AN INITIATIVE TO CLEAN LABEL: CAN WE REPLACE DOUGH STRENGTHENERS IN BREAD FORMULATIONS

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ABSTRACT

Dough strengtheners are the most common and largest group of chemicals used in bread formulations. This study evaluated the capability of hard red spring (HRS) wheat flour to replace commercial dough strengtheners in bread production. Doughs were prepared by blending different percentages (10%, 20%, 30% and 40%) of four different HRS wheat flours with hard red winter (HRW) wheat flour. In addition, doughs were prepared by adding ten commercially available additives with HRW wheat flour to compare the dough strengthening ability of HRS wheat flour. All the HRS wheat flour blends had significantly ($p<0.05$) strong dough rheological characteristics than most of the additives. The 40% blends of HRS wheat cultivar Glenn and Linkert had better bread making quality than other blends and all the additives. The SE-HPLC unextractable protein fractions of these two cultivars also showed a better correlation with bread making properties than all the additives.

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DEDICATION

I would like to dedicate this dissertation to my parents who has sacrificed a lot for my education.

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GENERAL INTRODUCTION

Bread is one of the most important sources of carbohydrates in the form of starch in the human diet (Dewettinck, et. al. 2008). It is a staple food in the United States and is consumed in-home during meals 97% of the time (International Markets Bureau 2012). According to U.S. Census data and Simmons National Consumer Survey data, 319.24 million Americans use bread in their diet (Statista 2017).

The major ingredients used in bread are flour, water, leavening agents (yeast or chemicals), salt (sodium chloride) and different types of sweeteners such as sucrose, corn syrups and dextrose, honey, malt and malt syrups, lactose, sorghum and maple syrups. Besides these components, some micro ingredients are also used for the overall quality improvement of bread. These are oxidizing agents, reducing agents, buffers, enzymes, gluten proteins, and dough improvers/ conditioners etc. (Pyler and Gorton 2008).

In a market survey, it was found that as many as fifty ingredients were used in bread formulations and of these, the greatest number (as many as ten) of chemical additives are dough conditioners. Among different types of dough conditioners, some are used for textural development of final product and others are used as dough strengtheners. Dough conditioners are generally, used to aid in production and to improve bread quality. These ingredients may bind with the protein to increase loaf volume, dough tolerance, structure and crust tenderness, and dough knockdown as well as bind with the starch to help in retard bread staling (Dubois 1979). However, many of these ingredients are associated with several health controversies. For example, potassium bromate is a much discussed subject since 1990 (Pyler and Gorton 2008) due to its carcinogenic effects (Kurokawa et al. 1990), Azodicarbonamide is irritating to the eyes and the respiratory tract (CDC 2015) while DATEM caused heart fibrosis and adrenal overgrowth in rats (Winter 2009).

Consumers' food choice is believed to be greatly influenced by their judgment of the available information on the label because they are becoming increasingly cautious about food safety (Aoki, et al. 2010). The long list of chemicals on ingredient labels often confuses consumers rather than enlightens them. That's why the demand for "clean label" formulations are steadily increasing (Watrous 2015). Although U.S. Food and Drug Administration (FDA) has no regulatory definition for clean label, it tends to involve the following views:

According to Edwards (2013), the clean label means food products are free from chemicals additives and produced with limited processing by traditional techniques with easy-to-understand ingredients. However, Ingredion (2014) ignored the processing technique and emphasized natural, organic and/or additives free food products. Later, Hutt and Sloan (2015) also explained that clean label includes all natural ingredients, organic, no artificial ingredients including preservatives and color, and no high fructose corn syrup.

In breadmaking, all the major and minor ingredients are mixed to form a viscoelastic dough. When wheat flour is mixed with appropriate amount of water, it forms strong and cohesive dough due to gluten formation, which involves the storage proteins glutenins and gliadins. Hydrated gliadin is extremely sticky, not resistant to extension and responsible for cohesiveness, viscosity, and extensibility in a dough system. On the other hand, glutenin is resilient and rubbery, responsible for elastic and cohesive properties of dough (Hoseney 1986). Glutenin proteins form a disulfide cross-linked protein polymeric network, while gliadins don't contribute to the protein matrix formation, but they interact with gluten in structures through noncovalent bonds and affect viscous properties of dough (Shewry and Tatham 1997).

Among the six distinct classes of wheat grown in the United States, hard red spring (HRS) wheat has the highest protein content (usually 13 to 16 percent) and strong gluten characteristics.

HRS wheat is used extensively as blending wheat to increase the dough strength in batches of flour. The strong dough helps to increase loaf volume and provides the strength needed for baked products etc. North Dakota is the highest producer of HRS wheat in the United States and produces approximately half of the country's production (North Dakota Wheat Commission).

Overall Objectives

In this context, the main objectives of this study were as follows:

1) To replace commercially available dough strengtheners by HRW: HRS wheat flour blend during bread making in order to provide a clean label.
2) To evaluate the properties of dough and bread made with HRW: HRS wheat flour as dough strengtheners.
3) To investigate protein molecular weight distribution (MWD) in doughs and bread made with HRW: HRS wheat flour blends and additives as well as the association of MWD with dough rheology and bread quality.

Need Statement

Consumers are becoming reluctant to purchase food products with a long list of chemicals on the label. The highest number of chemicals for a single purpose used in bread formulation are dough conditioners. Among different types of dough conditioners, some are used for the textural development of final product and others are used as dough strengtheners. However, many of these ingredients are associated with several health controversies. Additionally, HRS wheat has the highest protein content and strong gluten characteristics. The strong gluten helps to increase bread loaf volume and provides the strength needed for baked products. This study will compare the dough strengthening ability of HRS wheat flour with ten commercially available dough strengtheners. Some conclusions might be drawn on the influence of HRW: HRS flour blends by

examining the dough and bread properties after replacing commercial dough strengtheners. It will be of significant advantage to determine if the dough and bread quality remain the same or improved with flour blends containing HRS wheat flour compared to commercially available dough strengtheners.

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CHAPTER 1. LITERATURE REVIEW

1.1. Bread Production

Bread is a staple food (Cauvain 2004), and has been an essential part of our diet for thousands of years (Scanlon and Zghal 2001). Bread is a good source of carbohydrates as well as substantial amount of proteins, vitamins, and minerals (Collado-Fernández 2003). Breadmaking is a centuries old traditional craft and has gone through continuous innovation (Cauvain 2016). The existing geographical and cultural diversity in breadmaking are mainly attributed to variation in ingredients used in bread formulation. Thus, the differences among breads, for the most part, are a function of the ingredients used in formulations and the manner that ingredients are processed into the final products (Collado-Fernández 2003).

The earlier breads were made from grains mashed with water or milk. These were more like porridges or flat cakes and eaten raw or cooked. The leavened bread was introduced nearly seven thousand years after flat bread. Egypt began organized grain production after 5000 B.C.E. and bread became a staple food. Breadmaking was a status symbol in Britain during medieval times. The upper classe people preferred fine and white loaves while the whole wheat, bran, and coarser breads were left for poorer status.

In 1834, the steel roller mill was invented in Switzerland that broke the grain and separated the germ, bran, and endosperm. This invention drastically changed the milling technique around the world and increased the consistency of milled flour. At the beginning of the twentieth century, industrial revolution brought the mass production of white wheat breads that changed the taste and appearance of bread. By the early twentieth century, white flour was bleached and bromated by baking industry to get consistency in mass production as well as enrichment of flour was introduced to replenish the nutrients during milling. The hydrogenated oils, artificial preservatives,

emulsifiers, and other chemical additives had also been introduced in the twentieth century to soften the crumb and extend the shelf life (Bread 2003). Potassium bromate was first recommended in 1916 as a bread improver to increase loaf volume and improve texture (Bushuk and Hlynka, 1960).

Nowadays, the milling and baking industry produce homogenous bread in a mass scale and distribute over a wide geographical area. The dough conditioners make it possible by increasing machinability of dough without damaging it as well as softening the crumb to meet consumer demand. Emulsifiers help to strengthen the dough and also mold inhibitors delay the onset of bread mold (Bread 2003).

1.2. Ingredients Used in Bread Formulations

The principles of the breadmaking process have been established for thousands of years ago which involves a series of the interactions of bread raw materials, equipment, and people in a certain environment. There are chemical, physical, and biological activities which takes place during breadmaking due to interactions of the raw materials. Chemistry of dough is the interaction between carbohydrates, lipids and proteins; and physical science in dough making is rheology as well as the biological activities involve the fermentation process by yeast. There are many ingredients used in breadmaking namely: flour, water, leavening agents (yeast or chemicals), salt (sodium chloride), and different types of sweeteners such as sucrose, corn syrups and dextrose, honey, malt and malt syrups, lactose, sorghum and maple syrups. Besides these components, some ingredients are also used for the overall quality improvement of bread. These are oxidizing agents, reducing agents, buffers, enzymes, gluten proteins, salt, and dough improvers/ conditioners (Pyler and Gorton 2008). According to Pyler and Gorton (2008), bakers tend to group the ingredients into

three categories based on their level of use: major ingredients, minor ingredients, and micro ingredients.

1.2.1. Major ingredients used in bread formulations

Major ingredients are termed as bulk ingredients that makeup the majority of the formulations e.g. flour constitutes 55-60% (formula weight) or more in bread formulations. The major ingredients are wheat flour, non-wheat flour, masa, sweeteners, fats and oils, and water (Pyler and Gorton 2008).

Wheat flour contains three major components needed for baking namely protein, carbohydrates, and lipid (Bonomi et al. 2014). When wheat flour is mixed with appropriate amount of water, it forms a strong and cohesive dough due to the development of gluten network, which is considered as the basic framework for the baking process. One of the most versatile aspect of wheat flour is its storage proteins that develop gluten network during hydration and mixing. This gluten network is formed by two types of proteins found in wheat flour: polymeric glutenins and monomeric gliadins. The gliadin is extremely sticky, not resistant to extension, and is responsible for cohesiveness, viscosity as well as extensibility in a dough system. Conversely, the glutenin is resilient and rubbery which responsible for elastic and cohesive properties of dough (Delcour and Hoseney 2010). It is possible to define rheological properties of dough by the gluten network based on their quality, quantity, structure, conformation, and physical properties (Attenburrow 1990). Beside protein, wheat starch has play an important role in the rheological characteristics of dough by initially being entrapped as filler in the gluten network and followed by strengthening this network as they began to swell upon heating (Zeleznak and Hoseney 1986).

Although the role of water in baking process is neglected, the optimum amount of water controls the quality, texture, taste, smell, volume, flavor, and mouthfeel of bakery products

(Cauvain and Young 2009). The stickiness of dough partly depends on the amount of water added during mixing (Le-Bail 2011). In addition to that, water is required by dough to help yeast in gas production (Papasidero et al. 2014).

Sugar is used to hold moisture and prevent staleness in baked products. It tenderizes bakery products and provides a source of nourishment for the growth of yeast, which helps the leavening process. The residual sugars take part in browning reaction and help in the promotion of rapid crust color formation, enhancement of flavor and aroma by greater development of volatile acids and aldehyde, and improvement of crumb texture. Sugars of importance in bread making are sucrose, maltose, glucose, and fructose (Mondal and Datta 2008; Pyler and Gorton 2008).

The principal objective of fat in breadmaking is to act as a lubricant that helps the dough constituents to be moved by each other with greater ease. Fat also helps to retain the gases released during baking thus ensuring a well risen loaf, soft crumb, and stay fresh longer. Fat or shortening coat the gluten cells so as to make them more impervious to gas. Fats have a tenderizing effect on the crumb to produce a finer structure and crust to improve shelf life by slowing staling process (Brooker 1996).

1.2.2. Minor ingredients

Minor ingredients constitute 5-10% (or less) on formula weight. The minor ingredients are leavening agents (e.g. yeast or chemical), milk, eggs, starch, and fiber (Pyler and Gorton 2008). Yeast (*Saccharomyces cerevisiae*) ferment the sugar to alcohol and carbon dioxide (CO_2). Loaf volume depends on the amount of CO_2 produced and the ability of the dough to hold this gas (Salvador et al. 2006). Besides the CO_2, some other compounds also produced during fermentation like ethanol, succinic acid, and hydrogen peroxide that contribute to bread flavor and rheology (Razei et al. 2014).

Milk is not required but the addition of milk improves the nutritional value of the bread because milk contains lysine and calcium. Lactose is not fermented by yeast; however, lactose and protein take part in the browning reaction. In addition to milk, eggs added to raise the dough. The fats in egg yolk help to tenderize the crumb and lighten the texture a bit. Eggs also contain the emulsifier lecithin that adds the overall consistency of the loaf (Pyler and Gorton 2008).

1.2.3. Micro ingredients

Micro ingredients are typically used at 5% or less and usually used at 0.1% or less. These ingredients are often combined with other ingredients so that they are sometimes difficult to measure accurately. The micro ingredients are enzymes, gluten, salts, improvers, antioxidants, and gums. These ingredients typically are used for the overall quality improvement of bread (Pyler and Gorton 2008).

Salt is qualitatively the most important and the most common flavoring agent in processed food; it strengthens and tighten gluten but inhibits the yeast activity (Pyler and Gorton 2008). Salt in breadmaking increases dough development time, resistance to extension and extensibility, gelatinization temperature as well as maximum hot paste viscosity of dough (Linko et al. 1984). Additionally, gums are highly branched and long polysaccharides of plant or animal source. Gums act as water control agents to help to increase viscosity, remain sugar crystals small in size (Pyler and Gorton 2008).

1.2.4. Bakery improvers

Bakery improvers go by different names like dough conditioners, dough strengtheners, crumb softeners, emulsifiers and surfactants (Pyler and Gorton 2008). According to Dubois (1979), these ingredients may bind with the gluten to improve the machinability, gas retention of the dough, improve loaf volume, symmetry, texture, and grain as well as bind with the starch to retard

the rate of staling. In general, these ingredients perform one or more of the following functions in yeast raised bakery foods:

(I) Increase mixing, ingredient variations, and machining tolerances of the dough

(II) Lessen the knockdown time of dough during continuous mix systems and conveyor handling of proofed dough pieces

(III) Help the dough to achieve maximize water absorption which in turn helps in product yield and quality

(IV) Minimize the use of shortening

(V) Improve bread quality characteristics including loaf volume, grain structure, texture, crust tenderness etc.

(VI) Retard the rate of staling or soften the bread to lengthen self quality.

There are many chemical additives used as a dough conditioner, each has specific functions and limits. The approved limits and functions of these ingredients are illustrated in table 1.1.

Table.1.1. Dough conditioning agents with functions and use level (Pyler and Gorton 2008)

Dough conditioner ingredient	Function	Use level	Considerations
Vital wheat gluten	Structure	2 to 10%	Increases strength and absorption
Ammonium chloride	Yeast nutrient	0.04%	Nitrogen source
Ammonium sulfate	Yeast nutrient	0.04%	Nitrogen source
Ammonium phosphate	Yeast nutrient	0.04%	Nitrogen and phosphorus source
Calcium carbonate	pH regulator	0.1 to 0.5%	Rises pH
Monocalcium phosphate	pH regulator	0.1 to 0.3%	Lowers pH
Calcium sulfate	pH regulator	0.1 to 0.6%	Rises pH
Potassium bromate	Oxidizing agent	10 to 75 ppm	Slow oxidizer
Ascorbic acid	Oxidizing agent	10 to 100 ppm	Intermediate oxidizer
Calcium peroxide	Oxidizing agent	10 to 75 ppm	Dries dough surface
Azodicarbonamide	Oxidizing agent	10 to 45 ppm	Fast oxidizer
Potassium iodate	Oxidizing agent	10 to 75 ppm	Fast oxidizer
Calcium iodate	Oxidizing agent	10 to 75 ppm	Fast oxidizer
L-cysteine	Reducing agent	10 to 90 ppm	Chemical reducing agent
Non leavening (inactive) yeast	Reducing agent	0.25 to 1%	Natural source of glutathione
Protease	Enzyme	GMP*	Increases extensibility
Carbohydrase	Enzyme	GMP*	Improves oven spring and freshness
Oxidase	Enzyme	GMP*	Forms oxygen via hydrogen peroxide
Enzyme-active soy flour	Enzyme	0.25 to 0.5%	Lipoxygenase whitens crumb
Diastatic malt syrup	Enzyme	1 to 2%	Supplements flour enzyme activity
Malt flour	Enzyme	0.5 to 1%	Supplements flour enzyme activity
Lecithin	Emulsifier	0.25 to 1%	Natural softener
Sodium stearoyl lactylate	Emulsifier	0.25 to 0.5%	Strengthens and softens
Calcium stearoyl lactylate	Emulsifier	0.25 to 0.5%	Strengthens and softens
Diacetyl tartaric acid esters of mono- and diglycerides (DATEM)	Emulsifier	0.25 to 0.5%	Strengthens
Ethoxylated mono- and diglycerides	Emulsifier	0.25 to 0.5%	Strengthens
Polysorbate 60	Emulsifier	0.25 to 0.5%	Softens
Succinylated mono- and diglycerides	Emulsifier	0.25 to 0.5%	Strengthens and softens
Mono- and diglycerides	Emulsifier	0.25 to 1%	Softens
Distilled diglycerides	Emulsifier	0.25 to 1%	Softens

*Good manufacturing practice

1.2.5. Functions dough strengtheners and reaction with protein

According to Dubois (1979), the dough strengtheners help to improve not only dough but also final product. In the dough, they increase water absorption, improve mixing tolerance and gas retention, increase resistance to collapse and shorten the proof time. In addition, they improve loaf volume, help with robust texture, fine grain and strong side walls, and improved slicing characteristics of the finished product.

Although many of the dough strengtheners are emulsifiers, only a few emulsifiers are really dough strengtheners (Table 1.1). The dough strengtheners became important when bakers started to use bread flours with low protein and high ash content. Emulsifiers can form an intimate and stable mixture by reducing the surface tension of two normally immiscible components. The surfactant generally contains a hydrophilic and a lipophilic component, dough strengtheners tend to be more hydrophilic than lipophilic while crumb softeners tend to be more lipophilic than hydrophilic (Strouts 1979). Although there are several mechanisms for improving the action of dough strengtheners, the general conclusion is that improving action is a result of the colloidal binding of the additive to protein (Stutz 1973).

As mentioned earlier, the gluten network is formed by polymeric glutenins and monomeric gliadins. The gliadin is responsible for cohesiveness, viscosity, and extensibility in a dough system whereas glutenin responsible for elastic and cohesive properties of dough (Delcour and Hoseney 2010). Both the gliadin and glutenin contain disulfide bonds. The intramolecular disulfide bond in gliadin promote globular or folded molecule; however, intramolecular and intermolecular disulfide bonds between polypeptide chains make larger molecular aggregates in glutenin. These differences in the bond formation between gliadin and glutenin are due to different amino acid sequence. The three major amino acids that play a major role in gluten proteins characteristics are glutamine,

proline, and cysteine. During oxidation of cysteine to cystine, the thiol group of cysteine converts to SS group and this SS bond links two adjacent polypeptide chains. The oxidation of SH group or thiol group to SS group thus increases SS formation which enhances dough strength and improves the gas holding capacity of dough (Pyler and Gorton 2008). Oxidizing agents promote these disulfide bond formation between proteins by facilitating the formation of disulfide bonds between glutenin subunits, which improves dough strength (Bloksma 1972).

1.2.6. Effect dough additives on dough rheology and loaf volume

Among the dough conditioners in Table 1.1, the most widely used dough strengtheners are Azodicarbonamide (ADA), Ascorbic acid (AA), Sodium stearoyl lactylate (SSL), Calcium stearoyl lactylate (CSL), Diacetyl tartaric acid esters of mono- and diacylglycerides (DATEM), Potassium bromate (PB), Ethoxylated mono- and diacylglycerides (EMG), Vital wheat gluten. Among these commercially available dough strengtheners, ADA, AA, and PB are functionally classified as oxidants while CSL, SSL, DATEM, and EMG are functionally classified as surfactants.

The surfactants interact with gluten protein and make the gluten matrix becomes more elastic and extensible which eventually strengthen the dough. Surfactants also can form a clathrate with amylose thus limits swelling of starch and amylose leaching. (Science of Baking 2010). In addition to surfactants, the oxidants facilitate the interchange between the thiols and disulfide bonds in protein (Goldstein 1957), inhibit the protease enzyme (Kulp 1981), oxidize thiol group (Sullivan 1936), and affect protein aggregation (Bernardin 1987) to improve dough rheology and bread quality (as cited in Pyler and Gorton 2008).

1.2.6.1. Potassium bromate

Potassium bromate (PB) was one of the most commonly used dough improvers, which removes sulfhydryl groups by oxidizing the disulfide groups of gluten. The resulted in cross-linked molecule impacts dough structure and rheology, mainly increases the strength of dough during mixing and extensibility of dough during molding. PB affects the dough handling, changes the gas cell structure, and maintains carbon dioxide level as well as the loaf volume, grain and texture of dough (Cogswell 1997). PB at 5-10 ppm gives maximum loaf volume in 2.5 min of constant mixing time; however, more than 30 ppm resulted in toughening of dough and decreased loaf volume (Panozzo et al. 1994). PB is also used for flour maturation and matured flour gives more elastic, less sticky, and resistant to expansion dough which exhibits superior oven spring (Pyler and Gorton 2008).

1.2.6.2. Azodicarbonamide (ADA)

Azodicarbonamide (ADA) is one of the fastest oxidants used as a dough improver in breadmaking. ADA is a flour maturing agent which improves dough properties and baking qualities through oxidation of SH group. This maturation process requires 2-45 ppm of ADA depending on the grade of flour. Over-treatment with ADA is characterized by grey, a streaky crumb of poor volume resulting from a tight, extensible dough. Although ADA does not bleach the flour pigment, a little variation in color occurs with a high-dose treatment. ADA oxidizes the sulfhydryl (-SH) groups to exert this improving effect and this oxidation is rapid and almost completed during mixing. ADA forms a cohesive and dry dough that can tolerate high water absorption and shortened mixing times. Bread made from ADA mixed flour is characterized by increased loaf volume, improved grain and texture (Tsen 1963).

1.2.6.3. Ascorbic acid

L-Ascorbic acid (AA) is a unique dough improver. It shows an intermediate rate of oxidation and therefore its action sustained through most of the dough phase. Due to oxidation of ascorbic acid, L-ascorbic acid turned into dehydro-L-ascorbic acid by ascorbic acid oxidase where copper and iron may act as a catalyst. Then, dehydro-L-ascorbic acid oxidizes the gluten's thiol group, which consequently improves the dough. AA also improves gas retention ability of dough, increases oven spring, and gives a finer bread with good crumb cell structure. AA gives resiliency to recovery of its original shape after compression which conveys the impression of freshness to the consumer. AA of 30 to 120 ppm exhibits a nearly constant effect on the dough, however, treatment with 70 to 100 ppm of AA promotes the development of dough at high mixing speed (Pyler and Gorton 2008).

