EXAMINING THE BUBBLE STRUCTURE AND ANTIOXIDANT ACTIVITY OF PEA FIBRE-ENRICHED BREAD USING IMAGE AND TEXTURE ANALYSIS, ULTRASOUND TECHNIQUES AND ANTIOXIDANT ASSAYS

by

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GENERAL ABSTRACT

The addition of pea fibre to wheat bread supports the growing trend of improving health through diet. Various evaluation tools (bread scoring, C-cell, texture profile analysis, and ultrasound) were used to monitor the sensory and mechanical quality of air bubbles in bread. Different pea fibre particle sizes (250, 125, 180, 90 μm) were added at 0, 2, 4, 6, 8 g/serving into a bread formulation. Improvements in specific loaf volume were observed when water absorption was optimized. Adequate water absorption mitigated the high dough viscosity effect of pea fibre. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was used to measure antioxidant activity of pea fibre. All particle sizes of pea fibres had substantially greater antioxidant scavenging ability compared to wheat flour. The DPPH scavenging activity of pea fibre approached that of ascorbic acid by the end of the assay. Fibre-enriched bread had higher antioxidant activity compared to that of the control bread.

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CHAPTER 1: INTRODUCTION

There has been growing interest in adding dietary fibre to bread as a way of boosting the fibre intake of Westernized populations. The recommended daily fibre intake is 20 to 35 grams, whereas most people only consume 10 to 15 grams per day (Peressini and Sensidoni 2009). A diet rich in fibre has been associated with reduced constipation, lower risks of rectal and colon cancer, and lower levels of cholesterol and glucose in the blood (DeVries and others 2001, Vitaglione and others 2008). Fibre consumption increases fecal bulk and promotes satiety, which can help with weight management. As well, fibre is a prebiotic that promotes the growth of beneficial bacteria in the colon, which has been shown to improve gastrointestinal health (Gómez and others 2003).

Adding yellow pea fibre (*Pisum sativum* L.) to bread supports the growing trend of improving health through the diet. However, the general addition of fibre tends to affect the structural and mechanical properties of dough by disturbing the interactions of air bubbles with other dough ingredients, resulting in destabilization of the gas bubble structure (Pham and others 2007, Pomeranz and others 1977, Wang and others 2002). The disruption of dough structure due to fibre addition is most apparent in the fibre's physical effect on the dough matrix; fibre dilutes the gluten and thereby disrupts the gluten-starch matrix formation during dough mixing (Pham and others 2007). This matrix is responsible for providing a framework in which the gas cells can expand (Gan and others 1990). The disrupted dough structure causes detrimental changes in bread

quality, such as decreased specific loaf volume, denser air bubble distribution in the crumb, and a firmer crumb texture (Dalgetty and Baik 2006, Indrani and others 2010).

Various analyses can be done to assess the effect of pea fibre on bread quality. The sensory and mechanical qualities of air bubbles in bread can be monitored through evaluation tools like subjective bread scoring, C-cell analysis and texture profile analysis. Bread scoring is an examination of loaf appearance, crumb fineness (open versus closed cells), uniformity, cell shape, and cell wall thickness (Kamman 1970, Pyler 1988). C-cell analysis is an imaging system that evaluates bread crumb by quantifying its cell characteristics and external features (Whitworth and others 2004). The analysis provides information on cell quality such as the number of cells, average cell diameter, and the range of cell sizes (Calibre Control International Ltd 2006). Bread crumb texture is evaluated using a compression test in which bread crumb is indented and the amount of force is measured to provide a quantification of bread softness (AACC International Method 1969).

Ultrasound has also been used to observe the development of gas bubbles during proofing, as it is sensitive in distinguishing gas bubbles from the solid phase in a non-invasive manner. Ultrasonic velocity and attenuation is affected by the presence and expansion of gas cells in the dough (Elmehdi and others 2003a) and was used to characterize changes observed as the dough rose in the proofing cabinet. Because ultrasound is a novel way of monitoring dough fermentation (Elmedhi and others 2003a) it may have the potential to predict the effect of fibre on bread quality prior to the dough being baked.

Yellow field peas are a major pulse crop in Canada, and one of Canada's main exports (Wang and others 2003). Currently, pea fibre is a low cost product; greater value can be added by finding uses for it in other products which will provide economic returns to farmers and seed processors (Hughes 2011).

The health benefits of dietary fibre in pea fibre are supported by the presence of antioxidants. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is a common method of demonstrating the antioxidant activity of pea fibre (Molyneux 2004), and so it would be a good means of determining the antioxidant potential of pea fibre, and in particular to determine if the fibre's antioxidant activity is still present when it is baked into bread. In this way, pea fibre-enriched bread can potentially provide the health benefits associated with antioxidants while consuming dietary fibre in a common dietary staple, white bread.

In light of this research and economic background, the purpose of this thesis research was to investigate the effect of pea hull fibre on the conventional quality and nutritional quality of wheat bread from two main perspectives: its mechanical effect, by using traditional baking methods as well as low intensity ultrasound, and its chemical effect, by using antioxidant assays. The specific goals are as follows:

- To characterize the interaction of pea hull fibre with dough ingredients and how that affects air bubble stability through assessment of dough and bread quality parameters and using ultrasound as a novel non-invasive interrogation of dough fermentation;
- To determine the DPPH free radical scavenging abilities of pea fibres of different particle sizes, and to compare these to the DPPH activity of wheat flour;

- To determine the optimal amount of pea fibre in bread while still producing a loaf that has desirable volume and crumb appearance

CHAPTER 2: REVIEW OF LITERATURE

2.1. Dough processing

2.1.1. Breadmaking process

The three main steps in breadmaking are: mixing or dough formation, fermentation, and baking (Kent and Evers 1994). In the sponge and dough method, the mixing and fermentation steps are broken into two parts. First, the sponge is made by mixing yeast and part of the flour and water. This is fermented for up to five hours, and then the remaining flour and water and other ingredients (e.g. salt, sugar, fat, milk solids) are mixed to form the dough. The dough is allowed to relax during an intermediate proof (20-30 min), and then it is divided, moulded, proofed, and baked. The advantage of the sponge and dough method is that it produces soft bread with fine cell structure and good flavour (Hoseney 1998, Kilborn and Preston 1981).

The purpose of the mixing stage is to create a homogenous dough, to allow for flour hydration, to develop the gluten proteins, and to incorporate air into the dough. Dough is made of a continuous gluten phase with a discontinuous phase of starch granules and air cells dispersed through it (Hoseney 1998). Mechanical work input is required to achieve the goals of mixing (Kent and Evers 1994). Most of the water is absorbed by hydrophilic groups on the protein molecules, but there is some water absorbed by the starch (Hoseney 1998). Mixing also develops the gluten into a viscoelastic material that can entrap air and other dough components. Optimal gluten development is vital for subsequent bread crumb structure development. Since no new

gas cells are nucleated after the mixing step, it is important to produce a dough structure that is strong enough to encapsulate gas cells, but not so strong that it prevents their expansion during fermentation (Hoseney 1998, Scanlon and Zghal 2001).

The purpose of fermentation is to allow the existing gas cells to expand due to the production of carbon dioxide by the yeast. This carbon dioxide (CO_2) exists as a gas within the gas cells, as well as a dissolved species within the aqueous phase (from where it diffuses into the gas cells) (Hoseney 1998, Scanlon and Zghal 2001).

The dividing, moulding, and proofing steps redistribute the gas cells and other dough ingredients, and make the gas cell size more uniform by subdividing the cells to produce a greater amount of smaller cells (Tipples 1975). The moulding or sheeting step can influence how the gas cells expand during baking. The sheeting procedure rolls the dough into a cylinder, and the gas cells elongate in the direction of this rolling. This elongation continues during proofing if the dough is strong. During baking, the crust "breaks" where the crust meets the pan, allowing for greater elongation at this location (Hoseney 1998).

Baking stabilizes the porous structure by converting the liquid dough to a solid mass (Scanlon and Zghal 2001). There is an initial, fast expansion in dough size known as oven spring. The temperature rise in the oven results in dough expansion, which is attributed to several factors: the expansion of carbon dioxide in the gas cells, the diffusion of carbon dioxide from the aqueous phase into the gas cells, and the vaporization of water and ethanol (Hoseney 1998). Eventually expansion stops due to the increased dough viscosity caused by starch gelatinization and gluten aggregation, which sets the bread into a solid form (Mills and others 2003, Singh and Bhattacharya 2005).

2.1.2. Gas bubble stability during fermentation

2.1.2.1. Disproportionation and coalescence

Gas cell distribution and stability change throughout the breadmaking process, but the two fundamental mechanisms that cause destabilization are disproportionation and coalescence. Overall these occurrences contribute to coarsening, whereupon there are a diminishing number of gas cells while the remaining cells increase in size (Weaire and Hutzler 1999).

Disproportionation is the mass transport of gas from small to large bubbles, and is the main cause of foam destabilization during fermentation and proofing. A pressure difference (Laplace pressure) exists across the gas-liquid interface due to the curvature of the gas cell. The pressure difference is greater for smaller bubbles, so there is a net transfer of gas from small to large bubbles (Mills and others 2003, Weaire and Hutzler 1999). The rate of transfer depends on the difference in bubble sizes, gas solubility, and possibly the packing density of surfactants and surface elasticity (Mills and others 2003). In bread dough, there is the added complication of CO_2 being constantly produced by the yeast (Mills and others 2003). Some researchers do not accept that disproportionation occurs during fermentation, because gas from the small bubbles would have to go into the liquid phase to move into a larger bubble. This movement would require the gas to travel against the concentration gradient, as gas bubbles inflate only when the dough is saturated with CO_2 (Chiotellis and Campbell 2003).

Coalescence occurs when the film between two gas cells ruptures to form a larger cell. It usually takes place during the later stages of proofing and the early stage of

baking, when bubbles undergo a faster expansion phase due to increased CO_2 production and formation of steam (Babin and others 2006). As the bubbles expand and push against each other, this increases the tendency for them to coalesce as surface-active agents spread and surface tension increases. The rupture of thin dough films results in the loss of gas and leads to the creation of irregular crumb structure (Mills and others 2003).

Preventing the rupture of gas cells is necessary for obtaining good volume and finer crumb texture. Originally it was thought that only the starch-gluten matrix's viscoelastic properties were responsible for gas cell stabilization, but it has been proposed that gas cell stability is supported by a secondary mechanism: a liquid lamella that envelops the gas cells (Gan and others 1990, Gan and others 1995, Sroan and MacRitchie 2009. Scanning electron microscopy images appeared to show that individual gas cells were present even when the starch-gluten matrix was "discontinuous" – that is, there were holes forming interconnections between cells (Gan and others 1995). These gas cells remain separate due to the presence of surface-active materials in the liquid lamellae (Gan and others 1995, Sroan and others 2009).

2.1.2.2. Effect of gluten-starch matrix on gas cell stability

Air bubbles in bread dough are stabilized by two major mechanisms: surface-active materials, and the properties of the gluten-starch matrix. The matrix provides physical support for the air bubbles by preventing gas wall failure through strain hardening, the localized increase of stress in response to strain. Pressure produced by fermentation gases and gas expansion during baking causes gas cells to expand biaxially, resulting in gas cell wall thinning, and if gas continues to expand along the thin region

separating two cells, cells rupture. However, due to strain hardening, the stress in the thin region increases proportionately more than strain, so the thin region of the cell wall or starch-gluten matrix resists further deformation and cells expand along thicker (but less resistive) parts of cell walls (Dobraszczyk 1997, Dobraszczyk and Roberts 1994, Sroan and others 2009, van Vliet and others 1992).

Strain hardening originates from the entanglement of large glutenin molecules with molecular weights (MWs) greater than a threshold MW (M_t), which is the minimum MW at which stable entanglements can be formed (MacRitchie and Lafiandra 1997, Singh and MacRitchie 2001). When more high MW glutenin molecules are present, there are more entanglements per chain, which increases dough strength (Gupta and others 1990, Termonia and Smith 1988). However, this strength must be balanced with extensibility – if there is too much high MW glutenin present, there is too much resistance to expansion, which would limit volume (Singh and MacRitchie 2001, Sroan and others 2009).

2.1.2.3. Effect of surface-active components on gas cell stability

The other major mechanism of gas cell stabilization in bread dough is the liquid lamellae, the gas-liquid interfaces which contain surface-active agents that adsorb in this region to reduce surface tension (Gan and others 1990, MacRitchie 1976, Sahi 1994). The major surface-active agents are lipids and proteins, but their stabilization mechanisms are different. Lipid molecules are smaller, and diffuse faster to adsorb quickly. Lipids function under a concentration effect known as the Gibbs-Marangoni effect (Primo-Martín and others 2006). When gas cell deformation occurs, it causes local

thinning and depletes local surfactant concentration. Molecules migrate to the thinned area to reduce surface tension (Mills and others 2003, Walstra 1996).

Proteins or polymers unfold to expose hydrophobic amino acids and are strongly adsorbed at the liquid lamella to form a viscoelastic layer. When the lamella is deformed, the elastic deformed layer stretches and deforms, but remains intact if deformation is within the elastic limit (Mills and others 2003). Proteins have a larger contact area per molecule at the interface than lipids, so they are able to develop strong intermolecular interactions and form elastic networks (Primo-Martín and others 2006). Protein-stabilized doughs have been shown to produce breads with higher loaf volume and more uniform and finer crumb structure (Sroan and MacRitchie 2009).

Due to their different adsorption mechanisms, proteins and lipids compete for adsorption, so when both are present surface elasticity drops and stability is diminished (Mills and others 2003, Paternotte and others 1993, Primo-Martín and others 2006). It has been proposed that puroindolines, cysteine-rich lipid-binding proteins found in wheat, strongly interact with lipids to prevent lipid adsorption at the foam interface. The action of the puroindolines encourages only protein adsorption and thus improved foam stability (Dubreil and others 1998, Dubreil and others 2002).

Different cultivars of wheat have been shown to have different degrees of surface tension reduction due to the endogenous surface-active materials present (Sahi 1994). Bread formula ingredients such as fat and diacetyl tartaric acid esters of monoglycerides can also affect surface tension, and subsequently affect crumb structure and loaf volume due to changes in gas cell stability (Sahi 2003). For instance, lipids have varying effects on gas cell stabilization depending on their polarity. Polar lipids such as saturated free

fatty acids (e.g. palmitic, stearic) form condensed monolayers at the gas-liquid interface that do not easily desorb. The lipids pack closely together with the polar head oriented towards the aqueous phase, and the non-polar tail towards the air. The monolayer's high surface elasticity allows the gas cell to resist lamellae collapse with changes in interfacial area, thereby improving bread volume and crumb grain. Non-polar lipids like unsaturated free fatty acids (e.g. linoleic acid) form expanded monolayers, which pack loosely at the interface, thereby reducing gas cell stability and bread quality (Sroan and MacRitchie 2009).

2.1.3. Effect of baking on air bubble stability

While maintaining gas cell stability during breadmaking prior to baking is essential, exposure to heat in the oven causes the dough to undergo structural changes that affect the final structure and bread quality. During baking, the viscous dough expands its volume four to five times (Singh and Bhattacharya 2005). The separate gas cells in the dough rupture due to the increase of surface tension, forming an interconnecting network of gas cells, allowing steam to escape. The rapid increase in viscosity caused by starch gelatinization essentially freezes the dough's foam structure and halts bubble expansion, transforming the dough into a solid sponge-like structure (Mills and others 2003).

To maintain foam stability during baking, enough surfactant must be present to cover the expanding gas cells as pressure increases and water evaporates. Aeration in the solid foam is greatly affected by how soon starch gelatinization occurs – the earlier

gelatinization takes place, the smaller the bubbles and the lower the volume (Singh and Bhattacharya 2005).

2.2. Determination of loaf quality

Bread quality can be determined by subjective and objective methods. While the traditional method is subjective inspection by a trained baker, this has been shown to be inconsistent (Wang and Coles 1994). As such, there has been a move towards developing objective methods of bread evaluation such as C-cell image analysis that would be more reliable and precise.

2.2.1. Bread scoring

Bread crumb scoring is an examination of crumb fineness (open versus closed cells), uniformity, cell shape, and cell wall thickness (Kamman 1970, Pyler 1988). Physical characteristics of the crumb are strongly related to the cellular structure of the bread crumb; for instance, a softer, more elastic textured crumb would have finer, thin-walled, uniformly-sized cells (Pyler 1988). As such, bread crumb scoring not only gives an indication of visual appearance, but also gives an indication of the physical structure of the loaf of bread.

2.2.2. C-cell analysis

C-cell analysis is an imaging system to evaluate bread by quantifying its cell characteristics and external features. The analysis provides 48 different data values, including information on cell quality such as the number of cells, average cell size, and

cell wall thickness. It is asserted to be objective, reproducible, and fast (Calibre Control International Ltd 2006). C-cell analysis is intended to mimic the baker's assessment, but provide consistency (Salmon 2004). The analysis applies greater consideration to cells and cell walls closer to the centre, similar to how bread is usually visually assessed (Whitworth and others 2004). High correlation coefficients were found between subjective average crumb grain scores with average cell number, cell wall thickness, average coarse/fine cluster, and average crumb fineness for bread (Chen and others 2007). This indicates the potential of C-cell to determine important bread attributes that coordinate with subjective analysis.

C-cell can be used to determine bread quality due to different ingredients and processing changes (Stojceska and Ainsworth 2008). Besides bread, C-cell can be used to evaluate the structure of other baked goods such as cakes, buns and pastries. Sugar snap cookies were evaluated using C-cell to compare the relationship of the objective analysis to the subjective one (Stevenson and others 2010).

2.2.3. Crumb firmness analysis

The analysis of the mechanical properties of bread crumb is often done using a compression test in which bread crumb is indented and the amount of force is measured to provide an understanding of bread softness. Compression tests are simple, valid, require a small sample size, and highly correlated to sensory measurements and specific loaf volume (Ponte and others 1962, Scanlon and Zghal 2001). Bread crumb behaves as a non-linear viscoelastic material (Hibberd and Parker 1985). In the elastic linear region, Young's modulus can be obtained and used to characterize the mechanical response of

bread crumb (Keetels and others 1996, Scanlon and Zghal 2001). When measured parallel to the long axis of the bread, Young's modulus is 8-18 kN/m^2. This is 2 to 2.5 times greater than when measured parallel to loaf height, which demonstrates the anisotropic nature of bread crumb (Hibberd and Parker 1985).

2.3. The effect of fibre on gas cell stability in dough and bread quality

2.3.1. Destabilization of dough matrix due to fibre addition

Dough consists of a gluten-starch matrix, which provides a framework in which the gas cells expand. Dough structure is affected when fibre is incorporated (Gan and others 1990). For instance, in the production of whole wheat bread, the outer layers of the wheat caryposes (bran, epicarp hairs, and aleurone-pericarp particles) are included in the dough because they are rich in fibre. Due to their addition, structural differences in the resulting crumb have been noted between white and whole wheat breads. For instance, white breads tend to have large gas cells, thin gas cell walls, and a fine and even gluten matrix. In contrast, whole wheat breads have gas cells of varying size, thick gas cell walls, and a discontinuous gluten matrix (Gan and others 1989, Gan and others 1992). Changes in bread behaviour as a result of fibre enrichment are evidenced by increased water absorption in the dough, and decreased loaf volume and increased crumb firmness in the bread (Indrani and others 2010).

Most of the work addressing the disruption of dough structure due to fibre addition focuses on the physical effect of the fibre on the dough matrix. The destabilizing effect of fibre addition has been attributed to gluten dilution (Anil 2007,

Czuchajowska and Pomeranz 1993, Pomeranz and others 1977). Scanning electron microscopy (SEM) revealed that doughs made with waxy (non-amylose) wheat flours had discontinuous irregular matrices of fibre and gluten surrounding the starch granules, whereas dough made with regular wheat flour had a continuous gluten matrix covering all starch granules. It was concluded that the fibre diluted the gluten and thereby disrupted the gluten matrix formation during dough mixing. This dilution affected the formation of gluten cross-links, resulting in poor loaf volume (Pham and others 2007).

SEM was used to investigate how the inclusion of non-endosperm components of wheat physically affected the dough structure of whole wheat bread (Gan and others 1989). The epicarp hairs were found to interrupt the gluten matrix; the hairs were either closely aligned with the surface of the gas cell wall, protruding at angles from the gluten matrix, or puncturing the gas cell wall. These particulate components acted as points of weakness in an expanding dough system which decreased the stability of gas cells, leading to their rupture. This physical disruption changed crumb morphology, resulting in bread with a denser crumb (Gan and others 1989).

Similarly, Pomeranz and others (1977) found that at high (15%) replacement values of flour, the use of wheat bran (147-701 μm) and cellulose (20-225 μm) significantly decreased loaf volume due to a physical disruption of the crumb structure. The degree of loaf volume reduction was much greater than anticipated from the gluten-diluting effect of the fibre. While SEM revealed a control bread with a fine crumb structure consisting of thin sheets and filaments, this was not present in fibre-enriched bread. However, Kock and others (1999) found that a coarse bran (>1.8 mm) produced bread of greater volume. The particle size of fibre may affect the degree of

destabilization of dough structure and its bread quality. Fibre particle size was not stated in the work of Gan and others (1989).

2.3.2. Effect of pea hull fibre on bread quality

Different types of fibre have been shown to impart varying effects on gas cell stability in bread doughs due to differences in molecular structure. Fibres that have a greater number of hydroxyl groups would allow for more water interactions through hydrogen bonding. Dietary fibre mainly consists of saccharides, and thus the main hydrogen bond that affects structure is the hydroxyl-hydroxyl bond. When the hydroxyl groups of adjacent saccharides align closely, the saccharide polymers have greater shear and tensile strength. This makes it difficult for water to penetrate the molecular chains, resulting in a fibre having low water solubility (Cho and others 1997).

Wang and others (2002) investigated the effect of different fibres (pea and inulin) on wheat dough performance and bread quality. The most significant compositional difference was insoluble dietary fibre (IDF). Both fibres had similar amounts of total dietary fibre (TDF), but pea fibre had double the amount of insoluble fibre compared to inulin. Inulin consisted of oligo- and polysaccharides of fructose, while pea fibre was mostly cellulose. The study found that dough tenacity (resistance to extension) increased with the addition of fibres, but especially for inulin. Dough elasticity decreased with pea fibre, but not with inulin. The changes in viscoelastic behaviour were thought to result from interactions between the fibre and gluten (Wang and others 2002).

The pea hull fibre discussed in this thesis contains 89.3% total dietary fibre (8% soluble, 82% non-soluble) according to the manufacturer (Best Cooking Pulses Inc.,

Portage la Prairie, Manitoba). Sosulski and Wu (1988) found that field pea hulls contained 82.3% (dry moisture basis) total dietary fibre, with 8.2% hemicellulose and 62.3% cellulose. Fibre content likely differs depending on method of processing and the method used for fibre analysis (Tosh and Yada 2010).

Dalgetty and Baik (2006) found that increasing amounts of insoluble pea fibre resulted in greater water absorption, whereas increasing amounts of soluble pea fibre decreased water absorption. Regardless of fibre type, its addition resulted in decreased specific loaf volume, increased loaf moisture, and decreased overall bread quality (Dalgetty and Baik 2006, Sosulski and Wu 1988). Breads with soluble fibre had more desirable crumb uniformity and appearance than bread made with insoluble fibre and control white bread (Dalgetty and Baik 2006). While bread enriched with increasing amounts of pea fibre was found to have decreasing subjective bread scores, the scores were not drastically lower than the control loaf (Kasprzak and Rzedzicki 2010). Sosulski and Wu (1988) found that loaf shape and crumb texture were rated as very good in breads fortified with 10% pea hulls. Loaves enriched with pea fibre had a lighter and more yellow crust colour according to analysis with a Hunterlab colour meter, and a formulation containing 15% (by weight) of pea hulls was recommended as acceptable by the researchers.

The main concerns regarding the detrimental effect of adding fibre to bread on sensory quality is decreased loaf volume, gritty texture, and poor taste and mouthfeel (Pomeranz and others 1977). Various approaches have been used to mitigate these issues, such as adding vital gluten, surfactants, and surfactant/shortening blends, using high-grade flour, or using hemicellulase enzymes (Collar and others 2007). The type of

fibre and how it was processed also affect bread quality; factors such as solubility, viscosity, and water-binding capabilities changes the interactions between fibre and the other dough ingredients (Collar and others 2007, Nelson 2001). The optimal level of fibre addition in bread formulation remains dependent on a variety of factors, such as wheat quality and pea fibre quality, including the ratio of soluble/insoluble fibre present, and pea fibre particle size (Dalgetty and Baik 2006, Kasprzak and Rzedzicki 2010, Tosh and Yada 2010).

2.3.3. Effect of fibre particle size on bread quality

The investigation of the effect of fibre particle size on bread quality has produced various conclusions. Rosell and others (2009) found no relationship between particle size and hydration properties of a variety of fibres, including cellulose and wheat flour. Meanwhile, others have found that a reduction in fibre particle size of sugarcane bagasse was associated with lower water absorption and better breadmaking properties (Sangnark and Noomhorm 2003, Zhang and Moore 1997). In contrast, others hypothesize that a reduction in particle size would result in the exposure of greater surface area, as well as more polar groups with water binding sites, and thus more water absorption (Chau and others 2006, Rosell and others 2006). Moreover, water absorption is also affected by chemical structure of the molecules in the fibre and particle shape (Robertson and Eastwood 1981), so it is difficult to generalize the effect of particle size on water absorption for different fibres. For instance, it was found that cellulose fibres >154 μm produced loaves with normal breadmaking properties, whereas cellulose fibres <154 μm

interfered with gluten formation and resulted in poor loaf height (Seguchi and others 2007).

An additional consideration is that fibres swell in water and thus increase in particle size, which would affect their interactions with other dough ingredients (Rosell and others 2009). As such, the degree of fibre hydration would affect the behaviour of fibre in a dough system.

2.4. Ultrasound analysis

2.4.1. General ultrasound background

Ultrasound has been a very beneficial tool in material characterization as it can monitor the food material's physical properties quickly in real time in a non-destructive manner (Povey 1997). Compared to other non-intrusive methods (e.g., light scattering), it can observe optically opaque materials at low ultrasonic frequencies (Elmedhi and others 2003b, Saggin and Coupland 2001). Ultrasound propagates low-intensity ultrasonic pulses into a material; the interaction of the ultrasonic waves and the material is determined by monitoring the transmitted/reflected sound waves as changes in ultrasonic phase velocity and attenuation (Bellido and Hatcher 2010, Strybulevych and others 2007). Ultrasonic waves are at frequencies larger than 16-20 kHz, the upper limit of the human ear's ability to hear sound. Ultrasound is very sensitive to minute internal structural changes, like air pockets and fractures (Elmehdi and others 2003b).

Ultrasound can be used to examine food systems in a frequency range from 20 kHz up to the MHz range (Elmehdi and others 2003b, McClements and others 1993).

The passage of ultrasonic waves through a heterogeneous material is greatly influenced by the material's physical structure (concentration, size and distribution of phases or particles). The ability to ultrasonically distinguish between the multiple phases depends on how different the acoustic properties of the phases are (Elmedhi and others 2003b).

Ultrasound has also been used to improve the efficacy of material extraction, but its use in this manner has been done with high intensity ultrasound (frequently referred to as sonification).

2.4.2. Use of ultrasound in cereal science

The use of ultrasound is still an emerging technique in cereal science. Particularly for bread baking, the prevalence and established nature of other methods of dough analysis (e.g. farinograph, alveograph, and mixograph) has slowed down its application (Bloksma and Bushuk 1988, Hoseney 1998).

Regardless, ultrasound has been used in various ways to characterize material behaviour in cereal science. Its application to monitor air bubble formation has been used in bread baking. The technique's inherent sensitivity at distinguishing air bubbles was used to observe changes in gas cell behaviour of wheat doughs (Elmehdi and others 2004, Létang and others 2001, Mehta and others 2009, Ross and others 2004). Bread crumb porosity has also been evaluated (Elmedhi and others 2003b, Lagrain and others 2006). Some work has been done to characterize changes in mechanical properties of yellow alkaline noodle dough (Bellido and Hatcher 2010).

Ultrasound has been used to characterize flour quality in relation to water absorption in dough (Álava and others 2007). García-Alvarez and others (2011) also

demonstrated that ultrasound could be used to detect changes in dough consistency as a method of discriminating flour quality.

The use of high-intensity ultrasound has also been shown to assist in reducing the extraction time of wheat bran arabinoxylans (Hollmann and others 2009). Extraction of phenolic compounds from wheat brans using ultrasound (40 kHz) has also been investigated (Wang and others 2008). The improved extraction is attributed to acoustic cavitations produced by the ultrasound in the extraction solvent, and improved penetration of solvent into sample material. Similarly, high-intensity ultrasound was used to improve the extraction of rice starch without the use of chemicals (Wang and Wang 2004). Soaking legumes in combination with ultrasound treatment (47 MHz) appeared to cause greater leaching of oligosaccharides, and thus the technique can be used to reduce the required soaking time to remove oligosaccharides (Han and Baik 2006).

2.4.3. Use of ultrasound in fermentation studies

An important characteristic of bread dough is its gas content (Hoseney 1998). Bubbles exhibit resonance at low frequency (i.e. when the frequency wavelength is greater than bubble radii) (Leroy and others 2008). Resonance arises because of significant differences in sound propagation between gases and solids due to the difference in material density and the velocity of sound through these two phases. The change in sound propagation over a range of frequencies due to resonance can illustrate the change in development of gas cells during proofing (Strybulevych and others 2007). Velocity and attenuation are affected by the presence and expansion of gas cells in the

dough (Elmehdi and others 2003a). Moreover, ultrasound's non-invasive nature and sensitivity to the numbers and sizes of gas cells within a solid or liquid matrix (Leighton 1997) makes it a useful tool for monitoring the process of fermentation.

Hoche and others (2011) investigated an ultrasound inline sensor system that would monitor dough fermentation that would provide real-time monitoring of the breadmaking process. Skaf and others (2009) used ultrasound to monitor dough fermentation at low frequency (<50 kHz). The effect of changes in temperature, flour type, and amount of yeast was evaluated. It was shown that the ultrasound technique was an effective online monitoring and quality control tool that was sensitive to changes in the aforementioned parameters.

2.5. Antioxidants analysis

Both dietary fibre and antioxidant consumption have been associated with preventing chronic diseases such as cardiovascular disease, cancer, obesity and diabetes (Agboola and others 2010, Saura-Calixto and others 2009). Yellow peas are a rich source of dietary fibre and polyphenols (Wang and others 2003), and thus can provide these mutual health benefits.

The double health benefit of adding dietary fibre and antioxidants to baked goods has been explored using other food sources, such as the use of mango peel powder as a healthful ingredient in soft dough biscuits (Ajila and others 2008), and apple skin powder (Rupasinghe and others 2007) and dietary fibre extracted from peaches (Grigelmo-Miguel and others 1999) used as ingredients in muffins.

