CHARACTERIZATION AND EVALUATION OF HEAT TREATED WHEAT FLOURS

by

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B.S., Alexandria University, Egypt, 2006
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AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

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Department of Grain Science and Industry
College of Agriculture

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Abstract

The concept of thermal processing of foodstuffs has been used extensively since 1920 when the first scientific basis for calculating the minimum safe sterilization process was developed. There are several methods used in thermal processing of dry foods including infrared, microwave, hydrothermal treatments such as annealing and heat-moisture treatment, thermomechanical treatments (extrusion), and indirect (hot air) and indirect (steam) heating. Thermal processing has been the most widely used method for preserving and extending the shelf-life (via microbial reduction and enzyme inactivation, and for improving quality and functionality. In 2009 the Centers for Disease Control and Prevention released a report of an *Escherichia coli* outbreak resulting from consumers eating raw refrigerated cookie dough which brought attention to heat treatment of flours and powders. Chlorination of wheat flour in the European Union countries has been replaced in recent years by heat-treated flour which is used to produce high ratio cakes. By applying heat treatment, it is possible to modify the physical and rheological properties. The primary effect of heat treatment is denaturation of the proteins, partial reduction or inactivation of alpha-amylase, and partial gelatinization of the starch. Understanding of relationship between heat transfer, thermal properties of food, heating medium, thermodynamics and the functionality of the resulting heat-treated flour is of critical importance. Research reported in this dissertation has five chapters. Chapter 1 provides a general overview on the state-of-knowledge in the area. Chapter 2 focuses on developing a thermomechanical treatment (extrusion) for improving the functionality of low quality (ash > 1.3%) wheat flour. Chapter 3 deals with developing a direct, rapid and continuous thermal processing technique for treating whole wheat flour and whole wheat grain, and investigates physicochemical changes of heat-treated samples at various moisture-time-temperature combinations. Chapter 4 explores the mixing and development of composite flours in the presence of gluten fractions of at varying proportions, mixing speed and temperatures. Chapter 5 highlights general conclusions and identifies areas for future research.

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Approved by:

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Dedication

First I want to thank God for his love, provisions, guidance, mercy and blessings. I wish to dedicate this dissertation to my beloved family, relatives, and friends. I wish to give special tribute and gratitude to my loving parents Pilista Jokudu Mada and Eliaba Mada. Mom, how I wish you were here to share this special moment. Your spirit kept me through. Just like you, always smile even in tough times. If there is one thing I am certain, I inherited your beautiful smile, thank you mom! Dad, you always believed in education. Thank you for investing in our education. Special thanks to uncle Yoele Dilla, aunt Joyce Liyong, uncle Jacob Gonda, aunt Yunia Konga, Lucia Sebit Bolo Alex and Moses Leju. You are my real heroes. Daniel Leju Lomoloro, thank you. I could not have made this far without your timely advices. Edward Gwolo, I can only say, may God reward you abundantly. I don't have enough words to thank you. Ira Emmanuel and I owe it all to you. To Enike Poni, Susan Kabang, Emmanuel Ali, Betty Iya, Emmanuel, Pendo Duku, Urbe Woli, Knight Joseph, Patrick Joseph, Anthony Kenyi, Grace Juan Soma and so many others. You are the best family ever. To my African Student Union family, thank you Vuyiswa Bushula, Bruce Kamanga and families and everyone. Thank you for always checking on me and your sincere encouragement. To all those I have not been able to mention, you are all special to me. You are always in my thoughts and prayers. You have all been my best cheerleaders. God bless you in special ways.

Chapter 1 - Literature Review

1.1. Wheat

Wheat (*Triticum spp*) belongs to the grass family whose other members include maize, rice, millet, sorghum, barley, and oats. It is one of the most important food crops that man has cultivated for thousands of years. Wheat is unique in that it forms viscoelastic dough which is able to retain gas bubbles formed by fermentation. As a result, aerated baked products with desirable texture can be made from wheat flour (Wrigley 2009). Wheat is an important source of energy, carbohydrates, protein, fiber, B vitamins, iron, calcium, phosphorus, zinc, potassium and magnesium. This is true especially for whole wheat flour (Gooding 2009).

There are 15 species of wheat, however only three are grown on a commercial scale. Generally wheat is classified based on growth season, kernel color, hardness, quantity of protein and application. In the US, there are 7 commonly known wheat classes. These are hard red spring, hard red winter, soft red winter, hard white, soft white, durum and mixed wheat. The hard type refers to force required to crush the kernel. Spring and winter wheat types define their planting seasons and white or red to the color of pigments on the kernel. In addition, there are black and purple varieties of wheat as well (Carson and Edwards 2009).

Wheat is grown in diverse climatic regions. In addition, its kernel are hull free therefore easy to handle at harvest. It is nutritious and its unique functional protein make suitable for making baked products. All these factors make attractive for humans (Gooding 2009; Shewry 2009). The common wheat or hexaploid (*Triticum aestivum* L) is the dominant (~95%) cultivated wheat type. Today, more than 90% of wheat grown in the US and the world is the common wheat (Gooding 2009; Shewry 2009; Wrigley 2009). The common wheat varieties have wide ranges of protein contents, endosperm texture and growth seasons. They have excellent functional qualities and are used for making various products such as leavened bread, cakes, cookies or pastries. Hard wheat types such as hard red winter, hard red spring and hard white wheat have moderately high protein content and are used for making bread. Hard red winter is the most important and it's grown on the largest scale in the US and in state of Kansas in particular (Carson and Edwards 2009). Soft wheat such as soft red winter and soft white are lower in protein content and are used for making cookies, biscuits and pastries. The mixed class or blended wheat flour is used for making Chinese noodles, chapattis and Japanese noodles or to

obtain flour of desired protein content (Carson and Edwards 2009). Durum (*Triticum durum*) wheat makes less than 5% of total wheat grown in the US. It is mostly grown in North and South Dakota, Minnesota, Montana and California (Carson and Edwards 2009). Durum is grown in spring season, has very hard endosperm and the highest protein content. Durum wheat is milled to semolina and used for making pasta and bulgur (Carson and Edwards 2009; Gooding 2009). Lastly only about 3% of club *(Triciticum compactum)* wheat is grown in the North West of US, either in winter or spring seasons. Its kernels are soft and low in protein. Club wheat is not suitable for bread making and its flour is mostly for making cakes and pastries. Sometimes club wheat is blended with soft wheat to get low protein content desired flour (Gooding 2009).