1.2.6.4. Lactylates

The stearoyl lactylates, both sodium stearoyl lactylate (SSL) and calcium stearoyl lactylate (CSL), are widely used dough strengtheners and crump softeners. They form a complex compound with protein during mixing to strengthen the dough as well as form a complex compound with starch during baking to soften the crumb. Although both the SSL and CSL work as dough strengtheners and crumb softners, SSL is slightly more effective in anti-staling whereas CSL imparts more in sidewall strength and gives the bread a more resilient crumb.

Both of the stearoyl lactylates increase the pasting properties of the dough which is then delayed the crumb firming process. SSL increases the mixing stability and mixing tolerance, improves gas retention, improves bread volume, creates finer crumb grain, and develop slicing characteristics. Although these compounds do not reduce the proof time of dough, they give

significant improvement in the rheological properties during storage frozen dough (Ahmed et al. 2014; Pyler and Gorton 2008).

1.2.6.5. Diacetyl tartaric acid esters of mono- and diacylglycerides

Diacetyl tartaric acid esters of mono- and diglycerides (DATEM) the anionic emulsifier, also serves as dough strengthener. It increases the percent water absorption, overall dough rheological characteristics and baking quality of bread. This anionic compound can react with flour protein to increase the elasticity and extensibility of gluten when thinner gluten strands with fewer starch granules embedded in them. It improves loaf volume, gives resilient texture to crumb, fine crumb grain, and also improves slicing characteristics by forming strong hydrogen bonds by interacting with glutamine and starch fractions. DATEM can help in the production of strong protein networks by tight linkages with the hydrophobic protein surfaces which in turn give a better texture to the crumb and also enhance the volume. DATEM is added to the bread and other fermented products at the concentration of 0.3% on the flour weight basis (Ahmed et al. 2014; Pyler and Gorton 2008). Tri acyl gly cerides

1.2.6.6. Ethoxylated mono- and diacylglycerides

Ethoxylated monoglycerides (EMG) is made by chemically combining one part of mono- and diglycerides with about 20 parts of ethylene oxide and the end product is an oily liquid paste. However, the product is almost always used in combination with regular monoglycerides. It works as an emulsifier and helps to lower the tension between two liquids or a liquid and a solid.

Ethylene oxide reacts with the OH group of glycerides to form an oily liquid or paste and gives excellent dough strength as well as loaf volume. Although EMG improves crumb grain texture and dough strengthening effect, very low crumb softening. It also decreases the harmful effects of the extra fiber on loaf volume and helps on better air incorporation to improve bread

quality. However, overdosage of EMG collapsed the bread in the oven and form a gel with the water (Ahmed et al. 2014; Pyler and Gorton 2008).

1.2.6.7. Non-Fat Dry Milk

Non-Fat Dry Milk (NFDM) is not required but the addition of milk improves the nutritional value of the bread because milk contains lysine and calcium. Lactose is not fermented by yeast, but lactose and milk protein take part in the browning reaction. NFDM uses up to 6% of flour weight basis. NFDM influences the dough absorption, mixing requirements, fermentation rate, baking time and temperature as well as physical qualities of bread. Milk protein, casein improves water absorption and interaction with gluten. However, milk contains serum which must be denatured by high heat treatment otherwise the formation of lactoglobulin fraction of serum protein would weaken the gluten matrix, causes slack dough, and reduce loaf volume (Pyler and Gorton 2008).

1.2.6.8. Vital wheat gluten

Vital wheat gluten is a protein concentrate contains 72.5% protein (77.5% dry basis) and is extracted from wheat flour. Commercial gluten is in light-brown powder form and usually used to fortify flours that has poor breadmaking quality. Vital gluten can improve chemical, physical properties of weak flour to its requirements to reach better breadmaking performance. Vital wheat gluten is usually used for protein enrichment which eventually add elasticity and extensibility to the dough due to the presence of gliadins and glutenins.

Vital wheat gluten also increases water absorption, improves grain and texture, improves the softness of crumb, as well as prolong shelf-life. It also provides the extra gluten to low-gluten content whole grain flours, such as rye, oat, teff, spelt, or buckwheat to improve their breadmaking properties (Ortolan and Steel 2017; Pyler and Gorton 2008).

1.2.6.9. Fat

Among the three major food constituents (proteins, fats, and carbohydrates) and fat has the highest caloric value (Varela and Fiszman 2013). Bread usually contains 2% fat based on flour weight in the form of shortening. Shortening adds plasticity to the dough when attenuating into the film during breadmaking. Shortening helps to entrap air bubbles into the dough during mixing as well as leaven, tenderizes the crumb, contributes moistness, and enhances mouthfeel. Edible fats refer to triacylglycerides those are available in three forms, namely liquid oil, hard fats, and shortenings at room temperature. All of these three are similar in chemical makeup; however, differ in the types of fatty acids and the arrangement of those fatty acids in the fat molecule. The degree of hydrogenation of fat affects the stability and amount of saturated fat (Pyler and Gorton 2008).

1.3. Health Effect and Controversies with Dough Strengtheners

Most of the commercially available dough strengtheners are associated with some heath controversies. It has been demonstrated that PB induces renal cell tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid. PB is also carcinogenic in rats and nephrotoxic in both man and experimental animals when given orally (Kurokawa et al. 1990). At first, PB was found to cause tumors in rats in 1982, then the subsequent studies on rats and mice confirmed that it causes tumors of the kidney, thyroid, and other organs (Cronin 1999).

In addition, AA is an essential dietary nutrient that acts as an antioxidant, but it also involves in the development of various chronic diseases due to its role in maintaining oxidative balance (Daud et al 2016). Another widely used oxidants, ADA is irritating to the eyes and the respiratory tract and prolonged inhalation exposure may cause asthma and dermatitis (CDC 2015).

In addition, Joint FAO/WHO Expert Committee on Food Additives (JECFA) found the possible toxicity of DATEM in rats and found heart fibrosis and adrenal overgrowth (Winter 2009).

Among the three major food constituents, fat has the highest caloric value which is related to nutrition-related diseases such as obesity, heart disease, and diabetes. These public health concerns have caused the food industry to develop products with less fat and more complex carbohydrates (Varela and Fiszman 2013). Moreover, the monoglycerides of ethoxylated monoglycerides made of one fatty acid attached to glycerol. This ingredient contributes to *trans* fat levels in foods which is a factor for cardiovascular disease. The FDA labeling regulations require labeling of *trans* fat only when coming from triglycerides, but not when coming from emulsifiers like mono and diglycerides (Enig 2004).

1.4. What is Clean Label?

Consumers' food choice is believed to be greatly influenced by their judgment of the available information on the label because they are becoming increasingly cautious about food safety (Aoki, et al. 2010). These long list of chemicals on ingredients label often confuse consumers rather enlighten. Since 1990, potassium bromate was a much-discussed subject (Pyler and Gorton 2008) and FDA has urged bakers to voluntarily stop using potassium bromate since 1991, instead of banning. However, California requires warning of cancer on the label to use a certain level of potassium bromate in baking (Cronin, 1999). Later, many bakeries eliminated this chemical upon consumer demand (Pyler and Gorton 2008) and the demand for "clean label" formulations are steadily increasing (Watrous 2015).

1.4.1. What makes a clean label?

There is no established definition of a clean label to date, but market trend reports often provide several definitions or interpretations which are not supported by consumer behavior

research (Osborne 2015). According to National Starch Food Innovation (Skarra, 2006), clean label is-

(I) a preference for natural, organic or wholesome constituents

(II) the name of the constituents is not chemical sounding and familiar to consumer,

(III) constituents that are considered ingredients rather than additives for example food rather than chemicals,

(IV) fewer total ingredients,

(V) constituents that are contributing to a healthy lifestyle and absence of constituents that are contributing to a unhealthy lifestyle like fat,

(VI) constituents which has no known or perceived health risks.

According to Edwards (2013), clean label means chemicals additives free, easy-to-understand ingredient lists, and produced with limited processing by traditional techniques. However, Ingredion (2014), ignored the processing technique and emphasized natural, organic and/or additives/preservatives free. Later, Hutt and Sloan (2015) also explained that clean label includes all natural ingredients, organic, no artificial ingredients including preservatives and color, as well as no high fructose corn syrup. In 2017, Asioli et al. proposed the latest definition of clean label (Fig 1.1)

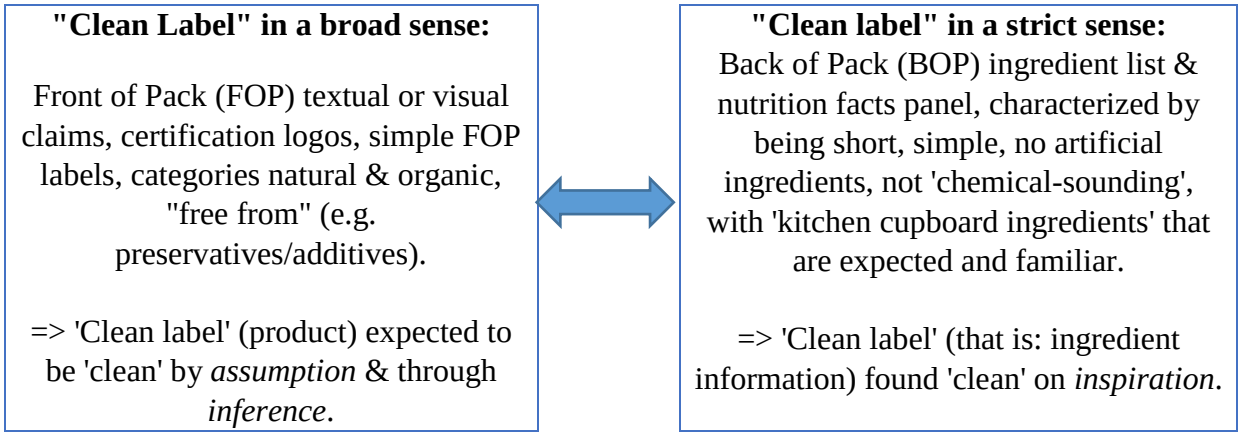

Fig. 1.1. A proposed definition and concept of ‘clean label’ (adopted from Asioli et al. 2017).

1.4.2. Ingredient selection for clean label

Wheat flour mixed with appropriate amount of water to form a strong and cohesive dough that can retain gas and produce the light aerated baked product. The dough can exhibit these properties due to gluten which comprises the storage proteins: glutenins and gliadins. Hydrated gliadin is extremely sticky, not resistant to extension and responsible for cohesiveness, viscosity, and extensibility in a dough system. On the other hand, glutenin is resilient and rubbery, responsible for elastic and cohesive properties of dough (Delcour and Hoseney 2010).

Among the six distinct classes of wheat grown in the United States, hard red spring (HRS) wheat has the highest protein content (usually 13 to 16 percent) and strong gluten characteristics. HRS Wheat is used extensively as blending wheat to increase the gluten strength in batches of flour. The strong gluten helps to increase loaf volume and provides the strength needed in baked products etc. (North Dakota Wheat Commission). In this experiment, HRS wheat flour will be used as clean label dough strengtheners.

1.4.2.1. Dough properties and baking quality

HRS wheat is called as the aristocrat of wheat for its bread baking quality. The high protein content and superior gluten quality gave some of the world's finest baked goods. Millers extensively use as blending wheat to increase the gluten strength in batches of flour in order to improve water absorption, dough handling, and mixing characteristics. (U.S. HRS crop quality report 2017).

According to this report, the dough properties and baking properties of hard red spring wheat flour are following:

Table 1.2. The dough and baking properties of hard red spring wheat flour (U.S. HRS crop quality report 2017)

Farinograph		
	Peak Time (min)	7.1
	Stability (min)	10.8
	Absorption (%)	62.1
Alveograph		
	P (mm)	81
	L (mm)	113
	P/L Ratio	0.71
	P (mm)	312
Extensograph		45min/135min
	Resistance (BU)	442/783
	Extensibility (cm)	16.9/13.5
	Area (cm^2)	109/154
Baking Properties:		
	Pan Bread: Bake Absorption (%)	67.9
	Loaf Volume (cc)	997
	Crumb Grain and Texture (1-10)	7.9

1.5. Wheat Flour Protein and Bread Quality

During bread making, complex (bio-) chemical and physical transformations occur and proteins play a very important role in bread-making quality (Payne et al. 1987). Wheat flour proteins contain mixtures of glutenins, gliadins, albumins, and globulins (Mendichi et al. 2008). The unique gluten-forming storage proteins of wheat are responsible for viscoelastic characteristics (Delcour et al. 2012). According to Osborne type fractionation procedures, the gluten forming gliadins are extractable in aqueous ethanol whereas glutenins are unextractable in aqueous ethanol. In contrast, the nongluten-forming albumins are extractable in water whereas globulins are extractable in salt solutions, but not in water. The gliadins are monomeric and have molecular weights between 30,000 and 60,000 Da whereas glutenins are polymeric with molecular weights ranging from approximately 80,000 Da to more than twenty million (Veraverbeke and Delcour 2002). Gliadin proteins are mainly responsible for the dough cohesiveness; however, the

addition of gliadins has been shown to decrease dough strength, decrease mixing time and loaf volume (Uthayakumaran et al. 1999). The gliadins are known as dough weakening agents because gliadins weaken the gluten network in terms of microstructural, thermal and rheological characteristics (Khatkar et al. 2013). Gliadin proteins act as a plasticizer, providing viscous flow and extensibility to the dough during mixing with the help of hydrophobic interactions and hydrogen bonds (Barak et al. 2015). Structurally, there are three distinct groups of gliadins, i.e., α-, γ-, and ω-types and are all involved in intrachain SS bonds (Muller and Wieser 1997). Among these types, γ-gliadins caused the greatest decrease in mix time and resistance to an extension because of hydrophobic in nature (Uthayakumaran et al. 2001), ω-gliadins cause the greatest decrease in mix time and the α- gliadins were thought to have an intermediate effect (Fido et al. 1997). Later, Khatkar et al. (2002) suggested that ω-gliadins are responsible for viscous properties in the wheat dough; however, Ohm et al. (2009; 2010) found that ω-gliadin proteins may have a positive effect on loaf volume and water absorption.

The glutenin is largely insoluble in most common solvents due to the polymeric structure but its subunit building blocks (GS) are more soluble than those of gliadins. The high molecular weight glutenin subunits (HMW-GS) have a molecular weight of 70,000 to 90,000kDa and low molecular weight glutenin subunits (LMW-GS) have a molecular weight of 30,000 to 45,000 kDa. Although HMW-GS are minor components in terms of quantity, they are major determinants of gluten elasticity and functionality hence are key factors in the breadmaking process (Gianibelli et al. 2001). A flow diagram illustrating the effect of protein on dough rheological properties shown in Fig. 1.2. There are several explanations on the molecular structure of glutenin, HMW-GS is the backbone of the molecule whereas LMW-GS are present as lateral attachments (Graveland et al. 1985), a cross-link between a HMW-GS and a LMW-GS (Keck et al. 1995), and HMW-GS and

LMW-GS for linear polymer separately in vivo (Wieser et al. 2006) etc. (as cited in Declour et al. 2012).

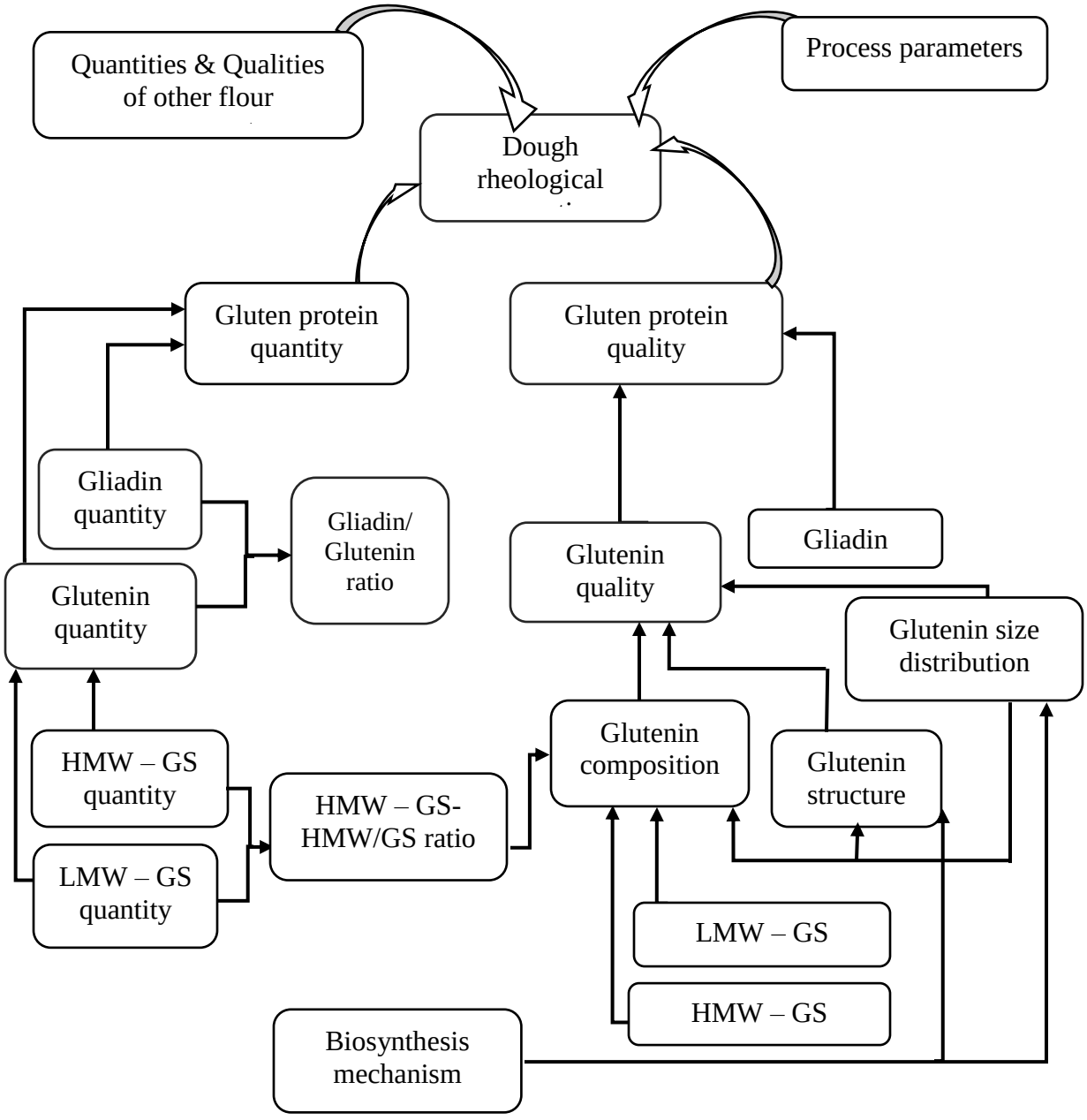

Fig. 1.2. Factors governing the dough rheological properties (adopted from Veraverbeke and Delcour 2002).

1.5.1. Dough strengtheners and protein molecular weight

As mentioned earlier in this chapter, surfactants react with gluten matrix which becomes more elastic and more extensible to strengthen the dough, and form a clathrate with amylose thus limits swelling of starch and amylose leaching. The oxidants facilitate the interchange between the thiols group and disulfide bonds, inhibit the protease enzyme, oxidize thiols group, and affect protein aggregation to improve dough rheology and bread quality (Pyler and Gorton 2008). Oxidizing agents promote oxidation of sulfhydryl bond and facilitating the formation of disulfide bonds between glutenin subunits, which improves dough strength (Bloksma 1972). The interchain disulfide bonds in glutenin are then less than half of total and each glutenin subunit of average 44,000 Da molecular weight contains two reactive thiol groups which oxidized to form two interchain disulfide bonds between two adjacent polypeptide bonds (Ewart 1972). The intramolecular disulfide bond in gliadin promotes globular or folded molecule; however, intramolecular and intermolecular disulfide bonds between polypeptide chains make larger molecular aggregates in glutenin. These disulfide-mediated cross-linking and resulting protein network increase the molecular weight and decrease in the solubility of proteins (Pyler and Gorton 2008).

The reducing agents give rise to a decline in molecular weight of gluten proteins mainly glutenin fractions due to the reduction of disulfide bonds. However, the oxidizing agent cannot give rise in molecular weight of gluten rather by oxidizing low molecular weight thiol groups to disulfide as well as stop thiol-disulfide exchange reaction between high molecular weight protein and low molecular weight thiol to prevent the loss of molecular weight (Stear 1990).

1.5.2. Protein Molecular weight distribution by SE –HPLC and bread quality

There is a linear relationship between protein content, gluten protein quality and breadmaking performance (Finney and Barmore 1948; Veraverbeke; Delcour 2002). There are some significant associations between protein molecular weight distribution with dough mixing and breadmaking characteristics (Ohm et al. 2010). Both the proportions of polymeric and monomeric gluten-forming proteins and their size distribution contribute to protein quality (Wrigley et al. 2006). Thus, this proportion defines the relationship between protein content and loaf volume. Gupta et al. (1993) found a strong correlation between molecular weight distribution of glutenin polymers and baking quality.

The biochemical basis for predicting flour dough strength was the proportion of SDS-insoluble polymeric protein in total protein or in total polymeric protein however the extractable polymeric proteins were associated with dough weakening characteristics (Gupta et al 1993; Ciaffi et al 1996; Bangur et al 1997; Borneo and Khan 1999; Morel et al 2000; Tsilo et al 2010).The high molecular weight SDS-unextractable polymeric proteins had greater positive correlations with dough properties than other polymeric protein fractions. (Ohm et al 2010). The SDS-unextractable polymeric proteins were positively associated with dough strength; however, the extractable polymeric proteins were associated with dough weakening characteristics (Gupta et al 1993; Ciaffi et al 1996; Bangur et al 1997; Borneo and Khan 1999; Morel et al 2000; Tsilo et al 2010). The unextractable high molecular weight polymeric protein had a positive effect on mixing time, and bread loaf volume whereas a high proportion of extractable polymeric protein had negative effects on mixing time and bread loaf volume (Ohm et al 2010).

The proportions of polymeric and monomeric components as well as the proportions of large polymers can be determined by size-exclusion high-performance liquid chromatography

(SE-HPLC) (Gupta et al., 1993). SE-HPLC have been used extensively to separate wheat flour proteins according to protein molecular weight distribution (Ohm et al., 2010). The unextractable polymeric protein can be determined using a two-step extraction procedure, followed by SE-HPLC separation of the polymeric and monomeric proteins (Gupta et al., 1993). The amounts of the polymeric and monomeric components in the two fractions are used to calculate the amount of unextractable polymeric protein as the percentage of polymeric protein content.

Size-exclusion chromatography (SEC) is the separation of mixtures based on the molecular size of the components i.e. protein, carbohydrate etc. This separation is achieved based on the size differential of the components as they pass through the stationary phase with different pore sizes. When the solutes pass through stationary phase mixture components get separation based on their size. In the case of proteins, large molecules are excluded from the pores so they pass through space in between the gel particles and will elute first. In contrast, smaller proteins can now enter the pores of these beads thus they move slower through the stationary phase and elute later (Size Exclusion Chromatography Principles and Methods, 2014).