2.5.1. Antioxidants in wheat

Total antioxidant capacity of cereals is mainly attributed to polyphenols, particularly phenolic acids, most of which are associated with dietary fibre (Saura-Calixto and others 2009, Serpen and others 2007). It is proposed that the association of polyphenols with dietary fibre results in the slow and continuous release of phenolic acids from dietary fibre in the large intestine, thereby improving antioxidant absorption by the body (Saura-Calixto and others 2009, Vitaglione and others 2008).

Wheat flour has been demonstrated to contain antioxidants (Beta and others 2005). Moreover, baking has been shown to significantly increase the concentration of phenolic compounds in bread. Increased baking time and temperature resulted in an increase in 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of pizza crusts containing wheat bread (Moore and others 2009). The increased phenolic concentration may be due to the production of Maillard reaction products during baking (Gélinas and McKinnon 2006).

2.5.2. Antioxidants in legumes

The enhancement of wheat bread's antioxidant activity with other ingredients has been explored, such as with barley flour (Holtekjolen and others 2008), yam flour (Hsu and others 2004) and buckwheat (Lin and others 2009). The use of pea fibre would provide another possibility in this pursuit. For instance, Kaack and Pedersen (2005a, 2005b) incorporated yellow pea hull flour into low-energy chocolate cakes and low-fat high-fibre sausages, while the addition of pea fibre to tortillas and puffed extruded snack foods has also been investigated (Maskus 2008).

Total antioxidant activity of legumes is significantly correlated with their total phenolic content (Amarowicz and others 2004). Phenolic acids (benzoic and cinnamic acids, and cinnamic derivatives), flavone and flavonol glycoside were extracted from pea hulls (Troszyńska and others 2002). Most of the antioxidants in peas are concentrated in the hull, where they would be needed to protect the plant against environmental stress. Antioxidant content varies according to variety, growing and harvesting conditions, growing location, and other environmental factors (Troszyńska and others 2002).

The effect of processing on legumes may affect their antioxidant activity. Boateng and others (2008) found that soaking and toasting dry beans decreased DPPH activity. Jimenez-Monreal and others (2009) found that baking fresh peas reduced their antioxidant activity (lipoperoxyl and hydroxyl radical scavenging). Xu and Chang (2009) also found that thermal processing (conventional boiling, conventional steaming, pressure boiling and pressure steaming) also significantly reduced ferric reducing antioxidant power and peroxyl radical scavenging activity in yellow peas.

2.5.3. Factors affecting 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was first published by Blois (1958). DPPH is a stable free radical that can accept an electron or hydrogen radical to achieve a reduced form. In its free radical state, DPPH absorbs strongly at 517 nm and has a deep violet colour. When an antioxidant is present, it reduces the DPPH free radical. The reduced form has no colour and hence no absorption. As a result, an antioxidant's DPPH scavenging activity is quantified as a change in absorbance.

2.5.3.1. Extraction method

The method of extraction and choice of solvent can affect the quantity and type of antioxidants removed from the raw material. Unbound phytochemicals were extracted from legumes by homogenization in 80% ethanol. However, bound phytochemicals are only extracted by stirring the residue of the unbound phytochemicals with 4 N NaOH (Han and Baik 2008).

Differences in phenolic acid compounds extracted were found depending on the extraction procedure. Acid hydrolysis of dry beans released compounds that were not released during base hydrolysis. A more aggressive base hydrolysis (10 N NaOH) released more phenolic acids than a mild base hydrolysis (2 N NaOH). The addition of ascorbic acid and ethylenediaminetetraacetic acid had a protective effect, preventing phenolic degradation during hydrolysis (Ross and others 2009). As such, extraction solvent and method can greatly affect the results of the DPPH assay.

2.5.3.2. DPPH assay method

While the DPPH assay method is asserted to be simple and reproducible (Sánchez-Moreno 2002), there are various factors in how the assay is conducted that can affect results. Agboola and others (2010) noted that solvent type affected antioxidant activity. Differences were found in scavenging activity for standard antioxidants (e.g. ascorbic acid) depending on whether the organic solvent was pH buffered (Sharma and Bhat 2009). DPPH stock solutions slowly deteriorate with exposure to light and oxygen, and should be protected from both (Molyneux 2004). Udenigwe and others (2009) found

that sample concentration, type of buffer and its pH all affected DPPH scavenging activity when analyzing antioxidants in flaxseed proteins.

The reaction kinetics of the DPPH interaction with antioxidants must also be considered, as the structural conformation of the substrate (antioxidant) affects its interaction with DPPH. Generally, the rate of reaction with DPPH increases as the number of hydroxyl groups present in the antioxidant increases. Thus it is important to choose a standard that has similar reaction kinetics to the samples. For instance, ascorbic acid has been found to quickly react with DPPH (less than 5 min) and reached a constant absorbance almost immediately (Brand-Williams and others 1995, Sharma and Bhat 2009), whereas other studies found that constant absorbance took a longer time to reach (Sánchez-Moreno and others 1998).

The original Blois method recommended a reaction time of 30 min, but more recent work has shown that shorter reactions times are possible depending on the reaction kinetics. As such, it has also been suggested to observe the change in scavenging activity over time until a steady state has been reached (Molyneux 2004, Sánchez-Moreno and others 1998, Sharma and Bhat 2009).

CHAPTER 3: THE EFFECT OF PEA FIBRE ADDITION AND PARTICLE SIZE ON BREAD QUALITY

Abstract

An emphasis on food and health relationships has driven bread manufacturers to pursue strategies for enriching products with healthful ingredients while maintaining a focus on optimal product quality. The effect of yellow pea hull fibre on bread quality arising from fibre interactions with bubbles in the dough was studied. Four different fibre particle sizes (250, 125, 180, 90 μm) were added at 0, 2, 4, 6, 8 g/serving into a sponge and dough formulation. When water absorption was not altered from that of the control flour, the loaves' specific volume significantly decreased as fibre content increased and particle size decreased. C-cell analysis of the crumb showed that for a given fibre loading, cell size homogeneity increased as particle size decreased, suggesting that volume depression arose from high dough viscosity limiting gas cell expansion rather than from fibre particles promoting gas cell coalescence. In support of this observation, work input during dough mixing increased with fibre loading and decreasing particle size. Breads were rebaked using optimal water levels determined by farinograph absorption. Mixer work input was essentially equivalent amongst treatments. No particle size effect on crumb cell size heterogeneity was evident. Significant improvements in specific volume were observed overall, except at high fibre loadings and smaller fibre particle size. In both optimized and non-optimized loaves, the effect of pea fibre on bread quality is attributable to enhanced dough viscosity rather than to greater coalescence arising from fibre particles interacting with bubbles in the dough.

3.1. Introduction

There has been growing interest in adding dietary fibre to bread as a way of boosting consumers' fibre intake so they can reap its health benefits. Fibre consumption has been associated with improved gastrointestinal health and lower risks of cancer and other aging-related diseases (Gómez et al 2003). Most Westernized populations consume less than half of the recommended dietary fibre intake (Peressini and Sensidoni 2009); as such, finding ways to enrich conventional foods with fibre would improve consumption. Like many food industries, the bread industry is pursuing strategies to enrich their products with fibre, but consumers still demand the same sensory quality of the products they are already familiar with (Adams and Engstrom 2000).

The major drawback of adding fibre to bread is that it detrimentally affects dough handling and bread quality, resulting in a significant decrease in volume and adverse change in crumb texture (Gómez et al 2003). There are many possible mechanisms through which fibre can be affecting the dough: gas cell puncture, interference with gluten development, or perhaps an effect on dough viscosity (Collar and others 2007, Gan and others 1989, Mills and others 2003, Nelson 2001). The work described in this chapter aimed to improve the understanding of the physical effects of adding pea fibre to wheat bread, particularly the fibre's effect on the gas cell structure of the loaf of bread.

3.2. Objectives

The objective was to study the effect of pea hull fibre on bread quality arising from the interactions of the fibre with the air bubbles in the dough. Different particle sizes were used at increasing addition levels of pea fibre to investigate the mechanism of

different physical effects of fibre. The effect of particle size and fibre addition level was studied in two sets of experiments: the first set was the addition of pea fibre without modifying the control formula, while in the second set, doughs of optimized water absorption were used. Optimizing water content was explored in an attempt to mitigate the detrimental effect of pea fibre on bread quality as observed in the first set of baking experiments.

3.3. Materials and methods

3.3.1. Materials

Pea fibre was obtained from Best Cooking Pulses, Inc. (Portage la Prairie, Manitoba, Canada). The product specification sheet indicated that the screen size (US standard) was 95% through 125 mesh, or <125 μm. Pea bran meal was also obtained, and milled and sieved into three fractions (see 3.3.2).

Canadian red spring wheat flour and other dough ingredients were provided by the Canadian Grain Commission (Winnipeg, Manitoba). The flour's protein content was 13.3%, moisture was 13.0%, and farinograph absorption was 66.7% (Lysenko 2011).

3.3.2. Milling method of pea fibre bran meal

Pea bran meal was milled using a hammer mill Retsch SR 300 (Retsch GmbH, Haan, Germany) with a 35 mesh screen (500 μm). Feed rate was kept at 51.75 g/min (± 3.75) to avoid overheating of the mill, and thus to prevent antioxidant degradation, as the same milled material was used for baking and antioxidant assays (see Chapter 4).

The milled pea fibre was sieved using a Ro-Tap testing sieve shaker (Tyler Industrial Products, Mentor, Ohio, US) and three particle sizes were used: >250 - <250 μm, >180 - <250 μm, and <90 μm. Henceforth these fractions are referred to as 250, 180, and 90, respectively. The 180 fraction was considered comparable to the commercial fibre (125) in terms of mean particle size, and it was considered useful for analysis of particle size effects to have larger and smaller particle sizes than the commercial pea fibre.

3.3.3. Pea fibre addition levels

In order to test pea fibre addition levels that were realistic and relevant, fibre content was chosen according to Health Canada's labelling regulations regarding dietary fibre (Health Canada 2010). Table 3.1 outlines the labelling regulations and the subsequent levels of fibre added to the test loaves, which correspond to the minimum amount of fibre required per serving to meet the labelling requirement. While baking loaves containing ≥ 8 g fibre per serving exceeded the labelling requirements for a "very high source of fibre", this was done to observe and emphasize the effect of fibre on baking quality. Control loaves containing no fibre were also baked.

Table 3.1. Pea fibre addition levels according to Health Canada dietary fibre labelling regulations.

Health Canada Label	g fibre per serving (serving = 50 g, or 1-2 slices)	g pea fibre for 100 g loaf
"Source of fibre"	≥ 2	6.26
"High source of fibre"	≥ 4	12.52
"Very high source of fibre"	≥ 6	18.77
	≥ 8	25.03

To determine the amount of pea fibre to add to the dough formula to fulfill the label requirements, the following example is used to illustrate the case of the addition of 2 g fibre per 50 g bread:

> If Best Cooking pea fibre contains 89.3% total dietary fibre (wet basis), then to determine g of pea fibre for 50 g bread:
>
> g pea fibre = (100)(2 g) / 89.3 = 2.24 g
>
> Thus for 100 g bread, 4.48 g pea fibre is required
>
> But the average control loaf weight resulting from a bread formula with a 100 g flour basis is 139.7 g. So, for a 100 g flour basis formula:
>
> g pea fibre = (4.48 g)(139.7 / 100) = 6.26 g

3.3.4. Sponge and dough baking procedure

The bread was baked using a sponge and dough procedure (Kilborn and Preston 1981). The original formula included potassium bromate, which was not included here. Table 3.2 outlines the formula for a control loaf. Figure 3.1 is a flowchart that outlines the baking procedure.

Table 3.2. Formula for control loaf using sponge and dough process (100% flour weight basis).

	Sponge	Dough
Sugar solution (500 g/L)	-	10.0 ml
Salt solution (225 g/L)	0.65 ml	10.0 ml
Malt solution (43.25 g/L)	1.0 ml	0.5 ml
Ascorbic acid solution (4 g/L)	1.0 ml	-
Phosphate solution (100 g/L)	1.0 ml	-
Skim milk powder	-	2.0 g
Shortening	-	3.0 g
Active yeast	2.4 ± 0.2 g	-
Flour (hard red spring wheat)	69.2 g	29.7 g
Water	41.0 g	4.8 g

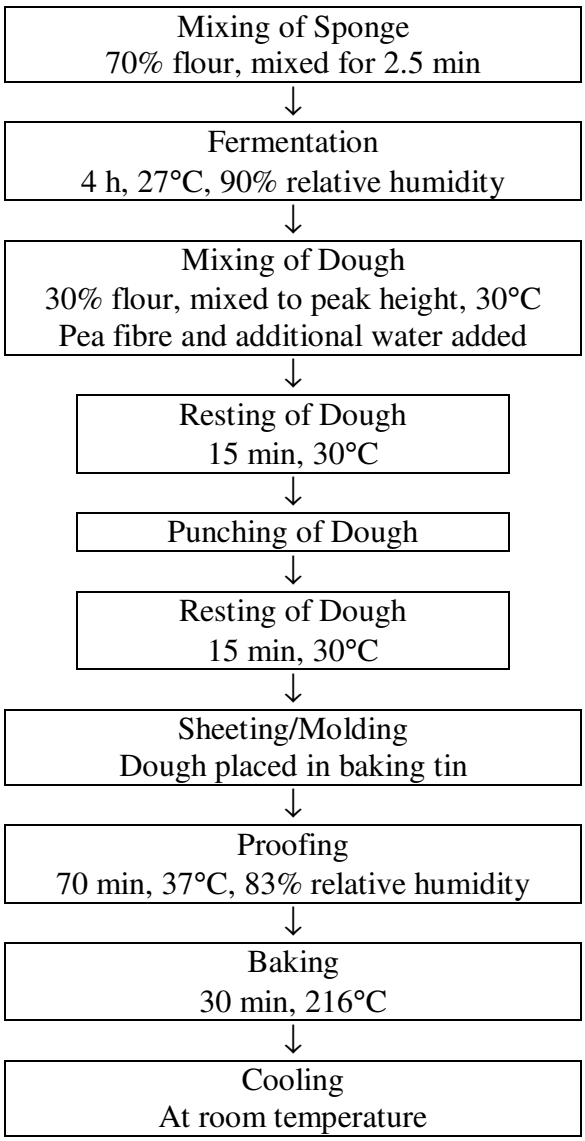


Figure 3.1. Flow diagram of sponge and dough baking procedure (Kilborn and Preston 1981).

Pea fibre was added at the dough mixing stage in addition to the ingredients required for a 100 g control loaf. Tests were kept at a consistent flour weight basis in order to maintain constant absolute gluten content.

For the first set of baking experiments, no additional water was added to observe the interaction of pea fibre with the other dough ingredients without the complication of changing other variables. For the second set of baking experiments, water absorption was optimized according to farinograph water absorption (see 3.3.12). For this purpose, two methods of fibre addition were analyzed. First, the fibre and additional water were directly added at the dough stage. The second method consisted of making a fibre slurry, where the additional water was mixed with the pea fibre prior to addition of the remaining dough ingredients and was left to sit for two hours at room temperature prior to dough mixing. This was an attempt to pre-hydrate the fibre so that its hydration would not interfere with gluten hydration. A similar method had been explored when examining the addition of barley (Jacobs and others 2008) and wheat bran (Nelles and others 1998) to bread. Sosulski and Wu (1988) found that prehydration of pea hull fibre 20 hours before breadmaking improved loaf volume and bread quality. The same levels of additional water as determined by the farinograph were used when adding the fibre directly and when using the slurry method.

3.3.5. Mixer work input

Mixing was accomplished using a GRL 200 mixer (Canadian Grain Commission, Winnipeg, Manitoba). Doughs were mixed to peak mixing time, which was monitored with RAR-P2M Power to Mixer software (RAR Software Systems, Winnipeg, Manitoba). Total work input (W·h/kg dough) was derived from the total area under the power-time curve to the end of the run.

3.3.6. Dough sheeter work input

The dough sheeter measured the force exerted on the rollers by the dough. A successive reduction of dough was accomplished in three steps, with decreasing gaps between the rollers (3/8", 7/32" and 5/32") (Lysenko 2011). Only the second and third reductions were analyzed. The area under the curves of the second and third peak forces as a function of dough travel through the sheeter was translated into work:

$$\text{Specific work input} = \text{Area} / \text{Dough mass}$$

3.3.7. Specific loaf volume and weight

Bread quality analysis included determination of loaf weight and volume (determined by rapeseed displacement in a loaf volume meter). Bread was weighed immediately after the loaf was removed from the oven, while volume was determined after bread had cooled. Specific loaf volume was determined from these measurements.

3.3.8. Subjective bread scoring

Subjective bread scoring was carried out using the Canadian Grain Commission's Total Bread Score which accounted for loaf appearance, crumb texture and crumb colour (Preston and others 1982). Middle slices were used for scoring. Slicing and scoring was done the day after baking. A machine slicer was used. Loaf appearance was mostly based on the size of the break: the greater the size, the greater the score (1-10). The score was modified according to the appearance of oldness/greenness, indicating over-/underoxidation. Eight was considered to be a good score for loaf appearance. Crumb texture was modified by cell size – the presence of gas holes or too many openings in the

crumb were indicated by point deductions. A typically good score was 6.5/10. Crumb colour for the control standard was always 8.0, and test loaves were ranked in relation to the colour of the control. Crumb sheen (i.e., reflection of light) was also considered for this score. Table 3.3 outlines the score corrections which are subtracted from the original score.

Table 3.3. Score corrections subtracted from original score for bread scoring.

Loaf appearance	Oldness	Greenness	Keyholing
	Very old = 1.5 Old = 1.0 Slightly old = 0.5 Very slightly old = 0.2	Very green = 1.5 Green = 1.0 Slightly green = 0.5 Very slight green = 0.2	Keyholing = 1.0 Slight keyholing = 0.5 Very slight keyholing = 0.2
Crumb texture	Openness	Closeness	Gas-holing
	Very open = 1.0 Open plus = 0.75 Open = 0.5 Slightly open = 0.25	Very close = 1.0 Close = 0.5	One gas hole = 2.0 Gas hole = 1.0
Crumb colour	Greyness	Dullness	
	Grey = 1.0 Slightly grey = 0.5	Dull = 0.5 Slightly dull = 0.2	

3.3.9. C-cell crumb analysis

C-cell imaging was completed using C-Cell Version 2.0 software (Campden & Chorleywood Food Research Association Group, Chipping Campden, United Kingdom). A middle slice was used. The analysis applies greater consideration to cells and cell walls closer to the centre, similar to how bread is usually visually assessed (Whitworth and others 2004). While the software provides many analysis parameters, only cell diameter, cell density, and relative volume range were evaluated as they were considered more relevant to gas cell behaviour in relation to fibre addition.

3.3.9.1. Cell diameter

Cell diameter gives an indication of the ratio of coarse versus fine cells (Edwards 2011). It is the average diameter of cells based on measurements of average cell area. Average cell diameter gives an overall indication of coarseness of texture, but does not consider cell depth.

3.3.9.2. Cell density

The C-cell analysis provides the number of cells and slice area, which is used to calculate cell density. Cell density is calculated as the number of cells per slice area. Cell density gives an indication of the fineness of the crumb, and hence the degree of dough expansion during proofing and baking (Edwards 2011). A greater cell density suggests a tighter crumb that had many smaller cells.

3.3.9.3. Relative volume range

Relative volume range is the ratio of cell volume range to cell volume. Cell volume range is a measure of the degree of variation in cell volumes within the slice. Relative volume range is a dimensionless measurement that indicates the degree of variation in cell volumes, normalized for average cell volume. Relative volume range indicates cell homogeneity; a lower range indicates greater homogeneity (Edwards 2011).

3.3.10. Texture profile analysis

Crumb firmness was determined by texture profile analysis using a T1-XT2i Texture Analyzer (Texture Technologies Corp., New York). Pre-test, test, and post-test

speed was 60.0 mm/min. A "Two-bite test" was used where the probe was triggered at 1.0 gf and then went down 50% of the distance between the trigger point and the base of the plate to determine "strain." The probe (2.5 cm diameter, made of Perspex) went down twice and measured the recovery in between each cycle. Peak force was measured. Hardness was measured as the peak force of the first compression. The slices were cut to a consistent thickness for all loaves by the machine slicer, and then the crusts were cut off by hand so that all non-height dimensions were approximately identical. Three crustless slices were stacked to create one specimen. The total thickness of the slices was 39 mm (Figure 3.2).

Crumb firmness was determined according to AACC International Method 74-09.01. The compression force value (N) was calculated at the point where the probe had traveled 9 mm, which was equivalent to 25% compression of the material.

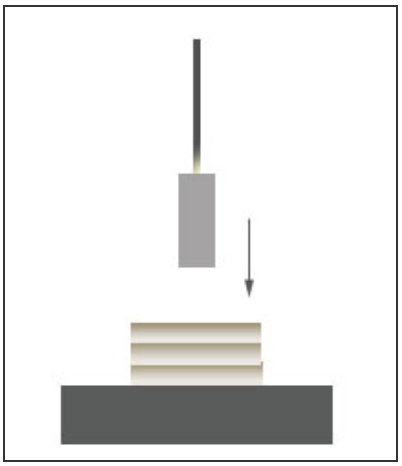


Figure 3.2. Setup of texture profile analysis technique with probe compressing three crustless bread slices.

While the texture profile analyzer software produced evaluations of hardness, cohesiveness, chewiness, and resilience, this study focused on discussing compression force value as it is the standard AACC method for assessing crumb firmness.

3.3.11. Determination of moisture content of flour and pea fibre

Moisture content for fibre-flour mixtures was determined using the AACC method (44-15.02) for an air-oven. Fibre-flour mixtures were made in all combinations for both particle size and fibre level. Moisture content was determined in triplicate.

3.3.12. Determination of farinograph water absorption

Optimal water absorption was determined using a Brabender farinograph (Brabender GmBH & Co., Duisburg, Germany). Absorption was determined in duplicate. Absorption was deemed optimized when the middle of the curve intersected the 500 BU line during mixing. The AACC method (54-21.01) was followed, but, this particular instrument operated at faster revolutions per minute (rpm): 64 for the slow blade and 96 for the fast blade (1:1.5) compared to the standard. This was double what was indicated by the AACC method and instrument manual (31.5 and 63 rpm (1:2) for the slow and fast blade, respectively).

Baking water absorption was calculated as 2% greater than the farinograph absorption (see Table 3.4). Preliminary bake tests using the modified water absorption indicated that the dough was slightly dry, so absorption was increased by 0-4% according to dough feel at sheeting based on the subjective assessment of an experienced baker. Table 3.4 indicates the farinograph absorption of the pea fibre/wheat flour blends, and the calculated water amount added to the dough, including the final determined water amount added. Water absorption optimization baking experiments were done only for 2, 4, and 6 g/serving for all fibre particle sizes.

Table 3.4. Moisture content and farinograph absorption of pea fibre/wheat flour blends.[a]

Particle Size	Pea fibre/ serving (g)	Average moisture content of flour/fibre blend (%)	Average farinograph absorption (%)	Baking absorption (%)	Water added to dough as determined by baking absorption needs (g) [ab]	Additional water added to dough after baker assessment (g)[b]
-	0	12.9	64.6	66.6	4.8	-
250	2	12.4	67	69	8.8	-
250	4	12.2	69	71	10.8	4.0
250	6	11.8	71.4	73.4	13.3	3.0
125	2	12.3	69.8	71.8	11.7	1.0
125	4	11.6	73.8	75.8	15.7	4.0
125	6	11.4	88	90	29.8	3.0
180	2	12.2	66.75	68.75	8.7	-
180	4	12.3	68.8	70.8	10.7	4.0
180	6	11.9	70.8	72.8	12.1	4.0
90	2	12.5	73.1	75.1	15	-
90	4	12.1	79.6	81.6	21.4	4.0
90	6	12.2	86.3	88.3	28.1	5.0

[a]Usually when water absorption is increased, the sponge and dough water content are both increased. However, the sponge water content was kept the same (41.0 g) since pea fibre was added to the dough.
[b]Based on 100 g flour formula

3.3.13. Determination of particle size distribution of pea fibres

The particle size distribution of each pea fibre fraction was determined using the Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, United Kingdom). This instrument used the principle of light scattering to determine particle size. Particles are passed through a focused laser beam, and the light diffraction is measured. Photosensitive detectors measure the angle at which the particles scatter the light, which is inversely proportional to their size. From this the particle size is predicted based on the Mie scattering model (Malvern Instruments Ltd., 2005). Pea fibre was placed into a trough from which the particles were sucked up and fluidized through the laser beam. A histogram showing the percent volume distribution of the subclasses of particle diameter was produced. Duplicate readings were completed for each pea fibre fraction.

3.4. Results and discussion

3.4.1. Deterioration of bread quality caused by addition of pea fibre

The addition of pea fibre without additional water caused a noticeable decrease in bread quality. This was evident for a wide range of quality parameters including specific loaf volume, bread score, and loaf and crumb appearance.

3.4.1.1. Specific loaf volume

The effect of increasing pea fibre on specific loaf volume depression was evident for all particle sizes when water content was constant. This was particularly noticeable

for small particle sizes and higher fibre loadings (Figure 3.3), as indicated by Tukey's multiple range test (see letters in figure to denote significant difference between loaves).

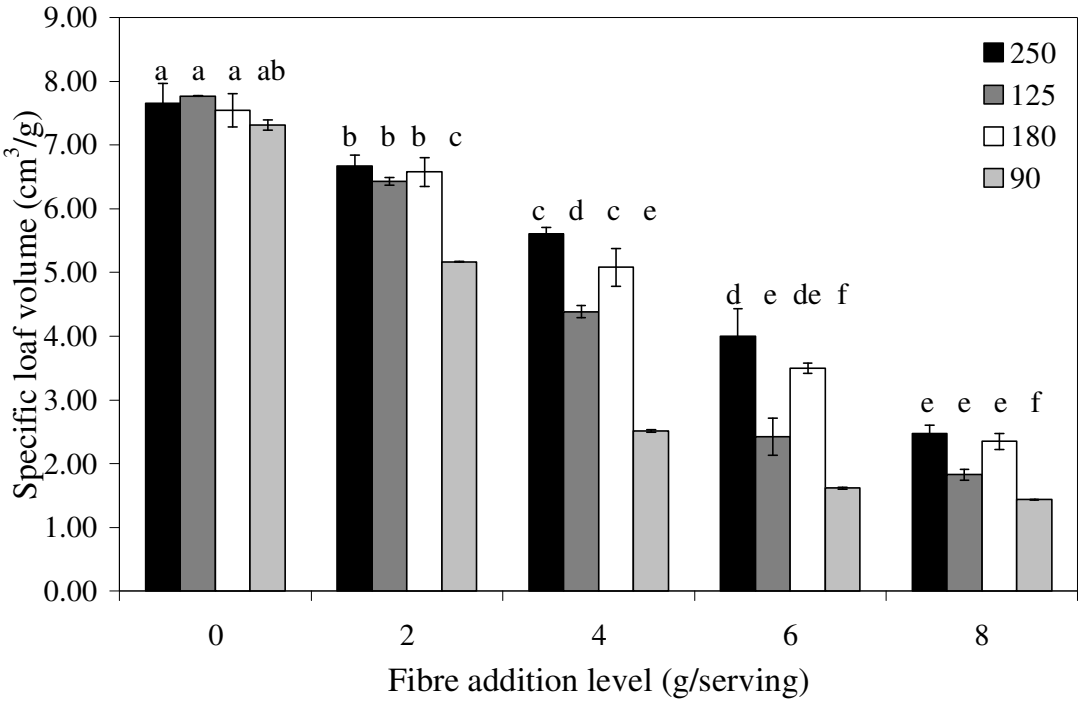


Figure 3.3. Effect of fibre addition level and particle size on specific loaf volume of non-optimized loaves.*

*The control loaves for each particle size contained no pea fibre, but correspond to the same day that the fibre-containing loaves were baked. As such, the control data was not grouped and averaged, so the control loaves can be directly compared to the fibre-enriched loaves baked on the same day.

Other types of fibre have been shown to decrease loaf volume, such as when wheat bran (147-701 μm) and cellulose (20-225 μm) were added to bread at 15% replacement (Pomeranz and others 1977). Similarly, increasing amounts of waxy (non-amylose) wheat flour in a dough formulation resulted in decreasing specific loaf volume (Pham and others 2007). The effect on specific loaf volume has been attributed to gluten dilution by the fibre, resulting in a physical disruption of the dough and crumb structure (Anil 2007, Czuchajowska and Pomeranz 1993, Pomeranz and others 1977). Scanning electron microscopy revealed that doughs made with added waxy (non-amylose) wheat

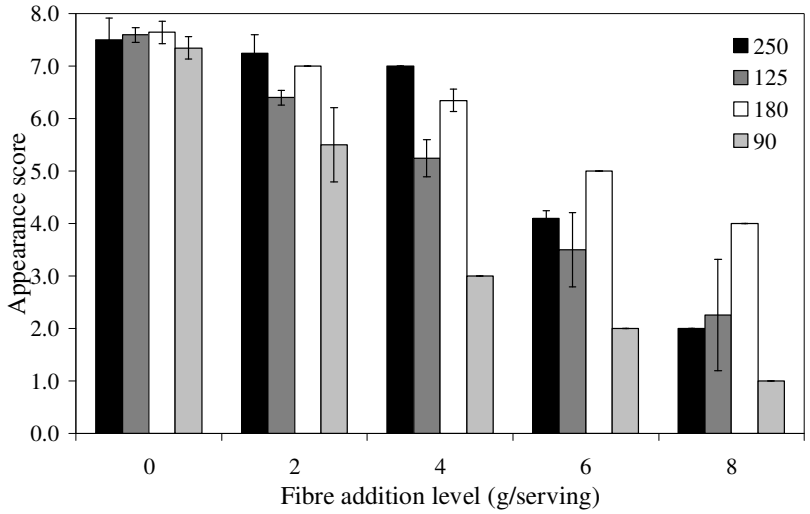
flours had discontinuous irregular matrices of fibre and gluten surrounding the starch granules, whereas dough made with regular wheat flour had a continuous gluten matrix covering all starch granules. It was concluded that the fibre diluted the gluten and thereby disrupted the gluten matrix formation during dough mixing. This dilution affected the formation of gluten cross-links, resulting in poor loaf volume (Pham and others 2007).

It is also possible that the presence of antioxidants affected specific loaf volume (see Chapter 6 for antioxidant activity work). The presence of phenolic acids has been shown to reduce the maximum resistance to extension and increase dough extensibility due to their interactions with high-molecular-weight gluten (Han and Koh 2011). These interactions potentially decreased bread volume. Phenolic antioxidants have been shown to form complexes with proteins via hydrogen bonding and hydrophobic interactions (Almajano and others 2007).