1.1.1. Origin

Wheat was first reported to have been cultivated in the Fertile Crescent in the South Eastern part of current Turkey. The two wheat ancestors that were domesticated were einkorn (*Tricticum monococcum*, genome A) and emmer (*Triticum turgidium* ssp durum genome BBAA). Einkron was the first to be cultivated although it has not been grown for a long time (Shewry et al. 2009). The emmer might have resulted from hybridization between *T. monococcum* (A genome) and the ancestral grass *Aegilops speltoides* (B genome). From its center of origin, emmer (*T. turgidium* ssp dicoccon) was disseminated to Europe, Africa and Asia. Today's common wheat (*Triticum aestivum)* resulted from hybridization between emmer wheat *(T. turgidium* ssp) dicoccon (genome BBAA) and the grass (*Triticum tauschii*, genome DD) (Dubcosvky and Dvok 2007; Shewry 2009; Wrigley 2009). The common wheat is reported to have first appeared in northwestern Iran or northeastern Turkey (Gooding 2009). Due to this geneneological development, unlike other cereal, wheat exhibits polyploidy traits. It may have diploid (einkorn type, 2 genome AA), tetraploid (emmer, 4 genomes BBAA) or hexaploid (common wheat, 6 genomes BBAADD) sets of chromosomes (Dubcosvky and Dvok 2007).

1.1.2. Production and Economics

Wheat is a very important economic crop in the world. It's a major source of carbohydrates which are utilized in feed and industrial applications. It's a staple food for a large portion of world's population (Shewry 2009; Dubcosvky and Dvok 2007; Joye et al. 2009). It's the third most produced cereal grain (670 million metric tons) after paddy rice (719 million metric tons) and maize (872 million metric tons) (FAOSTAT 2012). In 2012, among the three

major cereals, it's has the largest cultivated hectares (215 million hectares) in World (FAOSTAT 2012). In the United States in the same year, more than 22 million hectares of land (~10% of world's more than 218 hectares) was planted with wheat. Close to 61.6 million metric tons (9.4% of worlds) of wheat was produced. More than 40% of the wheat produced was the hard red winter, the rest were hard red spring, soft red winter, white and durum. In the world trade, about 137 million metric tons or one fifth of world wheat production was exported. The United States exported more than 27 million metric tons (44% of its total wheat production) and contributing to more than ~20% of worlds exported total. Of the total wheat produced, a small portion, about 136 (~20%) metric tons were used for animal feed production. Data from the Kansas City Board of Trade Wheat market show that it costs ~$378/metric ton to produce milled flour. The market price for bakery flour was $369/metric ton and $58/metric ton for byproduct. The net profit was approximately $49/metric ton, a very narrow margin for millers (http://www.ers.usda.gov/data-products/wheat-data.aspx).

1.1.3. Structure, Composition and Nutrition

Kernel structure is important as it influences its processing, especially at milling where the major objective is to separate the anatomical structures (Bechtel et al. 2009). The wheat kernel is completely covered with pericarp which has several layers. The pericarp is part of bran, and the bran makes up about 14% of total weight of wheat kernel (Bechtel et al. 2009). Bran is rich in cellulose with minor amounts of protein (6%), ash (2%), fat (5%) and the rest being non-starch polysaccharides. The aleurone, which is technically part of endosperm, is commonly classified as part of the bran because it is removed along with bran. It's rich in enzymes, protein, phosphorus, lipids and vitamins. The endosperm makes about 83% of kernel weight and is mostly composed of storage/functional protein, has traces of vitamin B complex (niacin, thiamine and riboflavin), soluble fibers (arabinoxylan, β-glucans and other hemicelluloses) and iron. The germ makes up about 3% of total kernel weight, and is very rich in protein (25%), sugar (18%), oil (16%), ash (5%), vitamins (especially E) and enzymes. The germ has the embryo which can germinate to new plant given right conditions. The germ is separated during milling due to its high level of fat and high enzyme activity. The fat can easily be oxidized and become rancid affecting the quality of flour (Delcour and Hoseney 2010).

1.2. Wheat Milling

1.2.1. Cleaning

Wheat cleaning is used to remove both non-wheat material and wheat material that is unsuitable for milling. This could be metals, stones, grains other than wheat, smaller/larger wheat grain, infested, shrived/cracked kernels or chaff. Effective wheat cleaning is critical for human safety, flour quality, dust control, increased flour yield and durability of milling equipment. Physical properties of wheat kernels such as mass, size, shape, density, color, and friability are the major principals that are used in wheat cleaning. For effective/thorough cleaning, several equipments have been designed and are used in the process. These include gravity tables, magnets, disk separators, scourers, and entoleter among others (Dexter and Sarkar 2003; Posner 2009; Delcour and Hoseney 2010).