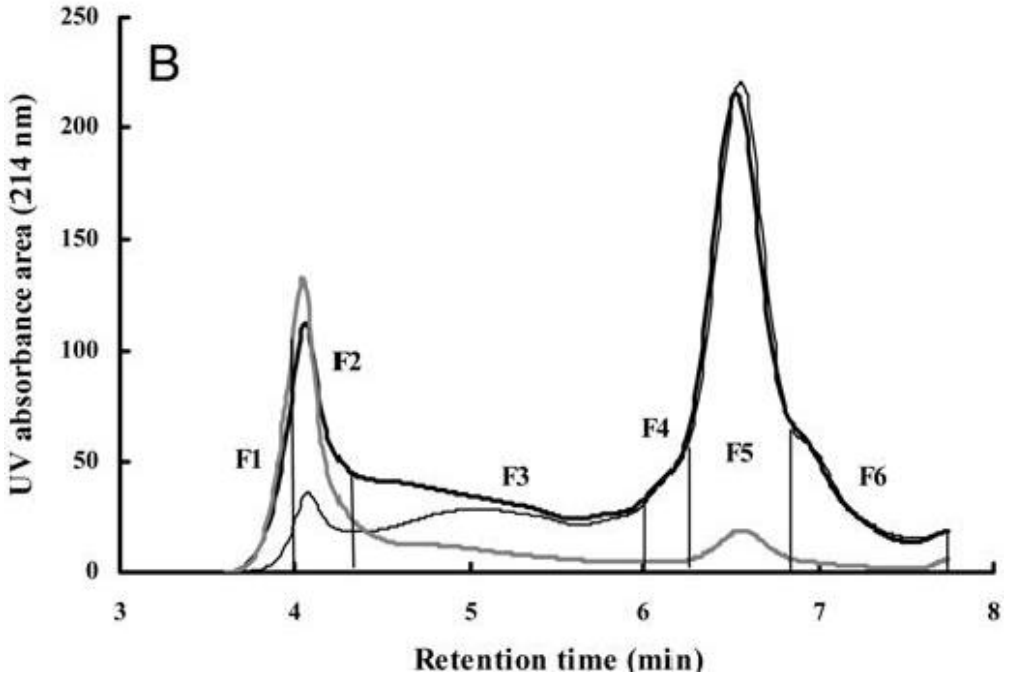

Fig. 1.3. Typical size exclusion HPLC profiles of protein extracts separated by a narrow bore column. Fl=very high molecular weight polymeric protein; F2=high molecular weight polymeric protein; F3=low molecular weight polymeric protein; F4=gliadins; F5=gliadins; and F6=albumins and globulins (Adopted from (Ohm et al., 2009).

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CHAPTER 2. REPLACEMENT OF COMMERCIAL DOUGH STRENGTHENERS WITH HARD RED SPRING WHEAT FLOUR

2.1. Abstract

Consumers are becoming increasingly reluctant to purchase food products with long lists of chemical additives on ingredient labels. Dough strengtheners are the most common and largest number of chemicals used bread formulations. The goal of this study was to replace chemical dough strengtheners with natural ingredients. High protein content hard red spring (HRS) wheat flour was selected as a natural dough strengthening ingredient in bread formulations where hard red winter (HRW) wheat flour was used as base flour. Doughs were prepared by blending HRW wheat flour with different percentages (10%, 20%, 30%, and 40%) of each of HRS wheat flour from wheat cultivars of Glenn and Linkert as well as two additional commercial HRS wheat flour. Additionally, doughs were prepared by adding each of the ten commercially available dough strengtheners to HRW wheat flour to compare the dough strengthening function of HRS wheat flour blends. Dough prepared from each of the treatments were analyzed for rheology by farinograph and extensograph. Then, test baking by straight dough method was performed for loaf volume and other quality traits for bread quality studies. Increasing percentages of HRS flour significantly ($p<0.05$) increased the farinograph water absorption, stability, and bread loaf volume. All of the HRS blends had significantly ($p<0.05$) higher farinograph stability and extensograph resistance at 135 mins than most of the dough additives. Bread flour with 40% Glenn and 40% Linkert showed the highest loaf volume of 920 cm^3 and 950 cm^3 with the firmness of 1553.50 and 1525.50 mN, respectively. Therefore, HRS as a replacement for dough strengtheners had better dough and bread properties compared to commercial strengtheners.

2.2. Introduction

Bread is one of the most important sources of carbohydrates in the human diet (Dewettinck, et. al. 2008). The major ingredients used in bread formulations are flour, water, yeast, salt, sugar. The three major components of flour are protein, carbohydrates, and lipid. Wheat flour is the most important for its storage proteins that develop gluten network during hydration. Moreover, the visco-elastic properties of gluten network are unique for breadmaking properties of wheat flour. Besides protein, starch also plays an important role in the rheological characteristics of dough by strengthening this network as they begin to swell upon heating (Zeleznak and Hoseney 1986).

Besides the major ingredients, there are some micro ingredients used in bread formulations for overall quality improvement. Among micro-ingredients, bakery improvers are used to aid production and improve certain quality factors of bread. These bakery improvers include dough conditioners, dough strengtheners, crumb softeners, emulsifiers, and surfactants (Pyler and Gorton 2008). Dough strengtheners may bind with the gluten to improve the machinability and gas retention of dough which subsequently affect loaf volume, symmetry, texture and grain of bread. Moreover, they bind with the starch to retard the rate of staling (Dubois 1979).

Dough strengtheners are typically oxidants, reductants, and surfactants. Surfactants interact with gluten to strengthen the dough and help the gluten matrix becomes more elastic and extensible. Surfactants also can form a clathrate with amylose, thus limiting swelling of starch and amylose leaching (Science of Baking 2010). Oxidants facilitate the interchange between the thiols and disulfide bonds in protein (Goldstein 1957), inhibit the protease enzyme (Kulp 1981), oxidize thiols group (Sullivan 1936), and affect protein aggregation (Bernardin 1987) to improve dough rheology and bread quality (as cited in Pyler and Gorton 2008).

Food products made from these chemicals often confuse consumers rather enlighten and the judgment of food choice mainly depends on the available information on the label (Aoki, et al. 2010). Many of these chemicals also associated with health controversies, for example, potassium bromates is a much-discussed subject since 1990 (Pyler and Gorton 2008) due to its carcinogenic effects (Kurokawa et al. 1990). In addition, azodicarbonamide irritates eyes and the respiratory tract (CDC 2015), and DATEM is found to be a cause of heart fibrosis and adrenal overgrowth in rats. Considering all of these issues many bakeries already eliminated potassium bromate and azodicarbonamide upon consumer demand to clean label (Pyler and Gorton 2008). Although there is no official definition of the clean label, it means using all natural ingredients, no artificial ingredients, no artificial preservatives, no high fructose corn syrup, and no artificial colors in food processing (Hutt and Sloan 2015).

In this experiment, high protein content HRS wheat flour was selected as a clean label ingredient. HRS wheat, exclusively grown in the Northern Plains (Montana, Minnesota, North Dakota and South Dakota), constitutes about 25% of the crops in the United States. HRS wheat cultivars are known to have a high protein content (13 to 16 %) and superior gluten quality among the bread wheats hence extensively used as a blending wheat to increase the gluten strength in batches of flour (U.S. HRS crop quality report, 2017). Maghirang et al. (2006) compared the HRS and HRW wheat flour qualities and found that the quantity of insoluble polymeric proteins, mixograph mix time, crumb grain score were higher for HRS wheat flour whereas HRW wheat flour had higher free polar lipids content, falling number, and farinograph tolerance. They also concluded that HRS wheat generally exceeds the grain and flour quality of HRW even in the similar range of protein content. Therefore, using HRS wheat flour as dough strengthening

ingredients would provide a natural ingredients as well as a solution for the consumers to avoid the above stated chemical dough strengtheners.

The objective of this study was to replace commercially available dough strengtheners with HRS wheat flour during bread making in order to provide a clean label.

2.3. Materials and Methods

2.3.1. Sampling

One hard red winter (HRW) wheat flour sample and four hard red spring (HRS) wheat flour samples used for this experiment. The HRW wheat flour was collected from Ardent Mills, Denver, CO. Among the hard red spring (HRS) wheat cultivar 'Glenn' was collected from producer field of Fessenden, ND. Cultivar 'Linkert' was collected from research field of Casselton, Langdon, Minot, Hettinger and Williston, ND then blended them before use. Wheat grain was cleaned and milled using Buhler laboratory mill by HRS Wheat Quality group, Department of Plant Sciences, North Dakota State University. Additional, commercial HRS wheat flour samples were collected from Swany White Mills, Freeport, MN and from ND State Mill, Grand Forks, ND (Table 2.1).

Table 2.1. Description of samples

Sample ID	Description
Base flour	Commercial hard red winter wheat flour, Ardent Mill
HRS1	Hard red spring wheat flour, Swany White Mill
HRS2	Hard red spring wheat flour of cultivar 'Glenn'
HRS3	Hard red spring wheat flour of cultivar 'Linkert'
HRS4	Hard red spring from wheat flour, ND State Mill

The HRW flour was used as base flour and HRS flours were used to compare dough strengthening ability with commercially available dough additives. The chemical dough

strengtheners were potassium bromate (PB), azodicarbonamide (ADA), ascorbic acid (AA), sodium stearoyl lactylate (SSL), calcium stearoyl lactylate (CSL), diacetyl tartaric acid esters of mono- and diglycerides (DATEM), and ethoxylated mono- and diglycerides (EMG). Moreover, three additional ingredients were non fat dry milk (NFDM), vital wheat gluten (VWG) and fat. The doughs were prepared by blending 10, 20, 30 and 40% (w/w) of each of the HRS wheat flour with HRW flour. Besides, doughs were prepared by adding each ten chemical dough additives at their optimum quantities to 100% HRW wheat flour (Table 2.2).

Table 2.2. Dough and bread were prepared by blending HRS flour with HRW flour and by adding additives to HRW flour[a]

Sample no	**Name/ Blending ratio**	**Dough strengthener/ additives**	**HRW**
1	HRW	None	100%
	HRS wheat flour blends[b]		
	HRW/HRS		
2	0/100	100% HRS	0%
3	90/10	10% HRS	90%
4	80/20	20% HRS	80%
5	70/30	30% HRS	70%
6	60/40	40% HRS	60%
	Additives		
7	Sodium stearoyl lactylate	50 ppm	
8	Calcium stearoyl lactylate	50 ppm	
9	Diacetyl tartaric acid esters of mono- and diglycerides	25 ppm	
10	Ethoxylated mono- and diglycerides	0.50%	
11	Ascorbic acid	150 ppm	100%
12	Azodicarbonamide	30 ppm	
13	Potassium bromate	10 ppm	
	Additional ingredients		
14	Vital wheat gluten	5%	
15	Fat	2%	
16	Non-Fat Dry Milk	2%	

[a]HRW= Hard red winter wheat flour, HRS= Hard red spring wheat flour
[b] This blending ratio was maintained for all four red spring wheat flour

2.3.2. Proximate composition of flours, dough, and bread crumbs

Dough was prepared by following the same procedure of baking and then freeze-dried (Labconco FreeZone 6 liter, Kansas City, MO, USA) to grinded with a ball mill (Mixer Mill MM 400, Haan, Germany) to obtain uniform particle size and stored in plastic bags at 4–5^{o}C until used. Bread crumbs were air-dried for 48 h at room temperature (temperature range 18-20^{o}C, RH range 15-18%) then ground like dough with a ball mill to obtain uniform particle size. The moisture content of each of the flour, dough, and bread samples were determined with air-oven drying at 135°C according to AACCI Approved Method 44-19.01. Then the protein content of flour, dough, and bread samples were determined according to AACCI Approved Method 46-30.01 with a LECO FP 528 nitrogen/protein analyzer (LECO, St. Joseph, MI, USA).

2.3.3. Dough rheology measurement

Water absorption and dough mixing properties of the samples were investigated by farinograph (C. W. Brabender Instruments, Hackensack, NJ, USA) according to the AACCI Approved Method 54-21.02. The flour 50 g (14% moisture basis) was added to the mixing bowl. Then water was added by biuret to reach a consistency of 500 Brabender units at the peak of the mixing curve. Data was recorded electronically. Dough extensibility was measured using an Extensigraph (C.W. Brabender Instruments Inc., Hackensack, NJ) according to AACCI Approved Method 54-10.01with some modifications. Flour (100 g, 14% moisture basis) salt (2%) and water were mixed in a pin mixer placed in temperature and humidity controlled extensograph chamber. The dough was rest and be pulled after 45 minutes, 90 minutes and 135 minutes of total rest time (AACCI, 2009). Data was recorded electronically.

2.3.4. Baking and bread quality

Loaves based upon 100 g flour per loaf were baked using straight dough method according to AACCI Approved Method 10-09.01 with the following modifications: fungal α-amylase (15 SKB) instead of dry malt powder, instant yeast (1.0%) instead of compressed yeast, and the addition of 10 ppm ammonium phosphate. A two-hour fermentation schedule with two punches was used for bread baking. After baking, bread loaf volume was measured in a volumeter by rapeseed displacement according to AACCI Approved Method 10-05.01. The bread was then evaluated for crust color, crumb color, crumb grain, and symmetry using a scale of 1-10, with ten being the best and one being the worst.

2.3.5. Bread firmness

Loaves were sliced using an electric bread slicer after one day of baking. The firmness of the bread slice was done using texture analyzer (Texture Technologies Corp., Scarsdale, NY) attached with an acrylic probe of 2.5 cm diameter according to AACCI Approved Method 74-09.01.

2.3.6. Statistical analysis

The experimental design was randomized complete block design (RCBD) with three replications. Statistical analysis was performed using the SAS statistical methods (Version 9.3, SAS Institute; Cary, NC). An analysis of variance (ANOVA) was performed to assess the effect of treatments on quality characteristics and treatment means were separated by Fisher's protected Least Significant Difference (LSD) test at P=0.05.

2.4. Results and Discussion

2.4.1. Proximate analysis of flour, dough, and bread crumb

HRW wheat is commonly used in pan bread production while HRS wheat is extensively used as a blending wheat and also used in specialty products where strong gluten profile is needed. In this study, HRW wheat was selected as the base flour to investigate the dough strengthening capability of HRS wheat. All of the flours were tested for moisture and protein (Table 2.3). HRW wheat flour showed a protein content of 9.25 % and moisture of 12.7%. Among the HRS wheat flours, HRS 2 showed the highest protein content of 14.8% and HRS 4 showed the lowest 13.0%. However, HRS 4 showed the highest and HRS 3 showed the lowest moisture content. Protein is likely the most important quality determining the factor of HRS wheat, and the average protein content of North Dakota grown HRS wheat flour is 13.8 % (14% mb) and average moisture content of grain is 12.6%, respectively (U.S. HRS Regional Quality Report 2017).

Table 2.3. Proximate compositions of flours

Sample ID	Moisture (%)	Protein (As is, mb) (%)
Base flour	12.7	9.25
HRS1	12.4	13.6
HRS2	12.8	14.8
HRS3	12.1	14.3
HRS4	12.9	13.0

2.4.2. Effect of HRS wheat blends and additives on dough rheology

Dough rheology is recognized to be central to the successful manufacturing of bakery products (Menjivar 1990). Dough rheology of each of the thirty-one doughs were studied by farinograph and extensograph, and presented in Table 2.4 and 2.5. The farinograph is the most important and commonly used flour quality test that also used to evaluate the effects of ingredients on mixing properties and flour blending requirements. The farinograph determines dough strength

by measuring the resistance of dough against the mixing action of blades (Wheat and flour testing methods 2004).

For the farinograph parameters, HRW wheat flour with AA showed the lowest water absorption of 52.3%, and 100% HRS 2 showed the highest of 63.6%, respectively. All of the HRS wheat flour blends showed significantly ($P<0.05$) higher water absorption than HRW wheat flour as well as the 40% HRS 2 and 40% HRS 3 showed significantly higher water absorption than their respective 30% blending. Therefore, results from Table 2.4 indicate that protein content affects the farinograph water absorption, with higher protein content increasing water absorption.

In addition to water absorption, farinograph peak time and stability are also very important parameters which are strongly correlated with baking quality (Confort and Johnson 1992). In this experiment, all of the HRS wheat flour blendings showed significantly ($P<0.05$) higher farinograph stability than HRW wheat flour, and the 40% blending of HRS 3 and HRS 4 showed significantly ($P<0.05$) higher stability than their respective 30% blending. There was also an increasing trend in stability with increasing percentages of HRS wheat flour for all the HRS in stability blends, and in peak time for most of the HRS wheat flour blends. Similar findings also specified by Maghirang et al. (2006) during the comparison of HRS wheat and HRW wheat. They also found higher protein quantity and quality as well as higher quantities of glutenin and gliadin in HRS wheat flour, which eventually increased the farinograph quality characteristics for HRS wheat flours and HRS wheat flour blends.

Besides farinograph stability, both the 30% and 40% blending of HRS 3 and HRS 4 showed significantly ($P<0.05$) lower mixing tolerance index (MTI) than HRW wheat flour. This 30% and 40% blending of HRS 3 and HRS 4 also showed higher MTI than other blending and most of the

commercial dough strengtheners. MTI indicates the degree of softening, and high water absorption with a low degree of softening indicates good quality flour (Hadnadev et al. 2011).

Among commercial dough strengtheners, SSL, CSL, DATEM, EMG, ADA, and PB had significantly ($P<0.05$) higher water absorption than HRW wheat flour. Among the oxidants, AA had significantly ($P<0.05$) lower water absorption and significantly ($P<0.05$) higher stability compared to HRW wheat flour. The ADA showed higher water absorption and stability than slow acting PB and AA. Because of fast acting rate, ADA exerted most of its effect during mixing whereas due to slow acting rate PB has no effect during mixing. Similar results are indicated by previous study (Tsen 1963; Bushuk and Hlynka 1960). Among the surfactants, only EMG showed significantly ($P<0.05$) high peak time and stability than HRW wheat flour, because surfactants were mainly used to improve machinability of dough (Pyler and Gorton 2008) which was not shown in farinograph.

For the farinograph parameters, almost all of the HRS wheat flour blending showed significantly ($P<0.05$) higher water absorption, peak time, and stability than commercial dough conditioners except VWG. These findings suggest that HRS wheat flour blends showed better dough characteristics than commercial dough strengtheners.

Table 2.4. Farinograph parameters of doughs made by blending HRS flour and by adding additives to HRW flour[a]

Flour blends (ratio)	Water Absorption (14% MB)	Peak Time (Min)	Stability (Min)	MTI (BU)	Quality Number (mm)
HRW	53.7	1.5	8.1	23.0	69.0
HRS wheat flour blends					
HRS1 / HRW					
100/0	61.7	13.0	21.3	14.7	247.3
10/90	55.9	1.6	10.6	11.7	112.0
20/80	56.4	6.8	13.5	21.0	139.3
30/70	58.3	6.4	13.7	16.7	146.0
40/60	58.2	7.8	13.9	21.7	148.0
HRS 2 / HRW					
100/0	63.6	10.3	20.1	8.0	285.3
10/90	54.3	7.2	13.7	26.0	136.3
20/80	55.0	7.6	14.5	19.0	152.3
30/70	56.5	7.3	15.3	16.7	156.7
40/60	57.2	6.9	16.5	15.0	177.7
HRS 3 / HRW					
100/0	62.3	8.2	27.6	13.3	250.3
10/90	55.9	1.6	11.8	13.7	118.7
20/80	56.3	7.2	14.6	20.3	155.0
30/70	57.3	7.0	14.6	19.3	151.0
40/60	58.2	7.2	16.3	13.3	168.7
HRS 4 / HRW					
100/0	62.2	15.2	19.8	21.3	220.3
10/90	55.7	7.7	12.4	25.0	133.7
20/80	56.5	9.1	13.0	31.0	140.3
30/70	57.2	9.9	13.6	30.3	147.3
40/60	57.4	11.1	15.0	27.3	164.7
100% HRW +Additives					
50 ppm SS	54.6	1.4	5.3	44.7	28.3
50 ppm CS	54.7	1.9	7.5	29.3	32.3
25 ppm DATEM	55.6	1.5	6.7	30.7	41.0
0.50% EMG	55.4	5.5	9.1	37.7	96.0
150 ppm AA	52.3	1.6	10.2	23.3	86.0
30 ppm ADA	56.1	1.7	5.8	26.3	56.3
10 ppm PB	54.5	1.6	9.3	16.3	94.7
5% VWG	62.3	7.9	17.6	18.3	182.7
2% Fat	53.9	1.5	11.0	7.0	115.7
2% NFDM	56.0	1.9	10.6	11.0	113.0
LSD	0.5	1.0	1.3	7.9	27.1

[a]LSD=least significant difference at P=0.05, MB= Moisture basis, HRS= Hard Red Spring Wheat flour, HRW = Hard Red winter Wheat flour, SSL = Sodium steroyl lactylate, CSL = Calcium steroyl lactylate, DATEM = Diacetyl tartaric acid ester of mono and diglycerides, EMG = Ethoxylated monoglycerides, AA = Ascorbic Acid, ADA = Azodicarbonamide, PB = Potassium bromate, VWG = Vital wheat gluten, NFDM = Nonfat dry milk, BU= Brabender Units

In this experiment, most of the HRS wheat flour blending showed significantly (P>0.05) higher extensibility than HRW wheat flour at 45 mins; however, there was no significant (P>0.05) difference in extensibility at 135 mins. The highest extensibility and resistance at 45 mins showed by 100% HRS2 and 100 % HRS 4, and the highest extensibility and resistance at 135 mins showed by 100% HRS3 and 100 % HRS 4. All of the blendings showed good increasing trends in resistance at 135 min with increasing amount of HRS wheat flour, but there was no increasing trends in extensibility. The 30% and 40% of HRS 2 as well as 30% and 40% of HRS 3 blends showed much better extensograms than their other respective blends. HRS wheat flours contained higher protein contents and strong gluten quantities than HRW wheat flour, which helped HRS flours and blends to show better dough properties in extensograph. These results also comply with a previous comparison study of HRS wheat and HRW wheat (Maghirang et al. 2006).

Among commercial dough additives, ADA showed significantly (P>0.05) lower extensibility at 45 mins than HRW wheat flour, besides AA and ADA showed significantly (P>0.05) lower extensibility at 135 than HRW wheat flour. However, AA and ADA showed significantly (P>0.05) higher resistance at 45 mins, and AA showed significantly (P>0.05) higher extensibility at 135 than HRW wheat flour. Among the surfactants, only SS showed significantly higher extensibility both at 45 mins and 135 mins than HRW wheat flour; however, there were no other significant (P>0.05) differences among the surfactants as well as between surfactants and HRW wheat flour. The ADA is fast acting oxidizing agents that increases resistance, but decreases extensibility than the medium acting AA and slow acting PB. These findings are aligned with a previous experiment by Tsen (1963). In another study, AA and PB showed a significant decrease in extensograph parameters, but extensibility increase with the increased label of PB (Celik et al.

