Effect of wheat bran on gluten network formation as studied through dough development, dough rheology and bread microstructure

by

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B.Tech., Osmania University, India, 2003
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Abstract

The overall hypothesis underlying this study is that the nature and extent of bran interactions with the gluten protein matrix play a dominant role in both 'in-process' dough and final product quality of whole grain baked goods. Therefore, the purposeful manipulation of those interactions should be able to minimize adverse processing or product characteristics resulting from bran inclusion/presence. The approach we took was to study the effects of bran milled to different particle sizes on dough development during and after dough mixing using fundamental rheology combined with traditional cereal chemistry approaches and x-ray microtomography (XMT). The research outcomes were used to create a better picture of how the bran is effecting the dough development and to suggest strategies that allow for the control of that effect.

Study-I focused on characterization of the chemical properties, empirical rheological properties and baking performance of flours and dough with different bran contents from different sources. The development of dough microstructure and the resulting crumb texture in the presence of different bran were studied using XMT. HRW and SW bran additions resulted in higher water absorptions (WA) irrespective of the flour type and bran source. Fine bran caused slightly higher WA followed by coarse and as is bran. Both HRW and SW bran decreased the dough stability of HRW flour, while it improved the stability of SW flour doughs. Macro and microstructure of baked products were significantly affected both bran type and addition level. HRW bran added to HRW flour resulted in 8-23% decrease in loaf volume while SW bran added at the same level caused 3-11% decrease. XMT indicated that bran decreased the total number of air cells significantly. SW flour resulted in harder crumb texture than that of HRW flour breads. Overall, SW bran had less detrimental effects on mixing and baking performance of HRW flour.

Study-II focused on specific bran particle size and composition on small and large deformation behavior of strong and weak flour doughs. Small deformation behavior was characterized using frequency and temperature sweep tests, while the large deformation behavior was studied using creep–recovery and uniaxial extensional testing. The results revealed that the rheological behavior of bran-enriched doughs depend on type of base flour, bran type, bran replacement level (0, 5, 10%), and the dough development protocol. Weak flour doughs benefited from inclusion of bran as inherently low peak height and stability of these doughs improved in the presence of bran. Temperature sweeps indicated a slight decrease in G′ and G" until around 55-60°C. In the same temperature range, presence of bran increased the moduli of composite four compared to that of the control flours. Creep compliance parameters indicated that both bran source and bran replacement had significant effect on maximum compliance (J_{max}) and elastic compliance (J_e). Finally, the bran type affected uniaxial extensional properties, maximum resistance (R_{max}) and elasticity (E), significantly independent from the type of base flour.

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Dedication

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Chapter 1 - Background and Goals

1.1 Bread-making

Wheat flour with high protein quality is used for wide range of baked products especially bread due to the unique viscoelastic properties of the dough. Wheat contains the gluten forming proteins which forms viscoelastic dough. By forming this viscoelastic dough, it allows the air cell incorporation and resists their coalescence. This property of the dough is very important for bread making and production. The bread-making process consists of series of stages including mixing, fermentation, dividing, proofing and baking. The changes in the rheological properties of the dough in each of these stages are the consequences of changes in dough structure at both the molecular and microscopic levels.

The **mixing process** involves mixing of flour and other ingredients with water and has significant influence on a final product texture (Angioloni and Rosa 2005). The objective of mixing is to form and develop the gluten network into a viscoelastic dough, with the ability to retain incorporated air. The mixing energy input and power are critical in proper dough development and creating tiny gas nuclei in the dough (Hanselmann and Windhab 1998). These gas nuclei serve as nucleation points for the diffusion of carbon dioxide, produced by yeast during fermentation. Four important factors affect the air incorporation during mixing process; the gas content of the dough, the rate of turnover of gas during mixing, the distribution of gas in terms of the bubble size distribution, and the gaseous composition of the bubbles (Campbell 2003). These factors are found to be dependent on the headspace pressure applied and type of the mixer and blade design used in the mixing process (Bloksma 1990a). The size and number of gas cells are important as this determines whether they grow or not during proving process. The other important role of mixing is to give stability to the gas cells occluded. Size of uniformity is important for gas cell stability (vanVilet 1995). After mixing, dough is subjected to large extensional strains in sheeting process, is another development stage. In this stage, the gas cells subdivide increasing their concentration and uniformity of size this enhances the viscoelastic properties of the dough. Aeration during mixing changes the dough properties physically and chemically, affecting the rheological properties of dough.

Fermentation follows mixing. The objective of fermentation is to bring the dough to an optimum condition for baking. Fermentation allows the dough to relax, yeast to begin fermenting and producing carbon dioxide gas. Most of the carbon dioxide produced by the yeast dissolves in the liquid dough phase. At a concentration of 4.3×10^{-2} kmoles/m^3, the liquid phase of the dough becomes fully saturated with carbon dioxide and subsequent production diffuses into gas cells at the same rate that is produced consequently the cells begin to expand. The number of gas cells can be increased by punching and molding during fermentation process. In punching and molding, the gas cell walls subdivide to increase both their numbers and concentration. Expansion of the dough is caused by pressure above atmospheric in the gas cells (Bloksma 1990). After fermentation, the dough rests for 15-20 minutes. Immediately after proofing and moulding the cylinder is fitted into the bread pan. This newly rounded cylinder is proofed allowing the gas cells to expand in size nearly 2x times before baking. During proofing, the dough undergoes changes in height and volume as well as texture and density (Rosell 2011).

Baking is the final step in the bread-making process. Fermentation ends when dough is baked in an oven (200-250°C, 12-45 min). In the initial stages of baking, as the oven heat penetrates the dough, gases in the dough expands. A final increase in volume occurs and the crust sets (Rosell 2011). As the temperature rises, the increase in dough volume is created by yeast producing carbon dioxide, evaporation of water vapor, and resulting in more expansion of existing gas cells (Campbell 2003). The gas cells continue to expand until the structure sets or the stress on thin bubble cell walls becomes to great and they rupture. The rupture of the cell walls separating the bubbles is called coalescence. During baking, starch gelatinization occurs, protein denatures, the gluten strands surrounding the individual gas cells become an porous network of cells, and referred as bread crumb. Chemical reactions including Maillard or nonenzymatic browning reactions are responsible for the brown color of the crust (Rosell 2011).

1.2 Fiber and Its Health Benefits

Fiber intake has increased after the food industry promoted the merits of including fiber in food products (Redgwell and Fischer 2005). Fiber is known as roughage, and is derived from plant cell walls. Fiber is composed of soluble dietary fiber and insoluble dietary fiber. According to the AACC Dietary Fiber Definition Committee, “Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and adsorption in the human small intestine with complete or partial fermentation in the large intestine”. Some of the primary sources of fibers include polysaccharides, oligosaccharides, lignin, cellulose and its derivatives;

fruits, vegetables, and oil seed fractions (defatted meals and hulls); and certain fractions of cereal grains (corn bran, soy hulls, rye bran, spent grains, triticale bran and wheat bran).

The reason for introducing fiber into the human diet includes the health benefits associated with fiber consumption. Several epidemiological research observations show that dietary fiber consumption promotes beneficial physiological effects including maintenance of health and protection from diseases such as constipation, blood cholesterol attenuation, blood glucose attenuation, (Boyer and Liu 2004; Scott et al 2008; Redgwell and Fischer 2005). One important health effect of dietary fiber is it is a modification of the colonic microflora which consequently decreases the pH in the colon. This inhibits pathogenic bacteria, and reduces the risk of colon cancer (Gibson et al 2004b). Other nutrition and dietetic studies found that low intake of dietary fiber is linked with health problems such as diverticular disease, coronary heart disease, obesity and rectal cancer (Kendall et al 2010; Sivam et al 2010). Fiber enrichment of foods has become a hot topic of research.

1.2.1 Fiber-enriched Foods: Impact on the Industry

The recommended daily dose of dietary fiber for a healthy diet is between 25-30 g per day (Jones 2004). In previous years, most of the population chose convenient foods that were palatable and lower in cost than healthy foods such as whole grains, fresh vegetables and fruits (Adams 1997). The consumer surveys found that public awareness of the benefits of consuming dietary fiber in their diets is increasing (Sloan 2001; Krystallis et al 2008). The industry had an obligation to improve the nutritional benefits of the products ever since the Healthfocus International Trends reported that European and American people claimed the food label “high fiber” is an extremely important (Sloan 2001). The Nutritional Labeling and Education Act made a mandatory field for ‘dietary fiber’ on nutrition labels (Singh et al 2012). The food industry has technological challenges in incorporating dietary fiber into foods because fiber imparts functional properties to the finished products. The functional properties include increased water holding, gel forming, stabilizing and thickening capacities (Gelroth et al 2001; Dikeman et al 2006). The addition of fiber also affects the rheological properties of the dough systems yielding higher water absorption and lower extensibility (depending on the type of baked products being made) (Gomez et al 2003; Sivam et al 2010). Dietary fiber addition to products did not appeal the consumers.

1.2.2 Dietary Fiber in Baked Products

Baked products are one of the most widely consumed foods, so dietary fiber in baked goods would be an easy way of consumption to achieve the daily intake goals. Various research studies has been conducted on dietary fiber sources in bread making to develop basic and applied information on the use of available, natural and relatively inexpensive fiber-rich materials. There are several fiber ingredients available to the baker and researchers have reviewed their available forms, functionality and applications in the wheat flour. Most fiber type breads contain two or more of these materials and the breads can be placed into four general classifications (Table 1.1). Dissertation will focus on wheat bran fiber.

Table 1.1 Classifications of fiber enriched breads (adapted from Dubois 1978)

Fiber Source	Marketing Approach	Relative Fiber Content
Multiple Grain Multiple fiber source No cellulose	All natural	Slightly more than whole grain
High amounts of grain or legume fiber No cellulose	Relatively high crude fiber All natural	Slightly higher than above class
Whole grain and/or grain fiber and/or legume fiber plus cellulose	Calorie reduction High crude fiber Fiber-rich appearance	Fiber content several times that of whole wheat bread
Cellulose as major fiber source	Calorie reduction Highest crude fiber	Highest of this type bread, several times that of whole wheat bread

1.3 Wheat Bran

Wheat bran is a by-product of milling wheat. Roller milling produces a clean separation of the bran and germ from endosperm through consecutive steps including grinding, sieving, and purifying steps (Hemdane et al 2016; Campbell 2007; Peyron et al 2002). Bran actually has several layers but is removed with all the layers intact. It is aleurone cells, along with the more peripheral layers plus the embryo, that constitutes the bran fraction. Wheat bran contains 2% bioactive compounds including multiple vitamins, phenolic compounds, and phytochemicals, within a strong fibrous structure (Shewry 2009). These phytochemicals significant in whole grain cereal (wheat) and include *n*-3 fatty acids, sulfur amino acids, oligosaccharides, lignin, minerals, trace elements, vitamins B and E, carotenoids, polyphenols (especially phenolic acids such as ferulic acid and smaller amounts of flavonoids and lignans), alkylresorcinols, phytic acid, betaine, total choline-containing compounds, nositols, phytosterols, policosanol and melatonin. The health benefits of the phytochemicals are listed in Table 1.2 (Fardet et al 2010).

The potential health benefits of high fiber food products have been the subject of several years. Still, some processing is needed for palatability and nutrient bioavailability (Topping 2007).Evidence accumulated from epidemiological and experimental research shows that wheat bran may reduce the risk of certain chronic diseases such as cardiovascular disease, body weight maintenance, type 2 diabetes, blood pressure, circulatory health and certain cancers (Montonen et al 2003; Koh-Banergee et al 2004; Erkkila et al 2005; Behall et al 2006; Schatzkin et al 2007; Mellen et al 2009). Researcher suggest that the consumption of fiber with a combination of components in the wholegrain matrix may work together to impart health benefits (Fardet 2010).

Wheat bran is a complex biological material characterized by a specific histological **structure** and a diverse **chemical composition** (Shetlar et al 1947). The bran makes up about 13% of the total wheat kernel. There are seven layers in the bran as is shown in Figure 1.1. Many layers are incomplete or unrecognizable at maturation so composition of the bran has been debated. The epidermis and hypodermis together constitute the outer pericarp, which is the outermost layer. Bran is commonly divided into three regions: outer, immediate, or inner layers (Jerovic et al 2010; Barron et al 2007). Adjacent to the outer pericarp is the inner pericarp which is composed of several compressed cell layers, the cross cells and tube cells (Bohm et al 2002). The next layer inwards is the seed coat or testa layer, which is strongly pigmented, in red wheat. Tightly bound to the internal surface of the seed coat is the hyaline layer. The next layer of bran is aleurone layer. It is considered anatomically a part of endosperm. The miller regards the aleurone as the innermost layer of the bran. The majority of mineral matter located in bran is found in the aleurone layer. The aleurone contains one third of the wheat grain's thiamin content. The aleurone granules are proteineous and rich in basic amino acids (Bechtel et al. 2009).

Table 1.2 Major health benefits of bioactive compound found in whole-grain cereal (adapted from Fardet et al 2010)

Health Benefits	Bioactive compound
Body-weight regulation and obesity	Insoluble fiber, fructans, resistant starch, Zn, Ca, tocotrienols, phenolic acids, flavonoids, choline, p-aminobenzoic acid
CVD and heart health	α-Linolenic acid, methionine, oligosaccharides, soluble fiber, resistant starch, phytic acid, Mg, Mn, Cu, Se, K, thiamin, riboflavin, nicotinic acid, pyridoxine, folates, tocopherols, tocotrienols, phylloquinone, b-carotene, lutein, zeaxanthin, phenolic acids, flavonoids, lignans, phytosterols, betaine, choline, inositols, policosanol, p-aminobenzoic acid, g-oryzanol, avenanthramides, saponins
Type 2 diabetes	Soluble fiber, resistant starch, phytic acid, Mg, Zn, Se, K, Ca, tocopherols, tocotrienols, phenolic acids, flavonoids, betaine, inositols, phytosterols, g-oryzanol, saponins
Cancers	α-Linolenic acid, oligosaccharides, soluble fiber, insoluble fiber, resistant starch, lignin, phytic acid, Zn, Mn, Cu, Se, P, Ca, riboflavin, nicotinic acid, pyridoxine, folates, tocopherols, tocotrienols, β-carotene, β-cryptoxanthin, phenolic acids, flavonoids, lignans, alkylresorcinols, betaine, choline, inositols, phytosterols, melatonin, p-aminobenzoic acid, saponins
Gut health	α-Linolenic acid, oligosaccharides, soluble fiber, insoluble fiber, resistant starch, riboflavin, pantothenic acid, phenolic acids, policosanol, g-oryzanol
Mental/brain/nervous system health and neurodegenerative disorders	α-Linolenic acid, methionine, oligosaccharides, Fe, Mg, Zn, Cu, P, Ca, Na, K, thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folates, tocotrienols, phenolic acids, choline, inositols, policosanol, melatonin, g-oryzanol, saponins
Skeleton health (i.e. bone, tendon, cartilage, collagen, articulation and teeth)	α-Linolenic acid, Fe, Mg, Zn, Mn, Cu, P, Ca, K, nicotinic acid, tocotrienols, phylloquinone, β-cryptoxanthin, flavonoids, lignans, p-aminobenzoic acid
Antioxidant protection (development of diseases in relation to increased oxidative stress)	Reduced glutathione, methionine, cystine, lignins, phytic acid, Mg, Fe, Zn, Mn, Cu, Se, thiamin, riboflavin, tocopherols, tocotrienols, β-carotene, lutein, zeaxanthin, β-cryptoxanthin, phenolic acids, flavonoids, lignans, alkylresorcinols, betaine, choline, policosanol, melatonin, g-oryzanol, avenanthramides, saponins

The complete separation of bran from endosperm in flour milling is due to differences in the mechanical properties of bran and endosperm (Peyron et al 2002). The ability to separate components of the kernel may rely solely on bran layer chemical differences (Evars and Millers 2002). In wheat bran, aleurone cells are high in proteins, ferulic acid, and lipids, and are composed of thick nonlignified cell walls, and the pericarp has thick lignified cells (Fulcher and Duke 2002; Cheng et al 1987). Bran has large concentrations of branched heteroxylans, cellulose and lignins (Hemery et al 2011).

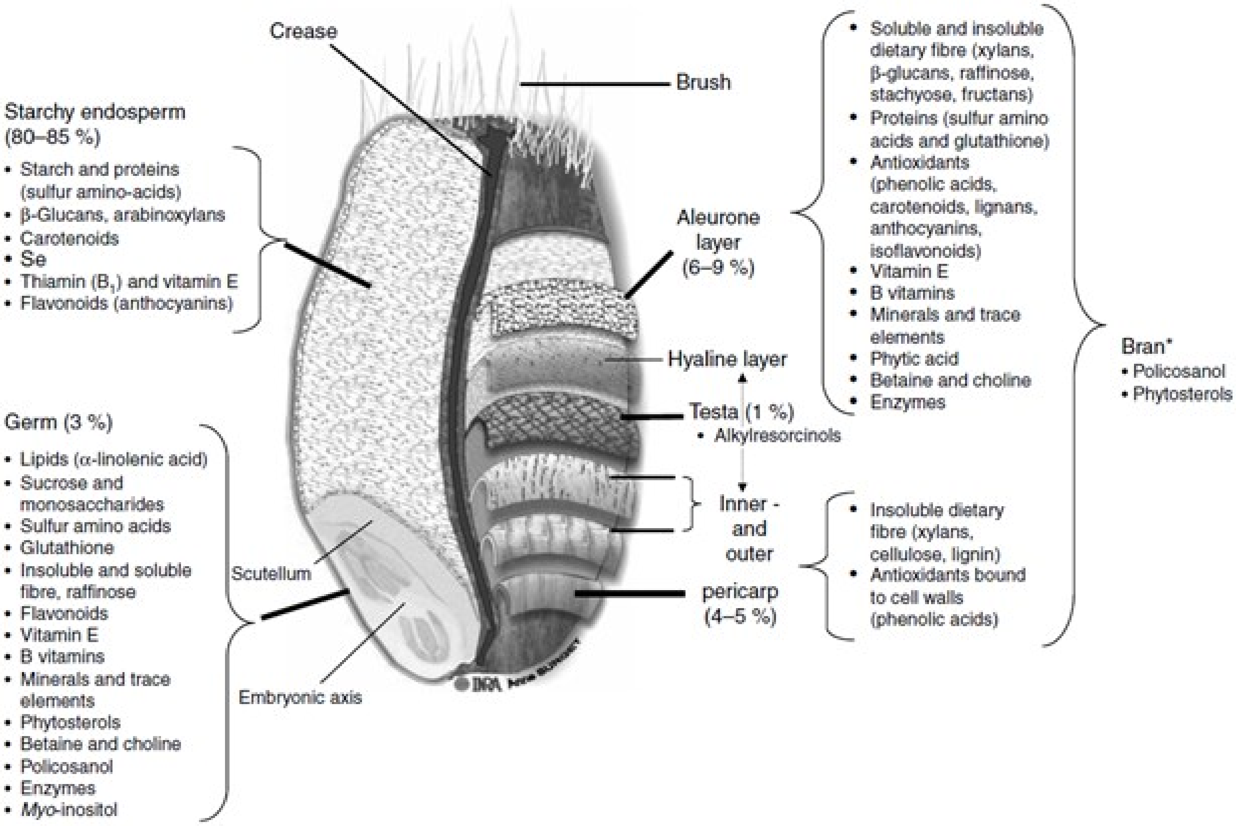

Figure 1.1 The seven layers constituting of wheat bran (adapted from Fardet 2010)

1.3.1 Wheat Bran in Bread-making

Wheat bran is used in the feed industry as livestock feed. Due to expanding market for health foods, wheat bran is considered as a nutritionally valuable ingredient and widely used as source of fiber for incorporating into processed foods, mainly bread. However, incorporating wheat bran in bread generally decreases its structure and sensory quality. This can cause reduced consumer acceptance. As a result, bran brings challenges in to the production of fiber-rich products: maintaining functionality and quality equivalent to traditional products available in the market. Besides affecting final product quality characteristics, incorporating wheat bran into flour systems also results in changes in dough properties and processing behaviors (Chassagne-Berces et al 2011). Studies have reported that wheat germ, red-dog and bran fractions of diverse wheat varieties have different effects on bread quality (Lai et al 1989a; Sidhu et al 1999; Noort et al 2010; Hemdane et al 2015).

1.4 Technological Challenges-Bran in Baked Goods

The first challenge to bran incorporation begins with the current diversity of mill-derived bran products. Depending on the mill, wheat bran contributes to by-products including coarse bran, fine bran, middling or shorts and red-dog. The different varieties of bran fractions may have their own specific properties which affect the wheat bran behavior in bread making. Hemdane et al (2015) reported that fine bran and red-dog fractions have impact that is more negative effects on bread making than do coarse bran fractions.

1.4.1. Interaction with Water

Bran interacts with water and has the ability to absorb a considerable amounts of water. Bran retains water by a variety of macro-, micro- and nanoscale, and molecular level mechanisms. At the macro level retention is attributed to filling of void spaces between particles; while on a micro level, the void space in pericarp cells and space in between the tissue layers is filled. Capillary mechanisms are involved in the water binding on the nanoscale. Chaplin(2003) stated that bran is rich in polysaccharides which can bind water on a molecular level by formation of hydrogen bonds. This mechanism contributes to water uptake phenomena and considered as a function of wheat bran particle size (Hemdane et al 2016). Jacobs et al (2015) reported that large particle size bran was able to retain more water during unconstrained hydration, due to its higher potential to bind water in its intact micropores.

In bread making, bran is hydrated during mixing and exposed to kneading and the hygroscopic forces applied by various flour components. Water bound through these mechanisms is relatively weakly bound and releases in the presence of these external force because stacking and micropores do not contribute to hydration (Zhang and Moore 1999). The tendency of bran to absorb water might result in competition between bran and flour constituents for water. This view is consistent and has been found to be correlated to the effect of wheat bran on bread making. (Roozendaal et al 2012; Schmiele et al 2012; Hemdane et al 2016). However, it is difficult to estimate the exact significance of bran hydration behavior in bread making functionality because no clear scientific evidence is available to support the hypotheses.

The bran addition has detrimental effects on bread quality in terms of functional and as well as sensory properties of bran. Addition of wheat bran to wheat flour generally increases required water absorption. The additional water is retained by the loaf during baking, giving a heavier loaf and decreased bread volume. The studies reported that this additional water is also available for starch gelatinization during baking, which lowers the starch gelatinization temperature and reduces gas retention during baking and thereby lowers the loaf volume. This mechanism implies that the effect of bran on gas retention is mainly during baking. The adverse effects of the wheat bran increased with increasing levels of bran in the bread (Zhang and Moore 1999; de Kock et al 1999; Campbell et al 2008; Seyer and Gelinas 2009). Schmiele et al (2012) reported a decrease of specific volumes when wheat flour was substituted with wheat bran at higher concentrations (20%, 30% and 40%) and decreased loaf volume. Other studies reported that effect of bran addition on crumb texture was not due only to reduced loaf volume. Majzoobi et al (2013) observed that in flat breads where loaf volume is not important, the quality of crumb texture decreased and darker crumb color was observed by incorporating bran. de Kock et al (1999) found that the deleterious impact of bran in bread can have both physical and chemical causes.

1.4.2 Physical Hindrance and Disruption Effect

The detrimental effect of bran addition on bread making can be attributed to the dilution of gluten proteins. Bran might interrupt gluten development by preventing proper contact between flour particles. There might be additional negative effects not amply covered by gluten dilution. Farinograph studies found longer dough development times when higher levels of bran were replaced with flour (Sanz Penella et al 2008). It may also be that the incorporation of bran particles into the gas cell walls limits their ability to expand resulting in the collapse, leading to coalescence, and leading to low gas retention in the dough, low bread volume and dense crumb texture of the final product (Gan et al 1992).

Several workers have considered the mechanical effect of bran is it physically disrupts gluten films during the dough formation in the mixer. Gluten is stretched into thin films during the later stages of proving and early stages of baking. (Zhang and Moore 1997; Campbell et al 2008). The authors suggested that in no-time breadmaking processes, bran could affect the initial air content and bubble size distribution and finally the baked loaf volume and crumb structure. The bran particles might force gas cells to expand in particular ways on pierce the gas cells leading to coalescence (Cauvain et al 1999; Campbell 2003). The rheological properties of bran supplemented doughs may be attributed to a weak and gluten network due to disruption by bran particles.

There can be clear cut beneficial effects on dough properties of bran addition. Ozboy and Koksel (1997) reported that soft white winter wheat coarse bran added flour increased the dough resistance to overmixing and to extension as measured by Farinograph and Extensigraph respectively. Later, when the coarse bran was added to strong flour from hard red winter wheat, a similar trend was observed.

1.5 Previous Studies

Extensive research has been done in bran addition to dough systems and has focused mainly on bread. As discussed earlier, bread is highly aerated and bran physically disrupts the gluten films during mixing. Incorporating bran into the bread products is not an ideal vehicle for delivering bran into diet. Several authors have worked on including bran into less or non-aerated products such as cakes, cookies, biscuits, pizza (Majzoobi et al 2014; Gujral et al 2003; Protonotariou et al 2016; Pacheco de Delahaye et al 2005).

1.5.1 Physical and Structural Properties of Dough Systems

The deleterious effect of bran can depend on type and level of bran added to the dough. Increased level of bran addition generally increases the water absorption of the doughs increased loaf weight; increased dough stickiness, decreased mixing tolerances, dough strength and extensibility. Bran additions reduced the loaf volume and specific volume, produced coarser structure, and reduced crumb structure with dark crumb color (Gan et al 1992; Zhang and Moore, 1997, 1999; de Kock et al., 1999; Campbell et al 2008; G´omez et al 2011; Schmiele et al 2012).

Other sources of fiber such as oat bran, inulin, date fiber, pea and broad bean pod fiber have been studied in relation to wheat flour dough formulation. The addition of these fibers also modified bread making performance of wheat doughs, affected mixing properties (Campbell et al 2008), and rheological behavior (Peressini et al 2009; Bonnand-Ducasse et al 2010) and viscometric patterns (Fendri et al 2016). Addition of fiber at lower levels strengthened the structure of the dough and improved its quality (Sivam et al 2011); however, excessive amounts of fiber had a negative effect on gluten network formation and reduced the quality of bread (Noort et al 2010; Ahmed et al 2015).

The structural properties of the bread crumb can be assessed by C-Cell Image analysis. Various other techniques such as image analysis and X-ray microtomography (XMT) have been used to evaluate the microstructural properties of bread crumb to determine the relationship between air incorporation, expansion and cell structure. Studies have been done on wheat flour doughs using XMT analysis to evaluate at the changes in gas cell expansion during proofing (Babin et al 2006). The analysis determined that air cell growth was unrestricted and average cell wall thickness was similar to dough during the initial stages of the proofing. The air cells begin to grow closer and more susceptible to integrate as proofing continued and larger cells were created causing coalescence.

Perez-Nieto et al (2010) studied dough structure changes and their relationship to dough temperature, mass loss and loaf height during baking by using image analysis techniques. The results showed dough expansion and coalescence of the bubbles during initial stages of baking. The greatest dough disruption was significant on crumb dough structure during the initial stage of baking. Besbes et al (2013) evaluated the cellular structure of bread under different baking conditions using XMT analysis. The studies showed that the structural properties of the crust and the crumb in terms of air cell size were influenced by heating rate. Van Dyck et al (2014) studied the physical property effects of bran addition to pan baked white bread. The results revealed that bran caused reductions in loaf volume, an increased cell wall thickness and an increased number of closed pores. Structure thickness and number of closed pores were found to be higher in crust areas and lower in crumb areas in bran addition to pan bread.

1.5.2 Rheological Properties

Dynamic rheological testing has been the preferred approach for examining structural and fundamental properties of wheat flour doughs and proteins (Song and Zheng 2007). This rheological testing has become popular because of its characteristic and sensitive response to the structural variations (Tronsmo et al 2003). Dough rheological properties have been investigated using empirical and dynamic rheological measurements (Mani et al 1992; Rouille et al 2005; Dobraszczyk 2004a). Studies on wheat doughs show that not all rheological measurements can predict the baking quality of different flours (Amemiya and Menjivar 1992; Safari-Ardi and Phan-Thien 1998), as measurements can differ in terms of magnitude and the type of applied deformation. Amirkaveei et al (2009) studied the effect of treated and untreated bran on linear and non-linear behavior using dynamic oscillatory tests. The studies showed that untreated bran weakens the gluten matrix and on the other hand the treated bran strengthens the protein matrix to some extent. Several other researchers have studied the influence of other dietary fibers such as inulin, β-D-glucan on dough systems (Peressini and Alessandra 2009; Ahmed 2014).

Research done by Bonnand-Ducasse et al (2010) studied the effect of wheat dietary fibers on bread dough development and its rheological properties. Several water-unextractable and water extractable arabinoxylans fractions of wheat fiber were isolated from starchy endosperm, aleurone layer and bran when added up to 10%, to standard flour and studied through mixing tests, and rheological tests at small and large deformations. Water-unextractable arabinoxylan in the dough led to a decrease in peak time and to a slight increase in peak bandwidth. Shorter development times suggested that dough was homogenized more rapidly by the presence of water-unextractable arabinoxylans whereas as the increase of bandwidth suggests that dough displayed more instantaneous resistance to elongation. Similar studies by Gomez et al (2003) and Peressini et al (2009) observed that water absorption by dough increased and dough tenacity increased with increasing insoluble wheat fiber content. The small deformation tests results showed on increase in viscoelastic moduli with fiber addition. The increase of viscoelastic moduli may be attributed to the reduction of polymer lubrication by water due to the competition of water absorption between gluten and fiber (Izydorczyk et al 2001; Wang et al 2003a), or to the fibers acting as a filler in a viscoelastic matrix (Uthayakumaran et al 2002). Viscoelastic properties of doughs at different water absorption levels were also evaluated with this method as the Farinograph water absorptions do not allow the decoupling of the effects of hydration and fiber (Peressini et al 2009). The storage modulus of inulin dough samples was lower than that of the control doughs and a trend of decreasing G′ with increase of dietary fiber content was identified. Similar research conducted by Rouillé et al (2005) reported an appreciable decrease in the linear study-state creep compliance of doughs at fixed water addition as low molecular weight sugar content of soluble fractions increased.

Large deformation tests such as creep and creep recovery were performed on the water-unextractable and water extractable supplemented doughs (Bonnand-Ducasse et al 2010). This test allows characterization of the viscoelastic behavior of the dough at long times and the determination of the steady flow viscosity of highly viscoelastic materials. For creep stress values $\sigma < 200$ pa, creep curves were close to each and showed a linear limit region. Above the creep stress value, the doughs exhibited time-dependent flow behavior with yield stress.

Large deformation measurements are more suitable for testing dough quality as food product as it can be related to bread eating quality. The large deformation tests are conducted where the stress exceeds the yield value. The most commonly adapted method for large deformation testing of dough is extension. Various instruments are available for performing extension tests on dough such as the extensograph, alveograph, Kieffer rig dough extensibility test and Instron. In the past, much research has been done on dough and gluten extensibility using attachments on the Universal Testing Machine on the Texture Analyzer (Kieffer et al 1998; Suchy et al 2000; Tronsmo et al 2003; Sliwinski et al 2004a, b).

Tronsmo et al (2003) showed the difference in the breadmaking performance of six different wheat flours and determined than maximum resistance to extension and total extensibility using Kieffer dough and gluten extensibility rig on the TA.TX2i texture analyzer. They performed extensibility tests on wheat flour doughs and gluten with and without salt. Results showed that the extensibility of unsalted doughs was more influenced by flour protein content than the extensibility of salted doughs. Measurement on gluten gave a purer indication of protein properties. Fresh gluten from flours of high protein content was less elastic than was gluten from flours of similar mixing strength but lower protein content.

Another recent study done by Ahmed et al (2015) using small amplitude oscillatory rheology and creep behavior found the mechanical rigidity of the β-glucan concentrate containing doughs strongly influenced by particle size, particle-to-water ratio and temperature. The studies suggested that large particles occupy more space during aggregation than do small particles due to less efficient particle packing. This leads to more volume occupation by large particles and an increase in flow resistance (Quemada 1998). In addition, the smallest particles would be expected to have lower mechanical strength because cellular structure breakdown of the particles increases due to milling. The heating temperatures had least effected on oscillatory measurements. The solid-like property of the dough gradually decreased with increasing dough water content. The finest particle dough showed the maximum strain during creep test confirming its viscoelastic nature.

Most researchers have studied the rheological properties such as storage modulus (G′), loss modulus (G"), and loss tangent (tan δ) of wheat flour doughs and good and poor protein quality flours (Miller and Hoseney 1999; Khatkar et al 1995 and Toufeli et al 1999; Peressini et al. 2009; Bonnand-Ducasse et al. 2010). In wheat doughs, oscillatory measurements in the linear viscoelastic region have not been able to predict the baking quality of different flours. The results of large deformation measurements correlate well with the properties of the overall network structure (Stadig 1993). Wheat bran addition has adverse effects on dough quality, depending on bran’s composition and particle size. Several researchers have studied the bran addition effect on bread quality and found that bread volume is highly correlated with the gluten yield. The constituents of the wheat bran interact with the gluten physically or chemically, negatively influencing gluten aggregation (Wang et al 2003a; Noort et al 2010) The previous research findings on the rheological properties of dough and gluten used rheometric techniques. Very little information is available on the effects of bran particle size addition using fundamental dough rheology and structural properties. Further investigation of the bran particle size effect on wheat flour dough mixing behavior, and small and large deformation measurements is needed.

1.6 Hypothesis

Previous studies explained the effects of bran on bread quality with widely different hypothesis. In a series of studies, Lai et al (1989, 1989a, b) proposed that presence of bran particles changes the appearance and the handling properties of the dough. They also attribute the effects to the actions of enzymes and reducing components in the short fractions. Gan et al (1992) hypothesized that the addition of fiber dilutes gluten network formation. In addition, the physical disruption of the gas cells or by a biochemical mechanism by lipase action causes the effects on loaf volume. Courtin and Delcour (2002) hypothesized that effects of water unextractable arabinoxylans present in minor wheat flour components such as lipids, endogenous enzymes, ash and non-starch polysaccharides destabilizes the dough structure by piercing the gas cells and forming physical barriers for the gluten network development. In another series of research papers, Wang et al (2003a, b, 2004a, b) explained the effects of water arabinoxylans and water insoluble solids from wheat flour has being due to effects on gluten formation. They further hypothesized that presence of bran particles in dough can act through a combination of both physical and chemical mechanisms. The physical mechanism was correlated to water binding and particle size; whereas the chemical mechanism was linked to the presence of ferulic acid.

1.7 Scope and Approach

Introduction of cereal grain bran into human diet has proven to have significant health benefits (reducing the coronary heart disease, some colon diseases, and type 2 diabetes risks). A fiber-rich material like wheat bran is added to the wheat flour in the production of high-fiber breads to improve the nutrition availability. The production of whole wheat breads has grown to become a major portion of the bread industry in few decades. The addition of bran fiber causes major changes in processing behavior and quality characteristics of bread such as loaf volume, texture and flavor.

Research has discovered that addition of bran fiber into flour causes weakening of cell structure and reduces gas retention, as the fibrous materials tend to cut the gluten strands. Studies examined the rheology of wheat bran flour dough components such as starch and protein, since they have the ability to form continuous macromolecular network which gives rise to viscoelastic behavior. There has been no combined effort to reveal the effect of bran on dough physical properties that are attributed to disruption of the gluten protein matrix.

Based on the above, the overall *hypotheses of this study* is that the nature and extent of bran interactions with the gluten matrix play a dominant role in both ‘in-process’ dough and final product quality. Therefore, the purposeful manipulation of the bran interactions which gluten protein network, should minimize the adverse processing or product characteristics resulting from bran inclusion. Based on the hypothesis, this dissertation seeks to provide that detailed fundamental understanding of biochemical, rheological and micro-structural roles of bran in wheat dough systems using traditional cereal chemistry approaches combined with fundamental rheology and x-ray microtomography.

The specific **objectives** of this dissertation study were:

> **Chapter-2.** Study of effect of hard and soft wheat bran sources, varying bran size and bran inclusion levels on dough development, crumb structure and texture of the end product.

Dough development was evaluated by empirical methods using Farinograph, Mixograph and Mixolab. The baking performance was assessed through C-Cell imaging (macro imaging) and by x-ray microtomography (XMT) (micro imaging). The bread texture was studied using the TAXT2 Texture Analyser.

> **Chapter-3.** Study of the effects of bran fractions of varying anatomical origin and size at varying replacement levels on dough development, used small and large deformation rheological techniques.

The bran fractions were characterized into two categories, ‘different particle size with equivalent composition’ and ‘equivalent particle size with different composition’. The empirical rheological methods such as Farinograph and Mixograph was used to study dough development. The visco-elastic properties of bran doughs were evaluated by small and large deformation tests. Small deformation tests including, frequency sweeps and temperature sweeps were performed to understand the nature of bran and starch-gluten interactions in the doughs. Large deformation tests including creep recovery test and Kieffer rig dough extensibility tests were performed to understand the dough quality and its effect on the end product.