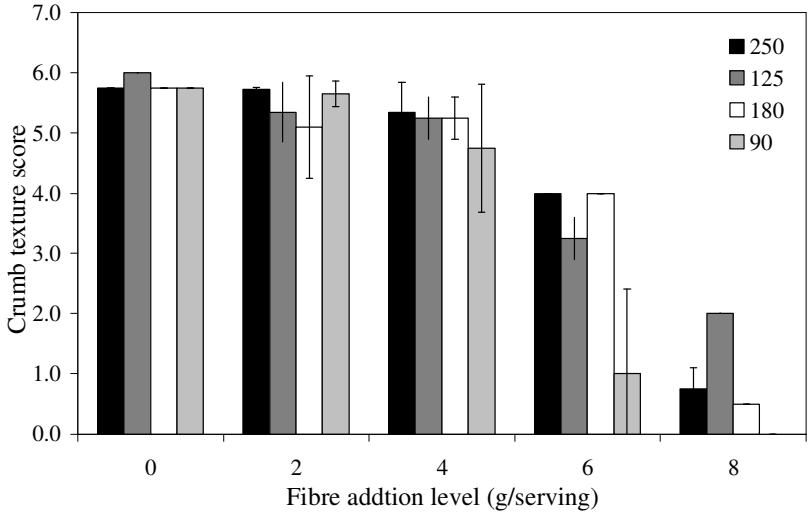
3.4.1.2. Subjective bread scoring of non-optimized loaves

Figure 3.4 shows the bread scoring results for the loaves baked with optimized water absorption levels.

(a)



(b)



(c)

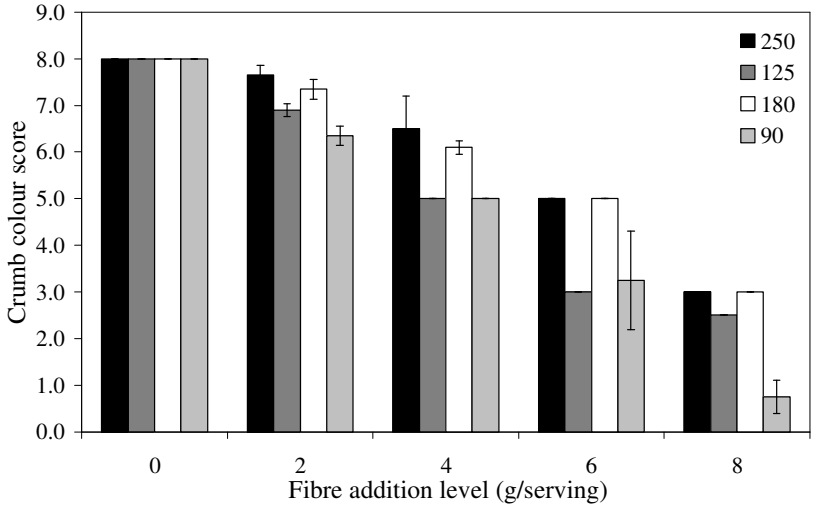
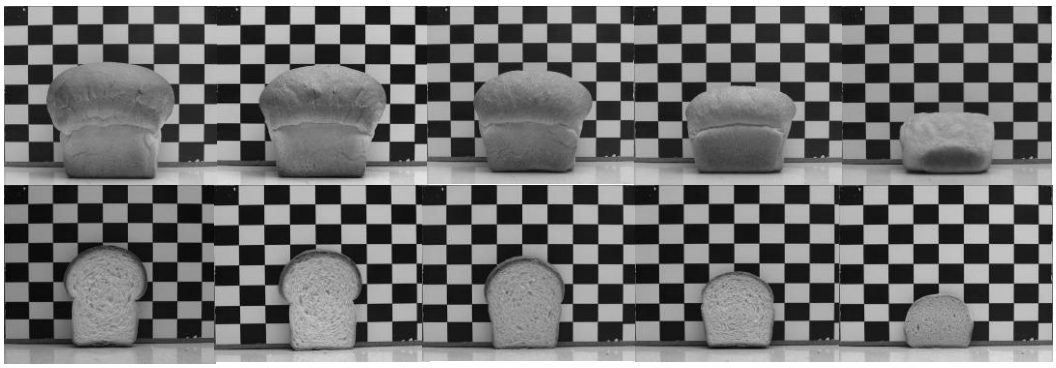


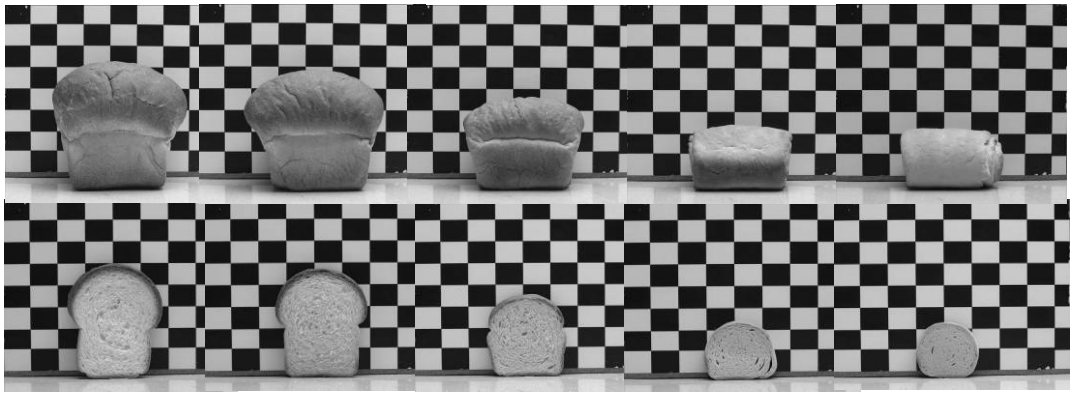
Figure 3.4. Bread scoring for non-optimized loaves: (a) Appearance, (b) Crumb texture, and (c) Crumb colour* (see Figure 3.3 for control loaf footnote).
*No error bar indicates that the scores for the loaf replicates were the same. Crumb colour for control loaves was always scored as an 8.

For all loaves of a given particle size, appearance score decreased as fibre was added, mainly due to the decreasing loaf size (Figure 3.4(a)). Moreover, the break in the loaf was apparent at only low fibre levels (2 g/serving), and here it was smaller than the control break of the loaves. The break is where the crust starts to elongate where it meets the baking pan (Hoseney 1998). This can be seen in Figure 3.5, which shows photos of the loaves and slices for bread made with each particle size at every fibre addition level. Figure 3.6 shows side profiles of the middle slice for loaves made with each particle size at increasing fibre loading, and it is indicative of the difference in loaf volume due to fibre loading. Previous studies have also shown that the addition of pea fibre to bread resulted in decreased specific loaf volume, increased loaf moisture, and decreased overall bread quality (Dalgetty and Baik 2006, Sosulski and Wu 1988).

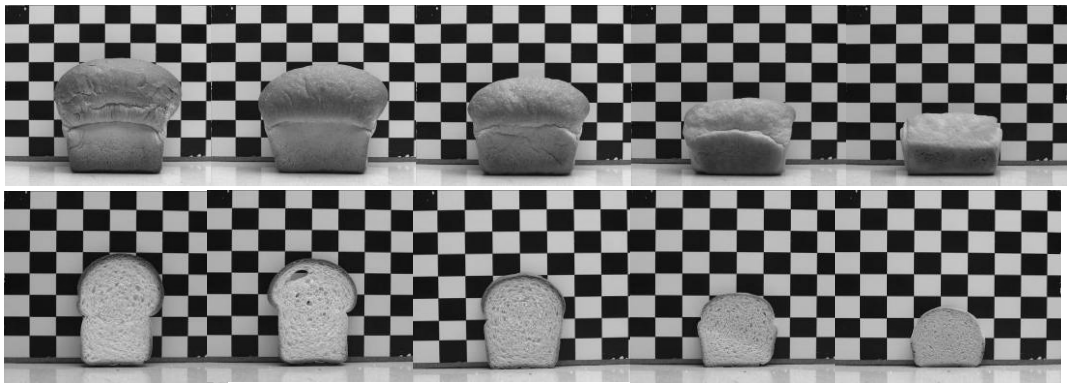
(a)



(b)



(c)



(d)

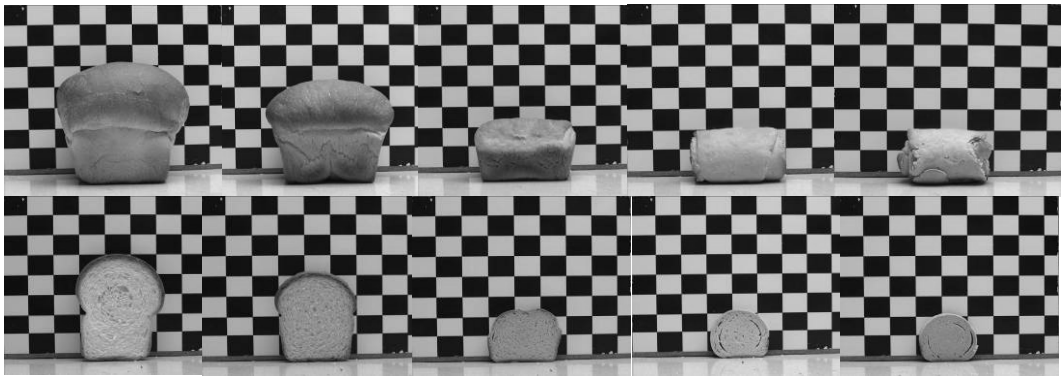


Figure 3.5. Photos of loaves and slices of non-optimized loaves: (a) 250 μm, (b) 125 μm, (c) 180 μm, (d) 90 μm. From left to right: 0, 2, 4, 6, 8 g/serving.

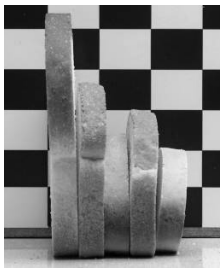
(a)  (b)  (c) 

Figure 3.6. Side profiles of non-optimized loaves: (a) 2 g/serving, (b) 4 g/serving, (c) 6 g/serving. Slices from left to right are: control, 250, 125, 180, 90 µm.

As fibre addition increased and particle size decreased, crumb texture score decreased overall (Figure 3.4(b)). This was due to the gas cells getting smaller, resulting in a denser and tighter crumb appearance (Figure 3.5). Crumb tightening was apparent at 4 g/serving and higher for all particle sizes, but it was especially apparent for 90 µm fibre. The 125 µm fraction also had a tighter crumb texture than the 180 µm fraction and looked more similar to 90 µm loaves. The original intention of sieving a 180 µm fraction in the lab setting was so that it could be compared to the commercial 125 µm, since their apparent particle sizes should be similar. However, commercial 125 µm fibre had a greater fraction of smaller particles, whereas the 180 fibre had a narrow particle size distribution (see Masterizer work in 3.4.2.3 for more discussion on particle size effect). As such, the smaller particles present in the 125 µm fibre appear to be responsible for the loaves looking more similar to the 90 µm loaves than the 180 µm loaves.

As more pea fibre was added, the crumb colour became less white and more yellow (Figure 3.4). The loaves containing 8 g/serving of 90 µm had a greyish tinge. For all particle sizes, there was a noticeable decrease in sheen at 4 g/serving and greater. These changes in colour were reflected in decreasing crumb colour score (Figure 3.3.(c)). Sosulski and Wu (1988) found that the addition of pea hull fibre had minimal effect on

the whiteness of the crumb even in a formulation containing 15% (twb) of pea fibre. This is comparable to loaves containing 4-6 g/serving of pea fibre. However, their loaves had optimized water hydration. The dilution of the fibre with water appeared to lessen the yellow colour of the crumb (see 3.4.3.2.3).

3.4.2. Effect of pea fibre on dough viscosity

3.4.2.1. Work expended in mixing and sheeting

As more pea fibre was added and, particularly at smaller particle sizes, there was an increasing effect on dough viscosity. Figure 3.7 shows the mixer work input for doughs prepared with different fibre formulations. As fibre content increased and particle size decreased, mixer work input increased and specific loaf volume decreased. In previous work, strong correlation had been found between specific loaf volume and dough extensibility (Tiapale-Valdivia and others 2010). So as dough viscosity increased (as indicated by increasing mixer work input), gas cell expansion during proofing was inhibited, which would negatively affect specific loaf volume.

The addition of fibre to bread dough has previously been found to increase dough tenacity and decrease dough elasticity. The changes in viscoelastic behaviour were thought to result from interactions between fibre and gluten (Wang and others 2002). A previous study (Bonnand-Ducasse and others 2010) found that insoluble wheat fibres led to an increase of dough resistance to extension. This was attributed to the fibres behaving like a filler component in a viscoelastic matrix, or the reduction of lubrication by water because the gluten and fibre compete for water absorption. Other high-fibre doughs have

exhibited similar behaviour: doughs made with additional waxy (non-amylose) wheat flours had lower elasticity than normal wheat dough (Pham and others 2007), and dough made with added arabinoxylans were found to have decreased extensibility and increased resistance to extension compared to fibre-less wheat dough (Courtin and others 1999).

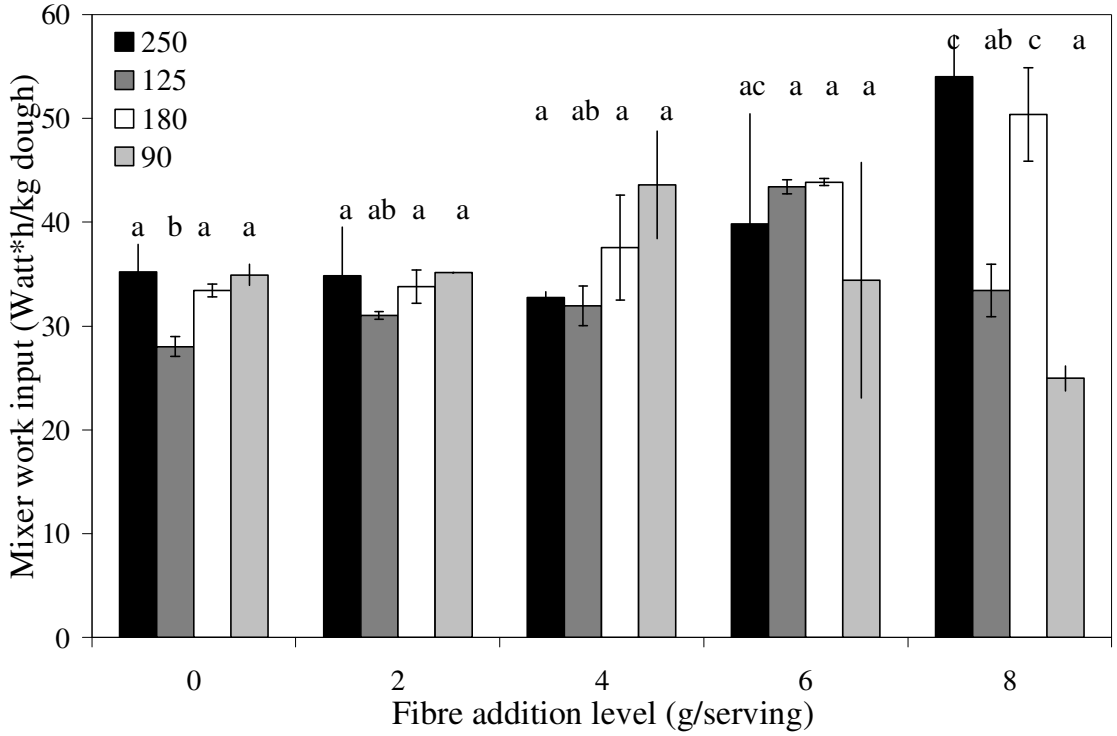


Figure 3.7. Effect of fibre addition level and particle size on mixer work input for non-optimized loaves (see Figure 3.3 for control loaf footnote).

Tukey's multiple range test does not show a significant change in mixer work input due to fibre content and particle size because of the large standard deviation of some of the doughs. As well, there was some discrepancy at 8 g/serving with the 125 and 90 μm loaves and at 6 g/serving for the 90 μm loaf – while one would anticipate an increase in work input at this high level of fibre loading, the work decreased. This was due to the dough being very dry and dense, such that the dough hook did not grab the dough, resulting in an apparently reduced work input (Figure 3.8). The poor contact

between the dough and the dough hook is likely the reason for the large standard deviation.

Figure 3.8. Dough containing 90 µm fibre at 6 g/serving after mixing.

For the non-optimized loaves, there was a trend of increasing sheeter work input as fibre content increased and particle size decreased, but it was not significant as indicated by the large standard deviation (Figure 3.9).

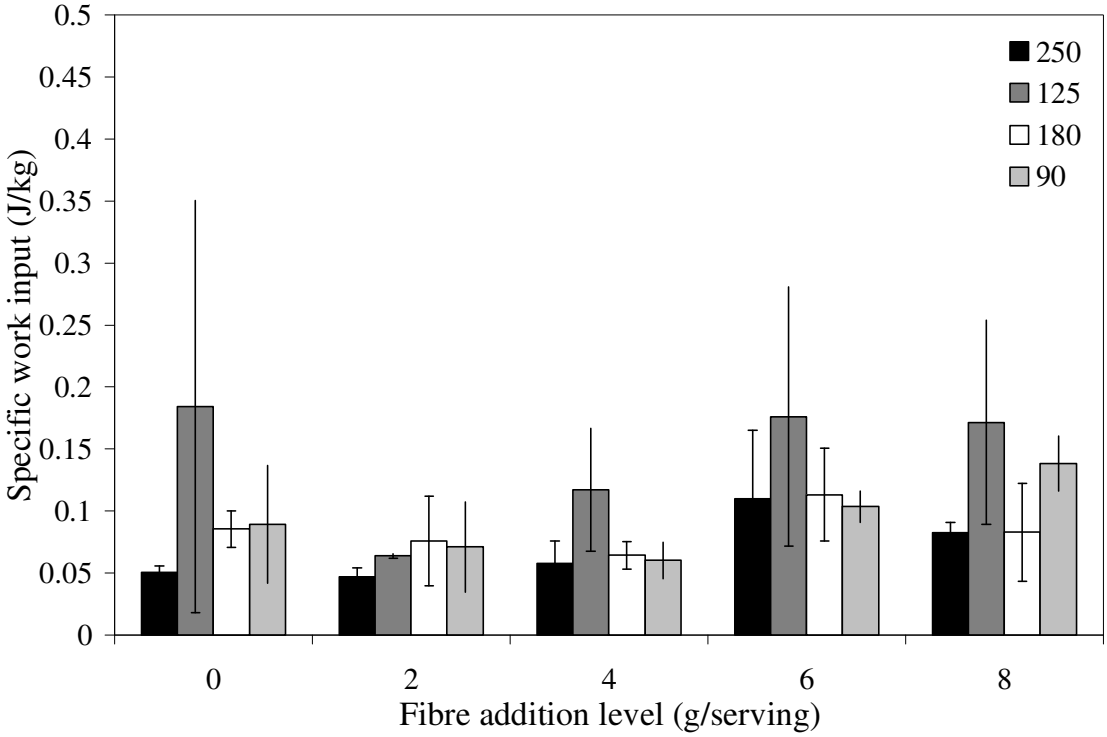


Figure 3.9. Effect of fibre addition level and particle size on sheeter work input for non-optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.2.2. C-cell parameters demonstrate effect of dough viscosity on gas bubble structure

3.4.2.2.1. Cell diameter of non-optimized loaves

The depression of specific loaf volume with increasing fibre content and decreasing particle size was reflected in the decrease of cell diameter measured by C-cell analysis. As particle size decreased and fibre content increased, there was a general decrease in cell diameter (Figure 3.10). The results for cell diameter corroborate the crumb texture results, where the crumb became denser and tighter as particle size decreased and fibre content increased. This was particularly noticeable for high fibre addition levels for the 125 and 90 μm fractions.

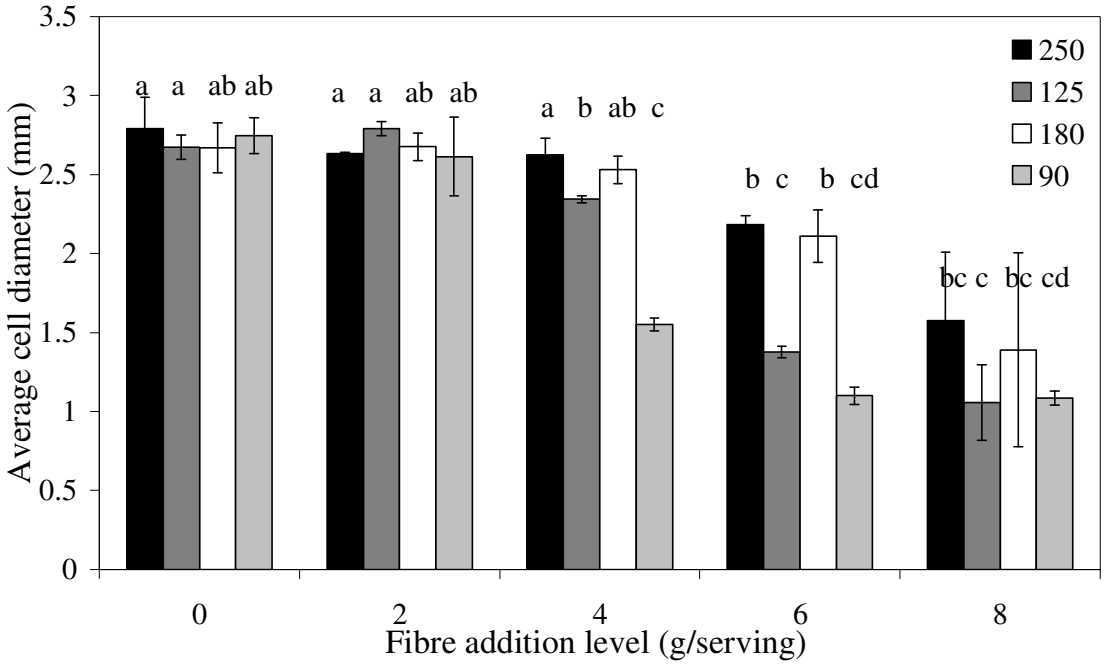


Figure 3.10. Effect of fibre addition level and particle size on cell diameter of non-optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.2.2.2. Cell density of non-optimized loaves

The cell density (number of cells per slice area) increased with increasing fibre content for all particle sizes (Figure 3.11). This suggests that the cells that nucleated did not coalesce; if cell coalescence had occurred then cell density would have decreased.

The cell densities of 250 and 180 are more similar than 250 and 125. This may be due to the large fraction of small particles present in the 125 commercial pea fibre, thereby making its behaviour more similar to that of the 90 μm fraction (see Masterizer work in 3.4.2.3).

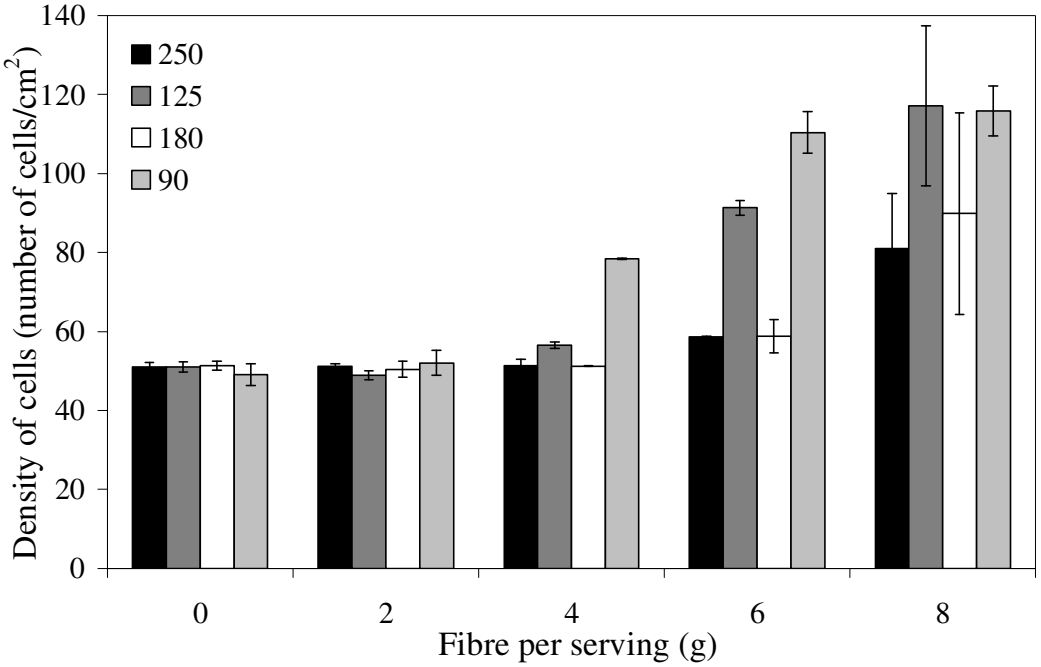


Figure 3.11. Effect of fibre addition level and particle size on cell density of non-optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.2.2.3. Relative volume range of non-optimized loaves

Within each level of fibre addition, relative volume range decreased with decreasing particle size, which indicated an increase in homogeneity, or a smaller range of gas cell size (Figure 3.12). Though the decrease in relative volume range is not

significant, there is a visible pattern seen, especially in the 2, 4, and 6 g/serving loaves from 125 to 180 to 90 μm. This suggested that volume depression arose from high dough viscosity limiting gas cell expansion rather than from fibre particles promoting gas cell coalescence. It was proposed by Hayman and others (1998) that the presence of starch granules larger than the thickness of the cell wall would destabilize the wall. However, when the gas cell wall was thick compared to starch granules, the wall was stabilized against coalescence. While the shape and size of a particle embedded in the gas cell wall would influence its effect on gas cell stability (Robertson and Eastwood 1981), the high dough viscosity of the non-optimized loaves would likely result in a thicker cell wall, thereby stabilizing the gas cells against coalescence and resulting in tighter crumb grain.

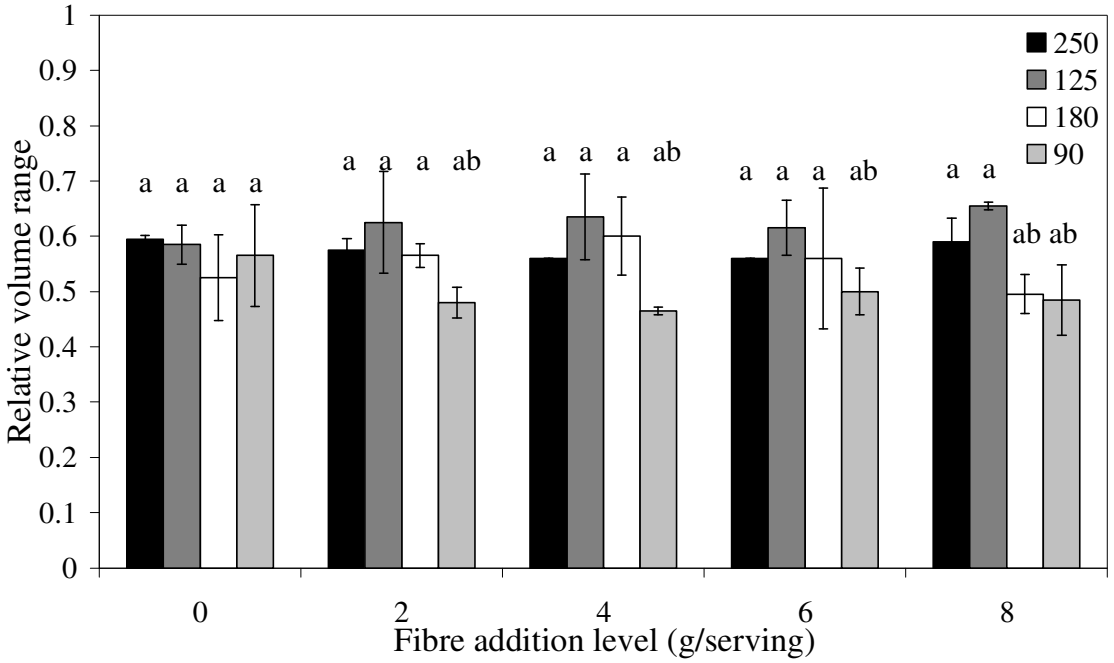


Figure 3.12. Effect of fibre addition level and particle size on relative volume range of non-optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.2.3. Texture profile analysis demonstrates effect of high dough viscosity on compression force value of bread slices

A dramatic increase in hardness was seen as fibre addition level increased, particularly with the 125 and 90 μm fractions (Figure 3.13).

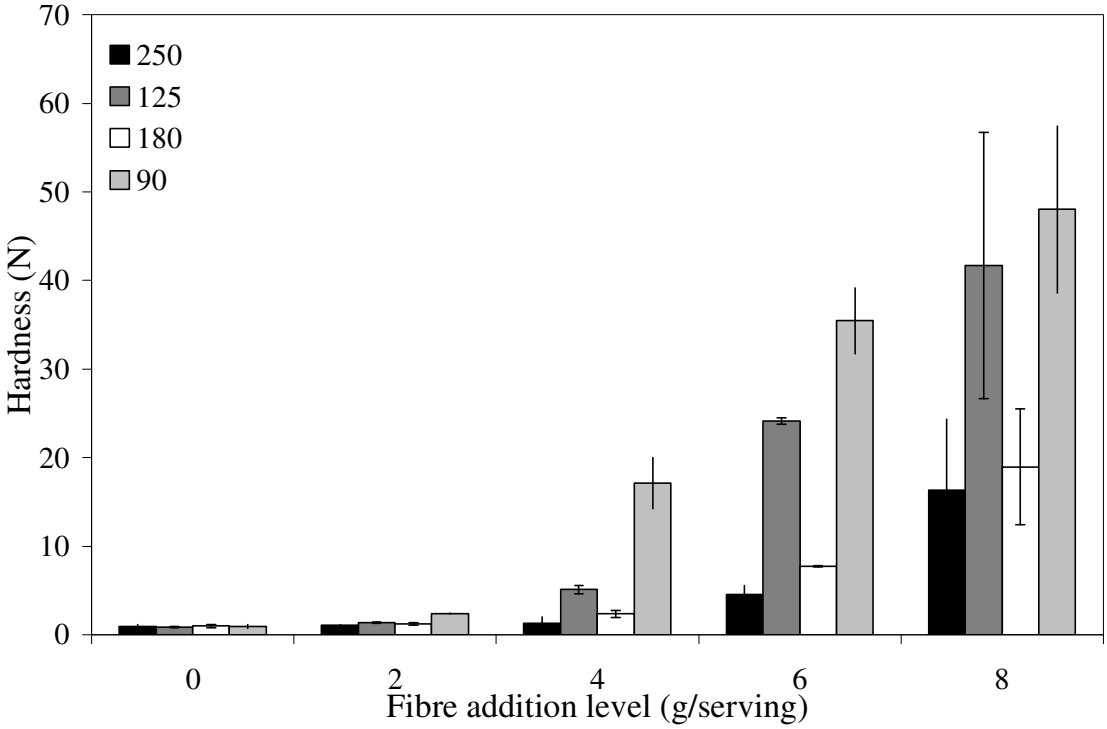


Figure 3.13. Effect of fibre addition level and particle size on bread crumb hardness of non-optimized loaves (see Figure 3.3 for control loaf footnote).