2000). They also concluded that there is no significant difference in extensograms with 0.5% SSL and DATEM, but SSL showed higher extensibility than DATEM.

These findings clearly indicate that both the 30% and 40% HRS 3 as well as 30% and 40% HRS 4 blends showed significantly ($P<0.05$) higher results for almost all the parameters than commercial dough additives excepting VWG. VWG add gliadins and glutenins to the dough that increase both extensibility and resistance. The extensograph is used to study the effects of ingredients on physical properties of dough as well as classification and assessment of flour by determining the gluten strength (Wheat and flour testing methods 2004). Gliadins are responsible for the viscosity and extensibility of dough whereas glutenins are responsible for strength and elasticity of dough (Wang et al., 2006 and Wieser, 2007).

Table 2.5. Extensograph parameters of doughs made by blending HRS flour and by adding additives to HRW flour[a]

Flour blends (ratio)	Extensibility at 45 min (cm)	Resistance at 45 min (BU)	Area at 45 min (cm^2)	Extensibility at 135min (cm)	Resistance at 135min (BU)	Area at 135 min (cm^2)
HRW	11.8	476.3	79.0	10.6	686.7	96.3
HRS wheat flour blends						
HRS1 / HRW						
100/0	17.1	679.0	145.7	12.7	1011.3	170.0
10/90	13.6	408.7	78.7	11.4	639.3	98.7
20/80	13.8	455.0	85.3	11.5	694.7	107.3
30/70	13.8	459.3	84.7	12.1	723.0	116.0
40/60	14.5	490.0	95.0	12.0	779.7	131.7
HRS 2 / HRW						
100/0	17.3	660.0	143.3	13.5	1124.7	208.0
10/90	13.0	495.3	89.3	11.7	686.7	106.7
20/80	13.1	543.7	97.7	11.6	760.0	117.7
30/70	14.2	516.7	99.0	12.2	815.3	133.3
40/60	14.2	556.3	106.3	11.8	947.3	145.7
HRS 3 / HRW						
100/0	16.8	677.7	146.0	14.0	1115.7	208.7
10/90	13.2	447.3	82.0	11.1	612.7	91.7
20/80	13.4	453.0	82.7	11.6	714.0	109.3
30/70	14.1	518.3	98.3	11.2	812.0	120.0
40/60	14.4	534.3	101.7	11.6	868.3	132.0
HRS 4 / HRW						
100/0	14.3	928.0	164.7	7.9	1500.0	138.0
10/90	12.3	533.0	89.3	10.1	796.3	106.7
20/80	12.9	579.7	98.0	9.1	946.7	110.7
30/70	12.1	639.0	102.3	8.5	1142.3	121.7
40/60	13.0	597.3	98.3	7.4	1186.7	104.0
100% HRW + Additives						
50 ppm SS	13.5	418.7	80.3	12.2	591.3	97.7
50 ppm CS	12.2	435.3	76.0	11.1	570.3	87.0
25 ppm DATEM	12.5	438.7	75.3	11.6	591.3	91.7
0.50% EMG	12.7	413.0	74.0	10.7	572.0	83.0
150 ppm AA	10.9	663.3	95.3	7.1	1263.7	105.7
30 ppm ADA	7.8	763.3	72.3	6.7	763.7	60.7
10 ppm PB	12.3	462.3	80.7	11.1	715.0	106.3
5% VWG	13.7	551.7	99.0	9.5	1034.0	122.3
2% Fat	12.2	451.7	78.3	11.3	635.0	97.7
2% NFDM	13.1	354.3	68.0	11.9	534.0	89.0
LSD	1.4	84.3	15.6	1.4	102.7	19.1

[a]LSD=least significant difference at P=0.05, HRS= Hard Red Spring Wheat flour, HRW = Hard Red Winter Wheat flour, SSL = Sodium steroyl lactylate, CSL = Calcium steroyl lactylate, DATEM = Diacetyl tartaric acid ester of mono and diglycerides, EMG = Ethoxylated monoglycerides, AA = Ascorbic Acid, ADA = Azodicarbonamide, PB = Potassium bromate, VWG = Vital wheat gluten, NFDM = Nonfat dry milk, BU= Brabender Units

2.4.3. Correlation between farinograph and extensograph data

For this experiment, extensibility of the doughs made of HRS wheat flour blends showed significant ($P<0.05$) and negative correlation with only MTI; however, there was no other extensograph parameters significantly correlated with MTI. Farinograph absorption, stability, and FQN showed significant ($P<0.05$) correlation with all the extenosgraph results, except the extensibility at 135 mins. On the other hand, there was no significant ($P<0.05$) correlation among almost all of the parameters of farinograph and extensograph for the commercial dough additives, whereas only EAR was significantly ($P<0.05$) correlated with farinograph stability (Table 2.6). Therefore, it can be concluded that HRS wheat flour blends showed much better correlations with dough rheology than commercial dough additives.

The farinograph and extensograph are used to measure rheological properties of dough. These rheological data are used to predict the performance of flour during processing and quality control of final products (Dobraszczyk and Morgenstern 2003). Therefore, a good correlation between farinograph and extensograph data is desirable.

Table 2.6. Correlation between farinograph and extensograph data among HRS blending and chemical strengtheners[a]

	FAB	FPT	FST	MTI	FQN
			HRS wheat flour blends		
EXT 045	0.854***	0.413^{NS}	0.83***	-0.628**	0.877***
ERS 045	0.72***	0.856***	0.694***	0.148^{NS}	0.717***
EAR 045	0.886***	0.788***	0.872***	-0.171^{NS}	0.899***
EXT 135	0.238^{NS}	-0.284^{NS}	0.348^{NS}	-0.728***	0.315^{NS}
ERS 135	0.718***	0.854***	0.663***	0.193^{NS}	0.702***
EAR 135	0.857***	0.529*	0.898***	-0.411^{NS}	0.918***
			Dough additives		
EXT 045	0.282^{NS}	0.363^{NS}	0.407^{NS}	0.03^{NS}	0.305^{NS}
ERS 045	0.064^{NS}	0.019^{NS}	0.009^{NS}	-0.027^{NS}	0.032^{NS}
EAR 045	0.319^{NS}	0.468^{NS}	0.633*	-0.079^{NS}	0.485^{NS}
EXT 135	-0.032^{NS}	-0.105^{NS}	-0.091^{NS}	0.05^{NS}	-0.112^{NS}
ERS 135	0.134^{NS}	0.276^{NS}	0.492^{NS}	-0.176^{NS}	0.408^{NS}
EAR 135	0.286^{NS}	0.375^{NS}	0.693*	-0.27^{NS}	0.558^{NS}

[a]FAB= Farinograph water absorption, FPT= Farinograph Peak Time, FST= Farinograph Stability, FQN=Farinograph Quality Number, EXT 045= Extensograph Extensiblity at 45 mins , ESR 045 = Extensograph Resistance at 45 mins , EAR 045 = Extensograph Area at 45 mins , EXT 135= Extensograph Extensiblity at 135 mins, ESR 135 = Extensograph Resistance at 135 mins , EAR 135 = Extensograph Area at 135 mins. NS= non-significant, *= $p \leq .05$, **= $p \leq .01$, ***= $p \leq .001$.

2.4.4. Quality traits of the bread made of HRS wheat flour blends and additives

Bread baking qualities are characterized by baking absorption, dough handling properties, bread loaf volume, specific volume, crust and crumb score, and firmness (Table 2.7). As water is the cheapest ingredient in breadmaking, higher baking absorption give higher economic return (Maghirang et al. 2006). In this experiment, baking absorption showed significantly ($P<0.05$) higher value for all of the HRS wheat flour blends (without 10% HRS 2) then HRW wheat flour. However, among commercial dough additives only 5% VWG showed significantly ($P<0.05$) higher baking absorption value than HRW wheat flour. All most all of the HRS wheat flour blends showed significantly ($P<0.05$) higher baking absorption than commercial dough additives whereas

VWG showed significantly ($P<0.05$) higher baking absorption than all most all of the HRS wheat flour blends. Due to high protein content, HRS wheat flour and blends had significantly ($P<0.05$) higher baking absorption than HRW wheat flour. Maghirang et al. (2006) also found similar results.

In addition to baking absorption, mixing time is an important factor for bakers, because too short mixing time is responsible for easily overdone whereas too long mixing time takes valuable time and energy (Alava, et. al. 2001). In our findings, there is no significant ($P<0.05$) difference in mixing time within the HRS wheat flour blends, and within most of the dough additives. However, HRS wheat flour blends showed significantly higher absorption than HRW wheat flour, which aligned with the results of Maghirang et al. (2006). Besides HRS wheat flour blends, the ADA and fat showed significantly ($P<0.05$) lower mixing time than other commercial dough strengtheners. The ADA showed lowest mixing time because of it's fast acting rate and almost complete by 2.5 mins of mixing (Tsen 1963); however, medium acting ascorbic acid and slow acting PB exert most of their effects after mixing (Pyler and Gorton 2008).

Oven spring is the sudden increase in the volume of fermented dough during the first 10-12 min of baking, due to increased rate of fermentation and expansion of gases in oven (Bender 2005). In this experiment, oven spring was increased with increasing percentage of HRS 2 flour and HRS 3 flour, moreover almost all the HRS wheat flour blends showed significantly ($P<0.05$) higher oven spring than almost all of the commercial dough additives. Among the HRS wheat flour blends, the highest oven spring showed by the loaf made with 40% HRS 3, and there was an increasing trend in oven spring with increase HRS wheat flour percentage. Furthermore, AA showed the highest oven spring among oxidants and ADA did not show any oven spring. Among the surfactants, DATEM and EMG showed significantly ($P<0.05$) higher value than SSL and CSL

and also HRW wheat flour. Oven spring increased with higher protein content of flour (He and Hoseney 1992) and there was no significant difference in oven rise among PB (75ppm), ADA (40ppm), and AA (125ppm) (Yamada and Preston 1992). However, Yamada and Preston (1994) found that PB (5ppm) showed higher than ADA (10ppm), and AA (15ppm) showed significantly lower oven rise in sponge dough method.

Loaf volume and crumb firmness are the main quality characteristics of bread (Katina et al. 2006). There was a very good increasing trend in the loaf volumes with increasing percentages of HRS wheat flour for all of the HRS wheat flour blending's (Table 2.7). The highest loaf volume of 1065 cc was showed by 100% HRS 3. Among the blends the highest loaf volume (950 cc) was showed by 40% HRS 3 blend followed by second highest loaf volume (920 cc) was showed by 40% HRS 2 blend. There was no significant difference in the loaf volume between the 30% and 40% HRS wheat flour blends for all the HRS wheat flour blendings. The reason of the increasing loaf volumes with increasing percentages of HRS wheat flour is that loaf volume is a function of flour protein as well as linearly increase with increasing protein content and protein quality for pan breads (Finney and Barmore 1948; Finney 1984).

Furthermore, VWG and EMG showed significantly ($P<0.05$) higher loaf volume than all other commercial dough additives, whereas ADA showed the lowest loaf volume of 450cc. Among surfactants, there was no significant ($P<0.05$) difference in the loaf volume between SS and DATEM; however, CS showed significantly ($P<0.05$) lower loaf volume and EMG showed significantly ($P<0.05$) higher loaf volume than SS and DATEM. Similar loaf volume increasing action of DATEM and SSL were found in a previous study (Rogers and Hoseney 1983). In another study, EMG showed highest loaf volume followed by SSL and DATEM with 0.50% treatments (Junge and Hoseney 1981).

Besides surfactants, there was no significant ($P<0.05$) difference in loaf volume between PB and AA; however, ADA was significantly ($P<0.05$) lower than those. In a previous study, Yamada and Preston (1992) did not find any significant difference in loaf volume among PB (75ppm), ADA (40ppm), and AA (125ppm). Later, they found that PB (5ppm) showed significantly ($P<0.05$) higher than ADA and PB, and there was no difference between ADA (10ppm), and AA (15ppm) in loaf volume using sponge dough method (Yamada and Preston 1992). The differences in the loaf volume are the due to rate of activity of the oxidants. The ADA is a fast acting, and completed the action during mixing; however, the ascorbic acid is medium acting and PB is slow acting, and these can exert most of the effects after mixing (Pyler and Gorton 2008).

To summarize the findings of loaf volume, bread made with the HRS wheat flour blends showed significantly ($P<0.05$) higher loaf volume than HRW wheat flour, and all the commercial dough additives except EMG and VWG. The 30% and 40% of HRS 2 as well as 30% and 40% of HRS 3 blends showed significantly ($p<0.05$) higher volume than respective blends of HRS 1 and HRS 4. The 30% and 40% of HRS 2, as well as 30% and 40% HRS 3 showed significantly ($P<0.05$) higher loaf volume than the highest loaf volume (850 cc) among the dough additives given by VWG. (Fig. 2.1).

Additionally, the specific volume is critical for consumer acceptance, is commonly used to assess bread quality and texture characteristics (Belz et al. 2012). In our experiment, specific volume showed very similar increasing trends of loaf volume. The highest specific volume among the blends showed by bread with HRS 3 blend, and that was significantly ($p<0.05$) higher than the highest specific volume given by bread made of commercial dough additives.

Once again, the firmness increase is the most widely used indicator of bread staling (Gray and Bemiller, 2003) which directly affects the consumer preference (He and Hoseney, 1990). In this experiment, bread with the HRS wheat flour blends showed lower firmness then all the commercial dough additives, except EMG and VWG. There was also a decreasing trend in the firmness with increasing amount of HRS wheat flour percentages for all the HRS wheat flour blends. The lowest firmness of 690 mN showed by 100% HRS 2; however, among HRS wheat flour blends, the lowest firmness of 1525 mN and 1553 mN showed 40% HRS 3 and 40% HRS 2, respectively. With the higher protein content decreasing bread firmness, and there is a negative correlation between gluten index and firmness (Barak et al. 2013).

Although there was no significant difference in between firmness and commercial additives, EMG showed the lowest whereas ADA showed the extremely high firmness of 10072 mN. In addition, emulsifiers (without CSL) showed lower firmness than oxidants. (Fig. 2.2). Emulsifiers are more efficient in reducing bread firmness by forming a complex with amylose, and monoacylglycerols has the higher than DATEM complex forming power. Both SSL and DATEM exerted similar crumb softening effect for having one stearic acid in their structure (Eduardo et al. 2014)

For the crust color, crumb color, and crumb texture of bread, almost all the HRS wheat flour blends showed better value than commercial dough strengtheners as well as bread with 30% and 40% of all the HRS wheat flour blends showed the highest value. Bread with the 30% and 40% of HRS wheat flour blends also showed much better symmetry and texture than all other HRS wheat flour blending (Fig. 2.3). Among the surfactants, EMG showed the highest value and CSL showed the lowest; however, among oxidants, AA showed the highest color and texture, and ADA

showed the lowest value. Among the additional ingredients, NFDM showed significantly higher value than almost all of the surfactants and oxidants excepting EMG.

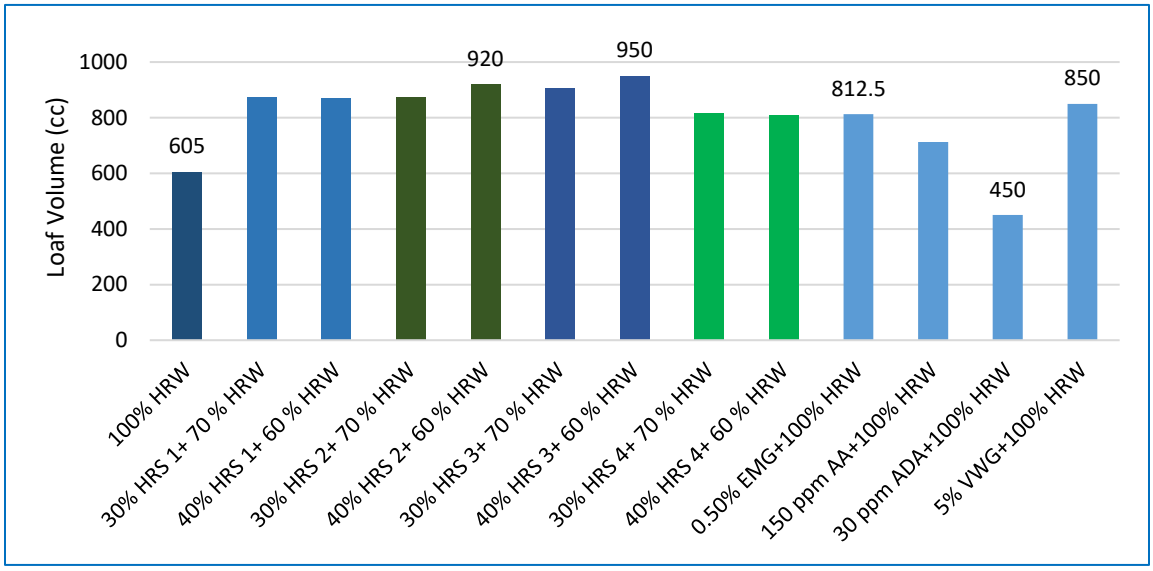

Fig. 2.1. Loaf volumes of bread made from HRS wheat flour blends and commercial additives

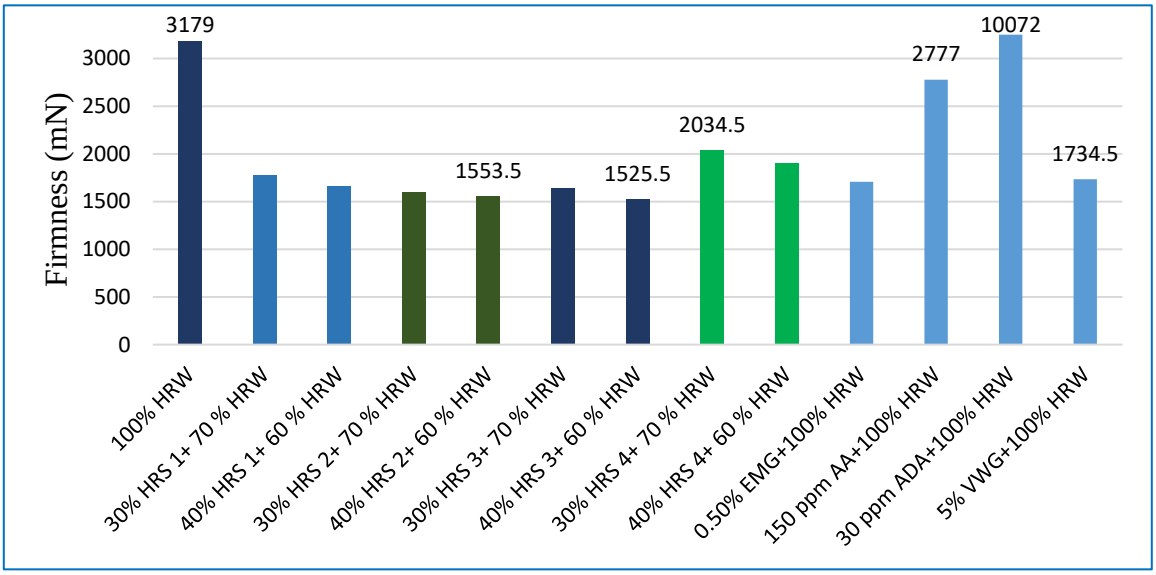

Fig. 2.2. The firmness of bread made from HRS wheat flour blends and commercial additive

Table 2.7. Baking quality characteristics of the bread made from HRS blends and commercial additives

	BAB (%)	MT (Min)	DS (0-10)	OS (cm)	LV (cc)	SV (0-10)	SY (0-10)	CRC (0-10)	CT (0-10)	CBC (0-10)	FM mN
HRW	60.1	3.6	8.0	1.0	605.0	4.7	3.0	4.5	4.0	4.5	3179.0
HRS wheat flour blends											
HRS1 / HRW											
100/0	69.8	5.0	9.0	4.2	1002.5	7.7	8.0	10.0	7.0	10.0	1293.5
10/90	62.5	4.5	9.0	2.3	785.0	6.1	5.5	8.0	6.0	8.0	2306.5
20/80	63.0	4.4	9.0	2.9	847.5	6.6	6.5	9.0	6.0	9.0	2083.5
30/70	65.0	4.5	9.0	3.1	875.0	6.8	6.5	10.0	7.0	10.0	1781.0
40/60	65.8	4.6	10.0	2.9	870.0	6.7	7.0	10.0	7.0	10.0	1658.0
HRS 2 / HRW											
100/0	72.0	5.0	8.0	4.1	1055.0	7.9	8.5	10.0	8.0	10.0	690.5
10/90	60.8	4.0	9.0	2.1	757.5	5.8	5.5	8.5	6.0	8.5	2163.5
20/80	63.1	4.1	9.0	2.7	815.0	6.3	6.5	9.5	6.0	9.5	1943.5
30/70	63.9	4.4	9.0	3.0	875.0	6.7	7.0	10.0	7.0	10.0	1599.5
40/60	65.0	4.4	9.5	3.5	920.0	7.1	7.5	10.0	7.5	10.0	1553.5
HRS 3 / HRW											
100/0	72.0	5.3	8.0	4.1	1065.0	8.2	8.0	10.0	8.0	10.0	919.5
10/90	63.5	4.3	9.0	2.7	802.5	6.2	5.5	8.5	6.5	8.5	2018.5
20/80	64.0	4.4	9.5	2.8	837.5	6.5	6.5	9.0	7.0	9.0	1971.5
30/70	65.0	4.5	9.5	3.0	905.0	7.0	7.5	10.0	8.0	10.0	1641.5
40/60	66.0	4.5	10.0	3.7	950.0	7.3	7.5	10.0	8.0	10.0	1525.5
HRS 4 / HRW											
100/0	69.4	5.0	8.0	3.7	992.5	7.5	8.0	9.5	7.5	9.5	1197.5
10/90	62.5	4.3	9.0	2.9	715.0	5.4	5.0	8.0	6.0	8.0	2222.5
20/80	63.8	4.5	9.0	2.3	762.5	5.7	6.0	9.5	6.5	9.5	2141.5
30/70	64.5	4.6	9.0	2.4	815.0	6.2	6.5	10.0	7.0	10.0	2034.5
40/60	65.2	4.6	10.0	3.0	807.5	6.1	7.0	10.0	7.5	10.0	1900.5

Table 2.7. Baking quality characteristics of the bread made from HRS blends and commercial additives (Continued)

