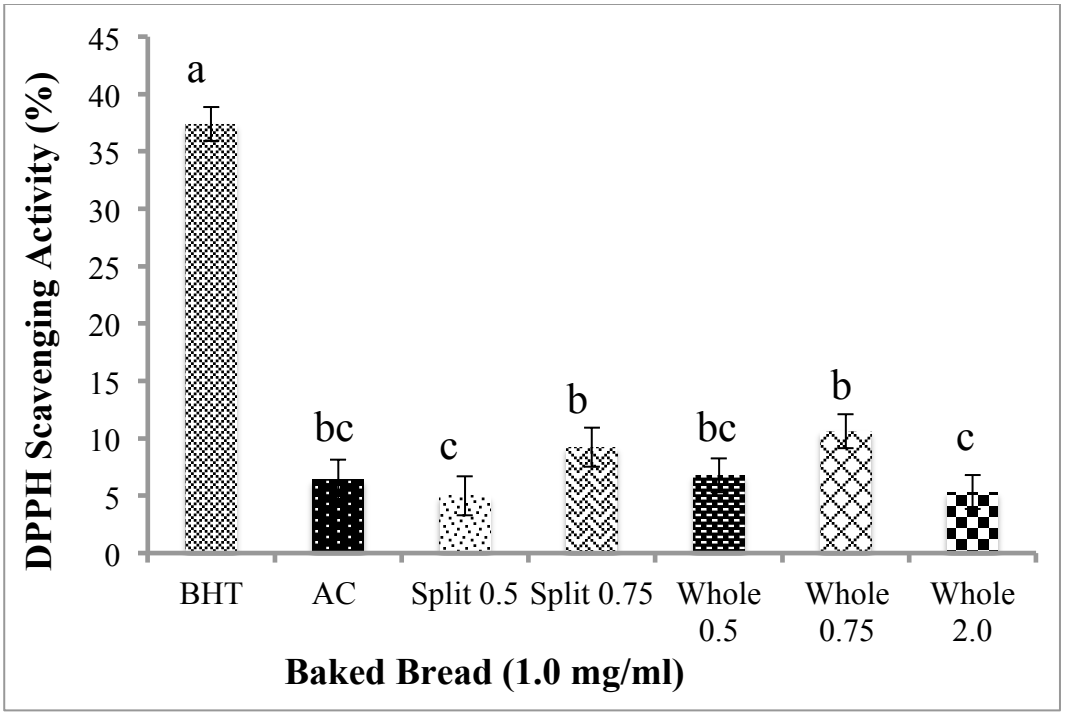
4.4.3. DPPH radical scavenging activity assay

DPPH is not specific to any one particular antioxidant component; thus, it reflects the overall antioxidant capacity of a sample. In the presence of an antioxidant, DPPH radicals are reduced, and the reduction reaction results in the decolorization of the solution. The loss of color, which can be monitored spectrophotometrically, indicates the reducing capacity of the antioxidant toward the DPPH radical (Apak et al., 2013). The DPPH scavenging activity results of the raw material samples are presented in Table 4.3. It is evident that there was less variation between fractions for this assay compared with the free TPC. Reduction in particle size of the yellow split pea flours resulted in a

decrease in DPPH scavenging activity. These results are in agreement with a study in which a reduction in wheat bran particle size produced a decrease in DPPH scavenging activity (Zhu et al., 2010). However, no significant statistical differences in scavenging activity were observed for the parent and reground yellow whole pea flours. This suggests that, on the whole, surface area changes play a minor role in extractability of antioxidants from both types of pulse flours that influence the DPPH scavenging activity of the samples.

The bread samples using a 1 mg/mL concentration produced a similar pattern in DPPH scavenging activity as the flours (Figure 4.2). The DPPH scavenging activity of the AC control bread was 6.4%, compared with 30% scavenging activity of the raw flour, with the DPPH scavenging activity of the split and whole pea composite flour breads not being significantly different from that of the AC control. The DPPH antioxidant activity results for the bread samples illustrate a substantial reduction in scavenging activity in comparison with their respective flours. Nayak et al. (2011) also showed a reduction in DPPH scavenging activity for processed purple potato and yellow pea flour mixes compared with their non-processed forms. As noted in the free TPC section, a potential reduction in antioxidant activity is attributable to a "dilution" effect in the bread when compared with the flour. Even so, the approximately fivefold reduction indicates that substantial elimination of DPPH scavenging ability occurs during processing. Investigation of the dough samples for antioxidant activity prior to baking could be used to confirm the effects of thermal processing on antioxidant activity.

Figure 4.2 DPPH Scavenging Activity for Baked Bread



1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity for baked bread. Data illustrated as least squares mean ± standard error of the population mean. Means with the same letter are not significantly different ($P > 0.05$). Bread sample concentration used in the assay was 1.0 mg/mL. BHT = butylated hydroxyl toluene; and AC = wheat cultivar AC Carberry.

4.4.4. MC activity assay

Metal ion chelating capacity plays an important role in reducing the concentration of transition metals that are available to catalyze lipid peroxidation (Mohan et al., 2012). The MC activities of the samples are presented in Table 4.3. The MC activity was 25, 52, and 31% for the AC control, yellow whole, and yellow split pea flours, respectively.

A significant difference existed between the yellow whole and reground flour samples and their split pea counterparts. This difference suggests that the seed coats play an important role in the metal chelation ability of the yellow pea flour. A reduction in particle size did not influence the antioxidant activity in the yellow whole or split pea

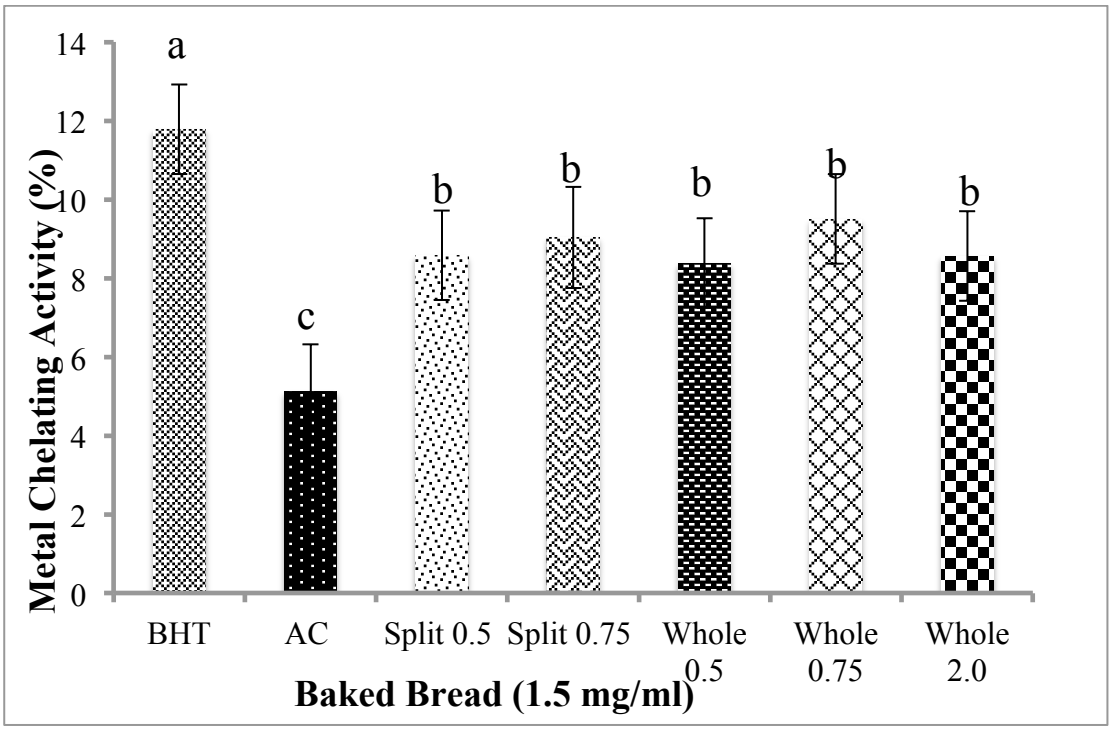
flour samples. It is conceivable that the temperature during grinding could be a factor in the reduced activity (Ragaee et al., 2013; Liu et al., 2015).

All yellow pea flour samples showed good MC activity compared with the BHT standard. This was an expected result because BHT functions to scavenge free radicals rather than chelate metal ions. An alternative positive control for the MC assay is EDTA because it chelates metal ions. Although EDTA is frequently reported as a positive control for the MC assay (Kozarski et al., 2011), BHT was used as a positive control to allow valid comparison for all our assays. The results reported here show a similar trend to a study conducted by Pownall et al. (2010) evaluating yellow pea seed fractions and their MC activity. Optimal MC activity has been attributed to aliphatic compounds where a five-member ring is formed, which consists of the metal ion and two chelating ligands (Pownall et al., 2010). The chelation of metal ions has the potential to decrease the amount of free iron and other metal ions available to participate in the Fenton reaction and reduce the formation of hydroxyl radicals (Pownall et al., 2010). In many foods, iron-promoted lipid oxidation can be decreased by removal of metals from raw materials and by avoiding metal contamination from sources such as processing equipment, food ingredients, and packaging.

The bread samples did not produce similar MC activity as the raw flours (Figure 4.3). The MC activity of the AC control bread was 5% compared with 25% MC activity in the flour. Thus, the magnitude of reduction in metal chelating capacity with processing is similar to the diminishment of DPPH radical scavenging brought about by processing. Although the bread samples were all significantly different than the AC control bread

sample, no statistically significant differences were observed among the bread samples, in line with results on the flours themselves.