1.2.2. Tempering

After thorough cleaning, the wheat is conditioned and tempered. Conditioning is application of water and temperature to wheat to raise moisture content to the desired level. The major purpose of tempering is to toughen the bran to prevent it from fragmenting to small pieces at crushing since the major objective is to separate the anatomical parts (endosperm, bran and germ) as clean as possible to improves milling efficiency. It also softens the endosperm by weakening the internal structure therefore less energy is required for milling. Factors that influence tempering are the wheat class/type, bran thickness, temperature, humidity, initial moisture content, protein content, target moisture and time. For example, hard-vitreous wheat may take up water much slowly than opaque wheat or low moisture grain requiring more water to be added and longer time for tempering (Posner 2009). The desired target moisture content for tempering is generally between 15 and 17 percent. However hydration level generally depends on class of wheat (6-12% for soft wheat). Tempering time, to allow water to penetrate the kernels is between 12 and 24 h with hard wheat requiring longer time (Dexter and Sarkar 2003; Delcour and Hoseney 2010).

1.2.3. Wheat Dry Milling Principles

The major objective of wheat dry milling is to separate the anatomical parts and grind the endosperm to flour. High concentrations of bran in flour are traditionally related to low milling

efficiency and therefore low quality flour. There are six systems in a commercial milling setup; break system, purification system, sizing system, residue, reduction, and low grade (Posner 2009). Each system plays a critical role in obtaining good quality flour. The dry milling of grains with crease such as wheat is done with roller mills (Delcour and Hoseney 2010). In the break system, the rolls are corrugated. Because of the differential speed and spiral corrugation on the roll, the rolls act like scissors, shearing and cutting the kernels. As the break system steps proceed (from 1BK to 4BK, or 5BK), the differential decreases and rolls become smoother. The objective of the break/dust system is to open up the kernel and remove clean endosperm. The products of break-systems are grouped to coarse and fine bran, sizing and germ, which are then sent to purifiers and sizing rolls via grading system.

The purpose of the grading system is to separate compound endosperm according to size. The "pure" endosperm is sent to the reduction system. The major objective of purifications system (purifiers) is to remove any bran fragments and group the endosperm chunks according to their size, density and air flow characteristics. The purifiers effectively support sizing and midds reduction systems. Overall, they diversify the flour milling products, grade the stocks and increase flour yield. The two principles used by the purifier to achieve this are resistance of material to air flow and stratification by moderate vibrations. Large endosperm fragments from break or purification systems are then sent to the sizing system (Posner 2009).

In the sizing system, smooth rolls are used to break down large endosperm chunks to smaller size flour particles. In addition there is continued bran separation. The products of sizing system are classified as coarse and fine fractions. In the midds reduction system, the endosperm is intentionally ground to flour (Posner 2009). Theoretically, the midds reduction should have the purest endosperm. The residue system produces flour rich in bran which makes up the 1^{st} and 2^{nd} tailings and quality flour. Finally, the low grade has endosperm from break/dusting and middling systems.

1.3. Wheat Flour Composition and Functionality

Wheat flour is composed primarily of starch, protein and lipid. Other important constituents include vitamins, minerals, fiber, bioactive compounds and enzymes. Starch is the major constituent (70%), protein (9-16%) and lipids (2.0-2.5%) in composition of wheat flour. Wheat protein is unique because of their viscoelastic property that makes it suitable for baked

products. Lipid has significant influence dough functionality. The minor constituents of vitamins, minerals, fiber and bioactive components play an important role in nutrition.

1.3.1. Proteins

Proteins are polypeptides of amino acids that are linked through peptide bonds. The peptide bond is flexible and stretchable to some extent. Amino acids, the subunits of protein, are classified as basic, acidic, neutral hydrophilic and neutral hydrophobic based on their R group. Hydrophobicity of the amino acid residues of protein plays a major role in influencing its physical and chemical properties such as solubility. Hydrophilic amino acids have a high degree of polarity. Glutenin and gliadin when compared to other protein such as myoglobin, hemoglobin and ovalbumin, have similar hydrophobicities. However, due to their high level of diversity and their relatively large molecular size, the relative surface hydrophobicity of cereal proteins has not yet been determined (Delcour and Hoseney 2010).

The primary, secondary and tertiary structures of protein are used for classification. Some R groups are chemically active such as sulfhydryl groups. They can react with (intra) or another (inter) cysteine group to form disulfide bonds. In addition, polypeptides are able to form weak non-covalent bonds. However, because they are numerous and stable bonds, these weak non-covalent bonds are able to maintain the 3-dimensional structures of protein. It's this tertiary structure of proteins that determines their functionality. Any physical, chemical or biological alternation in tertiary structures of protein results in changes in their functionality (Voet et al. 2011).

1.3.1.1. Cereal Proteins

Proteins have traditionally been classified according to the method of Osborne (1907). This classification is based on protein solubility and extraction in water, dilute salt, aqueous alcohol, dilute acid or alkali (Delcour et al. 2012; Delcour and Hoseney 2010). Cereal proteins are classified as albumins, globulins, prolamins (gliadin in wheat) and glutelins (glutenin in wheat) (Delcour and Hoseney 2010). Although this classification technique is simple and easy to follow, the solubility and extractability of protein in these four solvents overlaps to varying degrees. The low solubility of gluten protein is attributed to presence of weakly ionizable residues. Gluten has high amounts of glutamine and non-polar amino acids, mostly proline and glycine (Delcour et al. 2012; Lagrain et al. 2010).

Other current cereal protein classification techniques are based on their molecular size. This includes the use of size exclusion HPLC (SE-HPLC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation techniques (Delcour and Hoseney 2010; Shewry et al. 2002). Wheat protein can also be classified according to their functionality in bread-making: non-gluten (15-20%) proteins and gluten (80-85%) proteins (Goesaert et al. 2005). The non-gluten proteins are metabolic and found mostly in aleurone, germ and traces in endosperm. Nonetheless because many enzymes are composed of non-gluten protein, there is no doubt that they can influence quality of wheat product through their enzymatic action (Veraverbeke and Delcour 2002). The non-gluten wheat proteins include albumin, globulin and traces of triticin (Shewry and Halford 2002) and their contribution to bread quality is unknown (Uthayakumaran and Wrigley 2010; Goesaert et al. 2005).