	BA (%)	MT (Min)	DS (0-10)	OS (cm)	LV (cc)	SV (0-10)	SY (0-10)	CRC (0-10)	CT (0-10)	CBC (0-10)	FM mN
100% HRW + Additives											
50 ppm SS	61.0	4.4	9.0	1.5	720.0	5.5	5.0	6.0	6.0	6.0	2149.5
50 ppm CS	61.1	5.1	8.5	1.2	615.0	4.8	3.0	4.0	5.0	4.0	2931.5
25 ppm DATEM	62.0	4.3	9.0	2.2	712.5	5.5	4.5	7.0	6.5	7.0	2678.0
0.50% EMG	62.8	4.1	8.5	2.7	812.5	6.3	5.5	8.0	6.0	8.0	1708.0
150 ppm AA	61.2	4.3	9.5	2.0	712.5	5.5	4.0	7.5	6.5	7.5	2777.0
30 ppm ADA	58.8	3.5	5.0	0.0	450.0	3.5	0.0	1.0	0.5	1.0	10072.5
10 ppm PB	62.4	4.5	8.5	1.9	707.5	5.4	5.5	6.0	6.5	6.0	2793.5
5% VWG	70.4	4.4	9.0	2.5	850.0	6.1	6.5	9.0	7.5	9.0	1734.5
2% Fat	60.3	3.6	8.0	1.9	677.5	5.2	4.0	6.0	5.5	6.0	2586.5
2% NFDM	63.1	4.1	8.5	1.4	727.5	5.5	5.5	8.0	7.5	8.0	2602.0
LSD	2.6	0.5	1.1	0.7	45.5	0.4	1.0	1.2	0.9	1.2	1818.9

[a]LSD=least significant difference at P=0.05, HRS= Hard Red Spring Wheat flour, HRW = Hard Red Winter Wheat flour, BAB= Baking Absorption, MT= Mixing Time, DS= Dough Score, OS=Oven Spring, LV= Loaf Volume, SV=Specific Volume, SY=Symmetry, CRC=Crust Color, CT=Crumb Texture, CBC=Crumb Color, FM=Firmness, SSL = Sodium steroyl lactylate, CSL = Calcium steroyl lactylate, DATEM = Diacetyl tartaric acid ester of mono and diglycerides, EMG = Ethoxylated monoglycerides, AA = Ascorbic Acid, ADA = Azodicarbonamide, PB = Potassium bromate, VWG = Vital wheat gluten, NFDM = Nonfat dry milk, DS= commercially available dough strengtheners

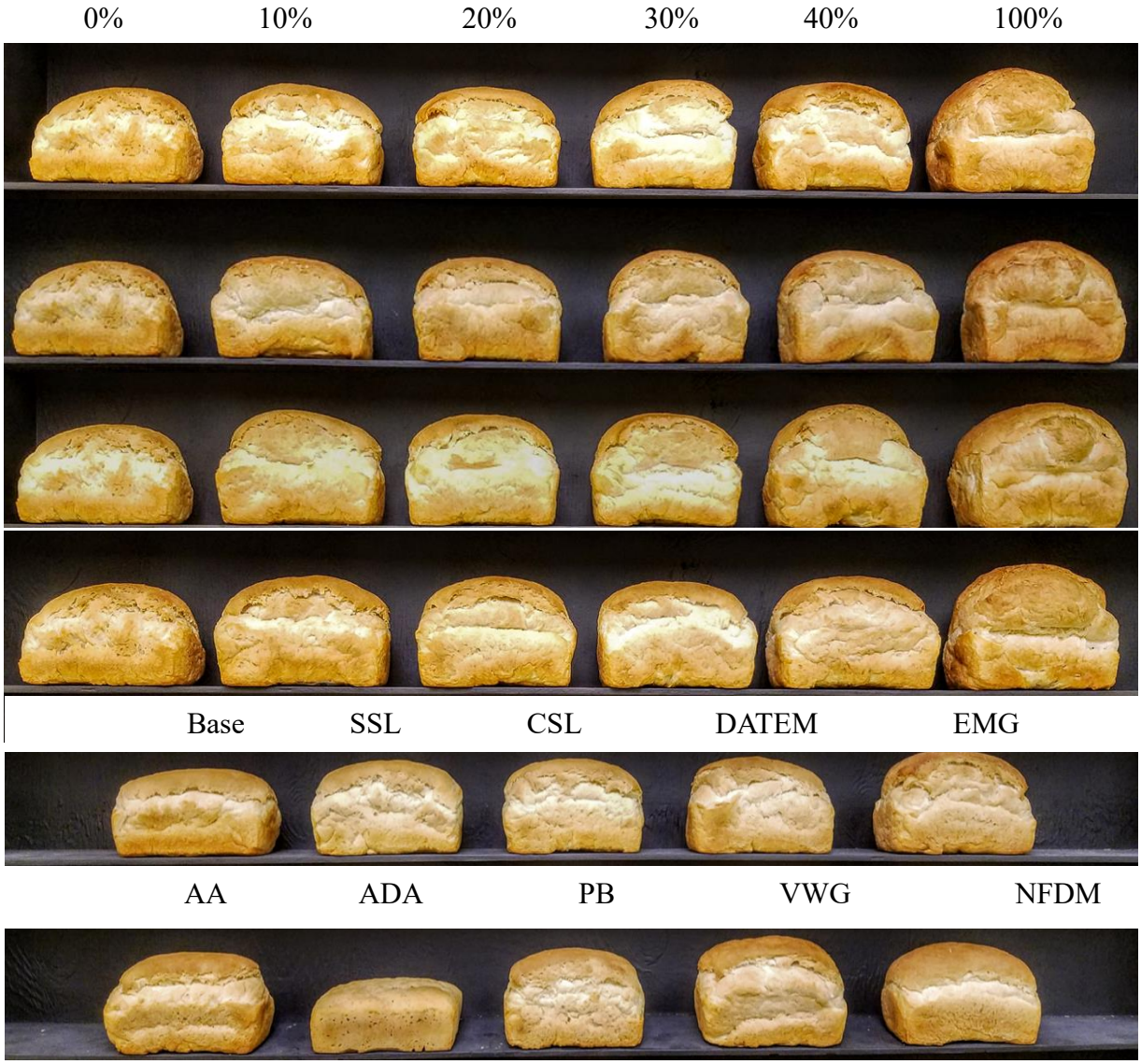

Fig. 2.3. Bread loves made from HRS blends (HRS1, HRS2, HRS 3, HRS 4 respectively) and Commercial dough strengtheners (CSL = Calcium steroyl lactylate, DATEM = Diacetyl tartaric acid ester of mono and diglycerides, EMG = Ethoxylated monoglycerides, AA = Ascorbic Acid, ADA = Azodicarbonamide, PB = Potassium bromate, VWG = Vital wheat gluten, NFDM = Nonfat dry milk)

2.4.5. Correlation between dough rheology and bread quality characteristics

For HRS wheat flour blends, all the farinograph parameters excepting MTI showed significant ($P<0.05$) correlation with all baking quality characteristics without dough score. The farinograph stability showed significant ($P<0.05$) and positive correlation with loaf volume ($r=0.894$); however, significant ($P<0.05$) and negative correlation with firmness ($r=-0.882$). These findings are very similar to a previous study (Barak et al. 2013). Similar to farinogragh, most of the extensograph parameters showed significant ($P<0.05$) correlation with all baking quality characteristics without dough score. However, ERS045 was not correlated with crumb color and texture, and ERS135 did not show any significant correlation with baking quality characteristics (Table 2.8).

For the commercial dough additives, there was no significant ($P<0.05$) correlation between almost all the dough quality characteristics and baking quality characteristics. The farinograph stability showed significant ($P<0.05$) and positive, but not strong correlation with loaf volume ($r=0.62$). Only EXT045 and EAR135 showed significant ($P<0.05$) correlation between almost all the dough quality characteristic.

There is good correlation between dough rheological measurements and baking performance found in many studies. Even many researchers have attempted to predict bread quality through prediction models made from grain quality, flour quality, and dough quality (Autio, et. al. 2001; Lee et al. 2006; Dowell et al. 2008). The farinograph has been traditionally used to measure flour quality (Shuey 1984), and there were strong significant correlations found between peak time and stability in relation to baking quality (Confort and Johnson 1992).

Table 2.8. Correlation coefficients between dough and bread qualities among HRS blends and chemical dough strengtheners[a]

	BAB	MT	DS	OS	LV	SV	SY	CRC	GT	CBC	FM
HRS blends											
FAB	0.982***	0.922***	-0.31^{NS}	0.826***	0.896***	0.866***	0.815***	0.559**	0.672***	0.837***	-0.878***
FPT	0.634**	0.68***	0.008^{NS}	0.663***	0.598**	0.56**	0.7***	0.622**	0.453*	0.671***	-0.66***
FST	0.901***	0.843***	-0.237^{NS}	0.858***	0.894***	0.883***	0.814***	0.605**	0.634**	0.891***	-0.882***
MTI	-0.411^{NS}	-0.241^{NS}	0.226^{NS}	-0.363^{NS}	-0.481*	-0.5*	-0.323^{NS}	-0.089^{NS}	-0.36^{NS}	-0.342^{NS}	0.389^{NS}
FQN	0.944***	0.86***	-0.244^{NS}	0.886***	0.912***	0.885***	0.867***	0.635**	0.662***	0.938***	-0.93***
EXT045	0.875***	0.77***	-0.205^{NS}	0.809***	0.887***	0.881***	0.776***	0.515*	0.581**	0.837***	-0.847***
ERS045	0.697***	0.681***	-0.34^{NS}	0.607**	0.602**	0.564**	0.587**	0.385^{NS}	0.357^{NS}	0.664***	-0.637**
EAR045	0.881***	0.811***	-0.3^{9NS}	0.774***	0.81***	0.78***	0.738***	0.463*	0.483*	0.842***	-0.817***
EXT135	0.29^{NS}	0.122^{NS}	-0.17^{NS}	0.297^{NS}	0.395^{NS}	0.429*	0.224^{NS}	0.097^{NS}	0.157^{NS}	0.347^{NS}	-0.335^{NS}
ERS135	0.703***	0.724***	-0.207^{NS}	0.596**	0.591**	0.546**	0.629**	0.469*	0.471*	0.668***	-0.634**
EAR135	0.897***	0.771***	-0.33^{NS}	0.796***	0.859***	0.842***	0.746***	0.501*	0.542**	0.925***	-0.863***
Additives											
FAB	0.85***	0.106^{NS}	-0.031^{NS}	0.241^{NS}	0.387^{NS}	0.233^{NS}	0.344^{NS}	0.306^{NS}	0.199^{NS}	0.045^{NS}	-0.033^{NS}
FPT	0.857***	0.137^{NS}	0.183^{NS}	0.563^{NS}	0.634*	0.538^{NS}	0.494^{NS}	0.524^{NS}	0.321^{NS}	0.279^{NS}	-0.289^{NS}
FST	0.845**	0.063^{NS}	0.336^{NS}	0.537^{NS}	0.62*	0.501^{NS}	0.569^{NS}	0.638*	0.535^{NS}	0.441^{NS}	-0.405^{NS}
MTI	-0.164^{NS}	0.277^{NS}	0.088^{NS}	0.026^{NS}	0.02^{NS}	0.093^{NS}	-0.076^{NS}	-0.123^{NS}	-0.159^{NS}	-0.038^{NS}	-0.004^{NS}
FQN	0.759**	-0.155^{NS}	0.165^{NS}	0.478^{NS}	0.564^{NS}	0.451^{NS}	0.53^{NS}	0.588^{NS}	0.438^{NS}	0.297^{NS}	-0.285^{NS}
EXT045	0.593^{NS}	0.487^{NS}	0.809**	0.7*	0.833**	0.819**	0.897***	0.774**	0.864***	0.904***	-0.922***
ERS045	-0.138^{NS}	-0.354^{NS}	-0.544^{NS}	-0.416^{NS}	-0.497^{NS}	-0.545^{NS}	-0.622*	-0.464^{NS}	-0.609*	-0.687*	0.734*
EAR045	0.573^{NS}	0.198^{NS}	0.454^{NS}	0.428^{NS}	0.435^{NS}	0.364^{NS}	0.333^{NS}	0.411^{NS}	0.349^{NS}	0.368^{NS}	-0.307^{NS}
EXT135	0.091^{NS}	0.312^{NS}	0.457^{NS}	0.321^{NS}	0.396^{NS}	0.43^{NS}	0.55^{NS}	0.338^{NS}	0.517^{NS}	0.588^{NS}	-0.644*
ERS135	0.321^{NS}	-0.022^{NS}	0.18^{NS}	0.163^{NS}	0.144^{NS}	0.09^{NS}	0.021^{NS}	0.211^{NS}	0.096^{NS}	0.033^{NS}	0.045^{NS}
EAR135	0.672*	0.337^{NS}	0.759**	0.638*	0.707*	0.646*	0.739**	0.693*	0.751**	0.773**	-0.728*

[a]BAB= Baking Absorption, MT= Mixing Time, DS= Dough Score, OS=Oven Spring, LV= Loaf Volume, SV=Specific Volume, SY=Symmetry, CRC=Crust Color, GT=Crumb Texture, CBC=Crumb Color, FM=Firmness, FAB= Farinograph Water Absorption, FPT= Farinograph Peak Time, FST= Farinograph Stability, FQN=Farinograph Quality Number, EXT= Extensograph Extensiblity, ESR= Extensograph Resistance , EAR= Extensograph Area, NS= non-significant, *= $p \leq .05$, **= $p \leq .01$, ***= $p \leq .001$

2.5. Conclusions

In this study, different percentages of four hard red spring (HRS) wheat flour were used to replace commercial dough strengtheners. To compare the strengthening ability of HRS flour blends, ten commercially available dough additives were used. For both the dough and bread properties, HRS wheat flour blends as a replacement for dough strengtheners had better characteristics in comparison with commercial strengtheners. Almost all of the HRS flour blends showed significantly ($P<0.05$) higher farinograph absorption, peak time, and stability than the commercial dough strengtheners. There were positive and significant correlations among almost all of the parameters of farinograph and extensograph for the HRS flour blends; however, there was no significant relationship between the farinograph and extensogrph parameters for the chemical dough strengtheners. The 30% and 40% of Linkert and Glenn flour blends showed much higher water absorption than the surfactants and oxidants as well as higher loaf volume and lower firmness than all the commercial dough additives. The highest loaf volume and the lowest firmness were shown by 40% Linkert followed by 40% Glenn. For the crust and crumb color, almost all of the blends showed better value than any of the additives, and all of the 30% and 40% of HRS wheat flour blends showed better loaf symmetry and texture than all other blends. For the commercial dough additives, there was no significant ($P<0.05$) correlation between the dough quality characteristics and baking quality characteristics whereas HRS what flour blends showed significant ($P<0.05$) correlation between almost all the dough quality characteristics and baking quality characteristics. Therefore, we can conclude that addition of 40 % HRS wheat flour to HRW wheat flour can be used as a replacement for almost all of the dough strengtheners in bread production.

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CHAPTER 3. MOLECULAR WEIGHT DISTRIBUTION OF PROTEIN EXTRACTED FROM DOUGH AND BREAD MADE OF HRS WHEAT BLEND AND ADDITIVES, AND ITS CORRELATION WITH DOUGH AND BREAD CHARACTERISTICS

3.1. Abstract

Hard red spring (HRS) wheat is extensively used as a blending wheat for its high protein content and strong gluten quality whereas hard red winter (HRW) wheat is used mainly for pan bread production. The objective of this study was to investigate molecular weight distribution (MWD) of protein extracted from dough and bread made of HRS wheat flour blends and additives as well as its correlation with dough and bread quality characteristics. Doughs were prepared by blending different percentages (10%, 20%, 30%, and 40%) of each of the HRS wheat flour to HRW wheat flour as well as by adding each of the ten different additives to HRW wheat flour. Each of the dough was measured for dough rheology followed by test baking. In addition, MWD of protein extracted from each of the dough and bread sample was measured. The 30% and 40% blends of most of the HRS wheat flour showed better absorbance area and area percent values of extractable and unextractable protein fractions than all the additives. The absorbance area value of unextractable F1 protein fraction from bread showed significantly ($P<0.05$) higher value for most of the HRS wheat flour blends than HRW wheat flour. Absorbance area of all four extractable and unextractable protein fractions showed significant ($P<0.05$) and positive correlation with all most all of the dough rheology parameters of HRS wheat flour; however, there was no significant ($P<0.05$) difference with all most all of the dough rheology parameters of additives. The area percent of the extractable F1 protein fraction showed significant ($P<0.05$), strong, and negative correlation with dough quality parameters for HRS wheat flour. Therefore, HRS wheat flour blends

had a better association between protein MWD with dough and bread properties in comparison with commercial additives.

3.2. Introduction

Bread is a staple food (Cauvain 2004), and a good source of carbohydrates as well as substantial amounts of the protein, vitamins, and minerals (Collado-Fernández 2003). During breadmaking flour, water, salt, and yeast with some nonessential ingredients are mixed into dough followed by fermented and baked (Goesaert et al. 2005). Wheat flour contains nongluten forming protein fractions namely albumins and globulins as well as gluten forming protein factions namely gliadins and glutenins. Gliadins and glutenins are responsible for viscosity and extensibility, respectively, in a dough system (Delcour and Hoseney 2010) and these visco-elastic properties are ascribed as the unique breadmaking properties of wheat flour. The gliadins are monomeric and have molecular weights between 30,000 and 60,000 Da (Veraverbeke and Delcour 2002). The intermolecular hydrogen and hydrophobic bonds occur between non-polar amino acid chains during dough mixing, and these bonds contribute towards the formation of the gluten network. Based on to mobility on low pH electrophoresis, gliadins are classified into four groups: α-, β-, γ- and ω- (Wieser 2007). Gliadins are mainly responsible for the dough cohesiveness (Delcour and Hoseney 2010) and the addition of gliadins has been shown to decrease dough strength, mixing time, and loaf volume (Uthayakumaran et al. 1999).

Glutenin is the major polymeric protein in wheat flour and is a highly heterogeneous mixture of a different number of low to high molecular weight glutenin subunits that are linked by disulfide bonds (Veraverbeke and Delcour 2002). The molecular weight of low molecular weight glutenin subunits (LMW-GS) is 30,000 to 45,000 Da, and the molecular weight of high molecular weight glutenin subunits (HMW-GS) is 70,000 to 90,000 Da. The HMW-GS are minor

components in terms of quantity; however, they are key factors in the process of bread making because they are major determinants of gluten elasticity and functionality (Gianibelli et al. 2001). Payne et al. (1987) found that the presence of certain HMW-GS has a positive influence on gluten strength. However, both the insufficiently elastic gluten and too elastic gluten leads to low bread loaf volume, therefore, an adequate balance of viscosity and elasticity is required for quality breadmaking. The glutenin and gliadin ratio is also believed to largely affect the bread making. The increases in the glutenin-to-gliadin ratio in a constant protein content resulted in the increase of mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume (Uthayakumaran et al. 1999).

There is a linear relationship between protein content, gluten protein quality, and breadmaking performance (Finney and Barmore 1948; Veraverbeke and Delcour 2002). Moreover, there are some significant associations between protein molecular weight distribution with dough mixing and breadmaking characteristics (Ohm et al. 2010). Among the six distinct wheat classes of United States, hard red spring (HRS) wheat has the highest protein content of 13 to 16 percent, and strong gluten characteristics (North Dakota Wheat Commission). Ohm et al. (2010) investigated the associations of MWD of proteins of HRS wheat and breadmaking quality characteristics by extracting MWD of proteins by size-exclusion HPLC and found that certain protein fractions were responsible for this associations. SDS-unextractable high molecular weight polymeric proteins had a positive partial correlations with breadmaking parameters, including mix time and bread loaf volume.

The objectives of this study were to investigate the molecular weight distribution (MWD) of protein extracted from dough and bread made of HRS wheat flour blends and additives. In

addition, to investigate the correlation between MWD of dough protein and dough quality characteristics as MWD of bread protein and bread quality characteristics.

3.3. Materials and Methods

3.3.1. Sampling

Five flour samples were used in the experiment: one hard red winter (HRW) wheat flour and four hard red spring (HRS) wheat flour. The HRW wheat flour was collected from Ardent Mills, Denver, CO. The HRS wheat cultivar 'Glenn', was collected from producer field near Fessenden, ND, and cultivar 'Linkert' was collected from research field near Casselton, Langdon, Minot, Hettinger and Williston, ND. The grain was blended, cleaned and milled by HRS Wheat Quality group, Department of Plant Sciences, North Dakota State University. Additional, two HRS wheat flour were commercial samples were obtained, one from Swany White Mills, Freeport, MN and another one from ND State Mill, Grand Forks, ND. In this experiment, HRW wheat flour was used as base flour, and HRS1 was from Swany White Mill, HRS2 was from Glenn, HRS3 was from cultivar Linkert, and HRS4 was from ND State Mill. The HRS flour sample were used to compare the dough strengthening functions with commercial dough additives. The ten additives were potassium bromate, azodicarbonamide, ascorbic acid, sodium stearoyl lactylate, calcium stearoyl lactylate, diacetyl tartaric acid esters of mono- and diglycerides, ethoxylated mono- and diglycerides, vital wheat gluten, nonfat dry milk, and fat.

3.3.2. Proximate analysis, dough rheology, baking, and bread quality analysis

The doughs were prepared by blending 10, 20, 30 and 40% of each of the HRS wheat flour with HRW flour. Doughs were prepared by adding each of the ten chemical dough strengtheners at their optimum quantities to 100% HRW wheat flour (Table 3.1). Proximate analysis of the

samples, dough rheology, test baking, and bread quality analysis were done according to AACCI Approved Methods. Details of the methods were described in chapter 2.

Table 3.1. Dough and bread were prepared by blending HRS flour with HRW flour and by adding additives to HRW flour[a]

Sample no	Name/ Blending ratio	Dough strengthener/ additives	HRW
1	HRW	None	100%
	HRS wheat flour blends[b]		
	HRW/HRS		
2	0/100	100% HRS	0%
3	90/10	10% HRS	90%
4	80/20	20% HRS	80%
5	70/30	30% HRS	70%
6	60/40	40% HRS	60%
	Additives		
7	Sodium stearoyl lactylate	50 ppm	
8	Calcium stearoyl lactylate	50 ppm	
9	Diacetyl tartaric acid esters of mono- and diglycerides	25 ppm	
10	Ethoxylated mono- and diglycerides	0.50%	
11	Ascorbic acid	150 ppm	100%
12	Azodicarbonamide	30 ppm	
13	Potassium bromate	10 ppm	
	Additional ingredients		
14	Vital wheat gluten	5%	
15	Fat	2%	
16	Non-Fat Dry Milk	2%	

[a]HRW= Hard red winter wheat flour, HRS= Hard red spring wheat flour
[b] This blending ratio was maintained for all four hard red spring wheat flour

3.3.3. Protein extraction and Size-Exclusion High Performance Liquid Chromatography (SE-HPLC)

Dough was prepared by following the same procedure of baking and then freeze-dried (Labcoco Freezone 6L) and ground with a ball mill to obtain uniform particle size and stored in plastic bags at 4–5ºC until used. Bread crumbs were air dried for 48 h at room temperature (temperature range 18-20^{0}C, RH range 15-18%) then ground with a ball mill to obtain uniform

particle size and stored in plastic bags at 4–5ºC until used. All the flour samples were stored in plastic bags at room temperature.