Figure 4.3 Metal Chelation Activity for Baked Bread



Metal chelating actvity for baked bread. Data illustrated as least squares mean ± standard error of a population mean. Means with the same letter are not significantly different (P > 0.05). Bread sample concentration used in the assay was 1.5 mg/mL. BHT = butylated hydroxyl toluene; and AC = wheat cultivar AC Carberry.

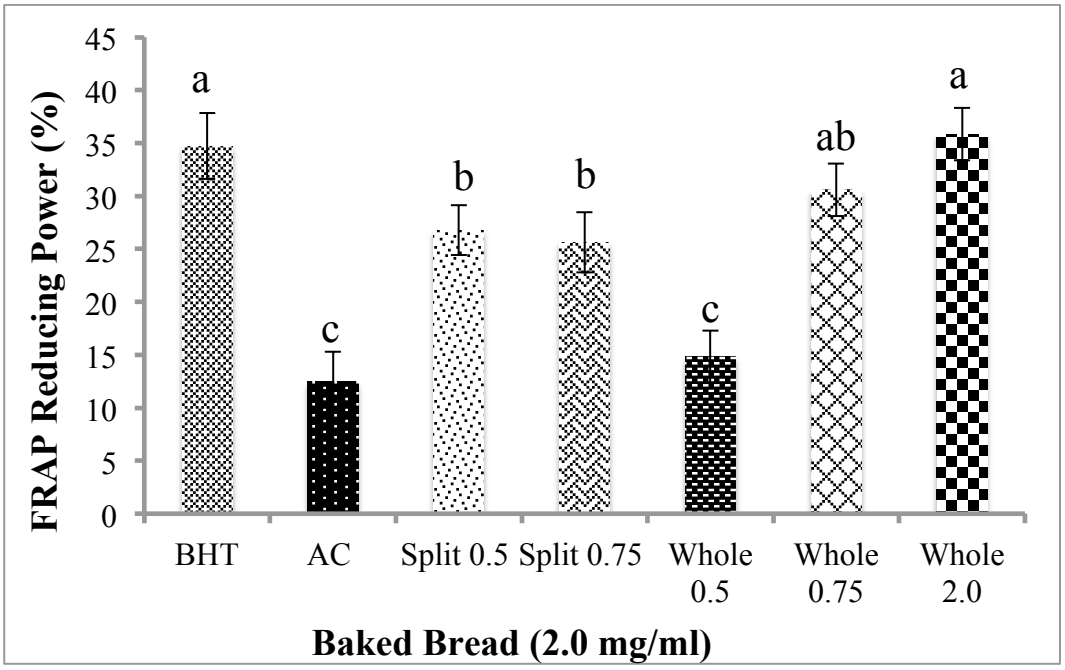
4.4.5. FRAP reducing power assay

The FRAP assay uses antioxidants as reductants. The assay evaluates a transformation from a ferric (III) to a ferrous (II) form by measuring its absorbance. The absorption readings are related to the reducing power of the electron-donating antioxidants present in the sample. The FRAP reducing power sample results are presented in Table 4.3. The FRAP reducing power was 29, 31, and 33% for the AC control, yellow whole pea flour, and yellow split pea flour, respectively. The yellow split

pea, 0.5 and whole pea samples (parent and reground materials) were not significantly different from the positive control, BHT. Brewer et al. (2014) also showed a nonlinear response to changes in particle size and reducing power of the FRAP assay in wheat bran.

A reduction in FRAP reducing power was shown in the bread samples (2.0 mg/mL) (Figure 4.4). Because the FRAP of the AC control bread was 12% compared with 29% in the flour, both dilution and processing effects may have been responsible for reduced activity. The FRAP reducing power in the composite bread samples mirrors that of the flour samples, but with substantial retention of FRAP reducing power in the composites. In particular, the decrease in FRAP reducing power of more finely ground yellow whole samples is essentially replicated in the bread baked from these composite samples. Because the bread samples were minimally impacted by the dilution with white flour, this outcome implies that processing of these bread samples enhanced their FRAP reducing power (Lu et al., 2014).

Figure 4.4 FRAP Reducing Activity for Baked Bread



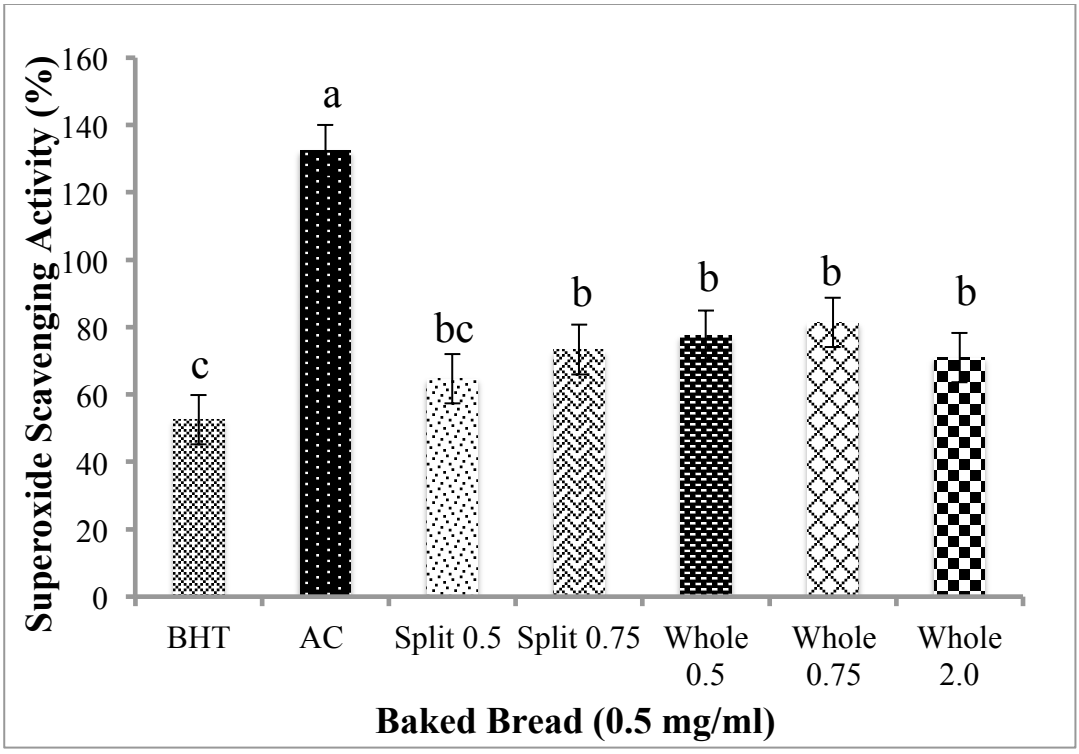
Ferric reducing antioxidant power (FRAP) for baked bread. Data illustrated as least squares mean ± standard error of a population mean. Means with the same letter are not significantly different (P > 0.05). Bread sample concentration used in the assay was 2.0 mg/mL BHT = butylated hydroxyl toluene; and AC = wheat cultivar AC Carberry.

4.4.6. SO radical scavenging activity assay

The SO assay evaluated the autoxidation reaction rate of pyrogallol. The SO scavenging activities of the samples are presented in Table 4.3. The scavenging activity was 45, 64, and 101% for the AC control, yellow whole pea flour, and yellow split pea flour, respectively. The yellow split pea flour and its reground materials generally exhibited greater SO scavenging activity. The AC control exhibited the least SO scavenging activity among all the samples. However, the yellow pea flour samples showed good SO activity when compared with the BHT standard. The results reported here show a similar trend to a study conducted by Pownall et al. (2010), who evaluated yellow pea seed fractions and their scavenging activity.

The bread samples (0.5 mg/mL concentration) produced similar or better SO activity compared with the flours (Figure 4.5). The SO activity of the AC control bread was 133% (this is a % of the control over 4 min), whereas those of the 10% wheat and yellow whole pea composite breads ranged from 71 to 81%. The SO scavenging activity of the 10% wheat and yellow split pea composite breads ranged from 65 to 73%. The bread samples showed greater SO scavenging activity than the positive control, BHT, but less than the AC control bread sample. The AC control showed the most SO scavenging activity within the sample set. The bread samples showed an increase in SO scavenging activity compared with their raw flours. The increase in SO scavenging activity is likely attributable to the yeast in the bread formulation. The enzyme superoxide dismutase is naturally found in nutritional yeast (Li, 2012). It is a reasonable assumption therefore that the same enzyme is also present in baker's yeast used for breadmaking. The reagent pyrogallol used in the SO assay may have been affected by the activity of the superoxide dismutase and other antioxidants present in the samples (Li, 2012). Yeast, as an ingredient, appears to have positively affected the SO scavenging activity in the bread samples. Therefore, it is postulated that the yeast in the bread formulation affected SO scavenging activity. However, it would appear that the yellow pea flour additions somewhat depress the effect of the yeast in the composite bread samples. This depression effect could be attributed to specific antinutritional factors present in the yellow pea flours (Gilani et al., 2012). Antinutritional factors found in pulses alter both the protein digestibility and the amino acids in foods, potentially impacting the availability of active compounds involved in the SO assay.

Figure 4.5 Superoxide Scavenging Activity for Baked Bread



Superoxide scavenging activity for baked bread. Data illustrated as least squares mean ± standard error of the population mean. Means with the same letter are not significantly different (P > 0.05). Bread sample concentration used in the assay was 0.5 mg/mL. BHT = butylated hydroxyl toluene; and AC = wheat cultivar AC Carberry.