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Chapter 2 - Effects of hard and soft wheat brans at varying size and inclusion levels on dough development and crumb structure and texture of the end products

Abstract

The effects of bran source, bran size (as is, coarse and fine) and addition level (0-10%) on water absorption and rheological properties of hard and soft wheat dough systems of different strength, and the loaf volume, crumb texture, and microstructure of the resulting baked products were studied. Hard red winter (HRW) and soft winter (SW) flours and their respective bran fractions were used. The dough rheological properties were studied using the Farinograph, Mixograph and MixoLab. HRW and SW bran additions resulted in higher water absorptions irrespective of the flour type and bran source. Fine bran additions caused slightly higher water absorption values followed by coarse and as is bran sizes. Both HRW and SW bran additions decreased the dough stability of HRW flour, while it improved the stability of SW flour. The peak time and peak value were higher in HRW flour doughs compared to SW flour, as expected. Macro and microstructure of baked products were significantly affected by bran addition levels as well as by bran type. Five and 10 % HRW bran addition in HRW bread formulations resulted in 8-23% decrease in loaf volume while SW bran added at the same level caused only 3-11% decrease in loaf volume. X-ray microtomography data indicated that bran addition resulted in a significant decrease in total number of air cells and slight increase in their average sizes irrespective of bran type and addition level. Increased bran addition irrespective of bran source and size caused a gradual shift in the cell wall thickness and air cell size distributions towards higher values. SW flour resulted in harder crumb texture than that of HRW flour breads as expected from lower their loaf volume. Bran addition further increased the hardness values irrespective of bran type and size. Cohesiveness scores of HRW flour breads decreased dramatically with the addition of HRW bran while SW bran showed an insignificant effect. Overall, SW bran additions had less detrimental effects on mixing and baking performance of HRW flour. Added bran slightly improved mixing, baking and end quality parameters of SW flour systems.

2.1 Introduction

There is an increased demand and interest in food systems containing whole grains due to the health benefits attributed to the various phytochemicals and nutrients associated with the non-endosperm portions (bran and germ) of cereals. Studies reported and documented the positive effects of whole grain consumption on type 2 diabetes risk (Montonen et al 2003), body weight maintenance (Koh-Banergee et al 2004), blood pressure and circulatory health (Behall et al 2006; Erkkila et al 2005).

Whole grain products are a rich source of fiber. The nutritional benefits of fiber have led to increases in the production of high-fiber products. Wheat bran, in particular, is one of most essential dietary fiber sources used in the bread making industry (Vetter 1998). Several studies have been done on nutritional benefits of dietary fiber sources and especially dietary fiber in wheat bran and it has been proven that it reduces the risk of colon cancer (Zhang and Moore, 1999). Several other studies have related consumption of dietary fiber and whole grains with a reduction in serum cholesterol, and a lower risk for coronary artery disease and certain forms of cancer (Burkitt 1971; Kantor et al 2001; Decker et al 2002).

The cereal foods industry has responded to the consuming public's interest in whole grain foods by developing and introducing new or reformulated products. In 2005, this accounted for more than 650 introductions (Neilsen 2005). A significant number of major US food companies have launched such new or reformulated products, which in turn, have affected the sales of cereal products positively. The inclusion of the non-endosperm components to dough systems, however, presents technological challenges. Incorporation of wheat bran into bread-making flour results in many major changes in dough properties, processing techniques and bread quality characteristics (Prentice and D'Appolonia 1977; Lai and Hoseney 1989; Lai et al 1989). Research by Pomeranz et al (1977) has shown that the addition of wheat bran to a bread formulation increased water absorption, decreased loaf volume, impaired crumb texture, darkened crumb color and reduced crumb softness. The adverse effects of the wheat bran increased with higher levels of bran substitution in the formula.

The effects of particle size reduction on the physical and functional properties of wheat bran have been studied extensively (Shetlar and Lyman 1944; Moder et al 1984; Galliard and Gallagher 1988; Zhang and Moore 1997). However, bran particle size is still a controversial issue as it regards the bread-making performance. Shetlar and Lyman (1944) reported that the loaf volume of wheat bran bread is negatively correlated to bran particle size when the bran particle size was obtained by a sifting process. The different particles sizes produced by sifting may differ in composition because bran consists of many layers including the underlying aleurone cells. Moreover, different bran particle size distributions can be produced by sifting and grinding processes. The method of bran preparation appears to be of critical importance and could be the reason for differences in the published information. Zhang and Moore (1997) reported that bran particle size produced by a grinding process affected dough rheological properties. Coarse wheat bran particle size had better baking quality as compared to fine bran particle size. Moder et al (1984) reported that fine wheat bran had better baking performance compared to coarse bran, while Galliard and Gallagher (1988) indicated that fine wheat bran particle size reduced the bread quality.

The effects of bran on dough physical properties, which are attributed to disruption of the gluten protein matrix, are not well understood on a fundamental level. The nature and extent of bran interactions with the gluten protein matrix play a dominant role in both "in-process" dough and final product quality of whole grain baked goods. With the growing appreciation by the general public that baked goods containing whole grains provide health benefits, there is a need to understand the specific effect of whole grain components on dough rheology. Processing and handling of doughs with a high ash content due to bran is known to be more difficult (Faridi 1990), and a more complete understanding of the effect of whole grain components such as bran on dough rheology will allow cereal scientists to understand the source of the difficulties so that they can be controlled.

Antoine et al (2004) reported the relative distribution of outer grain layers in relation to the particle sizes of bran fractions. The differences in performance of bran-added flour systems needs to be further explored with regard to their specific chemical composition, water absorption, and other physical properties as well as linked to their functional properties in dough systems. The objectives of this research were; to evaluate the effects of bran source, inclusion level, and bran size on water absorption and rheological properties of wheat flour dough systems of different strength, and to study the loaf volume, crumb texture, and microstructure of the resulting baked products.

2.2 Materials and Methods

2.2.1 Materials

Two wheat varieties of different baking strengths were used for all experiments. Karl 92, hard red winter (HRW) wheat, was obtained from KSU Foundation Seed, Kansas State University. Soft wheat, a combination of club wheat and soft white wheat varieties was obtained from the pilot mill in the Department of Grain Science and Industry. Straight-grade flours of Karl 92 (HRW) and soft white (SW) wheat varieties were obtained by milling on Buhler model MLU-202 experimental mill (AACC Approved Method 26-21A). The HRW and SW wheat samples were tempered to 16% and 14% moisture content, respectively, for 24 h before milling (AACC Approved Method 26-10A). HRW and SW bran samples were collected from their respective milling.

Original (as is) bran samples were fractionated based on their particle size to obtain coarse (1358-1190 µ) and fine (900-630µ) bran sub-fractions using a sieve stack of 1358, 1190, 1000, 900, and 630 µ. Each bran fraction (as is, coarse and fine) was added to both base flours at 0%, 5% and 10% levels resulting in flour/bran systems summarized below:

Base flour/bran type*	**Objective**
HRW/HRWa HRW/SWa SW/HRWa SW/SWa	To study the effect of different bran sources on breadmaking performance of hard and soft wheat flours
HRW/HRWc HRW/HRWf HRW/SWc HRW/SWf	To study the effect of bran size on breadmaking performance of hard wheat flour

* a: as is, c: coarse, f: fine bran

2.2.2 Chemical Composition

All flour and bran samples were subjected to compositional analysis. Moisture content was measured by the oven-air method (AACC 44-15A). Protein content was determined by the nitrogen combustion method using LECO Fp-2000 nitrogen/protein analyzer using a factor of 6.25 to convert nitrogen to protein (AOAC 990.03). Ash content was measured using the muffle furnace overnight method (AOAC 942.05). Lipid content was measured using AOAC method (920.39).

2.2.3 Water Sorption Behavior

Water sorption behaviors of HRW and SW bran samples (as is) were characterized by equilibrating them to water activity (Aw) levels of 0.11, 0.33, 0.58 and 0.75 using saturated salt solutions (lithium chloride, magnesium chloride, sodium bromide and sodium chloride, respectively). The moisture contents of equilibrated samples were determined and sorption isotherms (moisture content *vs.* equilibrium Aw) were plotted.

2.2.4 Mixing and Pasting Behavior

2.2.4.1 Farinograph

Flour and water were mixed in a 50-g Farinograph bowl for 20 min according to the standard procedure. AACC method 54-21 was used to determine Farinograph consistency, water absorption (adjusted to 14% MC), development time, stability, mixing tolerance index (MTI), time to breakdown, and Farinograph quality number.

2.2.4.2 Mixograph

The 10 g mixograph (National Manufacturing Co., Lincoln, NE, USA) was used to study the mixing properties of dough systems as described by AACC Approved Method 54-40A. The flour and water were mixed in a 10-g mixograph bowl for 10 min according to the standard procedure. The peak time and peak height values were obtained from the mixogram curve. Peak development time was used as a basis to determine optimum mix times for test baking.

2.2.4.3 MixoLab

Dough mixing and pasting properties were studied using a MixoLab (Chopin Technologies, France) as described by ICC No. 173-standard method. According to the Chopin+ protocol, flour and water were mixed in a 50-g MixoLab bowl for 45 min at constant speed of 80 rpm. The amount of water to be added to HRW and SW control flours was previously determined from the farinograms, and then adjusted to result in a peak torque of 1.1 Nm. These water addition levels were then kept constant to study the effect of bran addition on mixing and pasting behavior of the base flours. The resulting MixoLab curves were analyzed for the following parameters: Hydration capacity, development time (C1), protein weakening as a function of mechanical work and temperature (C2), starch gelatinization and peak viscosity (C3), stability or break-down viscosity (C4), and set-back of gelatinized starch (C5). In addition, the angles between ascending and descending curves α (protein breakdown), β (gelatinization) and γ (cooking stability rate) were calculated. A detailed explanation of these MixoLab parameters can be found in Rosell et al. (2006).

2.2.5 Test Baking

The straight-dough procedure (AACC method 10-10B) using 100 g (flour weight) was used. Each dough system was mixed in a 100 g pin mixer (National Manufacturing Co., Lincoln, NE, USA) using the optimized mixogram water absorptions and mix times as described in the method. Proofing was performed at 30 ºC and 95% relative humidity for 40 min. Doughs were punched twice, and then molded on a specialized pup loaf molder (National Manufacturing Co., Lincoln, NE, USA). After molding, panning, and final proofing, the doughs were baked for 24 min at 210 ºC in a reel oven (National Manufacturing Co., Lincoln, NE, USA). The samples were test baked in three replicates.

2.2.6 Bread Macrostructure

Loaf volume was measured with a calibrated rape seed displacement meter (AACC Method 10-05) immediately after the loaves were removed from the oven. Crumb structure of baked laves was characterized using C-Cell image analyzing software and equipment (Calibre Control International Ltd., UK). Loaves were sliced using a rotary disc food slicer. Image analysis was performed on central slices that were 1.5 cm thick. Image analysis parameters (number of cells, average cell wall thickness, cell diameter) were used to compare crumb grain differences in bread samples.

2.2.7 Bread Microstructure

Bread samples were scanned using a high resolution desktop X-ray microtomograph (XMT) (Skyscan1072, Aartselaar, Belgium) consisting of a microfocus sealed X-ray tube with a spot size of 5 µm, an X-ray detector and a CCD-camera (1024 x 024 pixels) with a maximum sample field of view of 20×20 mm. Bread specimens of approximately 12×12×12 mm were carefully cut from the center slice using a pair of sharp scissors while the bread was frozen. The cut specimen was then placed in a clear plastic tube (15 mm diameter) and sealed to prevent drying of the bread during scanning. The bread samples were adhered to a double sided selfadhesive disc to stabilize the specimen. Then the plastic tube was secured on the rotatable sample stage using double sided tape. Scanning was performed at 40 kV/248 µA at 20x magnification (resulting in 13.7 µm/pixel resolution) at 1.35º scan steps with an exposure of 1.88 sec through 180º of rotation. Total scanning time was around 15 min/sample. The scanning process was controlled by SkyScan 1072-TomoNT control software (version 3N.5). Sets of 138 2-D radiographs (shadow images) per sample were rendered into 3-D objects using a filtered back-projection algorithm by the NRecon reconstruction software (V1.5.1.) which was subsequently digitally sliced to create hundreds of 2-D cross-sectional images that were used for quantitative analysis. A dynamic image range of 0.015-0.0601/mm (attenuated coefficient) was selected in the gray-scale histogram to give an optimized clear reconstruction of the object.

Image analysis was performed using CT-analysis processing and analysis software (CTAn, v.1.7). A 5·6 mm rectangular region of interest (ROI) was defined in the center of the bottom slice. This rectangular section was then interpolated across the selected 600 slices with a total thickness of 8 mm to define the volume of interest (VOI). Images were then converted from grayscale to pure black and white (binary images) for analysis. Grayscale images have pixels with values that range from 1-255. The range of 0-64 was converted to pure black, representing void areas or gas cells. Pixels in the range of 65-255 were converted to pure white to represent cell wall structures. The following morphometric parameters were calculated for quantitative analysis:

Structure Separation: Average of the thickness of the spaces between solid structures (cell walls) i.e. measure of air cell size (mm). Analysis also provides air cell size distributions in the form of histograms.

Structure Thickness: Average of the thicknesses of solid structure, used as a measure of average cell wall thickness (mm). Analysis also provides cell wall thickness distributions in the form of histograms.

Percent void volume: Percentage of black pixel counts (void areas or gas cells) to total pixel counts (black + white pixels).

2.2.8 Texture Profile Analysis (TPA)

A TA.XT Texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with 30 kg load cell and a 1.53 cylindrical probe was used. Two center slices each 1.25 cm thick were stacked together to result in a sample thickness of 2.5 cm. The test was conducted on three replicates of the center of each sample using return to start in compression mode, a trigger force of 5 g and strain of 40% with pre-test speed of 1.0 mm/s, test speed of 1.7 mm/s, relaxation of 5 sec and post-test speed of 10.0 mm/s (AACC approved method 74-09). The bread was compressed twice to give a two bite texture profile curve, from the textural parameters were obtained.

2.2.9 Statistical Analysis

The experiments were factorial design. Results were analyzed using analysis of Variance (ANOVA) in SAS (version 9.2) software (SAS Institute Inc., Cary, NC). Means and standard errors were obtained by using proc GLM procedure. One way ANOVA was performed using a Tukey test procedure to determine differences among treatments. Duplicates were prepared for the proximate analysis and rheological tests. Triplicates were prepared for test baking, crumb structure, TPA and bread microstructure.

2.3 Results and Discussion

2.3.1 Composition and Water Absorption Behavior

The physico-chemical properties of Karl 92 (HRW) and soft wheat (SW) flours and their respective bran fractions (as is, coarse and fine) are shown in Table 2.1. HRW flour has higher protein (17.81 *vs* 11.06%) and slightly higher ash content (0.59 *vs* 0.46%) than does SW flour. The lipid and fiber content for HRW and SW flours are not significantly different. HRW bran has higher protein (22.28 *vs* 18.01%), higher lipid (3.97 *vs* 2.84%), higher ash (5.59 *vs* 5.48%), and less fiber content (8.51 *vs* 8.74%) than does SW bran. The difference in composition of HRW and SW flours and brans fractions might be affected by several factors including wheat class, variety and growth conditions including environmental factors, location and agricultural practices. Studies have shown that the variance in baking qualities among wheat samples can be attributed to both genotype and environmental conditions (Hazen et al 1997; Bergman et al 1998).

As coarse and fine bran particle sizes were obtained by sieving this could have affected their compositions. The HRW coarse bran has higher protein (21.67 *vs* 17.46%), lipid (3.31 *vs* 2.28%), fiber (12.42 *vs* 12.31%) and ash (6.41 *vs* 6.27%) contents as compared to SW coarse bran. Similarly, HRW fine bran had higher protein (23.18 *vs* 19.16%), lipid (4.24 *vs* 3.37%) and ash (5.54 *vs* 5.09%) content except for fiber (9.65 *vs* 9.82%) content than did SW fine bran. In comparison to the coarse bran sample, fine bran has higher protein and lipid, and lower fiber and ash content than does coarse sizes of both HRW and SW brans. The sieved fine particles might be mainly from shorts and residual starch attached to bran pieces which usually have less fiber content than do coarse particles (Blasi et al 1998). Because the original bran (as is) was a combination of coarse and fine bran particles, the compositional data of as is bran are expected to lie between those of coarse and fine bran. This was the trend observed in Table 2.1 except for fiber content. This can explained by the particle size distribution in as is bran indicating high contribution of particles smaller than the fine bran fraction (i.e. <630μ).

Peyron et al (2002) reported that the bran particle size, obtained from wheat grains by milling is correlated with the extensibility of the outer grain layers. The outer pericarp, which surrounds the outer grain layers, is a thin tissue weakly attached to the intermediate layer. Its fragile mechanical nature, low extensibility and friability have been reported to lead a marked decrease in bran particle size during milling. The intermediate layer, containing different tissues (nucellar epidermis, testa and inner pericarp), has a complex and heterogeneous structure. The testa exhibits high plasticity, and tends to break into larger particle sizes during milling. Friability of the inner pericarp has been shown to be similar to that of the outer pericarp, resulting in a smaller particle size during milling. The strong association between the aleurone layer and the extensible intermediate layer (Peyron et al 2002) was reported to be responsible for the presence of aleurone layer mostly in intermediate sized bran particles. This supports the observed differences in ash contents of coarse and fine bran samples as shown in Table 2.1.

Water sorption behavior of HRW and SW bran samples (as is) was compared using equilibrium moisture content *vs.* water activity plots at room temperature. The protein content of HRW bran was observed to be higher than that of SW bran (Table 2.1) which could imply that HRW bran has a higher potential to absorb water. However, there was no significant difference in sorption isotherms of HRW and SW bran for all water activity levels as indicated by overlapping nature of the curves (data not shown). Other chemical constituents, such as the lipid content (3.97 *vs* 2.84%) and damaged starch content of HRW and SW bran should be taken into consideration to understand the sorption isotherms.

2.3.2 Dough Mixing Properties

The Farinograph water absorptions and mixing properties of HRW and SW flours in the presence of HRW and SW bran samples (0-10%) are shown in Figure 2.1. HRW base flour has higher water absorptions compared to SW flour, which was an expected difference between strong and weak wheat flours. Generally, high water absorption of flour is considered an indication of good baking performance. One reason could be that high protein content leads to both good baking performance and higher water absorptions. The other reason is the increased amount of damaged starch in the HRW flour, which, along with the protein content, is known to increase the water absorption capacity (Bloksma 1972; Greer and Stewart 1959; Meredith 1966).

Bran has high protein content as compared to flour and is expected to increase the water absorption capacity of flour with bran additions. In all four combinations of flour/bran systems (i.e. HRW/HRW, HRW/SW, SW/HRW and SW/SW) water absorptions increased gradually with increased level of bran additions independent of bran type and size. In general, HRW and SW bran additions resulted in up to 5.7% higher water absorptions in HRW flour while the increase in water absorption rates in SW flour was up to 1.5%.

HRW and SW as is brans displayed an identical water sorption behavior. Their moisture content increased from 7.5% to 16.0% as the storage relative humidity (RH) increased from 11 to 75%. Although the water sorption behavior of two bran sources was observed to be identical, inclusion of HRW and SW brans in base flours had differing degrees of influence on water absorptions of HRW and SW flour. Addition of SW bran to HRW flour caused significantly higher water absorptions compared to HRW flour/HRW bran systems. This could be explained by the type and level of interactions between different source of brans and the starch, protein and lipid components of the base flour. SW bran has less protein (18.0 *vs*. 22.3%), more lipid (2.8 *vs* 4.0%), more fiber (8.7 *vs* 8.5%) and slightly less ash (5.5 *vs.* 5.6%) content than HRW bran. Different wheat cultivars have been reported to produce bran with different chemical compositions and to exhibit different hydration capacities which inherently influence end product quality and crumb texture of breads (Nelles et al 1998; de Kock et al 1999).

HRW and SW bran additions to HRW flour decreased the arrival time and stability while increasing the mixing tolerance index (MTI). In HRW bran/HRW flour systems, while 5% bran addition decreased the arrival time slightly, bran addition of 10 % resulted in almost 3 min decrease in arrival time. SW bran addition to HRW flour resulted in 4.4 min shorter arrival time at 5% and 5.2 min shorter at 10% addition level. However, bran additions to weak flour were found to be somewhat beneficial leading to improved mixing properties in SW flour. Arrival time and stability of SW flour increased with increased bran addition, while the MTI values decreased significantly. HRW bran was observed to improve such properties slightly more than did SW bran. In SW flour systems arrival time doubled at 10% bran addition along with a significant increase in stability values from 2.6 min to up to 4.5 min. MTI values decreased from 76.5 to 49.0 and 42.0 with the addition of 10% SW and HRW bran, respectively.

Figure 2.1a shows the water absorption and mixing characteristics of HRW flour doughs with coarse and fine bran additions. The water absorptions increased for all combinations of flour and bran systems with coarse and fine particle sizes for the reasons discussed previously. In HRW flour/HRW bran systems, the coarse and fine bran particle sizes resulted in up to 4.0 *vs* 4.2% increase in water absorptions while for HRW flour/SW bran systems, the increase in water absorption was up to 5.5 *vs* 5.2%, respectively. Bran source was observed to have a greater effect than bran size. The HRW flour with SW coarse and fine bran additions exhibited higher water absorptions as compared to HRW flour with HRW coarse and fine bran additions. The trend for HRW flour/SW coarse and fine bran additions is similar to HRW flour/SW as is bran water absorptions.

The addition of HRW and SW coarse and fine brans to HRW base flour decreased the arrival time while increased the stability (Figure 2.1 b and c). The mixing tolerance indexes (MTI) were lower (data not shown) for all coarse and fine bran additions in HRW flours except for SW fine bran inclusion. The MTI increased from 3.0 to 14.0 BU in HRW/SWf systems which was much lower than that of HRW flour with bran as is additions. The coarser particles size had higher water absorption with the magnitude of the effect increasing with the level of bran addition. The decreased mixing stability may be caused by the disruption of the gluten networks by the bran particles. The effect on the gluten network might be greater for fine bran which has more particles than does coarse bran at equal bran substitution levels.

The change in mixing behavior of dough systems can be explained by the non-starch polysaccharide (arabinoxylan) content of the fractions. Wang et al (2006) reported that among bran and shorts fractions, total pentosan and water unextractable pentosan (WUP) content increased in the order of coarse bran > fine bran > shorts; while water extractable pentosan (WEP) content decreased in the order of shorts > fine bran > coarse bran with increasing ash content. Pentosans are known to have an effect on the water absorption of dough. Wang et al (2002) reported that WEP interfered with gluten formation indirectly by competing for water and thus changing conditions for gluten development. Similarly, the presence of WEP has been reported to delay the development time of gluten, suggesting a competition for water during the first stage of dough formation (Labat et al 2002).

The mixing properties of HRW and SW flours with HRW and SW bran additions (0-10%) were also studied using the Mixograph. The Mixograph water absorptions were correlated with Farinograph water absorptions. The peak time and peak value were higher for HRW base flour as compared to SW base flour, as expected. Usually, flours with good gas-holding properties and machinability have higher water absorptions, take longer times to mix, and have a better tolerance to over mixing than do poor quality flours. HRW flour doughs with HRW and SW bran inclusions had higher water absorptions which led to higher peak time. The peak time increased up to 0.5% in the HRW flours with HRW bran inclusions (0-10%) while the peak time decreased up to 0.5% with SW bran inclusion. The peak time for SW flour/HRW bran systems increased from 2.3-2.8 min with the increase of bran level while the peak time decreased up to 1.2 % in SW flour/SW bran systems with the exception of the 5% SW bran inclusion level. There was no significant difference in peak values for all combinations of HRW and SW flour and bran inclusion. In relation to bran size, HRW flours with HRW coarse and fine bran inclusions (0-10%), the peak mixing time increased up to 1.0% and 0.5%, respectively.

2.3.3 Mixing and Pasting Properties

Mixing and pasting properties of HRW and SW base flour doughs with and without bran inclusion (0-10%) were studied using the MixoLab which measures in real time the torque (expressed in Nm) produced by passage of the dough between the two kneading arms, thus allowing the study of the physic-chemical behavior of the dough. The method measures mixing behavior of dough samples subjected to both mixing and controlled heating. Thus it has the capability to measure dough properties such as dough strength and stability and starch pasting properties.

The initial stages of mixing involve the distribution of material and the hydration of the flour leading to stretching and alignment of the storage proteins and ultimately formation of a viscoelastic structure with gas retaining properties. Thus the initial phase of MixoLab curves provided clear information on the hydration capacity and dough development time of HRW and SW base flours with and without bran. During this stage, an increase in the torque (C1) was observed until a maximum was reached after which the dough was able to resist the deformation for some time. HRW base flour had higher hydration capacity (93.1% *vs* 86.3%) and longer development time compared to SW base flour. The higher hydration capacity and development time of HRW flour were expected and probably the result of higher protein content of HRW flour. Effects of bran inclusion were studied at constant hydration levels. Thus the increase in C1 torque values for bran containing dough systems indicated their higher hydration capacity compared to control flours. Dough development time increased as the bran level increased in all flours. The MixoLab dough stability results were correlated with the Farinograph stability values. Dough stability of HRW base flour systems decreased slightly (from 11.7 min to 11.3 min) with 5% bran addition. It further decreased to 8.9 min when 10% bran was added. However, in SW (weaker) flour systems, bran addition improved the mixing quality by increasing the dough stability from 4.5 min to 7.0-7.2 min at 5-10% bran level.

The next MixoLab mixing stage, where dough temperature gradually increased from 30 to 90°C, resulted in a decrease in torque (C2), which is attributed to polymer softening due to mixing combined with heating. A decrease in the degree of this change was observed with an increase in the addition of HRW and SW bran to base flours. C2 values of HRW base flours increased from 0.57 Nm to 0.64 Nm (5% bran) and 0.71 Nm (10% bran) indicating less gluten network weakening due to prolonged mixing and temperature increase. This effect was less pronounced for SW base flours with an increase from 0.49 Nm to 0.54 Nm and 0.60 Nm with 5 and 10% bran addition, respectively. Moreover, a decrease in slope α (degree of network softening) with bran addition was observed.

Temperature increase and shear also contribute modifications of physico-chemical properties of the starch known as gelatinization and pasting. During heating, HRW and SW base flours contributed to higher starch pasting peak (C3), better cooking stability (C4) and set-back viscosity of starch (C5). With increasing levels of HRW or SW bran inclusions, C3, C4 and C5 shifted to larger values in parallel to with increased C1 values due to competition for water at constant water absorption. In both HRW and SW flour systems, peak viscosity (C3) values increased by only 0.03-0.04 Nm at 5% bran inclusion while the difference was up to 0.10-0.12

Nm at 10% addition. Similar trends were observed for C4 and C5 values with a gradual increase in torque readings with increasing levels bran additions.

In HRW flour systems, increases in C3 were accompanied by a reduction in C3-C4 difference indicating starch stabilization occurred with bran addition. In SW flour systems an opposite trend was observed. The above results are consistent with studies examining formulated bread doughs during mixing and heating (Collar et al 2007), and the effect of hydrocolloids on the thermo-mechanical properties of wheat dough (Rosell et al 2006).

2.3.4 Bread Macrostructure

The loaf volumes of HRW and SW flour breads with and without added bran are shown in Figure 2.2a. The HRW control bread had higher loaf volumes than did SW bread, as expected. The control breads (0% bran) in general had higher loaf volumes than their bran added counterparts. The loaf volume of HRW breads decreased up to 26.5% with bran addition and significant differences were observed with respect to source and size of bran. The loaf volume of HRW breads decreased from 1017 to 753 cc (26.0%) when 5-10% percent HRWa was bran added while HRWf and HRWc bran addition caused decreases up to 20.8 and 32.8%, respectively.

SW bran addition resulted in relatively less detrimental effect on the loaf volumes of HRW breads compared to breads containing HRW bran. The loaf volume of HRW breads decreased in the range of 10.2-11.5% when 5-10% percent SW bran was present. For HRW/HRW and HRW/SW systems, coarse bran was observed to be the most detrimental, followed by as is and fine bran sizes. Small particle size seemed to be physically less disruptive to gluten network during dough development. The basis for differences between HRW and SW bran source additions in HRW flours was not clear and will be further investigated.

Bran addition influenced the loaf volume of SW base flour breads only to a limited extent. Bran added at 5% level was observed to be slightly beneficial as the loaf volume increased up to 4%, while 10% bran addition caused around 8% decrease in the loaf volume. Observed decreases in loaf volumes with bran additions are in agreement with previous work (Shetlar and Lyman 1944; Pomeranz et al 1977; Dubios 1978; Shorgen et al 1981; Dreese and Hoseney, 1982; Lai et al 1989a-c; Zhang and Moore, 1999). Dreese and Hoseney (1982) and Rao and Rao (1991) reported that addition of bran to dough formulations generally increases the level of water required.

In addition to bread volume, crumb grain and crumb texture are also important quality factors in wheat-based bread products. In this study, C-cell data including numbers of cells, cell area and wall thickness were used for macrostructural characterization of HRW and SW breads (Figure 2.2 b, c, d). In these analysis, number of cells reflects the number of discrete cells detected within the slice while cell diameter reflects the coarseness of crumb texture. At constant loaf volume, a high number of cells may indicate a fine crumb structure. Breads with lower loaf volume were observed to have fewer and thicker cell walls and larger cell size (Zghal et al 2001). Cell number decreased with increased bran addition irrespective of bran source or size. Overall, decreasing cell numbers in combination with increasing cell size and cell wall thickness indicate coalescence of air cells during fermentation and/or baking resulting in low loaf volumes.

2.3.5 Bread Microstructure

Microstructural features of HRW and SW breads were studied through non-destructive 3D analysis of bread specimens taken from the loaf center using an x-ray microtomograph (XMT). XMT analysis provided quantitative information on void volume, cell size and cell size distribution, cell wall thickness and cell wall thickness distribution and cell connectedness. Cell wall thicknesses and air cell size distributions in HRW and SW breads with and without HRW and SW bran are shown in

Figure 2.3. Bran addition, irrespective of the source and size, increased the mean cell wall thickness and air cell sizes and produced a gradual shift in distribution curves towards higher values. Bubbles entrained in the HRW base flour bread (no bran) were dispersed over a narrower size range than were those in the bran-containing dough. Higher mean bubble size values were observed in bran-containing dough samples probably due to weakening effect of bran on the gluten network causing gas bubble coalescence. Increased levels of bran addition increased the average cell wall thickness of both HRW and SW breads. These observations were in accordance with the macroscopic properties of the bread. Loaf volumes decreased as the bran addition increased in HRW and SW flours as discussed above. Bran particles interfere with gluten development during mixing which weakens the gluten network and leads to gas bubble coalescence during proofing and baking. The cell wall thickness distribution of SW/HRW and

SW/SW flour/bran systems had larger shifts towards right as compared to HRW flours with HRW and SW bran additions.

Consistent with the loaf volume data, SW bran addition resulted in relatively less detrimental effect on the void volume. HRW flour breads with SW bran had relatively higher void volume, smaller average cell size and cell wall thickness, which collectively indicate a less cell coalescence during proofing and/or baking (Figure 2.4a, b and c). Bran type did not have a significant effect on the microstructure of SW flour breads as the SW flour was intrinsically lower in breadmaking quality flour due to its low protein content. No large differences were observed in bread microstructure with respect to bran size (Figure 2.4). All bran sizes caused a significant change in cell wall thickness and cell size distributions towards the larger end of the scale. The larger gas cells are likely the result of coalescence caused by the instability of bubble walls. Overall, the effect of wheat bran inclusion in HRW and SW flours was on increase in water absorption which further affected the loaf volumes. This indicates that at least some the additional water required when bran is included is retained during baking. With increased bran substitution, the external appearance of the loaves became coarser. The observations are in agreement with Dreese and Hoseney (1982), and Rao and Rao (1991).

2.3.6 Bread Texture Quality

Figure 2.5 shows the texture profile analysis (TPA) hardness values for HRW and SW breads with and without bran. Hardness of HRW base flour breads was lower than that of SW bread. This was expected from the macro- and microstructural differences detailed above. SW flour breads had poor textural quality and lower loaf volumes as compared to HRW flour breads.

TPA hardness increased as the bran level increased in both base flours. With increased bran addition, the loaf volumes decreased and the increased average cell wall thickness and air cell size led to poor texture quality. Both the bran source and particle size affected the texture quality of HRW flour breads. SW bran additions (0-10%) to HRW flour were less detrimental to texture as minimal or no increase was found in hardness values of HRW/SW flour/bran systems compared to control bread. SW bran addition up to 5% has improved the texture as indicated by a slight decrease in hardness values. As explained above, SW bran particle size had higher water absorptions, higher resulting loaf volumes with slightly less increased cell wall thickness which resulted in improved texture quality in HRW/SW systems as compared to HRW/HRW systems. HRW/SWf flour systems had higher hardness values compared to that of HRW/SWc which is in accordance with other studies. Collins (1983), Collins et al (1985), Collins and Young (1986), and de Kock et al (1999) reported that fine wheat bran gave a denser crumb structure, and smaller loaf volumes than did coarse brans.

2.4 Conclusions

Addition of wheat bran to bread dough formulations had significant effects on mixing properties, test baking and crumb structure. Increased bran content increased water absorptions. HRW base flour performed better compared to its counterpart SW both during dough mixing and baking, which was expected. In general, bran addition affected the performance of HRW flour negatively. However, SW and HRW bran additions into SW flour doughs systems slightly improved their mixing time as indicated by increased mixing tolerance indices. SW bran addition to HRW flour, resulted in relatively higher loaf volumes and better crumb texture compared to HRW/HRW flour/bran systems. Increased bran inclusion increased the baked crumb hardness of both HRW and SW flour systems**.** Bran particle size affected mixing and baking properties. Coarser particles gave better mixing properties and larger loaf volumes than did finer particles, but also gave open textures at higher bran levels. Increased bran additions increased the cell wall thickness of resulting breads baked from both HRW and SW flour dough. Microstructural analysis of bran containing bread samples indicated a gradual shift in air cell size distributions towards higher values. However, the experiments done using different particle sizes produced results that are contradictory with some of the previously published studies. This aspect is worthy of more detailed investigation.

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Table 2.1Compositional analysis of HRW and SW Flour and bran

Chemical composition (%)	Flour samples		Bran samples					
	HRW	SW	HRWa	SWa	HRWc	SWc	HRWf	SWf
Protein	17.81 ± 0.02^{e}	11.06 ± 0.01^{g}	22.28 ± 0.02^{b}	18.01 ± 0.03^{e}	21.67 ± 0.07^{c}	17.46 ± 0.01^{f}	23.18 ± 0.18^{a}	19.16 ± 0.17^{d}
Lipid	1.04 ± 0.03^{g}	1.04 ± 0.01^{g}	3.97 ± 0.02^{b}	2.84 ± 0.03^{e}	3.31 ± 0.01^{d}	2.28 ± 0.02^{f}	4.24 ± 0.01^{a}	3.37 ± 0.03^{c}
Crude fiber	0.04 ± 0.01^{f}	0.05 ± 0.06^{f}	8.51 ± 0.09^{e}	8.74 ± 0.01^{d}	12.42 ± 0.07^{a}	12.31 ± 0.07^{a}	9.65 ± 0.01^{c}	9.82 ± 0.07^{b}
Ash	0.59 ± 0.01^{f}	0.46 ± 0.01^{g}	5.59 ± 0.02^{c}	5.48 ± 0.01^{d}	6.41 ± 0.02^{a}	6.27 ± 0.03^{b}	5.54 ± 0.02^{c}	5.09 ± 0.03^{e}

*Protein (Flour)= n x 5.27 factor
*Protein (bran)= n x 6.25 factor
*HRW= Hard Red Winter, SW= Soft Wheat, a=as is, c=coarse, f= fine
Data are mean of duplicates ± standard deviation.

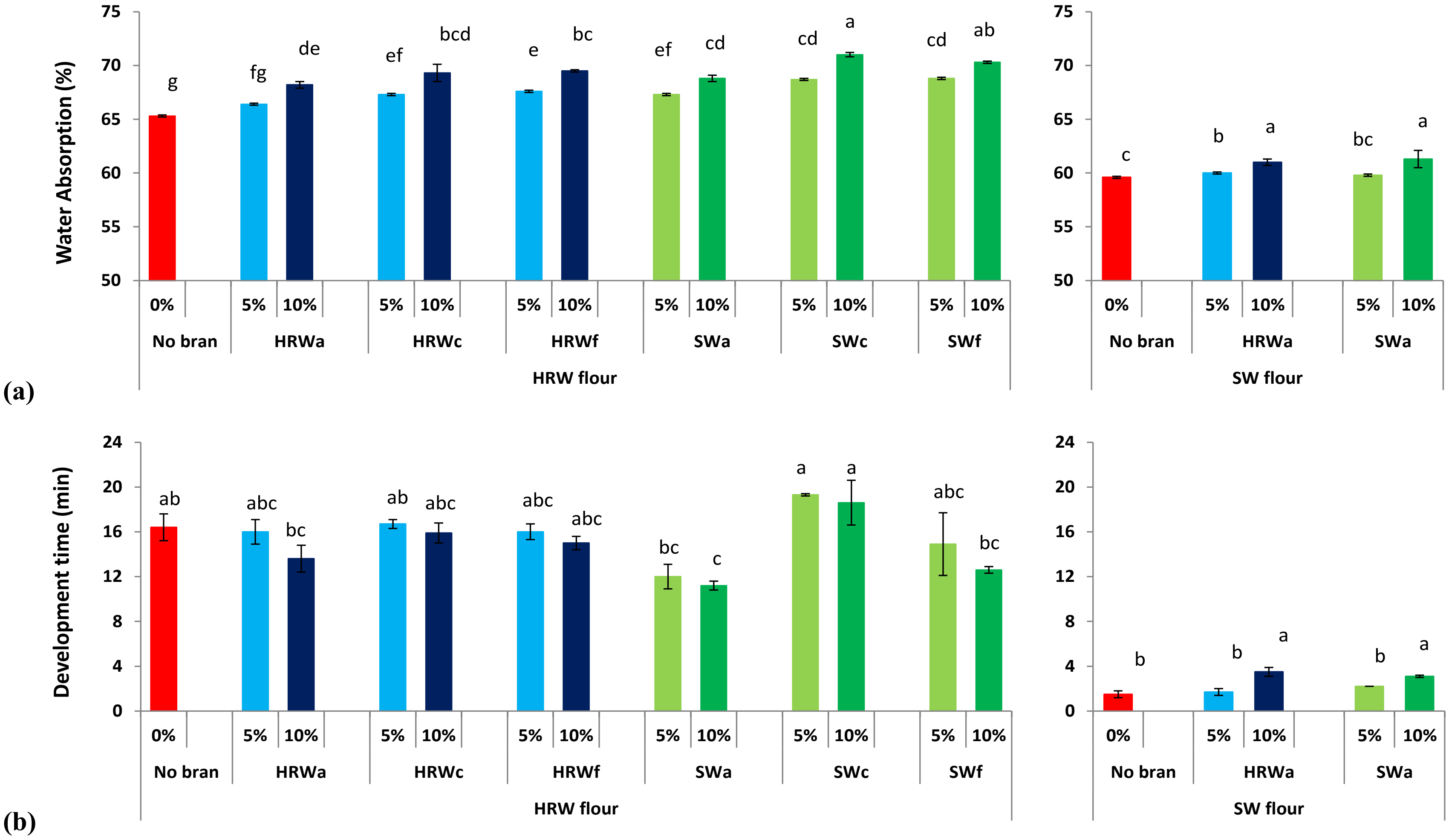
Values within the row with the same letter are not significantly different from each other at $p \leq 0.05$.