Gluten is a polydisperse system of wheat storage and functional proteins found in the endosperm. They are differentiated as gliadin and glutenin (Shewry 2009; Veraverbeke and Delcour 2002). The quantity, quality and proportionality of gliadin to glutenin have significant influence on their functionality in bread-making. The functional performance of wheat gluten has been strongly related to gliadin/gluten ratio, low molecular weight/high molecular ratio and composition of high molecular weight (HMW) gluten (Delcour et al. 2012; Shewry 2009; Wieser et al. 2007). Their ratio and composition has been shown to influence bread loaf volume and crumb structure (Mejia et al. 2012; Millar 2004; Don et al. 2003; Ververbeke and Delcour 2002). The HMW gluten has been associated with dough strength and baking performance (MacRitchie and Lafiandra 1997). There appears to be a critical ratio of glutenin to gliadin for good bread making wheat flour. For example, if amounts of glutenin are high, the dough is likely to be strong resulting in low loaf volume and dense crumb and vice versa. Gluten sub-classes of gliadin and glutenin are heterogeneous and there are numerous subunits (Goesaert et al. 2005). Optimally hydrated and mixing of wheat flour results in dough that is viscoelastic. The glutenin is responsible for elasticity of dough and the gliadin is responsible for viscosity (Ververbeke and Delcour 2002).

Gliadins are polymorphic and monomeric polypeptides soluble in 70% ethanol (Lagrain et al. 2010; Veraverbeke and Delcour 2002). They are composed primarily of two amino acid residues, glutamine and proline. Gliadins are sub-classified into α, γ and ω subunits (Lagrain et al. 2010; Uthayakumaran and Wrigley 2010; Wieser 2007). These subclasses have different

numbers of cysteine residues and are gropued to sulfur rich and are sulfur poor (Shewry and Halford 2002). For example α-gliadins, γ-gliadins and ώ-gliadins have 6, 8 and 0 cysteine residues, respectively (Lagrain et al. 2010). Gliadins have molecular weight in range of 30K to 60K (Veraverbeke and Delcour 2002), gliadin influences dough viscosity and extensibility, and functions as a plasticizer by weakening bonds between glutenin (Joye et al. 2009; Goesaert et al. 2005).

Glutenins are the largest heterogeneous polymeric proteins found in nature. The gluten macro polymer (GMP) contributes to wheat protein's functional properties (Delcour et al. 2012; Goesaert et al. 2005; Wieser 2007). Glutenins have molecular weights in the range of 80K to over 10 million (Veraverbeke and Delcour 2002). They are only soluble in acid or base and are classified as low molecular weight (LMW) and high molecular weight (HMW) subunits (Lagrain et al. 2010; Joye et al. 2009).

The amino acids residues and Mw of α-, γ-gliadins, and those of LMW-glutenin subunits are similar in having six cysteine residues (Wieser 2007). However unlike in gliadin, additional free cysteines (sulfhydryl groups) are only found in LMW-glutenin subunits. Therefore the LWM subunits can form polymeric intra-chain or inter-chain disulfide bonds (Delcour et al. 2012; Wieser 2007; Ververbeke and Delcour, 2002). The LMW subunits are further sub-classified to B, C and D types based on their mobility in SDS-PAGE. Overall, their molecular weight is lower than that of HMW and ranges from 30K to 60K (Delcour et al. 2012; Joye et al. 2007; Ververbeke and Delcour 2002). The B-type is further classified into LMW-s (serine), LMW-m (methionine), and LMW-i (isoleucine) according to the first amino acid residue (Delcour et al. 2012).

The high molecular weight (HMW) are grouped according to their coding genome to A, B or D (Wieser 2007; Shewry and Halford 2002). These are further sub-classified as x-type (Mw 83-88K) and y-type (Mw 67-74K) subunits (Delcour et al. 2012; Shewry 2009). Common wheat is a hexaploid with 6 genomes (1Ax, 1Ay, 1Bx, 1By, 1Dx, 1Dy). On the other hand durum wheat is a tetraploid with 4 genomes (1Ax, 1Ay, 1Bx, 1By). This corresponds to six and four HMW subunits for common and durum wheat, respectively (Shewry et al. 2002).

1.3.1.2. Gluten Structure and Models

There are several models that have been used to describe the gluten network:

Branch Model: Graveland et al. (1985) in their model proposed that HMW gluten subunit make up the backbone and LMW subunits are lateral side branches providing strength to the structure. After numerous analyses, Lindsay and Skierritt (1999) suggested that in addition to Graveland's model, the backbone is made of HMW subunits and or branches of LMW as well as HMW subunits linked through covalent and non-covalent bonds. The dough network is held by covalent and non-covalent bonds that are formed when wheat flour is optimally hydrated, and kneaded. HMW and LMW-subunits contain both inter- and intra-molecular bonds in their polymers. Because these covalent bonds are responsible for gluten network formation and thereby elasticity, their quantity is important for gluten quality. There is a good correlation between glutenin strength and non-extractable protein. The non-extractable glutenin are generally of large Mw (Veraverbeke and Delcour 2002).