4.4.7. Pearson correlation coefficient test

The Pearson correlation coefficient test was selected to determine if free TPC was correlated with the antioxidant activity as measured by the DPPH, MC, FRAP, or SO assays for both the raw flours and the bread samples. Many researchers have postulated that a relationship exists between TPC and antioxidant assay capacity (Xu et al., 2007; Han & Baik, 2008; Dziki et al., 2014). In other words, the total phenolics present in a given sample should be related to its antioxidant capacity. Table 4.4 illustrates the results of the correlation test for each of the assays. Most of the correlations are statistically significant, so to quantify the strength of a correlation, the following scale was used to

express the weight of its significance: greater than 0.50 is considered a strong correlation and less than 0.50 a weak correlation, and it applies to both positive and negative correlations. The MC assay showed a weak positive correlation to the free TPC values (0.27); FRAP showed a stronger positive correlation to the free TPC values (0.47); and SO scavenging showed a weak negative correlation to the free TPC values (–0.39). On the basis of these results, weak but significant relationships between TPC and antioxidant assay capacity were seen for the MC and FRAP assays.

In addition to evaluating possible correlations between free TPC and the antioxidant activity of the raw flour samples, correlations were also explored between the antioxidant activity assays themselves. The DPPH assay showed a weak positive correlation with the FRAP assay (0.40), while showing a weak negative correlation with the SO scavenging assay (–0.41); both of these correlations were statistically significant. The MC assay showed a strong negative correlation with the FRAP assay (–0.89) while showing a strong positive correlation with the SO scavenging assay (0.82), both of these correlations being highly statistically significant. The FRAP assay showed a weak negative correlation with the SO scavenging assay (–0.41) that was nevertheless statistically significant. Because the nature of each antioxidant assay is unique, we would not necessarily expect to see positive correlations among these antioxidant assays (Halliwell, 2013). The chemistry behind each assay is different, and so the results of each assay on the same compound differ. It is likely that protective mechanisms do not act independent of one another but rather function in the form of a cascade. The specificity and sensitivity of a single method does not lead to complete examination of all

compounds present in an extract; therefore, a combination of several tests could provide a more reliable assessment of the antioxidant profile of the food (Halliwell, 2013). However, the principles of one assay may be shared with another assay. For example, the MC and SO activity assays both involve metal ions. The SO anion functions to inactivate iron-sulfur cluster-containing enzymes that release iron in the cell. This iron would be able to undergo Fenton chemistry and generate the highly reactive hydroxyl radical. The ferrous ion chelators may also afford protection against oxidative damage by removing iron that would otherwise participate in Fenton-type reactions. In both cases, the metal ions are being removed to eliminate oxidation damage reactions from occurring in the cells (Gulcin, 2010). Therefore, we would expect to see a positive correlation between these two assays, and the results confirmed this perspective.

Table 4.4 Pearson Correlation Coefficient Testing of Free TPC and Antioxidant Assays

Assay	Free TPC	DPPH	MC	FRAP	SO
Free TPC	1.00	0.12	0.27**	0.47***	-0.39***
DPPH	0.12	1.00	-0.22	0.40***	-0.41***
MC	0.27**	-0.22	1.00	-0.89***	0.82**
FRAP	0.47***	0.40***	-0.89***	1.00	-0.41***
SO	-0.39***	-0.41***	0.82**	-0.41***	1.00

** and *** indicate correlation coefficient test is statistically significant at $P < 0.01$ and 0.001, respectively. TPC = total phenolic content; DPPH = 1,1- diphenyl-2-picrylhydrazyl; MC = metal chelation; FRAP = ferric reducing antioxidant power; and SO = superoxide radical scavenging.

4.5. Conclusion

Both the yellow whole and split pea flour results indicate that these materials are good chelators of metal ions. The bread sample results for the DPPH and MC assays showed a reduction in antioxidant activity when compared with their respective raw

flours, which is attributed to a reduction in activity owing to dilution with the white flour. The FRAP and SO assay results showed minor changes in the antioxidant activity present in the bread samples compared with their respective raw flours, which could be attributed to an increase in activity owing to Maillard reaction products generated during thermal processing. Particle size had little influence on antioxidant activity with the exception of the DPPH assay. However, it should be noted that the 80% methanolic extract contained mainly free phenolic compounds, which means that the unextracted bound polyphenols did not contribute to data presented in this work. Further investigation of the final product(s) is desirable to quantitatively ascertain whether the protein or the fibre fractions or the bound polyphenols have stronger influence on the antioxidant functionality of the wheat–pea composite bread products.

4.6. Acknowledgements

The authors are grateful to the Natural Sciences & Engineering Research Council of Canada Discovery grants program and Manitoba Agri-Food Research and Development Initiative for funding and to Best Cooking Pulses (Portage-la-Prairie, MB, Canada) for supplying samples.

4.7. Literature cited

Adiotomre, J., Eastwood, M. A., Edwards, C. A., & Brydon, W. G. (1990). Dietary fiber: In vitro methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition*, *52*,128-134.

Agboola, S., Mofolasayo, O., Watts, B., & Aluko, R. (2010). Functional properties of yellow field pea (Pisum sativum L.) seed flours and the in vitro bioactive properties of their polyphenols. *Journal of Food Research International*, *43*, 582-588.

Aluko, R., & Monu, E. (2003). Functional and bioactive properties of quinoa seed hydrolysates. *Journal of Food Science , 68*, 1254-1258.

Apak, R., Gorinstein, S., Bohm, V., Schaich, K., Ozyurek, M., & Guclu, K. (2013). Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure Applied Chemistry*, *85*, 957-998.

Bojnanska, T., Francakova, H., Liskova, M., & Tokar, M. (2012). Legumes—The alternative raw materials for bread production. *Journal of Microbiology and Biotechnology and Food Sciences*, *1*, 876-886.

Borrelli, R., Mennella, C., Barba, F., Russo, M., and Russo, G. (2003). Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food and Chemical Toxicology*, *41*, 1367-1374.

Brewer, L., Kubola, J., Siriamornpun, S., Herald, T., & Shi, Y.-C. (2014). Wheat bran particle size influence on phytochemical extractibility and antioxidant properties. *Food Chemistry , 152*, 483-490.

Buthmann, A. (2011). *Six Sigma Tools & Templates.* Retrieved 08 5, 2015, from iSixSigma: www.isixsigma.com

Delcour, J., Rouau, X., Courtin, C., Poutanen, K., & Ranieri, R. (2012). Technologies for enhanced exploitation of the health-promoting potential of cereals . *Trends in Food Science and Technology , 25*, 78-86.

Diziki, D., Rozylo, R., Gailik-Dziki, U., & Sevieca, M. (2014). Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science and Technology , 40*, 48-61.

Epstein, B. (1948). Logarithmico-normal distribution in breakage of solids. *Industrial and Engineering Chemistry*, *40*, 2289-2291.

Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., and Liardon, R., eds. (1990). The maillard reaction in food processing, human nutrition and physiology. In P. A. Finot (Ed.), *4th International Symposium on the Maillard Reaction.* Cambridge: Birkhauser Publishing Ltd.

Gelinas, P., & McKinnon, C. 2006. Effect of wheat variety, farming site and bread-baking on total phenolics. *International Journal of Food Science and Technology, 41*, 329-332.

Ghanachorloo, M., Tarzi, B., & Bahaninia , M. (2013). The Effect of germination on phenolic compounds and antioxidant activity in pulses. *Journal of American Oil Chemistry Society , 90*, 407-411.

Gilani, S., Xiao, W., Cockell, K. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition*, *108*, S315-S332.

Gulcin, I. (2010). Antioxidant properties of resveratrol: A structure-activity insight. *Innovative Food Science and Emerging Technologies , 11*, 210-218.

Halliwell, B. (2013). The antioxidant paradox: less paradoxial now? *British Journal of Clinical Pharmacology* , *75*, 1365-2125.

Han, H., & Baik, B-K. (2008). Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quanititative changes during processing. *International Journal of Food Science and Technology , 43* (11), 1971-1978.

Han, H. M., & Koh, B.K. (2011). Antioxidant activity of hard wheat flour, dough and bread prepared using various processes with the addition of different phenolic acids. *Journal of Science and Food Agriculture*, *91*, 604-608.

Jensen, S., Ostdal, H., Skibsted, L., & Thybo, A. (2011). Antioxidants and shelf life of whole wheat bread. *Journal of Cereal Science, 53*, 291-297.

Kozarski, M., Klaus, A., & Niksik, M. (2011). Extract from wild strain of mushroom Ganoderma lucidum as natural antioxidants. *Zbornik Matice srpske za prirodine nawke , 120*, 289-297.

Li, X. (2012). Improved pyrogallol autooxidation method: A reliable and cheap superoxide-scavenging assay suitable for all assays. *Journal of Agricultural and Food Chemistry , 60* (25), 6418-6424.

Li, Y., Jiang, B., Zhang, T., Mu, W., & Liu, J. (2008). Antioxidant and free radical scavenging activity of chickpea protein hydrolysate (CPH). *Food Chemistry , 106*, 444-450.

Liu, C., Liu, L., Li, L., Hao, C., Zheng, X., & Bian, K. (2015). Effects of different milling processes on whole wheat flour quality and performance in steamed bread making. *LWT-Food Science and Technology , 62*, 310-318.

Lu, Y., Luthria, D., Fuerst, E., Kiszonas, A., Yu, L., & Morris, C. (2014). Effect of processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties. *Journal of Agricultural and Food Chemistry , 62*, 10431-10436.

Malvern Instruments Ltd. 2007. Mastersizer 2000 user manual. Accessed on 7 Sep 2013. <https://www.malvern.com/en/support/resource-center/user-manuals/MAN0384EN>

Mohan, S., Balamurugan, V., Salini, S., & Rekha, R. (2012). Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant Kalanchoe pinnata. *Journal of Chemical Pharmaceutical Research*, *4,* 197-202.

Nayak, B., Berrios, J., Powers, J., & Tang, J. (2011). Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. *Journal of Food Science , 76* (6), C874-C883.

Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*, *72*, R21-R32.