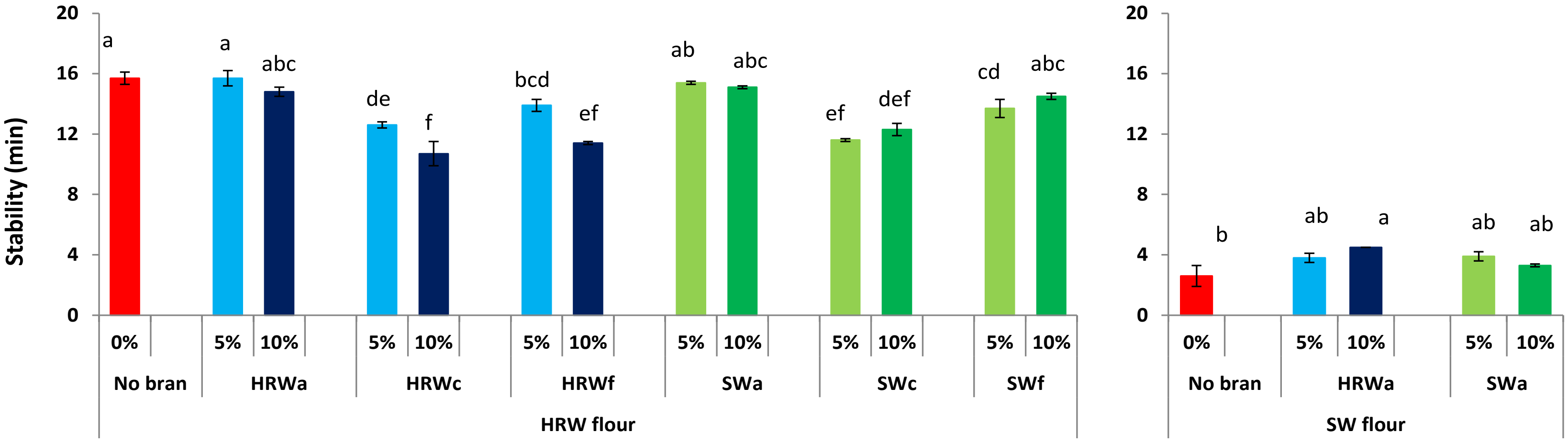
Table 2.2 MixoLab mixing and pasting profile parameters of Hard Red Winter (HRW) and Soft White (SW) flour doughs in the presence of bran

	HRW flour				
	No bran	**+5% HRWa**	**+10% HRWa**	**+5% SWa**	**+10% SWa**
Water absorption (% db)	93.1	93.1	93.1	93.1	93.1
Amplitude (Nm)	0.07 ± 0.01^{bc}	0.09 ± 0.00^{bc}	0.095 ± 0.01^{bc}	0.09 ± 0.00	0.095 ± 0.01^{bc}
Stability (min)	11.67 ± 0.99^{ab}	12.1 ± 0.47^{a}	9.44 ± 0.11^{cd}	10.52 ± 1.27^{bc}	8.43 ± 0.71^{de}
C1 (Nm)	1.15 ± 0.01^{e}	1.28 ± 0.00^{bc}	1.38 ± 0.03^{a}	1.31 ± 0.01^{b}	1.435 ± 0.02^{a}
C2 (Nm)	0.565 ± 0.01^{ef}	0.645 ± 0.04^{b}	0.71 ± 0.00^{a}	0.64 ± 0.01^{bc}	0.705 ± 0.01^{a}
C3 (Nm)	1.835 ± 0.01^{e}	1.89 ± 0.03^{d}	1.99 ± 0.00^{b}	1.85 ± 0.03^{e}	1.92 ± 0.00^{cd}
C4 (Nm)	1.745 ± 0.04^{d}	1.875 ± 0.02^{bc}	1.93 ± 0.01^{ab}	1.84 ± 0.06^{c}	1.865 ± 0.01^{bc}
C5 (Nm)	2.65 ± 0.00^{f}	2.83 ± 0.06^{de}	2.985 ± 0.05^{b}	2.822 ± 0.06^{de}	2.815 ± 0.01^{de}
Alpha (-)	-0.091 ± 0.00^{a}	-0.056 ± 0.06^{a}	-0.049 ± 0.12^{a}	-0.058 ± 0.04^{a}	-0.072 ± 0.10^{a}
Beta (-)	0.373 ± 0.02^{ab}	0.427 ± 0.02^{a}	0.393 ± 0.06^{ab}	0.345 ± 0.06^{ab}	0.379 ± 0.01^{ab}
Gamma (-)	-0.026 ± 0.02^{ab}	**0.001 ± 0.01^{a}**	0.003 ± 0.02^{a}	-0.011 ± 0.001^{ab}	-0.031 ± 0.00^{b}

	SW flour				
	No bran	**+5% HRWa**	**+10% HRWa**	**+5% SWa**	**+10% SWa**
Water absorption (% db)	86.3	86.3	86.3	86.3	86.3
Amplitude (Nm)	0.155 ± 0.04^{a}	0.1 ± 0.01^{b}	0.075 ± 0.01^{bc}	0.095 ± 0.01^{bc}	0.065 ± 0.01^{c}
Stability (min)	4.49 ± 0.65^{g}	7.41 ± 0.16^{ef}	7.66 ± 0.06^{ef}	6.6 ± 0.66^{f}	6.71 ± 0.09^{f}
C1 (Nm)	1.21 ± 0.04^{de}	1.18 ± 0.01^{de}	1.295 ± 0.05^{b}	1.225 ± 0.04^{cd}	1.315 ± 0.04^{b}
C2 (Nm)	0.49 ± 0.00^{g}	0.54 ± 0.01^{f}	0.585 ± 0.01^{de}	0.54 ± 0.01^{f}	0.605 ± 0.04^{cd}
C3 (Nm)	1.935 ± 0.01^{c}	1.99 ± 0.01^{b}	2.07 ± 0.00^{a}	1.95 ± 0.00^{c}	2.005 ± 0.02^{b}
C4 (Nm)	1.865 ± 0.01^{bc}	1.905 ± 0.02^{abc}	1.965 ± 0.01^{a}	1.835 ± 0.01^{c}	1.85 ± 0.08^{a}
C5 (Nm)	2.78 ± 0.01^{e}	2.925 ± 0.06^{bc}	3.085 ± 0.02^{a}	2.74 ± 0.03^{ef}	2.88 ± 0.06^{cd}
Alpha (-)	-0.063 ± 0.00^{a}	-0.05 ± 0.01^{a}	-0.074 ± 0.01^{a}	-0.067 ± 0.01^{a}	-0.075 ± 0.00^{a}
Beta (-)	0.37 ± 0.01^{ab}	0.36 ± 0.07^{ab}	0.405 ± 0.00^{ab}	0.334 ± 0.03^{b}	0.37 ± 0.00^{ab}
Gamma (-)	-0.021 ± 0.01^{ab}	-0.018 ± 0.01^{ab}	-0.027 ± 0.00^{ab}	-0.016 ± 0.03^{ab}	-0.035 ± 0.01^{b}

Values within the row with the same letter are not significantly different from each other at $p \leq 0.05$.
Please refer to the *Materials and Methods* for the description of the parameters listed.

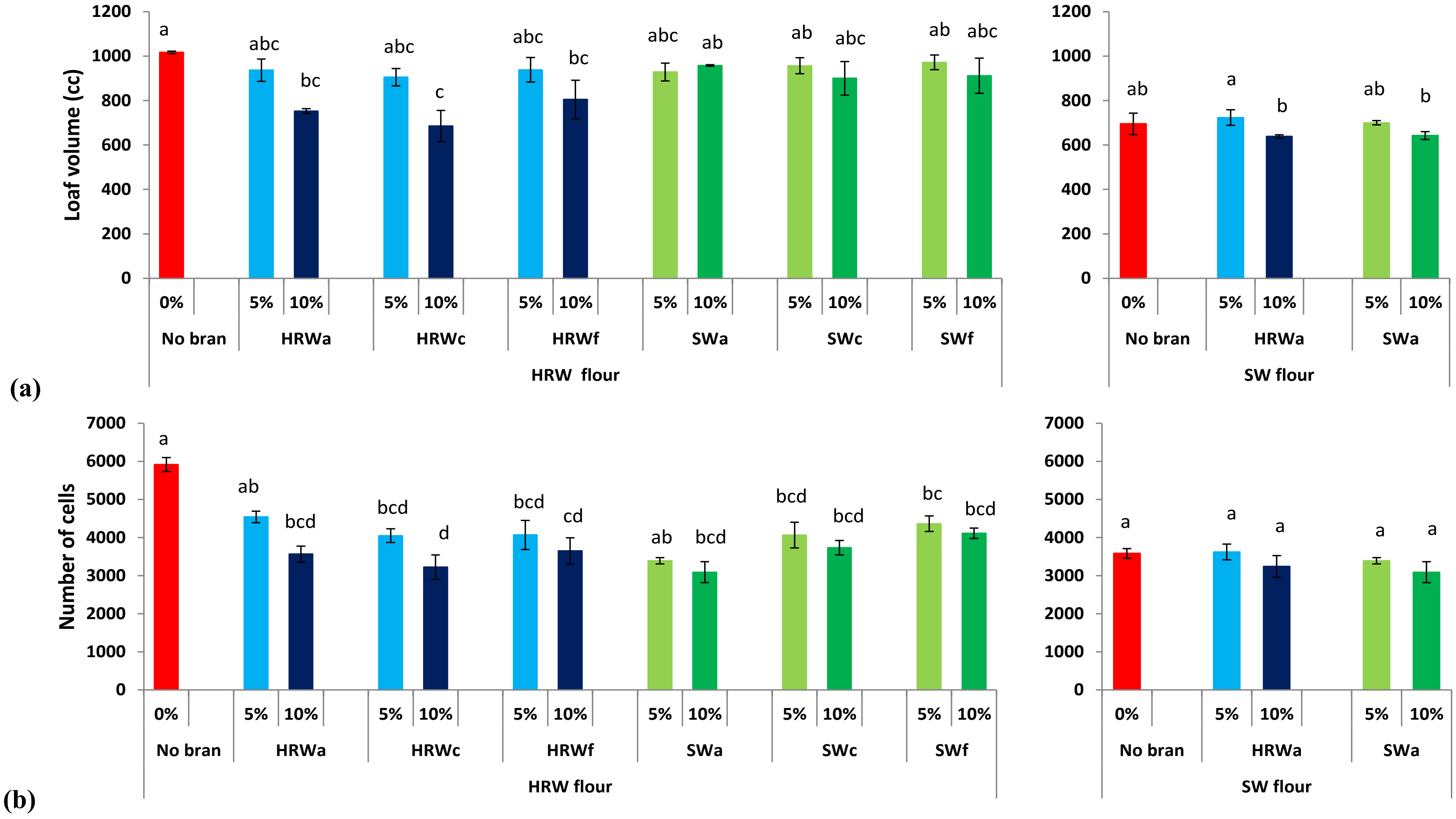

Water Absorption (%)
75
70
65
60
55
50
g
fg
de
ef
bcd
e
bc
ef
cd
cd
a
cd
ab
0%
5%
10%
No bran
HRWa
HRWc
HRWf
SWa
SWc
SWf
HRW flour
c
b
a
bc
a
SW flour
(a)
Development time (min)
24
20
16
12
8
4
0
ab
abc
bc
ab
abc
abc
abc
bc
c
a
a
abc
bc
b
b
a
b
a
(b)



(c)

Figure 2.1 Dough development properties of Hard Red Winter (HRW) and Soft White (SW) flour doughs in the presence of bran. (a) Water absorption, (b) dough development time, (c) stability.

a: as is, c: coarse, f: fine
Values within the HRW flour compared to no bran with the same letter are not significantly different from each other at p≤0.05.
Values within the SW flour compared to no bran with the same letter are not significantly different from each other at p≤0.05.


(a)
Loaf volume (cc)
1200
1000
800
600
400
200
0
a
abc
bc
abc
c
abc
bc
abc
ab
ab
abc
ab
abc
ab
a
b
ab
b
0%
5%
10%
No bran
HRWa
HRWc
HRWf
SWa
SWc
SWf
HRW flour
SW flour
(b)
Number of cells
7000
6000
5000
4000
3000
2000
1000
0
a
ab
bcd
bcd
d
bcd
cd
ab
bcd
bcd
bcd
bc
bcd
a
a
a
a
a
0%
5%
10%
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HRWf
SWa
SWc
SWf
HRW flour
SW flour

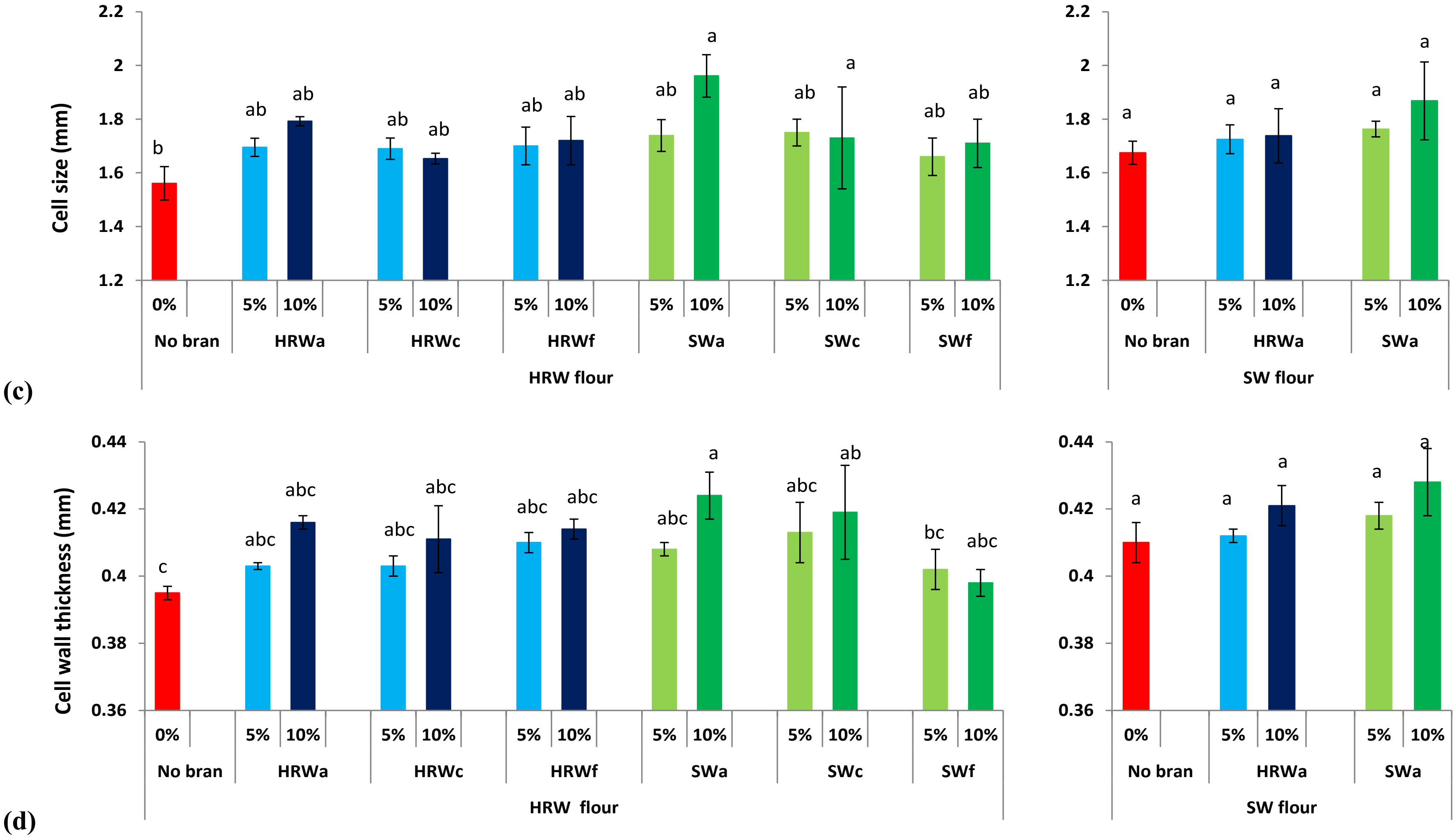


Figure 2.2 Macrostructure of Hard Red Winter (HRW) and Soft White (SW) flour breads in the presence of bran. (a) Loaf volume, (b) total number of cells, (c) cell size, (d) cell wall thickness

a: as is, c: coarse, f: fine
Values within the HRW flour compared to no bran with the same letter are not significantly different from each other at p≤0.05.
Values within the SW flour compared to no bran with the same letter are not significantly different from each other at p≤0.05

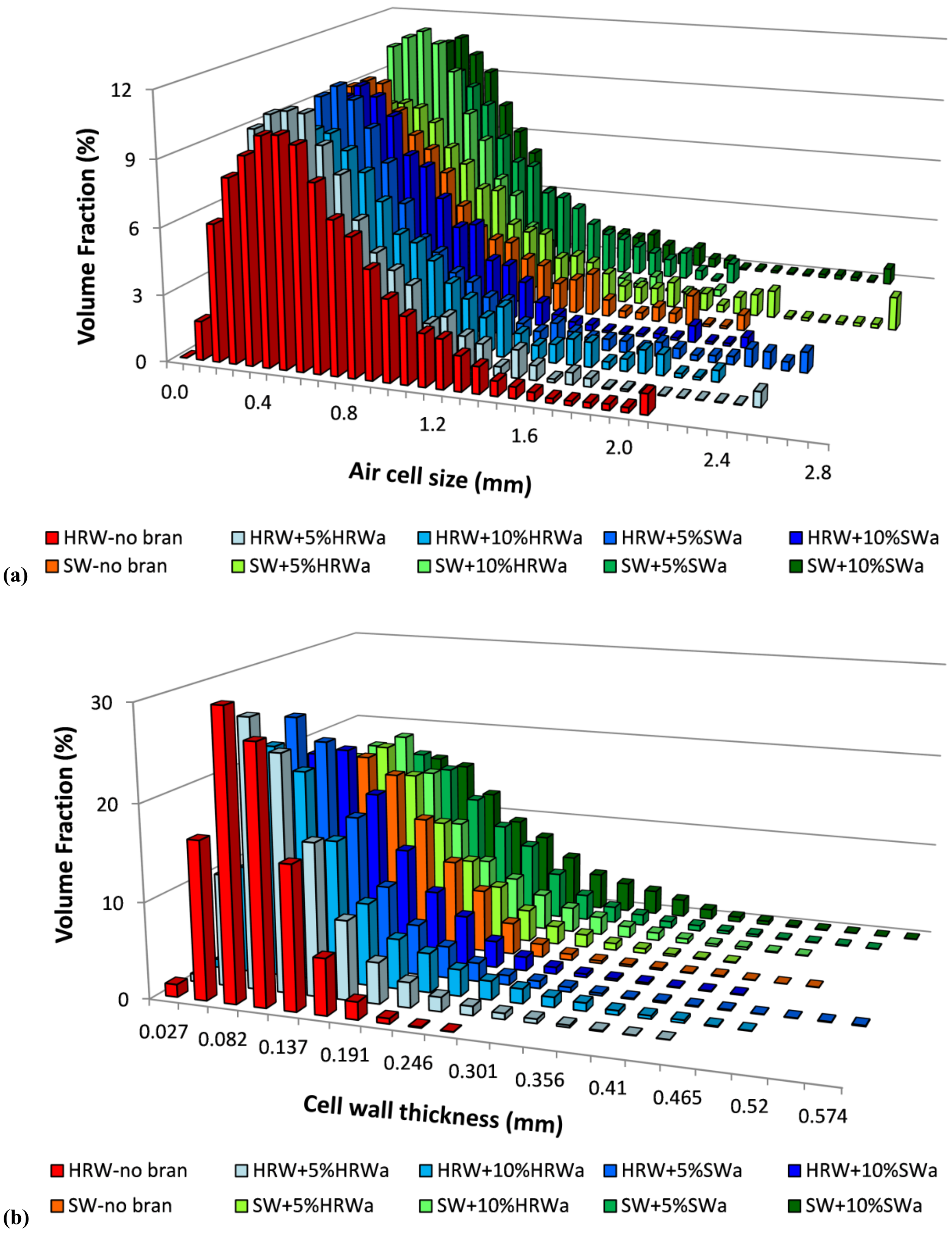
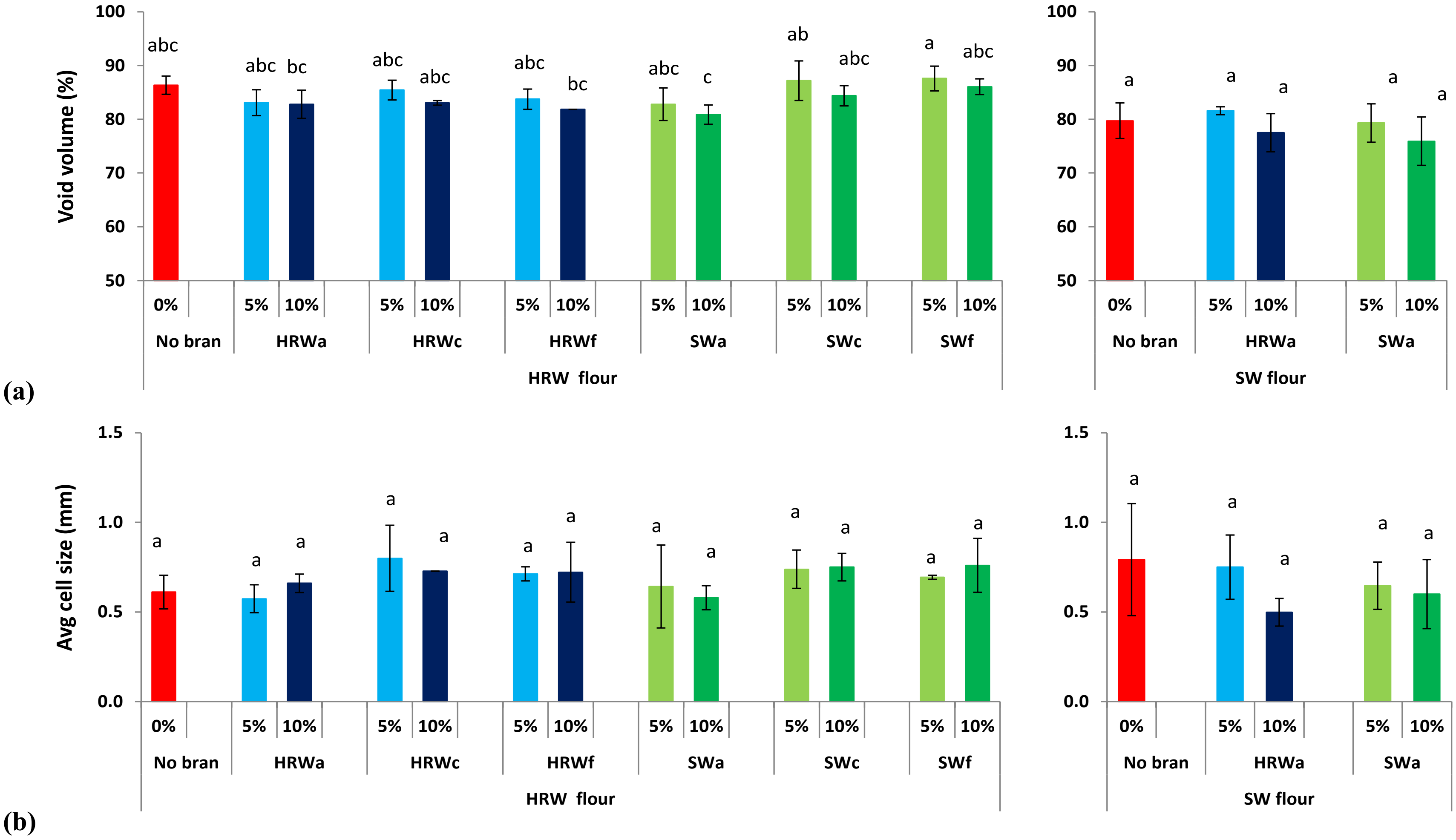
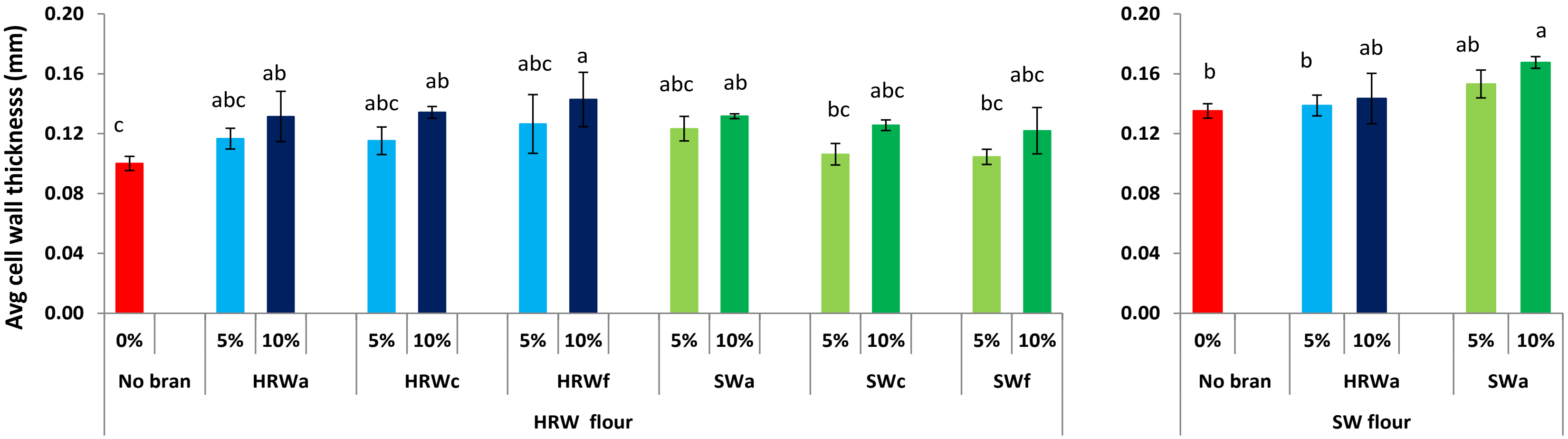


Figure 2.3 Microstructure of Hard Red Winter (HRW) and Soft White (SW) flour breads in the presence of bran. (a) Air cell size distribution, (b) cell wall thickness distribution.


(a)
Void volume (%)
100
90
80
70
60
50
abc
abc
bc
abc
abc
abc
bc
abc
c
ab
abc
a
abc
0%
5%
10%
No bran
HRWa
HRWc
HRWf
SWa
SWc
SWf
HRW flour
a
a
a
a
a
No bran
HRWa
SWa
SW flour
(b)
Avg cell size (mm)
1.5
1.0
0.5
0.0
a
a
a
a
a
a
a
a
a
a
a
a
a
HRW flour
SW flour



(c)

Figure 2.4 Microstructure of Hard Red Winter (HRW) and Soft White (SW) flour breads in presence of bran. (a) Void volume, (b) average cell size, (c) average cell wall thickness

a: as is, c: coarse, f: fine
Values within the HRW flour compared to no bran with the same letter are not significantly different from each other at $p \leq 0.05$.
Values within the SW flour compared to no bran with the same letter are not significantly different from each other at $p \leq 0.05$.

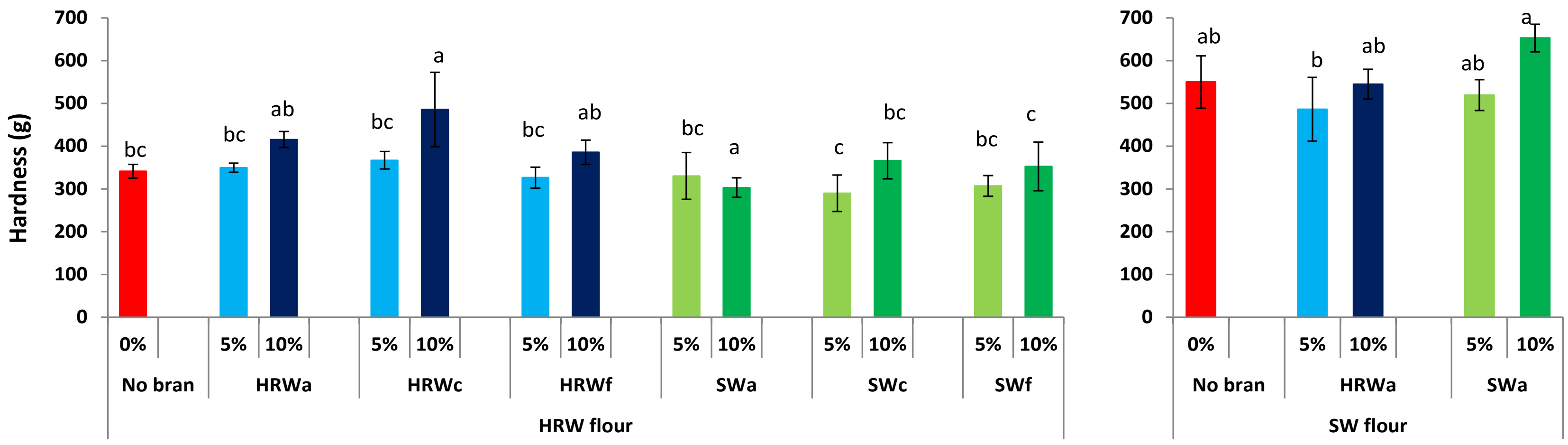


Figure 2.5 Texture Profile Analysis (TPA) hardness of Hard Red Winter (HRW) and Soft White (SW) in the presence of bran

a: as is, c: coarse, f: fine
Values within the HRW flour compared to no bran with the same letter are not significantly different from each other at p≤0.05.
Values within the SW flour compared to no bran with the same letter are not significantly different from each other at p≤0.05.

Chapter 3 - Effect of bran fractions of varying anatomical origin and size at varying replacement levels on dough development, and small and large deformation rheological properties

Abstract

The effect of adding brans of different particle size and anatomical origin in two wheat flours of different breadmaking quality were studied. Hydration behavior, mixing and dough development using empirical rheological methods, as well a fundamental rheology at small and large deformation rates were explored. The results revealed that the rheological behavior of bran enriched doughs depend on type of base flour (strong and weak), bran type, bran replacement level (0. 5, 10%), and the dough development protocol (constant versus optimum water absorption). Addition of bran did not affect dough hydration in excess water as measured by water solvent retention capacity, while the water absorption during dough development increase significantly independent from the bran type and replacement level. In general, a delay in dough development time of strong wheat flour was observed in the presence of bran. Weak flour doughs benefited from inclusion of bran as inherently low peak height and stability of these doughs improved in the presence of bran. Mechanical spectra provided set of rheological parameters including storage modulus (G′), loss modulus (G″), complex modulus (G*), tangent delta (tan δ), and complex viscosity (η*) in the linear viscoelastic range. Temperature sweeps indicated a slight decrease in G′ and G" until around 55-60°C. In the same temperature range, presence of bran increased the moduli of composite four compared to that of the control flours, however, upon the onset of gelatinization this difference diminished completely and resulted in not significant differences in peak G′and peak G″values. Creep compliance parameters indicated that both bran source and bran replacement had significant effect on maximum compliance (J_{max}) and elastic compliance (J_e). In general, J_{max} values of composite flour doughs were lower than that of control dough indicating that doughs in the presence of bran exhibit greater resistance, show smaller creep strain than their counterpart. Finally, the bran type affected uniaxial extensional properties, maximum resistance (R_{max}) and elasticity (E), significantly independent from the type of base flour.

3.1 Introduction

Wheat flour dough is a composite, incompressible, viscoelastic, soft-solid material with complex rheology. It is considered as hydrated protein network that behaves nonlinearly during large deformations (Tanner et al 2008). Gluten protein is made up of glutenin and gliadin proteins of different molecular weights, and the entanglements of these proteins during mixing and their subsequent interaction with starch polymers have the greatest effect on the final bread quality (Bloksma 1990a). Starch granules in the protein network of dough absorb water and swell at temperatures below its gelatinization temperature. Above the gelatinization temperature (i.e. during baking), the starch granules continue uptake of water until granules that are fully hydrated rupture and increase the dough viscosity by forming a starch network (Olkku and Rha 1978). Lipids native to the wheat flour can affect the starch gelatinization temperature by complexing with amylose to inhibit swelling, thereby increasing starch gelatinization temperature (Morrison 1995). Along with components of flour, processing conditions are important for bread quality.

The purpose of determining dough rheology is to have better control over each processing stage as well as over the final products. This can be accomplished by relating rheological behavior to predict functionality during mixing, sheeting, and baking (Dobraszczyk and Morgenstern 2003). By understanding how each component of dough contributes to the overall material properties, it becomes possible to manipulate the dough and get consistent, desirable results (van Vliet et al 1992). The production of good quality bread requires a balance of three requisite rheological properties: Extensibility, viscosity, and strain hardening. Extensibility refers to the dough's ability to stretch (lengthen) without rupture, viscosity is the resistance to flow. Strain hardening is a more complex property; it is the increase of stress at a rate larger than proportional to the strain rate (van Vliet et al 1992). In other words, strain hardening is the ability of a cell to undergo biaxial extension and not rupture and it has a large influence on the stability of gas cells (Sroan and MacRitchie 2009). Extensibility must persist throughout baking to minimize gas cell membranes from fracturing prematurely. Dough viscosity must be high enough to arrest gas cell ascension (Bloksma 1990b), and strain hardening must surpass a certain level to ensure proper retention of gas cells that expand during fermentation and baking.

Viscoelasticity describes a rheological behavior combining that of a viscous liquid and an elastic solid. Dough viscoelasticity has a great influence on the dough machinability, texture characteristics, and final product stability (Uthayakumaran et al 2000). Viscoelastic behavior has been attributed mainly to the gluten protein fraction of dough. A great number of concurrent processes during mixing and resulting gluten network development have been proven to affect the dough performance (Cornec et al 1994; Dobraszczyk and Morgenstern 2003; Song and Zheng).

Rheological tests have been devised that attempt to simulate the breadmaking processing in order to reveal how flow behavior relates to material (dough) composition, as well as describing mechanical properties of the dough for quality control purposes. Data obtained from rheological characterization can be useful in the development of food products through ingredient selection, product improvement and optimization, choosing and optimizing manufacturing techniques, and developing packaging and storage methods. The use of both small and large deformation measurement of dough is critical for a complete understanding of dough rheology. Large deformation methods simulate stress-strain conditions found in commercial processing and therefore can disclose food textural properties under those conditions as well as indicate final breadmaking quality (Dobraszczyk and Morgenstern 2003; Davidou et al 2008). Small deformation techniques are most useful for measuring viscoelastic properties of dough (Angioloni and Collar 2009). Empirical descriptive techniques are generally more accepted than fundamental method. Although fundamental rheometry provides a great deal of important knowledge in dough rheology, these fundamental techniques are often considered to be time-consuming and labor intensive.

Good baking performance is dependent on several rheological properties of wheat flour doughs. In the whole bread making process, bakers assess the properties of the dough at various process stages. The assessment of the dough properties is done using specialized equipment such as the Farinograph, Mixograph, Extensograph and Alveograph. These instruments imitate the deformation experienced by the dough during processing and provide measurements of the physical properties of the dough. These measurements are empirical and generally give good correlations with bread making performance. However, the results cannot be interpreted in terms of material properties (Menjivar 1990). Fundamental rheological methods are used to characterize those material properties. Several studies have been reported on small deformation tests for wheat flour doughs and correlated results with breadmaking quality. Dynamic testing has become a powerful approach for examining structure and fundamental properties of wheat flour doughs and proteins because of its sensitive response to the structure variations (Tronsmo et al 2003). Several authors have studied the rheological properties (storage modulus, G′; loss modulus, G″; and loss tangent, tan δ) of flours of different strengths. The good quality flours have lower loss tangent values when dynamic rheology testing is performed (Miller and Hoseney 1999).

In spite of the large amount of information available on the empirical and fundamental rheological properties on wheat doughs and gluten, very little information is available on small deformation tests on bran substituted flour doughs. Previous work reported a general deterioration occurs in dough rheological properties when bran is added (Shetlar and Lyman 1944; Pomeranz 1977; Lai et al 1989; Zhang and Moore 1997). Bran addition causes reduction of dough strength especially at high bran levels. Most of the research has been done on flour-water doughs; not much information is available on the effect of the bran particle size on dough using fundamental rheology.