Proteins from flour, dough, and bread crumbs were extracted as described by Gupta et al. (1993) with minor modifications (Ohm et al. 2009). 10 mg (14%mb) powder was suspended in 1 mL of 0.5% SDS and 0.05 M sodium phosphate buffer (pH 6.9) followed by stirring for 5 min (at 2,000 rpm) using pulsing vortex mixer (Fisher Scientific). No defatting was done for dried bread crumbs flour. The supernatant was separated after centrifuging the mixture for 15 min at 17,000 g (Eppendorf Centrifuge 5424). The residue was sonicated in the 1 mL of extraction buffer for 30 sec at 10 W output to solubilize SDS unextractable proteins using a Sonic Dismembrator 100 (Fisher Scientific) (Gupta et al. 1993; Ohm et al. 2009) and the sonicated mixture was also centrifuged as described for the extractable fraction. The supernatants from extractable and unextractable fractions were individually filtered by a membrane (0.45 mm PVDF, Sun Sri, Rockwood, TN) and then heated in a water bath at 80°C for 2 min (Larroque et al. 2000) to remove any enzyme activity.

SE-HPLC was performed on a narrow-bore size exclusion column (BioSep SEC S4000, 300 x 4.5mm, Phenomenex, Torrance, CA) with a guard cartridge (BioSep SEC S4000) using an Agilent1100 Series chromatograph (Agilent Technologies, Santa Clara, CA) (Batey et al. 1991; Ohm et al. 2009). The SE-HPLC settings were as follows: injection volume,10 μL; eluting solution, 50 % acetonitrile in aqueous 0.1 % trifluroacetic acid solution; flow rate of 0.5 mL/min; and detection, UV 214 nm absorbance (Photodiode array detector, 1200, Agilent Technologies).

The SE-HPLC profiles were also divided into four fractions for flour, dough, and bread samples for both extractable and unextractable fractions. The retention time intervals are F1 (3.5–5.0 min), F2 (5.0-5.9 min), F3 (5.9–6.6 min), and F4 (6.6-7.8 min) for flour, dough, and bread

samples (Morel et al. 2000; Ohm et al. 2009; Park et al. 2006). Primary components constituting individual fractions were high molecular weight polymeric proteins for F1; low molecular weight polymeric proteins for F2; ω-gliadin for F3; γ-, β-, and α-gliadins for F3; and albumin and globulins for F4 (Larroque et al. 1997).

3.3.4. SE-HPLC data analysis

The SE-HPLC chromatograms were transformed and processed using an in-house program that was developed using MATLAB (version 6, The MathWorks, Natick, MA). UV absorbance values were interpolated to 0.002 min intervals by the 'spline' methods and then used to calculate absorbance area by mean absorbance × time interval (0.002 min). The sum of absorbance area (AA) for each retention time interval of 0.01 min between 3.0 and 8.0 min of run time are used for data analysis. The percentage of absorbance area (A%), for each 0.01 min time interval, was calculated by the % of absorbance area of each time interval over the sum of all the AAs within runtime, represent the same level of protein content. Simple linear correlation coefficients (r) were calculated between both absorbance area and A% values and quality parameters for each 0.01 min retention interval, and shown as a continuous spectrum over retention time (correllogram)

3.3.5. Statistical analysis

All of the HPLC experiments were done in duplicate and statistical analysis was performed using the SAS statistical methods (Version 9.3, SAS Institute; Cary, NC). The experimental design was randomized complete block design. Simple linear correlation coefficients were calculated using the CORR procedure in SAS. An analysis of variance (ANOVA) was performed to assess the effect of treatments on quality characteristics and treatment means were separated by Fisher's protected Least Significant Difference (LSD) test at P=0.05.

3.4. Results and Discussion

3.4.1. Effect of HRS wheat blends and additives on dough rheology and bread quality

Details of these results were discussed in chapter 2.

3.4.2. Protein molecular weight distribution of flour samples

All samples were subjected to protein molecular weight distribution (MWD) analysis. Primary components of each fraction (F1-F4) are known to be high molecular weight protein (HMW) polymeric protein for F1; low molecular weight (LMW) polymeric protein for F2; gliadins for F3; and albumins and globulins for F4 (Larroque et al., 1997; Morel et al., 2000; Ohm et al., 2009).

SE-HPLC absorbance area (AA) and area percent (A %) values of extractable and unextractable protein fractions in all the flours samples shown in Table 3.3. The AA values of all four protein fractions were significantly ($P<0.05$) higher in HRS wheat flours than HRW wheat flour. For extractable F1, there was no significant ($P<0.05$) difference in AA values of HRS 2, HRS 3, and HRS 4, nor was there any significant ($P<0.05$) difference in A% values of HRS 1, HRS 2, and HRS 3. For extractable F2, there was no significant ($P<0.05$) difference in AA value of HRS 3 and HRS 4, as well as there was no significant ($P<0.05$) difference in A% values among all of the samples, only HRS1 flour was significantly ($P<0.05$) lower. For extractable F3, HRS 2 flour showed significantly ($P<0.05$) higher AA value and HRS 3 flour showed significantly higher A% value than all other flour.

For unextractable F1, there was no significant difference ($P<0.05$) in AA values and A% of HRS 2 and HRS 3 flour; however, both of these two samples showed significantly ($P<0.05$) higher value than others. For F2, F3, and F4 fractions, all of the HRS flours showed significantly ($P<0.05$) higher value than HRW wheat flour; however, there was no significant difference

(P<0.05) in AA values and A % values among HRS wheat flours. These results are similar to a comparison study of HRS and HRW by Maghirang et al. (2006). They found higher insoluble polymeric protein, soluble polymeric protein, and gliadin content in HRS than HRW. Therefore, it can be concluded from Table 3.3 that HRS wheat flours contain a significantly (P<0.05) higher value for all of the protein fractions than HRW wheat flour, and with higher protein and higher AA value for all the protein fractions. A previous study also indicated that flour protein content had high correlations with AA values of protein fractions (Ohm 2009).

Table 3.2. SDS-Extractable and SDS-Unextractable protein fractions in flours[a]

	Absorbance Area (mAu*min)				Area percent (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
				Extractable				
Base flour	30.80	22.34	107.67	37.24	11.32	8.21	39.58	13.69
HRS1	36.91	30.89	156.90	42.88	9.63	8.06	40.95	11.19
HRS2	43.42	38.16	185.44	50.61	9.48	8.33	40.49	11.05
HRS3	41.48	35.92	160.21	45.40	9.97	8.63	38.51	10.91
HRS4	42.56	34.60	166.08	45.79	10.23	8.32	39.92	11.01
LSD	2.80	2.51	11.51	2.91	0.64	0.50	2.61	0.49
				Unextractable				
Base flour	31.89	21.52	15.22	5.42	11.72	7.91	5.59	1.99
HRS1	52.20	31.99	24.08	7.29	13.62	8.35	6.29	1.90
HRS2	69.02	36.88	26.31	8.23	15.07	8.05	5.75	1.79
HRS3	65.67	35.04	24.71	7.56	15.80	8.42	5.94	1.82
HRS4	57.31	34.70	27.02	8.16	13.76	8.33	6.49	1.96
LSD	6.53	7.12	8.90	2.60	0.97	1.16	1.82	0.54

[a]LSD=Least Significant Difference at P=0.05, HRS= Hard Red Spring Wheat flour, HRW = Hard Red Winter Wheat flour

3.4.3. Protein molecular weight distribution of dough samples

The biochemical basis for predicting flour dough strength was the proportion of SDS-insoluble polymeric protein in total protein or in total polymeric protein; however, the extractable polymeric proteins were associated with dough weakening characteristics (Gupta et al. 1993; Ciaffi et al. 1996; Bangur et al. 1997; Borneo and Khan 1999; Morel et al. 2000; Tsilo et al. 2010).

Moreover, the high molecular weight SDS-unextractable polymeric proteins had greater positive correlations with dough properties than other polymeric protein fractions (Ohm et al. 2010).

In this experiment, all of the 31 dough samples were subjected to protein molecular weight distribution (MWD) analysis. For the extractable protein fractions of dough samples, there was an increasing trend in AA values with increasing amount of HRS wheat flour blends for almost all of the HRS wheat flour blends. However, there was no significant ($P<0.05$) difference in AA and A % values within the blends for all of the HRS wheat flour blends. Almost all of the HRS wheat flour blends showed significantly ($P<0.05$) higher AA value then HRW wheat flour for all of the extractable protein fractions (Fig. 3.1).

For the unextractable protein fractions of dough samples, there was an increasing trend in AA values of F1 with increasing amount of all HRS wheat flour blends, except for 30% HRS 4. The 40% HRS2, 40% HRS3, and 40%HRS4 blends showed significantly ($P<0.05$) higher AA values of F1 than their respective other blends; however, there was no significant difference ($P<0.05$) in AA value of F2, F3 and F4 fractions among the HRS wheat flour blends. For the A% values of the F1 protein fractions, the 40% HRS2 and 40% HRS4 blends showed significantly ($P<0.05$) higher than 10% of their respective blends; however, there was no significant difference ($P<0.05$) in A% value of F2, F3 and F4 fractions among most of the HRS wheat flour blends (Figure 3.2). The F1 fraction is the high molecular weight polymeric protein which had greater positive correlations with dough properties than other polymeric protein fractions (Ohm et al 2010). The AA values of protein fractions had higher correlations with flour protein content (Ohm et al 2009) and the high protein content HRS wheat flour showed higher AA values of the F1 fraction. Therefore, the blending with high protein flour showed higher AA values of the F1 fraction.

In addition to HRS wheat flour blends, there was no significant ($P<0.05$) difference in the AA and A% value of extractable F1 among PB, ascorbic acid, and HRW wheat flour; however, only ADA showed significantly ($P<0.05$) higher value than other oxidants. In addition, there was no significant ($P<0.05$) difference in the AA and A% values of extractable F2, F3, and F4 between oxidants and HRW wheat flour. The AA and A% of unextractable F1 of oxidants showed very high value than HRW wheat. Although there was no significant ($P<0.05$) difference in the AA and A% value of unextractable F2, F3, and F4 among PB, ascorbic acid, and HRW wheat flour, ADA showed significantly ($P<0.05$) lower value than other the oxidants. Oxidizing agents promote disulfide bond formation between proteins. Intramolecular and intermolecular disulfide bonds between polypeptide chains make larger molecular aggregates and resulting protein network increase the molecular weight (Pyler and Gorton 2008). The effects of fast-acting ADA upon dough properties are during or just after mixing, the intermediate AA and the slow-acting PB effect after mixing in dough or in the oven (Pyler and Gorton 2008).

Besides oxidizing agents, there were no significant ($P<0.05$) differences in the AA and A% value of extractable fractions among the surfactants. However, both the AA and A% value of extractable F1 fractions of dough containing CS and EMG showed significantly ($P<0.05$) higher values than those of HRW wheat flour alone. Among the unextractable protein fractions, only DATEM showed significantly ($P<0.05$) higher value than HRW wheat flour for all the protein fractions, and CS showed significantly ($P<0.05$) higher value than HRW wheat flour for F1 protein fractions. SSL interact with gluten through ionic bonds due to it's high hydrophilic/lipophilic balance; however, DATEM interacts with hydrophobic domains of gluten due to it's low hydrophilic/lipophilic balance (Armero and Collar 1996). Surfactants generally react with protein causing gluten to become more elastic and extensible, and to strengthen the dough (Pyler and

Gorton 2008). Emulsifiers reduce the repulsing charges between gluten proteins; hence, help them to form an aggregate (Collar et al 1998).

Among other additives, dough containing fat showed lowest AA values for the extractable protein fractions whereas dough containing VWG showed the highest. For the A% values of all of the extractable protein fractions, there was no significant difference ($P<0.05$) within the blends, and even among almost all of the HRS wheat flour blends. The 30% and 40% blends of HRS 2, HRS 3, and HRS 4 showed better protein fraction value than all the additives without VWG. The ADA showed significantly lower value for most of the unextractable fractions whereas DATEM showed highest, but that value was lower than 40% blends of HRS 2, HRS 3 and HRS 4 flour. Therefore, HRS wheat flour blends showed better unextractable protein fractions value than commercial additives.

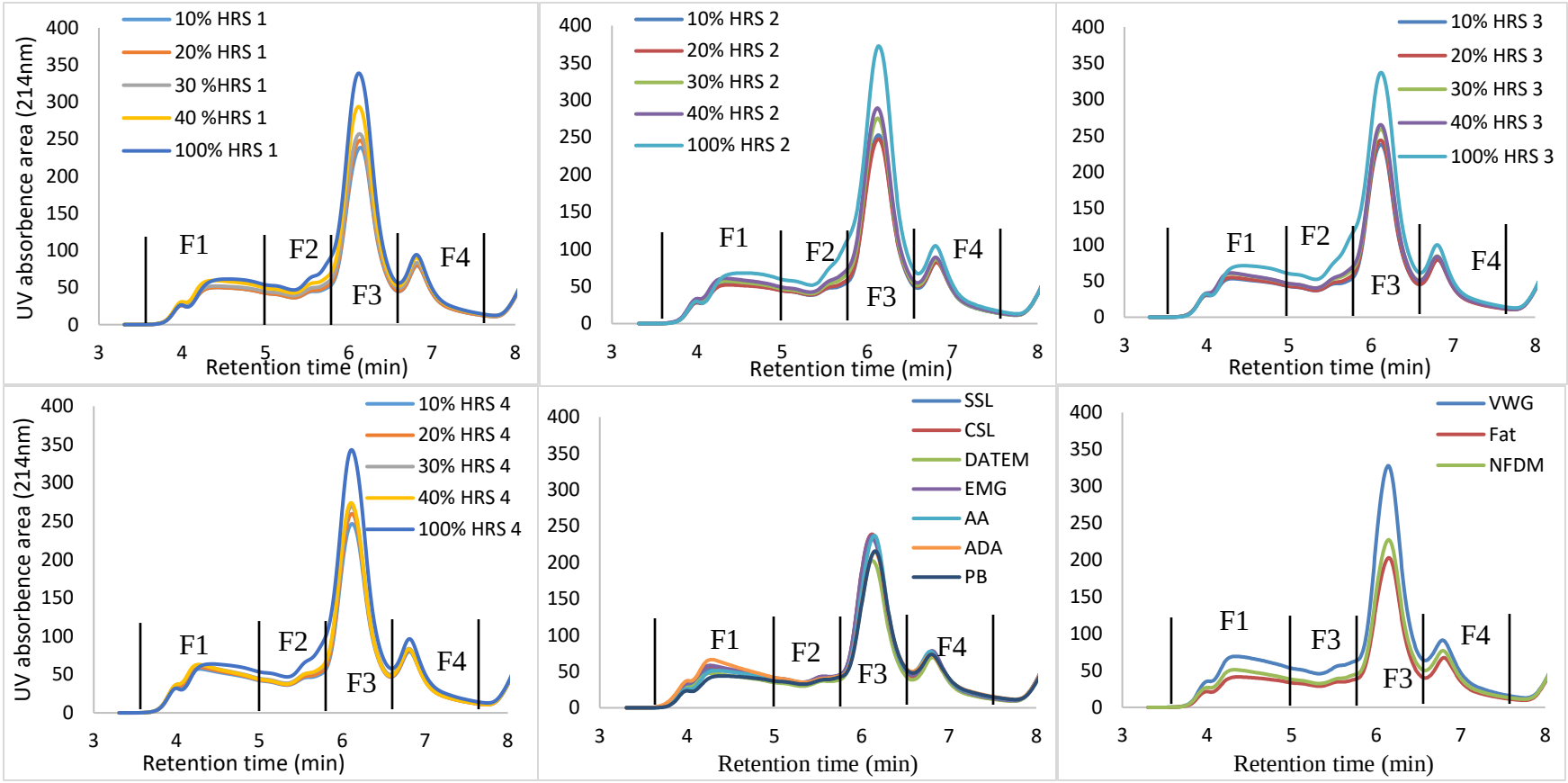

Fig. 3.1. Size Exclusion HPLC profile of protein extracted from dough: Extractable protein of dough made of HRS wheat flour blends and additives

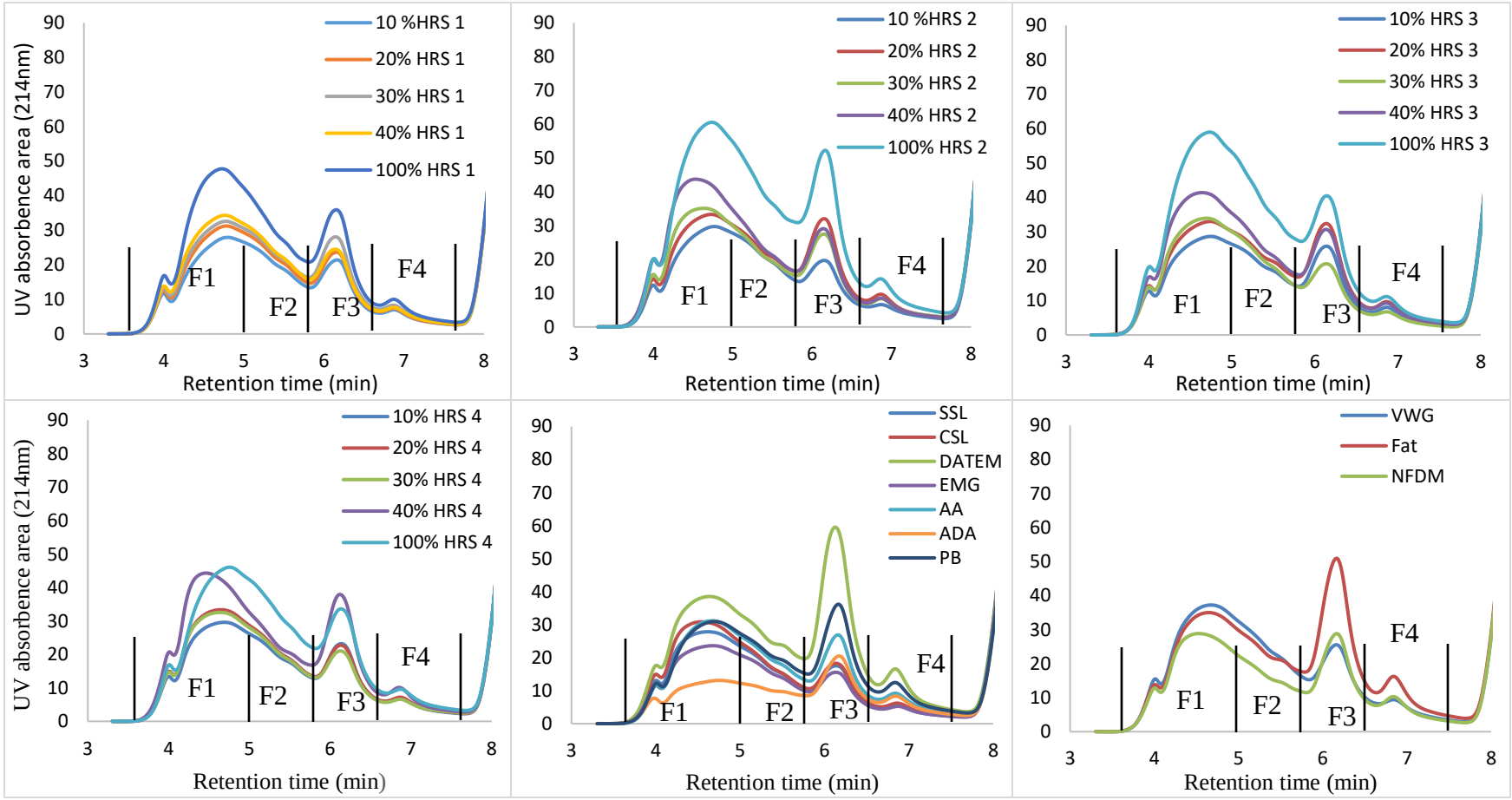

Fig. 3.2. Size Exclusion HPLC profile of protein extracted from dough: Unxetractable protein of dough made of HRS wheat flour blends and additives

3.4.4. Protein molecular weight distribution of bread samples

The molecular weight distribution of proteins extracted from bread crumb made of HRS wheat flour blend and additives were analyzed. SDS-unextractable high molecular weight polymeric proteins had positive correlations with breadmaking parameters (Ohm et al 2010). Although HMW-GS are minor components in terms of quantity, they are major determinants of gluten elasticity and functionality and hence are key factors in the process of bread making (Gianibelli et al. 2001).

For the extractable protein fractions of bread samples, there were no significant ($P<0.05$) differences ($P<0.05$) in AA and A% values for almost all of the HRS wheat flour blends or additives (Figure 3.3). The AA and A% values of extractable F1 of HRW wheat flour bread showed significantly ($P<0.05$) higher value than all of the HRS wheat blends. However, AA value of unextractable F1 showed significantly ($P<0.05$) higher value for 10% and 30% of HRS 2, all the HRS3 blend as well as 30% and 40% of HRS 4 blends than HRW wheat flour. For the AA and A% values of extractable F2, F3, and F4, there was no significant ($P<0.05$) difference between 30% and 40% of HRS wheat flour blends for all the blends, besides there was no significant ($P<0.05$) difference in AA and A% value of unextractable F2, F3 and F4 within the blends for all the HRS blends (Figure 3.4). The HRS wheat flour showed higher protein content than HRW flour and blending of HRW wheat flour with high protein HRS wheat flour also increase the protein content of the blends. Similar findings were found in a previous study where the bread with high protein flour showed higher AA values of F1 fraction because the AA values of protein fractions had high correlations with flour protein content (Ohm et al 2010).

In addition to HRS wheat flour blends, the AA and A% value of extractable F1 of the bread made from surfactants showed significantly ($P<0.05$) lower than HRW wheat flour bread.

However, there was no significant difference in the AA and A % among the extractable F2, F2, and F4. Only AA value of extractable F3 and F4 of the bread made from SS and CS showed significantly ($P<0.05$) higher value than HRW flour bread. The bread containing CS and SS showed significantly ($P<0.05$) higher AA and A% value of unextractable F1 and F2 than HRW wheat flour, EMG, and DATEM. DATEM interacts with gluten hydrophobic domains and SSL interact with gluten through ionic bonds (Armero and Collar 1996). Surfactants react with gluten matrix become more elastic and more extensible to strengthen the dough, and form a clathrate with amylose thus limiting swelling of starch and amylose leaching (Pyler and Gorton 2008). The interaction of surfactants due to the high hydrophilic/lipophilic balance, which eventually promote gluten aggregation and form hydrogen bonds with glutamine (Hahnel et al 1995).

Besides oxidants, the AA and A% values of extractable F1 of bread made with ascorbic acid showed significantly ($P<0.05$) lower than HRW wheat flour bread; however, there was no significant ($P<0.05$) difference among other extractable protein fractions. Bread of PB showed significantly ($P<0.05$) higher AA value of unextractable F1, F2 and F3 than HRW wheat flour whereas ascorbic acid was not significantly ($P<0.05$) different than HRW wheat flour. A previous study explained that PB changed the molecular weight distribution and built an extra-large glutenin aggregate (Panozzo et al 1994). In addition, bread made of ADA showed extremely high value for all the extractable protein fractions and extremely lower value for unextractable protein fractions. The oxidants facilitates the interchange between thiols and disulfide bonds in a protein, inhibit the protease enzyme, oxidize thiols group, and affect protein aggregation which in turn affect molecular weight distribution and improve dough rheology and bread quality (Pyler and Gorton 2008).