Loop and Train Model: The loop and train dough formation model (Delcour et al. 2012) has been suggested to describe formation of dough and to explain its viscoelastic property. It states that HMW glutenin subunits are linked through inter and intra hydrogen bonds. During hydration, inter and intra chain are broken forming loops. At same time hydrogen bonds are formed between glutamine of one peptide, water and glutamine of second peptide. On further hydration, more loops are formed. However, beyond optimum hydration level the gluten elasticity weakens because hydrogen bonds are no longer strong enough. Because of this critical involvement of glutamine in hydrogen bonding and loops formation, the ratio of glutamine to other amino acids in Glutenin Macro-Polymer (GMP) is important and it is reported to be 1.1:1 (Delcour et al. 2012).

1.3.1.3. Wheat Gluten in Bread-making

Wheat gluten has unique and desirable functionality in that it can form continuous, cohesive, viscoelastic protein network which sets to form desirable texture characteristic of baked products (Delcour et al. 2012; Wieser 2007; Singh and MacRitchie 2001). The viscoelastic property of wheat flour dough enables it to retain carbon dioxide generated by yeast during sugar fermentation, from leavening agent or air that is incorporated at mixing (Dobraszczyk et al. 2003; Shewry et al. 2002).

The first step in bread-making is dough development, a mixing process which can have a significant influence on final product texture (Angioloni and Rosa 2005). The purpose of mixing is to have homogenous dough with 3-dimensional viscoelastic structures, and with the ability to retain incorporated/generated air (Angioloni and Rosa 2005). Also mixing disperses and incorporates bread-making ingredients: flour, water, salts, yeast and other minor ingredients (Goesaert et al. 2005).

Mixing, aeration, dough rheology, baking performance and texture of final products are closely related. This is because during dough processing, the rheology of viscoelastic dough changes (physically and chemically) and that has significant influence on final product quality (Dobraszczyk et al. 2003). In turn, the rheology of dough is influenced by optimum mixing time, optimum mixing energy, composition, protein quantity or quality (glutelin/gliadin ratio), environmental conditions (temperature) and type of mixer (Campbell and Shah 1999).

Rheology is the study of deformation and flow of material under well-defined conditions. Important properties of materials measured in rheology studies include viscosity, stiffness, hardness, strength, toughness of material, and modulus. In rheology studies, a force is applied for a given time, and the stress/deformation as a result of the material is measured or a deformation/stress is applied and the resulting force is measured (Dobraszczyk et al. 2003). The rheological properties of a material can be used to describe its physical characteristics, to understand composition, molecular and structural organization and finally predict its performance under given processing conditions. Predicting and having mathematical models becomes of greater significance since the sequential steps in bread-making of mixing, sheeting, proving and baking cannot be easily studied without disrupting these processes therefore making it tough to relate process steps to quality of final product (Dobraszczyk et al. 2003).

In cereal science, the two broad categories of rheological techniques are descriptive/empirical and fundamental measurements. In empirical measurements, the geometry of material changes during testing therefore stress and strains are complex and non-uniform. Consequently, empirical measurements are less precise. Generally, a single point from the test is randomly chosen to compare materials, for example peak torques under specified conditions. It becomes impossible to compare tests between machines or within tests in more accurate ways since these studies are time and condition specific. Nonetheless empirical measurement techniques are cheap, fast, and easy to operate so as to obtain properties that can be correlated

with product quality or processing. Some of the tools used in empirical measurements include Farinograph, mixograph, Alveograph, extensigraph, texturometer, consistometer (Goesaert et al. 2005; Dobraszczyk et al. 2003).

To overcome challenges faced in empirical measurements, fundamental measurements are used. Although they are expensive, can be delicate and require good training and skills to operate, fundamental measurements provide more accurate results. These fundamental rheological tests include small and large deformation shear, creep and stress relaxation, small deformation dynamic oscillation, large deformation extensional measurements and flow viscometry. In the literature, there are numerous studies on mixer designs and how they influence rheology and final texture of a product, empirical measurements of rheology during mixing, effect of rheology on mixing patterns and finally simulation and prediction of mixing flow rheology as function of mixer geometry (Dobraszczyk et al. 2003).

Fundamental rheology is widely used to understand the structure of gluten and more specifically the contributions of HMW subunits. However, complete comprehension of the relationship between gluten functionality performance and its structure has been difficult. Currently available methods to study molecular size of polymers such as gel permeation chromatography (GPC), SE-HPLC are not sufficient without disrupting their structure because gluten has low solubility and large molecular weight (Delcour et al. 2012; Goesaert et al. 2005; Dobraszczyk 2004). HMW subunits exhibit long chains, branches and entanglement (Delcour et al. 2012), which may explain its ability to exhibit elongational strain hardening (Dobraszczyk 2004).

1.3.2. Starch

Starch is a semi-crystalline biopolymer and the main storage form of carbohydrates in plants. It is an excellent and important component of the human diet, of animal feed and for industrial applications. Starch is synthesized in the amyloplast during photosynthesis (Jacobs and Delcour 1998; Bertoft et al. 2008; Belitz et al. 2009; Zavareze and Dias 2011), and stored mostly in grains, stem, tubers or roots. Major sources of the starch are cereal grains (corn, wheat, rice, barley, oats, sorghum and millet); root tubers (cassava); stems (sweet potatoes, potatoes), fruits (plantains) and legumes (lentils and peas) (Adebowale et al. 2005; Sajilata et al. 2006; Belitz et al. 2009).

Starch granules differ in size, size distribution, shape, crystallinity and composition. In general starch granules range from 0.5 to 175 μm in size. Wheat starches are 3-38 μm and corn starches 5-25 μm in size. Potato starches are 15-110 μm in size and are known to be the largest in size and rice starch (3-8 μm) are the smallest (Jacobs and Delcour 1998; Hoover 2001; Morikawa and Nishinari 2002; Delcour and Hoseney 2010). Commonest shapes are lenticular, polyhedral, spherical, oval, kidney-shaped and elliptical and irregular shapes (Jacobs and Delcour 1998; Hoover 2001).