Treated and untreated bran flour doughs studied using dynamic oscillatory tests found that G′ values were higher than that of control flour dough (Amirkaveei et al 2009). The loss tangent of untreated bran flour doughs was higher than that of control flour doughs indicating that untreated bran weakens the gluten matrix while the treated bran strengthens the protein matrix to some extent (Amirkaveei et al 2009). Bonnand-Ducasse et al (2010) studied the effect of wheat dietary fiber fractions from starchy endosperm, aleurone layer and bran on bread dough development and rheological properties. The results showed that wheat dietary fiber fraction in the dough led to a decrease in mixing peak time and to an extent to an increase of peak bandwidth. Similar peak time results were observed decrease in peak time with increasing levels of wheat dietary fiber added to the flours (Jelaca and Hlynka 1971; Courtin et al 1999). Small deformation tests found increases in viscoelastic moduli with increased fiber content. This could be explained by two hypotheses: (i) gluten and fiber compete for water in the mixing process, or (ii) the fiber acts as filler in the viscoelastic network (Izydorczyk et al 2001; Wang et al 2003a; Uthayakumaran et al 2002).

Peressini et al (2009) evaluated the influence of soluble dietary fibers such as inulin on the rheological and breadmaking properties of wheat doughs. The addition of inulin affected the viscoelastic properties indirectly by changing water-flour ratios or directly by affecting dough elasticity. The same researchers studied the viscoelastic properties of doughs at constant water absorption because the use of Farinograph optimum absorptions does not allow decoupling the effects of hydration and fiber. Storage modulus decreased as the amount of inulin in the wheat doughs increased. These results are in accordance with studies conducted by Rouillé et al (2005).

Ahmed et al (2015) studied the mechanical rigidity of β-glucan concentrate supplemented doughs. Rigidity was strongly influenced by the concentrate particle size and solid-to-water ratio. Their studies suggested that large particles occupy more space during aggregation than do small particles due to less efficient particle packing and that this leads to more volume occupied by large particles and a concomitant increase in flow resistance. Solid-like behavior gradually decreased with increasing dough water content. The finest particle dough showed the maximum strain during creep test which confirms its viscoelastic nature. Creep tests exhibited more pronounced effect of β-glucan on dough behavior compared to the oscillatory measurements. Schimele et al (2012) analyzed the influence of substituting refined wheat flour by wheat bran and whole grain wheat flour on dough rheological properties and pan bread quality characteristics. Wheat bran added composite flours or whole grain wheat flours increased water absorption and resistance to extension and decreased stability, extensibility and peak viscosity.

Although some recent studies have demonstrated the effects of different dietary fibers on the rheological properties of the dough, little has been done on the influence of its particle size on rheological properties. The objective of this study was to evaluate the effects of different wheat bran particle sizes and sources on small and large deformation rheological properties of dough systems of different protein strength.

3.2 Materials and Methods

3.2.1. Wheat Flour and Bran

Base flours: Hard red winter (good breadmaking) wheat flour was supplied by King Arthur Flour, Norwich, VT, USA. The flour contained 11.6 % (14% mb) protein, 0.48 % (14 % mb) ash and this flour is referred as strong (S) flour hereafter. The wheat flour was diluted with 100% pure wheat starch (MGP Ingredients, Inc., Atchison, KS) to obtain flour of 10 % protein. This is referred as weak (W) flour.

Bran samples: Hard red winter (HRW) bran was obtained from the Hal Ross Pilot Mill in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS. Sieve analysis indicated that 99.50% (by weight) of bran particles were below 2920 µm. Two categories of wheat bran samples were prepared on the basis of (i) same anatomical origin but different particle size, and (ii) same particle size but different anatomical origin as explained in Table 3.2 Classification of the bran samples.

“As is” wheat bran was divided into four equal portions. Extreme care was taken through adequate mixing to minimize segregation of bran and thus provide homogeneity. Each portion was passed through a Fitz mill (Fitzpatrick, Elmhurst, IL, USA) using four different screen sizes so as to obtain bran samples of “same anatomical origin but different particle size” (Category-I, Table 3.2). Screen sizes were selected to provide approximately 1000 µm intervals (i.e. 3000, 2000, 1000 µm). The fourth screen size (140 µm was chosen to reflect the particle size cut off for straight grade wheat flour.

In order to obtain bran samples of “same particle size but different anatomical origin” (Category-II, Table 3.2) “as-is” bran was sieved and collected in three sub-fractions: 2920-2030 µm, 2030-977 µm and below 977µm.The first two categories were passed through a Fitz mill (Fitzpatrick, Elmhurst, IL, USA) equipped with a 977 µm screen.

Each bran sub-fraction A, B, C, D, E, F, G was added separately to both base flours (S and W) at 5% and 10% replacement.

3.2.2. Experimental Design

A full factorial experimental design was used to test the parameters listed below:

2 base flours	: Strong (S) and weak (W) flour
7 bran fractions	: A, B, C, D and E, F, G.
2 replacement levels	: 5 and 10% (flour basis) + control (no bran)
2 dough development protocols	: Optimum and constant water absorption (WA)

3.2.3. Bran and Flour Characterization

3.2.3.1. Proximate Analysis of Flours and Bran Sub-fractions

Moisture content was measured by the oven-air method (AACC 44-15A). Protein content was determined by nitrogen combustion using a LECO Fp-2000 nitrogen/protein analyzer and a factor of 6.25 to convert nitrogen to protein (AOAC 990.03). Ash content was measured by the muffle furnace overnight method (AOAC 942.05). Fiber and lipid contents were measured using AOAC methods (962.09 and 920.39 respectively). Analyses were performed in duplicate.

3.2.3.2. Solvent Retention Capacity

AACC method 56-11 was used to measure the solvent retention capacity (SRC) of flour and bran samples alone, and that of composite flours at 5 and 10% replacement levels. Test results were reported as percentages of the mass of the flour gel after exposure to the solvent divided by the original flour weight. Analyses were performed in triplicate.

3.2.3.3. Particle Size

Mean particle size and particle size distribution were measured using a Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman-Coulter, Inc., Miami, FL). The flour and bran samples were placed into the load cell until it was approximately 2/3 full. The Tornado Dry Powder Dispersing attachment was used to load up the sample and measure its particle size. Analyses were performed in duplicate. Particle size distributions were expressed as of d-values (d10, d25, d50, and d90) corresponding to the maximum diameters of 10%, 50%, and 90% of the particles, respectively (in % of total volume).

3.2.4. Dough Development

3.2.4.1. Farinograph

The Farinograph (AACC Method 54-21) was used to determine water absorption (adjusted to 14% MC), development time, stability, mixing tolerance index (MTI), time to break down, and Farinograph quality number of the bran sub-fractions containing wheat flour doughs. Analyses were performed in duplicate.

3.2.4.2. Mixograph

The 10 g mixograph (National Manufacturing Co., Lincoln, NE, US) was used to study the mixing properties of the flour doughs as described as AACC Method 54-40A. Optimum water absorption values obtained for control flours (S and W flour) were kept constant and used for all 5 % and 10% bran substitutions to study the effect of bran replacement level at constant water absorption. Analyses were performed in duplicate. The peak development time and peak height were reported for all bran substituted doughs.

A series of mixograms were analyzed to determine the optimum water absorption of each bran substituted flour. Optimum absorption and time to reach peak were reported. Peak development time was used to determine mixing time for each sample. Analyses were performed in duplicate.

3.2.5. Dough Rheology (Small Deformation)

A stress-controlled rheometer (Stress Tech HR, ATS Rheosystems, Bordentown, NJ, US) equipped with a 40 mm parallel plate were used. Plate temperature held constant at 30°C, The gap between the two plates was set at 2.1 mm.

3.2.5.1. Sample Preparation

Dough samples were prepared by mixing 10 g of bran substituted flour in a mixograph pin mixer (National Manufacturing Co., Lincoln, NE, US) at optimum and constant water absorption levels with variable mixing times. Two small balls (approximately 3.8 g each) were made from each mixed dough sample, placed on a parchment paper, and rested in a humidity and temperature controlled cabinet set at 30°C and 75% RH for 15 min. After resting, the dough piece was placed gently on the bottom plate of the rheometer using a spatula to avoid excess deformation. The rheometer was lowered to a gap of 2.2 mm, and the excess sample was trimmed. Trimming was done with a sharp spatula in a downward motion to avoid excess deformation of the dough while cutting it even with the edge of the top plate. Mineral oil was used to keep the edges of the dough from drying. After trimming, the rheometer was lowered to the target gap of 2.1 mm, and sample was allowed to rest for 15 min prior to testing. The dough rheology was measured by strain sweep, frequency sweep, temperature sweep, creep recovery and stress relaxation testing.

3.2.5.2. Stress Sweep (Linear Viscoelastic Region)

Stress sweeps were performed to determine the linear viscoelastic region (LVR) of the doughs. LVR was determined for the samples at the extremes of 0% (i.e. control) and 10% bran replacement. Samples were prepared, rested and loaded on rheometer plate for testing. The instrument operated with a gap of 2.1 mm gap at 30°C with a sample loading method “to gap”.

The maximum loading force on the sample was 3.539E+4 Pa. The testing proceeded when force was below 1.770E+4 Pa. The final equilibrium time was 15 min, and all settings for number of measurements 1, measurement interval at 2.000E+1 s, constant frequency at 1.0 Hz, delay time of 10 sec, integration period 1.00, FFT size at 512, and strain range between 0.001 % and 10 %.

3.2.5.3. Frequency Sweep

Frequency sweeps were performed at a range from 0.1 to 100 Hz with a constant stress of 30 Pa at 30°C (as determined through stress sweep test described in section 3.2.5.2), final equilibration time 15 min, number of measurements 1, measurement interval 2.000E+0 s, delay time 10 seconds, integration period of 1.00, FFT size at 512. Analyses were performed at least in triplicate with separate dough batches for all bran-substituted flours. Elastic (storage) modulus (G′), viscous (loss) modulus (G″), complex modulus (G*), shear stress, phase angle (δ) and complex viscosity (ƞ*) were collected.

3.2.5.4. Temperature Sweep

Temperature sweeps were performed from 30-90°C for 2400 sec at a heating rate of 1.5°C/min with a constant stress of 30 Pa and a frequency 1.0 Hz. The temperature sweep tests were performed with the “auto tension” sample loading method, and a target tension of 8.85E+3 Pa. The final equilibration time was 15 min, delay time of 1.000E+0 sec, integration periods 1.00, Stress 30 Pa, FFT size 512. Temperature sweeps were performed at optimum water absorption levels for strong and weak flours and the bran sub-fraction substituted flours. Analyses were done in duplicates with separate dough batches. Flour doughs were compared using data for elastic modulus (G′), viscous modulus (G″), complex modulus (G*), shear stress, phase angle (δ) and complex viscosity (ƞ*).

3.2.6. Dough Rheology (Large Deformation)

3.2.6.1. Uniaxial Extensional Properties

Bran substituted flour doughs were analyzed for their uniaxial extensional properties using the Kieffer rig extensibility test method using TA.XT2 texture analyzer (Stable Micro Systems, UK). Dough samples were prepared in a pin mixer using Mixograph parameters. The dough was placed into a Teflon-coated block to prepare dough strips according to the method of

Kieffer at al (1981, 1998), and rested for 45 min at 30°C, 75% RH. 5-10 dough strips from each dough sample were tested at extension speed of 10.0 mm/sec. The parameters recorded were maximum resistance to extend the developed dough (R_{max}) and extensibility (E) until dough rupture.

3.2.6.2. Creep Recovery

Creep recovery measurements were performed on bran substituted flour doughs at a temperature of 30°C and shear stress of 50 Pa over a creep time of 1200 sec and recovery time of 1200 sec. The final equilibrium time was 15 min, and the set parameters were; number of measurements 1, measurement interval 2.000E+1 sec, and inertia compensation of 100 %. Analyses were performed at least in duplicate with separate dough batches for strong and weak flour with bran substitutions at optimum water absorption levels. Data for maximum creep strain (J_{max}), maximum recovery strain (J_e) and percent recovery (J_e/J_{max}), recovery strain expressed as a percent of maximum creep strain, were collected.

3.2.6.3. Stress Relaxation

Stress relaxation tests were performed in a non-linear viscoelastic region with strain of 5% for 250 sec at constant temperature of 30°C. The set parameters for the test were; number of measurements 1, measurement interval 2.000E+1 sec, relaxation strain rise time 0.100 sec, measurement interval 0.100 sec. Duplicate doughs were tested. Relaxation modulus G(t) was collected and analyzed to compare the effect of bran particle size on both strong and weak flour at optimum water absorption.

3.2.7. Statistical Analysis

A full factorial experimental design was used to analyze the rheological properties of wheat dough with or without bran of different particle sizes. Results were analyzed using Analysis of Variance (ANOVA) using SAS program (Statistical Analysis System Version 9.2). Means and standard errors were obtained by the proc GLM procedure. One way ANOVA was performed using Tukey test procedure to determine differences among treatments. Duplicates were prepared for the proximate analysis and rheological tests. Triplicates were prepared for uniaxial extension tests

3.3. Results and Discussion

3.3.1. Physical and Chemical Properties of Base materials

3.3.1.1. Proximate Analysis

Hard red winter (HRW) wheat flour (King Arthur Flour, Norwich, VT, USA) was used as the base of the "strong flours" with good breadmaking quality. The flour contained 11.6 % protein and 0.48 % ash at 14% moisture basis. This wheat flour was diluted with 100% pure wheat starch to obtain flour at 9.6 % protein, which designated "weak flour" with inferior breadmaking qualities (Table 3.1).

Bran of HRW wheat was obtained was used as the starting material for creation of two categories of bran samples. As explained in section 3.2.1. "as is bran" was divided into four fractions and passed through Fitz mill using four different screen sizes to obtain bran samples of "same anatomical origin but different particle size" at particle size cut off points of around 3000, 2000, and 1000 μm. The last portion was forced to pass through a 140 μm screen to match the particle size cut off of straight grade wheat flour. In order to obtain bran samples of "same particle size but different anatomical origin" as-is bran was first sieved and collected in three sub-fractions: 2920-2030 μm, 2030-977 μm and below 977μm.The first two categories of these brans were passed through Fitz mill (Fitzpatrick, Elmhurst, IL, USA) using 977 μm screen (Table 3.2). The proximate composition, mean particle size and particle size distribution of these seven bran samples (A, B, C, D, E, F and G) are presented in Table 3.3 and Figure 3.1.

Particle size: Sieve analysis indicated that 99.50% (by weight) of original bran (as is) was below 2920 μm. Particle size and size distribution analysis indicated that bran A has the highest mean particle size (1189 μm), as expected. 90% of bran A particles were below 1663 μm. This was closely followed by bran B with a mean particle size of 1097 μm, where 90% of particles were below 1620 μm. Bran C has much lower particle size (~ 666 μm). The last fraction of category-I, bran D, has mean particle size of ~ 109 μm, and 90% of particles were below 216 μm.

Two of the Category-II brans (E and F) have mean particle size of 663 μm and 654 μm, respectively. 90% of their particles were below 1237 and 1210 μm. Both mean particle size and particle size distribution of brans C, E and F were almost identical. Although bran G has the same particle size cut off (977 µm) as brans E and F, the mean particle size of the resulting population was found to be 1005 µm, with d90 value of 1568 µm. Although brans E and F were obtained through sifting followed by grinding to pass through a 977µm screen, bran G did not require further grinding as the screen size marches the target cut off size. This fraction corresponds to the fine bran particles (a.k.a shorts) obtained by commercial milling, as well as a small portion germ and floury endosperm particles as separated in the usual processes of commercial flour.

Chemical composition: The composition of all bran fractions varied little (Table 3.3) as they were obtained by sifting or grinding process from single source of the wheat bran. The sifting and grinding process of bran caused no structural difference and only resulted in subdividing the bran with minimal compositional change. Their protein content varied between 16.53% and 17.06% with no significant difference ($p \leq 0.05$) between type of brans Table 3.3. The compositional analysis of bran has been well established in numerous publications. The bran layers are chemically comprised of arabinoxylan (AX) (38%) > protein (25%) > cellulose (16%) > lignin (6.6%) (Brillouet and Mercier 1981; Brillouet et al 1982; DuPont and Selvendran 1987). Amrein et al (2003) and Maes and Delcour (2001) reported the average bran composition from commercial milling as 41-60% non-starch polysaccharides, 10-20% starch, and 15-20% protein.

Lipid content of Category-I brans (A, B, C and D) ranged from 0.56 to 0.86, although they all are originated from the same starting “as is” bran (Table 3.3). This could be explained by the dependence of lipid extraction efficiency on particle size. Luthria et al (2004) reported that crude fat content in corn varied with changes in grinding conditions and that was due to the differences in proportion of finer particles, which increased the crude fat extraction efficiency. Bran A and B have lipid content of 0.56 and 0.56% (no significant difference at $p<0.05$), while bran C and D have significantly higher lipid content at 0.84-0.86%. Further grinding from 1000 µm cut off to 140 µm cut off did not affect the extractable lipid content.

Bran E and F have the highest lipid content at 1.11-1.12%. Although bran E and F have mean particle size comparable to that of bran C, they had significant differences ($P \leq 0.05$) in their lipid contents. Brans E and F were obtained through sifting as is bran to form sub-fractions in the range of 2920-2030 µm and 2030-977 µm (Table 3.3).

In processing, the multiple layers comprising bran is usually removed as one component with some (minimal) starchy endosperm attached. As a whole, bran is high in branched heteroxylans, cellulose and lignin (Fincher et al 1974; Hemery et al 2011), even though compositional differences among the layers is known to exist. Studies have shown that breaking wheat bran layers into three parts (aleurone, intermediate, and pericarp), results in fractions with different compositions (Barron et al 2007; Jerkovic et al 2010; Nurmi et al 2012). Bran E and F resemble larger particles of the “as is” bran. Larger particle sizes are reported to be obtained from outer bran layers (Peyron 2002), and contain more lipid content compared to the smaller particle sizes. Bran G corresponds to predominantly shorts and residual floury endosperm particles. It has 0.79% lipid content, which is unique among the rest of the bran samples.

Crude fiber content ranged from 20.9 to 35.44% (Table 3.3). Bran A and B were again very similar to each other with a crude fiber content of 21.1%. Among the category-I brans, the crude fiber content increased with decreasing particle size (A=B < C < D). Bran E and F have higher fiber content, 32.6 and 35.4%, respectively. As explained above, these fractions correspond to larger particles of the “as is” bran, which is mostly composed of pericarp. In wheat bran, aleurone cells are high in proteins, ferulic acid, and lipids, and are composed of thick nonlignified cell walls (Fulcher and Duke 2002), whereas the pericarp has thick, lignified cells (Cheng et al 1987). This also explains the relatively low crude fiber content (20.9%) of bran G. In general, the sifted fine particle sizes had lower fiber content than the larger particle sizes as expected. Similar results had been reported by Protonotariou et al (2015). The fiber content increased when the bran particle sizes are obtained by milling process. The increase in fiber content was explained by possible interactions between protein and hemicellulose or cross-linking/oxidation among compounds during milling process (Protonotariou et al 2015; Drakos et al 2011).

Lastly, ash content of the bran samples varied between 4.93 and 7.57% (Table 3.3). Among the category-I brans, which are expected to have identical chemical composition, the ash content increased with decreasing particle size (A < B < C < D) indicating that ash content is influenced by the particle size. However, there was no significant difference ($p \leq 0.05$) in ash content of the brans except for bran D. Category-II brans, which originated from different sub-fractions of the as is bran, have significantly different ash contents ($p \leq 0.05$). Despite of the decreasing particle size, the ash content ranked as E > F > G. This could be explained by the origin of these bran samples. Outer bran layer or pericarp is composed of thick lignified cells that have high amount of inorganic material, compared to the inner layers of bran that typically form the shorts. Peyron et al (2002) reported that bran particle sizes were obtained from different bran layers (outer pericarp, nucellar epidermis, testa and inner pericarp) of the wheat grains during milling would be related to the decrease in bran extensibility measured and increase in bran friability. The strain rate applied on the bran layers during the milling process could modify the rheological behavior of the bran portion, which has high plasticity.

In short, it was concluded that, based on the similarities in their physical (particle size) and chemical properties, these seven bran samples can be regrouped in four: A≅ B, C ≅ E ≅ F, D and G which resulted in similar performances as discussed in future sections.

3.3.1.2. Solvent Retention Capacity (SRC) of the Bran Fractions

There are three main functional polymers in wheat: Protein, starch (damaged) and pentosans (Kweon et al 2011). Each of these polymers is able to absorb a particular solvent: 5% sodium carbonate for damaged starch, 50% sucrose for pentosans, and 5% lactic acid for glutenins. The working principle of solvent retention capacity (SRC) is to make flour swell into these solvents, then force the solvent out of the polymer. If the polymer has high functionality, it retains a higher quantity of solvent.

The water-solvent retention capacity (W-SRC) is related to the overall water holding capacity controlled by the flour functional components including gluten, damaged starch, and pentosans and represent the combined contributions of lactic acid-solvent retention capacity (LA-SRC), sodium carbonate-solvent retention capacity (SC-SRC), and sucrose-solvent retention capacity (Su-SRC) values (Kweon et al 2011; Gaines 2000). All the other three retention parameters are related to starch and non-starch polysaccharides components of wheat flour which can be affected by the process and the milling practice. This method has been selected to obtain an overall picture of the effect of different bran particle size and source levels on wheat flour quality concerning its potential in breadmaking performance.

In the study, the original bran was collected and fractionated into different sizes of same origin (A, B, C, and D) and different origins of same size (E, F, and G). The solvent retention capacity test, values of these wheat bran fractions in comparison to control flour are shown in Figure 3.2. In general, SRC of bran samples were significantly higher than that of base flour sample. This could be explained by high water holding capacity of wheat proteins and fiber. In category-I brans, the W-SRC values decreased with the decrease in bran particle size. All bran fractions (A, B, C, and D) were significantly different ($p<0.05$). Although bran E and F have protein contents similar to that of bran A and B, and have significantly higher fiber contents than that of bran A and B, their W-SRC values were significantly lower. This can be explained by the differences in the particle size, which surpasses the compositional differences. The porous matrix structure of the insoluble fiber chains can hold large amounts of water through hydrogen bonds (Kethireddipalli et al 2002). Noort et al (2010) reported that size reduction of bran from 1,000 to 75 µm, reduced water holding capacity from 500 to 250 %. Bran D, E and F, however, have similar W-SRC. Bran D was obtained by sifting process with the smallest particle size (cut off size < 140 µm, mean particle size of ~110 µm), whereas, bran E and F were obtained by grinding with similar resulting particle size (cut off size < 1000 µm, mean particle size of ~660 µm). Despite differences in their anatomical origins (thus chemical composition) they displayed comparable hydration properties. Bran G, obtained by sifting process, has a higher W-SRC (similar to bran A and B). The results are in consistent with earlier work of Zhang and Moore (1997), and Mongaeu and Brassard (1982) on wheat bran/fiber, and Ahmed et al (2015) on β-glucan fiber in flour doughs. The authors reported the water absorption index of wheat bran/fiber decreased with a reduction of its particle size. Thus bran particle size and shape are important factors, which will affect the water absorption index, cannot be generalized and must be assessed for each type of fiber (Perry and Chilton 1973). A larger particle bran fraction packs less efficiently by centrifugation than the medium and smaller particle bran. It has also been observed that smaller bran particle samples tend to have more water-soluble loss than do large bran samples and this might contribute to the differences between the water absorption indexes of the different particle size samples.

The rest of the SRC values (SC-SRC, LA-SRC, Su-SRC) of base flour, and category I and II brans followed the same trends with respect to different types of brans as the W-SRC profiles explained above. Since there is insignificant amount of residual starch in bran samples (except for Bran G) it is difficult to attribute these high SC-SRC values solely to the amount of damaged starch (Figure 3.2) in comparison to that of base flour. The sodium carbonate (SC-SRC) values of bran fractions increased with decrease in particle size. Bran A, B and C were significantly different; however, there was no significant difference in bran C, D, E and F (Figure 3.2).

The LA-SRC value reflects flour gluten in functionality (Kweon et al 2011). Since bran proteins are non-gluten, the observed differences between the bran samples are mostly related to their hydration and swelling behavior in water rather than their gluten functionality. All category-I brans (A, B, C and D) were observed to be significantly different in their LA-SRC due the differences in their particle size which affect the hydration rate and water holding ability as explained before. However, bran G displayed high LA-SRC despite of its small particle size due to significant differences in its composition in relation to its anatomical origin.

Lastly, the Su-SRC reflects flour pentosans in functionality. Pentosans, which are minor component of wheat flour, but play an important role in dough rheology. Pentosans are considered as the characteristic constituent of bran. They are hydrophilic and absorb as much as 10 times their weight in water (Kulp 1968; Jelaca and Hlynka 1971). The Su-SRC values of bran samples were the highest of all SRC profiles, typically 20-70% higher than the observed W-SRC values which can be attributed to 16-22% pentosans present in bran (D'appolonia 1976). Despite of the shift in SRC percentages, the trends remained the same as that of W-SRC with respect to the bran types.

3.3.1.3. Solvent Retention Capacity (SRC) of the Composite Flours

Target SRC profiles for hard wheat flour with good breadmaking quality have been reported to be 70% for W-SRC, 110% for Su-SRC, max 88% for SC-SRC and 150% for LA-SRC (Kweon et al 2011). The base flour used in this study partially justified these expectations with W-SRC of 61.3%, Su-SRC of 83.7%, SC-SRC of 75.8% and LA-SRC of 114.3% (Figure 3.3).

SRC of composite flours at 5 and 10% bran replacement levels was studied in comparison to the control flour (no bran) as shown in Figure 3.3. Presence of bran resulted in a slight increase in all SRC profiles except for LA-SRC, especially at 10% replacement level. The effects were not all statistically significant ($p \leq 0.05$). Base flour has W-SRC of 61.3% while W-SRC values in the presences of bran increased by 0.1-4.3%. Ten percent bran replacement resulted in larger shifts in W-SRC values of 2.5-4.3% compared to 0.1-4.2% increase at 5% replacement for all bran types except for bran G. The flours with excessive water retention require increased baking and energy cost in baking industries (Guttieri et al 2001). Thus, the observed changes in W-SRC up to 4.3% in this study were not concerning.

Su-SRC of base flour was 83.7% while presences of bran resulted in a mixed effect (Figure 3.3). At 5% bran replacement level, Su-SRC decreased up to 2.2%, except for bran E. At 10% replacement levels, almost all bran samples resulted in increase in Su-SRC values by 0.2-4.3%, were the highest increase was observed for Bran D. This is the bran sample that was ground to pass through the finest screen size (i.e. 140 µm) which possibly exposed more pentosans and made them available for hydration.

SC-SRC of base flour was 75.8% while the values in the presences of bran increased by up to 4.3%. The highest shift was observed when bran D was added at 10% replacement level. Bran D was ground aggressively to pass through the finest screen size (i.e. 140 µm) which possibly caused further damage in residual starch attached to bran particles. Similar to W-SRC values, The SC-SRC values in the presence of bran E and F were not significantly different ($p \leq 0.05$), but they were significantly higher as compared to bran G (Figure 3.3). The SC-SRC value measures the contribution of damaged starch in flour to its baking performance and well known that flour with higher SC-SRC value poses a negative effect on its baking performance.

The largest changes were observed in LA-SRC values. LA-SRC of base flour was 114.3%, which decreased significantly ($p \leq 0.05$) by 17.0-28.9% at 5% bran replacement, and further decreased up to 30.2-41.7% at 10% bran replacement level (Figure 3.3). This indicates that presence of bran decreased the gluten functionality as indicated by low LA-SRC values because of gluten dilution and interference of bran particles. The bran particles interfere with accurate LA-SRC measurement due to easy swelling of bran in lactic acid solvent. The LA-SRC values were lower for both bran source and size levels as compared to W-SRC and SC-SRC values, but there was no significant difference in regards to different categories of bran (Figure 3.3).

Protein content alone is an uninformative flour specification, because it includes both (functional) gluten and (non-functional) non-gluten proteins. Even with regards to gluten, its constituent proteins, gliadins and glutenins, manifest quite different functionalities (Slade et al 1989): gliadins are lower-MW, viscous, extensible, two-dimensional film formers, not network formers, whereas glutenins are higher-MW, elastic, three-dimensional network formers (Kweon et al 2011). Although SRC testing has been used to date mainly for evaluating soft wheat flour quality (Kweon et al 2011), it has been reported to be a good predictor for evaluation of hard winter wheat quality for bread making. Xiao et al (2006) showed that LA-SRC correlated with the quality of gluten protein relating to baked loaf volume over a wide range of flour protein contents. They also reported that their LA-SRC results were significantly correlated with SDS-sedimentation volume data and that the SRC test was reliable in predicting the loaf volume of breads for hard winter wheat flours with similar protein contents. Colombo et al (2008) reported a study on the use of SRC testing for quality prediction of different Argentinean wheat flours used for bread production. Their results showed a positive correlation between bread loaf volume and LA-SRC. Duyvejonck et al (2011) reported studies on the relative contributions of wheat flour constituents to SRC profiles for commercial European wheat flours. They concluded that SRC values are good, time-efficient, and simple bread quality predictors for European commercial wheat flours.

3.3.2. Dough Development

3.3.2.1. Mixograph

The mixograph is a widely used recording mixer. The quantitative information provided by the mixograph is similar to those defined for the Farinograph. Peak mixing time is similar to dough development time. Peak height (%) provides information about flour strength and absorption. Resistance to breakdown is similar to MTI.

In this study, effects of bran source, particle size and substitution levels on mixing properties were studied under 2 conditions; optimum water absorption and constant water absorption. Optimum and constant water absorption are two different approaches to dough water content. Optimal dough water absorption is critical in commercial bread production because variation in flour-water absorption dramatically affects dough handling and loaf volume. Constant (nonoptimized) water absorption is more common in engineering studies. Viscoelastic properties of the doughs at constant water absorption were evaluated as optimum water absorptions do not allow the decoupling of the effects of hydration and fiber. These two dough development protocols were used in this study to gain better understanding of effects of bran source and size on mixing properties and rheological properties.

Constant water absorption: The Farinograph water absorption of strong (S) flour and weak (W) flours was found to be 58.70% and 56.75%, respectively. These values were kept constant to study the Mixograph properties of for all bran substituted S and W flour doughs at constant WA protocol. To elucidate the effect of bran substitution, mixograph peak mixing time and peak height for strong and weak flour doughs were measured (Figure 3.4a, c). Presence of bran resulted in an increase in the peak time of S flour doughs independent of the bran type. Although the increase was not statistically significant at 5% replacement, bran A, B and G at 10% replacement resulted in significantly higher peak times in comparison to the control S flour dough sample (Figure 3.4a). However, W flour dough peak time remained fairly constant at both 5% and 10% replacement level for all bran samples (Figure 3.4c). Analysis of variance (ANOVA) results (Table 3.8b) indicated that there was no significant difference in peak time of bran substituted weak (W) flour doughs with regards to bran particle size and source ($p \leq 0.05$) and replacement level ($p \leq 0.05$) compared to control flour doughs. However, peak time of bran substituted S flour doughs was significantly ($p \leq 0.05$) affected both by bran source and replacement level (Table 3.8b).

In general, peak height of S flour doughs was negatively influenced by the presence of bran independent from bran type and replacement level (Figure 3.4b). All S composite flour dough samples, except for bran D contained samples, exhibited a significant decrease in their peak height compared to the control sample. Decrease in peak height was more pronounced at 10% bran replacement for category-I brans. The peak height of Category-II bran containing S flour doughs were not influenced by the replacement level. The peak height of W flour control dough was significantly lower than that of the S flour dough, as expected. Most of the W composite flour doughs had slightly higher peak height in comparison to W flour dough. This increase was significant ($p<0.05$) in bran D both at 5% and 10% replacement, for bran F and G at 10% replacement (Figure 3.4d). ANOVA results indicated that both bran type and replacement levels had significant effect ($p \leq 0.05$) on both S and W flour doughs (Table 3.8b).

Optimum water absorption: Figure 3.5 presents the peak mixing time and peak height measured for bran containing S and W flour doughs and control doughs (no bran) at optimum water absorptions. In general, the trends explained above with respect to bran type and replacement level were remained the same, although the peak mixing time values of optimum WA were higher than that of constant WA protocol. This implies that at constant water absorption bran and flour are competing for water and developing fairly quickly. Doughs developed through optimum WA protocol had enough water both for bran and flour, however it took slightly longer time possibly due the hydration behavior of the bran. In general, the peak time of S flour doughs increased in the presence of bran. The effect was significant ($p\leq0.05$) at

10% replacement for entire category-I and II brans (Figure 3.5a). There was no significant difference ($p \leq 0.05$) in peak mixing time of W composite flour doughs (Figure 3.5b). The bandwidth of the mixograph curves was wider for doughs made of strong protein quality flours than the doughs of weak protein quality flours (data not shown).

The increased replacement levels of both category-I and II brans decreased the peak height (%) of S flour doughs which represents decrease in flour strength and absorption. Decrease was more pronounced in category-I brans, especially bran A and B at a level up to 5% reduction in peak height. S flour doughs containing bran E and F of category-II brans had relatively higher peak heights indicating the least detrimental effect of these brans compared to the rest of the brans. Peak height of W flour dough was lower than that of S flour dough as explained before. The inclusion of category-I or II brans did not cause further decrease in the dough strength. Especially peak height of category-II bran including W flour doughs were comparable to that of the control sample.

ANOVA results at optimum WA (Table 3.8c) were similar to that were presented for the constant WA protocol. Both bran type and replacement levels had significant effect ($p \leq 0.05$) on peak height of both S and W flour doughs. Peak time was significant only for the S flour doughs.

3.3.2.2. Farinograph

Doughs were prepared from S and W base flours alone and in the presence of category-I and II brans at 5 and 10% replacement levels. Figure 3.6 presents the water absorption (WA), dough development time (DDT) and stability data measured for the bran dough formulations. The water absorptions of S and W flour doughs were 58.70% and 56.75%, respectively. These values are in accordance with the published data. Stojceska and Butler (2008) evaluated the water absorption of the flours of twenty-four wheat varieties and found values between 58.8% and 60.6% with varying protein content. Inclusion of bran resulted in significant increase in the WA values irrespective of bran type. The WA of S flour doughs increased significantly from 58.7% to 60.0-63.5% in the presence of bran.

Fiber substituted flour doughs are known for their ability to absorb significant amounts of water (Campbell et al 2008; Linlaud et al 2009). The presence of large numbers of hydrophilic groups which allows more water interactions through hydrogen bonding plays a major role here (Rosell et al 2001). Water absorption is mainly affected by source, structure, isolation method, porosity and particle size. Research on wheat cultivars reported that bran with different chemical compositions can lead to different hydration capacities, and that these differences product quality (de Kock et al 1999). No significant differences were seen for bran that was obtained by sifting and grinding process. Increased sucrose and sodium carbonate SRC values indicated increases in starch damage and pentosan content.

In our study, there was an incremental increase in WA when the bran replacement was increased from 5 to 10% both for S and W flour doughs (Figure 3.6 a, b). The bran particle size had high impact on both S and W flour compared to bran anatomical origin. The lower the particle size the higher the WA of category-I bran containing flour doughs. This is in contradictory to the hydration behavior of bran samples. As presented earlier, there was an inverse relationship between the particle size and W-SRC of category-I brans (Figure 3.2). W-SRC of composite flour samples, however, remained constant at for all bran types and both replacements levels of 5 and 10% (Figure 3.3). WA required to reach 500 FU consistency, as an indication of optimum mixing and dough development, displayed an opposite trend. There is a gradual increase in WA values as the bran particle size decreased from 3000 µm (bran A) to 140µm (Bran D). The water absorption of both flour doughs was the highest with an average of 63.5% in the 10% bran D replacement.

Dough development time (DDT) of bran supplemented S and W flours increased significantly for all bran particle sizes and sources. Similar increase in DDT with the addition of various bran sources (wheat, rice, rye) has been reported (Sudha et al 2007). In W flour doughs, except for brans C, D, E and F at 5% replacement, all bran samples at all replacement levels resulted in significant increase in DDT (Figure 3.6d).

Dough stability an important quality factor in dough development and machinability. Presence of bran, independent of type and replacement level, caused slight decrease in recorded values however it did not affect the stability of S flour dough significantly ($p \leq 0.05$) with the exception of A and B bran at 10 % replacement. W flour doughs, however, benefited from the inclusion of bran as the stability values increased from 8 min to up to around 12 min, which were comparable to that of S composite flour doughs (Figure 3.6f) except for bran A at 10% replacement. Ahmed et al (2013) and Mis et al (2012) reported similar observations for date fiber and carob fiber, respectively.

Analysis of variance (ANOVA) test results (Table 3.8d) indicated that both bran type and replacement level were significant for all three parameters (WA, DDT and stability) for both flour types at $p \leq 0.05$.

3.3.3. Dough Rheology

Multiple studies have evaluated the viscoelastic properties of wheat flour dough with the aim of studying the influence of flour quality, dough ingredients, and processing conditions on fundamental rheological properties (Amemiya and Menjivar 1992; Navickis et al 1982). The availability of increasingly rheometers created a great deal of interest in characterizing wheat flour doughs and glutens of varying quality by fundamental rheological tests (Safari-Ardi and Phan- Thien 1998; Wikström and Eliasson 1998). Fundamental rheological testing such as dynamic oscillatory measurements or creep recovery, is often used for dough characterization and to gain information on the structure of the composite materials. Elucidating the effects of wheat bran of varying anatomical origin and particle size on the rheological properties of wheat flour doughs with different breadmaking quality would be helpful in determining both dough handling properties during processing and the quality of the end products. The next sections explore and compare the behavior of two categories of bran samples in two different wheat flour doughs (strong and weak) at two bran replacement levels (5 and 10%) by dynamic oscillatory measurements in the linear viscoelastic region, and creep–recovery and uniaxial extensional testing at under high shear stress.