Among other additives, only bread of VWG showed the significantly higher value of unextractable F1 than HRW flour bread and all other unextractable protein fractions of bread with additives showed a higher value than HRW wheat flour bread. VWG contains 72.5% protein and provides the extra gluten to low-gluten content flours (Ortolan and Steel 2017; Pyler and Gorton 2008). Milk protein, casein improved water absorption and interaction with gluten (Pyler and Gorton 2008) and in this study NFDM showed better protein fraction value than fat and significantly ($P<0.05$) higher than HRW wheat flour.

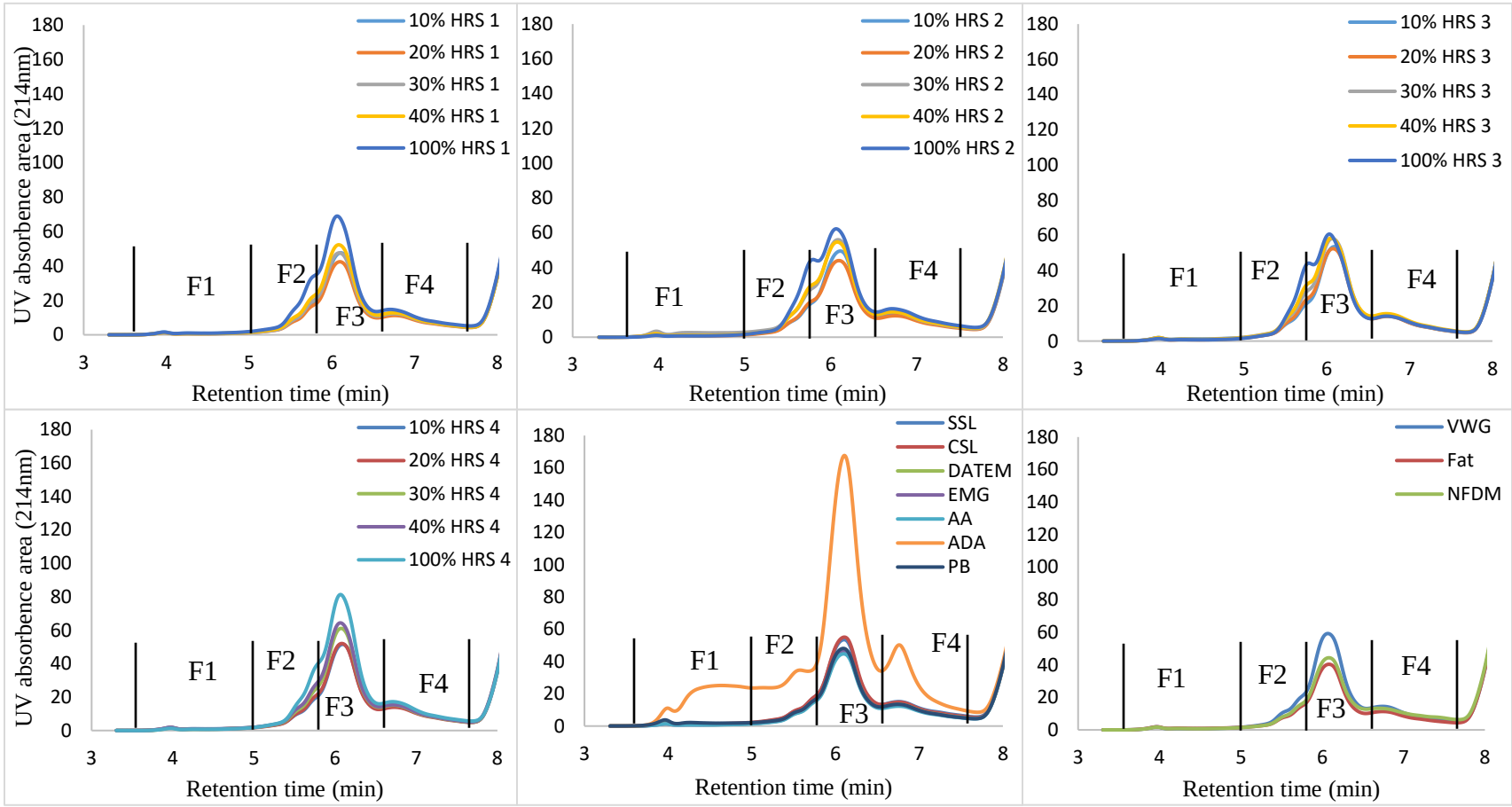

Fig. 3.3. Size Exclusion HPLC profile of protein extracted from bread: Extractable protein of bread made of HRS wheat flour blends additives

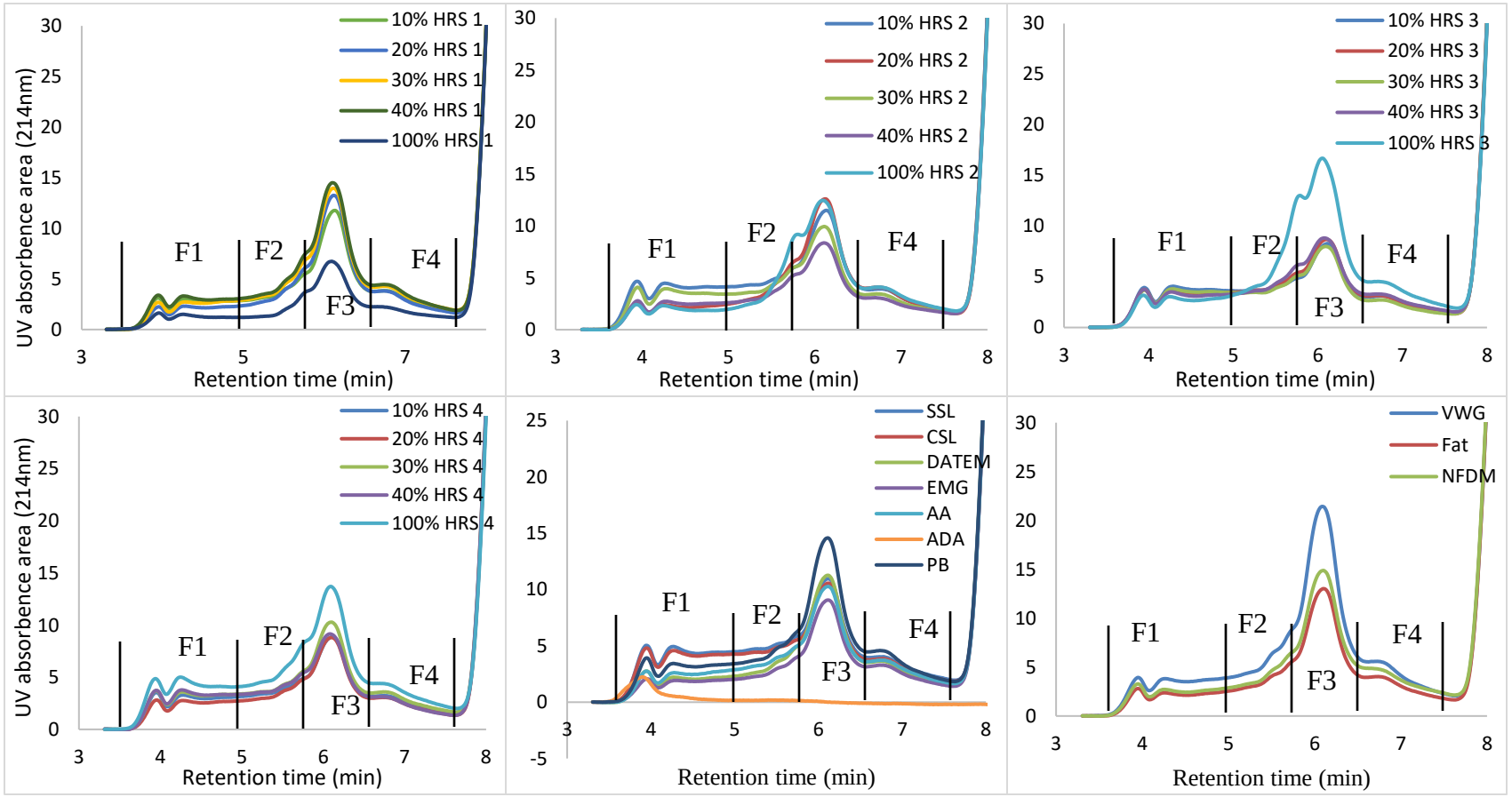

Fig. 3.4. Size Exclusion HPLC profile of protein extracted from bread: Unextractable protein of bread made of HRS wheat flour blends and additives

3.4.5. Correlation between dough protein fractions and dough characteristics

The SDS-unextractable polymeric proteins are positively associated dough strength; however, the extractable polymeric proteins were associated with dough weakening characteristics (Gupta et al 1993; Ciaffi et al 1996; Bangur et al 1997; Borneo and Khan 1999; Morel et al 2000; Tsilo et al 2010). The high molecular weight SDS-unextractable polymeric proteins had greater positive correlations with dough properties than other polymeric protein fractions. The unextractable high molecular weight polymeric protein had a positive effect on mixing time, and bread loaf volume whereas a high proportion of extractable polymeric protein had negative effects on mixing time and bread loaf volume (Ohm et al 2010).

For the HRS wheat flour blends in our experiment, all the farinograph parameters showed significant ($P>0.05$) correlation with the dough protein content. Absorbance area of all four extractable and unextractable protein fractions showed significant ($P>0.05$) as well as positive correlation with all the farinograph parameters without the mixing tolerance index. The AA of extractable F3 showed highest positive correlation with farinograph water absorption; however, AA and A% values of the extractable F3 section had significant ($P < 0.05$) and positive correlation with farinograph peak time. In a previous study, farinograph water absorption was significantly associated with gliadins in HRS wheat flour (Ohm et al 2009; 2010).

In addition, A% of extractable F1 and F4 showed significant ($P>0.05$) and negative correlation with farinograph absorption, stability, and mixing tolerance. However, A% of unextractable F1 showed significant ($P>0.05$) and positive correlation with all farinograph parameters without the mixing tolerance index and unextractable F3 showed significant ($P>0.05$) correlation with absorption, stability, and a quality number. Only AA value of unextractable F4 showed desirable significant ($P>0.05$) and negative correlation with mixing tolerance index.

Besides farinograph parameters, the AA value of all four extractable and unextractable protein fractions showed significant (P>0.05) and positive correlation with all the extensograph parameters without the extensibility at 135 mins. The A % value of extractable F1 showed significant (P>0.05) and negative correlation with all the extensograph parameters without the resistance at 135; however, unextractable F1 fraction showed significant (P>0.05) and positive correlation with all the extensograph parameters without the extensibility at 135 mins. The AA value of extractable F4 significant (P>0.05) and negative correlation with all the extensograph parameters without the extensibility at 135 mins; however, extractable F2 value showed significant (P>0.05) and positive correlation with all the extensograph parameters without the resistance at 135 mins (Table 3.3). For the additives, only AA of extractable protein fractions showed significant (P>0.05) and positive correlation with dough protein content, farinograph absorption, stability, and peak time. As well as there was no other significant (P>0.05) correlation with any other rheological characteristics.

3.4.6. Correlation between bread protein fractions and bread quality characteristics

For the baking quality characteristics, all the baking parameters of HRS wheat flour blends showed significant (P>0.05) correlation with the bread protein content excepting BDS. However, none of the baking parameters showed significant (P>0.05) and positive correlation with the AA of the extractable protein fractions. Both the AA and A% value of the extractable F2 showed significant (P>0.05), strong, and positive correlation with BAB, BMT, BLV, BSV, BSY, and BCC whereas significant (P>0.05), strong, and negative correlation with BFM. The A% value of the extractable F4 showed significant (P>0.05) and negative correlation with most of the baking parameters; however, AA and A% value of the unextractable F4 did not show any significant (P>0.05) and positive correlation (Table 3.4). For the additives, there was no positive and

significant (P>0.05) relationship between baking parameters and extractable protein fractions; however, only BAB showed positive and significant (P>0.05) with bread protein content. The AA and A% of the extractable F2, F3 and F4 showed significant (P>0.05) correlation with most of the baking parameters (Table 3.5).

Table 3.3. Correlation between dough protein fractions and characteristics of dough made of HRS wheat flour blends [a]

Protein fractions	Farinograph parameters					Extensograph parameters					
	FAB	FPT	FST	MTI	FQN	EXT045	ERS045	EAR045	EXT135	ERS135	EAR135
DPC	0.924***	0.608**	0.926***	-0.428*	0.968***	0.898***	0.681***	0.89***	0.402^{NS}	0.662***	0.956***
Extractable											
F1 (AA)	0.774***	0.706***	0.826***	-0.065^{NS}	0.822***	0.616**	0.714***	0.776***	0.033NS	0.784***	0.778***
(A%)	-0.707***	-0.348^{NS}	-0.625**	0.546**	-0.711***	-0.781***	-0.453*	-0.681***	-0.493*	-0.360^{NS}	-0.724***
F2 (AA)	0.913***	0.623**	0.927***	-0.361^{NS}	0.940***	0.870***	0.727***	0.913***	0.366^{NS}	0.695***	0.962***
(A%)	0.028NS	-0.176^{NS}	0.164^{NS}	-0.119^{NS}	-0.011^{NS}	0.189^{NS}	0.041^{NS}	0.144^{NS}	0.404^{NS}	-0.126^{NS}	0.235^{NS}
F3 (AA)	0.941***	0.706***	0.887***	-0.320^{NS}	0.959***	0.863***	0.771***	0.936***	0.281^{NS}	0.745***	0.931***
(A%)	0.336^{NS}	0.357^{NS}	0.158^{NS}	0.052^{NS}	0.246^{NS}	0.309^{NS}	0.383^{NS}	0.423*	0.056^{NS}	0.243^{NS}	0.255^{NS}
F4 (AA)	0.901***	0.619**	0.837***	-0.372^{NS}	0.908***	0.837***	0.719***	0.895***	0.353^{NS}	0.672***	0.921***
(A%)	-0.841***	-0.73***	-0.889***	0.215^{NS}	-0.919***	-0.751***	-0.697***	-0.815***	-0.128^{NS}	-0.759***	-0.821***
Unextractable											
F1 (AA)	0.849***	0.645**	0.893***	-0.318^{NS}	0.926***	0.779***	0.672***	0.819***	0.214^{NS}	0.739***	0.879***
(A%)	0.557**	0.529*	0.677***	-0.174^{NS}	0.681***	0.494*	0.457*	0.525*	0.015^{NS}	0.603**	0.579**
F2 (AA)	0.909***	0.567**	0.895***	-0.410^{NS}	0.932***	0.867***	0.672***	0.869***	0.391NS	0.644**	0.936***
(A%)	0.723***	0.362^{NS}	0.733***	-0.401^{NS}	0.730***	0.741***	0.489*	0.683***	0.446*	0.417^{NS}	0.767***
F3 (AA)	0.818***	0.484*	0.77***	-0.493*	0.872***	0.786***	0.535*	0.727***	0.353^{NS}	0.555**	0.816***
(A%)	0.395^{NS}	0.112^{NS}	0.357^{NS}	-0.494*	0.454*	0.421^{NS}	0.123^{NS}	0.266^{NS}	0.288^{NS}	0.153^{NS}	0.368^{NS}
F4 (AA)	0.774***	0.375^{NS}	0.696***	-0.592**	0.815***	0.775***	0.440*	0.657***	0.429*	0.445*	0.771***
(A%)	-0.076^{NS}	-0.378^{NS}	-0.157^{NS}	-0.498*	-0.072^{NS}	0.033^{NS}	-0.344^{NS}	-0.23NS	0.291^{NS}	-0.345^{NS}	-0.104^{NS}

[a]FAB= Farinograph water absorption, FPT= Farinograph Peak Time, FST= Farinograph Stability, FQN=Farinograph Quality Number, EXT 045= Extensograph Extensiblity at 45 mins, ESR 045 = Extensograph Resistance at 45 mins , EAR 045 = Extensograph Area at 45 mins , EXT 135= Extensograph Extensiblity at 135 mins, ESR 135 = Extensograph Resistance at 135 mins , EAR 135 = Extensograph Area at 135 mins.

Table 3.4. Correlation between bread protein fractions and baking characteristics of bread made of HRS wheat flour blends

Protein fractions	BAB	MT	DS	OS	LV	SV	SY	CRC	CT	CBC	FM
BPC	0.96***	0.867***	-0.261NS	0.863***	0.929***	0.899***	0.88***	0.649**	0.756***	0.938***	-0.941***
Extractable											
F1 (AA)	-0.393^{NS}	-0.508*	-0.141^{NS}	-0.386^{NS}	-0.403^{NS}	-0.392^{NS}	-0.463*	-0.520*	-0.457*	-0.180^{NS}	0.385^{NS}
(A%)	-0.530*	-0.662***	-0.187^{NS}	-0.559**	-0.572**	-0.553**	-0.659***	-0.721***	-0.669***	-0.377^{NS}	0.582**
F2 (AA)	0.825***	0.744***	-0.153^{NS}	0.756***	0.783***	0.753***	0.754***	0.518*	0.698***	0.875***	-0.807***
(A%)	0.651**	0.522*	-0.066^{NS}	0.637**	0.613**	0.598**	0.565**	0.331^{NS}	0.470*	0.783***	-0.614**
F3 (AA)	0.801***	0.793***	-0.095^{NS}	0.744***	0.754***	0.717***	0.770***	0.578**	0.695***	0.775***	-0.787***
(A%)	0.588**	0.603**	0.139^{NS}	0.658***	0.554**	0.535*	0.588**	0.452*	0.408^{NS}	0.610**	-0.560**
F4 (AA)	0.586**	0.515*	-0.122^{NS}	0.459*	0.488*	0.429*	0.546**	0.385NS	0.697***	0.598**	-0.546**
(A%)	-0.62**	-0.703***	0.061^{NS}	-0.635**	-0.706***	-0.713***	-0.662***	-0.600**	-0.479*	-0.555**	0.719***
Unextractable											
F1 (AA)	-0.160^{NS}	-0.046^{NS}	0.176^{NS}	-0.083^{NS}	-0.066^{NS}	-0.07^{NS}	0.002^{NS}	0.109^{NS}	0.198^{NS}	-0.122^{NS}	-0.014^{NS}
(A%)	-0.583**	-0.448*	0.305^{NS}	-0.434*	-0.456*	-0.44*	-0.387^{NS}	-0.164^{NS}	-0.161^{NS}	-0.516*	0.381^{NS}
F2 (AA)	0.206^{NS}	0.280^{NS}	-0.075^{NS}	0.155^{NS}	0.253^{NS}	0.243^{NS}	0.259^{NS}	0.308^{NS}	0.431*	0.148^{NS}	-0.306^{NS}
(A%)	-0.312^{NS}	-0.199^{NS}	0.088^{NS}	-0.265^{NS}	-0.205^{NS}	-0.189^{NS}	-0.182^{NS}	0.029^{NS}	0.017^{NS}	-0.346^{NS}	0.164^{NS}
F3 (AA)	0.387^{NS}	0.391^{NS}	-0.344^{NS}	0.187^{NS}	0.362^{NS}	0.350^{NS}	0.31^{NS}	0.265^{NS}	0.329^{NS}	0.209^{NS}	-0.352^{NS}
(A%)	-0.031^{NS}	-0.004^{NS}	-0.209^{NS}	-0.146^{NS}	-0.009^{NS}	0^{NS}	-0.045^{NS}	0.020^{NS}	-0.043^{NS}	-0.188^{NS}	0.045^{NS}
F4 (AA)	0.225^{NS}	0.234^{NS}	-0.326^{NS}	0.023^{NS}	0.198^{NS}	0.179^{NS}	0.167^{NS}	0.165^{NS}	0.264^{NS}	0.047^{NS}	-0.203^{NS}
(A%)	-0.315^{NS}	-0.291^{NS}	-0.172^{NS}	-0.419^{NS}	-0.300^{NS}	-0.290^{NS}	-0.325^{NS}	-0.206^{NS}	-0.247^{NS}	-0.459*	0.330^{NS}

[a]BAB= Baking Absorption, MT= Mixing Time, DS= Dough Score, OS=Oven Spring, LV= Loaf Volume, SV=Specific Volume, SY=Symmetry, CRC=Crust Color, CT=Crumb Texture, CBC=Crumb Color, FM=Firmness, BPC= Bread Protein content.

Table 3.5. Correlation between bread protein fractions and baking characteristics of bread made of additives[a]

Protein fractions	BAB	MT	DS	OS	LV	SV	SY	CRC	CT	CBC	FM
BPC	0.834**	0.168^{NS}	0.009^{NS}	0.255^{NS}	0.389^{NS}	0.228^{NS}	0.349^{NS}	0.341^{NS}	0.265^{NS}	0.042^{NS}	0.002^{NS}
Extractable											
F1 (AA)	-0.392^{NS}	-0.48^{NS}	-0.942***	-0.755**	-0.783**	-0.824**	-0.812**	-0.792**	-0.887***	-0.978***	0.987***
(A%)	-0.484^{NS}	-0.544^{NS}	-0.945***	-0.82**	-0.856***	-0.883***	-0.856***	-0.864***	-0.940***	-0.949***	0.958***
F2 (AA)	-0.317^{NS}	-0.422^{NS}	-0.918***	-0.720*	-0.732*	-0.783**	-0.773**	-0.751**	-0.851***	-0.958***	0.970
(A%)	-0.44^{NS}	-0.528^{NS}	-0.947***	-0.763**	-0.805**	-0.832**	-0.857***	-0.826**	-0.945***	-0.969***	0.965***
F3 (AA)	-0.249^{NS}	-0.351^{NS}	-0.881***	-0.678*	-0.68*	-0.739**	-0.728*	-0.710*	-0.807**	-0.929***	0.946***
(A%)	-0.358^{NS}	-0.361^{NS}	-0.711*	-0.433^{NS}	-0.557^{NS}	-0.535^{NS}	-0.720*	-0.597^{NS}	-0.793**	-0.817**	0.780**
F4 (AA)	-0.299^{NS}	-0.364^{NS}	-0.888***	-0.724*	-0.703*	-0.756**	-0.733*	-0.719*	-0.803**	-0.935***	0.951***
(A%)	-0.554^{NS}	-0.316^{NS}	-0.021^{NS}	-0.159^{NS}	-0.163^{NS}	-0.034^{NS}	-0.176N^{S}	-0.043^{NS}	-0.112^{NS}	-0.075^{NS}	-0.002^{NS}
Unextractable											
F1 (AA)	0.357^{NS}	0.794**	0.607*	0.246^{NS}	0.398^{NS}	0.360^{NS}	0.493^{NS}	0.281^{NS}	0.524^{NS}	0.651*	-0.572^{NS}
(A%)	0.25^{NS}	0.737**	0.788**	0.392^{NS}	0.486^{NS}	0.497^{NS}	0.581^{NS}	0.415^{NS}	0.656*	0.826**	-0.77**
F2 (AA)	0.598^{NS}	0.720*	0.795**	0.551^{NS}	0.690*	0.641*	0.767**	0.629*	0.812**	0.844**	-0.784**
(A%)	0.454^{NS}	0.63*	0.895***	0.640*	0.718*	0.714*	0.790**	0.702*	0.878***	0.930***	-0.894***
F3 (AA)	0.795**	0.49^{NS}	0.736**	0.692*	0.815**	0.737**	0.880***	0.802**	0.894***	0.812**	-0.775**
(A%)	0.635*	0.376^{NS}	0.801**	0.765**	0.825**	0.793**	0.886***	0.855***	0.937***	0.866***	-0.859***
F4 (AA)	0.625*	0.591^{NS}	0.865***	0.690*	0.812**	0.78**	0.890***	0.808**	0.946***	0.927***	-0.891***
(A%)	0.424^{NS}	0.422^{NS}	0.892***	0.731*	0.782**	0.802**	0.846**	0.828**	0.936***	0.94***	-0.936***

[a]BAB= Baking Absorption, MT= Mixing Time, DS= Dough Score, OS=Oven Spring, LV= Loaf Volume, SV=Specific Volume, SY=Symmetry, CRC=Crust Color, CT=Crumb Texture, CBC=Crumb Color, FM=Firmness, BPC= Bread Protein content.