Pownall, T. L., Udenigwe, C. C., & Aluko, R.E. (2010). Amino acid composition and antioxidant properties of pea seed (Pisum sativum L.) enzymatic protein hydrolysate fractions. *Journal of Agricultural and Food Chemistry , 58*, 4712-4718.

Preston, K. K. (1982). The GRL pilot mill: II physical dough and baking properties of flour streams milled from Canadian Red Spring Wheat. *Canadian Institute of Food Science Technology Journal, 15*, 29-36.

Ragaee, R., Gamel, T., Seethraman, K., & Abdel-Aal, E.-S.(2013). Handbook of plant food phytochemicals: sources, stability and extraction. In N. B. B.K.Tiwari (Ed.), Food Grains (pp. 138-162). Oxford, UK: John Wiley Sons Ltd.

Repetsky, J. A., & Klein, B. P. (1982). Partial replacement of wheat flour with yellow field pea flour in white pan bread. *Journal of Food Science*, *47*, 326-327, 329.

Rizello, C., Calasso, M., Campanella, D., De Angelis, M., & Gobetti, M. (2014). Use of sourdough fermentation and mixture of wheat, chickpea, lentil, and bean flours for enhancing the nutritional, texture, and sensory characteristics of white bread. *International Journal of Food Microbiology, 180*, 78-87.

Sardesai, V. (1995). Role of antioxidants in health maintenance. *Nutrition in Clinical Practice*, *10*, 19-25.

Sharma, P., & Gujral, H. (2014). Antioxidant potential of wheat flour chapattis as affected by incorporating barley flour. *LWT- Food Science and Technology, 56*, 118-123.

Singleton, V., Orthofer, R., & Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin ciocalteau's reagent. *Methods of Enzymology , 299*, 152-178.

Stanisavljevic, N., Jovanovic, Z., Cupic, T., Lukic, J., Dukie, J., & Radovic, S., (2013). Extractability of antioxidants from legume seed flour after cooking and in vitro gastrointestinal digestion in comparison with methanolic extraction of the unprocessed flour. *International Journal of Food Science and Technology , 48*, 2096-2104.

Xie, Z., Huang, J., Xu, X., & Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry , 111* (2), 370-376.

Xu, B., Yuan, S., & Chang, S. (2007). Comparative analysis of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *Journal of Food Science , 72* (2), S167-S177.

Zhu, K., Hueng, S., Peng, W., Qian, H., & Zhou, H. (2010). Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fibre. *Food Research International , 43*, 943-948.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

5.1. Introduction

This research project evaluated the properties of both yellow split and whole pea flours and their incorporation into wheat pan bread. Two wheat control flours were used in the composite wheat-pea dough samples. AC Carberry was the experimental pure wheat flour, and was used as the control flour during the antioxidant activity assessment of the bread samples. Both the PFM P500 commercial and AC Carberry wheat flours were used as control flours to evaluate the effect of yellow pea flour additions in the composite wheat-pea bread samples from a quality perspective. Quality deteriorations were expected due to a dilution of the wheat flour with the yellow whole and split pea flour additions. The dilution of the wheat flour resulted in a relative decrease of the gluten proteins, which negatively influenced the gluten matrix and gas retention of the dough leading to a reduction in loaf volume **(**Sosulski & Wu, 1988). Testing was conducted to determine quality changes, the influence of particle size as well as different substitution levels (10, 15, 20 and 25%) on: mixing and dough properties, bread quality parameters, bread staling, total phenolic content and antioxidant activity.

5.2. Chemical composition of the yellow split and whole pea flours

A chemical analysis was conducted to quantify the protein, moisture and ash content of the flour samples. The results of this chemical analysis suggest that the yellow split pea flour was a richer source of ash and protein content compared to the yellow whole pea flour. The yellow split pea flour is a superior choice for a composite wheat-pea blend when the formulation objective is to increase protein as well as mineral content.

5.3. Predictors of bread quality

5.3.1. Mixing as a predictor of bread quality

Researchers have concluded that the addition of non-wheat proteins into wheat flour impacts mixing parameters such as water absorption, which in turn influences loaf volume (Fenn et al., 2010 and Mohammed et al., 2012). Based on the reported results for the mixing parameters, the testing being conducted on the farinograph and mixograph instruments, a definitive relationship between the mixing and bread quality parameters could not be established. Pollard and colleagues (2002) also reported a lack of statistical correlation between loaf height and mixing parameters for composite flour blends. The statistical analysis identified that the type of base control wheat flour, type of yellow pea flour and the level of substitution in the composite wheat-pea flour blends negatively influenced mixing properties, while particle size of the yellow pea flours did not have a statistically measurable effect. Interestingly, the level of yellow pea flour substitution did not have an effect on the mixograph development time but negatively influenced the other reported mixing parameters including: water absorption, farinograph dough development, stability, mixing tolerance index and mixograph peak dough resistance. The study also showed that the yellow split pea flour had a larger negative effect on the results compared to the yellow whole pea flour.

5.3.2. Thermal analysis as a predictor of bread quality

The RVA parameters peak viscosity, set back value, trough, breakdown and final viscosity for the yellow split and whole pea flours were statistically lower than those for both control wheat flours. Angioloni and Collar (2012) attributed the poor pasting behavior

of the non-wheat flours to their lower carbohydrate content. The yellow split and whole pea flours had similar RVA results; however, the yellow split pea flour had less of an effect than the yellow whole pea flour for the above noted RVA parameters. The peak time and pasting temperatures did not exhibit the same result. No statistical difference was reported amongst the yellow pea flour samples with the exception of the yellow split 0.5 mm flour sample for the pasting temperature parameter.

The yellow split and whole pea flour blend RVA results report lower set back values compared to the control wheat flours. Based on these reported set back values, we would expect to see a bread firmness benefit with the wheat-pea composite flour bread samples compared to the control wheat flour bread samples. A lower set back value at the peak of the curve indicates higher diastatic activity, which has been shown to correlate with lower retrogradation. Amongst the yellow pea flour samples, the 0.75 mm yellow whole pea flour reported the lowest value for the set back value parameter. The bread firmness results confirm that the type of yellow pea flour influences this bread quality parameter. A bread firmness benefit was statistically validated for the yellow whole pea flour bread samples as well as the 0.50 mm yellow pea flour particle size. A bread firmness benefit would have also been expected for the yellow split pea flour given the reported set back values. The RVA test may not be a good predictor of bread quality for these composite wheat-pea flour blends.

5.3.3. Dough measurements as a predictor of bread quality

The addition of yellow pea flour into wheat flour dough compromised dough quality parameters such as oven spring, resistance to extension, extensibility and the area under the curve. The statistical analysis confirmed that the base control wheat flour, type of yellow pea flour and the level of substitution negatively influenced these dough quality

parameters as the level of yellow pea flour substitution increased in the wheat-pea composite dough samples. However, there was no statistical difference amongst the control base wheat flours for oven spring. Autio and Laurikainen (1997) reported that a larger loaf volume was related to an improved oven spring. These researchers attributed the improved oven spring to an increase in the volume of the gluten fraction, which permits an increase in the extensibility of the dough sample (Autio & Lauikainen, 1997). Dietary fibre when present in dough makes significant changes to the extensional behavior of hydrated flour-fibre blends (Collar et al., 2007). The addition of fibre from a range of sources has been reported to decrease the extensibility of dough, whereas it has been reported to have a variable effect on the resistance to extension (Ktenioudaki & Gallagher, 2012). In this study, the oven spring results for the yellow pea flours showed a linear decrease as the substitution levels increased compared to the control wheat flours. The results for loaf volume followed the same trend.

5.4. Bread quality

Many bread quality parameters were investigated in this project. However, many researchers might argue that the loaf volume and the texture/firmness of the bread product may be the most important factors to consider where an end-product is concerned.

As expected, the PFM P500 commercial control wheat flour had a greater loaf volume compared to the AC Carberry experimental control wheat flour. Many commercial bread wheat flours contain improving agents and dough conditioners to produce consistent leavened bread products. The loaf volumes reported in this study suggest that additional improvers will need to be explored when any addition of yellow pea flour is added to produce wheat-pea composite bread samples. Aside from the bread quality parameter loaf volume, a decrease in the whiteness index and breadscore was reported, while the firmness

and cell density increased with increasing yellow pea flour additions in the wheat-pea composite bread samples.

5.5. Bread staling evaluated using a DSC instrument

The DSC instrument was used to evaluate staling of the bread dough samples. The parameter enthalpy change reported for Day 0 was significantly different than Days 1, 2 and 3 and this parameter decreased as the sample storage time increased. Day 1 was also significantly different than Days 2 and 3, while Days 2 and 3 were not significantly different from each other. No statistical difference was reported for the parameter enthalpy change between the PFM control wheat flour and the 10% yellow whole-PFM P500 composite flour blends, while a statistical decrease was shown between the 10% yellow split-PFM P500 composite flour blends and the 10% yellow whole-PFM P500 composite flour blends. The statistical difference between the yellow pea flours would suggest that a retrogradation reduction should be present in the 10% yellow split-PFM P500 composite flour blend samples. This study did show that a statistical difference existed between the types of yellow pea flour used in the wheat-pea composite bread samples and the bread quality parameter firmness; the yellow whole pea flour composite bread samples reported lower firmness values than the yellow split pea flour composite and control wheat flour bread samples. In addition to the type of yellow pea flour, a statistical difference was also shown between the 0.5 and 0.75 mm particle size for the yellow pea flours. Collar and colleagues (2015) reported that wheat control flour breads were softer and staled at a slower rate than non-wheat flour formulated breads. Unfortunately, the results from the DSC instrument and the TA XT2 analyzer for bread firmness were not in agreement. It is also important to note that the firmness parameter was only evaluated on Day 0 in this study. Further investigation

should be conducted to determine whether the data trend identified for the yellow whole pea flour bread samples carries over to Days 1, 2 and 3 and also whether the 0.5 mm particle size maintains a bread firmness benefit in the final product.