3.3.3.1. Small Deformation Behavior

3.3.3.1. Stress Sweeps (Linear Viscoelastic Region)

Stress sweeps at constant frequency of 1 Hz were performed to determine the dough's linear viscoelastic region (LVR) using a stress-controlled rheometer. The mechanical spectra (data not shown) of storage (G') and loss (G'') moduli were constant for stress values up to 30 Pa whereas moduli started to decrease at higher values, indicating the onset of non-linear behavior. Thus, 30 Pa was used in subsequent experiments.

In strain-controlled rheometers, however, the elastic modulus (G') has been reported to start decaying above 0.1% strain and display a large drop above 1% strain, indicating the breakdown of the dough structure beyond this deformation level (Phan-Thien and Safari-Ardi 1998; Weipert 1990).

3.3.3.2. Frequency Sweeps

Frequency sweeps involved increasing the frequency of oscillation from 0.1 Hz to 100 Hz while keeping stress constant at 30 Pa. Mechanical spectra provided set of rheological parameters from each test, namely storage modulus (G′), loss modulus (G″), complex modulus (G*), tangent delta (tan δ, G″/G′), and complex viscosity (η*) in the linear viscoelastic range (Figure 3.7 through Figure 3.16).

Mechanical spectra for the base flours (S and W) revealed that G′ > G″ over all frequencies indicating the viscoelastic nature of what flour dough, as expected. Phase angles that were below 45º (i.e. tan delta < 1.0) displayed slightly more solid-like material property of doughs. Both G′ and G″ were higher for W control flour dough than for the S control flour doughs, indicating the formation of a much stiffer dough in the case of the poor breadmaking quality flour. Lower values of storage and loss moduli (G′ and G″) for S flour dough over the entire frequency range fit with observations by other authors that higher dough strengths correlate with lower moduli values at small deformations (Safari-Ardi and Phan-Thien 1998; Uthayakumaran et al 2002). Similarly, Khatkar and Schofield (2002) and Petrofsky and Hoseney (1995) observed that doughs from poor breadmaking wheats had G′ values of greater magnitude than those of the good breadmaking wheat cultivars.

The frequency scans showed that both S and W flour dough samples displayed higher values of G′ and G″ at higher frequencies compared with low frequencies. These results indicate that the recovery of the stressed dough network was a slow process; that is, the network was not completely elastic. Dreese et al (1988) reported a similar trend that G′, G″, and tan delta of flour dough were frequency dependent and increased with increasing frequency. This behavior implies that the dough acts more like a solid, when subjected to slow rates of deformation, and this starts to reverse when it is deformed rapidly. A similar frequency dependence was noted by Baltsavias et al (1997) and Pedersen et al (2004) for cookie doughs.

Frequency sweep experiments were first conducted using *constant water absorption protocol* to single out the effect of bran inclusion on rheological properties (Figure 3.7, 3.11, 3.13, 3.15). Water absorption level plays a vital role in rheological properties of the dough, on dough handling and product quality. In dough, water interacts with gluten and starch to form the continuous gluten phase. The distribution of water varies among the flour constituents and strongly influenced by amount of protein, damaged starch and other components such as bran, which has high water holding capacity (Bushuk 1966).

In general, S and W composite flour dough samples showed a response typical of a cross-linked polymer network with predominant solid-like behavior (G′> G″). This is a true representation of viscoelastic network. Such behavior was earlier reported by various researchers for fiber-enriched dough (Mis 2011; Singh et al 2012). Both control and composite dough samples at 5 or 10% bran replacement showed an increase in G′ and G″ with increasing frequency from 0.1 to 10 Hz, with the elastic behavior dominating over viscous component throughout the frequency range. Bran enriched dough showed higher mechanical strength than the control samples indicating much stiffer dough. An increase in rheological moduli with fiber incorporation has been reported in the literature (Ahmed at el 2013). The higher G′ for bran enriched flour doughs could be attributed to the limited plasticization effect leading to increased dough mechanical property. Storage and loss moduli of S composite flour doughs containing category-I brans displayed a significant difference ($p \leq 0.05$) with respect to replacement level. Both G′ and G″ increased further at 10% replacement compared to 5% replacement. However, W composite flour dough samples did not have any distinction with respect to the bran replacement level. Although inclusion of category-I brans shifted the moduli to higher values, 10% replacement level did not cause a further increase in moduli. There was no distinction between the 5% and 10% replacement levels when category-II brans were included in both S and W flour doughs (Figure 3.7 and Figure 3.9).When the frequency sweep test was repeated for the dough samples prepared under *optimum water absorption protocol* (Figure 3.8 and Figure 3.10) the trends explained above did not change. However, the magnitude of shift in the moduli of S and W flour doughs in the presence of brans was much less significant. The excess water provided during dough development, following the optimum water absorption protocol, minimized the competition between flour (especially protein in flour) and bran and thus allowed gluten to develop fully and form a continuous network without creating stiff dough.

Since the mechanical spectra provide a continuous data set, it is often difficult to make comparisons between two different experimental data sets in a quantitative manner. To overcome this, it is a common practice to report G′ and G″ at 1 Hz constant frequency. Figure 3.11and

Figure 3.12 provide such information for dough samples prepared at constant WA and optimum WA, respectively. G′ at 1 Hz of S composite flour doughs increased by 40-90% at 5% bran replacement level, while the change was as high as 70-170% when bran was added at 10% replacement. Although similar trends were observed for W flour dough, the change in G′ at 1 Hz was 8-25% at 5% bran replacement, and 25-80% at 10% replacement. The G″ values of composite flour doughs experienced much higher increase in comparison to control doughs: up to 250% in S composite flour dough and 180% in W composite flour dough (Figure 3.11). Dough samples prepared under optimum WA protocol displayed similar trends with less pronounced effects. As explained earlier, excess water provided to satisfy optimum dough development minimized the competition between flour and created less stiff doughs (Figure 3.12).

The differences in protein content and particle size distribution can explain the higher moduli of bran substituted flour doughs versus the control flour dough. The overall protein content in bran flour dough is higher than the control flour dough, which means a smaller amount of starch present in bran substituted flour doughs. This can be explained as wheat flour protein contains gliadins and glutenins that provides viscoelasticity and strength are diluted when bran fractions are substituted in flour doughs. Thus, the bran substituted strong or weak flour doughs give higher moduli than flour doughs with no bran. The decrease in glutenin and gliadin ratio also affects the large deformation rheological properties such as extensibility of the doughs as it will be discussed in later sections of this chapter. The observations also support the previous studies done on flour reconstitution experiments and correlation studies have shown that the baking quality of various flours and rheological properties are primarily related to the gluten protein fraction of the flours (MacRitchie 1992; Magnus et al 2000). The increase of storage and loss moduli with other dietary fiber additions has also been reported (Izydorczyk et al 2001; Santos et al 2005; Peressini, and Sensidoni 2009; Bonnand-Ducasse et al 2010).

The viscoelastic nature of the dough can be further described by calculated slopes of the linear regression of the power-type relationship of ln G′ versus ln frequency (ω which is commonly used to predict the solid-like characteristics of polymers. The frequency dependency of biopolymers is reported to have three zones: the rubbery zone, the entangled zone, and the free-flow (or reaction) zone. The frequency dependency of G′, G″, and tan δ can be monitored to identify phase behavior of proteins, whether it is in the rubbery zone, entangled polymer flow region or the reaction zone (Dogan and Kokini 2007). The slope of logarithmic plots of G′ versus frequency (ω) above 0.3 is high enough to suggest that the material had a non-network structure capable of experiencing entangled polymer flow. A true gel is characterized by a zero slope of the power law model (Ross-Murphy 1984).

Incorporation of bran into wheat flour dough significantly affected the rheological properties. The slopes presented in Table 3.4 and Table 3.5 ranged from 0.109 to 0.229 indicating a good network development. The slope of curves of composite dough decreased significantly with the increase in the percentage bran additions in wheat flour dough. Typically, bran replacement at 5% resulted in lower slope values compared to control doughs, and 10% bran replacement led to further reduction, which was statistically significant for most bran samples. Slopes around 0.2 or lower have been reported to suggest shorter-range networking compared to entangled polymer flow zone (Dogan and Kokini 2007). When the slope gets closer to zero, reaching a plateau, it indicates a cross-linked network. Bran D (the smallest particle size) and bran G (the fine bran fraction obtain through sifting) added to strong flour at 10% replacement level resulted in the most dramatic change in slope values, from control value of 0.230 to 0.156 and 0.109, respectively. When added to W flour same category-I and II brans caused a lesser degree of change in slope since the base value for W flour control dough was inherently much below than that of the S flour dough. *Optimum water absorption protocol* resulted in smaller margin of change in slope values (from 0.230 to 0.170 in S flour composite doughs) due to lack of competition for water between bran and flour. Under optimized conditions wheat proteins have a better chance of hydrating and forming a well-developed network.

Analysis of variance (ANOVA) results (Table 3.8e) indicated that there was significant difference ($p \leq 0.05$) in G′ at 1 Hz and G′ slope bran substituted S flour doughs with regards to both bran source and replacement level. However, in W composite flours doughs only replacement level had a significant effect on these two parameters.

At *optimum water absorption*, increasing frequency from 0.1 to 10 Hz increased both storage (G′) and loss (G″) moduli (Figure 3.7 through Figure 3.10), with the elastic behavior dominating over the viscous component throughout the entire frequency range examined. At low frequencies, the tan delta (G″/G′) was around 0.5 whereas at high frequencies, the tan delta increased above 1.0 (Figure 3.13 and Figure 3.14). Lower values for phase angles are indicative of a more elastic nature. This implies that control (S and W) flour doughs have the higher elastic nature at small deformations across a range of frequencies. The results showed that all bran substituted flour doughs increased their phase angles with increasing frequency, and higher phase angle indicate that larger frequencies causes the viscous nature of the samples to increase. The increase in viscous nature of the dough can be explained since phase angle is the ratio of G" to G′, an increase in the phase angle reflects that G" has a greater slope than G′, and is therefore increasing at a faster rate.

The complex viscosity (η*) versus frequency plots are given in Figure 3.15 and Figure 3.16. In general, data indicated a steady decrease in η* with increasing frequency for all bran-substituted S and W flour doughs irrespective of bran type and replacement level, which indicates a shear thinning behavior. The dough viscosity increased with the addition of bran while the most significant change was observed in S composite flour dough containing category-I brans where the replacement level was observed to be critical. However, in dough samples containing category-II brans replacement level did not have a further effect on viscosity as both 5% and 10% data points overlapped in the same range. Viscosity of W composite flours were minimally affected by inclusion of bran samples, especially the category-II brans (Figure 3.15). In the dough samples prepared through optimum water absorption protocol (Figure 3.16) the aforementioned effects were much less pronounced.

3.3.3.3. Temperature Sweeps

Dough is a combination of starch and protein in aqueous medium. Upon heat treatment, starch gelatinizes and protein denatures in the dough, thus making the process is a complex one. Nevertheless, the rheological properties of wheat gluten do not change during heating, and the rheological properties of wheat dough are predominantly affected by starch gelatinization during thermal treatment (Dreese et al 1988; Ahmed et al 2013). The conditions applied in the rheometer did not simulate those prevailing in a real baking process (Sikora et al 2010), however, the rheometric studies are very useful in examining starch gelatinization in the dough. Furthermore, it is believed that the temperature range of 92-96°C is the controlling factor for the final quality of bread.

Initially, there was slight decrease in G′ and G" until around 55-60°C, as has been generally observed for all control flour and wheat bran substituted flour doughs (Figure 3.17 and

Figure 3.18). This might be attributed to the swelling of starch during initial stages of heating. Starch granules start to swell, leading to an increase of their volume and becoming closely packed in the system (Eliasson 1986). The decrease in moduli indicates softening of the dough until the gelatinization temperature is reached around 65°C. The decrease is likely due to α-amylase acting on starch and releasing some absorbed water thereby reducing dough interactions (Salvador et al 2006). Similar results were observed from previous workers (Lambert and Kokini 2001; Angioloni and Rosa 2005). The effect of bran was observed only in the initial stages of heating before onset of gelatinization. As the heating temperature was raised to above the threshold value of 50°C, the G′ and G" increased markedly up to 70°C, and then followed by a drop in their magnitudes. There was no significant difference ($p \leq 0.05$) in G′and G" with respect to bran particle size or source substitution levels to both strong and weak flour doughs. Storage and loss moduli within the bran particle size (A, B, C, and D) levels, did not show any significance between 5 and 10% bran substitution for both S and W flour doughs. However, 10 % bran substituted S flour doughs have higher G′ and G″ values as compared to S control flour dough. A similar trend was observed for 20% β-glucan fiber substitutions in the dough by et al (2014, 2015). Bale and Muller (1970) reported that the loss modulus (G″) decreased initially while heating the dough to 50-55 °C, and storage modulus decreased or remained constant (Legrys et al 1981; Bale and Muller 1970). Increase in storage modulus (G′) was attributed to cross-linking interactions induced in gluten during the formation of network structure (Kim and Cornillon, 2001). Protein-protein interactions provided highly cross-linked structure resulting in higher G′ and generally lower G″ values. Dreese et al (1988) explained that starch gelatinization, gluten cross-linking, or both would be the attribute for the thermally induced rheological changes during baking. Gélinas and Mckinnon (2004) reported that the effect of temperature on dough rheological properties is mainly due to the effect of temperature on gluten rheological properties as resistance to mixing increases in heated gluten.

He and Hoseney (1991) suggested that higher moduli and loss tangent value of the doughs from poor quality flours resulted either from fewer entanglements or from entanglements that were easily dissociated. The other research reported that the good breadmaking quality flours have lower G′ and tan delta values. The increase in G′ could be related to the protein composition of bran flour doughs. Eliasson (1983) showed that high protein content increases the starch gelatinization temperature because proteins soluble in water more easily interact with starch granule surface to slow starch swelling and shifts the gelatinization temperature. In limited water systems such as bread dough systems, starch gelatinizes can be affected by a slight change in water availability, the presence of a larger amount of proteins interact with water would likely shift the gelatinization temperature.

The temperature corresponding to maximum value of G′ during heating ramp (denoted as $T_{G'max}$) can be considered as peak gelatinization temperature. The $T_{G'max}$ for S and W control flour doughs were recorded as 69.3°C and 70.7°C, respectively. The $T_{G'max}$ of composite flours in the presence of category-I and II brans were not influenced significantly by the bran content, and ranged between 68.2 and 71.8°C for S composite flours (Table 3.6). Analysis of variance (ANOVA) results (Table 3.8g) indicated that bran type had significant effect ($p \leq 0.05$) on $T_{G'max}$ of S flour dough, while no significance was observed for peak G′, peak G″ and peak η^*. For W flour dough, both bran type and replacement level were observed to have significant effect ($p \leq 0.05$) on $T_{G'max}$, while other three parameters had no significance.

Figure 3.19 indicates that compared to their control flour doughs, tan delta values of composite flours were slightly lower for S flour doughs, while they were slightly higher for W flours dough independent from the bran type. It can be concluded that bran imparts strengthening effect in the W flour doughs while it weakens the S flour doughs. Ozboy and Koksel (1997) showed that by using empirical methods, coarse bran of some varieties has an unexpected strengthening effect on rheological properties but has somewhat reduced adverse effect on baking properties. In both S and W flour doughs, tan delta remained constant around 0.5 during the initial heating step until the temperature reached around 50°C. There was a dramatic drop to values around 0.4 to 0.3 in temperature range from 50 to 70°C, while tan delta remained constant around 0.3 from thereon until the end of the heating period.

3.3.4. Large Deformation Behavior

3.3.4.1. Creep and Recovery

A typical characteristic of viscoelastic materials is that they undergo creep, i.e. they continue to deform under constant stress or load. Since the behavior of dough is non-linear, measurements at a high rate of deformation do not simulate the resistance of dough against slow deformations, such as those occurring in bread making. However, such type of measurements can provide information about the pressure developing in the gas cells and the resultant stresses. The creep-recovery curves of doughs exhibit a typical viscoelastic behavior (Figure 3.21), combining both viscous-fluid and elastic components (Steffe 1992). Creep and recovery responses of S and W control doughs are similar to respective curves obtained for wheat doughs in previous studies (Edwards et al 1999; Lazaridou et al 2007; Sivaramakrishnan et al 2004). Wang and Sun (2002) defined that maximum creep strain could be used to describe dough rigidity (firmness). They also claimed that stronger doughs with greater resistance to deformation had smaller creep strain than softer doughs. In accordance with this view, S flour dough exhibited greater resistance, showing smaller creep strain than its counterpart W flour dough (Figure 3.21). Furthermore, the dough of the stronger flour exhibited greater strain recovery after removal of the load than its poor quality flour counterpart.

Three important creep compliance parameters were obtained from the creep-recovery test: J_{max}, the maximum compliance, J_e, the elastic compliance, and J_e/J_{max} ratio, the elastic part of the maximum creep compliance. The J_{max} values of S composite flour doughs were lower than that of control dough (Figure 3.21 a, b) except for bran C at 5% replacement level. J_{max} of s flour dough dropped from 0.631 Pa^{-1} to as low as 0.419 Pa^{-1} when bran D was added at 10%. Upon removal of applied stress, the percent recovery in S composite flour doughs as measured by the J_e/J_{max} ratio ranged from 51.5% to 59.4 %, control flour being at 56.0%. The J_{max} value of W control flour doughs was 1.250 Pa^{-1}, while that of W composite flours ranged between 0.561 Pa^{-1} and 1.200 Pa^{-1} (Table 3.7). Upon removal of applied stress, the percent recovery in W composite flour doughs as measured by the J_e/J_{max} ratio ranged from 55.6% to 62.4 %, W control flour being at 60.5%.

Ahmed et al (2015) studied the creep and relation behavior of β-glucan doughs with respective to particle size and found that creep curves of doughs containing fine particle size were close to the control dough. This might be explained as the finest particles produced softer dough with maximum strain values and believed that lower values of water absorption by finest particles are responsible for the dough softness. Tronsmo et al (2003a and 2003b) indicated that large strain measurements of creep recovery have given correlations with large molecular weight glutenin. This indicates that the creep recovery measurements are also related to protein composition. The protein-protein interactions play a significant role on the rheological properties of wheat flour doughs.

The creep recovery results can be associated with large deformation extensibility test and bread volume (Safari-Ardi and Phan-Thien 1998; Wikstrom and Eliasson 1998), and strength of durum wheat varieties (Edwards et al 1999, 2001). This probably indicates that the recovery strain represents elastic properties of doughs. Schofield and Scott Blair (1932) reported that the proper bread dough must not only be viscous or plastic, but also be elastic and recoverable. The creep recovery test provides the measurement of dough elasticity simply by recovery strain. This test can be used to classify and predict flour quality for breadmaking. Creep recovery tests may give more insight into the macro-structure of the dough. The recovery is an important factor for dough film stability. The higher the recovery strain, the better the stability against rupture of dough films between gas cells (Bloksma and Bushuk 1988). This implies that bran substituted flour doughs irrespective of bran particle size and bran source has lesser recovery strain and less stability compared to strong flour doughs.

3.3.4.2. Uniaxial Extensional Properties

The extensibility tests are typically conducted on doughs to evaluate their tensile strength and extensibility characteristics based on the wheat's protein and gluten quality. In this study, the extensibility tests were conducted first at the *constant water absorption* level to single out the bran effect from hydration effect. Figure 3.22 represents the effect of bran type and replacement level extensional properties of S and W flour doughs at constant water absorption. There was no significant difference ($p \leq 0.05$) in R_{max} of S composite flours irrespective of bran type except bran B at 5%, bran C and D at 10 % replacements levels. The R_{max} of these samples were higher than that of the control flour dough. In general, extensibility of S composite doughs decreased with the addition of bran, especially at 10% replacement level. Bran C, D and E imparted slightly higher E values in comparison to the control dough although the difference was not statistically significant.

Strong flour doughs with higher protein content represented higher strength (R_{max}) than weak flour doughs. The observations are in agreement with results reported previously on wheat bran addition of hearth bread (Aamodt et al 2004) and oat and bran fibers on wheat bread doughs (Rieder et al 2012). Aamodt et al (2004) suggested that flours containing higher proportions of large glutenin polymers require time to reach appropriate level of polymer size as part of developing gluten network is likely to be longer than for flours with less of the largest glutenin polymers. This might explain the longer mixer time requirement found for the strong flour doughs vs. weak flour doughs in the present study.

W control flour dough has lower R_{max} compared to S flour dough, as expected. W flour dough did benefit from inclusion of bran as the R_{max} values went higher in W composite flour doughs. All types of bran at all replacements levels were significantly higher in R_{max}, except for bran E at 5% replacement. However, the extensibility of W composite doughs decreased significantly with the addition of bran, especially at 10% replacement level, with the exception of bran C, E, and F at 5%.

The composite dough samples prepared at *optimum water absorption* exhibited much less dramatic differences in their R_{max} compared to their control flour counterparts (Figure 3.23). However, bran replacement had a strong effect on the extensibility data at optimum water absorption, as it was the case in constant water absorption.

Dough handling in commercial production lines includes mixing, molding, and resting (Weipert 1991). Extended dough resting time might help the bran substituted flour doughs to re-aggregate the gluten protein polymers and no significant difference was observed when compared to control flour doughs for both strong and weak protein qualities. Extensibility has affected with bran substitution in strong and weak flour doughs. There was larger difference in 10% bran substitutions in the flour. The higher percent of bran addition into the flour dilutes the protein quality and affects the viscoelastic properties of the dough. In addition, water absorption level in the dough also influences the rheological properties including tensile strength (Muller and Hlynka 1964) and the results are meaningful only at a specified level.

Aamodt et al (2004) studies showed that weak protein quality flours were more extensible than the doughs made from strong protein quality. The addition of bran to wheat flour doughs dilutes the concentration of gluten-forming proteins, and the bran particles disrupt the gluten network (Gan et al 1992). The dilution effect and the disruption of gluten network might contribute to decreased extensibility of the dough in the present study. The substitution of bran appears to hinder the gluten network formation and results in decreased extensibility.

3.3.4.3. Stress Relaxation

Stress relaxation test is used to determine the time dependence of the viscoelastic properties of doughs. The stress relaxation curves are plotted as $G(t)/G_0$ versus time, where $G(t)$ is the relaxation modulus at any time and G_0 is the initial modulus. Stress relaxation depends on the molecular weight of proteins; higher the molecular weight, longer time to relax than with fewer high molecular weight proteins. Larger proteins needed more time to rearrange into low energy conformations than small proteins. Stress relaxation data of wheat flour dough usually gives the bimodal behavior in the relaxation spectra. Rao et al (2000) attributed the bimodal distribution of relaxations times to a blend of low and high molecular weight polymers. They suggested that the first peak represents entanglements between low molecular weight proteins, possibly with some high molecular weight proteins. The second relaxation peak is related to the entanglements properties of molecular weight insoluble glutenin polymers. This has been shown to be directly related to insoluble fraction of the high MW glutenins.

Stress relaxation test was performed both for strong wheat flour alone and at 5 and 10% replacement levels for all seven bran samples. However, no data was not included to this thesis due to the anomalies in the test outcomes.

3.4. Conclusions

The study gave an overview of the relationships between variables obtained from a range of different measurements of bran substituted flour dough with respect to bran particle size and source. Farinograph data demonstrate a slight difference between all bran flour doughs; it has been shown that total bran increases the water absorption and the dough development time. The physical properties of the bran flour doughs were directly compared using uniaxial extension by the Kieffer rig test. The bran containing doughs were more dependent on the protein content. There is no difference between the category-I and category-II bran inclusions in the performance of strong flour doughs. The bran substitution in weak flour doughs causes a strengthening effect and while it weakened the strong flour doughs.

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3.6. References

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Table 3.1 Properties of flour samples

	Strong (S) flour	Weak (W) flour
Composition		
Protein content (%)	11.6±0.14	9.6±0.28
Moisture content (%)	12.02±0.66	11.7±0.12
Farinograph properties		
Water absorption (FU)	58.70 ± 0.14	56.75 ± 0.21
Development time (min)	2.55 ± 0.21	2.10 ± 0.14
Stability (min)	10.70 ± 0.14	8.20 ± 0.14
Degree of softening (FU)	13.00 ± 1.41	42.50 ± 2.12
Mixograph properties		
Water absorption (%)	61.00 ± 0.0	58.00 ± 0.0
Peak time (min)	4.98 ± 0.16	5.36 ± 0.06
Peak height (%)	44.38 ± 0.90	38.72 ± 0.19

Data are mean of duplicates ± standard deviation.
*Protein= n x 5.27 factor

Table 3.2 Classification of the bran samples

Categories	**Original particle size range**	**Ground and passed through**	**Designation and particle size cut off**	
Category-I	< 2920 µm	2920 µm screen	**A**	< ~3000 µm
		2030 µm screen	**B**	< ~2000 µm
		977 µm screen	**C**	< ~1000 µm
		140 µm screen	**D**	< ~140 µm
Category-II	2920 - 2030 µm	977 µm screen	**E**	< ~1000 µm
	2030 - 977µm		**F**	< ~1000 µm
	< 977µm		**G**	< ~1000 µm

Table 3.3 Physico-chemical properties of bran samples

		A	**B**	**C**	**D**	**E**	**F**	**G**
Particle size and distribution (μm)								
Mean size		1188.9±3.5a	1097.5±29.4b	666.5±5.6d	108.9±0.4e	663.8±8.5d	654.2±2.0d	1005.4±5.9c
Volume (%)	d_{10}	673.5±15.3a	506.6±6.3b	148.9±3.5d	11.4±0.0e	134.2±0.1d	131.6±2.6d	453.4±5.0c
	d_{25}	927.4±9.6a	814.8±16.0b	370.1±5.6d	31.9±0.2e	339.3±0.2d	342.2±4.1d	722.4±6.2c
	d_{50}	1212.3±0.6a	1134.2±24.4b	674.4±5.6d	106.4±0.2f	629.0±4.8e	623.2±2.6e	1004.0±5.1c
	d_{75}	1474.0±4.5a	1422.9±37.8a	936.8±6.7c	165.2±0.6d	925.5±11.2c	912.3±2.7c	1290.5±6.6b
	d_{90}	1663.1±7.2a	1620.3±49.6ab	1161.9±8.6c	216.5±1.0d	1236.8±23.4c	1209.9±13.8c	1568.0±9.4b
Chemical composition (%)								
Protein		16.72±0.35a	16.76±0.11a	16.57±0.20a	16.85±0.04a	17.06±0.05a	16.60±0.32a	16.53±0.01a
Lipid		0.56±0.06bc	0.58±0.06bc	0.86±0.06b	0.84±0.07b	1.11±0.18a	1.12±0.01a	0.79±0.02b
Crude fiber		21.13±0.16b	21.09±0.84b	30.93±0.04ab	34.36±6.42a	32.55±5.12ab	35.44±2.70a	20.91±0.31b
Ash		5.84±0.30c	6.04±0.05bc	6.12±0.22bc	7.57±0.20a	6.64±0.24b	5.71±0.17c	4.93±0.10d

*Protein= n x 6.25 factor
Data are mean of duplicates ± standard deviation.
Values within the row with the same letter are not significantly different from each other at $p \leq 0.05$.

Table 3.4 Slope of storage (G′) and loss (G″) moduli versus frequency curves of strong (S) and weak flour (W) doughs with respect to bran types and replacement level at constant water absorption.

Strong (S) Flour	Slope of G′	Slope of G″	Weak (W) Flour	Slope of G′	Slope of G″
No bran	0.230a	0.227c	No bran	0.181a	0.210b
SA05	0.185a	0.258abc	WA05	0.177a	0.256ab
SA10	0.201a	0.315a	WA10	0.114a	0.314a
SB05	0.209a	0.230bc	WB05	0.182a	0.291ab
SB10	0.188a	0.28abc	WB10	0.156a	0.28ab
SC05	0.206a	0.219c	WC05	0.177a	0.263ab
SC10	0.196a	0.272abc	WC10	0.185a	0.308a
SD05	0.174ab	0.242abc	WD05	0.212a	0.271ab
SD10	0.156ab	0.259abc	WD10	0.138a	0.276ab
SE05	0.204a	0.241bc	WE05	0.190a	0.273ab
SE10	0.178ab	0.270abc	WE10	0.185a	0.301a
SF05	0.192a	0.249abc	WF05	0.189a	0.264ab
SF10	0.154ab	0.260abc	WF10	0.186a	0.277ab
SG05	0.204a	0.285abc	WG05	0.195a	0.279ab
SG10	0.109b	0.301ab	WG10	0.193a	0.297a

Values with the same letter in a column are not significantly different from each other at $p \leq 0.05$.
S-Strong Flour, W-Weak flour, A-G- Bran types, G′-Storage modulus, G″-Loss modulus.

Table 3.5 Slope of storage (G′) and loss (G″) moduli versus frequency curves of strong (S) and weak flour (W) doughs with respect to bran types and replacement at optimum water absorption.

Strong (S) Flour	Slope of G′	Slope of G″
No bran	0.230a	0.232bcde
SA05	0.209ab	0.265ab
SA10	0.181cd	0.273a
SB05	0.217ab	0.238bcde
SB10	0.201bc	0.260abcd
SC05	0.212ab	0.229de
SC10	0.203bc	0.256abcd
SD05	0.206abc	0.241abcde
SD10	0.170d	0.264abc
SE05	0.219ab	0.243abcde
SE10	0.215ab	0.240bcde
SF05	0.222ab	0.240abcde
SF10	0.212ab	0.216e
SG05	0.207abc	0.231cde
SG10	0.206abc	0.256abcd

Weak (W) Flour	Slope of G′	Slope of G″
No bran	0.181a	0.210b
WA05	0.186a	0.259ab
WA10	0.141b	0.289a
WB05	0.193a	0.234ab
WB10	0.185a	0.264ab
WC05	0.182a	0.230ab
WC10	0.202a	0.278ab
WD05	0.171ab	0.248ab
WD10	0.190a	0.292a
WE05	0.189a	0.251ab
WE10	0.198a	0.272ab
WF05	0.191a	0.260ab
WF10	0.187a	0.265ab
WG05	0.190a	0.260ab
WG10	0.188a	0.301a

Values with the same letter in a column are not significantly different from each other at $p \leq 0.05$.

S-Strong Flour, W-Weak flour, A-G- Bran types, G′-Storage modulus, G″-Loss modulus.

Table 3.6 Peak storage (G′) and loss (G″) moduli, temperature at which peak G′ was attained ($T_{G'max}$) and peak viscosity (η*) of strong (S) and weak flour (W) doughs with respect to bran types and replacement level at optimum water absorption.

Strong (S) flour	Peak G′ (Pa)	Peak G″ (Pa)	$T_{G'max}$ (°C)	Peak η* (Pa.s)
No bran	1.26E+05ab	4.59E+04a	69.30c	2.15E+04a
SA05	1.05E+05ab	3.67E+04a	68.20d	1.75E+04a
SA10	1.25E+05ab	3.45E+04a	71.80a	2.07E+04a
SB05	1.20E+05ab	3.41E+04a	71.80a	1.99E+04a
SB10	1.23E+05ab	3.86E+04a	70.70b	2.05E+04a
SC05	1.26E+05ab	4.62E+04a	69.30c	1.85E+04a
SC10	1.13E+05ab	3.27E+04a	69.30c	1.88E+04a
SD05	1.27E+05ab	4.26E+04a	70.15bc	2.00E+04a
SD10	1.08E+05ab	3.32E+04a	69.50c	1.84E+04a
SE05	0.88E+05b	2.88E+04a	71.80a	1.48E+04a
SE10	1.10E+05ab	3.44E+04a	70.70b	1.83E+04a
SF05	1.02E+05ab	3.00E+04a	70.70b	1.69E+04a
SF10	1.30E+05ab	4.13E+04a	70.70b	2.17E+04a
SG05	1.05E+05ab	6.35E+04a	71.80a	1.73E+04a
SG10	1.47E+05a	4.51E+04a	70.70b	2.31E+04a

Weak (W) flour	Peak G′ (Pa)	Peak G″ (Pa)	$T_{G'max}$ (°C)	Peak η* (Pa.s)
No bran	1.15E+05a	3.77E+04a	70.70e	1.92E+04a
WA05	1.26E+05a	3.98E+04a	69.40i	2.08E+04a
WA10	1.43E+05a	4.27E+04a	70.80d	2.38E+04a
WB05	1.18E+05a	3.71E+04a	69.70f	1.94E+04a
WB10	1.29E+05a	4.62E+04a	70.70e	2.17E+04a
WC05	1.25E+05a	3.85E+04a	71.90b	2.08E+04a
WC10	1.19E+05a	3.82E+04a	70.80d	2.00E+04a
WD05	1.17E+05a	3.60E+04a	71.90b	1.92E+04a
WD10	1.23E+05a	3.57E+04a	71.90b	2.04E+04a
WE05	1.30E+05a	4.52E+04a	69.50h	2.56E+04a
WE10	1.21E+05a	3.14E+04a	71.80c	1.98E+04a
WF05	1.19E+05a	3.36E+04a	69.50h	1.97E+04a
WF10	1.05E+05a	3.10E+04a	69.60g	1.74E+04a
WG05	1.18E+05a	3.54E+04a	70.70e	1.95E+04a
WG10	1.42E+05a	4.32E+04a	74.30a	2.36E+04a

Values with the same letter in a column are not significantly different from each other at $p \leq 0.05$.

S-Strong Flour, W-Weak flour, A-G- Bran types, G′-Storage modulus, G″-Loss modulus, T-Temperature, η*-Complex viscosity.

Table 3.7 Creep and recovery parameters of strong (S) and weak flour (W) doughs with respect to bran types and replacement at optimum water absorption.

Strong (S) flour	**$J_{max} \times 10^{-3}$ (1/Pa)**	**$J_e \times 10^{-3}$ (1/Pa)**	**J_e/J_{max} ratio**	**Weak (W) flour**	**$J_{max} \times 10^{-3}$ (1/Pa)**	**$J_e \times 10^{-3}$ (1/Pa)**	**J_e/J_{max} ratio**
No bran	0.631abc	0.354abcd	0.560a	No bran	1.250a	0.752a	0.605a
SA05	0.638abc	0.364abc	0.571a	WA05	0.962abcd	0.571cde	0.594a
SA10	0.476cde	0.258cd	0.541a	WA10	0.770def	0.460ef	0.598a
SB05	0.585abcd	0.329abcd	0.561a	WB05	1.200ab	0.734ab	0.613a
SB10	6.090abcd	3.62abcd	0.594a	WB10	0.773def	0.469def	0.607a
SC05	0.752a	0.421a	0.560a	WC05	1.110abc	0.642abc	0.578a
SC10	0.590abcd	0.349abcd	0.593a	WC10	0.881cde	0.519cde	0.589a
SD05	0.516cde	0.283bcd	0.549a	WD05	0.975abcd	0.606bcd	0.624a
SD10	0.419e	0.236d	0.562a	WD10	0.561f	0.317g	0.572a
SE05	0.704ab	0.388ab	0.551a	WE05	1.06abcd	0.590cde	0.556a
SE10	0.633abc	0.376abc	0.594a	WE10	0.880cde	0.533cde	0.605a
SF05	0.558bcde	0.301abcd	0.539a	WF05	0.999abcd	0.579cde	0.579a
SF10	0.522cde	0.286bcd	0.546a	WF10	0.760def	0.453ef	0.596a
SG05	0.502cde	0.259cd	0.515a	WG05	0.884bcde	0.510cde	0.577a
SG10	0.462de	0.263bcd	0.569a	WG10	0.601ef	0.336g	0.559a

Values with the same letter in a column are not significantly different from each other at $p \leq 0.05$

J_{max}-Maximum compliance, J_e-Elastic compliance, J_e/J_{max}-Elastic part of the maximum creep compliance.