Starch is composed of amylose (mostly linear polymer of glucose linked through $\alpha(1\rightarrow4)$ glycosidic bonds) and amylopectin (glucose units linked in a linear way with $\alpha(1\rightarrow4)$ glycosidic bonds and branching with $\alpha(1\rightarrow6)$ bonds occurring every 24-30 glucose units) (Bertoft et al. 2008). Starch normally contains (17-28%) amylose although there are variety of cereals with high amylose (70%-amylomaize) and (~0% amylose-that is waxy) (Jenkins and Donald 1995; Buleon et al. 1998). The locations and ratios of amylose to amylopectin vary among starch botanical source types. This ratio influences their physical and chemical properties (Saibene and Seetharaman 2010; Zavareze and Dias 2011).

The α-D-glucosyl units associate in long linear chains linked through α-D-1, 4 glycosidic linkages, wherein α-D-1, 6 glycosidic linkages are formed as branch points. The contribution of α-D-1, 6 glycosidic linkages to total bonds is extremely low in amylose (less than 1%) and moderately extensive in amylopectin (~5–6%) (Hoover 2001; Bertoft et al. 2008; Zavareze and Dias 2011). Amylose, which is relatively extended, has a lower molecular weight (500–20,000 glucose units) than amylopectin (~10^6 glucose units) which has a compact shape. The glucose units in amylose are linked through α-1, 4 bonds, with degree of polymerization (DP) of 500-6000 glucose unit. The number of glucose units in amylose is variable. It is between 500 and 6000 glucose units in wheat starch and about 4500 glucose units in potato starch (Sajilata et al. 2006; Belitz et al. 2009). Amylose chains are able to re-associate after gelatinization to form a gel (Zavareze and Dias 2011). Retrograded amylose requires temperatures >120°C to disperse. Some compounds like iodine, fatty acids, fatty acid esters of hydroxy, carboxylic acids, phenols, are capable of forming clathrates (inclusion compounds) with amylose. Cereal starches are stabilized by these lipid clathrate resulting in lower swelling power (Tester et al. 2004; Jenkins and Donald 1995). However, alcohols can improve the swelling by removing the lipids from the helices (Delcour and Hoseney 2010). Amylose on its own specially, especially at DP>100, may

associate with itself or amylopectin to form double helices (Sajilata et al. 2006; Tester et al. 2004).

Amylopectin is much larger polymer with molecular weight of 10^7 to 10^9 (Jacobs and Delcour 1998; Hoover 2001; Bertoft et al. 2008; Zavareze and Dias 2011). The amylopectin branch chains are classified to A- and B-branch chains, which are in turn connected to the root C-chain (Bertoft et al. 2008). Branches in amylopectin have on average between 14-60 glucose residues (Sajilata et al. 2006; Hoover 2001; Carriere 1998). The A-chain is free of side chains (non-branched) has between 14-18 glucose residue units in length. The unit chain branches of amylopectin are divided into short (with 6-35 DP) and long (with >35 DP) chains (Bertoft et al. 2008). The majority of the shorts chains form clusters and the external chains/clusters form left hand double helices making up crystalline lamellae. The internal clusters found between the chains form amorphous lamellae (Bertoft et al. 2008). The crystalline and amorphous lamellae alternate, and are about 9 nm thick stacks and form the semi-crystalline rings (Jenkins and Donald 1995). The type of crystalline lamellae formed is function of these double helices (Bertoft et al. 2008). Amylopectin structure is arranged radially, with the reducing end pointing outwards.

The level of branching influences the efficiency of enzymes in digesting starch granules. The enzymes α-amylase, β-amylase and glucoamylase can easily hydrolyze amylose. Amylopectin can also be degraded, however, the β-amylase only degrades it to the branching point leaving limit-dextrin (Belitz et al. 2009). Amylopectin slurries when heated form a more viscous solution than amylose does, which are also more coherent and sticky. Retrogradation is seldom observed in amylopectin as it is in amylose except at high concentrations and it can be reversed at temperature of about 50^oC.

1.3.2.1. Starch Crystallinity

Native starch crystalline lamellae can be classified to A, B, C and V-types allomorphs based on their X-ray diffraction and are indicator of their crystalline structures (Bertoft et al. 2008; Jenkins and Donald 1995). The polymorphism of starch granules is influenced by length of amylopectin chains, its growth temperature, alcohol and fatty acids (Hoover 2001). Within the clusters, the most external chains form left handed double helices. One turn has 6 glucose residues. The structure is stabilized through hydrogen bonds. The type of crystalline lamellae formed is function of these double helices (Bertoft et al. 2008).The short A-chains are associated

with type-A crystalline and mostly found in cereal starches. The long A-chains associated with type-B crystalline which is found in potato starch and non-retrograded starch and intermediate A-chains associated with type-C crystallinity (Jenkins and Donald 1995).

Both type-A and B crystalline are similar in structure with hexagonal helices running antiparallel (Sajilata et al. 2006). In type-A crystalline the helix core is filled with another double helix resulting in a tight packing. In type-B crystalline channel core is filled with water (Tester et al. 2004). Type-C crystalline is mixture of type A and B, found in legumes (Tester et al. 2004; Hoover 2001). The V-type crystalline of starch crystalline is found in swollen granules and formed when amylose fraction forms a complex with compounds like alcohol, normal iodine (Hoover 2001).