Similar to hardness, a greater compression force value (CFV) indicates greater crumb firmness. The CFV of the non-optimized loaves got significantly larger with increasing fibre addition for all particle sizes (Figure 3.14). An increase in crumb firmness had also been found in loaves with 1-7% pea fibre substitution of wheat flour (Dalgetty and Baik 2006).

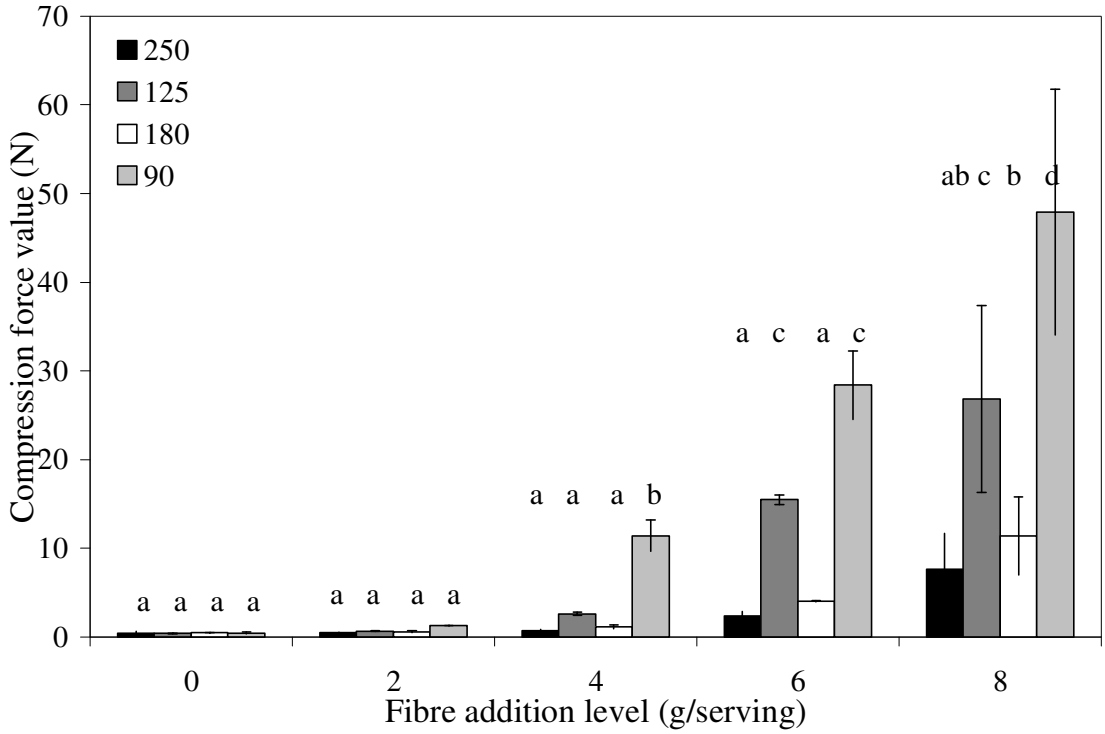


Figure 3.14. Effect of fibre addition level and particle size on compression force value of non-optimized loaves (see Figure 3.3 for control loaf footnote).

The increase in CFV was most dramatic for loaves containing 125 and 90 μm fibre at 6 and 8 g/serving. While the 125 and 90 μm fibres had CFVs closer to each other, the loaves made with the 250 and 180 μm fibres were closer to each other. A similar trend was seen in cell density (see 3.4.2.2.2). This was thought to be due to the effect of small particles present in the 125 and 90 μm particle size fractions (see 3.4.2.3).

3.4.2.4. Investigation of effect of pea fibre particle size on dough viscosity using Mastersizer

Due to the similarity in results between the 125 and 90 μm fibres, it was suspected that the commercial 125 μm fibre had a considerable fraction of small particles. According to Best Cooking Pulses Inc., their commercial 125 μm fibre has a particle size

of 95% through a 125 mesh screen. As an initial investigation, the 125 μm fibre was sieved using the Ro-Tap testing sieve shaker; 52% went through a 125 μm sieve, which suggested that a significant portion of it was smaller than 125 μm. To confirm this, the particle size distribution of the pea fibres was determined using a Masterizer to gain a greater understanding of the material's properties in order to explain its effect on baking properties.

Figure 3.15 shows the particle size distribution of all the pea fibre fractions. The two top fractions milled from the pea fibre bran (250, 180) had very narrow particle size distributions, whereas the 90 μm had a tail of small particle sizes in its distrubtion. This was also evident in the commercial fibre (125). This may explain how the behaviour of the 125 μm fraction was similar to the 90 μm fibre in baking, because it contained small particles that behaved like those in the 90 μm fraction.

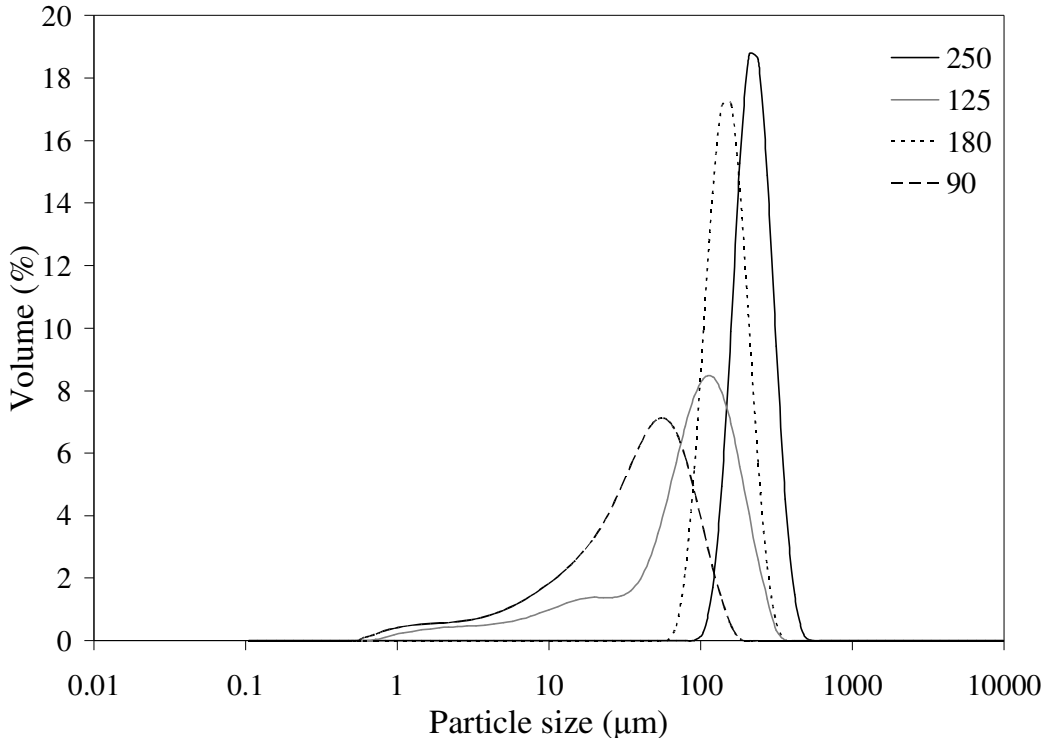


Figure 3.15. Particle size distribution of pea fibre fractions (250, 125, 180, 90 μm).

It was previously found that cellulose fibres >154 μm produced loaves with normal breadmaking properties, whereas cellulose fibres <154 μm interfered with gluten formation and resulted in poor loaf height (Seguchi and others 2007). Cellulose is an insoluble fibre (Goldstein and others 2010), and the pea fibre used contains 82% insoluble fibre according to the manufacturer. This would suggest that the loaves containing fibre of small particle size would have poorer quality, which we found particularly evident with the loaves containing the 90 μm fraction.

Particle size has been shown to affect water absorption of wheat flour (Zhang and others 2005) and barley flour (Izydorczyk and others 2007, Prasopsunwattana and others 2009). For whole barley flour and wheat flour, as particle size decreased, farinograph water absorption increased (Prasopsunwattana and others 2009, Wang and Flores 2000). It is hypothesized that a reduction in particle size would result in the exposure of greater surface area, as well as more polar groups with water binding sites, and thus require greater water absorption (Chau and others 2006, Rosell and others 2006). This would create competition for water absorption with protein and starch, thereby disrupting dough matrix development and resulting in poor loaf volume (Choi and others 2011).

In contrast, it had been previously found that fine grinding of wheat bran particles before their addition to a bread formula improves loaf volume (Lai and others 1989). The cause of this benefit has been attributed to improved bran hydration (Nelles and others 1998). Similarly, a reduction in fibre particle size of sugarcane bagasse and wheat bran was associated with lower water absorption and better breadmaking properties (Sangnark and Noomhorm 2003, Zhang and Moore 1997). Paradoxically, Rosell and others (2009) found no relationship between particle size and hydration properties of a variety of fibres,

including cellulose and wheat flour. Besides particle size, water absorption is also affected by chemical structure and shape of fibre particles (Robertson and Eastwood 1981), so it is difficult to generalize the effect of particle size on water absorption for different fibres. While a strong particle size effect was seen in this study, elucidating a mechanism for this is difficult because the literature is inconclusive.

3.4.3. Mitigation of pea fibre effect by optimizing water absorption

Due to the high dough viscosity effect that pea fibre addition had on the doughs, it was suspected that more water was needed in the bread formulas (Grigelmo-Miguel and others 1999, Izydorczyk and others 2008, Weightman and others 1995). The pea hull fibre used in this study contains 89.3% total dietary fibre (8% soluble, 82% non-soluble) according to the manufacturer. Wang and Toews (2011) determined commercial pea fibres to have 354.1-708.9 g/kg (dry matter) insoluble dietary fibre and 18.2-49.1 g/kg soluble dietary fibre. It has been found that increasing amounts of insoluble pea fibre resulted in greater water absorption in bread dough formulations (Dalgetty and Baik 2006 Gómez and others 2003, Wang and others (2002). It would follow that the pea fibres that we used would result in greater water absorption. The second set of my baking experiments involved optimizing water absorption levels in the loaves to observe if adequate hydration would mitigate the detrimental effect of pea fibre on gas bubble growth. Figure 3.16 illustrates the effect of optimized water absorption on the ability of gas cells to expand in the fermenting loaf.

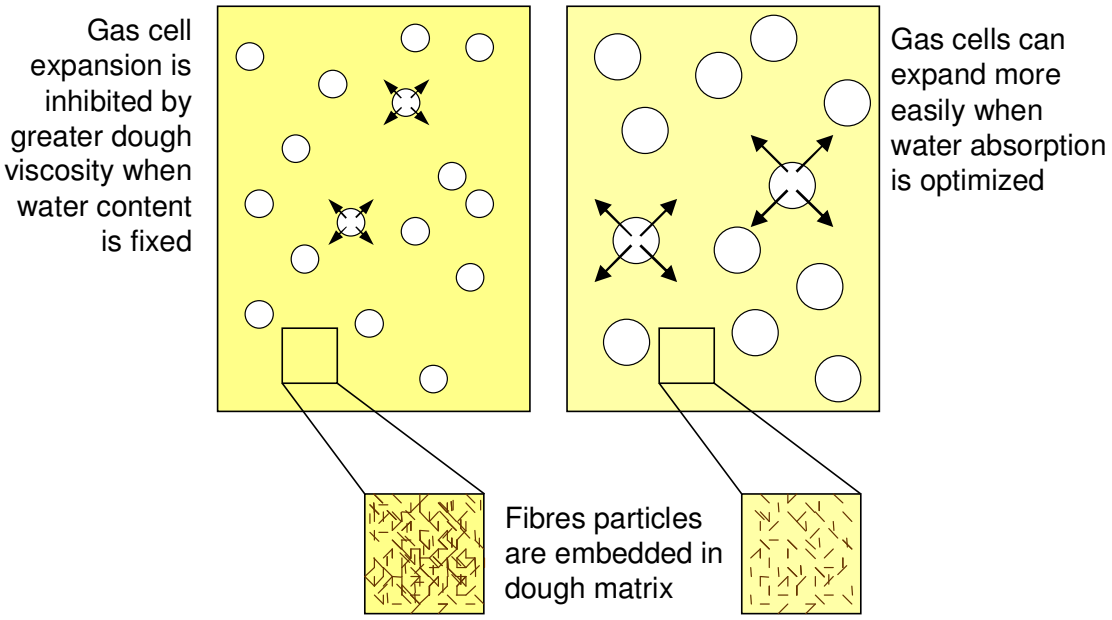


Figure 3.16. Schematic diagram demonstrating limited gas cell expansion due to high dough viscosity (left) and greater expansion when water levels optimized.

It was found that there was no insignificant difference between the properties of the bread made with the direct and slurry addition of fibre and water. Therefore, the analysis of optimized water absorption is based on direct addition (see 3.4.3.7 for more details). The improvement of bread quality through water addition was evident in measurements of specific loaf volume, mixer work input, C cell parameters, and texture profile analysis.

3.4.3.1. Specific loaf volume increase in optimized loaves

Significant improvements in specific loaf volume were observed overall when water absorption was optimized, except at high fibre loadings and smaller particle size (Figure 3.17). With the exception of the 90 μm fraction, all of the 2 g/serving loaves were similar to the control volume. There was also considerable volume depression for the 90 μm pea fibre-enriched loaves at the 4 and 6 g/serving. While it had been

previously found that fine grinding of wheat bran particles before their addition to a bread formula improves loaf volume (Lai and others 1989), others have found that small particle size results in poor loaf volume (Choi and others 2011). Besides particle size, other factors, such as the number of hydroxyl groups and particle shape, play a role in how the fibre particles affect bread quality (Almelda and others 2010, Robertson and Eastwood 1981).

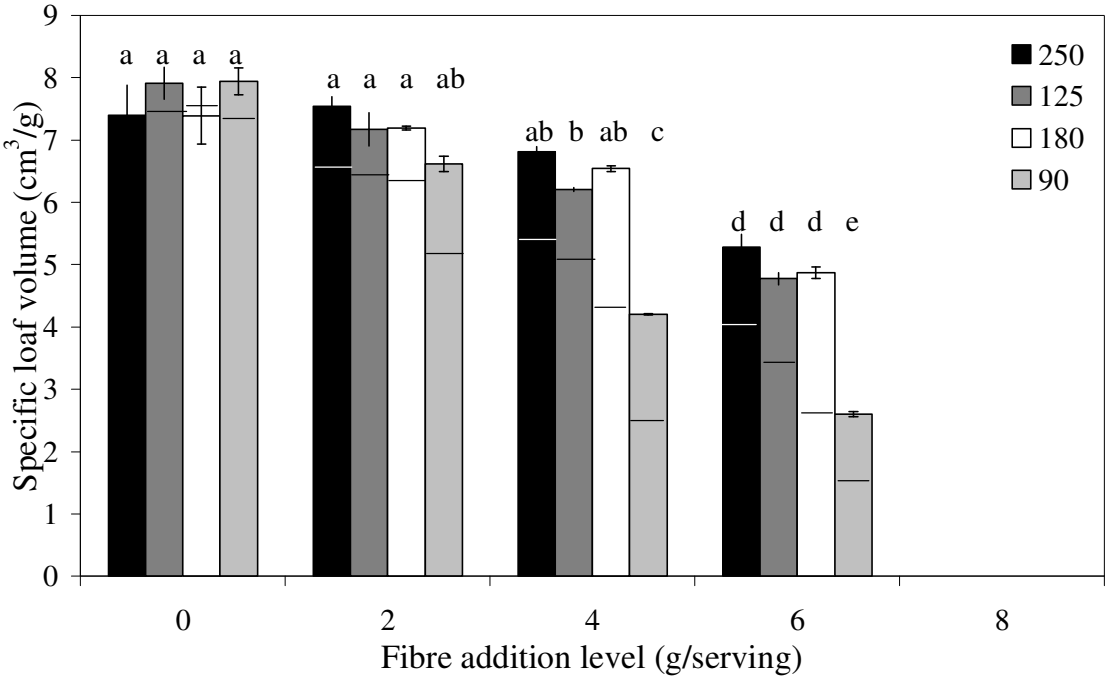


Figure 3.17. Effect of fibre addition level and particle size on specific loaf volume of loaves with optimized water absorption* (see Figure 3.3 for control loaf footnote). *Lines on bars indicate the average specific loaf volume of the non-optimized loaves.

3.4.3.2. Subjective bread scoring of optimized loaves

3.4.3.2.1. Loaf appearance of optimized loaves

There was a relative improvement in loaf appearance scoring when loaves were optimized compared to their non-optimized counterparts, particularly at 4 and 6 g/serving for all particle sizes (Figure 3.18). The scores for 90 μm loaves at 4 and 6 g/serving were

dramatically lower than the scores for the other optimized loaves, due to their smaller loaf volume.

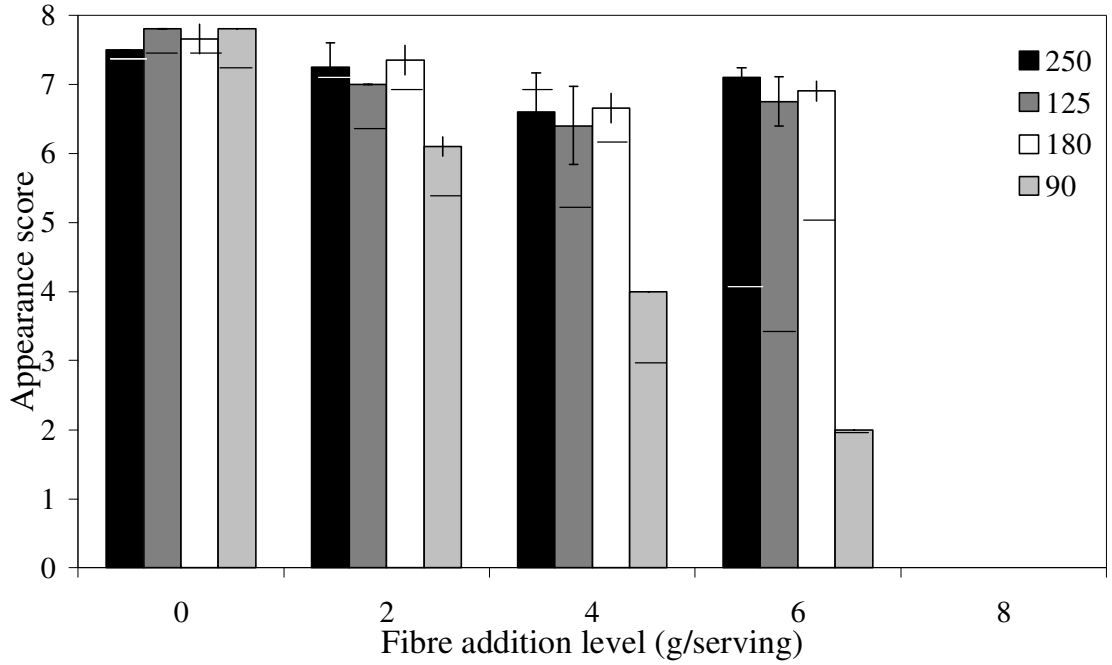


Figure 3.18. Bread score for appearance of optimized loaves (see Figure 3.3 for control loaf footnote).

Photos of the loaves are shown in Figure 3.19. Breaks were apparent on all 2 g/serving loaves, though they were smaller than those of the control. All 4 g/serving loaves except the 90 μm fraction had breaks but they were smaller than those at 2 g/serving. None of the 6g/serving loaves had breaks. A greater break indicates greater gas cell expansion (Hoseney 1998). Figure 3.20 shows side profiles of the slices for each particle size at increasing fibre loading, showing that there is less difference in loaf volume with increasing fibre content when water absorption was optimized.

(a)

(b)

(c)

(d)

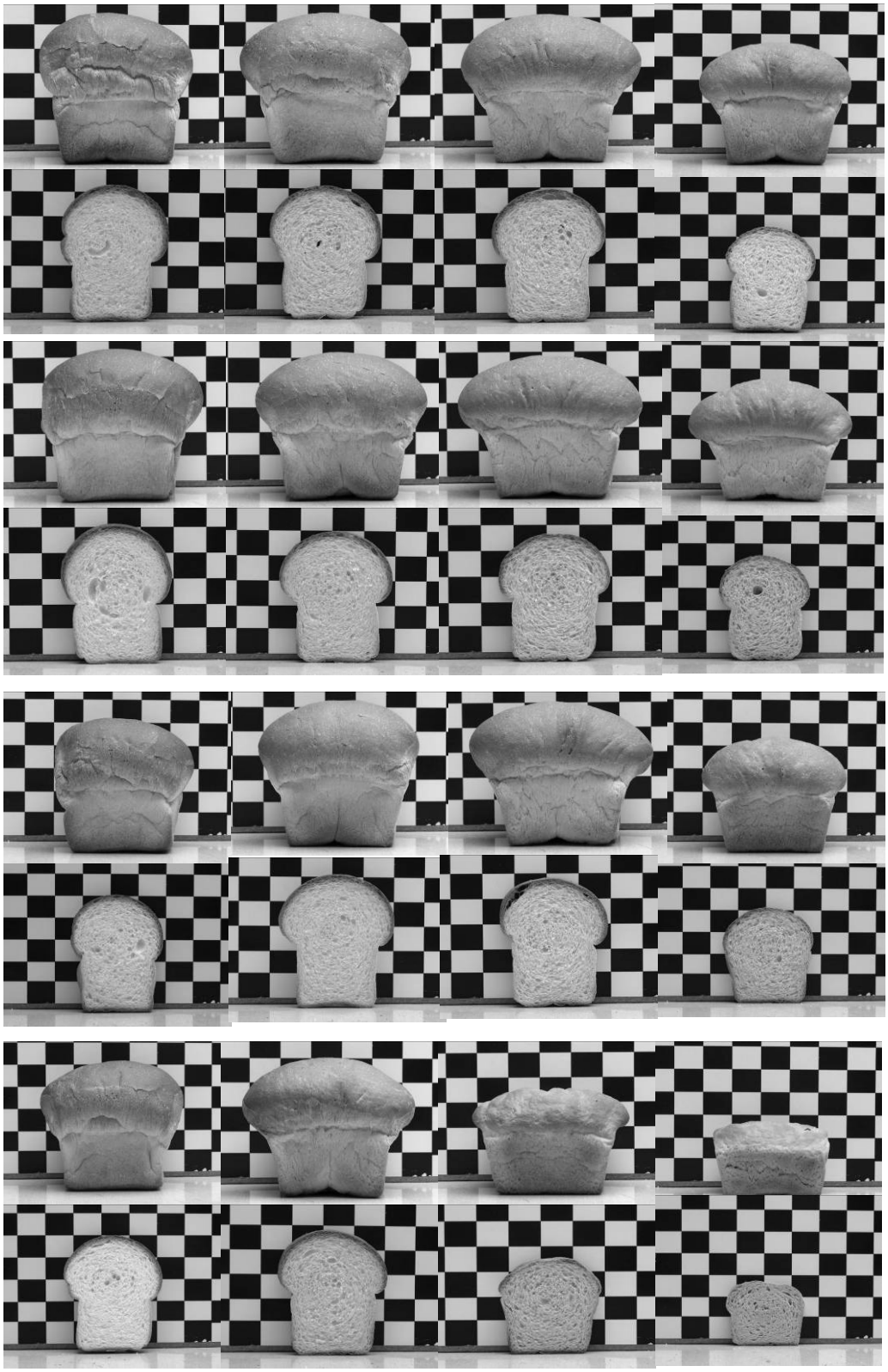
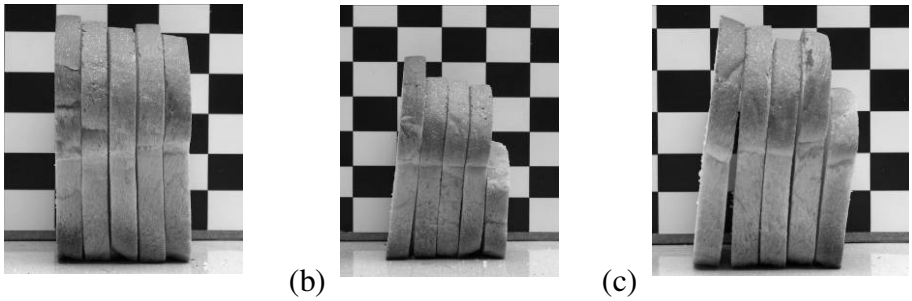


Figure 3.19. Photos of loaves and slices of optimized loaves: (a) 250 μm, (b) 125 μm, (c) 180 μm, (d) 90 μm. From left to right: 0, 2, 4, 6 g/serving.



(a) (b) (c)

Figure 3.20. Side profiles of optimized loaves: (a) 2 g/serving, (b) 4 g/serving, (c) 6 g/serving. Slices from left to right are: control, 250, 125, 180, 90 μm.

Fibres swell in water and thus increase in particle size, which would affect their interactions with other dough ingredients (Rosell and others 2009). As such, the degree of fibre hydration would affect the behaviour of fibre in a dough system. In the case of our pea fibre, the water addition has resulted in the fibre-enriched loaves appearing more like the control loaves.

3.4.3.2.2. Crumb texture of optimized loaves

Overall there was improvement in crumb texture in the optimized loaves; all the loaves had scores within one point of the control except for the 90 μm loaves at 4 and 6 g/serving and 125 μm at 6 g/serving (Figure 3.21).

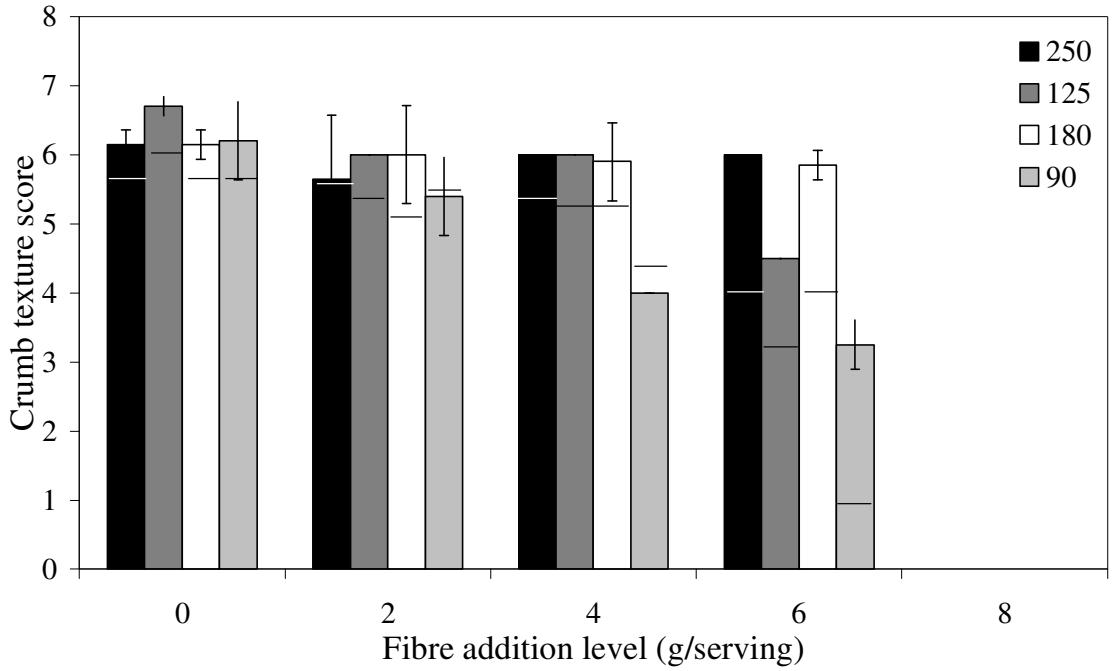


Figure 3.21. Bread score for crumb texture of optimized loaves (see Figure 3.3 for control loaf footnote).

Compared to the other particle sizes, the 90 μm loaves had smaller gas cells, and thus a tighter crumb structure. The 125 μm at 6 g/serving had a tighter crumb structure as well. The small particle size of 90 μm and the large fraction of smaller particles in the 125 μm loaf were likely the cause for the decrease in crumb texture due to their greater water hydration demand (Prasopsunwattana and others 2009, see Table 3.4). While the scores of these loaves were still low compared to the other optimized loaves, they were greater than the scores of their non-optimized counterparts (see comparison lines). Similarly, a previous study found that while bread enriched with increasing amounts of pea fibre had decreasing subjective bread scores, the scores were not drastically lower than the control loaf when optimized (Kasprzak and Rzedzicki 2010). Sosulski and Wu (1988) found that loaf shape and crumb texture were rated as "very good" in breads

fortified with 10% pea hulls (equivalent to 2-4 g/serving). As such, the improved quality of our optimized loaves seems to be similar to that of other researchers.

3.4.3.2.3. Crumb colour of optimized loaves

There was a general improvement in crumb colour in the optimized loaves: they were generally less yellowy and grey than the non-optimized loaves (Figure 3.22).

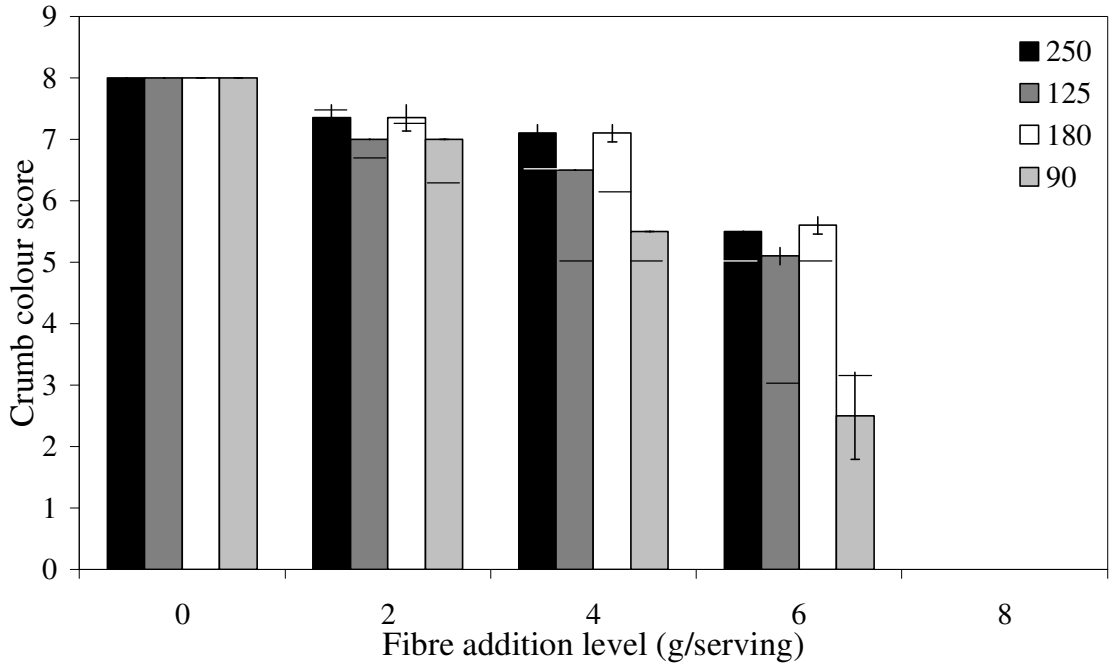


Figure 3.22. Bread score for crumb colour of optimized loaves (see Figure 3.3 for control loaf footnote).