Table 3.8 Analysis of Variance (ANOVA) Tukey test results

	a. Solvent Retention Capacity Test			
	W-SRC	**Su-SRC**	**SC-SRC**	**LA-SRC**
Source	**p value**			
Bran Effect	0.3888	**0.0904**	**0.0436**	**<.0001**
RL Effect	**0.0224**	**0.0063**	**<.0001**	**<.0001**
Bran*RL Interaction	0.4573	0.4575	0.244	0.0817

P values are significantly different at $p \leq 0.05$
RL-Replacement level
SRC-Solvent Retention Capacity, W-Water, Su-Sucrose, SC-Sodium carbonate, LA-Lactic acid.

	b. Mixograph Test (Constant WA)			
	Strong Flour		**Weak Flour**	
	Peak Time	**Peak Height**	**Peak Time**	**Peak Height**
Source	**p value**			
Bran Effect	**0.0127**	**<.0001**	0.1496	**<.0001**
RL Effect	**0.001**	**0.0005**	0.9705	**<.0001**
Bran*RL Interaction	**0.0264**	**<.0001**	0.3508	0.0556

P values are significantly different at $p \leq 0.05$
RL-Replacement level

	c. Mixograph Test (Optimum WA)			
	Strong Flour		**Weak Flour**	
	Peak Time	**Peak Height**	**Peak Time**	**Peak Height**
Source	**p value**			
Bran Effect	**0.0026**	**<.0001**	0.2614	**<.0001**
RL Effect	**<.0001**	**<.0001**	0.7927	**<.0001**
Bran*RL Interaction	**0.012**	**0.0272**	0.3952	**0.0002**

P values are significantly different at $p \leq 0.05$
RL-Replacement level

	d. Farinograph Test					
	Strong Flour			Weak Flour		
	WA	DDT	Stability	WA	DDT	Stability
Source	p value					
Bran Effect	**<.0001**	**0.0028**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
RL Effect	**<.0001**	0.3142	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Bran*RL Interaction	0.4812	0.3932	**<.0001**	0.2591	**<.0001**	**<.0001**

P values are significantly different at $p \leq 0.05$
RL-Replacement level, WA-Water absorption, DDT-Dough development time

	e. Frequency Sweep Test (Constant WA)							
	Strong Flour				Weak Flour			
	G′ at 1Hz	G′ slope	G″ at 1Hz	G″ slope	G′ at 1Hz	G′ slope	G″ at 1Hz	G″ slope
Source	p value							
Bran Effect	**0.0004**	**0.0251**	**0.0009**	**0.0111**	0.1563	0.2094	**0.0014**	0.8554
RL Effect	**<.0001**	**0.0016**	**<.0001**	**0.0002**	**<.0001**	**0.0289**	**<.0001**	**0.0152**
Bran*RL Interaction	0.0858	**0.0271**	0.4456	0.3852	0.4849	0.2157	0.1604	0.4089

P values are significantly different at $p \leq 0.05$
RL-Replacement level, G’-Storage modulus, G”-Loss modulus

	f. Frequency Sweep Test (Optimum WA)							
	Strong Flour				Weak Flour			
	G′ at 1Hz	G′ slope	G″ at 1Hz	G″ slope	G′ at 1Hz	G′ slope	G″ at 1Hz	G″ slope
Source	p value							
Bran Effect	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0096**	**<.0001**	0.2465
RL Effect	**<.0001**	**<.0001**	**<.0001**	**0.0028**	**0.0018**	0.6826	**<.0001**	**0.0004**
Bran*RL Interaction	**0.0001**	**0.0176**	**0.0137**	**0.0025**	0.6718	**0.0052**	**0.0005**	0.6654

P values are significantly different at $p \leq 0.05$
RL-Replacement level, G’-Storage modulus, G”-Loss modulus

	g. Temperature Sweep Test (Optimum WA)							
	Strong Flour				**Weak Flour**			
	Peak G′	**Peak G″**	**Peak T**	**Peak η***	**Peak G′**	**Peak G″**	**Peak T**	**Peak η***
Source	**p value**							
Bran Effect	0.2472	0.6619	**<.0001**	0.4021	0.504	0.3704	**<.0001**	0.3989
RL Effect	**0.0433**	0.6421	0.5898	**0.0186**	0.4573	0.8526	**<.0001**	0.8083
Bran*RL Interaction	0.0712	0.875	**<.0001**	0.3278	0.504	0.2091	**<.0001**	0.2282

P values are significantly different at $p \leq 0.05$

RL-Replacement level, T-Temperature, η*-Complex viscosity, G’-Storage modulus, G”-Loss modulus

	h. Creep and Creep Recovery Test (Optimum WA)					
	Strong Flour			**Weak Flour**		
	J_{max}	**J_e**	**J_e/J_{ma}x**	**J_{max}**	**J_e**	**J_e/J_{max}**
Source	**p value**					
Bran Effect	**0.0012**	**<.0001**	0.8147	**<.0001**	**<.0001**	0.1961
RL Effect	**<.0001**	**<.0001**	0.9625	**0.0002**	**0.0216**	**0.0277**
Bran*RL Interaction	0.2141	**0.0031**	0.7086	0.0554	0.1	0.2921

P values are significantly different at $p \leq 0.05$

RL-Replacement level, J_{max}-Maximum compliance, J_e-Elastic compliance, J_e/J_{max}-Elastic part of the maximum creep compliance.

	i. Uniaxial Extension Test (Constant WA)			
	Strong Flour		**Weak Flour**	
	R_{max}	**E**	**R_{max}**	**E**
Source	**p value**			
Bran Effect	**<.0001**	**<.0001**	**<.0001**	**<.0001**
RL Effect	0.2481	**<.0001**	**<.0001**	**<.0001**
Bran*RL Interaction	**<.0001**	**0.0057**	**<.0001**	**<.0001**

P values are significantly different at $p \leq 0.05$

RL-Replacement level, R_{max}-Maximum resistance, E-Extensibility.

	j. Uniaxial Extension Test (Optimum WA)			
	Strong Flour		**Weak Flour**	
	R_{max}	**E**	**R_{max}**	**E**
Source	**p value**			
Bran Effect	**<.0001**	**<.0001**	**<.0001**	**<.0001**
RL Effect	**0.0045**	**<.0001**	**<.0001**	**<.0001**
Bran*RL Interaction	**<.0001**	0.0557	**<.0001**	0.5452

P values are significantly different at $p \leq 0.05$

RL-Replacement level, R_{max}-Maximum resistance, E-Extensibility.

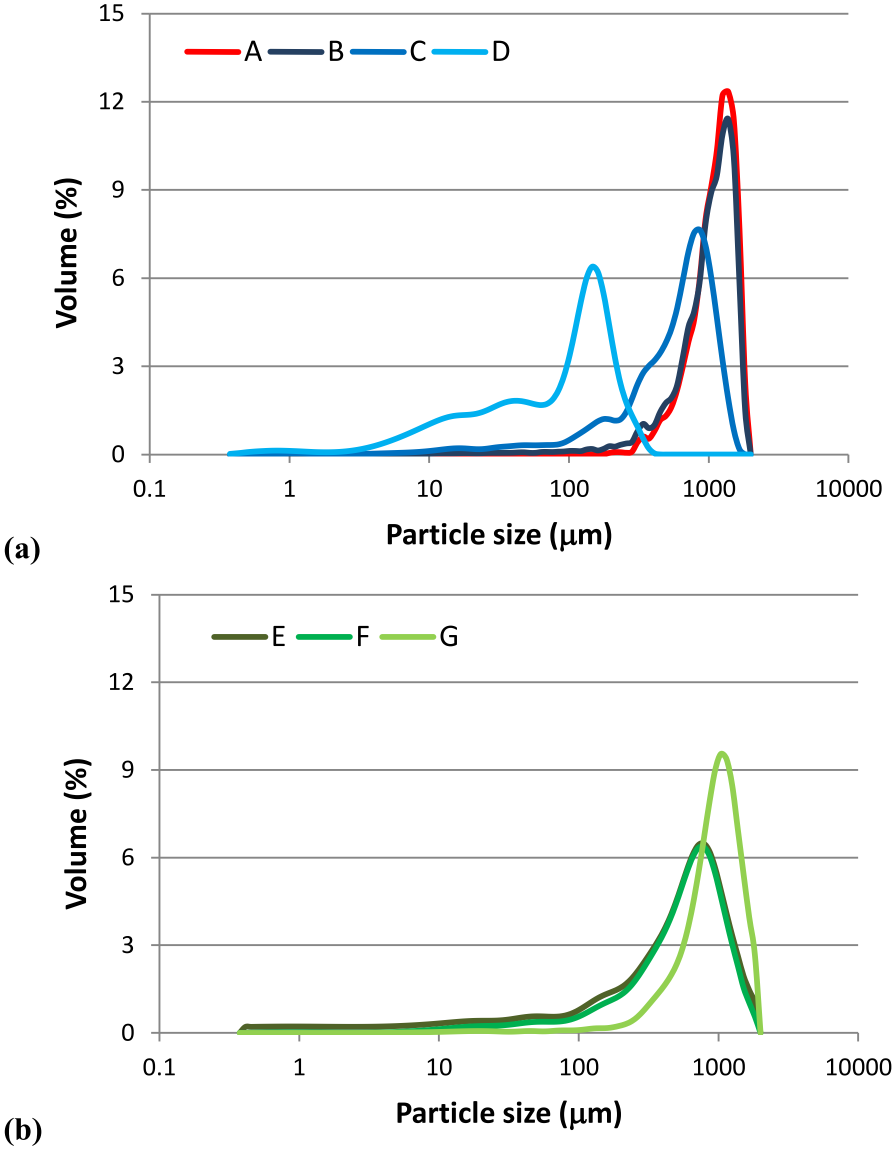


Figure 3.1 Particle size distribution of the bran samples.

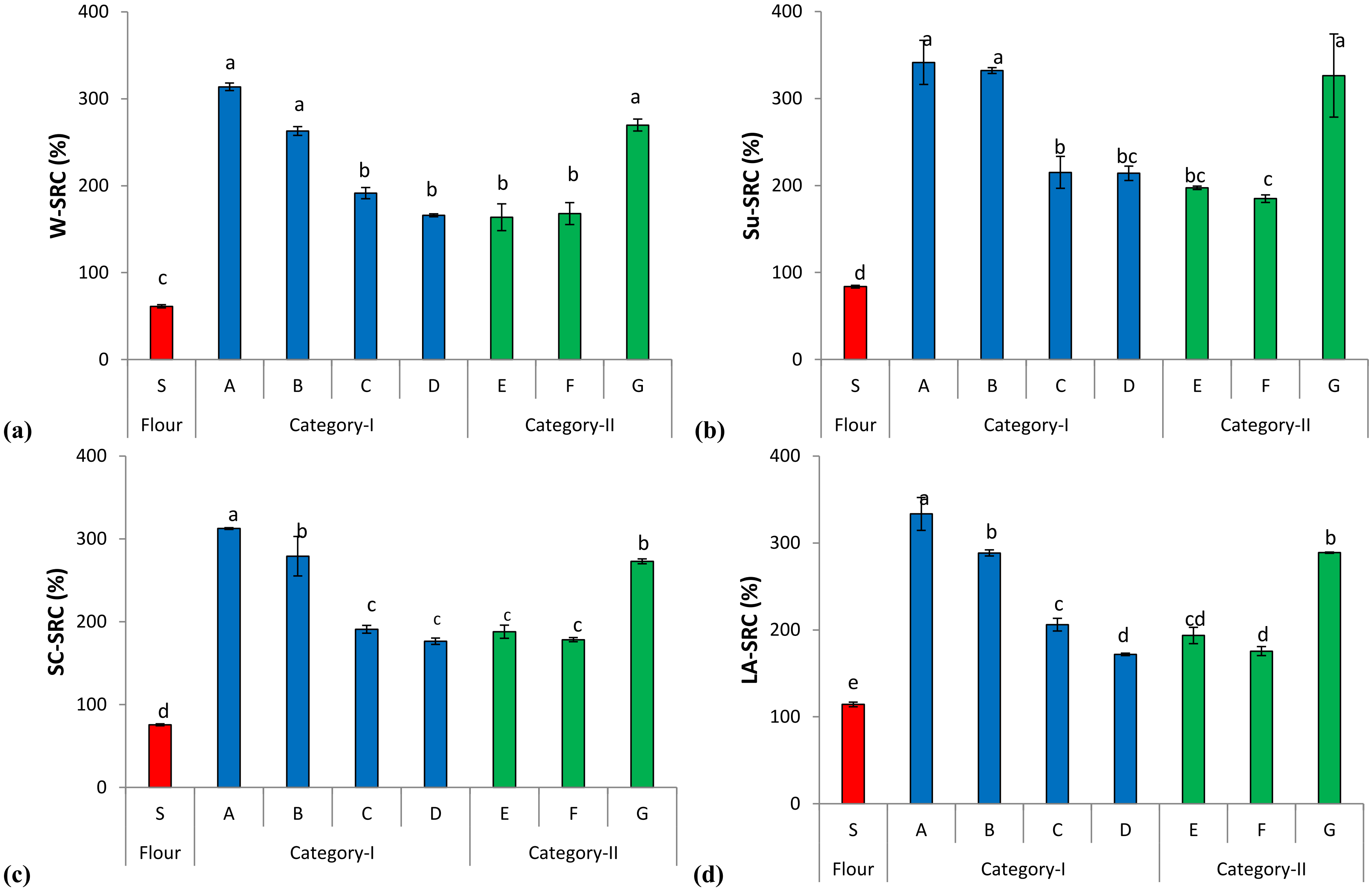


Figure 3.2 Solvent retention capacity (SRC) of strong (S) flour and bran samples. (a) Water SRC, (b) Sucrose SRC, (c) Sodium carbonate SRC, (d) Lactic acid SRC.

Values with the same letter are not significantly different from each other at $p \leq 0.05$.

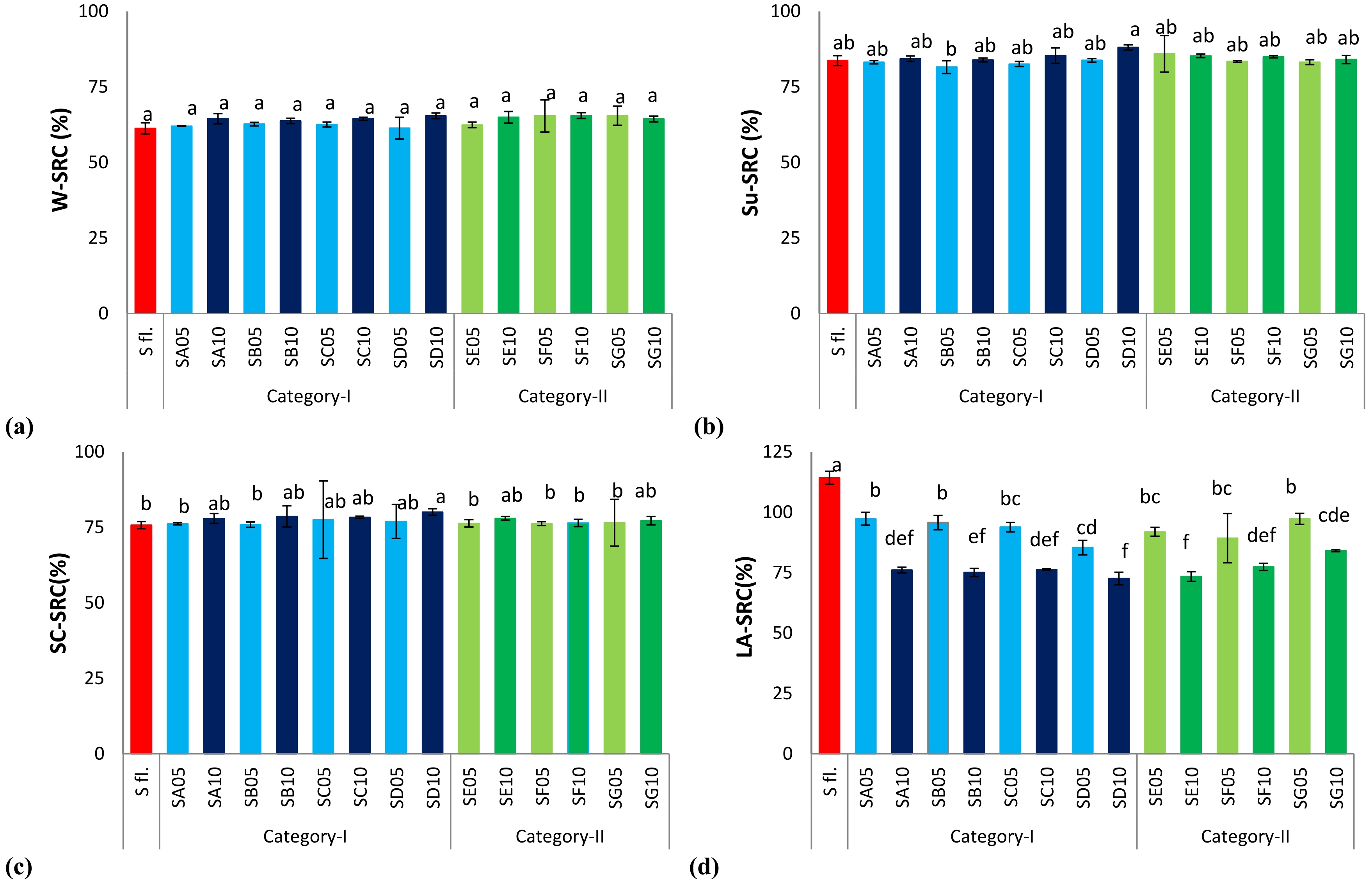


Figure 3.3 Solvent retention capacity (SRC) of strong (S) flour at 5 and 10% bran replacement levels. (a) Water SRC, (b) Sucrose SRC, (c) Sodium carbonate SRC, (d) Lactic acid SRC.

Values with the same letter are not significantly different from each other at $p \leq 0.05$.

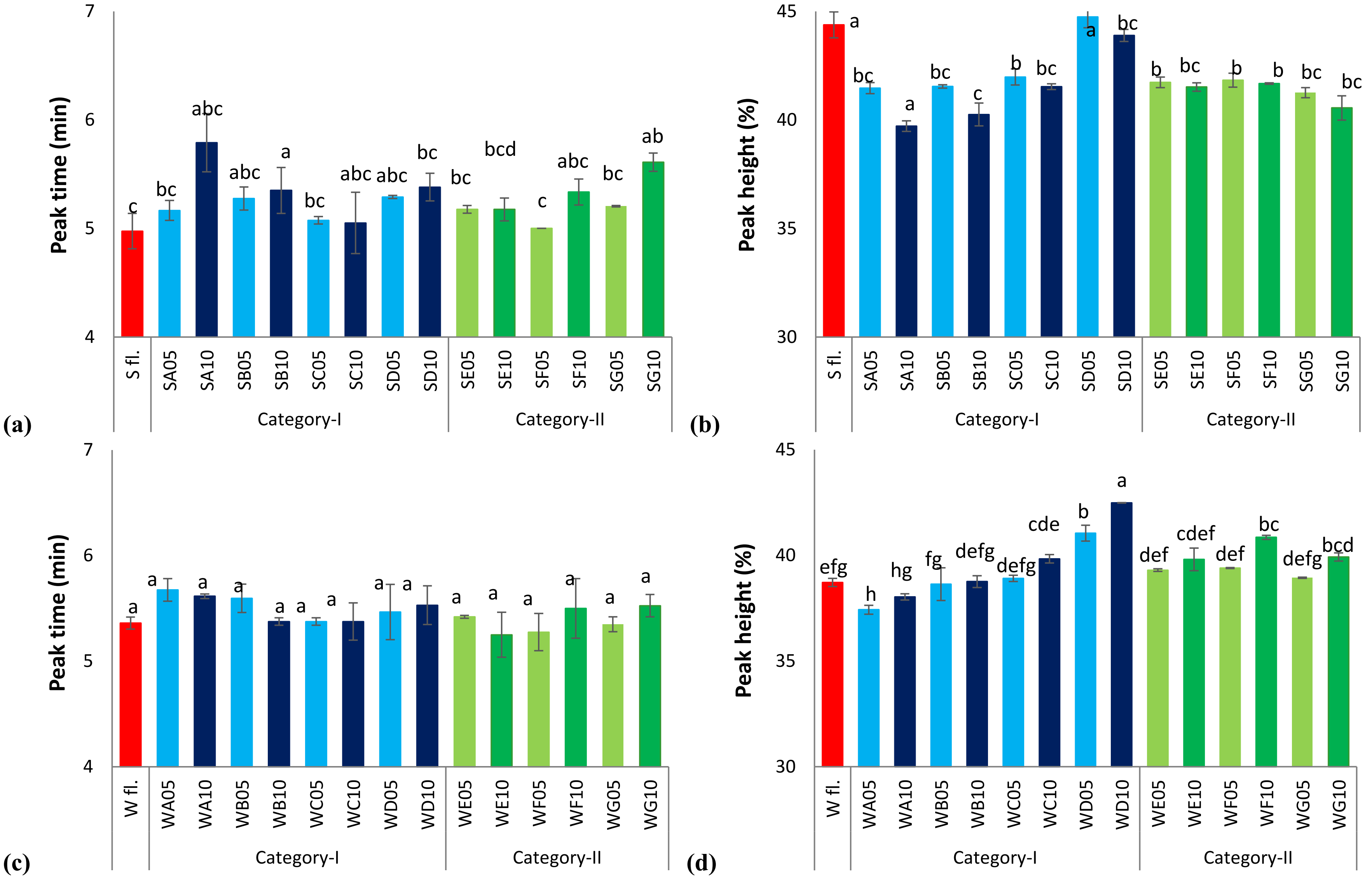


Figure 3.4 Mixograph parameters of flour samples at 5 and 10% bran replacement levels at constant water absorption. (a) Peak time, strong (S) flour, (b) Peak height, strong (S) flour, (c) Peak time, weak (W) flour, (d) Peak height, weak (W) flour.
Values with the same letter are not significantly different from each other at p≤0.05.

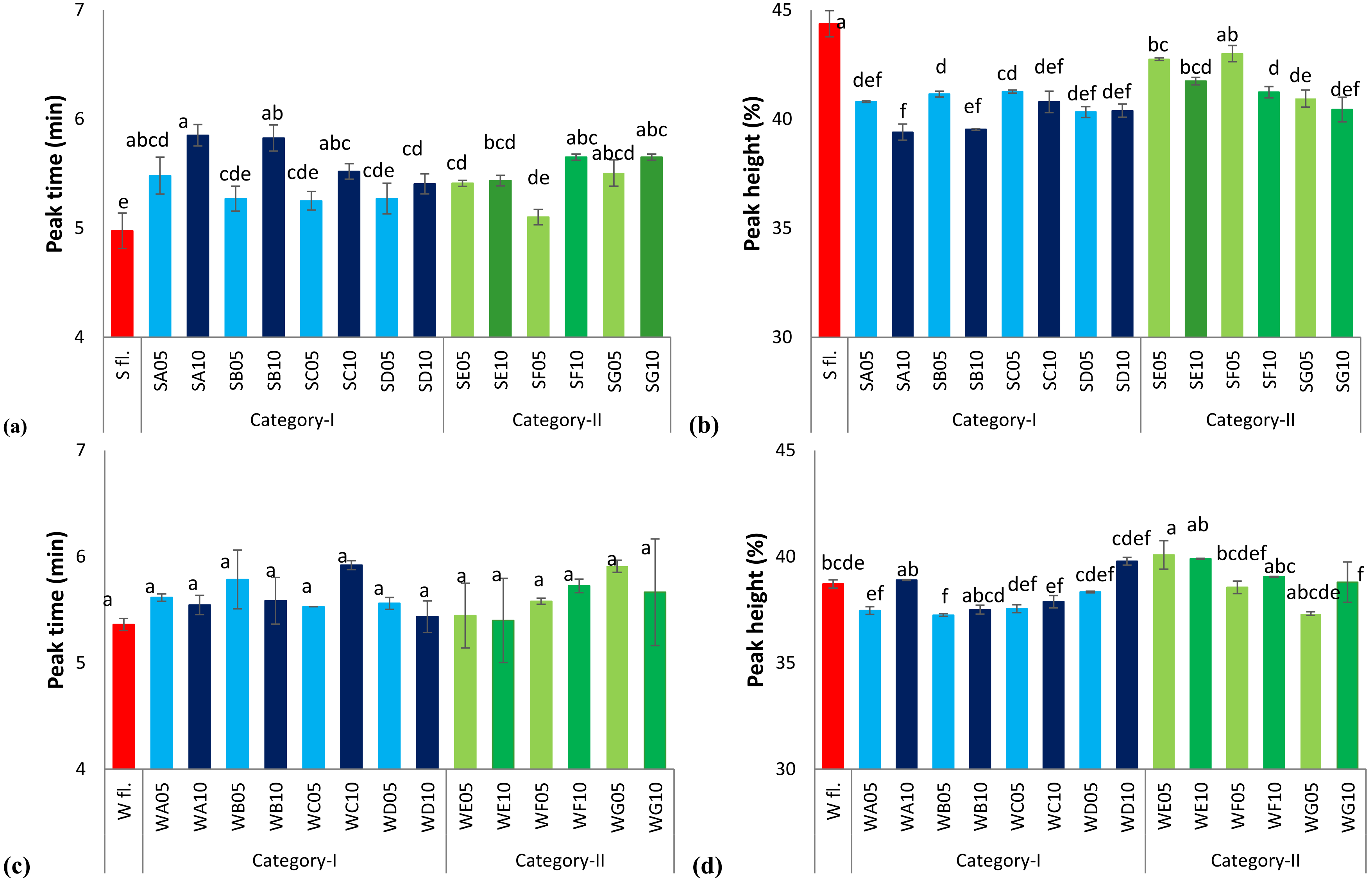
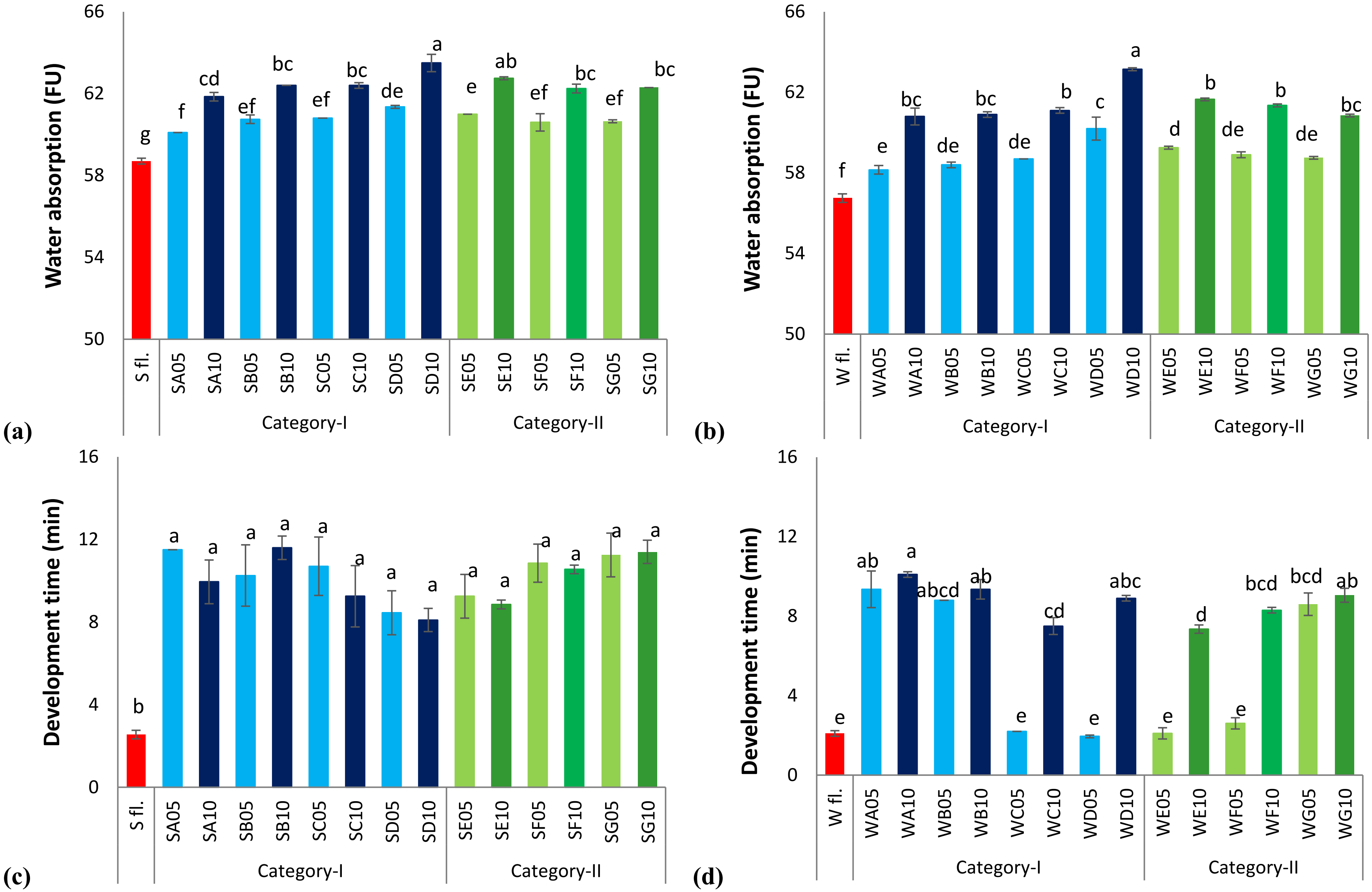


Figure 3.5 Mixograph parameters of flour samples at 5 and 10% bran replacement levels at optimum water absorption. (a) Peak time, strong (S) flour, (b) Peak height, strong (S) flour, (c) Peak time, weak (W) flour, (d) Peak height, weak (W) flour.
Values with the same letter are not significantly different from each other at $p \leq 0.05$.


(a)
Water absorption (FU)
66
62
58
54
50
g
f
cd
ef
bc
ef
bc
de
a
e
ab
ef
bc
ef
bc
S fl.
SA05
SA10
SB05
SB10
SC05
SC10
SD05
SD10
SE05
SE10
SF05
SF10
SG05
SG10
Category-I
Category-II
(b)
Water absorption (FU)
66
62
58
54
50
f
e
bc
de
bc
de
b
c
a
d
b
de
b
de
bc
W fl.
WA05
WA10
WB05
WB10
WC05
WC10
WD05
WD10
WE05
WE10
WF05
WF10
WG05
WG10
Category-I
Category-II
(c)
Development time (min)
16
12
8
4
0
b
a
a
a
a
a
a
a
a
a
a
a
a
a
a
S fl.
SA05
SA10
SB05
SB10
SC05
SC10
SD05
SD10
SE05
SE10
SF05
SF10
SG05
SG10
Category-I
Category-II
(d)
Development time (min)
16
12
8
4
0
e
ab
a
abcd
ab
e
cd
e
abc
e
d
e
bcd
bcd
ab
W fl.
WA05
WA10
WB05
WB10
WC05
WC10
WD05
WD10
WE05
WE10
WF05
WF10
WG05
WG10
Category-I
Category-II

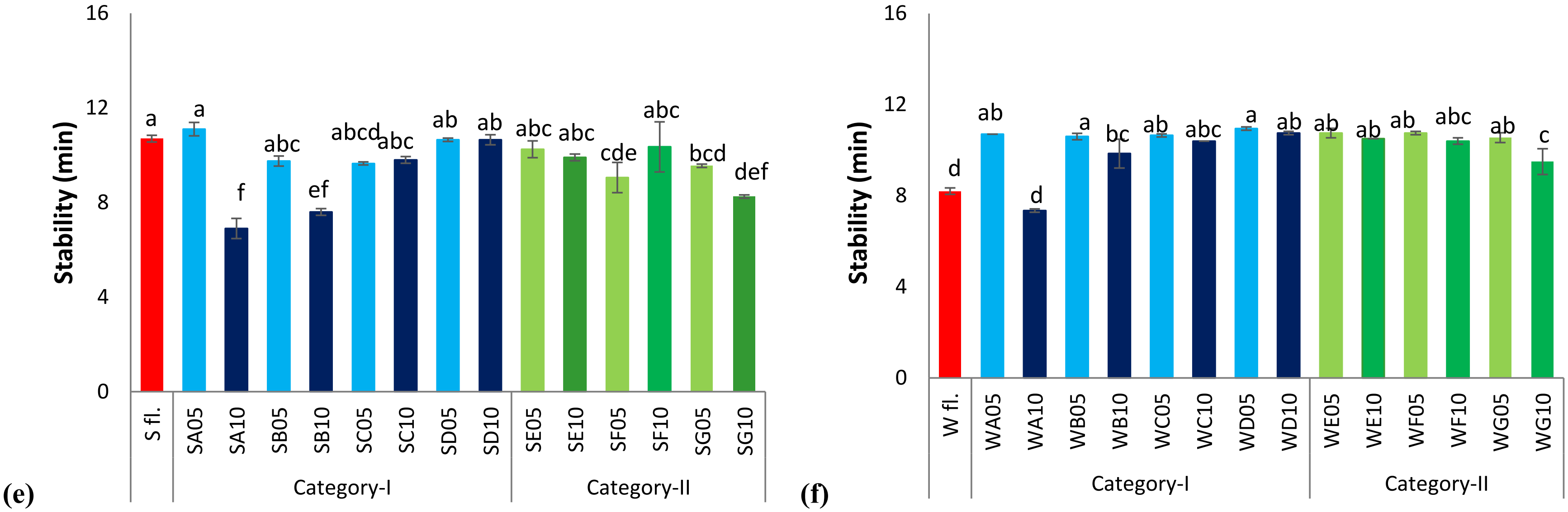


Figure 3.6 Farinograph parameters of flour samples at 5 and 10% bran replacement levels. (a) Water absorption, strong (S) flour, (b) Water absorption, weak (W) flour, (c) Development time, strong (S) flour, (d) Development time, weak (W) flour, (e) Stability, strong (S) flour, (f) Stability, weak (W) flour.

Values with the same letter are not significantly different from each other at $p \leq 0.05$.

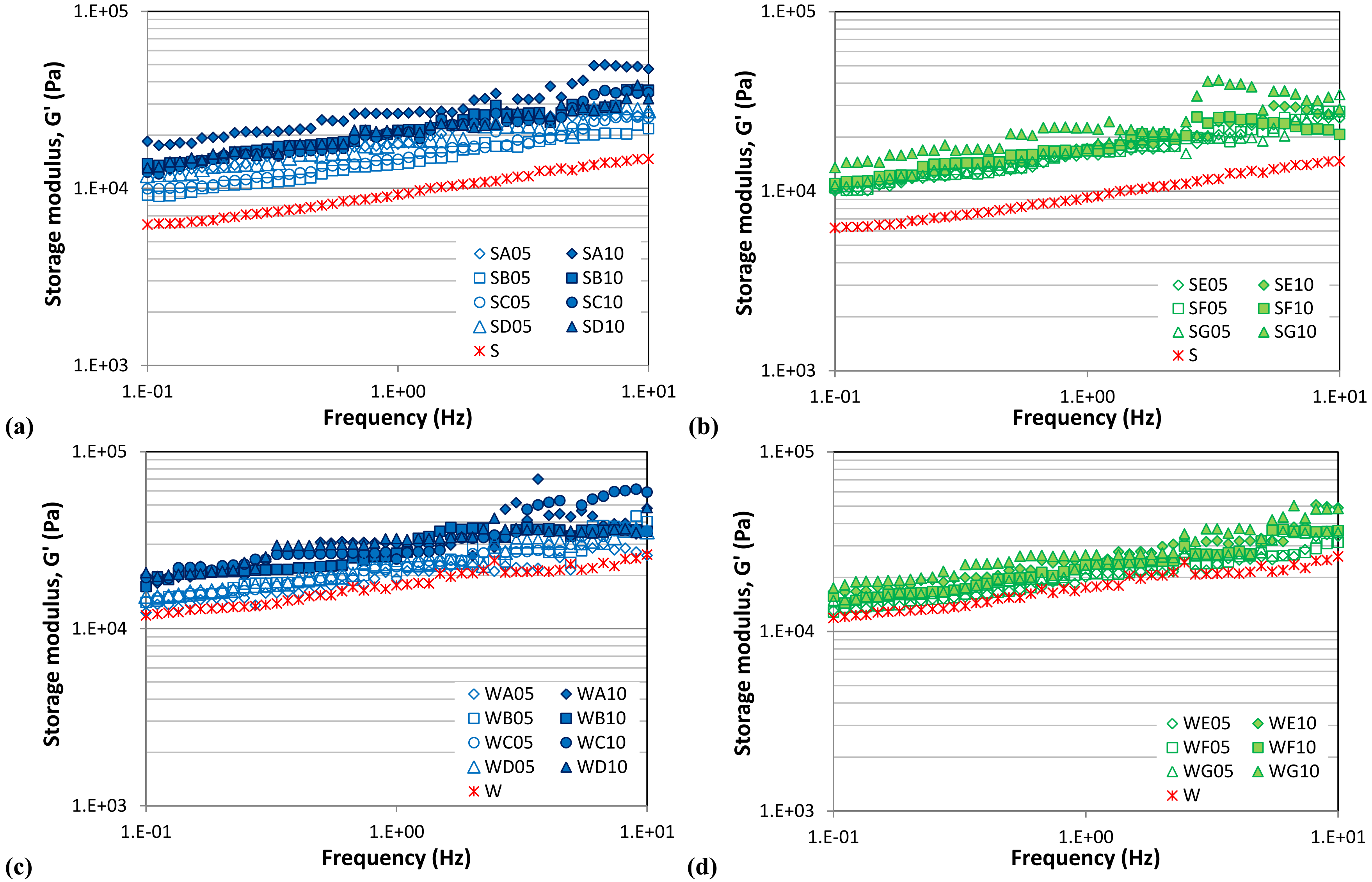


Figure 3.7 Storage modulus (G′) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at constant water absorption.