1.3.2.2. Starch Gelatinization and Retrogradation

Starch is insoluble in cold water, however, air-dried starches can absorb and/lose up to 30 to 40% water of its original mass. It can swell slightly which results about 5% increase in volume (Delcour and Hoseney 2010; Belitz et al. 2009; Goesaert et al. 2005). The numerous hydroxyl groups in starch give its high affinity for water. But, due to its large size, starch polymers tend to associate with each other and link through its hydroxyl groups via hydrogen bonds (Belitz et al. 2009). These strong hydrogen bonds and poor water molecules interactions between hydroxyl groups of amylose/amylopectin and water make starch insoluble (Zavareze and Dias 2011).

Heating starch in excess water to above its glass transition temperature makes it to undergo irreversible phase transition called gelatinization (Goesaert et al. 2005; Hoover 2001; Jacobs and Delcour 1998). Starch gelatinization is characterized by collapse of crystalline structure, loss in birefringence, swelling, water absorption and amylose leaching (Hoover 2001; Jacobs and Delcour 1998). The glass transition temperature (between 50 and 70°C range for any starch) of an amorphous material is temperature at which it changes from glassy to rubbery state in presence of a plasticizing solvent (Jacobs and Delcour 1998). Cereal starches generally have wider gelatinization temperature ranges than do root or tuber starches (Hoover 2001). When a starch solution is kept for some time below its gelatinization temperature before heating, the starch granule structure is reorganized and its gelatinization temperature is increased (Jacobs and Delcour 1998). In limited water, gelatinization occurs at higher temperature and vice versa. However, complete gelatinization and crystalline melt can never occur in insufficient water even at increasingly higher temperature (Hoover 2001). On the other hand, treating starch in the same

manner at lower moisture content and higher temperature results in stabilization of the crystallite as well as decreased swelling (Zavareze and Dias 2011).

The gelatinization process of starch starts when the amorphous region absorbs increasing water levels by hydration process as the starch granule swells (Goesaert et al. 2005; Hoover 2001; Jacobs and Delcour 1998). The starch granule loses birefringence (loss of 'Maltese' cross), takes up heat energy, the double helices dissociate, loss of crystalline, amylose leaching as well as increase in viscosity. The starch crystalline structure may also change from one type to another for example from type B to type A (Zhong et al. 2009; Jayakody and Hoover 2008; Hoover 2001). Gelatinization temperature is increased by polyhydroxy compounds like sugar and decreased by salts like common salt (sodium chloride). These compounds change the water activity which in turn affects gelatinization temperature. Lipids and free amino acids influence gelatinization through formation of complexes. They reduce swelling ability of starch and solubility in water (Belitz et al. 2009).

Gelatinization can be monitored by measuring changes in viscosity, energy changes, swelling, crystallinity etc. (Zhong et al. 2009; Jacobs and Delcour 1998). Swelling of starch results in increase in viscosity, however the melting is followed by a drop in viscosity. The viscosity can be measured as a function of heating or cooling temperatures under controlled conditions. Such systems allow the time when starch swelling starts to be detected. This is sometimes referred to as pasting temperature (Kuo and Wang 2006). Differential scanning calorimetry (DSC) is another technique used to determine gelatinization temperature. This method can detect transition phases, and provides enthalpy of gelatinization and crystallization (Zhong et al. 2009; Kuo and Wang 2006; Jacobs and Delcour 1998).

Retrogradation (amylopectin) and crystallization (amylose) are terms used to describe the irreversible changes when solubilized or highly swollen starch becomes insoluble, shrunken and microcrystalline (Goesaert et al. 2005). They are characterized by increases in turbidity, precipitation and gel formation as the first indicators. Retrogradation starts when soluble starch molecules start to associate, followed by formation of double helices which increases strength of the network. With time, these formed helices become partly crystalline (Belitz et al. 2009). Crystallization can only occur between the glass transition temperature of starch and melting temperatures. The melting temperatures of amylopectin and amylose starches are 50-60^{o}C and 150^{o}C, respectively. This implies that the temperature range for crystal nucleation and

propagation in starch retrogradation is narrower for amylopectin than amylose (Delcour and Hoseney 2010). The initial stiffness in retrogradation is attributed to amylose and latter stiffness which might take days or weeks to develop, to amylopectin (Delcour and Hoseney 2010; Goesaert et al. 2005). Starch composition and concentration, molecular structure of starch, pH, aging time and absence of surface active substance are some of the factors that influence starch retrogradation (Goesaert et al. 2005).

1.3.3. Non-starch Polysaccharides

Cellulose and hemicellulose are the most abundant biological, non-starch polysaccharides found in nature (Stone and Morell 2009). Cellulose is a linear biopolymer of glucose units linked through β-1, 4 glucosidic bonds, present at less than 2% in cereal grains and traces in flour (Stone and Morell 2009; O'sullivan 1997). Hemicelluloses are diverse in compositions and structures. Common sugar units constituents of hemicellulose include D-xylose, L-arabinose, D-galactose, D-glucose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid (Ebringerova et al. 2005).

Arabinoxylans are the majority and most important non-polysaccharide in cereal grains. They are classified as water-extractable (25-33%) or water-unextractable (Ebringerova et al. 2005; Swennen et al. 2006; Stone and Morell 2009). Arabinoxylans are composed of β-1,4 linked D-xylopyranosyl residues, with monomeric α-L-arabinofuranose substituted at carbon 3 and/or 2 position (Swennen et al. 2006). Ferulic acid can also be linked to carbon 5 of the arabinose by an ester linkage and more uronic acids, mostly glucuronic acid or its 4-methyl ether derivative, substitutions can occur at position C-(O)-2 of xylopyranosyl residues (Delcour and Hoseney 2010; Swennen et al. 2006). To a lesser extent, wheat endosperm cell walls also contain arabinogalactan peptide, whose functionality is not yet well known (Stone and Morell 2009).