5.6. Influence of particle size on total phenolic content and antioxidant activity of raw materials and bread samples

An evaluation of both the phenolic content and antioxidant activity was conducted on the raw materials and their respective bread samples to determine the influence of particle size.

5.6.1. Free total phenolic content (TPC)

A reduction in the particle size did show an effect on the TPC results for both the yellow split and whole pea flours. A size reduction in wheat bran led to an increase in chelating activity, reducing power, and TPC (Hemery et al., 2011). A reduction in flour particle size increases the surface area of flour particles potentially leading to increased antioxidant activity. The 0.5 mm particle size for the yellow split pea flour had a statistically significant increase in antioxidant activity compared to its parent material, while the 0.75 mm particle size for the yellow split pea flour had a decrease in antioxidant activity. All three particle sizes for the yellow whole pea flour had statistically significant increases in antioxidant activity compared to the parent material. The TPC for the yellow split parent material was higher than the yellow whole parent material; however, the reduction in size of the yellow whole pea flour improved the TPC values. The greatest TPC was reported for the whole 2.0 mm particle size.

Based on the reported results for the yellow split pea 0.5 mm particle size bread sample, thermal processing did not impact TPC. The yellow split pea 0.75 mm particle

size showed an increase in TPC content for the bread compared to the raw materials. The three yellow whole pea flour particle sizes showed a minor change in TPC due to thermal processing. The highest TPC in the bread samples was reported for both the yellow split pea flours and yellow whole 2.0 mm particle size. Processing clearly plays a role in the release of phenolic compounds that directly impacts the TPC and antioxidant activity (Nayak et al., 2011). However, the literature is not univocal regarding baking effects on TPC. In fact, while Gelinas and McKinnon (2006) showed an increase of TPC, Leenhardt and colleagues (2006) reported that phenolic compounds are destroyed during the baking process. Moreover, the methods used for extraction and evaluation of antioxidant capacity vary, making it rather difficult to make a comparison amongst the studies, and thus an ability to extrapolate to formulate general conclusions.

5.6.2. DPPH radical scavenging activity

The DPPH assay did not show a similar trend as the TPC. The 0.75 mm particle size yellow split pea flour had a statistically significant reduction in antioxidant activity compared to the parent material. No statistical differences were reported for the particle sizes of the yellow whole pea samples compared to their parent material. El-Sayed and colleagues (2013) evaluated particle size effects in whole-wheat pizza crusts. The research outcomes were in agreement with the work conducted by Moore and colleagues (2009) since they reported a reduced wheat bran particle size did not impact its antioxidant properties during processing (El-Sayed et al., 2013). Another study by Cheng and colleagues (2006) found that bran particle size reduction increased its antioxidant properties. Unfortunately, this reduction in particle size also contributed to greater losses in

antioxidant activity during storage and thermal processing. Evaluating the effect of particle size on antioxidant activity in finished products is complex and should consider all processing steps as well as flour storage. The yellow parent split and a 0.5 mm particle size yellow split pea raw flour sample had the highest DPPH activity, and was statistically the same as the AC Carberry control flour.

All yellow split and whole pea flour bread samples had a decrease in DPPH activity compared to their respective raw materials. Of the reported bread samples, the yellow split and whole 0.5 mm particle size had the highest TPC values. In a study conducted by Xu and Chang (2008), the DPPH antioxidant activity of legumes was decreased significantly by atmospheric and pressure boiling using a pressure cooker. In contrast, Stanisavljevi and colleagues (2013) reported that boiling legume flour for 45 minutes at 100°C increased its DPPH activity. Based on these study results, it is evident that the type of processing influences the antioxidant activity of the sample.

5.6.3. Metal chelation activity

The metal chelation assay results showed that there was a higher antioxidant activity for the yellow whole and split pea flours compared to the AC Carberry control wheat flour. The 0.5 and 0.75 mm particle sizes for the yellow split pea flour were reported to have a statistically negative effect on the antioxidant activity compared to the parent material, while a reduction in particle size for the yellow whole pea flour positively influenced the antioxidant activity compared to that of the parent material. As noted above, a size reduction in wheat bran led to an increase in chelating activity (Hemery et al., 2011). The split parent and three particle sizes for the whole yellow pea flours had the highest metal chelation capacity amongst the samples, whereas the metal chelation values for the whole

parent and two particle sizes for the split yellow pea flour were not statistically different.

All yellow split and whole pea flour bread samples had a decrease in metal chelating activity compared to their respective raw materials. However, the yellow whole and split pea bread samples reported higher metal chelating activity compared to the AC Carberry control flour. No statistical differences were reported amongst the yellow whole and split pea bread samples for this assay.

5.6.4. FRAP reducing power

For the FRAP assay, no statistical differences existed between the parent and the two particle sizes for the yellow split pea flour. The yellow whole pea flour showed a similar trend in their results, but the whole 0.5 mm particle size had a decrease in antioxidant activity compared to the parent as well as the 0.75 and 2.0 mm whole particle sizes. As noted above, a size reduction in wheat bran led to an increase in reducing power (Hemery et al., 2011). The yellow split and whole 2.0 mm particle size yellow pea flour samples had a significantly higher antioxidant activity compared to the AC Carberry control flour.

With the exception of the yellow whole pea 0.5 mm particle size, all yellow pea bread samples reported an increase in FRAP activity compared to their respective raw materials. The yellow whole pea 0.75 and 2.0 mm particle sizes were statistically the same and the yellow whole 0.75 mm was statistically the same as both yellow split pea particle size bread samples. Both the yellow whole 0.75 and 2.0 mm particle size bread samples had the highest FRAP activity, while the yellow whole 0.5 mm particle size bread sample had no statistical differences compared to the AC Carberry control bread sample.

5.6.5. SO scavenging activity

For the SO assay, a reduction in particle size negatively influenced the antioxidant activity for both the yellow whole and split pea flours. The yellow split pea flours had the greatest antioxidant activity followed by the yellow whole pea flours. All three particle sizes for the yellow whole pea flours were not statistically different than the AC Carberry control flour. Both parent yellow pea flours had significantly higher antioxidant activity compared to the AC Carberry control flour. For the bread samples, the AC Carberry control had the highest SO activity, which was the opposite effect compared to the results for the raw materials. The yellow split pea bread samples had minor changes compared to their respective raw materials. No statistical differences were reported amongst the yellow whole and split pea bread samples.

5.7. Conclusions and future research

The data collected and analyzed in this research project support the hypothesis that a reduction in particle size has a chemical and physical effect on the raw materials and their antioxidant activity. Therefore, the functionality of the antioxidant in the bread product should be determined when selecting the type of yellow pea flour as well as the particle size. The researcher should determine if the addition of the antioxidant is to increase the antioxidant load or to improve the shelf stability of the bread product. Further investigations would be necessary to determine the viability of pea flours from a shelf stability perspective; lipoxygenase and lipase assays would be required to evaluate lipid oxidation and shelf stability in the bread product. From a thermal processing perspective, the yellow whole pea 2.0 mm and yellow split pea 0.5 mm particle size had the most stable bread antioxidant activity results compared to their respective raw materials. However, the

metal chelation and SO assays did not exhibit a statistical difference amongst the composite wheat-yellow pea bread samples. Additional research should be carried out to evaluate the antioxidant molecules of the dough samples; this would help quantify the effects of thermal processing on the bread samples. As a special consideration, selection of EDTA as a control for the metal chelation assay should be considered. Additional testing should be conducted to determine the bioavailability of the antioxidants, and a nutritional evaluation of the bread samples should be completed. Quantification of micro and macronutrients would potentially shed light on the antioxidant results and highlight the nutritional benefits associated with composite wheat-pea bread products.

Based on the reported results for this project, the yellow split pea flour makes a good wheat-pea composite bread product from a source of protein, quality and antioxidant activity perspective, although, the yellow whole pea flour was reported to have an advantage where the bread quality parameter bread firmness was concerned. Therefore, future research should be conducted using the yellow split pea flour in the composite wheat-pea bread products. No statistical differences were reported between the 0.5 and 0.75 mm yellow split pea particle sizes in the 10% bread products for antioxidant activity. Further, antioxidant investigations could be conducted evaluating the yellow split parent material as well as the 15% composite wheat-yellow split pea bread products. If additional substitution levels were desired in the final formulation to further increase the protein and antioxidant content of the bread samples, dough-improving agents will need to be considered. And finally, a sensory evaluation should be conducted to determine consumer acceptability of the composite wheat-pea bread products to complement the bread quality results reported here.

REFERENCES

AACC. 1969. Approved Methods. American Association of Cereal Chemists. St. Paul, MN.

Adiotomre, J. E. (1990). Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition , 52*, 128-134.

Agboola, S., Mofolassayo, O., Watts, B., & Aluko, R. (2010). Functional properties of yellow field pea (Pisum sativum L.) seed flours and the in vitro bioactive properties of their polyphenols. *Journal of Food Research International , 43*, 582-588.

Almeida, E., Chang, Y. K., & Steel, C. J. (2010). Effect of adding different dietary fiber sources on farinographic parameters of wheat flour. *Cereal Chemistry , 87* (6), 566-573.

Aluko, R., & Monu, E. (2003). Functional and bioactive properties of quinoa seed hydrolysates. *Journal of Food Science , 68*, 1254-1258.

Angelidis, G., Protonotariou, S., Mandala, I., & Rosell, C. M. (2016). Jet milling effect on wheat flour characteristics and starch hydrolysis. *Journal of Food Science Technology , 53* (1), 784-791.

Angioloni, A., & Collar, C. (2008). Functional response of diluted dough matrixes in high-fibre systems: a viscometric and rheological approach. *Food Research International , 41*, 803-812.