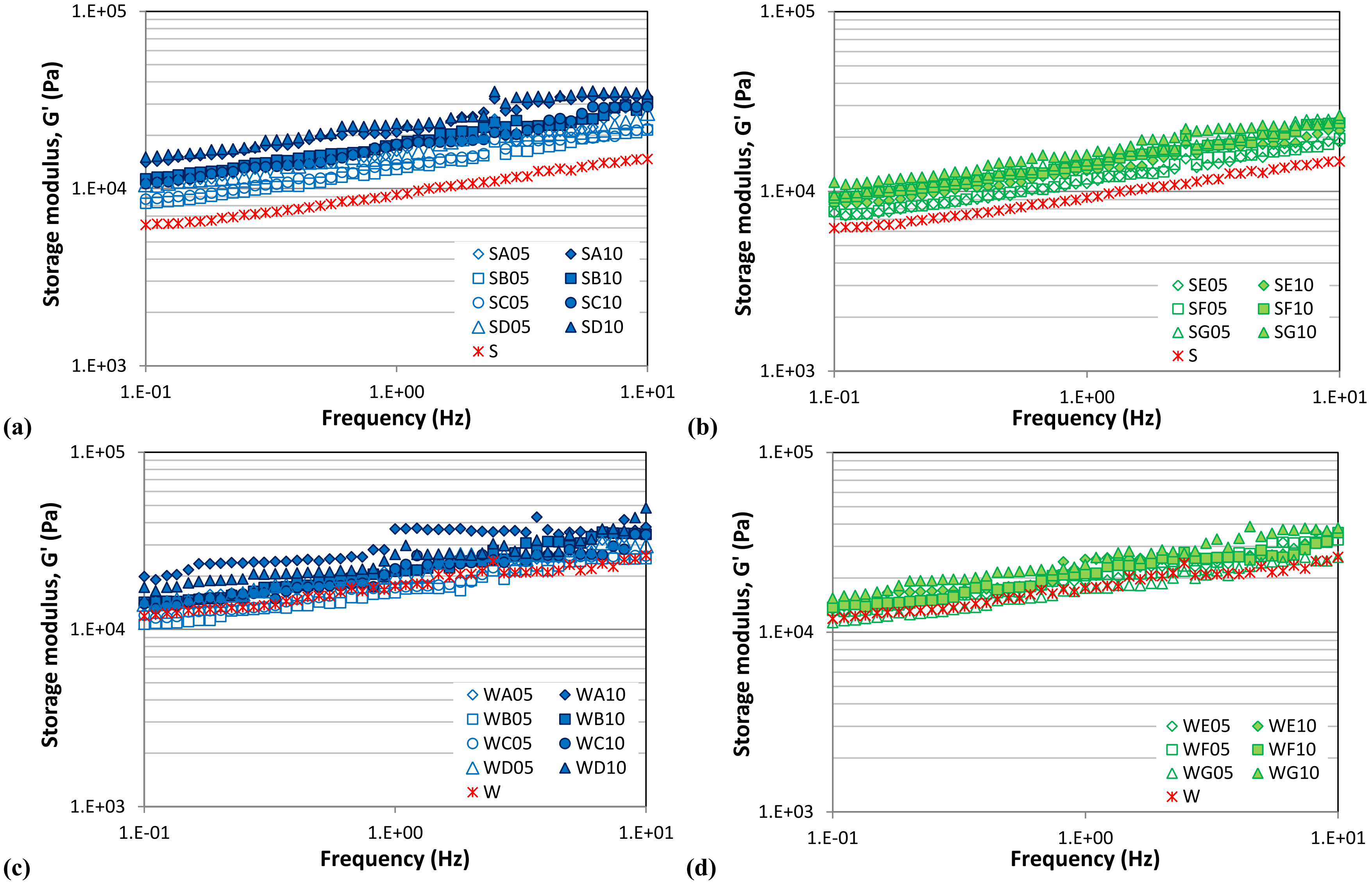


Figure 3.8 Storage modulus (G′) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.

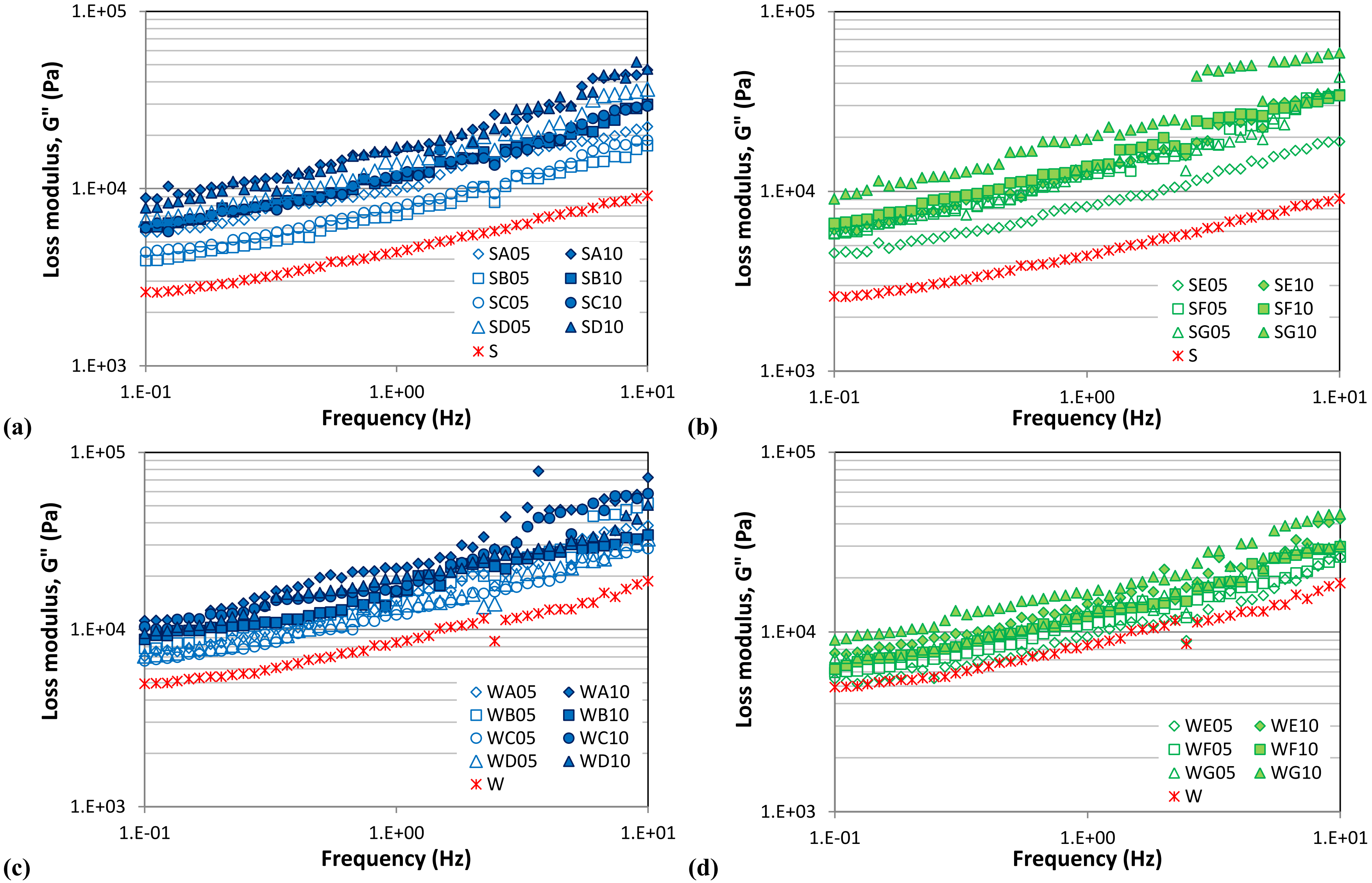


Figure 3.9 Loss modulus (G″) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at constant water absorption.

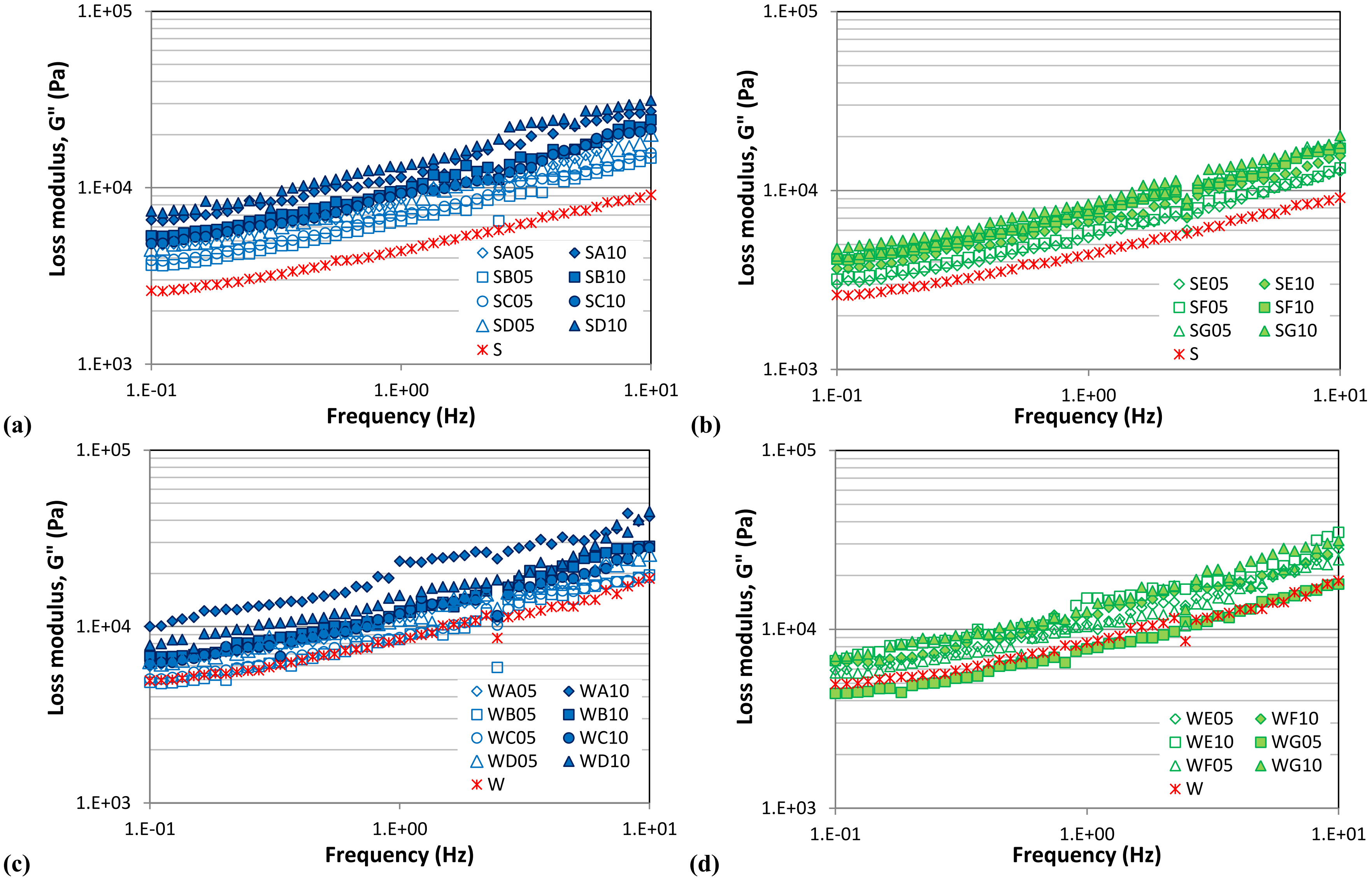


Figure 3.10 Loss modulus (G″) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.

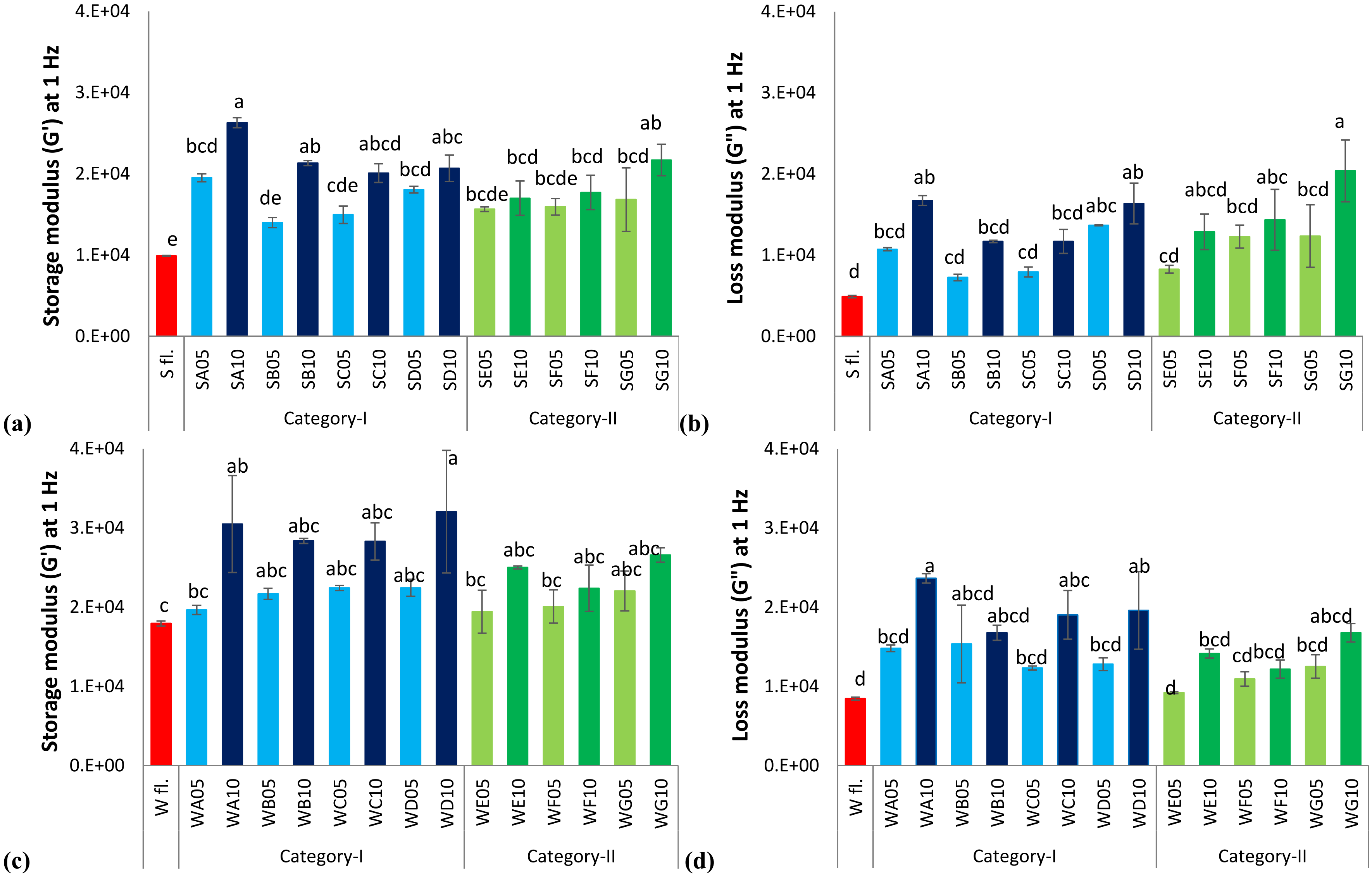


Figure 3.11 Storage (G′) and loss (G″) moduli at 1 Hz frequency. (a) G′ of strong (S) flour, (b) G″ of strong (S) flour, (c) G′ of weak (W) flour, and (d) G″ of weak (W) flour at 5 and 10% bran replacement levels at constant water absorption

Values with the same letter are not significantly different from each other at $p \leq 0.05$.

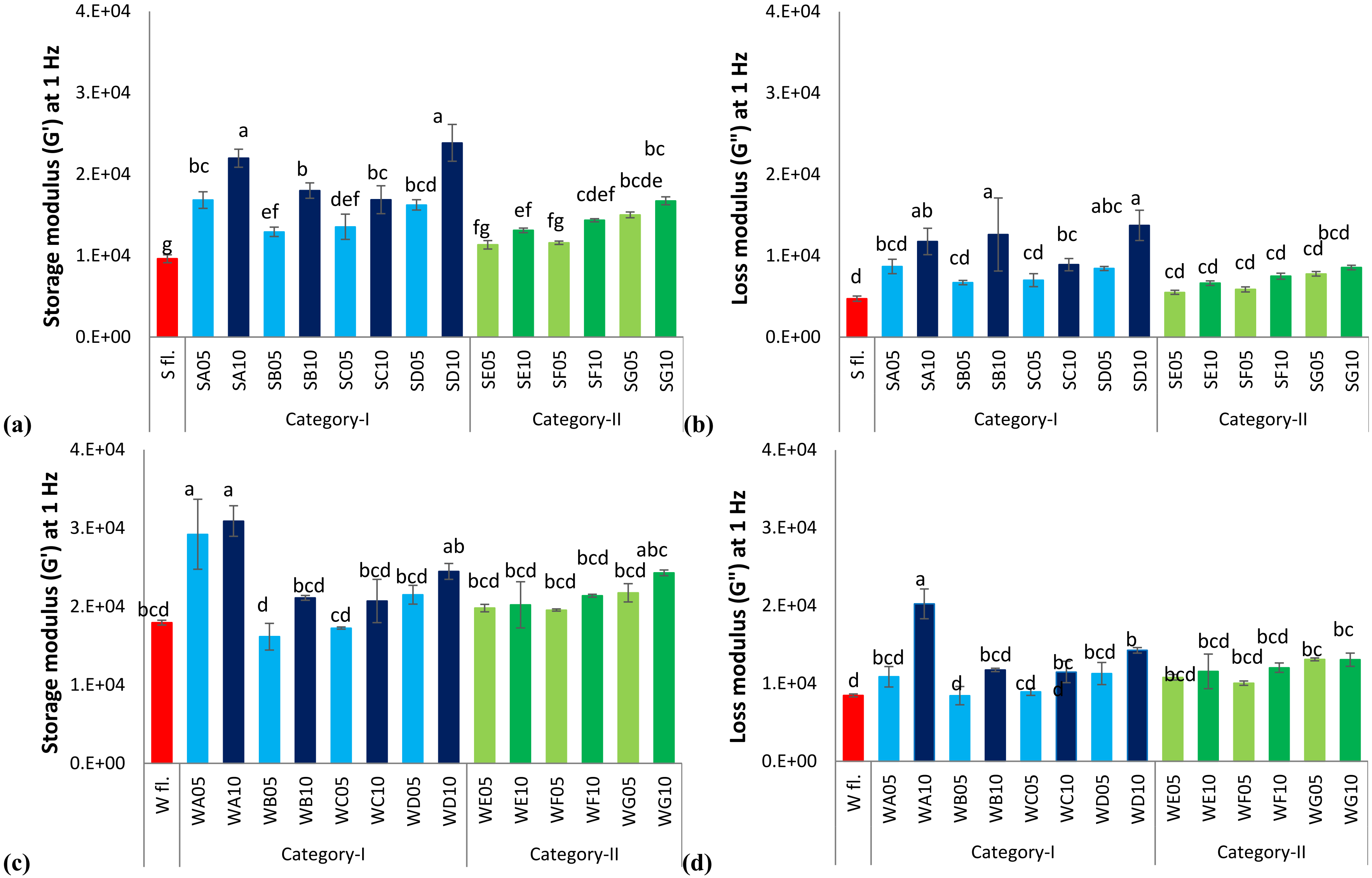
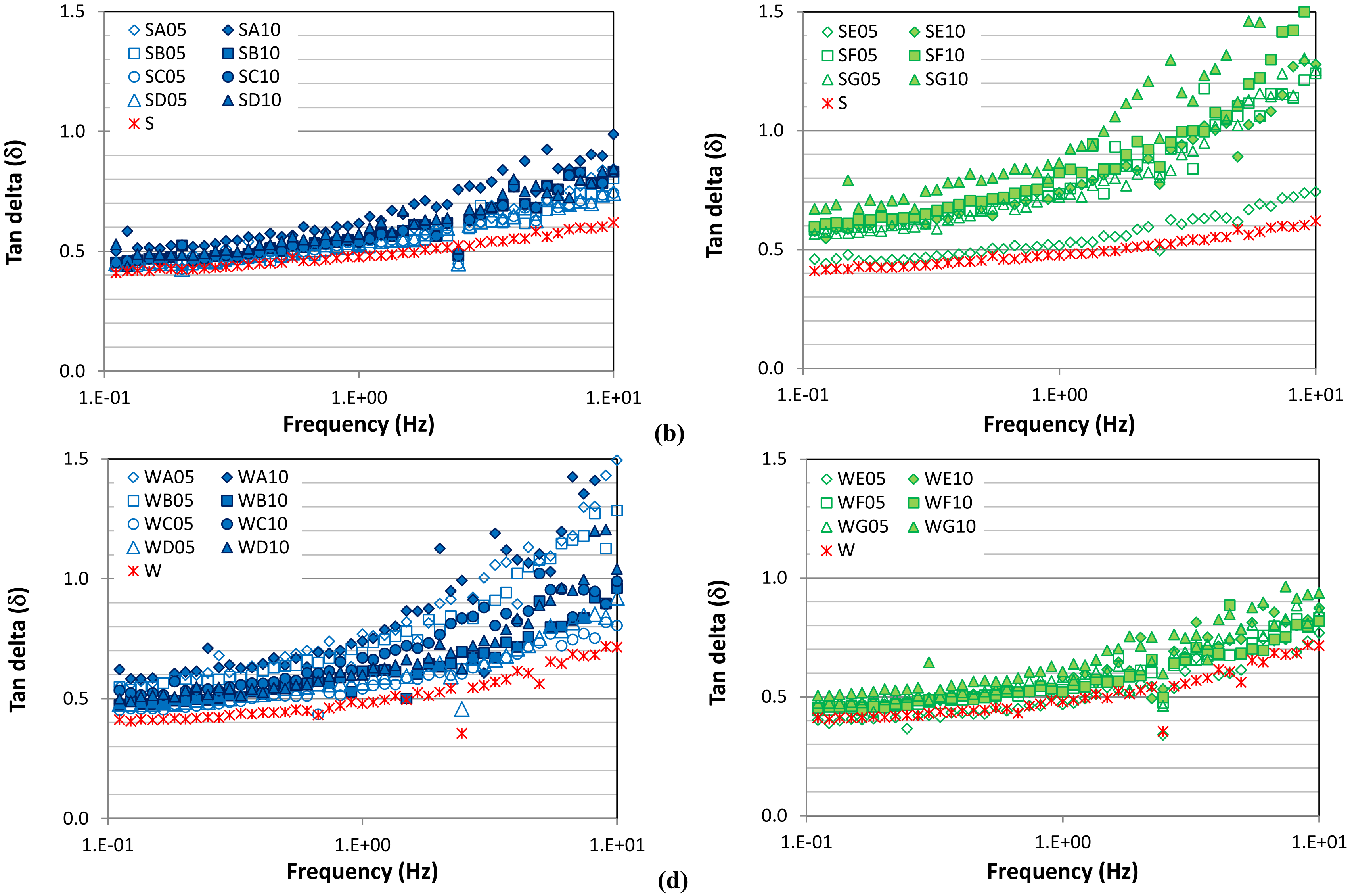


Figure 3.12 Storage (G′) and loss (G″) moduli at 1 Hz frequency. (a) G’ of strong (S) flour, (b) G” of strong (S) flour, (c) G’ of weak (W) flour, and (d) G” of weak (W) flour at 5 and 10% bran replacement levels at optimum water absorption.

Values the same letter are not significantly different from each other at $p \leq 0.05$.



(a) (b) (c) (d)

Figure 3.13 Tangent delta (δ) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at constant water absorption.

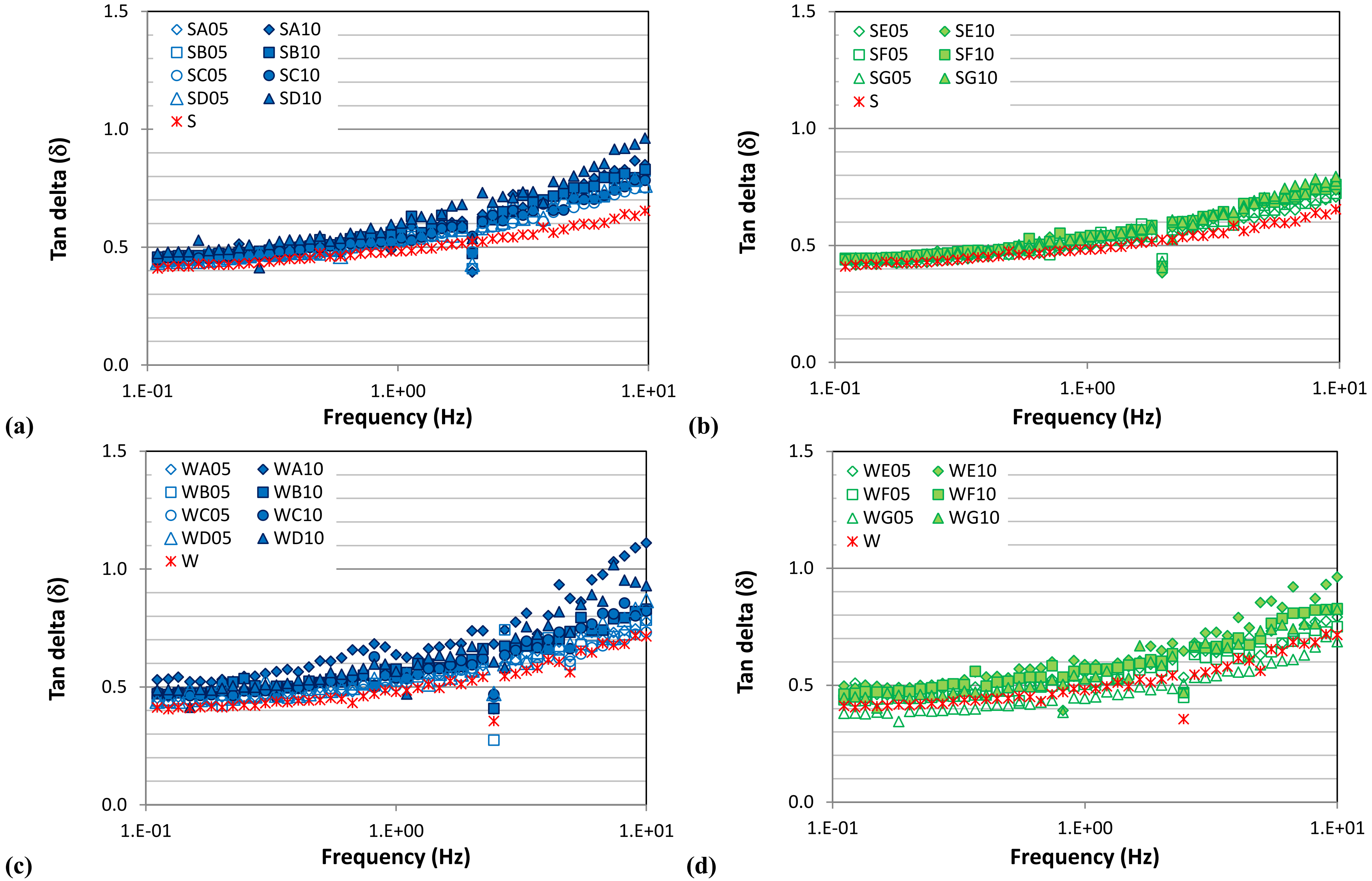


Figure 3.14 Tangent delta (δ) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.

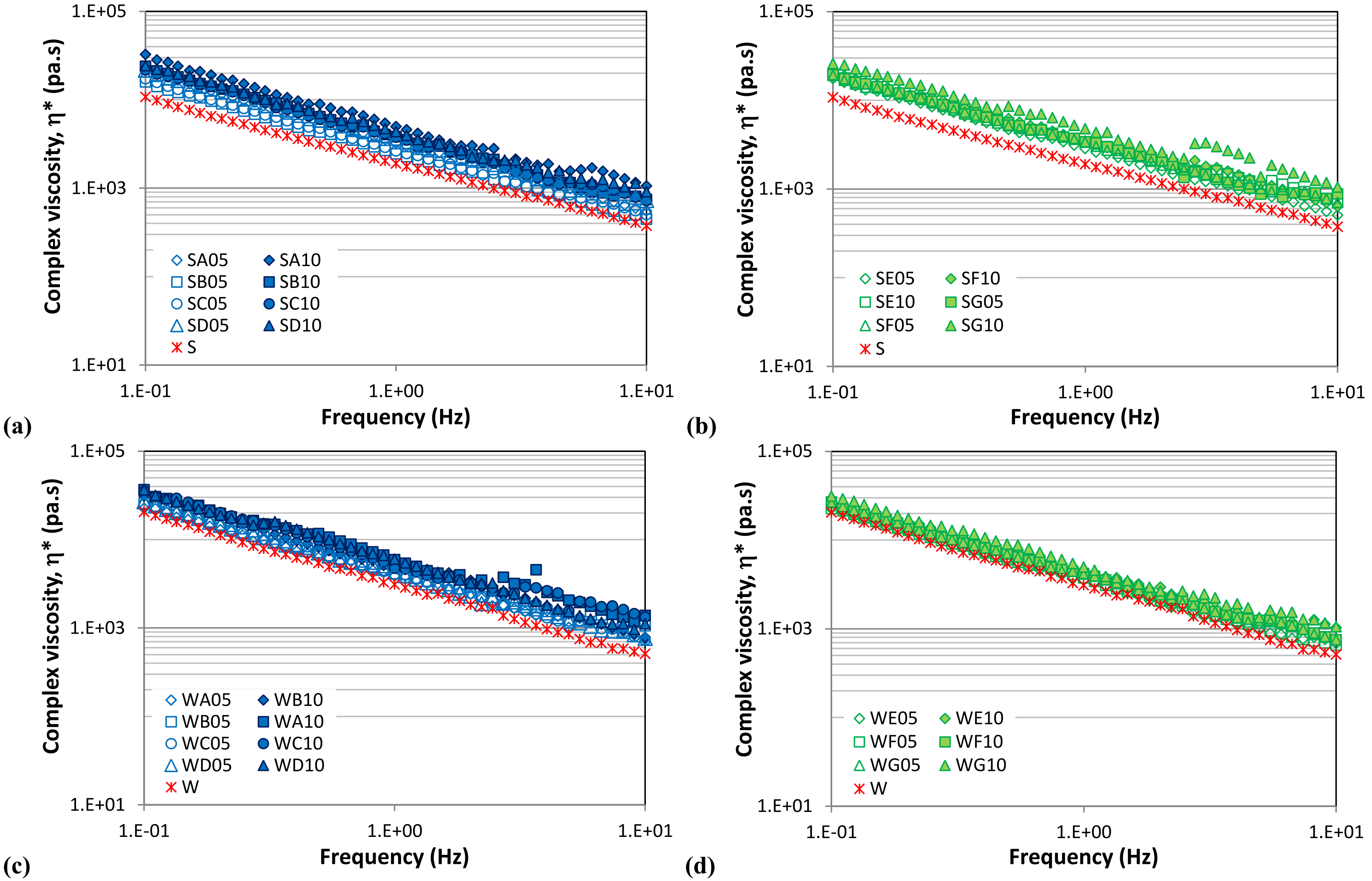


Figure 3.15 Complex viscosity (η*) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at constant water absorption.

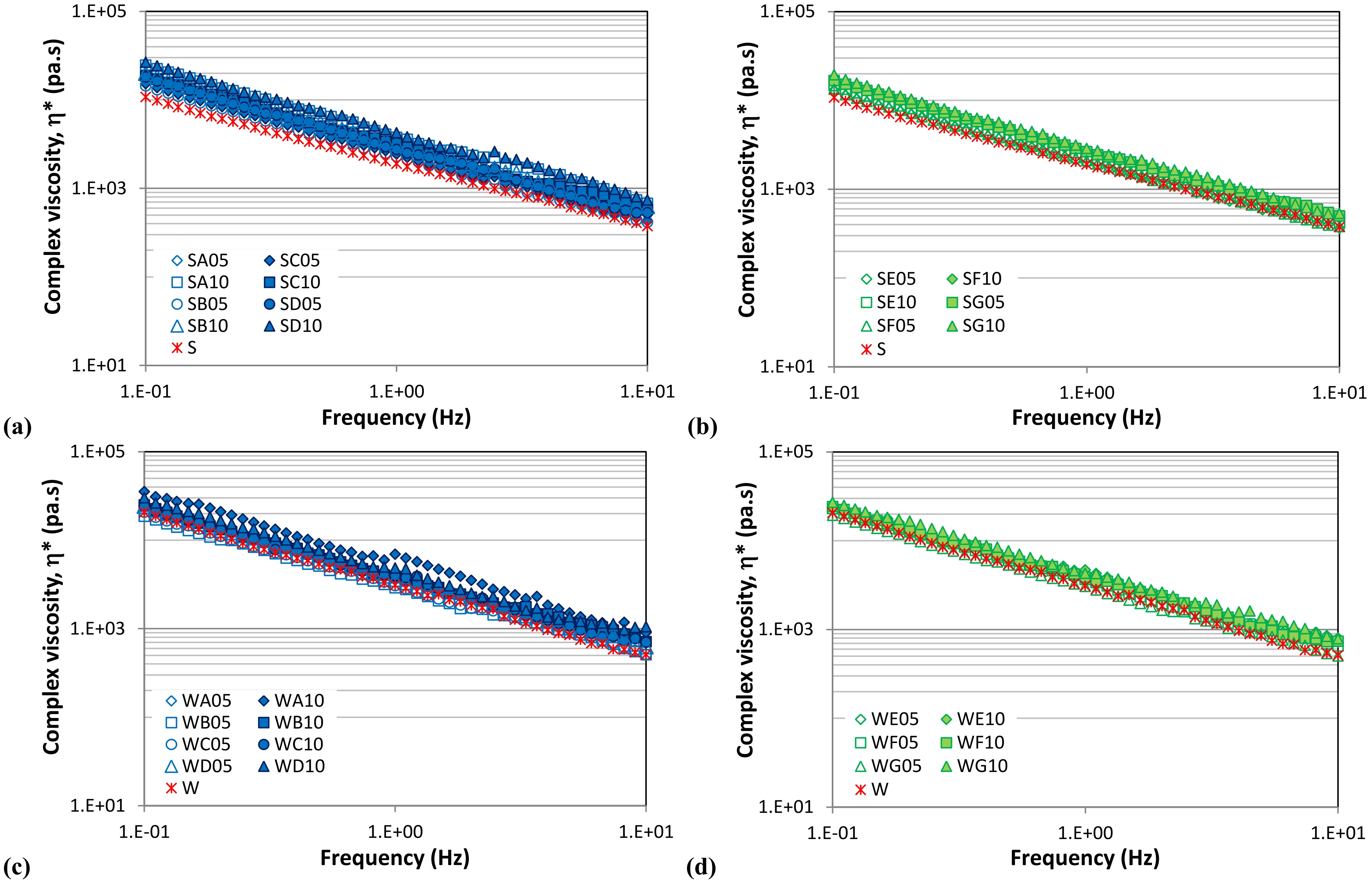


Figure 3.16 Complex viscosity (η*) versus frequency plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.

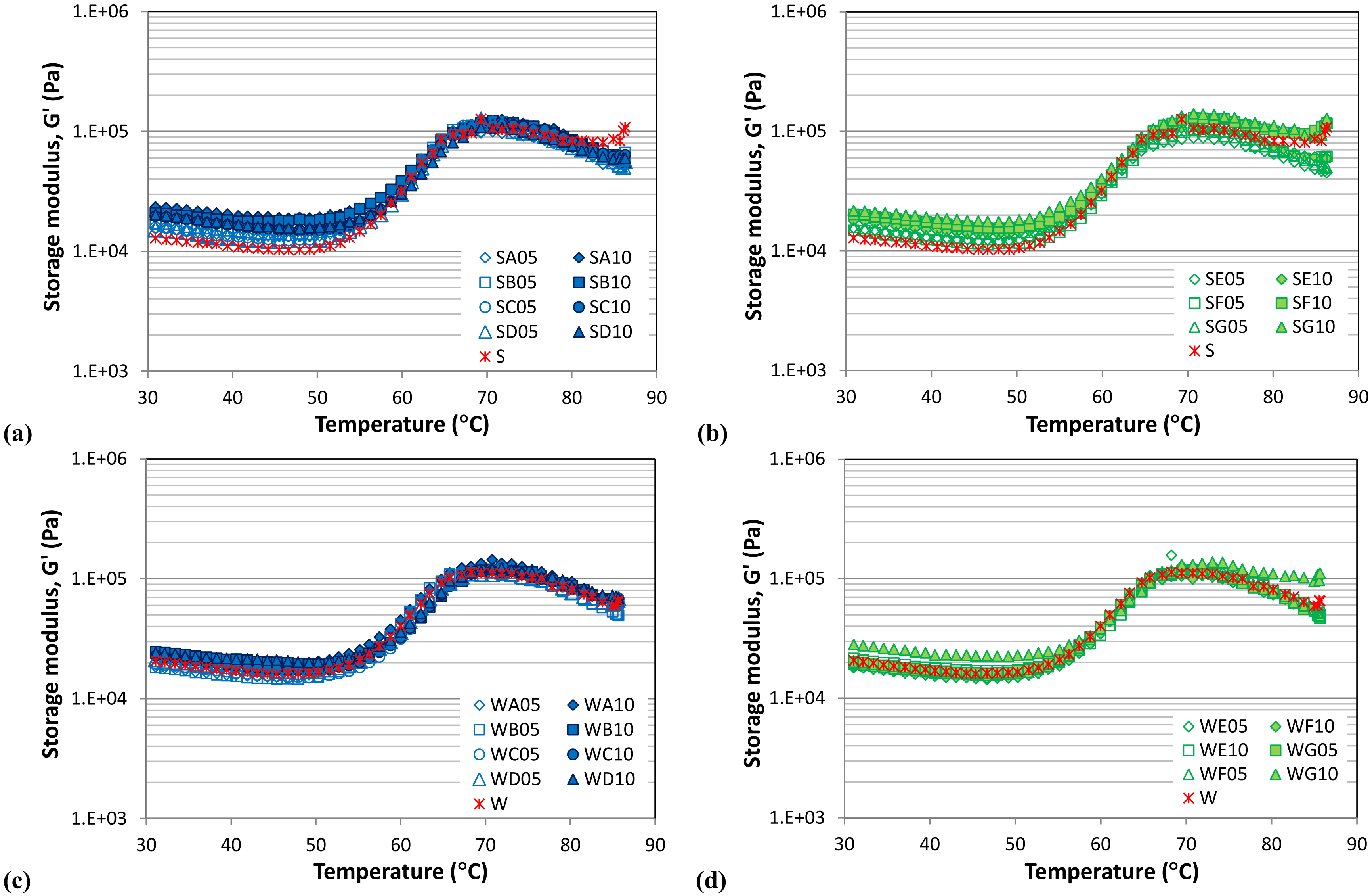
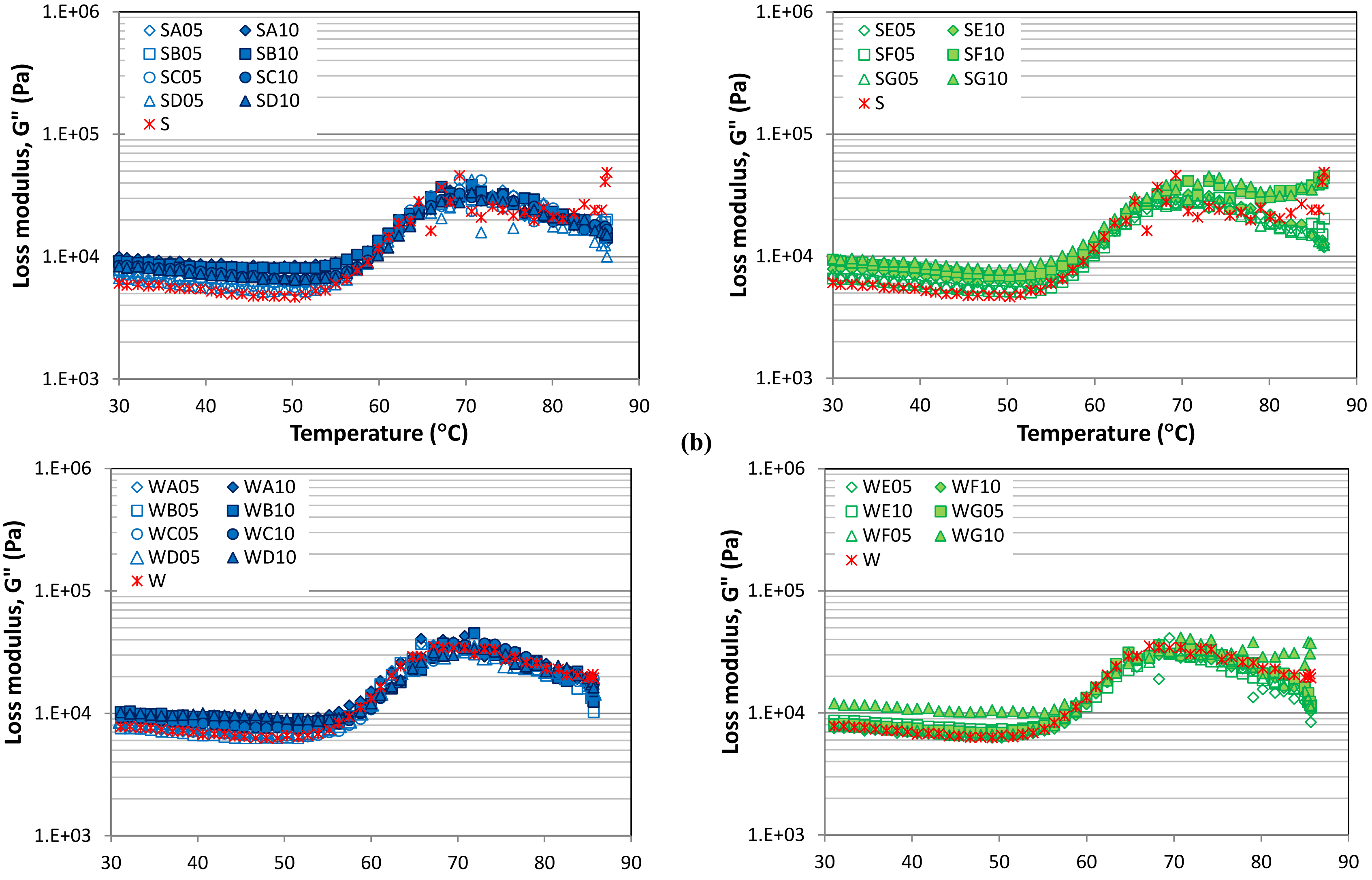


Figure 3.17 Storage modulus (G′) versus temperature plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption



(a) (b) (c) (d)

Figure 3.18 Loss modulus (G″) versus temperature plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.