The greatest improvement was seen in the effect of the 125 and 90 μm fractions at 4 and 6 g/serving (see Figure 3.5 and 3.19 for photos of non-optimized and optimized slices, respectively). A previous study found that loaves enriched with pea fibre had a lighter and more yellow crust colour when assessed with a Hunterlab color meter. Based on the color meter's results, the researchers concluded that the replacement of wheat flour with 15% (by weight) pea hull fibre resulted in a minimal effect of fibre on the whiteness

of the crumb (Sosulski and Wu 1988). Loaves baked with a 15% flour replacement with pea fibre would be similar to the loaves baked in this study to which fibre was added at 6 g/serving. All the particle sizes baked at this addition level had significantly lower crumb colour scores than the control – but crumb colour score was based on a baker's assessment, not from the data derived from a color meter.

3.4.3.3. Mixer work input of dough with optimized water absorption

Mixer work input was essentially the same amongst all breads when rebaked using optimal water levels (Figure 3.23). With adequate water addition to the formula, there was reduced competition between the gluten and fibre for water absorption, resulting in less dough resistance to extension (Bonnand-Ducasse and others 2010).

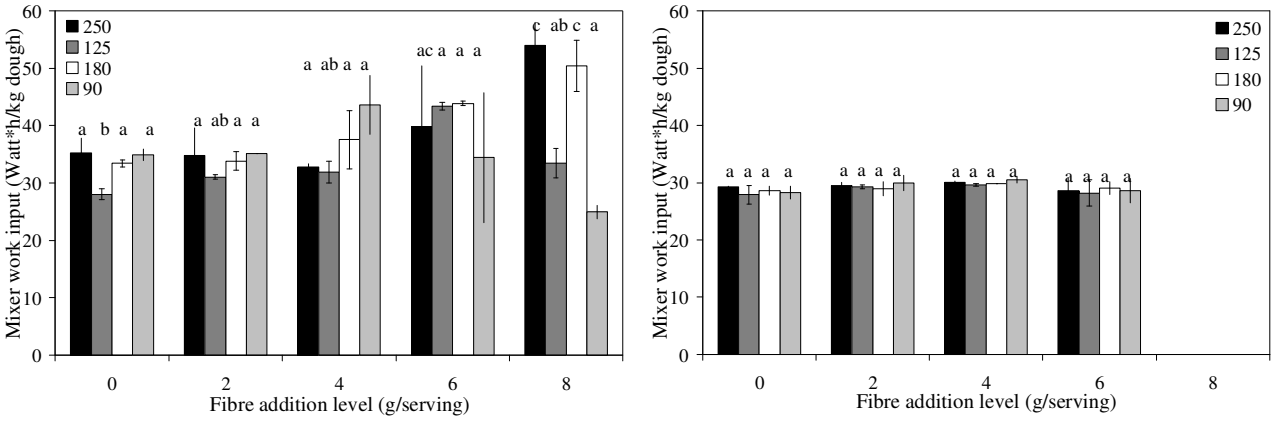


Figure 3.23. Effect of fibre addition level and particle size on mixer work input of non-optimized (left) and optimized loaves* (see Figure 3.3 for control loaf footnote).
*The values for the control loaves are different for the non-optimized and optimized loaves as control loaves were baked every day with the test loaves.

3.4.3.4. Sheeter work input of dough with optimized water absorption

Overall, there was essentially no difference in sheeter work input for the doughs made with different fibre fractions where water absorption had been optimized (Figure 3.24).

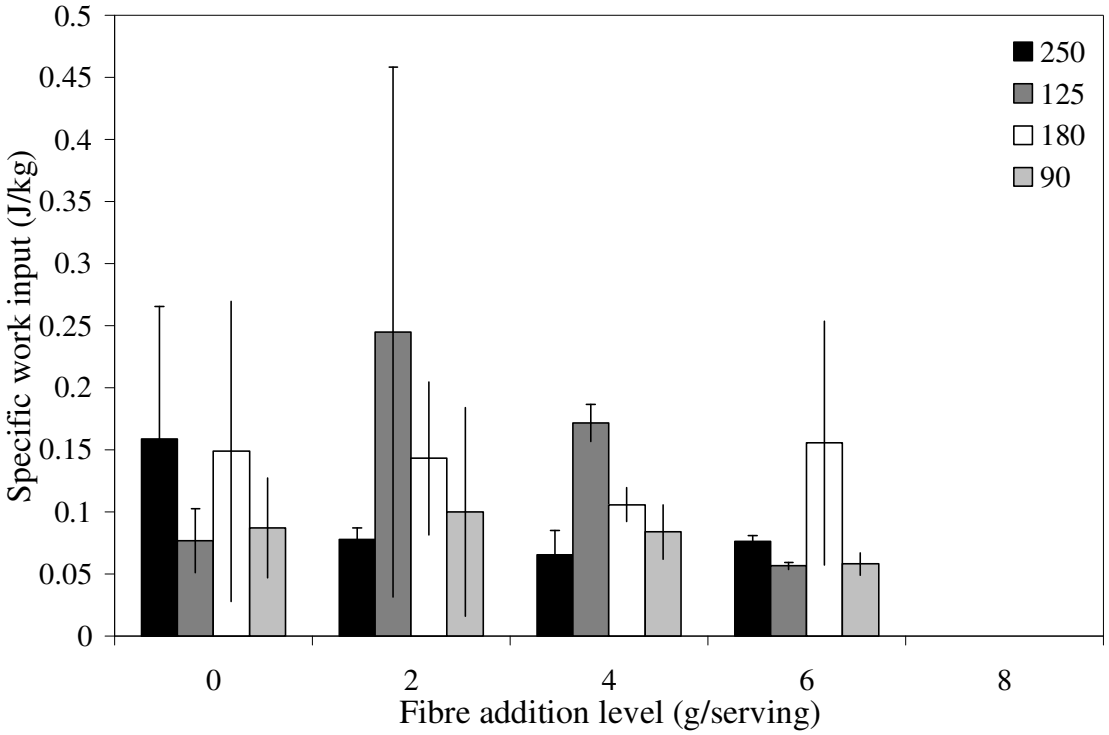


Figure 3.24. Effect of fibre addition level and particle size on sheeter work input of optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.3.5. C-cell analysis of optimized loaves

The c-cell analysis parameters discussed below demonstrated that there was a general improvement in crumb structure when water absorption was optimized.

3.4.3.5.1. Cell diameter of optimized loaves

Improvements in specific loaf volume were reflected in greater cell diameters (Figure 3.25), indicating a more open crumb structure. Except for bread containing 90

μm pea fibre at 6/serving, cell diameters for all pea fibre enriched loaves were similar to those of the control loaves. As smaller average cell diameter would indicate less gas cell expansion; the loaf containing 90 μm at 6 g/serving had a significantly smaller specific loaf volume.

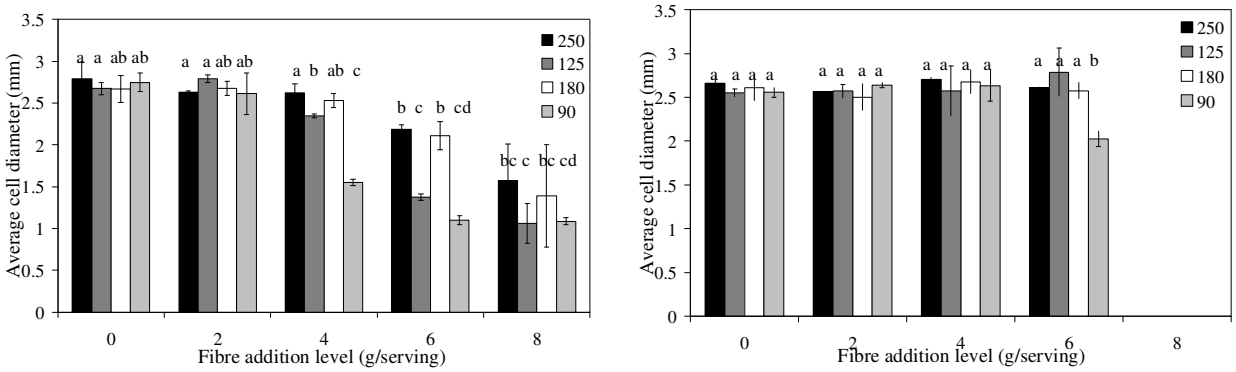


Figure 3.25. Effect of fibre addition level and particle size on cell diameter of non-optimized (left) and optimized loaves (see Figure 3.3 for control loaf footnote).

To date there is no literature studying the relationship between C-cell analysis and dough mechanical properties. Existing studies have used C-cell analysis to as an alternative to subjective crumb texture analysis (Alvarez-Jubete and others 2010, Lodi and Vodovotz 2008, Stojceska and Ainsworth 2008, Sun and others 2010). Other studies using C-cell analysis have compared its objective analysis to subjective ones (Day and Rogers 1996, Stevenson and others 2010).

3.4.3.5.2. Cell density of optimized loaves

Cell density was essentially invariant for all fibre level additions and particle sizes when water absorption was optimized except for the 90 μm fraction at 6 g/serving (Figure 3.26). At this high addition level, the cell density was greater than that of the other optimized loaves, but there was still significantly lower cell density compared to the non-

optimized version of the loaf. The overall decrease in cell density in optimized loaves gives an indication of larger, fewer cells, imparting a perception of a more open crumb structure.

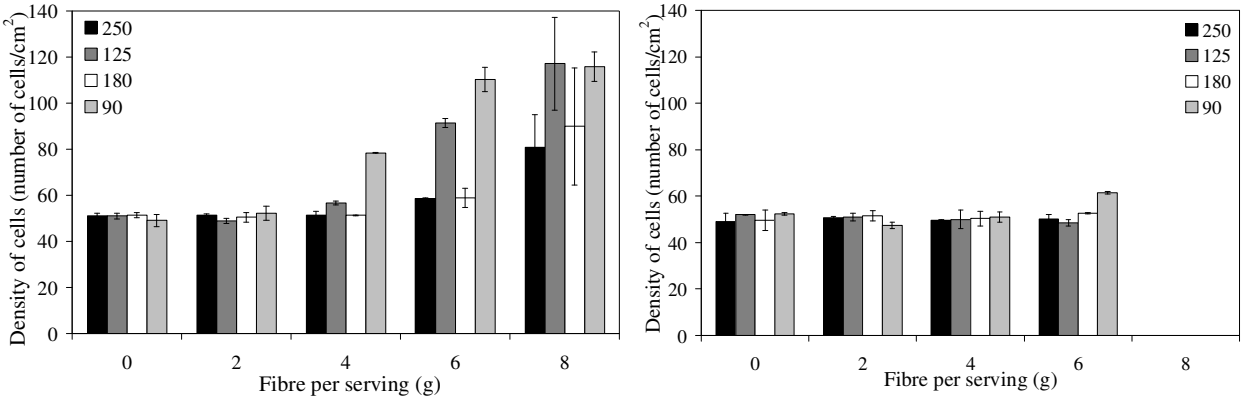


Figure 3.26. Effect of fibre addition level and particle size on cell density of non-optimized (left) and optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.3.5.3. Relative volume range of optimized loaves

For optimized loaves, there is no clear overall trend in relative volume range for particle size and fibre addition level (Figure 3.27). In contrast, the non-optimized loaves had decreasing relative volume range (and thus a decreasing incidence of coalescence) with decreasing particle size. For the optimized loaves, the relative volume ranges for the fibre-enriched loaves are similar to those of the control loaves, which suggest that the degree of coalescence for all the loaves is similar. It would appear that the addition of water allows the fibre-enriched dough to behave more like the control dough, likely because of a reduction in competition between the gluten and fibre for water absorption (Bonnand-Ducasse and others 2010).

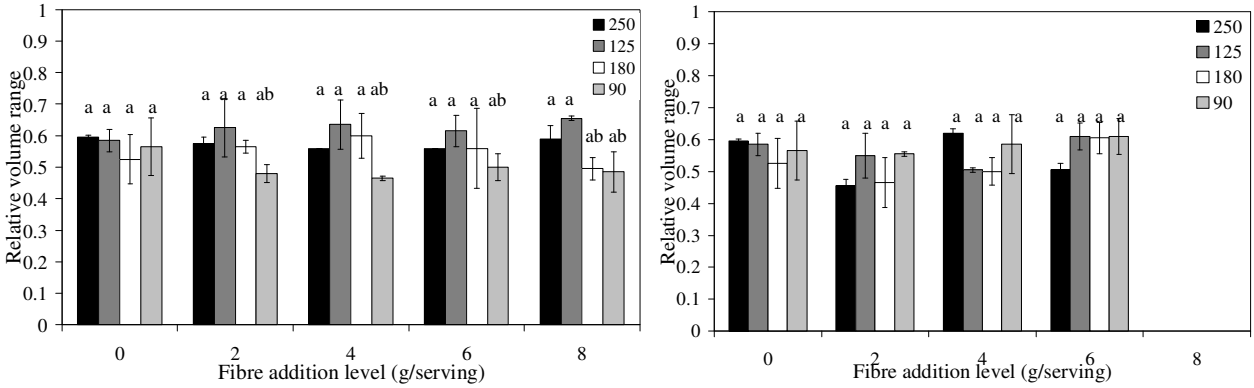


Figure 3.27. Effect of fibre addition level and particle size on relative volume range of non-optimized (left) and optimized loaves (see Figure 3.3 for control loaf footnote).

3.4.3.6. Texture profile analysis

The mitigating effect of optimizing water absorption was clearly seen in the improvement of CFV for all particle sizes and fibre addition levels (Figure 3.28). Note that the scale of the y-axis of Figure 3.28 is seven times smaller than that in Figure 3.14 which shows the CFV for the crumb obtained from non-optimized loaves; the lines indicating the non-optimized CFV for 90 μm fraction fibre addition at 4 and 6 g/serving do not fit on the y-axis scale of Figure 3.28. All the optimized loaves had a significant decrease in CFV, indicating a substantial decrease in crumb firmness. Similarly, bread crumb hardness greatly decreased when doughs were baked with optimized water addition levels (Figure 3.29, note a fourfold increase in the y-axis of the non-optimized loaves).

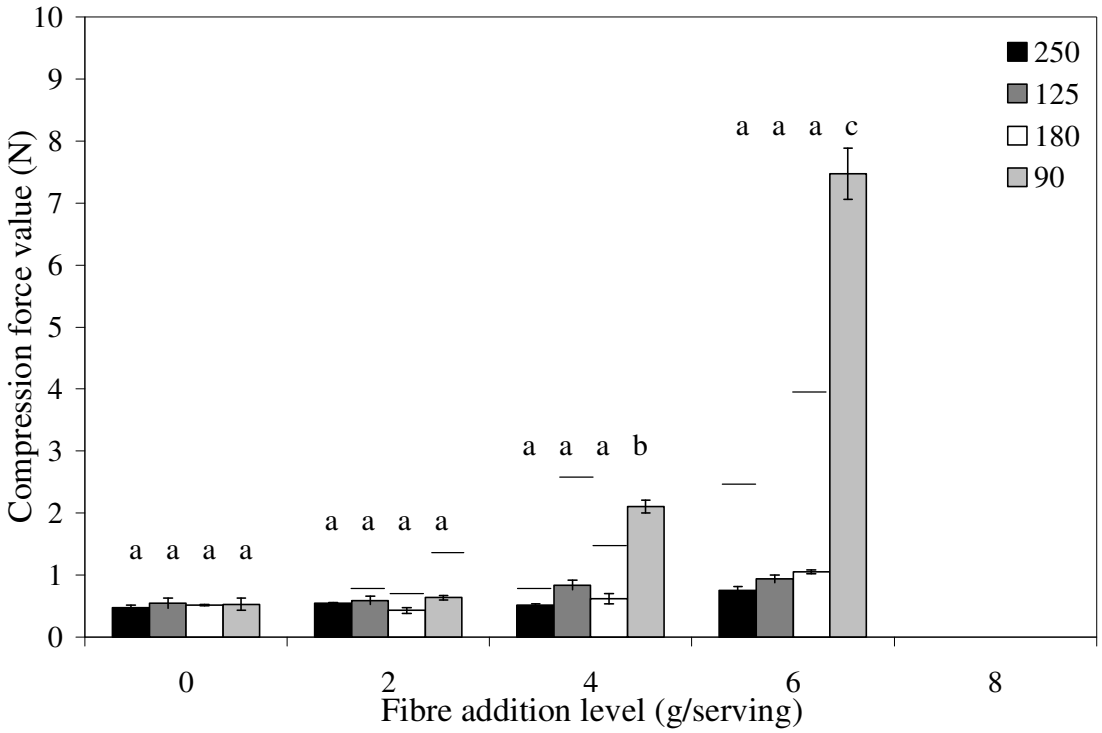


Figure 3.28. Effect of fibre addition level and particle size on compression force value of optimized loaves (see Figure 3.3 for control loaf footnote).

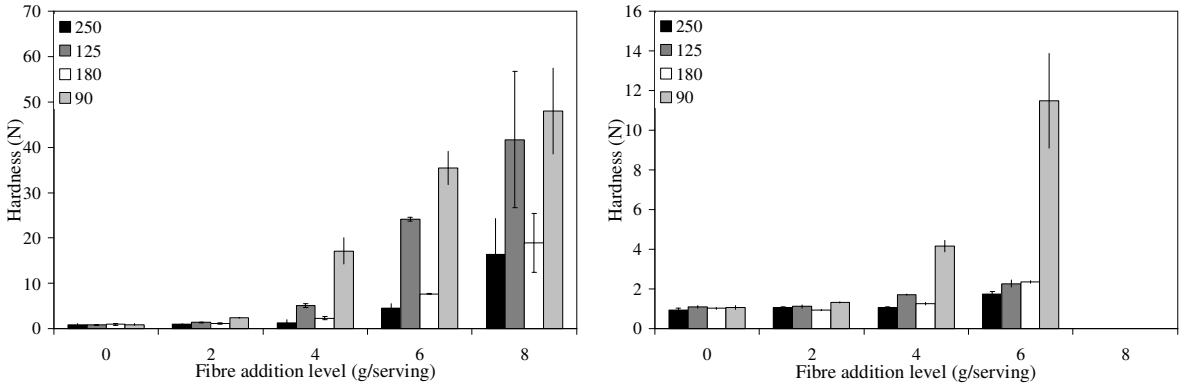


Figure 3.29. Effect of fibre addition level and particle size on bread crumb hardness of non-optimized (left) and optimized loaves (see Figure 3.3 for control loaf footnote).

The 90 µm loaf containing fibre at 4 and 6 g/serving had much higher CFVs than the other optimized loaves. Similarly, there was a decrease in specific loaf volume for these loaves. It would appear then that the upper limit of fibre addition level and particle size that would produce an acceptable loaf is 2 g/ serving at 90 µm; a greater fibre

loading and a smaller particle size would produce a loaf that cannot be optimized by water remediation alone. Different studies have proposed varying optimal levels of fibre addition (Tosh and Yada 2010); this likely depends on many factors such as wheat quality and pea fibre quality, including factors such as the ratio of soluble/insoluble fibre present, and variation in pea fibre particle size (Dalgetty and Baik 2006, Kasprzak and Rzedzicki 2010).

3.4.3.7. Differences in quality parameters due to method of fibre and water addition

Two methods of water addition were used: directly during mixing of the dough, and as a slurry mixture of pre-soaked pea fibre (see 3.3.4). To determine if there was a significant difference between the results for direct and slurry addition, t tests ($p<0.05$) were conducted comparing all the parameters measured. The specific loaf volume for the slurry loaves was significantly less than the volumes of loaves where the fibre was added directly. For the bread scoring parameters, only crumb colour was slightly significantly different. Similarly, there was no significant difference in the C-cell parameters. The compression force value was significantly greater for the slurry loaves. There was no significant difference in sheeter work input.

Overall, no substantial difference in quality improvement was found between the direct addition and slurry methods for almost all the parameters measured. If there was a difference, the slurry method resulted in poorer loaves compared to the direct addition method. As such, the results do not demonstrate any benefit in taking the extra step of pre-hydration. In a previous study, no significant improvement in specific loaf volume was found when pea fibre was pre-hydrated before being mixed into the dough (Sosulski

and Wu 1988). Similarly, Jacobs and others (2008) found no improvement in bread quality when barley fibre was pre-hydrated in a sponge-and-dough procedure. While Nelles and other (1998) found an improvement in bread quality when wheat bran was pre-hydrated, their hydration process involved agitating the bran in excess water to allow for complete water hydration.

3.5. Conclusions

In both optimized and non-optimized loaves, the effect of pea fibre on bread quality was due to enhanced dough viscosity rather than to greater coalescence arising from fibre particles interacting with bubbles in the dough. Increased dough viscosity was evident in greater mixer work input. The suppression of gas cell expansion due to dough viscosity was evident in decreased bread scores and C-cell parameters (cell diameter, cell density). Within each level of fibre addition, decreasing relative volume range with decreasing particle size indicated increasing homogeneity, inferring that coalescence was not responsible for depression in loaf volume. The effect of enhanced dough viscosity became more evident with increasing fibre addition level and decreasing particle size. The increasing effect of pea fibre on dough viscosity was mitigated when water absorption was optimized in bread formulation. This resulted in greater specific loaf volume and more open crumb structure as indicated by increased cell diameter and improved bread and crumb scores. Concomitantly, bread firmness decreased. The improvement in dough viscosity was seen in decreased mixer work input.

CHAPTER 4: RELATIONSHIPS BETWEEN DIFFERENT PARAMETERS USED TO ASSESS BREAD QUALITY

Abstract

It has been shown that the mechanical properties of bread are related to its structural properties, which are used to assess crumb quality. The relationship of Young's modulus (modulus of elasticity) with loaf density was determined for non-optimized and optimized loaves. No significant effect on the stiffness-density relationship was found due to changes in pea fibre particle size and fibre loading for any loaves. Crumb quality can be assessed subjectively by trained personnel or objectively using C-cell analysis. The correlations between subjective scoring and relevant C-cell parameters were determined. Overall the correlations were stronger for the non-optimized loaves than the optimized loaves, likely due to the greater diversity in data. In the future it may be possible to use C-cell analysis parameters to predict the subjective assessment of crumb quality. Similarly strong correlations found between compression force values and texture profile analysis parameters support the potential of predicting various crumb quality indicators with the use of one force value, simplifying the process of quality analysis.

4.1. Introduction

Crumb cell materials and crumb structure created by processing greatly affect the mechanical properties of bread. Having an understanding of the elastic properties of bread crumb would be desirable as it is considered a key factor of bread quality (Scanlon

and Zghal 2001). A compression loading test which indents a sample of bread crumb is an instrumental method of ascertaining subjective qualities such as mouthfeel and chewiness. One method of characterizing bread's mechanical properties is its Young's modulus, which can predict values for bread crumb's firmness and is determined from the linear (elastic) portion of the stress-strain curve (Scanlon and Zghal 2001).

Loaf and crumb appearance are also considerations when determining bread quality to predict consumer acceptability, especially the size of gas holes or cells (Day and Rogers 1996). The traditional method of assessing bread quality has been subjective, where trained personnel give a score to the sample loaves in relation to the control loaf based on loaf appearance, crumb texture, and crumb colour. The reliability of this is questionable, as different people often give a different score for the same loaves (Wang and Coles 1994). As such, there has been interest to move towards digital imaging techniques such as C-cell analysis, which would provide a more consistent, reliable and objective way of assessing bread quality (Day and Rogers 1996). C-cell analysis software has been designed to mimic how bread is visually assessed by trained personnel as it applies greater consideration to cells and cell walls closer to the centre (Whitworth and others 2004). To transition from subjective to objective scoring, it would be beneficial to know the relationship between subjective bread scoring and relevant C-cell analysis parameters.

4.2. Materials and methods

4.2.1. C-cell analysis and subjective bread scoring

Since C-cell analysis only looks at crumb and not loaf appearance, correlations were made on crumb scoring, not loaf appearances scores. The sections 3.3.8 and 3.3.9 have more details on how subjective bread scoring and C-cell analysis were conducted.

4.2.2. Texture profile analysis

Please see 3.3.10.

4.2.3. Calculation of Young's modulus

Young's modulus (E) was calculated as follows:

$$E = \frac{F(1-v^2)}{2b}$$

Where F (N) was the force of the first compression at 2 mm, v was Poisson's ratio (and assumed zero), and b was the radius of the indenter (0.025 m).

4.3. Relationship between structure and physical texture of bread

4.3.1. Correlation of Young's modulus with loaf density for non-optimized loaves

The physical texture and the cellular structure of bread crumb are highly correlated. Density gives an indication of the void fraction of the bread and can be related to its mechanical properties (Scanlon and Zghal 2001), and its relationship to the bread's modulus of elasticity is expressed by:

$$E/E_s \alpha (\rho/\rho_s)^m$$

Where E is Young's modulus (modulus of elasticity, E), E_s is Young's modulus of the solid material, ρ is the density of the material, ρ_s is the density of the solid material, and m is the power law index which depends on the type of foam. Since E_s and ρ_s were unknown, the correlation was simplified to:

$$E \alpha \rho^m$$

If m is 2, this indicates more open cells, whereas if n is 3, this indicates more closed cells (Gibson and Ashby 1988).

Figure 4.1 shows the correlation of modulus of elasticity with loaf density for the non-optimized loaves. There is no difference in this relationship between the different particle sizes overall, and thus no evidence that an increase in fibre content or change in fibre particle size affects loaf stiffness. The slope of the line is 2.8, indicating that there is more of a closed cell structure (Gibson and Ashby 1988), which was reflected in a decrease in average cell diameter as fibre content increased and fibre particle size decreased (see 3.4.2.2.1, Figure 3.10).

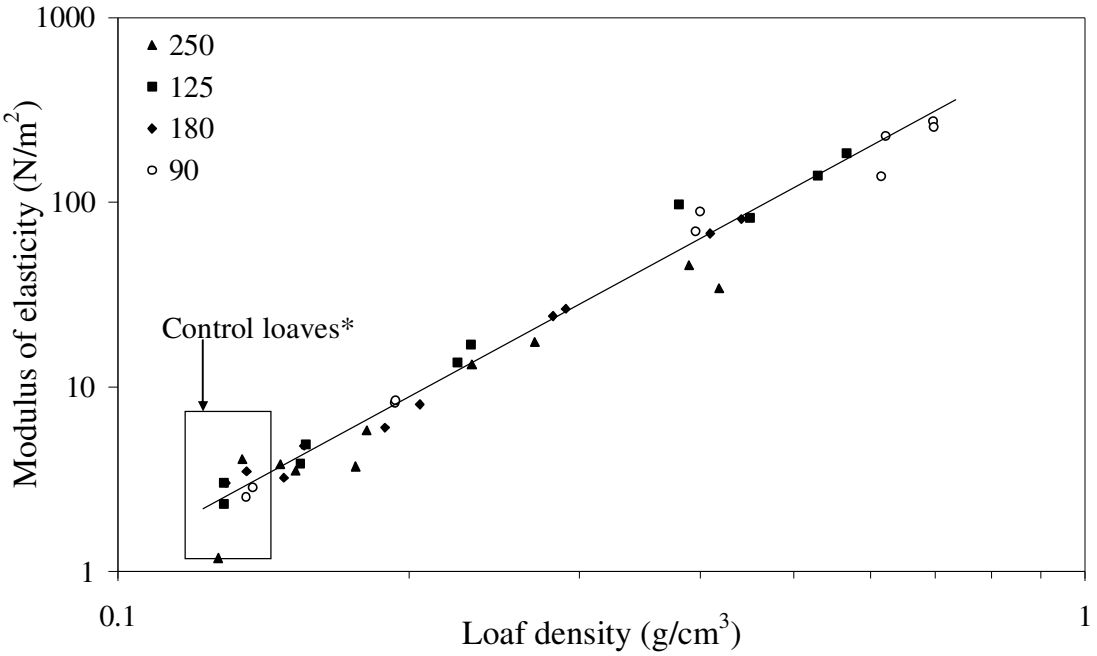


Figure 4.1. Correlation of modulus of elasticity with loaf density for non-optimized loaves.
*Box indicates control loaves associated with each pea fibre particle size.

4.3.2. Correlation of Young's modulus with loaf density for optimized loaves

Figure 4.2 shows the correlation of modulus of elasticity with loaf density for the optimized loaves. Similar to the non-optimized loaves, there was no difference in this relationship between the different particle sizes overall. The slope of the line is 2.2, indicating that there was more of an open cell structure (Gibson and Ashby 1988), which was reflected in the average cell diameter of all fibre-enriched loaves being similar to that of the control (see 3.4.3.5.1, Figure 3.25).

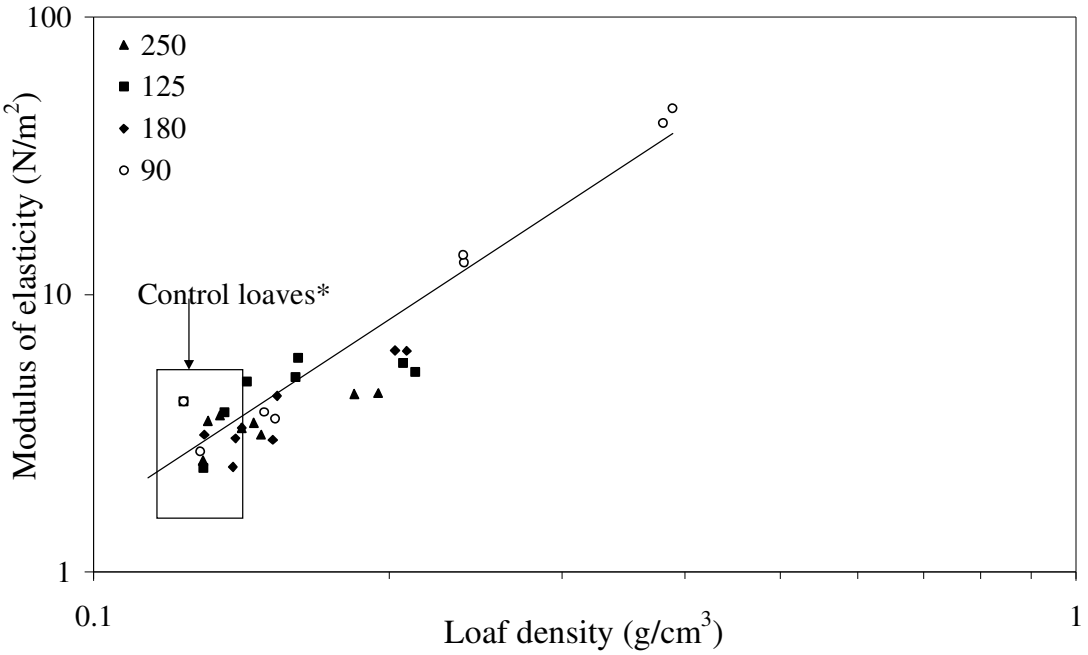


Figure 4.2. Correlation of modulus of elasticity with loaf density for optimized loaves. *Box indicates control loaves associated with each pea fibre particle size.