Angioloni, A., & Collar, C. (2012). High legume-wheat matrices: an alternative to promote bread nutritional value meeting dough viscoelastic restrictions. *European Food Research and Technology , 234* (2), 273-284.

Angioloni, A., & Collar, C. (2011). Nutritional and functional added value of oat, Kamut, spelt, rye and buckwheat versus common wheat in bread making. *Journal of the Science of Food and Agriculture , 91*, 1283-1292.

Antiochia, R., Movassaghi, K., Lipone, P., & Campanella, L. (2012). Determination of the antioxidant capacity of different types of bread and flour using a superoxide dismutase biosensor. *Journal of Chemistry and Chemical Engineering , 6*, 199-208.

Anton, A., Ross, K. A., Lukow, O. M., Fulcher, R. G., & Arntfield, S. D. (2008). Influence of added bean flour on some physical and nutritional properties of wheat flour tortillas. *Food Chemistry , 109*, 33-41.

Apak, R., Gorinstein, S., Bohm, V., Schaich, K., Ozyurek, M., & Guclu, K. (2013). Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure Applied Chemistry , 85*, 957-998.

Autio, K., & Laurikainen, T. (1997). Relationships between flour/dough microstructure and dough handling and baking properties. *Trends in Food Science and Technology , 8*, 181-185.

Bakare, A., Osundahunsi, F. O., & Olusanya, J. O. (2016). Rheological, baking, and sensory properties of composite bread dough with breadfruit (Artocarpus communis Forst) and wheat flours. *Food Science and Nutrition , 4* (4), 573-587.

Bjorck, I., & Elmstahl, H. L. (2003). The glycemic response: importance of dietary fibre and other food properties . *The Proceedings of Nutrition Society , 62* (1), 201-6.

Bojnanska, T., FrancaKova, H., Liskova, M., & Tokar, M. (2012). Legumes - The alternative raw materials for bread production. *Journal of Microbiology, Biotechnology and Food Sciences , 1*, 876-886.

Borrelli, R., Mennella, C., Barba, F., Russo, M., and Russo, G. (2003). Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food and Chemical Toxicology , 41*, 1367-1374.

Borsuk, Y. (2011). Incorporation of pulse flours with coarse and fine particle size milled from green lentils (Lens culinars), yellow peas (Pisum sativum L.), navy beans (Phaselous vulgaris L.), and pinto beans (Phaselous vulgari L.) into baked products. Winnipeg, MB, Canada.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews , 56* (11), 317-333.

Brewer, L., Kubola, J., Siriamornpun, S., Herald, T. J., & Shi, Y. C. (2014). Wheat bran particle size influence on phytochemical extractibility and antioxidant properties. *Food Chemistry, 152*, 483-490.

Buthmann, A. (2011). *iSixSigma*. Retrieved 08 5, 2015, from Six Sigma Tools and Templates: www.isixsigma.com

C., C., Conte, P., Fadda, C., & Piga, A. (2015). Gluten-free dough-making of specialty breads: Significance of blended starches, flours and additives on dough behaviour. *Food Science and Technology International , 21* (7), 523-536.

Cai, L., Choi, I., Park, K.-K., & Baik, B.-K. (2014). Bran characterisitcs and bread-baking quality of whole grain wheat flour. *Cereal Chemistry , 91* (4), 398-405.

Canadian Grain Commission. (2016, 03 29). Retrieved 07 20, 2015, from https://www.grainscanada.gc.ca

Canadian National Millers Association. (2015). Retrieved 06 20, 2015, from http://www.canadianmillers.ca

Charles, D. (2012). *Antioxidants properties of spices, herbs and other sources.* Springer.

Chau, C., Wang, Y. T., & Wen, Y. L. (2007). Different micronization methods significantly improve the functionality of carrot insoluble fibre. *Food Chemistry , 100*, 1402-1408.

Cheng, Z., Su, L., Moore, J., Zhou, K., Luther, M., Yin, J.-J., et al. (2006). Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *Journal of Agricultural and Food Chemistry , 54*, 5623-5629.

Choi, I., Kang, C.-S., Cheong, Y.-K., Hyun, J.-N., & Kun, K.-J. (2012). Substituting normal and waxy-type whole wheat flour on dough and baking properties. *Preventative Nutrition and Food Science , 17* (3), 197-202.

Collar, C. (2003). Significance of viscosity profile of pasted and gelled formulated wheat doughs on bread staling. *European Food Research Technology , 216*, 505-513.

Collar, C., Santos, E., & Rosell, C. M. (2007). Assessment of the rheological profile of fibre-enriched dough bread by response surface methodology. *Journal of Food Engineering , 78*, 820-826.

Collar, C., Santos, E., & Rosell, C. M. (2006). Significance of dietary fibre on the viscometric pattern of pasted and gelled flour fibre blends. *Cereal Chemistry , 83 (4)*, 370-376.

Craft, B., Kerrihand, A. L., Amarowicz, R., & Pegg, R. B. (2012). Phenol based antioxidants and the in vitro method used for their assessment. *Comprehensive Reviews in Food Science and Food Safety , 11*, 148-173.

Czuchajowska, Z., Pomeranz, Y., & Jeffers, H. C. (1989). Water activity and moisture content of dough and bread. *Cereal Chemistry , 66 (4)*, 128-132.

Dahl, W., Foster, L. M., & Tyler, R. T. (2012). Review of the health benefits of peas (Pisum sativum L). *British Journal of Nutrition , 103*, S3-S10.

Dalgetty, D., & Baik, B.-K. (2006). Fortification of bread with hulls and cotyledon fibers isolated from peas, lentils, and chickpeas. *Cereal Chemistry , 83* (3), 269-274.

D'Appolonia, B., & Morad, M. M. (1981). Bread staling. *Cereal Chemistry , 58* (3), 186-190.

Defloor, I., & Delcour, J. A. (1999). Impact of maltodextrins and antistaling enzymes on the differential scanning calorimetry staling endotherm of baked bread doughs. *Journal of Agricultural and Food Chemistry , 47*, 737-741.

Degen, A., Becker, K., Makar, H. P., & Borowy, N. (1995). Acacia saligna as a fodder tree for desert livestock and the interaction of its tannins with fibre fractions. *Journal of Food Science and Agriculture , 68*, 65-71.

Delcour, J., Rouau, X., Courtin, C., Poutanen, K., & Ranieri, R. (2012). Technologies for enhanced exploitation of the health-promoting potential of cereals. *Trends in Food Science Technology , 25*, 78-86.

Delgado-Andrade, C., Conde-Aguilera, J., Haro, A., Pastoriza de la, S., & Ruflan-Henares, J. (2010). A combined procedure to evaluate the global antioxidant response of bread. *Journal of Cereal Science , 52*, 239-246.

DePauw, R., Lukow, O. M., & Adams, K. (2011). Origin and continuity of glutenin and gliadin subunits in Canada Western Red Spring wheat. In INRA (Ed.), *Proceedings of the 10th International Gluten Works.*

Deshpande, S., Rangnekar, P. D., Sathe, S. K., & Salunkhe, D. K. (1983). Functional properties of wheat-bean composite flours. *Journal of Food Science , 48* (6), 1659-1662.

Dhinda, F., Jamuna Prakash, J. L., & Dasappa, I. (2012). Effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fibre and low carbohydrate bread. *Food Bioprocessing Technology , 5*, 2298-3006.

(1989). *Diet and health: implications for reducing chronic disease risk.* National Research Council. Washington: The National Academies Press.

Dishman, K.L. (2006). Sieving in particle size analysis. In *Encyclopedia of Analytical Chemistry*. Hoboken, NJ: John Wiley & Sons Ltd.

Diziki, D., Rozylo, R., & Gailik-Diziki, U. (2014). Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science Technology , 40*, 48-61.

Dominguez, H. (Ed.). (2013). *Functional Ingredients from Algae for Foods and Nutraceuticals.* Philadelphia, PA, USA: Woodhead Publishing.

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

Dubois, C. (1978). The practical application of fiber materials in bread production. *Baker's Digest , 52*, 30-33.

Durazzo, A., Turfani, V., Azzini, E., Maiani, G., & Carcea, M. (2013). Phenols, lignans, and antioxidant properties of legume and sweet chestnut flours. *Food Chemistry , 140*, 666-671.

El-Sayed, M., & Abdel-Aal, I. R. (2013). Effect of baking on free and bound phenolic acids in wholegrain bakery products. *Journal of Cereal Science , 57*, 312-318.

Epstein, B. (1948). Logarithmico-normal distribution in breakage of solids. *Industrial and Engineering Chemistry , 40*, 2289-2291.

Fadda, C., Angioloni, A., Piga, A., & Collar, C. (2010). Impact of sourdough, yeast and gluten on small and large deformation rheological profiles of durum wheat bread doughs. *European Food Research and Technology , 231*, 431-440.

FAOSTAT. (2015). Retrieved 06 15, 2016, from Food and Agriculture Organization of the United Nations: faostat@fao.org

Fearn, T., & Russell, P. L. (1982). A kinectic study of bread staling by differential scanning calorimetry. The effect of loaf specific volume. *Journal of the Science of Food and Agriculture , 33*, 537-548.

Fenn, D., Lukow, O. M., Humphreys, G., Fields, P. G., & Boye, J. I. (2010). Wheat-legume composite flour quality. *International Journal of Food Properties , 13* (2), 381-393.

Finley, J., Hintze, K. J., Jeffrey, E. H., Ji, L., & Lei, X. G. (2011). Antioxidants in food: state of the science important to the food industry. *Journal of Agricultural and Food Chemistry , 59*, 6837-6846.

Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., and Liardon, R., eds. (1990). The Maillard reaction in food processing, human nutrition and physiology. In P. A. Finot (Ed.), *4th International Symposium on the Maillard Reaction.* Cambridge: Birkhauser Publishing Ltd.