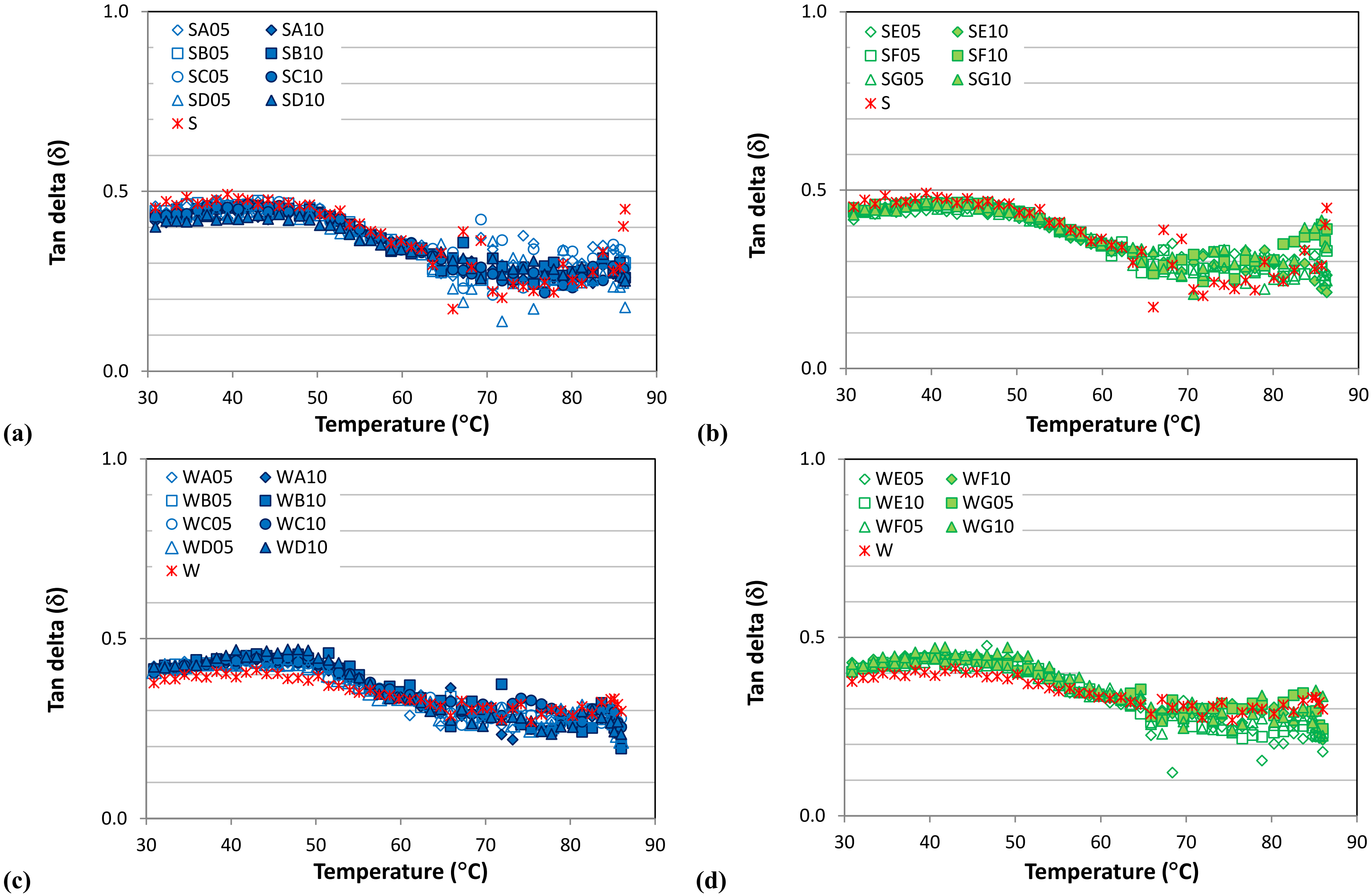


Figure 3.19 Tangent delta (δ) versus temperature plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.

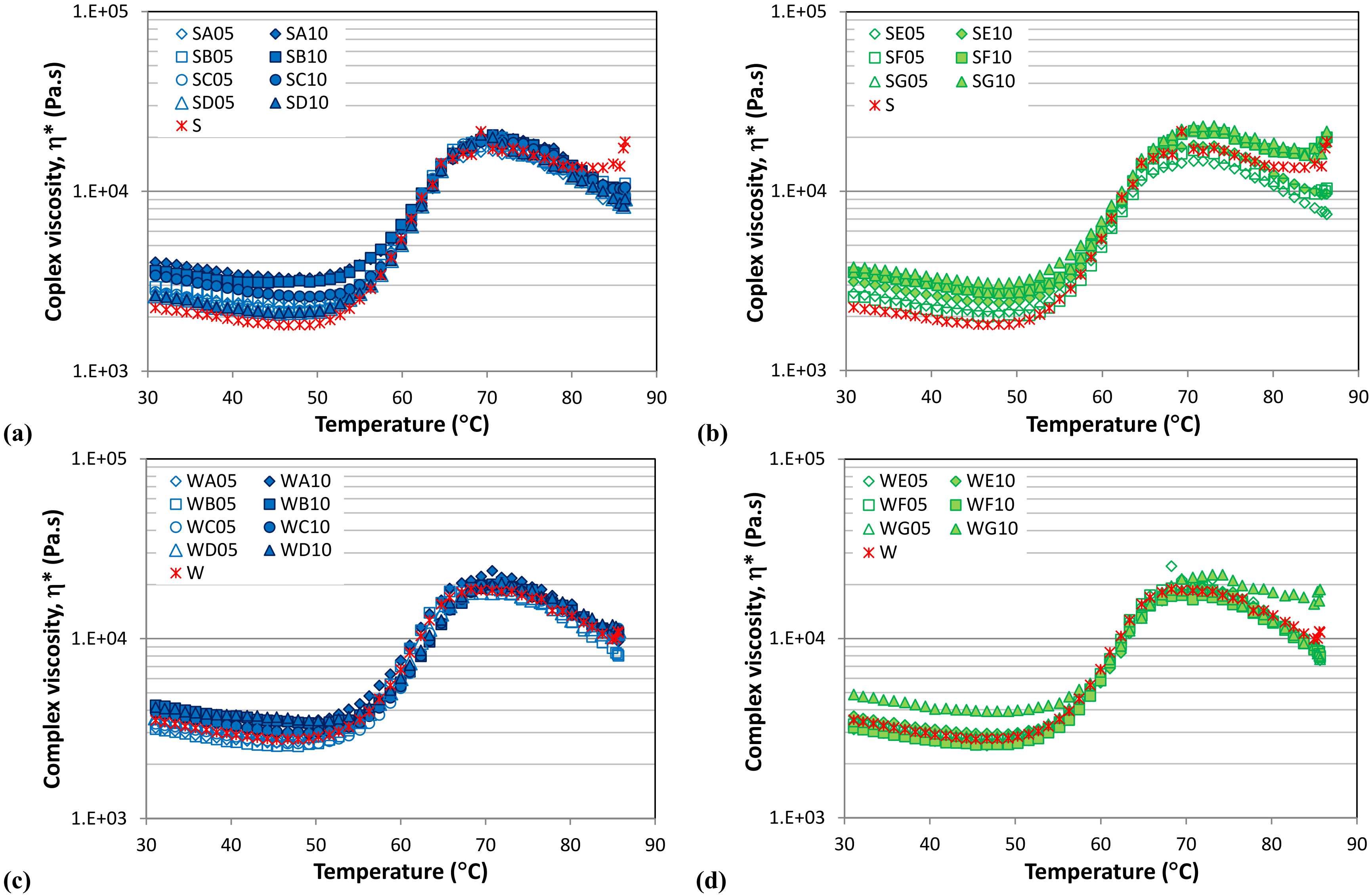
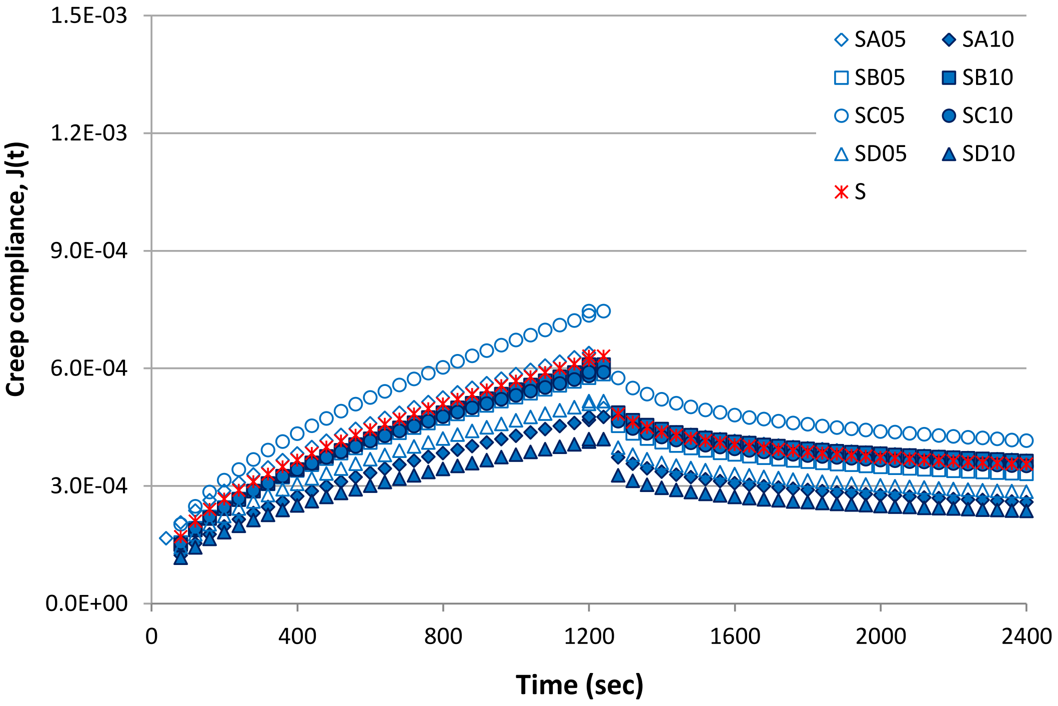
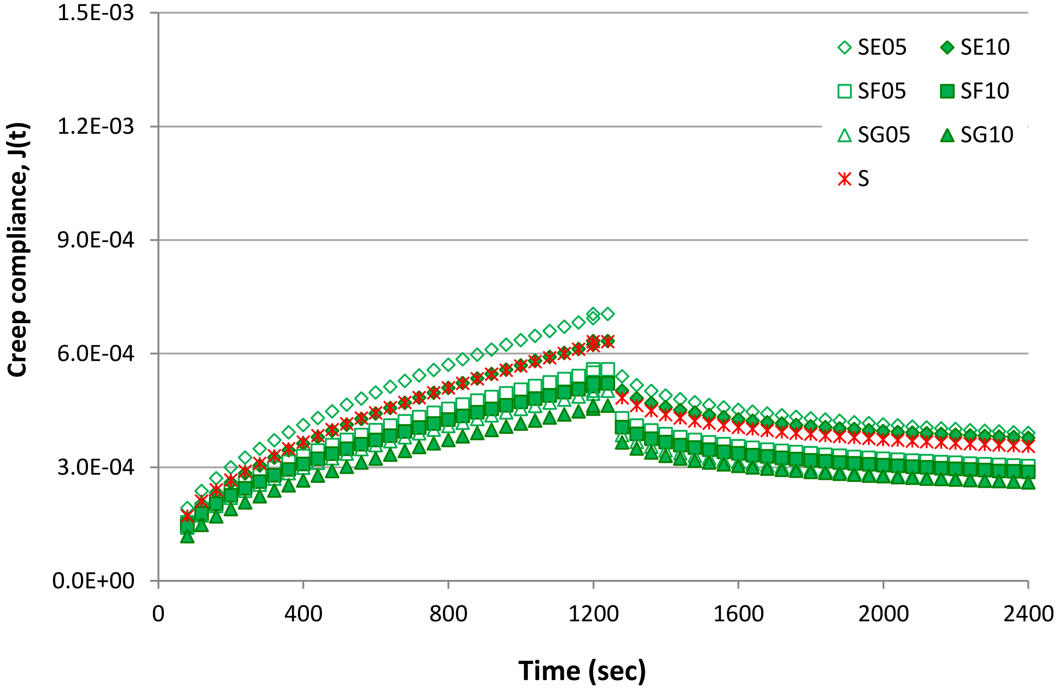


Figure 3.20 Complex viscosity (η*) versus temperature plots for (a) strong (S) flour, category-I brans, (b) strong (S) flour, category-II brans, (c) weak (W) flour, category-I brans, d) weak (W) flour, category-II brans at 5 and 10% replacement levels at optimum water absorption.


1.5E-03
1.2E-03
9.0E-04
6.0E-04
3.0E-04
0.0E+00
Creep compliance, J(t)
0
400
800
1200
1600
2000
2400
Time (sec)
SA05
SA10
SB05
SB10
SC05
SC10
SD05
SD10
S

(a)


1.5E-03
1.2E-03
9.0E-04
6.0E-04
3.0E-04
0.0E+00
Creep compliance, J(t)
0
400
800
1200
1600
2000
2400
Time (sec)
SE05
SE10
SF05
SF10
SG05
SG10
S

(b)

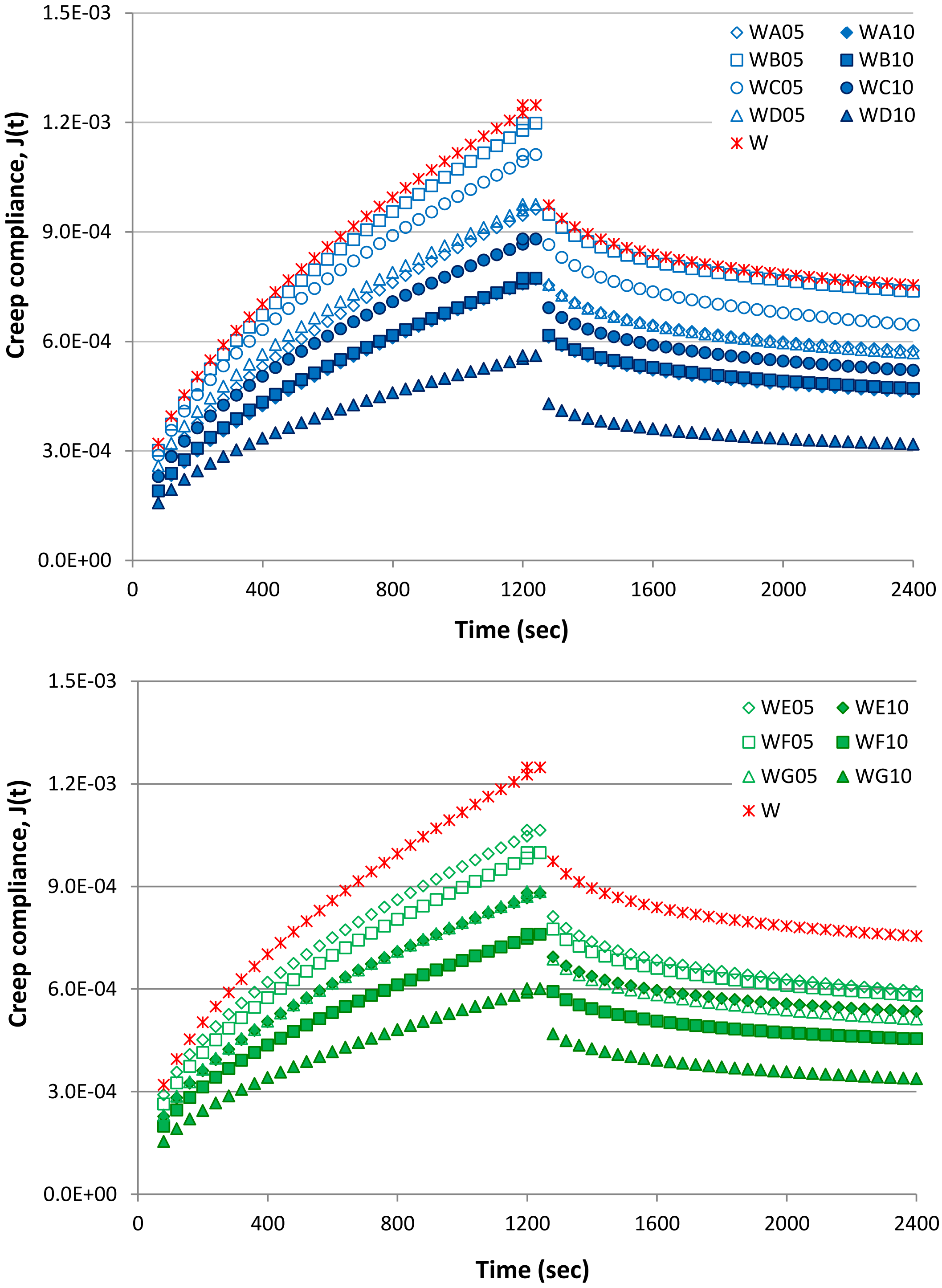


Figure 3.21 Creep and recovery profiles of strong and weak flours at 5 and 10% bran replacement levels at optimum water absorption. (a) Strong (S) flour, category-I brans, (b) Strong (S) flour, category-II brans, (c) Weak (W) flour, category-I brans, (d) Weak (W) flour, category-II brans

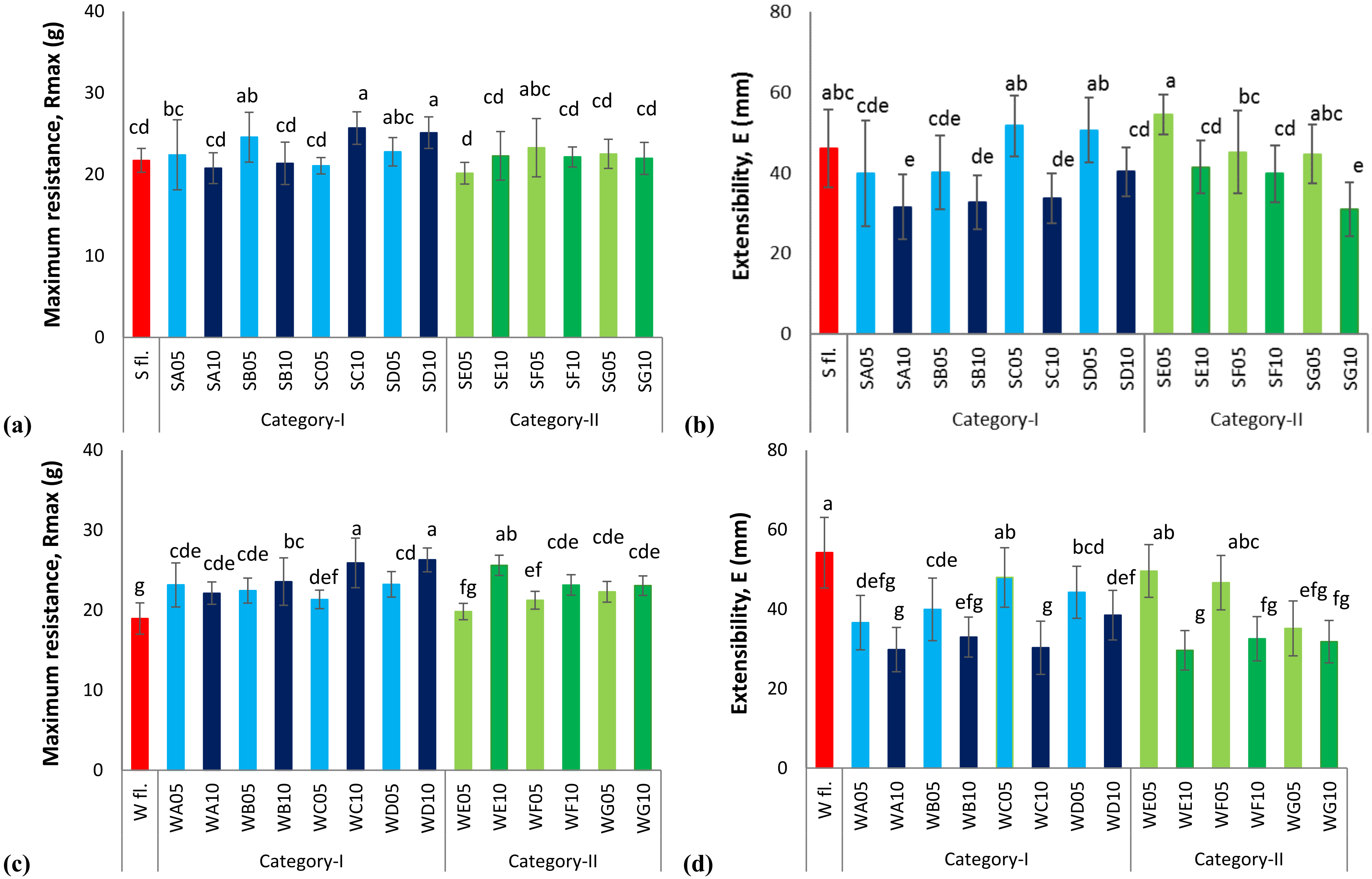


Figure 3.22 Uniaxial extensional properties. (a) Rmax of strong (S) flour, (b) E of strong (S) flour, (c) Rmax of weak (W) flour, (d) E of weak (W) flour at 5 and 10% bran replacement levels at constant water absorption

Values with the same letter are not significantly different from each other at p≤0.05.

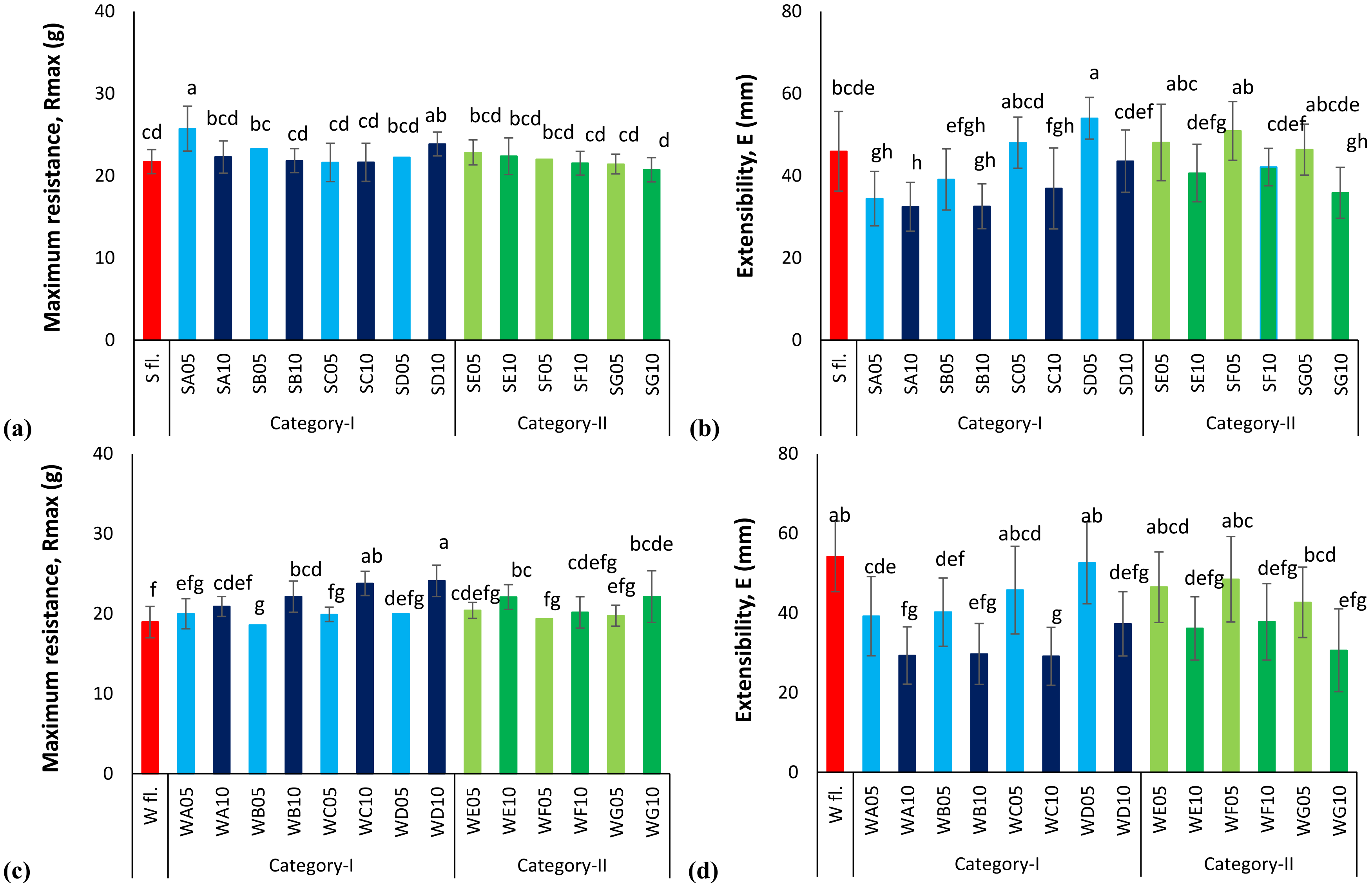


Figure 3.23 Uniaxial extensional properties. (a) Rmax of strong (S) flour, (b) E of strong (S) flour, (c) Rmax of weak (W) flour, (d) E of weak (W) flour at 5 and 10% bran replacement levels at optimum water absorption.
Values with the same letter are not significantly different from each other at $p \leq 0.05$.

Chapter 4 - Conclusions and Future Work

4.1 Research Summary

The fiber rich materials such as wheat bran has been added to the wheat flour to improve the nutrition availability, It also provides health benefits associated with fiber consumption. From past a few decades, the production of high-fiber bread has grown relatively a major portion of the bread industry. Several studies has been conducted on high-fiber bread production and reported that fiber affects the bread quality. The bran interactions with the gluten protein matrix play a dominant role in both ‘in-process’ dough and final product quality of whole grain bake goods. This study seeked to provide the fundamental understanding of the bran interactions with gluten matrix to minimize the adverse processing or product characteristics resulting from bran inclusions.

The *first study* compared hard red winter (HRW) and soft wheat (SW) cultivars of different protein contents and their respective brans, and their baking qualities using recording dough mixers, quantitative baking, textural and structural analysis of baked loaves. Addition of wheat bran to bread dough formulations appeared to have significant effects on mixing properties, quantitative baking and crumb texture. Increased level of bran substitution increased the water absorption. HRW flour performed better compared to SW both during dough mixing and baking. In general, bran addition affected the performance of HRW flour negatively. The bran additions to SW flour have strengthening effect by slightly improving the mixing time as indicated by increased mixing tolerance indices. The quantitative results shows that SW bran addition to HRW flours resulted in relatively higher loaf volumes and better crumb texture compared to HRW bran additions to HRW flour systems. Two particle sizes, coarse and fine, were also studied in HRW and SW flour systems. Bran particle size affected the mixing and baking properties. The fine particle size had better mixing properties and larger loaf volumes than coarse particle size. The fine particle size also gave an open texture at higher bran levels. X-ray microtomography analysis showed that increase in bran addition level increases the cell wall thickness of resulting breads baked from both HRW and SW flour doughs. The microstructural analysis of bran containing bread samples indicated a gradual shift in air cell size distribution towards higher values in comparison to that of control breads.

The *second study* focused on testing the effect of bran particle sizes and anatomical origin from single variety of wheat bran on dough development and rheology. Two flours of different strength (strong and weak) were used for this study. The original bran was grinded and sifted to have seven (A, B, C, D, E, F, and G) different fractions to study their effect at 0, 5 and 10% replacement levels. Constant and optimum water absorption protocols were used for dough development and sample preparation for subsequent tests. Hydration behavior of the bran samples alone seems to be affected mainly by their particle size; except for bran G. Solvent retention capacity (SRC) of bran samples was significantly higher than that of base flour due to their high protein and fiber content. In general, solvent retention capacity decreased with decreasing particle size. Water-SRC of composite flours increased insignificantly with increasing bran replacement levels, while sucrose-SRC, sodium carbonate-SRC remained same as the control sample. Lactic acid-SRC, which reflects general gluten functionality, decreased very dramatically even at 5% replacement level. Bran, irrespective of type and size, diminished the capacity of flour to form gluten network.

The Farinograph data demonstrated a slight difference between all bran flour doughs. Increase in bran substitution level increased the water absorption by 1-4%. At optimum water absorption protocol, there was no significant difference in mixing time for bran substituted strong flour with respect to particle size and origin, while the replacement level was a significant contributor. Bran substitution had significant effects on dough dynamic properties. Small deformation behavior was studied through frequency sweep and temperature sweep tests. The storage (G') and loss moduli (G'') were higher for all bran substituted flour doughs than the control flour doughs irrespective of bran particle size and source. Differences were more pronounced in constant absorption protocol compared to optimum water absorption. Higher elastic modulus and lower frequency dependent behavior with increased bran substitution indicated stable network development. Temperature sweep data reflects the biochemical and physical reactions that take place during baking. The storage and loss moduli and viscosity of bran substituted samples were significantly higher than those of control dough at cold stages of the process. After onset of gelatinization, no significant differences were observed in the peak G', peak G'' and peak viscosity values.

Large deformation behavior was assessed using creep-recovery test and uniaxial extension test. Bran inclusion resulted in lower creep compliance for all bran substituted flour doughs compared to their control dough counterparts. 10% substitution resulted in the lowest lower creep compliance which indicates higher resistance to deformation due to presence of bran. The decrease in creep compliance due to the presence of bran was less noticeable in strong flour doughs. Percent creep recovery upon removal of the applied stress was up to 6% higher in strong dough samples compared to the weak dough samples. Kieffer rig tests resulted in no significant difference in maximum resistance to extension and extensibility values for all the bran doughs irrespective of bran particle size or source for both optimum and constant water absorption protocols. However, the extensibility decreased significantly with increased bran substitution level. Bran substitution affected the extensibility of strong flour doughs in a lesser extent than the effect observed in weak flour doughs.

Based on the results from second study summarized above, seven (A, B, C, D, E, F, and G) types of bran were concluded to exhibit *four distinct groups* in terms of their overall functionality: A ≅ B, C ≅ E ≅ F, D, and G.

i. A and B – the largest particle size
ii. D – the smallest particle size
iii. C, E and F – mid particle size range; C represents the original bran while E and F are sub-fractions from outer layers of bran.
iv. G – close to large particle size range, represents the sub-fraction from inner layers of bran (a.k.a. shorts).

Published work on inclusion of non-endosperm fractions (e.g. fiber, bran) in wheat flour doughs suggest widely differing hypotheses to explain the effects of bran on bread quality:

(1) Dilution effect:
  - Dilution of gluten by the addition of fiber fractions (Gan et al 1992).
(2) Physical hindrance:
  - Fiber particles piercing the gas cells (Courtin and Delcour 2002).
  - Fiber particles hindering gluten network formation (Lai et al 1989a, b).
(3) Chemical mechanisms:
  - Presence of water extractable arabinoxylans (WEAX) and water insoluble solids (WUS) affecting dough development (Wang et al 2003a,b, 2004a,b).
  - Negative effects of enzymes and reducing components on the gluten network (Lai et al 1989).

Our experimental findings suggest that **dilution of gluten by addition of bran** and **physical hindrance of gluten network by bran particles** seem to be main cause for the negative effect of bran on dough development, and small and large deformation rheological properties. However, we are not completely ruling out the effect of chemical mechanisms. As explained earlier, our bran sub-fraction preparation protocols did not results in a clear distinction in the chemistry of the bran fractions obtained. The method used in creation of Category-II brans was intended to produce bran fractions from varying anatomical parts of the original bran. However, except for bran G, there was not enough distinction observed the chemistry of resulting bran fractions. This is still an area that needs further investigation, as it is highlighted in section 4.3.

4.2 Limitations

The nature and extent of bran interactions with the gluten protein matrix play a dominant role in both 'in-process' dough and final product quality. Purposeful manipulation of those interactions should be able minimize adverse processing or product characteristics resulting from bran inclusion/presence. This study suggested that dilution of gluten by addition of bran and physical hindrance of gluten network by bran particles are two major mechanisms affecting dough performance in the presence of bran. However, these findings are limited and empirical in nature as only one-year crop, and one type of bran source were used. In order to reach more universal conclusions, the seasonal and location-based variances in crop quality as well as genetic pedigree of the wheat used, the farming practices employed and several other factors have to put into consideration.

4.3 Future Work

The study provided understanding based on the hypothesis of "dilution of gluten by addition of bran" and "physical hindrance of gluten network by large bran particles". Bran reconstitution studies have shown that the baking quality of various flours is primarily related to gluten protein fraction. Therefore, the study also needs to be focused on hypothesis of "bio-chemical mechanism by addition of bran particles" which includes interactions of specific reactive components with gluten and the degree of liberation of these reactive components due to cell breakage during milling and bran processing.

Further research can be done in the following areas:

1. Studies needed to identify the exact components that are responsible for the negative effects of fiber fractions on breadmaking quality:
    - Studying interactions of specific reactive components with gluten (e.g. ferulic acid bound to insoluble cell wall material).
    - Studying degree of liberation of reactive components (e.g. ferulic acid monomers, reducing components, phytate) due to cell breakage.
    - Identifying the exact components by fragmenting wheat bran layer by layer by using electrostatic separation studies and quantified using biochemical marker method described by Hemery at al (2009, 2011).
2. Studies needed to explore evolution of air bubble formation and growth starting from dry mixing step followed by dough development until the end of proofing and baking.
    - A fast speed XMT technique can be used to have a closer look into dough microstructure in-real-time.
3. Studies needed to explore the dough rheology at extreme strain rates (both low and high) that mimic the deformation rates typical to breadmaking process.
    - Important rheological properties including stress relaxation, creep recovery, uniaxial and biaxial extension, strain hardening could be studied to better understand dough network development, gas cell stability, dough and end product microstructure and the resulting texture.
4. Studies needed to identify the sensory and consumer preference aspects of the bran containing baked goods.

4.4 References

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