4.4. Subjective versus objective bread quality assessment

4.4.1. Correlations of bread scoring results with C-cell parameters for non-optimized loaves

Table 4.1 shows correlation coefficient (r) values between bread scoring and C-cell analysis parameters for non-optimized loaves.

Table 4.1. Correlation coefficient values of bread scoring parameters and C-cell analysis parameters for non-optimized loaves.

	Specific loaf volume	Crumb texture	Crumb colour
Cell diameter	0.94	0.87	
Cell density	-0.86	-0.86	
Relative volume range		-0.18	
Slice brightness			0.70

There was a strong positive correlation between specific loaf volume and cell diameter. This suggests that specific loaf volume would be a predictor for cell diameter; a greater specific loaf volume, likely indicates a greater average cell diameter.

There was a strong negative correlation between specific loaf volume and cell density. This would be expected; as cell density increases, it indicates that the gas cells are not expanding, which would mean a lower specific loaf volume. No scientific literature was found exploring the relationship between specific loaf volume and C-cell parameters; most research published simply uses C-cell analysis as a replacement for human visual analysis of crumb texture (Alvarez-Jubete and others 2010, Lodi and Vodovotz 2008, Stojceska and Ainsworth 2008, Sun and others 2010).

There was a strong positive correlation between cell diameter and crumb texture score, which was similar to the relationship of cell diameter with specific loaf volume. This confirmed that greater average cell diameter gave an indication of a more desirable open crumb structure, as a greater crumb texture score indicates a greater similarity with the control bread. This was similar to the findings of Day and Rogers (1996), who found a high correlation between crumb fineness determined by computer imaging and crumb quality assessed by human scoring.

There was strong negative correlation between cell density and crumb texture. As cell density increases, it indicates that gas cells are not expanding, meaning the crumb structure would be more closed. This would result in decreasing crumb texture score, hence a negative relationship between cell density and crumb texture score.

A weak negative correlation was found between relative volume range and crumb texture. Relative volume range gives an indication of crumb heterogeneity – a decrease

in range indicates an increase in homogeneity, or a smaller range of gas cell size (Edwards 2011). Thus relative volume range was a poor predictor of human perception of crumb texture; crumb heterogeneity does not appear to factor highly in subjective bread scoring.

While slice brightness was not discussed in Chapter 3, there appears to be high positive correlation between slice brightness and crumb colour scoring. Slice brightness is measured as the mean grey level (0-255) of pixels within the slice. Lower brightness can be caused by a darker crumb as well as larger or deeper cells that cast more shadows. Slice brightness also indicates higher product reflectance (Edwards 2011, Sun and others 2010).

4.4.2. Correlations of bread scoring results with C-cell parameters for optimized loaves

Table 4.2 shows the correlations of bread scoring parameters with C-cell analysis parameters for the optimized loaves. As there was less diversity in the optimized loaves, the correlations were weaker overall.

Table 4.2. Correlation coefficient values of bread scoring parameters and C-cell analysis parameters for optimized loaves.

	Specific loaf volume	Crumb texture	Crumb colour
Cell diameter	0.37	0.33	
Cell density	0.45	-0.37	
Relative volume range		-0.42	
Slice brightness			0.90

There was weak positive correlation between specific loaf volume and cell diameter. Similarly, there was a weak positive correlation between cell density and

specific loaf volume. The positive correlation between the latter pairing is unexpected, since as cell density increases, it would follow that the gas cells are not expanding, indicating a lower specific loaf volume. However, the cell density of the optimized loaves was essentially invariant (see 3.4.3.5.2, Figure 3.26). The range of specific loaf volume of the optimized loaves was less than that of the non-optimized loaves. Perhaps it is more difficult to see a clear correlation if there was not a dramatic difference in the cell density and specific loaf volume. As a result, the correlation is weak. A similar explanation can be proposed for cell diameter, which was very similar between the control and all fibre-enriched breads when water absorption was optimized (see 3.4.3.5.1, Figure 3.25). Interestingly, the correlation between specific loaf volume and cell density is positive, contrasting with the sign in the non-optimized loaves. A positive relationship would be expected if cell density is lowered by gas cell coalescence.

Cell diameter was still positively correlated to crumb texture, but the correlation was weaker than with the non-optimized loaves. Similarly, cell density and relative volume range maintained their negative correlation to crumb texture. Relative volume range had a stronger negative correlation to crumb texture for optimized loaves versus non-optimized loaves. This may indicate that relative volume range is a minor consideration when the data set range increases, but is more important when the data set range decreases. Crumb colour had very high correlation to slice brightness.

4.4.3. Correlations of texture profile analysis parameters with compression force value for non-optimized and optimized loaves.

Texture profile analysis (TPA) software produced evaluations of hardness, cohesiveness, gumminess, chewiness, and resilience. However, the standard AACC method uses compression force value (CFV) to characterize crumb firmness. Figure 4.3 shows a typical TPA curve for a bread containing 2 g/serving of 125 µm pea fibre.

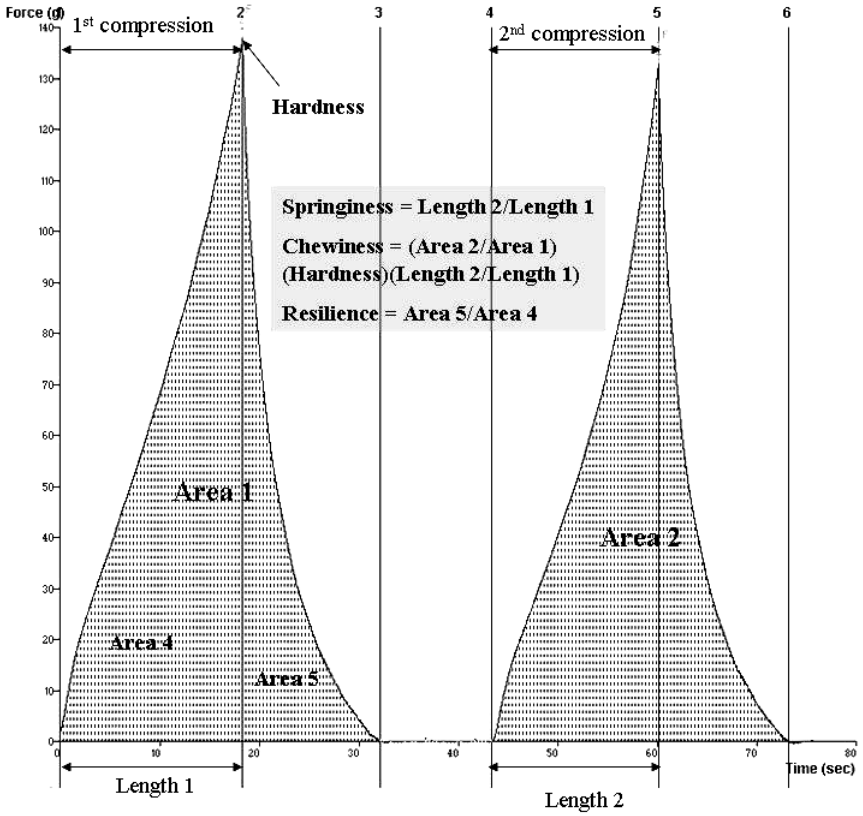


Figure 4.3. Typical TPA curve for a bread containing 2 g/serving of 125 µm pea fibre.

The relationship between the TPA parameters and CFV were assessed through their coefficient correlation values (Table 4.3).

Table 4.3. Correlation coefficient values of texture profile analysis parameters and compression force value for non-optimized and optimized loaves.

	Compression force value	
	Non-optimized loaves	Optimized loaves
Hardness	0.97	0.99
Springiness	0.68	-0.48
Cohesiveness	-0.91	-0.93
Chewiness	0.92	0.91
Resilience	-0.85	-0.86

The hardness value is the peak force of the bread's first compression (Bourne 1982). Hardness had a strong positive correlation with CFV for both non-optimized and optimized loaves. The compression force reading was taken at 25% of the first compression, while the hardness reading was taken at the maximum force of the first compression, so a strong positive relationship between the two readings would be logical.

Chewiness also had a strong positive correlation with CFV for both non-optimized and optimized loaves. A value for chewiness is based on cohesiveness, hardness, and springiness (Bourne 1982). CFV appears to be a strong predictor for crumb chewiness.

Springiness indicates how well the bread slices spring back after the first compression (Bourne 1982). Springiness had a weak positive correlation with CFV for non-optimized loaves, and a weak negative correlation for optimized loaves. CFV does not take into account the force of a second compression, which may explain the weak and inconsistent correlations. The weakness of the correlations indicate that CFV is not a dependable predictor of springiness.

Cohesiveness denotes the bread's ability to withstand a second compression after the first compression. It is measured as the area of work during the second compression divided by the area of the work of the first (Bourne 1982). Cohesiveness had a strong

negative correlation with CFV for both non-optimized and optimized loaves. While CFV does not take into account the force of a second compression, the strength of the correlation suggests that a greater CFV indicates poor cohesiveness. Similarly, resilience had a strong negative correlation with CFV for both non-optimized and optimized loaves. Resilience is the initial springiness, or how well a product regains its original position. It is determined as the area of the work of the first withdrawal of the probe divided by the area of the work of the first compression (Bourne 1982). A greater CFV indicates poor resilience.

The relationship of CFV with cohesiveness and resilience was particularly noticeable for non-optimized breads with high fibre loadings of low particle size (e.g. 6 g/serving of the 90 μm fraction); during the texture profile analysis, sometimes the slices broke due to the impact of the probe. These breads had very high CFVs, but poor cohesiveness and resilience, as was indicated by their fracturing.

The strong positive or negative relationships between CFV and TPA parameters can allow the prediction of bread crumb quality using just the CFV, thereby simplifying the bread quality analysis process.

4.5. Conclusions

For the relationship between structure and texture as determined by Young's modulus and loaf density, there was no evidence of a change in structure due to particle size or fibre content. The linear relationship between Young's modulus and loaf density indicated no effect due to particle size or fibre content on dough stiffness. This was seen in both the non-optimized and optimized loaves.

The correlations for the non-optimized loaves were stronger overall than the optimized loaves, likely due to the greater diversity of properties of the loaves. For all loaves, a positive correlation was found between cell diameter and crumb texture, and negative correlation was found for both cell density and relative volume range with crumb texture. Slice brightness correlated positively with crumb colour score. In the future it may be possible to use C-cell analysis parameters to predict the subjective assessment of crumb quality. Similarly, strong correlations between compression force values and texture profile analysis parameters allow for the potential to predict various indicators of bread crumb quality simply using only the compression force value.

CHAPTER 5: USE OF ULTRASOUND TO MONITOR EFFECT OF PEA FIBRE ADDITION AND PARTICLE SIZE ON DOUGH PROOFING

Abstract

Two ultrasonic parameters were used to characterize changes in dough structure: phase velocity and the attenuation coefficient since these are related to the properties of the dough. The objective was to determine the effect of pea fibre particle size, fibre addition level, and optimization of water absorption on gas cell development during dough proofing by monitoring changes in ultrasonic phase velocity and attenuation. For both the control and fibre-enriched doughs, attenuation increased while phase velocity decreased during the beginning of proofing, demonstrating substantial gas cell expansion. The fibre-enriched dough also had broader peaks in phase velocity than the control, indicating an occurrence of smaller bubbles, which suggests there was a low degree of coalescence. Fibre-enriched doughs with optimized water absorption were shown to be more similar to the control dough than doughs with non-optimized water absorption. More work is needed to improve the reliability of using ultrasonic parameters to discern the effect of pea fibre addition level, fibre particle size, and water addition level.

5.1. Introduction

Gas cells are an important characteristic of bread doughs, as they greatly affect crumb texture and structure (Hoseney 1998). As such, monitoring their development during dough proofing would be of great benefit in determining the changes in structure as the cells expand. Bubbles exhibit resonance at low frequency (i.e. when the

wavelength associated with the frequency is greater than bubble radii) (Leroy and others 2008). As such, the use of low-frequency ultrasound may be useful in monitoring bubbles. Ultrasound is a non-invasive method that is sensitive to the numbers and sizes of gas inclusions within a solid or liquid matrix (Leighton 1997), and thus would be appropriate for such an investigation.

Two ultrasonic parameters are typically used to characterize changes in dough structure: phase velocity and attenuation coefficient. Ultrasonic phase velocity is the velocity at which sound is propagated in the dough. Attenuation coefficient indicates how quickly the sound is absorbed (Mehta 2007).

Phase velocity is related to the longitudinal modulus (β) and the density (ρ) of a material and so is affected by dough rheology. Velocity would be greater when there are fewer air cells present. So as proofing continues and gas cells expand, changes in dough density and dough rheology (as manifest in the longitudinal modulus) would affect velocity. Velocity decreases as the size and number density of the bubbles increases (Elmedhi and others 2003b). It is also affected by the mechanical properties of the dough matrix. Elmehdi and others (2003a) found that as more carbon dioxide was produced by the yeast, the dough pH dropped, changing the intermolecular interactions between the side chains of the gluten polymers, and this altered velocity beyond any density effect.

Attenuation can be monitored by measuring the voltage corresponding to the peak height of a transmitted ultrasonic pulse. It is affected by the air and water content of the dough, which change during proofing, and so there should be a corresponding change in attenuation during this process. The attenuation coefficient is affected by the sum of two

acoustic loss mechanisms: absorption and scattering. As gas cells expand, attenuation increases (Elmehdi and others 2003a).

5.2. Objective

The objective was to determine the effect of pea fibre particle size, fibre addition level, and optimization of water absorption on gas cell development during dough proofing by monitoring changes in ultrasonic phase velocity and attenuation.

5.3. Materials and methods

5.3.1. Materials and dough preparation

Dough samples were prepared as discussed in Chapter 3. In addition to the control dough, the following samples were made with both the non-optimized (non) and optimized (opt) water absorption levels: 250 μm fibre at 2 g/serving (250-2), 250 μm fibre at 6 g/serving (250-6), 90 μm fibre at 2 g/serving (90-2), and 90 μm fibre at 6 g/serving (90-6). These samples were chosen to observe the extremes in particle size and fibre loading that had been previously examined in the baking section of this work.

5.3.2. Dough density measurements

Dough densities were determined using specific gravimetric bottles of 25 ml capacity (Kimble Glass Inc., New Jersey, USA) using 5 g subsamples of dough (Table 5.1). Immediately after mixing, the doughs were covered to protect from moisture loss. From the dough, subsamples were cut with a razor. The gravimetric bottle was cleaned,

dried, filled with distilled water and weighed after every density measurement. The subsample was weighed before being immersed in the water in the bottle. The outside of the bottle was dried before being weighed again. The volume of the dough subsample was determined by calculating the volume of the water displaced from the bottle when the subsample was put inside the bottle. Three subsamples were taken from each dough. Dough density was determined as:

$$\rho_{dough} = m_{dough} / V_{dough}$$

Where ρ_{dough} is the density of the dough (g/cm^3), m_{dough} is the mass of the dough subsample (g), and V_{dough} is the volume of the dough (cm^3):

V_{dough} = [Mass of (bottle + water + dough subsample) – Mass of (bottle + water)] / Density of water at water temperature

Table 5.1. Dough densities of control and fibre-enriched doughs.

Particle size (μm)	Fibre addition level (g/serving)	Water absorption optimization (NON = non-optimized, OPT = optimized)	Average dough density (g/cm^3)
0	0	-	1.031 ± 0.044
250	2	NON	1.029 ± 0.048
250	2	OPT	1.031 ± 0.018
250	6	NON	1.020 ± 0.008
250	6	OPT	1.035 ± 0.007
90	2	NON	1.036 ±0.037
90	2	OPT	1.035 ± 0.008
90	6	NON	1.037 ± 0.005
90	6	OPT	1.029 ± 0.012

5.3.3. Setup of ultrasound apparatus

A Panametrics broadband transducer with a central frequency of 3.5 MHz (Olympus NDT Canada Ltd., Alberta, Canada) was used in a normal incidence wave reflection set-up (Figure 5.1). The transducer was embedded in an acrylic block. The pulse from the transducer traveled along the delay line and was reflected back through the delay line and was detected by the same transducer. The reflected signal was averaged 200 times to improve the signal-to-noise ratio and displayed on a digitizing oscilloscope (Tektronix model TDS 544A, Tektronix Canada Inc., Toronto, Canada). The averaged signal was sent to a computer for later analysis of longitudinal velocity and attenuation.

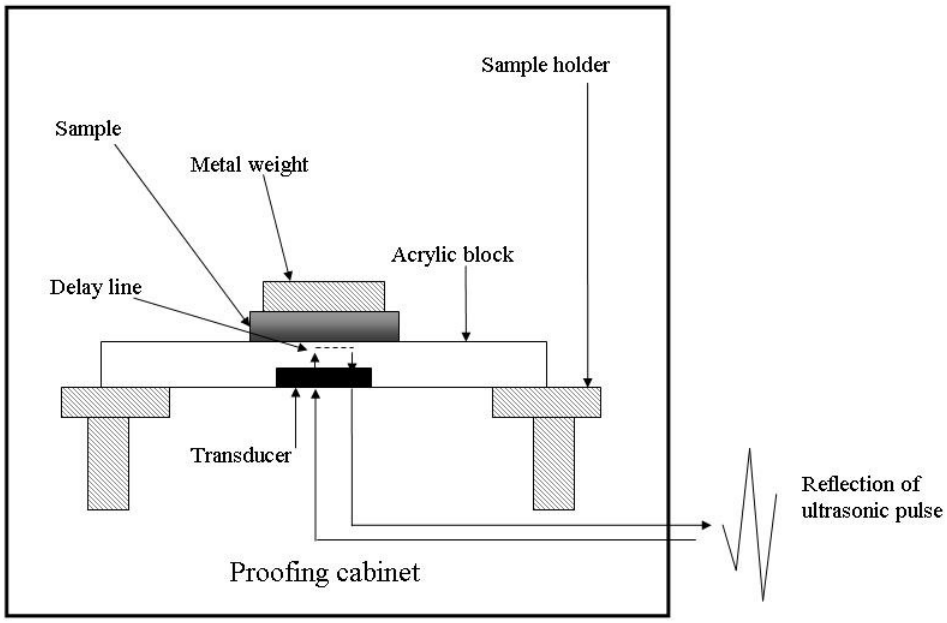


Figure 5.1. Block diagram of set-up used for reflectance of ultrasonic waves through dough.

The acrylic block was placed inside the proofing cabinet at normal proofing conditions (37°C, relative humidity 83%) for at least one hour before the dough was analyzed to let the block acclimatize to the proofing cabinet's temperature. To prepare a dough sample, a very sharp razor greased with mineral oil was used to cut a disc

approximately 9 mm thick from the center of the dough immediately after it was sheeted and moulded into a cylinder. The cut surface of the dough was greased lightly with mineral oil and placed greased side down on top of the acrylic block. A metal weight was placed on top of the dough to improve contact of the dough piece with the block.

5.3.4. Calculations of phase velocity and attenuation

The ratio *x* of the Fourier transform of the reference signal (*FT_ref*) and the sample signal (*FT_sam*) is measured to determine the sample impedance *Z* and the reference impedance Z_0:

$$x = FT\_sam / FT\_ref = - (Z - Z_0) / (Z + Z_0)$$

A reference signal was determined by the reflection of the ultrasonic pulse from an air interface with no sample on the acrylic block. The above equation can be rewritten to determine Z:

$$Z = Z_0\{(1 - x) / (1 + x)\}$$

Since impedance is a function of phase velocity ν and attenuation α, then:

$$1 / Z = (1 / \rho\nu) + i(\alpha / 2\omega\rho) = Re + Im$$

Therefore attenuation α and phase velocity ν are calculated as:

$$\alpha = (2\omega\rho)(Im[1/Z])$$

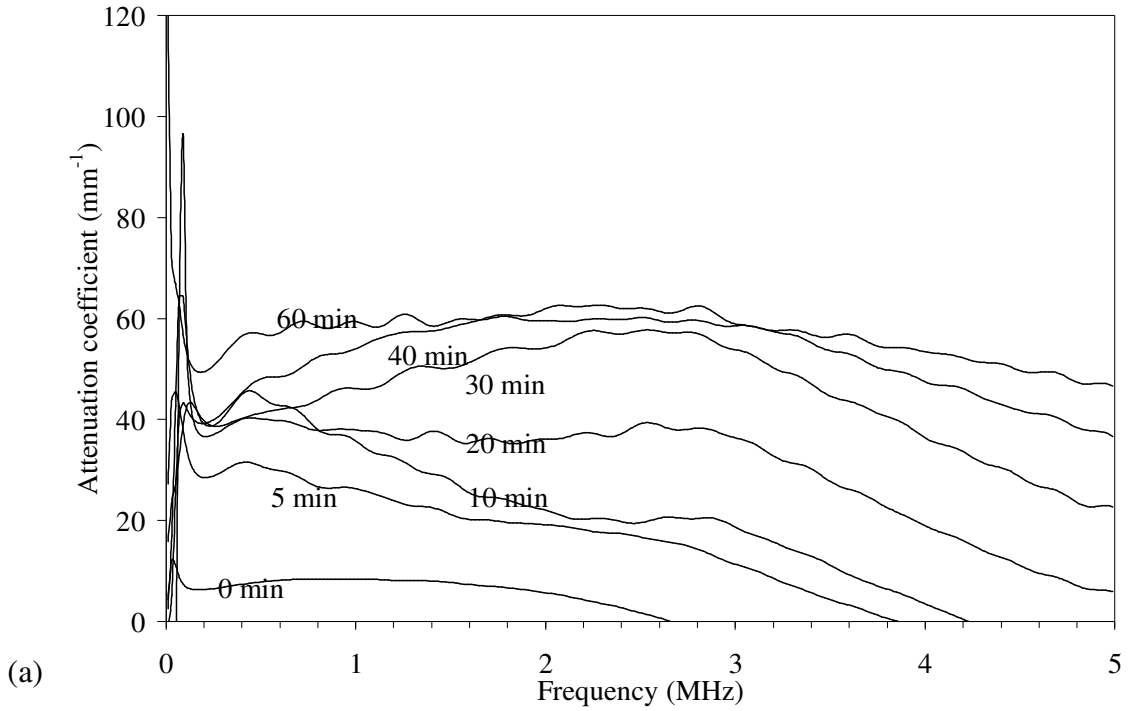
$$\nu = 1/(\rho Re[1/Z])$$

Where ρ is density of the sample, and ω is the angular frequency of the ultrasonic signal (Leroy and others 2010, Strybulevych 2011).

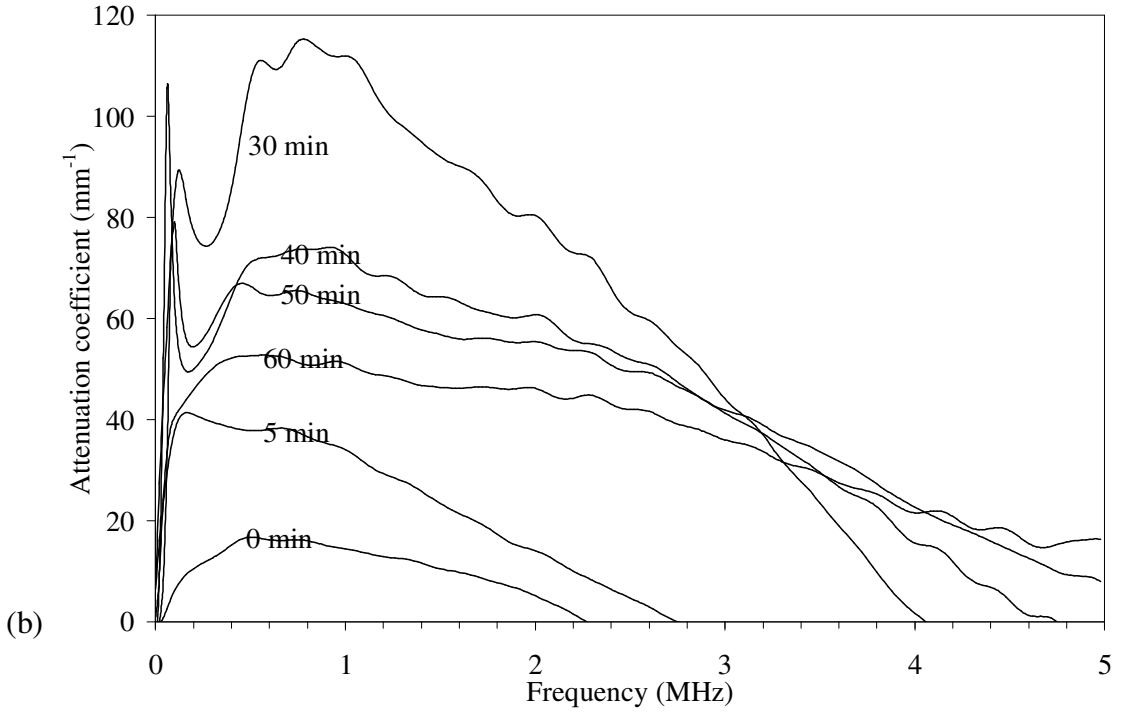
5.4. Results and discussion

5.4.1. Changes in attenuation during dough proofing

Figure 5.2 shows the changes in attenuation during dough proofing of the control dough and dough containing 6 g/serving of the 90 μm fraction (90-6) with optimized water absorption. For both types of doughs, attenuation increased steadily during the first 30 minutes of proofing, likely demonstrating attenuation increases due to substantial gas cell expansion. Higher attenuation is an indication of greater scattering of the ultrasonic waves due to a greater proportion of void fraction in the dough (Elmehdi and others 2003a, Leroy and others 2008). While attenuation peaked at around 50 mm^{-1} for the control, it peaked at approximately double that for the fibre-enriched dough. Similarly, all fibre-enriched doughs had maximum attenuations greater than the control.



(a)



(b)

Figure 5.2. Effect of frequency on the attenuation coefficient of dough for changes in proofing time: (a) Control dough, (b) Dough made with 6 g/serving of 90 μm fraction with optimized water absorption.

After 30 minutes, the attenuation became somewhat constant for the control, indicating that the void fraction remained essentially constant for the remainder of the proofing time. However, attenuation began to decrease for the 90-6 dough after 30 minutes. This may indicate a decrease in void fraction. Or, if the void fraction held constant, there may have been an increase in median radius, resulting in a decrease in maximum attenuation (Leroy and others 2008). Regardless, by the end of proofing, the 90-6 dough had an attenuation value similar to that of the control, which suggests that at this point the void fraction of both doughs were similar (Mehta and others 2009). This was reflected in the proof height of the doughs being approximately the same, which was measured just before the doughs were put in the oven.

The benefit of monitoring gas cell development in dough is that the gas cells play a pivotal role in the cellular structure of the baked bread (Scanlon and others 2008). In the end, the specific loaf volume of the control ended up being about double that of the 90-6 loaf. If the void fraction at the end of proofing was similar for the control and the 90-6 dough as indicated by attenuation, the substantial difference in specific loaf volume suggests that the control had much greater oven spring than the fibre-enriched bread, expanding dramatically during baking. Similar behaviour in oven spring was seen for all fibre-enriched doughs, where the proof heights were similar to the control, but specific loaf volume was less. This reflected previous work which showed poor correlation between expansion during proofing and baked loaf volume for doughs containing wheat bran, suggesting that the negative effect of the bran occurred during baking (Campbell and others 2008).

Perhaps it would be possible to use ultrasound to predict oven spring. The steady increase and eventual plateau of the attenuation coefficient of the control was potentially an indicator of substantial oven spring. In comparison, the large increase and then decrease in attenuation of the 90-6 dough resulted in poor oven spring. The steady change in the control attenuation may be indicative of a more stable dough matrix, whereas the inclusion of pea fibre resulted in disruption of the dough matrix (Seguchi and others 2007), which was reflected in dramatic changes in attenuation. More work would be needed to explore the possibility of using ultrasound as a predictor of dough behaviour during baking.

Peaks in attenuation and phase velocity are due to the bubbles responding in phase opposition to the incoming wave (Leroy and others 2008). The shape and position of peak phase velocity and attenuation as a function of frequency is an indication of bubble size distribution (Commander and Prosperetti 1989). The control dough had a broader peak in attenuation than the 90-6 dough. As well, the peak attenuation of the control dough occurred at a higher frequency (about 2.53 MHz) than the 90-6 dough, which occurred at about 0.91 MHz. The difference in peak shape and broadness demonstrates that the inclusion of pea fibre to the dough has an effect on ultrasonic attenuation that may reflect changes in the distribution of bubbles. The lower peak frequency seen in 90-6 dough is indicative of an increasing median radius (Leroy and others 2008). However, the eventual average cell diameter seen in the 90-6 bread crumb was smaller than the control; the greater oven spring of the control may have been responsible for the greater crumb cell size.

5.4.2. Changes in phase velocity during dough proofing

For the control dough, an initial decrease in phase velocity was seen for the first 10 minutes; after this period the phase velocity steadily increased (Figure 5.3). In the 90-6 dough, phase velocity decreased for the first 20 minutes and then steadily increased at essentially all frequencies. A similar pattern was seen for all other doughs analyzed that contained fibre. It would be expected for phase velocity to decrease as proofing continues due to gas cell expansion (Elmehdi and others 2003a), but the reason for the increase in phase velocity is unclear. It is possible that as proofing continued, the degree of contact between the dough and acrylic block changed due to the placement of the metal weight on top of the dough. As the doughs rose, their structure became less dense – but also more delicate. The metal weight might have squashed the dough, essentially decreasing the void fraction. This was observed at the end of proofing when the samples were removed; the doughs had grown outwards from underneath the metal weight and were no longer as thick as when they were initially placed on the acrylic block.