Frankel, E. (2007). *Antioxidants in food and biology.* California, USA: The Oily Press.

Frankel, E., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture , 80* (13), 1925-1941.

Gelinas, P., & McKinnon, C. (2006). Effect of wheat variety, farming site, and bread-baking on total phenolics. *International Journal Food Science and Technology , 41*, 329-332.

Ghanachorloo, M., Tarzi, B. G., & Bahaninia, M. (2013). The effect of germination on phenolic compounds and antioxidant activity in pulses. *Journal of American Oil Chemistry Society, 90*, 407-411.

Gilani, S., Xiao, W., & Cockell, K. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition , 108*, S315-S332.

Gomez, M., Jimenez, S., Ruiz, E., & Oliete, B. (2011). Effect of extruded wheat bran on dough rheology and bread quality. *LWT: Food Science and Technology , 44*, 2231-2237.

Gomez, M., Ronda, F., Blanco, C. A., Caballero, P. A., & Apesteguia, A. (2003). Effect of dietary fibre on dough rheology and bread quality. *European Food Research and Technology , 216*, 51-56.

Gray, J., & Bemiller, J. N. (2003). Bread staling: molecular basis and control. *Comprehensive Reviews in Food Science and Food Safety , 2*, 1-21.

Gritton, E. T., Pomeranz, Y., & Robbins, G. S. (1975). Protein content and amino acid composition of developing peas. *Journal of Food Science , 40* (3), 584-586.

Gulcin, I. (2010). Antioxidant properties of resveratrol: A structure-activity insight. *Innovative Food Science and Emerging Technology , 11*, 210-218.

Halliwell, B. (2013). The antioxidant paradox: less paradoxial now? *British Journal of Clinical Pharmacology , 75*, 1365-2125.

Han, H., & Baik, B-K. (2008). Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*) and their quantitative changes during processing. *International Journal of Food Science and Technology , 43* (11), 1971-1978.

Han, H. M., & Koh, B.K. (2011). Antioxidant activity of hard wheat flour, dough and bread prepared using various processes with the addition of different phenolic acids. *Journal of Food Science and Agriculture , 91*, 604-608.

Hayman, D., Hoseney, R. C., & Faubion, J. M. (1998). Bread crumb grain development during baking. *Cereal Chemistry , 75 (5)*, 577-580.

Helou, C., Jacolot, P., Niquet-Leridon, C., Gadonna-Widehem, P., & Tessier, F. J. (2016). Maillard reaction products in bread: a novel semi-quantitative method for evaluating melanoidins in bread. *Food Chemistry , 190*, 904-911.

Hemdane, S., Leys, S., Jacobs, P. J., Dornez, E., Delcour, J. A., & Courtin, C. M. (2015). Wheat milling by-products and their impact on bread making. *Food Chemistry , 187*, 280-289.

Hemery, Y., Chaurand, M., Holopainen, U., Lampi, A.-M., Lehtinen, P., Piironen, V., et al. (2011). Potential of dry fractionation of wheat bran for the development of food ingredients, part I: Influence of ultra-fine grinding. *Journal of Cereal Science , 53*, 1-8.

Ho, L., & Noor Aziah, A. A. (2013). Dough mixing and thermal properties including the pasting profiles of composite flour blends with added hydrocolloids. *International Food Research Journal , 20* (2), 911-917.

Hsu, D., Leung, H. K., Finney, P. L., & Morad, M. M. (1980). Effect of germination on nutritive value and baking properties of dry peas, lentils and faba beans. *Journal of Food Science , 45*, 87-92.

Ikegwu, O., Okechukwu, P. E., & Ekumankana, E. (2010). Physicochemical and pasting characteristics of flours and starch from achi brachystegia eurtcoma seed. *Journal of Food Technology , 8* (2), 58-66.

Jagannadham, K., Parimalavalli, R., & Surendra Babu, A. (2014). A study on comparison between cereal (wheat) and non cereal (chickpea) flour characteristics. *International Journal of Current Trends in Research , 3* (2), 70-76.

Jensen, S., Ostdal, H., Skibsted, L. H., & Thybo, A. K. (2011). Antioxidants and shelf life of whole wheat bread. *Journal of Cereal Science , 53*, 291-297.

Kanner, J. (2010). Metals and food oxidation. In *Oxidation in Food and Beverages* (Vol. Chapter 2, pp. 36-56). Woodhead Publishing.

Kerr, W., Ward, C. D., McWatters, K. H., & Resurreccion, A. V. (2000). Effect of milling and particle size on functionality and physiochemical properties of cowpea flour. *Cereal Chemistry , 77* (2), 213-219.

Khatkar, B., Bell, A. E., & Schofield, J. D. (1995). The dynamic properies of glutens and gluten sub-fractions from wheat of good and poor bread making quality. *Journal of Cereal Science , 22*, 624-628.

Kohajdova, Z., Karovicova, J., & Magala, M. (2013). Effect of lentil and bean flours on rheological and baking properties of wheat dough. *Chemical Papers , 67* (4), 308-407.

Kozarski, M., Klaus, A., & Niksik, M. (2011). Extract from wild strain of mushroom ganoderma lucidum as natural antioxidants. *Zbornik Matice srpske za prirodine nawke , 120*, 289-297.

Ktenioudaki, A., & Gallagher, E. (2012). Recent advances in the development of high-fibre baked products. *Trends in Food Science and Technology , 28*, 4-14.

Kumar, S., Kumar, N., & Garg, M. K. (2015). Pasting characteristics of stored wheat in hermetic bags and conventional methods. *Agriculture Engineering International , 17* (3), 278-286.

Leenhardt, F., Lyan, B., & Rock, E. (2006). Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *Journal of Agricultural and Food Chemistry , 54*, 1710-1715.

Li, X. (2012). Improved pyrogallol autooxidation method: A reliable and cheap superoxide-scavenging assay suitable for all assays. *Journal of Agricultural and Food Chemistry , 60*, 6418-6424.

Li, Y., Jiang, B., Zhang, T., Mu, W., & Liu, J. (2008). Antioxidant and free radical scavenging activity of chickpea protein hydrolysate (CPH). *Food Chemistry , 106*, 444-450.

Liu, C., Liu, L., Li, L., Hao, C., Zheng, X., & Bian, K. (2015). Effects of different milling processes on whole wheat flour quality and performance in steamed bread making. *LWT: Food Science and Technology , 62*, 310-318.

Lu, Y., Luthria, D., Fuerst, E., Kiszonas, A., Yu, L., & Morris, C. (2014). Effect of processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties. *Journal of Agricultural and Food Chemistry , 62*, 10431-10436.

Lukow, O., Suchy, J., Adams, K., Brown, D., DePauw, R., Fox, S., et al. (2011). Effect of wheat maturity and post-harvest temperature treatments on the quality of grain and end-products. *Journal of Agrocrop Science , 2* (2), 15-22.

Lyddon, C. (2014, 07 02). *Focus on Canada*. Retrieved 06 02, 2015, from World-Grain: http://www.world-grain.com/Departments/Country-Focus/Country-Focus-Home/Focus-on-Canada-2014.aspx

Ma, Z., Boye, J. I., Swallow, K., Malcolmson, L., & Simpsone, B. K. (2015). Techno-functional characterization of salad dressing emulsions supplemented with pea, lentil and chickpea flours. *Journal of Science and Food Agriculture, 96* (3), 837-847.

Malvern Instruments Ltd. 2007. Mastersizer 2000 user manual. Accessed on 7 Sep 2013. <https://www.malvern.com/en/support/resource-center/user-manuals/MAN0384EN>

Marcin, K., Jaroslaw, W., Monika, P., & Agnieszka, W. (2016). Application of the response surface methodology in optimizing oat fibre particle size and flour replacement in wheat bread rolls. *Journal of Food , 14* (1), 18-26.

Maskus, H., Bourre, L., Fraser, S., & Malcolmson, L. (2016). Effects of grinding method on the compositional, physical, and functional properties of whole and split yellow pea flours. *Cereal Foods World , 61* (2), 59-64.

Menga, V., Fares, C., Troccoli, A., Cattivelli, L., & Baia, A. (2010). Effects of genotype, location and baking on the phenolic content and antioxidant properties of cereal species. *International Journal of Food Science and Technology , 45*, 7-16.

Mohammed, I., Ahmed, A. R., & Senge, B. (2014). Effects of chickpea flour on wheat pasting properties and bread making quality. *Journal of Food Science and Technology , 51* (9), 1902-1910.

Mohammed, I., Ahmed, A., & Senge, B. (2012). Dough rheology and bread quality of wheat–chickpea flour blends. *Industrial Crops and Products , 36*, 196-202.

Mohan, S., Balamurugan, V., Salini, S., & Rekha, R. (2012). Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant Kalanchoe pinnata. *Journal of Chemical Pharmaceutical Research , 4*, 197-202.

Moore, J., Luther, M., Cheng, Z., & Yu, L. (2009). Effects of baking conditions on dough fermentation, and bran particle size on antioxidant properties of whole-wheat pizza crusts. *Journal of Agricultural and Food Chemistry , 57*, 832-839.

Naivikul, O., & D'Appolonia, B. (1978). Comparison of legume and wheat flour carbohydrates. I Sugar analysis. *Cereal Chemistry , 55 (6)*, 913-918.

Nayak, B., Berrios, J. J., Powers, J. R., & Tang, J. (2011). Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. *Journal of Food Science , 76*, C874-C878.

Nenadis, D., & Tsimoudou, M. Z. (2010). Assessing the activity of natural food antioxidants. In *Oxidation in Food and Beverages* (Vol. Chapter 14, pp. 332-367). Woodhead Publishing.