3.5. Conclusions

In this study, the molecular weight distribution (MWD) of protein extracted from dough and bread was measured to compare the correlation between the MWD of dough protein with dough characteristics as well as the MWD of bread protein with bread quality characteristics. For both the dough and bread properties, HRS flour blends as had better characteristics in comparison with commercial strengtheners. Almost all the HRS flour blends showed significantly ($P<0.05$) higher correlations with dough rheological charaterisctis. In addition, the highest loaf volume and the lowest firmness was shown by 40% Linkert followed by 40% Glenn. Doughs of both 30% and 40% blends of Glenn, Linkert, and one of commercial flour showed better absorbance area and area percent values of extractable and unextractable protein fractions than all the additives. It is widely accepted that the unextractable polymeric proteins were positively associated dough strength and gliadin associated with water absorption. The absorbance area value of unextractable high molecular weight polymeric protein showed significantly ($P<0.05$) higher value for 10% and 30% of Glenn, all the Linkert blends, 30% and 40% of commercial 2 blends than HRW wheat flour. Absorbance area of all four extractable and unextractable protein fractions showed significant ($P<0.05$) and positive correlation with all the farinograph parameters without the mixing tolerance index. The absorbance area and area percent of the extractable low molecular weight protein showed significant ($P<0.05$), strong, and positive correlation with most of the bread quality parameters. However, protein fractions of bread made with additives did not show positive and significant ($P<0.05$) relationship between baking parameters. Therefore, it can be concluded that molecular weight distribution (MWD) of protein extracted HRS wheat flour blends are more closely associated with dough rheology and bread quality characteristics than commercial additives.

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CHAPTER 4. OVERALL CONCLUSIONS

In this study, different percentages of four hard red spring (HRS) wheat flours were used to replace commercial dough strengtheners as well as ten commercially available dough additives also used to compare their strengthening ability with HRS flour blends. Almost all the HRS flour blends showed significantly ($P<0.05$) higher farinograph absorption, peak time, and stability than commercial dough strengtheners, excepting vital wheat gluten. There were positive and significant ($P<0.05$) correlations among almost all the parameters of farinograph and extensogrph for the HRS flour blends whereas there was no significant ($P<0.05$) relationship among the farinograph and extensogrph parameters for the chemical dough strengtheners. The 30% and 40% of Linkert as well as the 30% and 40% Glenn flour blends showed much higher absorption than the surfactants and oxidants as well as higher loaf volume and lower firmness than all the commercial dough strengtheners. The highest loaf volume and lowest firmness were shown by 40% Linkert followed by 40% Glenn. For the crust and crumb color, almost all the blends showed better value than available dough additives as well as all the 30% and 40% of HRS blends showed better symmetry and texture than their respective 10% and 20% blends. For the commercial dough strengtheners, there was no significant ($P<0.05$) correlation between the dough quality characteristics and baking quality characteristics whereas HRS showed significant ($P<0.05$) correlation between almost all the dough quality characteristics and baking quality characteristics. Doughs of both 30% and 40% blends of Glenn, Linkert and one commercial flour showed better absorbance area and area percent values of extractable and unextractable protein fractions than all the additives. The absorbance area value of unextractable high molecular weight polymeric protein showed significantly ($P<0.05$) higher value for 10% and 30% of Glenn, all the Linkert blends, and 30% and 40% of commercial 2 blends than HRW wheat flour. Absorbance area of all four extractable and

unextractable protein fractions showed significant ($P<0.05$) and positive correlation with all the farinograph parameters excepting the mixing tolerance index. The absorbance area and area percent of the extractable low molecular weight protein showed significant ($P<0.05$), strong, and positive correlation with most of the bread quality parameters. However, protein fractions of bread made with additives hadn't shown positive and significant ($P<0.05$) relationship between baking parameters. It is obvious form this study that HRS flour blends as replacement for dough strengtheners had better characteristics compared to commercial additives. Moreover, molecular weight distribution (MWD) of protein extracted HRS wheat flour blends are more closely associated with dough rheology and bread quality characteristics than commercial additives. Therefore, it can be concluded that the addition of 40 % HRS wheat flour to HRW wheat flour can be used as a replacement for almost all of the dough strengtheners in bread production.

CHAPTER 5. FUTURE RESEARCH

In this study, dough strengthening ability of HRS flour blends were compared to ten commercially available dough additives. However, bakers use more than one or a combination of dough strengtheners' in bread formulation. In the future, it would be interesting to compare dough strengthening ability of HRS flour blends with commercially available combination of the dough strengtheners. Besides, a commercial flour baking company could have been partnered in this study to show variation in dough and breadmaking quality. This would be very interesting to compare whether "lab scale" baking differ from a "commercial scale" baking. In addition, a careful cost analysis also would be done in order to compare the price of clean label bread with commercially available bread.

APPENDIX

Table A.1. Extractable protein fractions of dough made from HRS blends

	Absorbance Area (mAu*min)				Area Percent (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
HRW	41.72	26.99	101.87	39.19	15.87	10.28	38.75	14.93
HRS1 / HRW								
100/0	55.84	38.88	159.38	46.80	14.29	9.95	40.79	11.98
10/90	47.57	28.76	111.48	39.69	16.74	10.12	39.23	13.97
20/80	47.48	29.11	115.88	39.97	16.11	9.87	39.31	13.56
30/70	49.78	31.11	121.30	42.70	15.94	9.97	38.84	13.68
40/60	56.42	34.54	136.80	44.93	16.57	10.15	40.19	13.20
HRS 2 / HRW								
100/0	62.17	44.28	178.67	51.75	13.62	9.70	39.15	11.34
10/90	50.62	30.40	118.24	41.63	16.91	10.16	39.50	13.91
20/80	50.20	30.91	117.71	42.26	16.05	9.88	37.66	13.51
30/70	54.27	32.93	129.35	42.79	16.49	10.01	39.31	13.00
40/60	58.01	34.96	136.27	44.42	16.40	9.88	38.52	12.56
HRS 3 / HRW								
100/0	65.24	45.09	165.12	49.05	15.01	10.37	37.99	11.28
10/90	50.77	29.27	111.41	39.69	17.37	10.02	38.14	13.59
20/80	51.98	29.88	114.32	39.15	16.94	9.74	37.26	12.76
30/70	56.28	32.78	122.43	42.50	17.70	10.31	38.49	13.36
40/60	56.82	33.23	124.92	41.41	16.89	9.88	37.14	12.31
HRS 4 / HRW								
100/0	59.83	39.42	160.93	48.32	15.05	9.92	40.49	12.15
10/90	53.28	28.95	113.90	39.94	17.99	9.78	38.46	13.50
20/80	55.03	29.70	119.01	40.29	17.85	9.63	38.62	13.07
30/70	57.90	31.39	124.19	41.77	18.23	9.88	39.12	13.15
40/60	57.62	30.93	125.14	40.79	17.04	9.14	37.01	12.06

Table A.1. Extractable protein fractions of dough made from HRS blends (Continued)

	Absorbance Area (mAu*min)				Area Percent (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
100% HRW + additives								
50 ppm SS	49.85	27.49	106.70	38.42	18.11	9.98	38.77	13.95
50 ppm CS	53.26	27.08	107.89	36.88	18.90	9.61	38.28	13.08
25 ppm DATEM	44.37	23.51	92.67	34.08	15.32	8.12	31.99	11.79
0.50% EMG	53.92	27.72	108.31	38.32	19.61	10.08	39.38	13.93
150 ppm AA	47.31	25.93	104.83	39.58	16.91	9.26	37.45	14.13
30 ppm ADA	58.50	27.06	98.78	39.84	22.40	10.36	37.85	15.24
10 ppm PB	41.45	25.17	98.62	39.30	15.12	9.18	35.99	14.33
5% VWG	63.61	36.09	145.82	47.60	17.41	9.87	39.91	13.02
2% Fat	38.65	22.51	90.66	34.34	14.38	8.38	33.73	12.78
2% NFDM	46.84	25.46	103.36	39.46	17.05	9.26	37.62	14.36
LSD($p<0.05$)	8.48	4.66	17.47	5.44	2.32	1.25	4.68	1.44

Table A.2. Unextractable protein fractions of dough made from HRS blends

	Absorbance Area (mAu*min)				Area Percent (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
HRW	19.60	13.86	13.87	5.63	7.46	5.28	5.30	2.15
HRS1 / HRW								
100/0	38.95	22.08	21.37	7.30	9.98	5.64	5.48	1.87
10/90	23.06	14.84	13.37	5.36	8.12	5.23	4.71	1.89
20/80	25.85	16.25	14.67	5.63	8.77	5.51	4.97	1.91
30/70	27.19	17.06	16.93	6.13	8.71	5.47	5.43	1.97
40/60	28.99	17.51	15.36	5.88	8.52	5.14	4.51	1.73
HRS 2 / HRW								
100/0	49.27	29.53	30.73	9.89	10.80	6.48	6.75	2.17
10/90	25.22	15.32	12.72	5.19	8.42	5.12	4.25	1.73
20/80	29.08	16.50	18.50	6.66	9.39	5.31	6.02	2.16
30/70	31.48	15.80	16.21	6.20	9.57	4.80	4.93	1.89
40/60	39.31	17.62	17.13	6.17	11.10	4.97	4.83	1.74
HRS 3 / HRW								
100/0	48.66	28.35	25.06	8.03	11.20	6.52	5.77	1.85
10/90	25.30	14.72	15.21	5.88	8.65	5.03	5.19	2.01
20/80	29.10	16.81	18.69	6.75	9.50	5.49	6.11	2.20
30/70	29.95	16.01	13.05	5.08	9.41	5.03	4.10	1.60
40/60	36.66	18.69	18.06	6.65	10.89	5.55	5.36	1.97
HRS 4 / HRW								
100/0	38.06	23.02	20.77	7.15	9.58	5.79	5.22	1.80
10/90	26.66	14.13	13.67	5.08	9.04	4.81	4.67	1.73
20/80	30.04	15.14	13.67	5.24	9.76	4.92	4.44	1.70
30/70	29.47	14.83	12.88	5.07	9.30	4.67	4.05	1.60
40/60	39.86	16.71	20.52	6.66	11.80	4.94	6.06	1.97
100% HRW + DS								
50 ppm SS	25.50	12.33	10.71	4.28	9.28	4.47	3.88	1.55
50 ppm CS	28.47	12.63	11.03	4.63	10.10	4.48	3.91	1.64
25 ppm DATEM	35.24	18.36	30.12	9.88	12.31	6.41	10.60	3.47
0.50% EMG	21.91	11.19	9.60	4.06	7.97	4.07	3.49	1.47
150 ppm AA	26.47	14.24	14.98	6.46	9.47	5.10	5.36	2.31
30 ppm ADA	12.42	7.32	11.33	5.71	4.79	2.81	4.36	2.20
10 ppm PB	26.54	15.10	19.49	8.06	9.72	5.53	7.17	2.96
5% VWG	32.40	17.43	15.67	6.77	8.87	4.77	4.29	1.86
2% Fat	30.33	16.41	25.93	10.03	11.30	6.09	9.62	3.72
2% NFDM	25.57	11.72	15.43	6.61	9.36	4.27	5.66	2.42
LSD($p<0.05$)	7.10	4.30	12.38	3.59	2.63	1.48	4.37	1.29

Table A.3. Extractable protein fractions of bread crumb made from HRS blends

	Absorbance Area (mAu*min)				Area Percent (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
HRW	3.08	3.81	20.57	9.21	6.92	8.21	43.67	19.56
HRS1 / HRW								
100/0	1.34	6.03	34.91	10.95	2.18	9.74	56.29	17.66
10/90	1.25	3.63	24.25	9.66	2.29	6.65	44.43	17.70
20/80	0.98	3.34	21.84	8.79	1.95	6.65	43.51	17.46
30/70	0.92	3.66	24.51	9.37	1.66	6.54	43.87	16.75
40/60	1.06	4.20	26.84	9.72	1.77	6.96	44.48	16.10
HRS 2 / HRW								
100/0	1.05	6.81	35.16	12.30	1.49	9.58	49.36	17.27
10/90	1.45	3.88	25.61	10.18	2.53	6.53	42.91	17.12
20/80	1.22	3.86	23.48	9.52	2.32	7.18	43.50	17.64
30/70	2.96	5.73	29.65	10.76	4.58	8.82	45.57	16.53
40/60	1.52	5.37	29.59	11.02	2.53	8.87	48.85	18.18
HRS 3 / HRW								
100/0	1.16	6.89	33.30	10.79	1.59	9.44	45.55	14.75
10/90	1.63	4.63	27.84	10.74	2.75	7.74	46.58	17.96
20/80	1.25	4.83	27.87	10.55	2.10	8.14	47.01	17.80
30/70	1.58	5.77	30.95	11.02	2.51	9.12	48.90	17.40
40/60	1.42	6.05	31.82	11.59	2.15	9.18	48.22	17.55
HRS 4 / HRW								
100/0	1.15	6.79	41.38	12.54	1.39	8.22	50.10	15.19
10/90	1.53	4.41	26.66	10.90	2.64	7.63	46.09	18.85
20/80	1.37	4.48	26.93	10.56	2.41	7.92	47.66	18.69
30/70	1.23	5.18	31.34	11.15	1.91	8.01	48.37	17.23
40/60	1.39	5.65	33.07	11.39	2.10	8.51	49.84	17.17
100% HRW + DS								
50 ppm SS	1.59	4.41	27.37	11.69	2.54	6.91	42.85	18.35
50 ppm CS	2.01	4.58	28.01	11.20	3.21	7.19	43.85	17.59
25 ppm DATEM	1.42	3.60	23.75	10.11	2.66	6.78	44.86	19.12
0.50% EMG	1.16	3.66	23.95	9.93	2.30	7.22	47.19	19.56
150 ppm AA	1.03	3.40	22.85	9.44	1.99	6.62	44.46	18.36
30 ppm ADA	22.56	19.49	77.65	26.44	15.26	13.20	52.71	17.97
10 ppm PB	2.57	4.01	24.43	10.03	4.27	6.70	40.91	16.78
5% VWG	1.22	4.66	29.86	10.84	1.75	6.63	42.44	15.43
2% Fat	1.40	3.45	20.84	8.88	2.80	6.86	41.42	17.66
2% NFDM	1.21	3.68	23.43	11.35	2.14	6.36	40.27	19.59
LSD($p<0.05$)	1.40	1.21	4.78	1.69	1.82	0.83	3.11	1.75

Table A.4. Unextractable protein fractions of bread crumb from HRS blends

	Absorbance Area (mAu*min)				Area Percent (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
HRW	2.54	1.61	4.00	2.27	5.20	3.31	8.34	4.78
HRS1 / HRW								
100/0	1.58	1.17	3.92	2.02	2.57	1.91	6.37	3.29
10/90	3.34	2.49	6.66	3.30	6.13	4.57	12.19	6.05
20/80	2.56	2.33	7.31	3.14	5.02	4.61	14.56	6.24
30/70	3.12	2.72	7.88	3.60	5.58	4.90	14.23	6.47
40/60	3.65	2.89	8.29	3.68	6.05	4.80	13.74	6.11
HRS 2 / HRW								
100/0	2.42	2.43	7.66	3.45	3.36	3.39	10.72	4.83
10/90	4.99	3.19	6.75	3.30	8.42	5.42	11.45	5.60
20/80	2.72	2.50	7.22	3.27	5.02	4.66	13.57	6.10
30/70	4.24	2.79	5.94	2.93	6.51	4.29	9.17	4.53
40/60	2.98	2.27	5.13	2.70	4.90	3.74	8.45	4.48
HRS 3 / HRW								
100/0	3.39	3.73	10.05	3.78	4.58	5.10	13.81	5.18
10/90	4.39	2.71	5.02	2.76	7.35	4.55	8.43	4.65
20/80	4.11	2.76	5.31	2.61	6.92	4.66	8.96	4.40
30/70	4.14	2.60	4.86	2.38	6.52	4.11	7.68	3.77
40/60	3.90	2.80	5.56	2.85	5.89	4.24	8.44	4.32
HRS 4 / HRW								
100/0	5.25	3.60	8.15	3.76	6.34	4.35	9.85	4.55
10/90	3.66	2.56	5.29	2.83	6.33	4.42	9.15	4.89
20/80	3.07	2.31	5.19	2.62	5.42	4.09	9.18	4.64
30/70	3.96	2.75	6.02	3.10	6.11	4.25	9.31	4.81
40/60	4.03	2.72	5.48	2.63	6.06	4.09	8.27	3.96
100% HRW + DS								
50 ppm SS	5.39	3.44	6.52	3.52	8.27	5.32	10.21	5.54
50 ppm CS	5.12	3.24	6.24	3.37	8.00	5.08	9.79	5.30
25 ppm DATEM	2.62	2.15	6.30	3.00	4.94	4.05	11.91	5.67
0.50% EMG	2.25	1.83	5.16	2.76	4.40	3.59	10.25	5.49
150 ppm AA	3.03	2.48	5.97	3.19	5.90	4.84	11.63	6.20
30 ppm ADA	1.11	0.12	0.04	0.00	0.76	0.08	0.03	0.00
10 ppm PB	3.93	2.96	8.09	3.69	6.60	4.97	13.59	6.18
5% VWG	4.34	3.76	11.54	4.35	6.14	5.30	16.17	6.14
2% Fat	2.76	2.45	7.23	3.29	5.49	4.87	14.34	6.55
2% NFDM	3.20	2.79	8.40	4.03	5.46	4.79	14.42	6.97
LSD($p<0.05$)	1.23	0.77	2.34	0.64	1.46	0.98	3.49	0.92

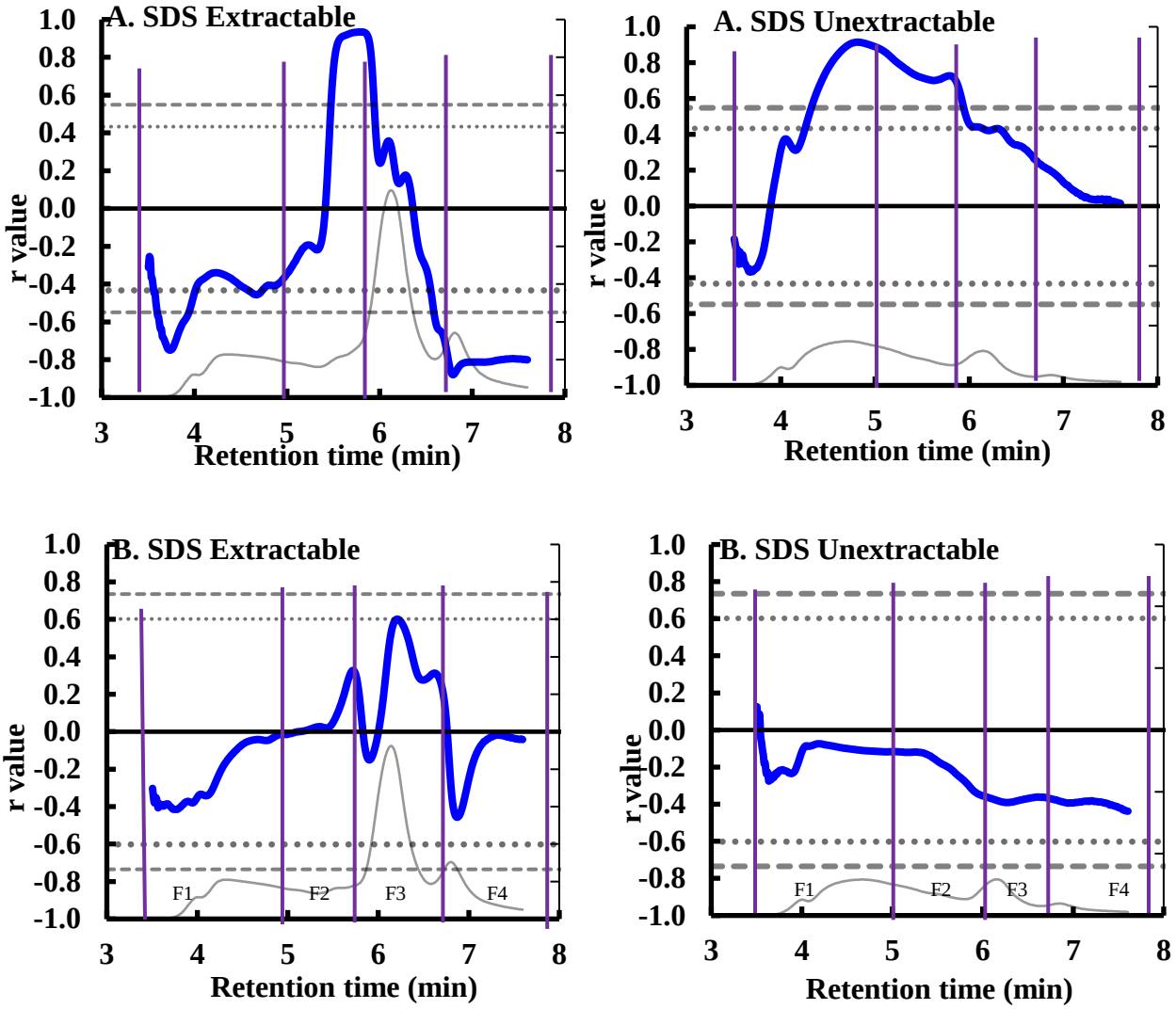

Figure A.1. Correlation coefficients (r) of farinograph stability with size-exclusion HPLC area % (A%) values of SDS-extractable and unextractable proteins extracted from (A) dough made of HRS wheat blends and (B) dough made of additives