In the control dough, peaks appear to shift left (i.e. decrease in frequency) as proofing continues, which indicates an increase in median radius (Leroy and others 2008). There is also the emergence of an additional peak at a higher frequency (~3.2 MHz) that shifts left, which may have been indicative of an increase in median radius of cells that are smaller than the peaks that occur at a lower frequency (Leroy and others 2008). There are also two peaks in phase velocity of the 90-6 dough (one at ~1.2 MHz, another at ~3.3 MHz), which could be indicative of two bubble sizes like in the control dough. Another possibility is that the higher frequency could be indicative of the pea fibre, as solid materials resonate at a higher frequency than gas bubbles (Strybulevych

and other 2007). The shift in peak velocity is less obvious in the 90-6 dough, suggesting that gas cell expansion was less dramatic for the fibre-enriched dough than the control. From the baking experiments of this project, it was determined using C-cell analysis that the addition of pea fibre did result in smaller average cell diameter than the control (see 3.4.3.5.1). The difference in peak phase velocity between the control and 90-6 dough appears to support this conclusion.

For a given void fraction, a lower mean radius results in a broader peak in phase velocity (Leroy and others 2008). The 90-6 dough resulted in broader peaks than the control, perhaps indicating an occurrence of smaller bubbles. As well, the phase velocity of the 90-6 dough was overall less than that of the control, indicating a greater density of bubbles (Elmehdi and others 2003a). This again reflects the conclusion drawn from the baking experiments, where it was seen that fibre inhibited gas cell expansion.

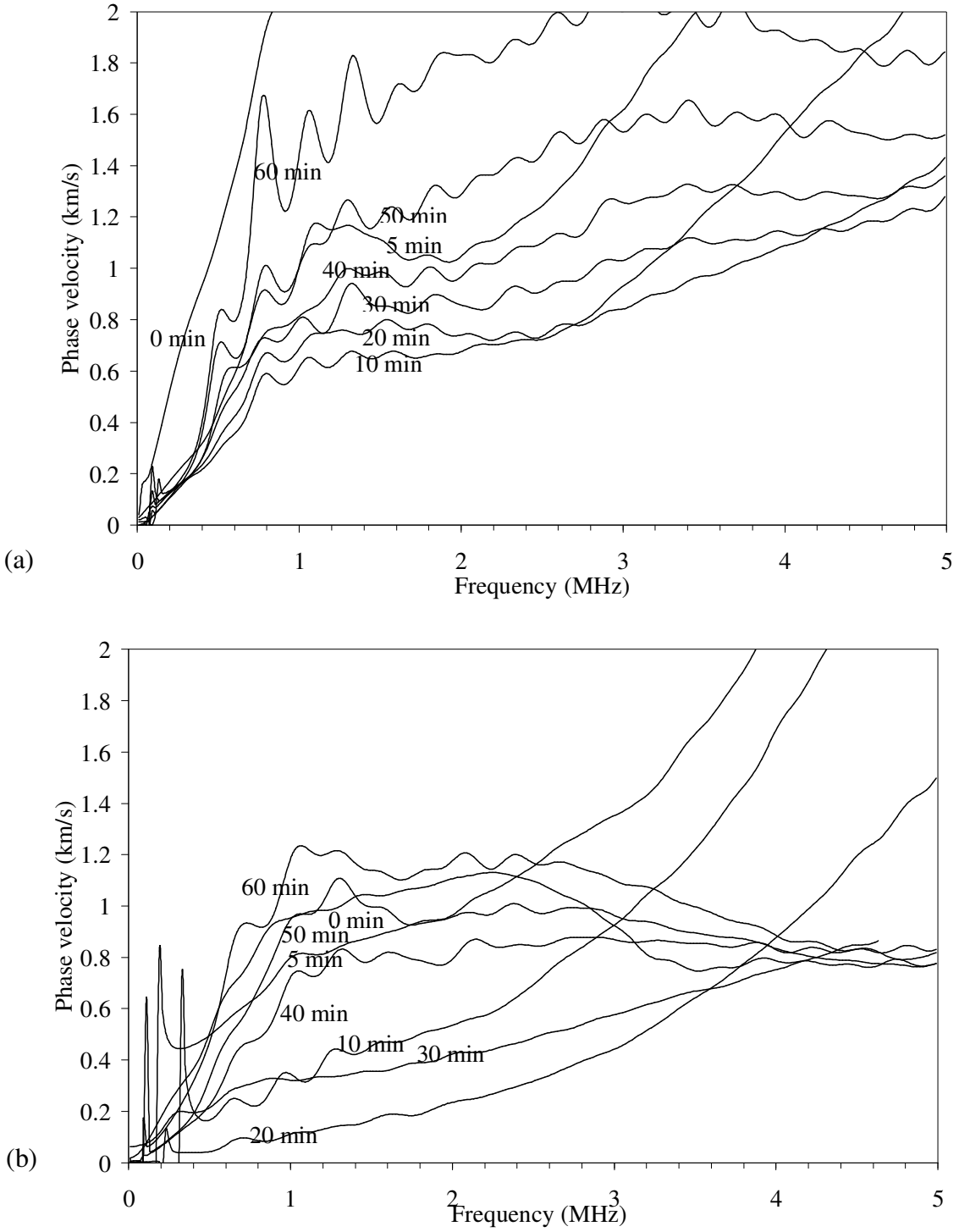


Figure 5.3. Effect of frequency on the phase velocity of dough for different proofing times: (a) Control, (b) 6 g/serving of the 90 μm fraction with optimized water absorption.

5.4.3. Changes in attenuation during proofing at various frequencies

The attenuation coefficient of the different doughs is similar at frequencies 0.5, 1.0 and 2.0 MHz. However at 4.0 MHz, almost all the attenuations of the fibre-enriched doughs are less than that of the control.

As proofing time increases, there should be an increase in void fraction, which would be reflected in an increase in attenuation (Skaf and others 2009). This is more clearly seen at 2.0 MHz (Figure 5.4c) particularly for the control, a frequency at which bubble detection is more sensitive (Elmehdi and others 2003a). A substantial increase in attenuation can be seen for all the fibre-enriched doughs for up to 30 minutes, but then attenuation begins to decrease. The 90-6 dough made with optimized water absorption has a particularly odd attenuation curve, which may have been due to poor contact with the acrylic block; even though it had greater water than the non-optimized 90-6 dough, it still was relatively dry compared to the control and doughs containing the 250 μm fibre fraction.

At lower frequencies (0.5 and 1.0 MHz), a peak in attenuation is seen at 10 minutes, whereas the peak comes at 30 minutes for the higher frequencies. This may have to do with different frequencies being more sensitive to different bubble sizes (Leroy and others 2008). Since lower frequencies are more sensitive to larger bubbles (Leroy and others 2008), a peak attenuation at 10 minutes suggests that the bubble size was greater at the beginning, and then decreased by 30 minutes as indicated by the peak attenuation at higher frequencies. This is puzzling as bubble size should increase during proofing, but perhaps the effect of the metal weight squishing the dough sample to a greater extent as proofing continued resulted in a bubble size decrease.

It is difficult to discern a clear pattern from the attenuation behaviour during proofing due to the effect of pea fibre particle size, as both the 250 and 90 μm fraction had attenuation curves above and below the control curve. The effect of water optimization is undetermined, as both non-optimized and optimized doughs had attenuation curves above and below the control. Overall, there is no clear trend in attenuation behaviour during proofing due to the pea fibre addition and particle size and water optimization level.

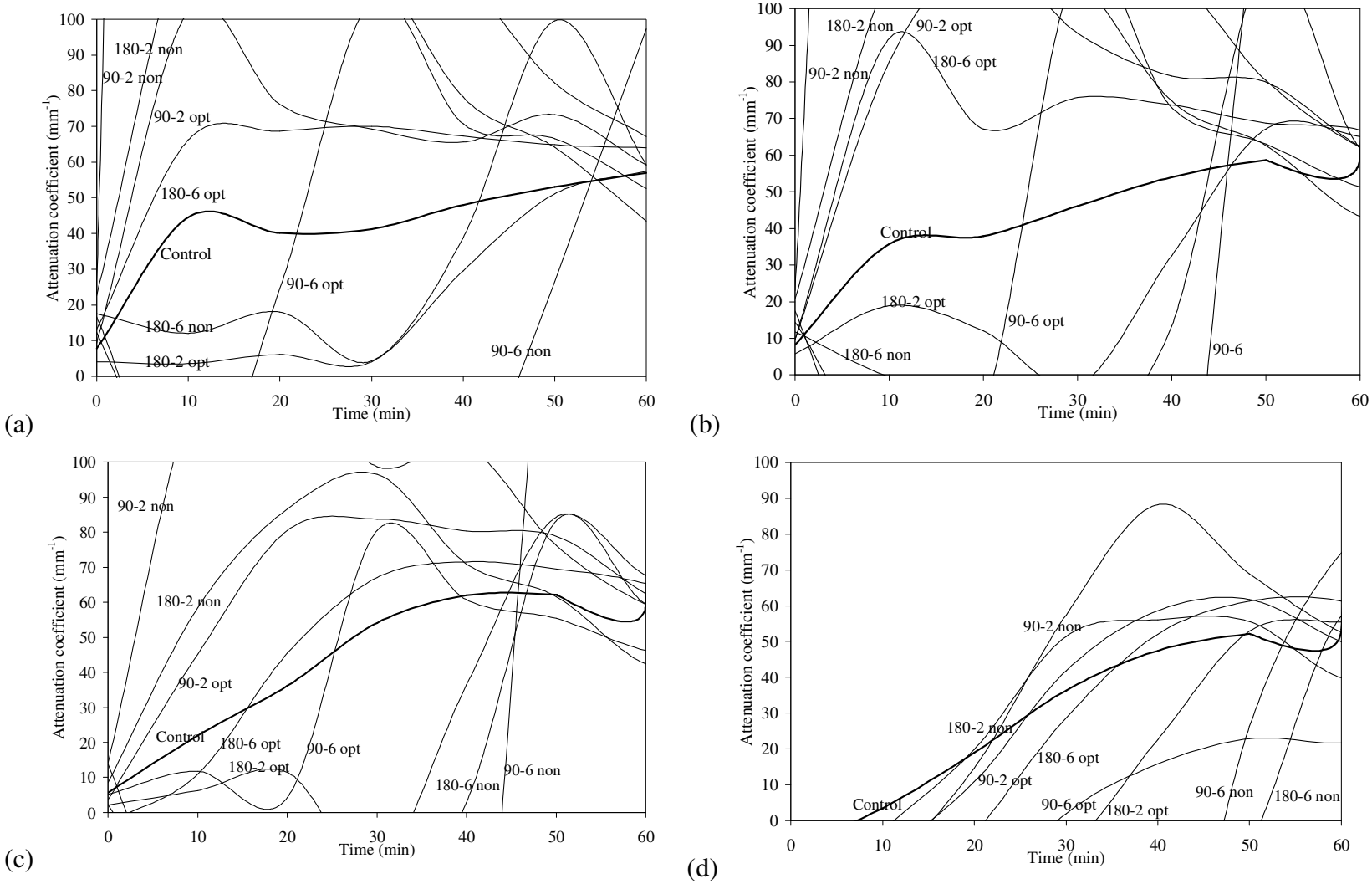


Figure 5.4. Change in attenuation coefficient during proofing of various doughs at different frequencies: (a) 0.5 MHz, (b) 1.0 MHz, (c) 2.0 MHz, and (d) 4.0 MHz.

5.4.4. Changes in phase velocity during proofing at various frequencies

The phase velocity of dough should decrease during proofing due to the increase in total void fraction (Elmehdi and others 2003a, Lee and others 2004). While this was seen for the first 20-30 minutes, phase velocity eventually increased (Figure 5.5). This would suggest the loss of bubbles during the latter half of proofing, resulting in decreasing dough density or the emergence of resonances due to differences in bubble sizes that affect ultrasonic velocity. It has also been suggested that changes in the elasticity of the dough matrix may also affect velocity (Elmehdi and others 2001, Lee and others 2004, Scanlon and others 2002). However, due to the inconsistency of the attenuation curves during proofing, it is not clear that reliable conclusions can be drawn from changes in phase velocity due to pea fibre addition, pea particle size and water optimization level.

It should be noted that the dough containing 6 g/serving of the 90 μm fraction was quite dry and therefore likely had poor contact with the transducer, which would affect the reflectance of the ultrasonic wave. Moreover, the densities of the doughs decreased as proofing continued, but calculations to determine attenuation and phase velocity were based on the initial density at the beginning of the proofing period. As such, more work is needed to optimize data acquisition so that ultrasound can be used to characterize changes in proofing doughs due to fibre addition.

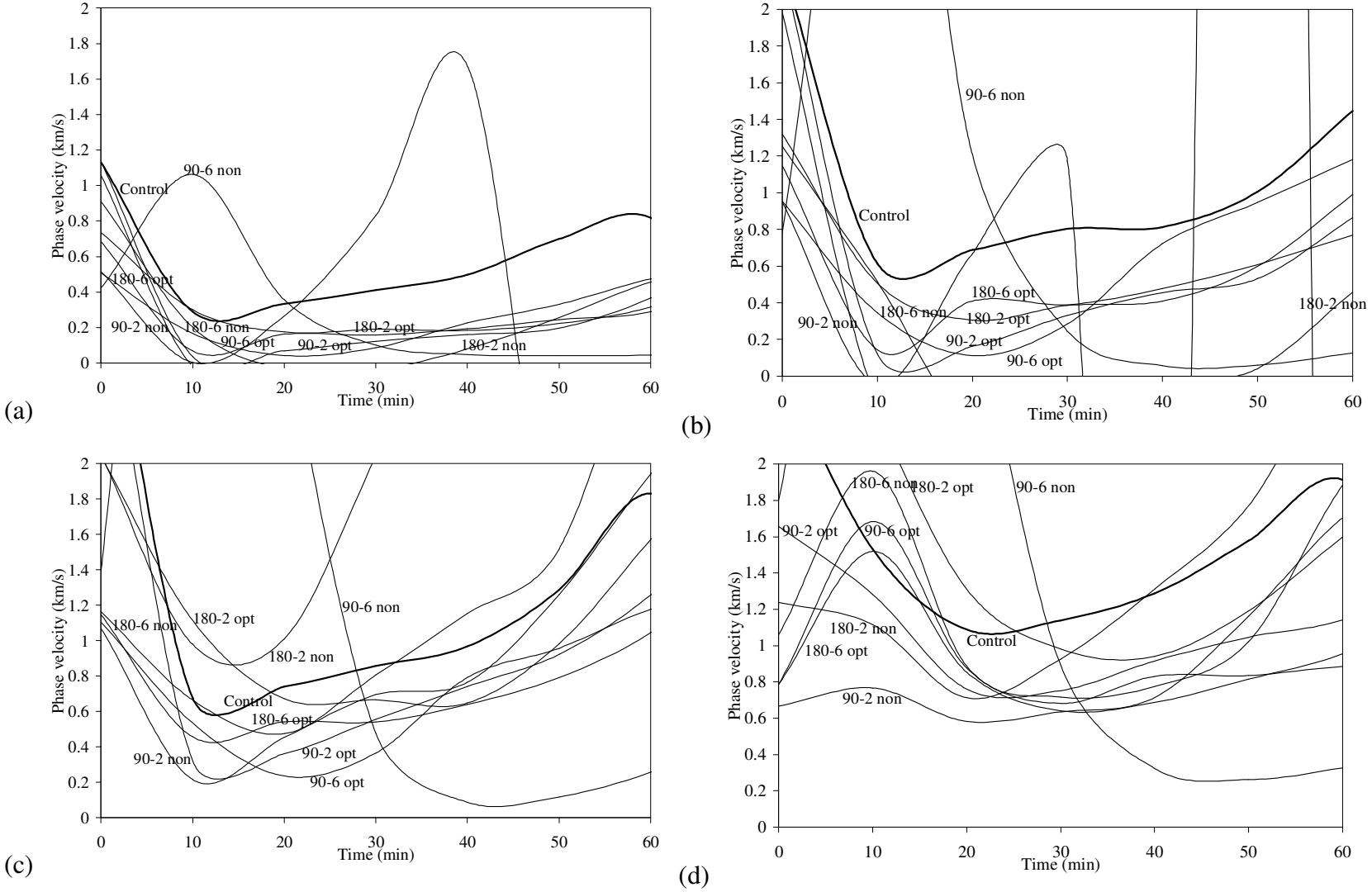


Figure 5.5. Change in phase velocity during proofing of various doughs at different frequencies: (a) 0.5 MHz, (b) 1.0 MHz, (c) 2.0 MHz, and (d) 4.0 MHz.

5.4.5. Absolute changes in attenuation and phase velocity during proofing between control dough and fibre-enriched doughs

By determining the absolute difference between the attenuation of the fibre-enriched doughs and the control dough, it was easier to see whether the ultrasonic technique could differentiate a difference between the dough treatments in terms of: non-optimized versus optimized water absorption, fibre addition level (2 versus 6 g/serving), and fibre particle size (250 versus 90 μm fraction). The change in attenuation coefficient was determined by:

$$\Delta\,\alpha = \Sigma_{(\text{all frequencies})} \left| \alpha_{(\text{fibre-enriched})} - \alpha_{(\text{control})} \right|$$

While the changes in attenuation do not demonstrate an obvious effect due to fibre addition level or particle size (Figure 5.6), it is clear that all the optimized doughs had attenuation coefficients that were more similar to the control than the non-optimized doughs. As such, the similarity in gas bubble expansion due to adequate water addition between the fibre-enriched doughs and the control is seen in the change in attenuation. This similarity is reflected in the quality of the baked fibre-enriched loaves being similar to that of the control bread (3.4.3).

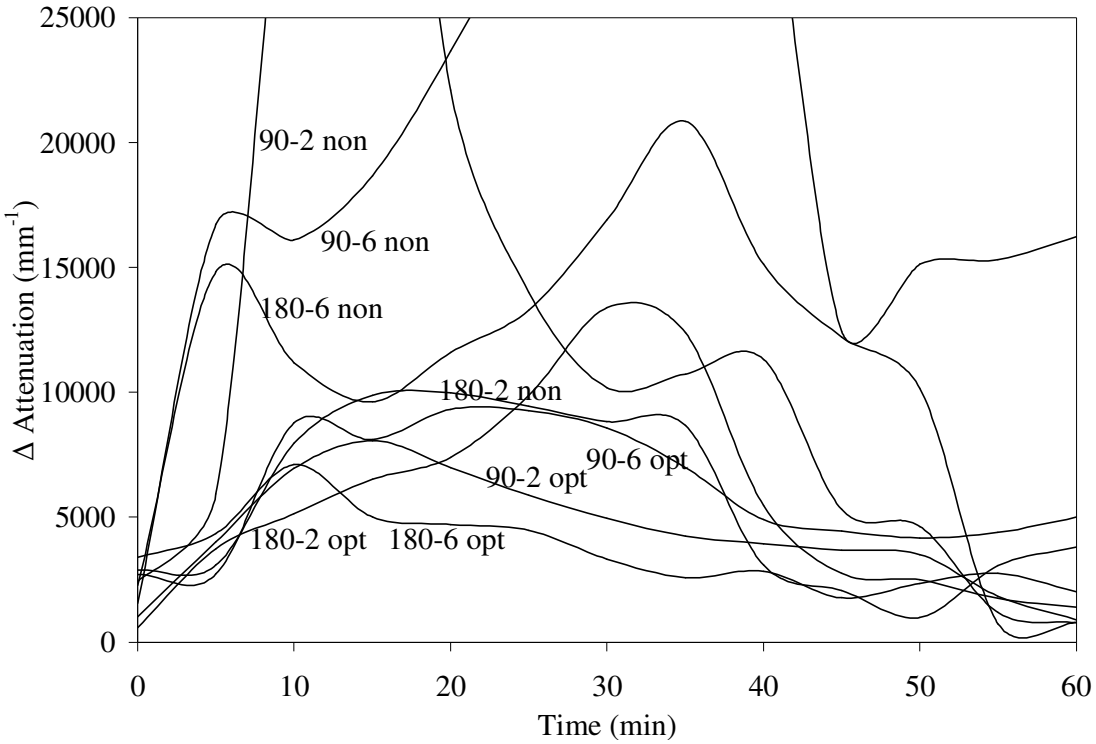


Figure 5.6. Change in attenuation coefficient during proofing of various doughs.

Likewise, the difference in phase velocity between the fibre-enriched doughs and the control dough was determined by:

$$\Delta v = \Sigma_{(\text{all frequencies})} \left| v_{(\text{fibre-enriched})} - v_{(\text{control})} \right|$$

While the changes in phase velocity do not demonstrate an obvious effect due to fibre addition level or particle size (Figure 5.7), it is clear that all the optimized doughs had phase velocities that were more similar to the control than the non-optimized doughs. As such, the similarity in gas bubble expansion due to adequate water addition between the fibre-enriched doughs and the control is seen in the change in phase velocity as well as the change in attenuation.

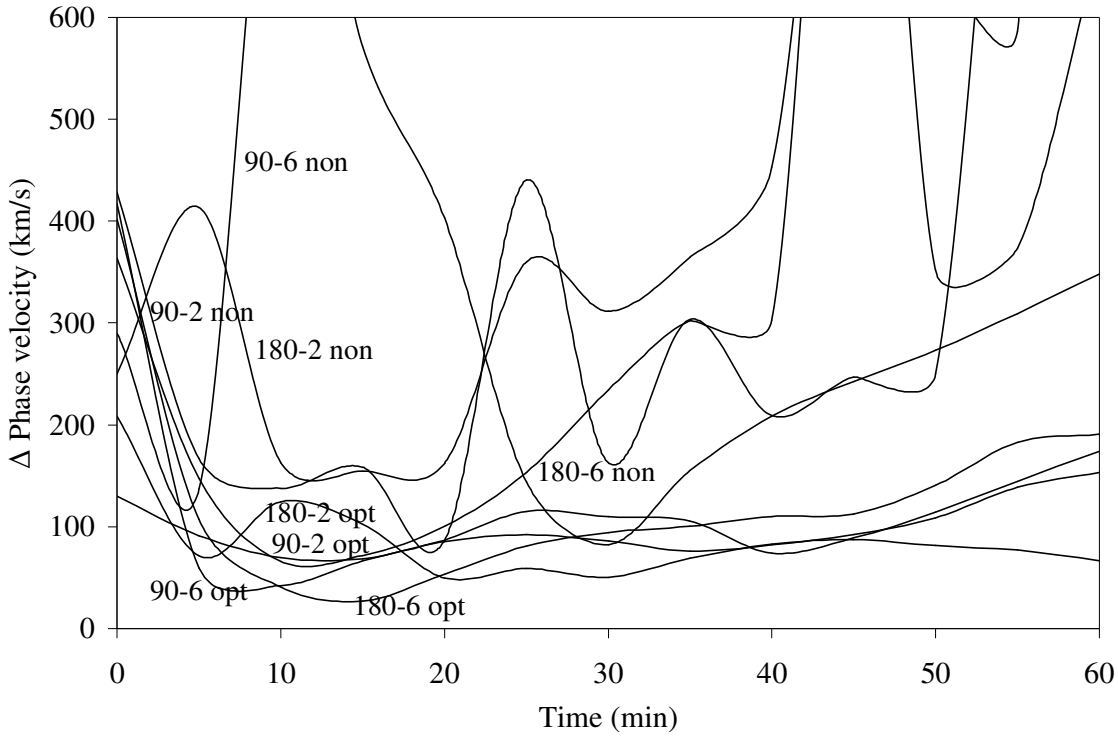


Figure 5.7. Change in phase velocity during proofing of various doughs.

5.5. Conclusions

The use of ultrasound to observe bubble development and size distribution in bread doughs has shown promise as a way of monitoring dough proofing. For both the control dough and dough enriched with 6/serving of 90 μm pea fibre, attenuation increased steadily during the first 30 minutes of proofing, demonstrating substantial gas cell expansion. Both of these doughs had similar attenuation coefficient values at the end of proofing, perhaps suggesting similar void fractions. In both the control and fibre-enriched dough, the peak in phase velocity shifted to a lower frequency during proofing, indicating an increase in median bubble radius. The behaviour of the fibre-enriched dough appeared to support a previous conclusion made from baking experiments that the addition of pea fibre did not promote gas coalescence. The change in attenuation and phase velocity between the fibre-enriched doughs and the control dough demonstrated

that the doughs made with optimized water absorption levels were more similar to the control than the non-optimized doughs in terms of time-dependent changes. More work is needed in finessing how this sort of experiment is conducted to produce reliable results.

CHAPTER 6: ANTIOXIDANT PROPERTIES OF PEA HULL FIBRE IN WHEAT BREAD

Abstract

Yellow field peas are known to contain antioxidants, which have been shown to promote health by fighting against cellular damage caused by free radicals. This study compared the antioxidant capacity of yellow pea hull fibre of different particle sizes (250, 125, 180, 90 μm) with wheat flour using ascorbic acid as the control standard. In addition, a comparison between wheat bread and pea fibre-enriched bread (250 μm pea fibre, 6 g/serving) was performed. The goal was to see whether the antioxidant capacity of bread could be improved by augmenting the natural antioxidants in wheat flour with those in pea fibre. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was used to measure antioxidant activity. Scavenging activity was reported as a function of time up to 30 minutes. All particle sizes of pea fibres had substantially greater antioxidant scavenging ability compared to wheat flour, but there was minimal difference in activity between the different particle sizes. The DPPH scavenging activity of pea fibre approached that of ascorbic acid by the end of the assay. Fibre-enriched bread had significantly higher antioxidant activity compared to that of the control bread. As such, pea hull fibre has a number of benefits for bakers seeking to improve the nutrient profile of their products.

6.1. Introduction

6.1.1. Antioxidant activity in whole legumes and wheat flour

Yellow peas are a rich nutrient source. Besides containing fibre, protein, vitamins, and minerals, their seed coats have a significant amount of polyphenols (Wang and others 2003). As such, ingredients derived from pea hulls are a source of both dietary fibre and polyphenols. Both dietary fibre and antioxidant consumption have been associated with preventing chronic diseases such as cardiovascular disease, cancer, obesity, and diabetes (Agboola and others 2010, Saura-Calixto and others 2009).

Besides health benefits, antioxidants in pea fibre have functional benefit. The demand for natural antioxidants like ascorbic acid and beta-carotene is increasing, as some synthetic antioxidants have been shown to be toxigenic, mutagenic, and carcinogenic (Nanditha and Prabhasankar 2009). Therefore, there is the potential for using plant fibre as a clearer label alternative to synthetic antioxidants (Jimenez-Escrig and others 2001, Mildner-Szkudlar and others 2011, Vergara-Valencia and others 2007).

Bread and wheat flour have also been shown to contain antioxidants, most of which originate from the outer parts of the wheat grain, the bran and the germ (Liyana-Pathirana and Shahidi 2007, Van Hung and others 2009). Canadian wheat red spring flour has less than half the DPPH scavenging activity of whole grain (Liyana-Pathirana and Shahidi 2007). As a consequence, whole wheat bread has over two times the total phenolic compounds content of white bread (Gélinas and McKinnon 2006). As such, the addition of pea fibre could potentially boost the antioxidant activity of white bread to elevate the activity to levels found in whole wheat bread.

6.1.2. Effect of processing on antioxidant activity

During processing, the extent of antioxidant activity can change. Some studies have shown that the smaller the particle size, the greater the yield and antioxidant activity of the extract (Gião and others 2009, Qu and others 2010). Smaller particle size has been shown to accelerate antioxidant degradation in wheat bran and germ (Galliard 1986). It follows that there may also be differences in antioxidant activity in pea fibres of different particle size. Moreover, baking processing steps tend to lower antioxidant activity, especially natural sources of antioxidants (Mahon and Chapman 1953, Nanditha and Prabhasankar 2009, Nicoli and others 1999).

6.1.3. Antioxidant extraction methods

Several types of antioxidants exist, such as phenolic and flavonoid compounds (Boateng and others 2008). Different extraction solvents can lead to differences in DPPH scavenging activity, as different antioxidants are extracted by differences in the polarity of the solvent used (Xu and Chang 2007). Various extraction methods can release different antioxidants, as phenolic compounds exist in both free and bound form. A simple solvent extraction only releases the soluble free phenols, but releasing bound phenols requires hydrolysis (Han and Baik 2008, Oboh and others 2009). Since this study was just a starting point for assessing the free antioxidant activity of pea fibre, a simple solvent extraction was used.

6.1.4. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was first published by Blois (1958). DPPH is a stable free radical that can accept an electron or hydrogen radical to achieve a reduced form. In its free radical state, DPPH absorbs strongly at 517 nm and has a deep violet colour. When an antioxidant is present, it reduces the DPPH free radical. The reduced form has no colour and no absorption. The quenching of the free radical is usually performed in ethanol or methanol, neither of which interferes with the reaction. DPPH scavenging ability is the ability of an antioxidant to reduce DPPH free radicals, and has been reported as the change in absorbance at 517 nm (A): $\{[A_{517\,nm})_c - (A_{517\,nm})_s]/(A_{517\,nm})_c\} \times 100$, where subscripts $_c$ and $_s$ represented blank control and sample, respectively (Pownall and others 2010).

The rate of reaction between antioxidant and DPPH can vary, such that reaching a constant absorbance value can be achieved in less than 5 min, or over an hour, depending on the substrate. The reaction is deemed finished when the absorbance no longer changes (Brand-Williams and others 1995, Sharma and Bhat 2009). The original method first published by Blois (1958) recommended a reaction time of 30 min, while more recently it has been suggested that the change in scavenging activity should be observed over time (Sánchez-Moreno and others 1998, Sharma and Bhat 2009).

In addition, there are several factors that have been identified that affect the reaction's outcome, such as sample concentration, pH buffering by the organic solvent, and solvent type (Agboola and others 2010, Sharma and Bhat 2009, Udenigwe and others 2009).

Ascorbic acid is a natural antioxidant already used in prolonging the shelf life of bread and bakery products (Madhavi and others 1996, Nanditha and Prabhasankar 2009, Park and others 1994). It has also been used as a standard in confirming the efficacy of the DPPH assay (Brand-Williams and others 1995, Sánchez-Moreno and others 1998). As such, it was chosen as the control antioxidant for assessing the free-radical quenching efficacy of pea fibre in these experiments.

Although there is no standard DPPH assay method, it is still asserted to be simple, accurate, and highly reproducible (Sánchez-Moreno 2002). While previous research evaluating antioxidant capacity in wheat breads was done using enzyme assays with lipoxygenase and lipase (Galliard 1986), we chose the DPPH assay because of its simplicity and accuracy for analyzing the antioxidant capabilities of pea fibre (Sánchez-Moreno 2002).

6.2. Objective

The objective of this research was to determine the DPPH free radical scavenging abilities of pea fibres of different particle sizes, and to compare these to the DPPH activity of wheat flour. The scavenging ability of pea fibre-enriched bread was also compared to a control wheat bread to determine if further processing steps altered scavenging potential and so to ascertain whether fibre enriched bread had improved scavenging ability over wheat bread.

6.3. Materials and Methods

6.3.1. Materials

Extraction of polyphenols was performed on wheat flour, pea fibres of various particle size (250, 125, 180, and 90 μm), control bread, and bread baked with pea fibre of 250 μm particle size at 6 g/serving. All materials and the baking method were the same as those used in the baking experiments discussed in Chapter 2.