North Dakota State University. (2015, 08 31). Retrieved 08 15, 2016, from Wheat quality & carbohydrate research: www.ndsu.edu/faculty/simsek/wheat/overview.html

Ohimain, E. (2015). Recent advances in the production of partially substituted wheat and wheatless bread. *European Food Research and Technology , 240*, 257-271.

Oomah, B., Caspar, F., Malcolmson, L., & Bellido, A. (2011). Phenolics and antioxidant activity of lentil and pea hulls. *Food Research International , 44*, 436-441.

Otto, T., Baik, B. K., & Czuchajowska, Z. (1997). Microstructure of seeds, flours, and starches of legumes. *Cereal Chemistry , 74 (4)*, 445-451.

Palafox-Carlos, H., Ayala-Zavala, J. F., & Gonzalez-Aguilar, G. A. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable. *Journal of Food Science , 76* (1), R6-R15.

Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science , 72*, R21-R32.

Paraskevopoulou, A., Provatidou, E., Tsotsiou, D., & Kiosseoglou, V. (2010). Dough rheology and baking performance of wheat flour-lupin protein isolate blends. *Food Research International , 43*, 1009-1016.

Peressini, D., & Sensidoni, A. (2009). Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. *Journal of Cereal Science , 49*, 190-201.

Pollard, N., Stoddard, F. L., Popineau, Y., Wrigley, C. W., & MacRitchie, F. (2002). Lupin flours as additives: Dough mixing, breadmaking, emulsifying, and foaming. *Cereal Chemistry , 79 (5)*, 662-669.

Pomeranz, Y., Shogren, M., Finney, K. F., & Bechtel, D. B. (1977). Fibre in breadmaking-effects on functional properties. *Cereal Chemistry , 54*, 25-41.

Pownall, T. L., Udenigwe, C. C., & Aluko, R.E. (2010). Amino acid composition and antioxidant properties of pea seed enzymatic protein hydrolysate fractions. *Journal of Agricultural and Food Chemistry , 58*, 4712-4718.

Preston, K. K. (1982). The GRL pilot mill: II physical dough and baking properties of flour streams milled from Canadian Red Spring Wheat. *Canadian Institute of Food Science Technology Journal , 15*, 29-36.

Ragaee, R., Gamel, T., Seethraman, K., & Abdel-Aal, E-S. (2013). Handbook of plant food phytochemicals: sources, stability and extraction. In N. B. B.K.Tiwari (Ed.), *Food Grains* (pp. 138-162). Oxford, UK: John Wiley Sons Ltd.

Ram, S. (2009). *Cereals: processing and nutritional quality.* Nipa.

Rawle, A. (n.d.). Retrieved 05 08, 2015, from Research Gate: www.researchgate.com

Repetsky, J., & Klein, B. (1982). Partial replacement of wheat flour with yellow field pea flour in white pan bread . *Journal of Food Science , 47*, 326-327, 329.

Ribotta, P., Arnulphi, S. A., Leon, A. E., & Anon, M. C. (2005). Effect of soybean addition on the rheological properties and breadmaking quality of wheat flour. *Journal of the Science of Food and Agriculture , 85* (11), 1889-1896.

Rizello, C., Calasso, M., Campanella, D., De Angelis, M., & Gobetti, M. (2014). Use of sourdough fermentation and mixture of wheat, chickpea, lentil, and bean flours for enhancing the nutritional, texture, and sensory characteristics of white bread. *International Journal of Food Microbiology , 180*, 78-87.

Roberfroid, M. (2000). Defining functional foods. In G. Gibson, & C. M. Williams (Eds.), *Functional Foods: Concept to product* (pp. 9-25). Cambridge, England: Woodhead Publishing Limited.

Roccia, P., Ribotta, P. D., Perez, G. T., & Leon, A. T. (2009). Influence of soy protein rheological properties and water retention capacity of wheat gluten. *LWT: Food Science and Technology , 42*, 358-362.

Roels, S., Cleemput, G., Vanderwalle, X., Nys, L., & Delcour, J. A. (1993). Bread volume potential of variable-quality flours with constant protein level is determined by factors governing the mixing time and baking absorption levels. *Cereal Chemistry , 70* (3), 318-323.

Sadowska, J., Blaszczak, W., Fornal, J., Vidal-Valverde, C., & Frias, J. (2003). Changes of wheat dough and bread quality of germinated pea flour addition. *European Food Research Technology , 216*, 46-50.

Sakhare, S., Inamdar, A., Soumya, C., Indrani, D., & Venkateswara Rao. (2014). Effect of flour particle size on microstructural, rheological and physico-sensory characteristics of bread and south Indian parotta. *Journal of Food Science and Technology , 51* (12), 4108-4113.

Sanz-Penella, J., Wronkowska, M., Soral-Smietana, M., Collar, C., & Haros, M. (2010). Impact of the addition resistant starch from modified pea starch on dough and bread performance. *European Food Research and Technology , 231*, 499-508.

Sardesai, V. (1995). Role of antioxidants in health maintenance. *Nutrition in Clinical Practice , 10*, 19-25.

Saunders, J. I. (2011). *Limitations and Challenges for Wheat-Based Bioethanol Production, Economic Effects of Biofuel Production*. Retrieved 11 09, 2014, from intrchweb: http://cdn.intechweb.org

Serna-Saldivar, S. (2012). Cereal grains laboratory reference and procedures manual. *Food Preservation Technology* .

Shahidi, F. (2007). Measurement of antioxidant activity in food and biological systems. In F. a.-T. Shahidi (Ed.), *Antioxidant measurement and application* (Vol. Chapter 4, pp. 36-66). Washington, D.C., USA: American Chemical Society.

Shahidi, F., & Naczk, M. (1995). Phenolic compounds in cereals and legumes. In *Food Phenolics: Sources, Chemistry, Effects, Applications* (pp. 13-18). Lancaster, PA: Technomic Publishing Company Inc.

Sharma, P., & Gujral, H. (2014). Antioxidant potential of wheat flour chapattis as affected by incorporating barley flour. *LWT: Food Science and Technology , 56*, 118-123.

Shogren, M., Pomeranz, Y., & Finney, K. F. (1981). Counteracting the deleterious effects of fibre in breadmaking. *Cereal Chemistry , 58* (2), 142-144.

Shum, A. (2011). Examing the bubble structure and antioxidant activity of pea fibre-enriched bread using image and texture analysis, ultrasound techniques and antioxidant assays. Winnipeg, MB, Canada.

Sievert, D., Pomeranz, Y., & Abdelrahman, A. (1990). Functional properties of soy polysaccharides and wheat bran in soft wheat products. *Cereal Chemistry , 67* (1), 10-13.

Simons, C., Hunt-Schmidt, E., Simsek, S., Hall, C., & Biswas, A. (2014). Texturized pinto bean protein fortification in straight dough bread formulation. *Plant Foods Human Nutrition , 69*, 235-240.

Singleton, V., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu's reagent. *Method Enzymology , 299*, 152-178.

Smith, C., Mollard, R. C., Luhovyy, B. L., & Anderson, G. H. (2012). The effect of yellow pea protein and fibre on short-term food intake, subjective appetite and glycemic response in healthy young men. *British Journal of Nutrition , 108* (1), S74-80.

Sosulski, F., & Wu, K. K. (1988). High-fiber breads containing field pea hulls, wheat, corn, and wild oat brans. *Cereal Chemistry , 65* (3), 186-191.

Stanisavljevic, N., Jovanovic, Z., Cupic, T., Lukic, J., Dukie, J., & Radovic, S. (2013). Extractability of antioxidants from pulse seed flour after cooking and in vitro gastrointestinal digestion in comparison with methanolic extraction of the unprocessed flour. *International Journal of Food Science Technology , 48*, 2096-2104.

Suchy, J., Lukow, O. M., & Ingelin, M. E. (2000). Dough microextensibility method using a 2-g mixograph and a texture analyzer. *Cereal Chemistry , 77* (1), 39-43.

Sweica, M. G.-D. (2013). The influence of protein-flavonoid interaction on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. *Food Chemistry , 141*, 451-458.

Torres-Fuentes, C., Alaiz, M., & Vioque, J. (2012). Iron-chelating activity of chickpea protein hydrolysate peptides. *Food Chemistry , 36*, 40-50.

USDA National Nutrient Database. (n.d.). Retrieved 07 5, 2016, from www.ars.usda.gov

Vucic, D., Petkovic, M. R., Rodic-Grabova, B. B., Stefanovic, O. D., Vasic, S. M., & Comic, L. R. (2013). Antibacterial and antioxidant activities of bilberry (Vaccinium myrtillus L.) in vitro. *African Journal of Microbiology Research , 7* (45), 5130-5136.

Wronkowska, M., Haros, M., & Soral-Smietana, M. (2013). Effect of starch substitution by buckwheat flour on gluten-free bread quality. *Food Bioprocessing Technology , 6*, 1820-1827.

Wu, S., Chien, P. J., Lee, M. H., & Chau, C. F. (2007). Particle size reduction effectively enhances the intestinal health-promotion ability of orange insoluble fibre in hamsters. *Journal of Food Science , 72*, 618-621.

Xie, Z., Huang, J., Xu, X., & Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry , 111*, 370-376.

Xu, B., & Chang, S. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science , 72*, S159-S166.

Xu, B., & Chang, S. (2009). Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. *Journal of Agricultural and Food Chemistry , 57*, 10718-10731.

Xu, B., Yuan, S. H., & Chang, S. K. (2007). Comparative analysis of phenolic composition, antioxidant capacity, and color of cool season pulses and other selected food pulses. *Journal of Food Science , 72*, S167-177.

Zhou, K., Laux, J. J., & Yu, L. (2004). Comparison of swiss red wheat grain and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry , 52*, 1118-1123.

Zhu, K.-X., Huang, S., Peng, W., Qian, H.-F., & Zhou, H.-M. (2010). Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber. *Food Research International , 43*, 943-948.