6.3.2. Antioxidant extraction method

Extraction of polyphenols was performed according to Agboola and others (2010). This was a simple extraction method in which only free antioxidants were removed from the raw material; no hydrolysis was done to liberate bound antioxidants. The wheat flour and pea fibres required no additional preparation prior to extraction.

The breads were baked and extraction was performed the next day. Bread crumbs were made by gently pulling apart the bread with tweezers. Wheat flour, pea fibre, or bread crumbs were added to 80% methanol at a 1:10 ratio and stirred for one hour. The mixture was then centrifuged at 10,000g for 20 min. The supernatant was decanted and the methanol was evaporated by blowing nitrogen over the samples. The concentrate was freeze-dried. The freeze-dried material was used in the DPPH assay.

6.3.3. DPPH assay

The DPPH assay was carried out according to Pownall and others (2010). Wheat flour, pea fibre, or freeze-dried extracts from the bread (control and pea fibre enriched)

were dissolved in 0.1 M sodium phosphate buffer, pH 7.0, containing 1% (w/v) Triton X-100 to specific concentrations depending on the final concentration evaluated. DPPH was dissolved in methanol to a final concentration of 100 μM. A blank control consisting of a mixture of DPPH in methanol (100 μL) and sodium phosphate buffer (100 μL) was used. Absorbance was read on a Cary 50 Microplate Reader (Varian Inc., Canada).

Ascorbic acid was used as the standard due to its quick reaction time with DPPH (less than 5 min), which appeared to match the reaction times of pea fibre and wheat flour (Brand-Williams and others 1995, Sharma and Bhat 2009).

To evaluate the effect of extract concentration, three final sample concentrations of wheat flour and pea fibre (125 μm) were used: 0.5, 0.25, and 0.125 mg/ml. The same ascorbic acid concentration was matched to the sample concentration. To evaluate the effect of particle size and the difference in antioxidant activity between pea fibre and wheat flour, four pea fibre particle sizes (250, 125, 180, 90 μm) and wheat flour were used at 0.25 mg/ml final concentration. To evaluate the difference in antioxidant activity in breads, control bread and bread with 250 μm fibre (6 g/serving) were analyzed at 0.25 mg/ml.

Dissolved extracts (100 μL) were mixed with 100 μL of DPPH solution and absorbance was read at 517 nm. To consider both methods of observing the absorbance at the end of 30 min as well as noting change in absorbance over time, absorbance was read every 2 min up to 10 min, and then every 5 min up to 30 min.

6.4. Results and discussion

6.4.1. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity (%) was determined as $\{[A_{517\ nm})_c - (A_{517\ nm})_s]/(A_{517\ nm})_c\} \times 100$, where subscripts $_c$ and $_s$ represented blank control and sample, respectively. This method of reporting antioxidant efficacy has been used by a number of research teams (Jao and Ko (2002); Kanatt and others (2007); Li and others (2008); Liyana-Pathirana and Shahidi (2007)).

6.4.2. Effect of sample concentration on DPPH scavenging activity

The effect of sample concentration on DPPH scavenging activity is shown in Figure 6.1 which demonstrates the change in scavenging activity for ascorbic acid of varying concentrations (1.0, 0.5, 0.25, 0.125 mg/ml). Overlapping scavenging activity was evident for the first four minutes, but this may have been due to air bubbles in the microplate wells.

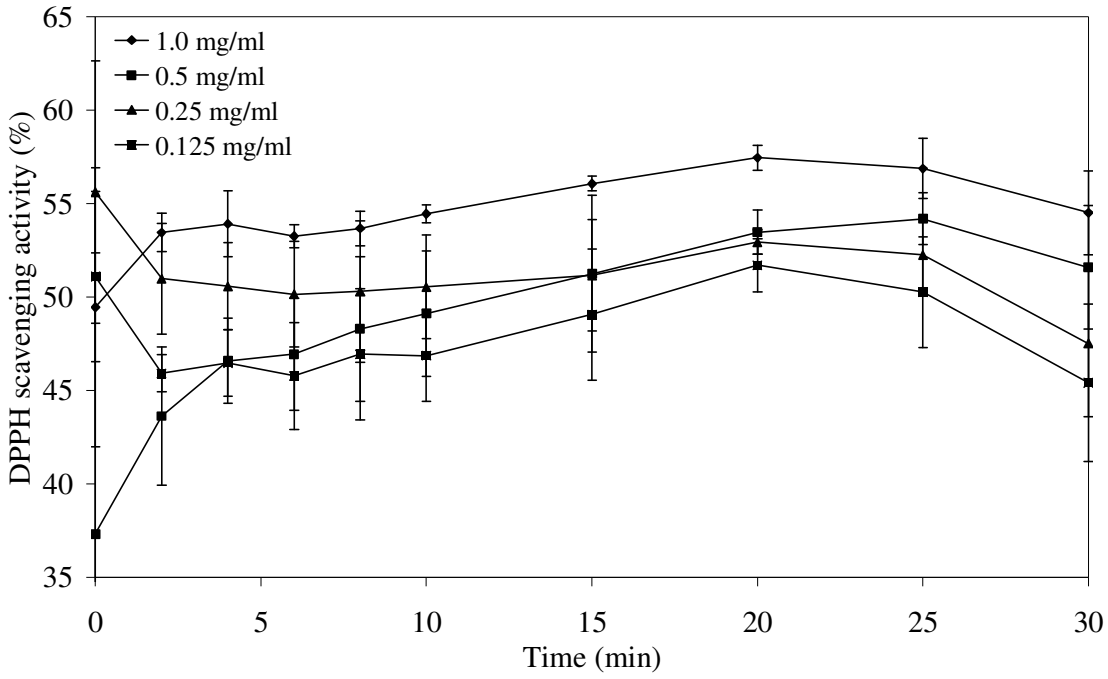


Figure 6.1. DPPH scavenging activity of ascorbic acid at various concentrations.

Similarly, scavenging activity decreased with decreasing sample concentration of pea fibre (125 μm) and wheat flour (Figure 6.2). Pea fibre scavenging activity decreased by almost half from 50% to 30% when concentration was halved from 0.5 to 0.25 mg/ml. Less change was noticed between pea fibre at 0.25 and 0.125 mg/ml. As such, the effect of sample concentration is certainly a factor in conducting the DPPH assay; this must be considered when comparing results to other sources.

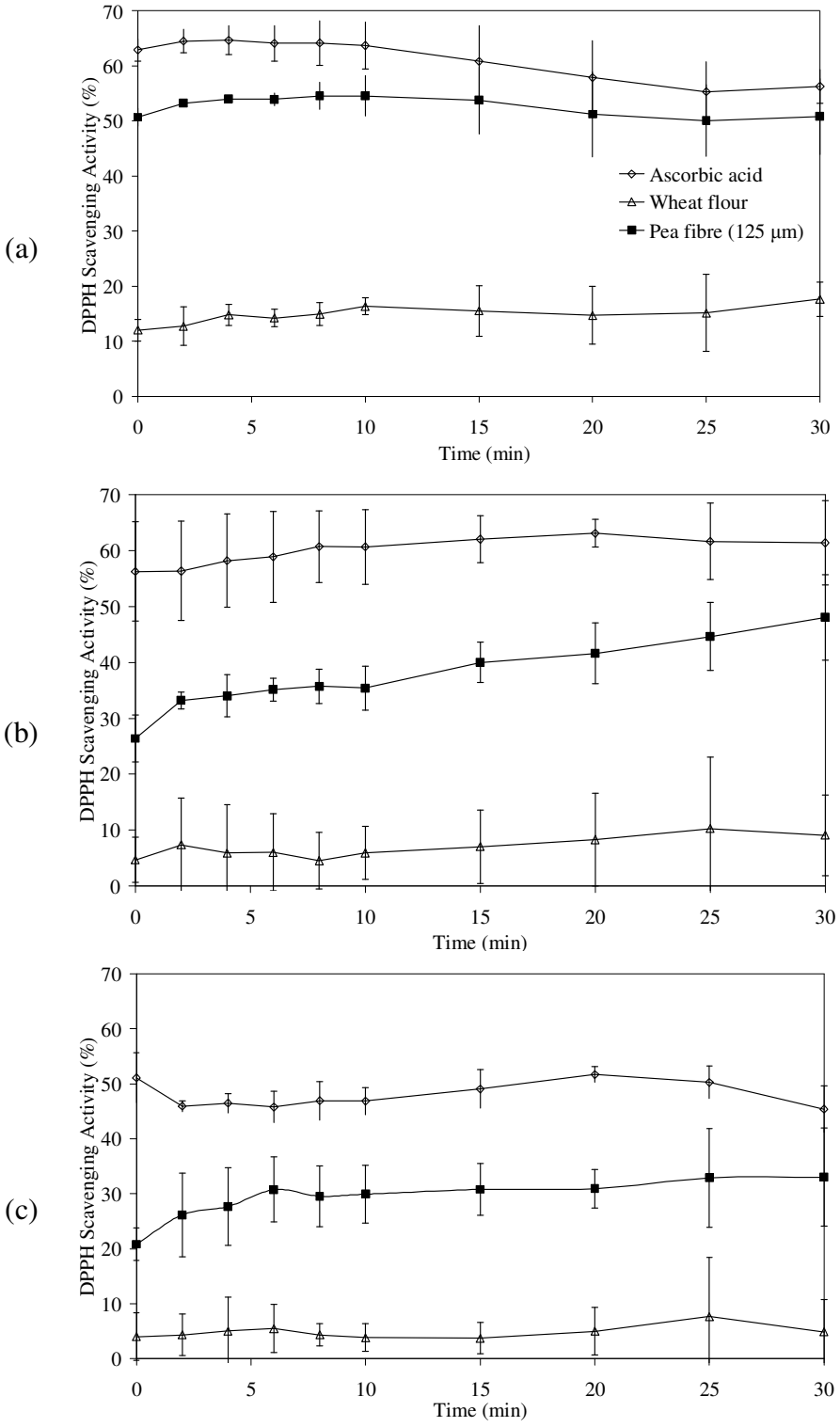


Figure 6.2. DPPH scavenging activity of wheat flour and pea fibre (125 µm) at (a) 0.5 mg/ml, (b) 0.25 mg/ml, and (c) 0.125 mg/ml final concentration.

The absorbance for wheat flour or pea fibre in a mixture of phosphate buffer (100 μL) and methanol (100 μL) was always less than 0.100, indicating that the colour of the extracted sample itself did not impart any significant effect to the reading. Moreover, absorbance was read at a specific wavelength corresponding to the violet hue of DPPH free radical, and the solutions containing dissolved sample material (pea fibre, wheat flour, and bread extracts) were colourless (Blois 1958). Hence the inherent colour of the extracted sample did not affect the absorbance, which was primarily dependent on the absorbance of the DPPH.

6.4.3. Comparison of DPPH scavenging activity of wheat flour and pea fibre

Regardless of sample concentration, the DPPH scavenging activity for wheat flour was poor compared to pea fibre (Figure 6.2). Pea fibre's activity was at least double wheat flour's activity for all concentrations. This demonstrated that the pea fibre had significantly greater DPPH scavenging ability than wheat flour. The high antioxidant activity of pea fibre indicates that it may be possible to augment the intrinsic antioxidant activity of wheat bread when pea fibre is added to the bread formulation (Fan and others 2007, Ragaee and others 2006).

6.4.4. Comparison of DPPH scavenging activity of pea fibres of different particle size

While all pea fibres had substantially greater antioxidant scavenging ability compared to wheat flour, there was minimal difference in activity between the pea fibres of different particle size (Figure 6.3). The pea fibre with the greatest activity was 250 μm, the largest particle size. Overall, as the particle size decreased, so did the scavenging

activity, though differences were slight, especially between 180 and 90 μm. While some studies have found that decreasing particle size resulted in increasing antioxidant yield and activity (Gião and others 2009, Qu and others 2010), some studies found that particle size had no effect on antioxidant activity (Moore and others 2009).

Regardless of particle size, all pea fibres had antioxidant activities approaching that of ascorbic acid by 30 min, the end of the assay when comparing average antioxidant ascorbic acid activities over the three concentrations evaluated (see Figure 6.2). This suggests that pea fibre has the potential to provide substantial protection against free radicals.

The ascorbic acid plot in Figure 6.3 is slightly different from that in Figure 6.2.b, even though the final solutions are the same. The plots within each Figure were from assays completed simultaneously, so all the plots were of reactions that started at the same time. The discrepancy between plots could be from the DPPH slowly degrading in solution – as a free radical, DPPH is unstable (Blois 1958). While the solution was stored at -30°C in a brown bottle and tightly sealed, the same solution was used over the two weeks in which the assays were performed. As such, DPPH breakdown could have been occurring over this time. The same solution was used instead of making fresh solution to avoid the effect of slight differences in concentration with different solutions.

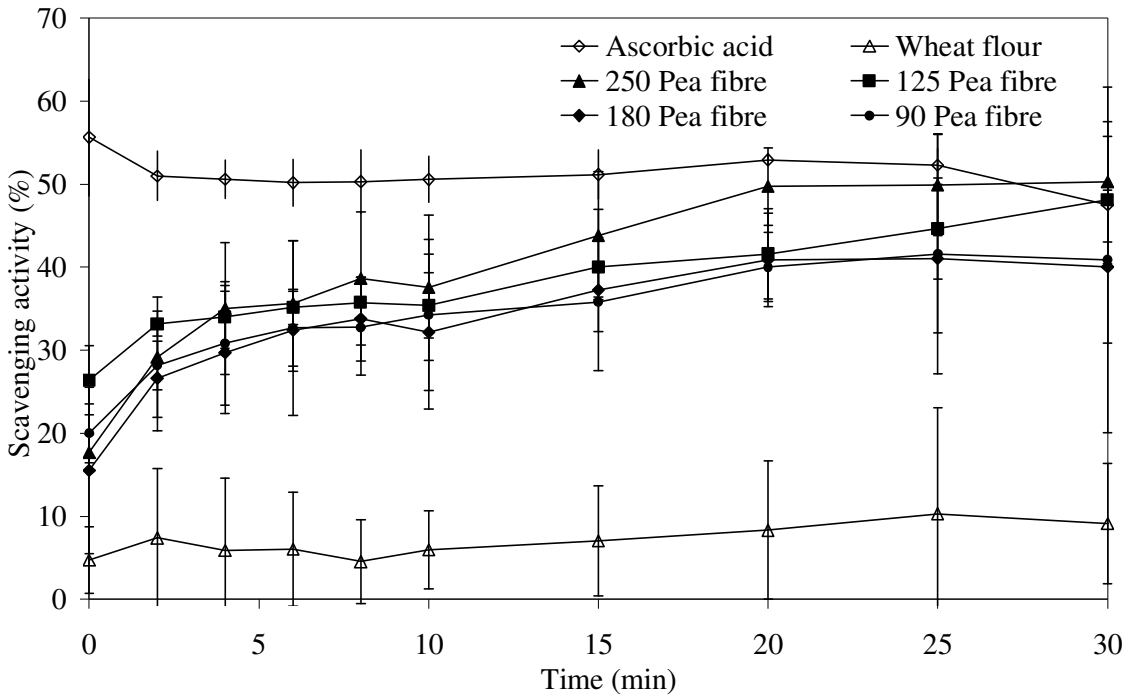


Figure 6.3. DPPH scavenging activity of wheat flour and pea fibres (250, 125, 180, 90 μm) at 0.25 mg/ml final concentration.

6.4.5. Comparison of DPPH scavenging activity of control bread and pea fibre enriched bread

As no significant difference in DPPH scavenging activity was found between the different pea fibre particle sizes, one particle size was chosen to demonstrate the difference in DPPH activity between a control bread and fibre-enriched bread. Bread containing 250 μm pea fibre at 6 g/serving was used as it produced a loaf of high specific volume at the highest fibre content evaluated when optimizing bread quality (see Chapter 2). Figure 6.4 shows that the fibre-enriched bread had 10% scavenging activity, slightly over double that of the control bread. This demonstrated that pea fibre-enriched bread had greater antioxidant activity than the control bread. This has very promising marketing implications. Whole wheat bread is generally seen as more desirable than white bread due to its higher antioxidant and fibre content (Gélinas and McKinnon 2006).

However, pea fibre enriched bread has a similar appearance to white bread (see Chapter 2), but can provide the antioxidant and potential health benefits of whole wheat bread. As such, pea fibre enriched bread can be marketed as being a healthy alternative to whole wheat bread, while retaining the sensory qualities of white bread.

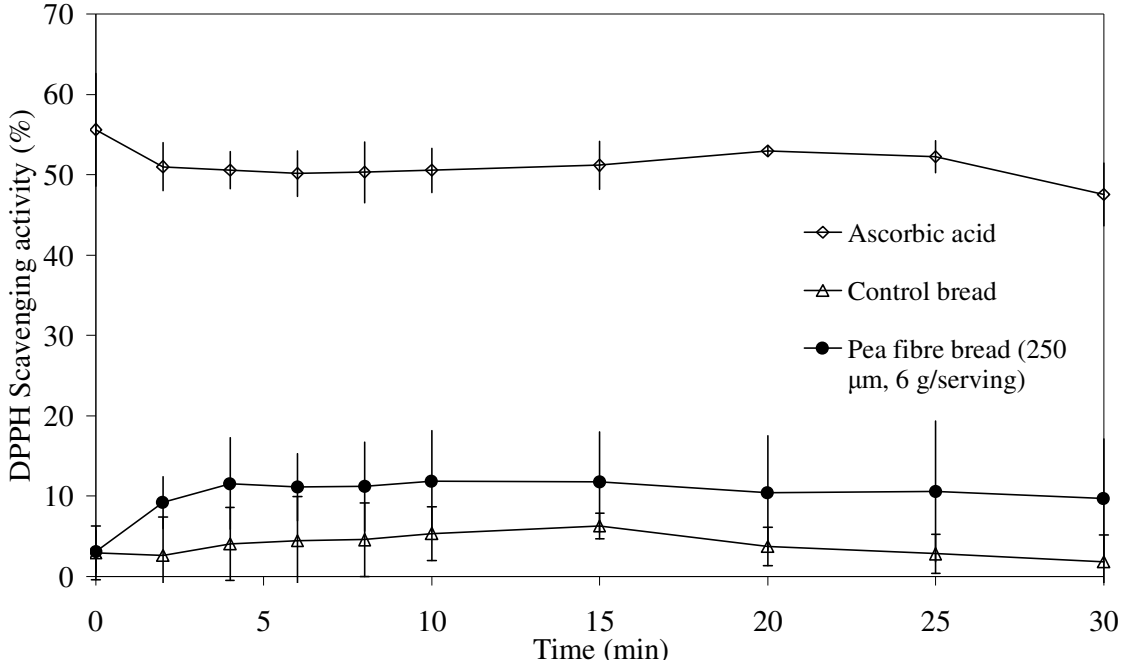


Figure 6.4. DPPH scavenging activity of control bread compared to pea fibre enriched bread (250 µm, 6 g/serving) at 0.25 mg/ml final concentration.

6.5. Conclusions

Regardless of particle size, pea fibre had substantially greater antioxidant scavenging ability compared to wheat flour, but there was minimal difference in activity between the pea fibres of different particle size. Pea fibre scavenging activity was close to that of ascorbic acid by the end of the reaction. Bread containing 6 g/serving of 250 µm pea fibre had higher DPPH activity compared to that of the control bread. As such, the prevention of aging-related diseases associated with the consumption of dietary fibre can be coupled with the health benefits of consuming antioxidants present in pea fibre.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

7.1. Introduction

To this point, the investigation of the effect of pea fibre on bread has been approached in several ways. The use of bread quality evaluation tools like subjective bread scoring, C-cell analysis, and texture profile analysis has shown that pea fibre addition to bread decreases specific loaf volume, densifies gas cells in the crumb, and firms crumb texture. Because each of these bread quality issues is a reflection of altered gas cell development, ultrasound was used to monitor gas bubble development during dough proofing as an additional method of investigation. As well, it has been demonstrated, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, that pea fibre contains antioxidants. An integration of the results from these techniques may point to a future direction where additional research could develop.

7.2. Effect of pea hull fibre on bread quality

7.2.1. Increased dough viscosity and inhibited gas cell expansion due to pea fibre addition

The addition of pea fibre without additional water caused a noticeable decrease in bread quality. This was evident in a wide range of quality parameters, such as the decrease of specific loaf volume, the decrease of bread scores for loaf appearance and crumb texture, the decrease of average cell diameter, and the increase in crumb firmness. The detrimental effect of pea fibre was attributed to increased dough viscosity, which became more severe with increasing fibre addition and decreasing particle size.

Within each level of fibre addition, relative volume range decreased with decreasing particle size, which indicated a smaller range of gas cell size. It was concluded that loaf volume depression arose from high dough viscosity limiting gas cell expansion rather than from fibre particles promoting gas cell coalescence. This conclusion was supported by the correlation between Young's modulus and loaf density, which showed no overall difference in the relationship of these two parameters between particle sizes.

The contribution of pea fibre to inhibited gas cell expansion was supported by the monitoring of ultrasonic phase velocity during dough proofing. In the control dough, peaks in velocity decreased in frequency during proofing, which indicated an increase in median radius (Leroy and others 2008). No clear pattern was seen in the dough containing 6 g/serving of the 90 μm pea fibre, suggesting limited gas cell expansion. Broader velocity peaks were observed in the fibre-enriched dough than the control, indicating a lower mean radius and hence a greater occurrence of smaller bubbles (Leroy and others 2008). The reduced gas cell expansion in the fibre-enriched doughs seen in the ultrasonic analysis reflects the conclusion drawn from the baking experiments that fibre inhibited gas cell expansion.

7.2.2. Additional methods of quality improvement of pea fibre-enriched bread

The increasing effect of pea fibre on dough viscosity was mitigated when water absorption was optimized in bread formulation. This resulted in greater specific loaf volume and more open crumb structure as indicated by increased cell diameter, improved bread and crumb scores, and decreased bread firmness. The improvement in dough

viscosity was seen in decreased mixer work input. For instance, an acceptable loaf was produced using 4 g/serving of the 250 μm pea fibre, which would allow a nutritional claim of "a good source of fibre" to be used. However, the upper limit of acceptable pea fibre loading was 2 g/serving with the 90 μm fraction. To further improve the quality of pea fibre-enriched bread for all fibre particle sizes, other strategies will have to be employed besides adequate water hydration of the dough.

The addition of fibre leads to gluten dilution, thereby disrupting the gluten matrix formation during mixing. This dilution would affect the formation of gluten cross-links, resulting in poor loaf volume (Pham and others 2007). As such, an additional strategy to improve bread quality is to include additional gluten in the bread formula (Sosulski and Wu 1988). The addition of non-wheat protein may also be of benefit; the addition of sodium caseinate was shown to improve the loaf volume and crumb texture of wheat bread (Kenny and others 2001).

The use of xylanase to break down the fibre in order to improve water absorption is a possible method of improving the quality of fibre-enriched bread (Dornez and others 2007, Edwards 2011, Harada and others 2005, Laurikainen and others 1998). The addition of xylanase to dough made with wheat flour has been shown to reduce mixing and sheeting work requirements, resulting in a softer and more pliable dough. The breads baked had greater loaf volumes and bread scores than the control (Harada and others 2005). Similarly, the addition of xylanase to whole wheat bread has been shown to decrease water absorption by 11% and increase dough rising by 58%. The specific loaf volume increased by 56%. A five-member sensory panel gave higher scores for flavour,

texture, and overall acceptability for loaves containing xylanase. Decreased crumb firmness was confirmed using texture profile analysis (Shah and others 2006).

The addition of dough improvers may also improve bread quality. It was found that the addition of potassium bromate (45 ppm) to bread containing a 15% replacement of flour with pea hull fibre increased loaf volume by a third compared to fibre-enriched bread containing no dough improver (Sosulski and Wu 1988).

7.2.3. Relationship between C-cell analysis parameters with other assessments of bread behaviour and quality

C-cell analysis is intended to mimic the baker's assessment, but provide consistency (Salmon 2004). The analysis applies greater consideration to cells and cell walls closer to the centre, similar to how bread is usually visually assessed (Whitworth and others 2004). High correlation coefficients have been found between subjective average crumb grain scores and average cell number, cell wall thickness, average coarse/fine cluster, and average crumb fineness for bread (Chen and others 2007). From the analysis of pea fibre-enriched bread, correlations between subjective scoring and relevant C-cell parameters were stronger overall for the non-optimized loaves than the optimized loaves, likely due to the greater diversity in data. This indicates the potential of C-cell to determine important bread attributes that coordinate with subjective analysis.

While there have been several studies that used C-cell analysis as a replacement for subjective crumb texture analysis (Alvarez-Jubete and others 2010, Lodi and Vodovotz 2008, Stojceska and Ainsworth 2008, Sun and others 2010), there are few studies that compared C-cell parameters to subjective scores (Stevenson and others

2010). More work will need to be done to establish a relationship between objective and subjective crumb analysis.

7.3. Prediction of bread quality using various techniques

7.3.1. The prediction of crumb quality using texture profile analysis

The compression force value (CFV) determined using the standard AACC method (74-09.01) gives an indication of crumb firmness. Various texture profile analysis (TPA) parameters (hardness, cohesiveness, chewiness and resilience) also attempt to describe crumb texture properties. Strong positive correlations were found between CFV and hardness and chewiness. Strong negative correlations were found between CFV and cohesiveness and resilience. The strength of these relationships between CFV and TPA parameters can allow for the prediction of bread crumb quality using just the CFV, thereby simplifying the bread quality analysis process. The relationships found for the pea fibre-enriched bread supported previous work with pan bread, where it was found that CFV had strong positive correlations with hardness and chewiness, and negative correlations with cohesiveness and resilience (Angioloni and Collar 2009).

7.3.2. Prediction of bread quality using ultrasound

Gas cells are an important factor in determining crumb texture and structure (Hoseney 1998). As low-frequency ultrasonic waves are sensitive to changes in the numbers and sizes of gas cells in a solid or liquid matrix (Leighton 1997), ultrasound

would potentially be an effective, non-invasive and in-line method of investigating bubble behaviour in a dough matrix.

Moreover, the ability to monitor gas cell development during proofing could provide the benefit of predicting the baked result, if relationships between ultrasonic parameters and final bread quality can be established. Besides giving insight on the relationship between dough matrix structure and the resulting bread, the ability to predict bread quality would improve the efficiency of bread product development. From the work previously described in Chapter 5, ultrasound may be used to predict oven spring. The steady increase and eventual plateau of the attenuation of the control dough resulted in substantial oven spring. In comparison, the large increase and then decrease in attenuation of the dough containing 6 g/serving of the 90 μm pea fibre with adequate water addition resulted in poor oven spring. The steady change in the control attenuation may be indicative of a more stable dough matrix, whereas the inclusion of pea fibre resulted in disruption of the dough matrix (Seguchi and others 2007), which was reflected in dramatic changes in attenuation. More work would be needed to explore the possibility of using ultrasound as a predictor of dough behaviour during baking. In addition, poor dough contact with the transducer would greatly affect the reflectance of the ultrasonic waves, so methods of improving contact could be explored.

7.4. Further developments in antioxidant evaluation of pea fibre and pea fibre-enriched products

Total antioxidant capacity of cereals is mainly attributed to polyphenols, particularly phenolic acids, most of which are associated with dietary fibre (Saura-Calixto

and others 2009, Serpen and others 2007). It is proposed that the association of polyphenols with dietary fibre results in the slow and continuous release of phenolic acids from dietary fibre in the large intestine, thereby improving antioxidant absorption by the body (Saura-Calixto and others 2009, Vitaglione and others 2008). It would therefore be a great health benefit to establish that pea fibre is a significant source of both dietary fibre and antioxidants.

Since only one type of antioxidant assay was done, it can only be concluded that pea fibre has good DPPH free radical scavenging activity – the total antioxidant potential cannot be elaborated beyond this. It would be interesting to perform other antioxidant assays; for instance, determining total antioxidant capacity and bioavailability of pea fibre, as well as its activity in combating specific disorders, such as high blood pressure (Agboola and others 2010). DPPH may not have physiological relevance, as it does not resemble the operations of the human absorption of antioxidants and as it lacks functional similarity to highly reactive peroxyl radicals involved in lipid peroxidation (Prior and others 2005, Udenigwe and others 2009).

As lipid oxidation is more relevant to determining the shelf life of baked goods (Galliard 1986, Nanditha and Prabhasankar 2009), doing lipoxygenase and lipase assays may prove beneficial in determining the effect of pea fibre antioxidants on shelf life of bread or other products they are added to. This could encourage the development of pea fibre as a "natural" antioxidant, expanding its uses. Determining the quantity and types of antioxidants may also help with identifying more uses for pea fibre.

While there are some drawbacks to using the DPPH assay method, it is still asserted to be simple, accurate, and highly reproducible (Sánchez-Moreno 2002), and

hence was considered a good starting point for investigating the antioxidant activity of pea fibre.

Another development would be to compare the antioxidant activity of cooked pea fibre with uncooked pea fibre to look at the effect of processing. It may be difficult to determine the effect of baking as the other ingredients in bread may interact with pea fibre and affect its activity, but quantifying the effect of cooking would be beneficial, as pea fibre is more likely to be incorporated into a product than be consumed on its own. In a similar regard, investigating the interactions between antioxidants and gluten proteins and how processing affects these interactions may shed more light on the effect of antioxidants on bread quality.

7.5. Conclusions

The effect of pea fibre addition level, fibre particle size and dough hydration was seen in the suppression of gas cell expansion due to enhanced dough viscosity. Within each level of fibre addition, decreasing relative volume range with decreasing particle size indicated increasing homogeneity, inferring that coalescence was not responsible for depression in loaf volume. The lack of coalescence appeared to be supported by observations with low-frequency ultrasound; the phase velocity of the fibre-enriched dough was overall less than that of the control, and this is a signature for a greater density of bubbles. The linear relationship between Young's modulus and loaf density indicated no effect due to particle size or fibre content on dough stiffness. Increasing levels of fibre addition and decreasing particle size resulted in poorer bread quality. This was particularly evident for fibre-enriched breads that contained no additional water. The

increasing effect of pea fibre on dough viscosity was mitigated when water absorption was optimized in bread formulation. This resulted in greater specific loaf volume, more open crumb structure and decreased bread firmness. There were strong correlations between C-cell analysis parameters and subjective bread scores, and also between compression force values and texture profile analysis parameters. Regardless of particle size, pea fibre had substantially greater antioxidant scavenging ability compared to wheat flour, but there was minimal difference in activity between the pea fibres of different particle size. Pea fibre scavenging activity was close to that of ascorbic acid by the end of the reaction. Bread containing 6 g/serving of 250 μm pea fibre had higher DPPH activity compared to that of the control bread. As demonstrated by the improved bread quality parameters when additional water was included in the dough, pea fibre-enriched bread has the potential to be an acceptable product that provides the health benefits associated with consuming dietary fibre as well as antioxidants.

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