Some non-starch polysaccharides found in cell walls of cereals have anti-nutritional effects. Phytic acid, an inositol hexaphosphoric acid, chelates divalent cations and makes them unavailable for nutrition (Delcour and Hoseney 2010; Stone and Morell 2009). Overall, non-starch polysaccharides especially soluble arabinoxylan and beta glucan in cereals are known to have positive health benefits. They are associated with reduction in blood cholesterol and regulation of blood glucose (McKevith 2004). Cereals grains are good sources of beta-D glucan and (glucurono) arabinoxylans (Stone and Morell 2009; Ebringerova et al. 2005). Beta-D-

glucans are linear polysaccharides of D-glucopyranosyl units linked by 1→4 and occasional 1→3 beta linkages found mostly in starchy endosperm and aleurone walls (3-7% in barley; 3.5-4.9% in oats; 0.5-2% in rye and wheat) (Belitz et al. 2009; Stone and Morell 2009). Both arabinoxylans and beta-D-glucans can influence the viscosity (Belitz et al. 2009; Stone and Morell 2009).

1.3.3. Lipids

Lipids in cereals are minor constituents (2.0-2.5%), however, they have a profound influence on functionality and application of wheat flour, bread-making and quality of end products (Pareyt et al. 2011; Chung et al. 2009; Sroan and MacRitchie 2009). Most of the lipids (70%) are non-polar and free lipids, which become bound or trapped within gluten. The glutenin fraction interacts with galactolipids whereas gliadin interacts with phospholipids (McCann et al. 2009). These interactions add a functional value to the gluten in that the lipids are able to provide stability to gas cells during baking (Pareyt et al. 2011; Sroan and MacRitchie 2009). In addition, lipids have an important but negative role in self-life due their oxidation or enzymatic hydrolysis. The important enzymes are lipases, phospholipases and lipoxygenases. Lipases hydrolyze lipids and release glycerol and free fatty acids, while phospholipases release phospholipids. Lipoxygenases promotes oxidation of unsaturated fatty acids (Delcour and Hoseney 2010).

There are several ways to classify cereal lipids: Simple or complex; non-polar (70%) lipids, glycolipids (20%) or phospholipids (10%); and according to location in the grain i.e. germ (26-29%), aleurone (24-31%), or endosperm (starch and non-starch) lipids; polar (25-30%) and nonpolar lipids (70-75%). The composition of polar and nonpolar lipids in these anatomical parts differs as well (Pareyt et al. 2011; Chung et al. 2009). The germ and aleurone have almost similar composition of polar and nonpolar lipids. They both have on average between 72 and 85% of non-polar lipids and 0 and18 % of polar lipids. The aleurone contains 6.7-9.8% glycolipids and 13.8-17.9% phospholipids. The germ contains 0-3.6% and 15.2-16.8% glycolipids and phospholipids, respectively. The endosperm (starch and non-starch) lipids are rich in polar lipids. The non-starch and starch glycolipids are 30.7-38.3% and 1.2-5.5%, respectively and non-starch and starch phospholipids are 23.6-34.4% and 90.1-94.4% respectively (Chung et al. 2009).

1.3.4. Minor Constituents

Whole wheat flour is a good source of vitamin, minerals and functional bioactive components (Delcour and Hoseney 2010; McKevith 2004). Wheat is nutritionally rich in vitamin B complex especially thiamin, niacin, vitamin B_6, folate and vitamin E. It is also an important source of bioactive components like phytosterols, phenolic choline and betaine; and minerals such as potassium, phosphorus, magnesium, calcium, manganese, iron, selenium and copper (Piironen et al. 2009; Shewry 2009). The influence of micronutrients on flour functionality is insignificant; however their nutritional value is very significant. These micronutrients are mostly distributed in germ and bran. In traditional milling, these two components are separated and removed from flour therefore refining of flour decreases nutritional benefits of grain, with exception of selenium which is closely associated with sulfur (Shewry 2009; McKevith 2004).

1.4. Heat Treatment

1.4.1. Theory

The two broad categories of energy are mechanical and internal (Bergman et al. 2011). Mechanical energy includes potential and kinetic energy. Internal energy includes thermal, chemical and nuclear energy. Thermal energy is defined as sensible and latent energy. In food processing, thermal energy is the one of interest (Bergman et al. 2011). Thermodynamics and heat transfers are complimentary, interrelated but different field of studies. The latter is a study that relates energy, heat and work done on materials using the four laws of thermodynamics. Internal energy, entropy, temperature, pressure and radiation are most important properties of materials that are studied in a thermodynamic system. On the other hand, heat transfer studies focus on understanding quantity and rate of thermal energy exchange among materials using rate equations (Bergman et al. 2011). Fundamental knowledge of these two concepts help food processors understand, predict and evaluate performance of heat exchangers, cooking processes and how they influence final quality attributes of the food. In addition, a better understanding in the performance of heat exchanges leads to better equipment design and costs savings (Singh and Heldman 2001).

1.4.1.1. Heat and Food Processing

Thermal energy has been used in processes like heating and cooling to preserve and protect food for a long time. Sterilization is mostly used to kill pathogens, spoilage microbes, inactivate enzymes. Other beneficial functions of cooking include improvement in flavor, texture and color. Of greater importance to science is the understanding of relationship between thermal properties of food, heating medium, thermodynamics and heat transfer. Central to application of thermal energy in food processing are conventional heat transfer types which include conduction, convection, radiation. Transfer of heat can be described as contact (direct) and noncontact (indirect). In contact heating, the food material and the heating medium are in contact while in noncontact, the heating medium and food materials are separated by a partition. Contact heating methods include the use of steam and hot air. Noncontact methods include use of heat exchanger plates, tubular tubes and scraped-surface heat exchangers (Singh and Heldman 2001).

1.4.1.2. Thermal Properties

Each material has unique thermal properties. Among these are specific heat transfer capacity, thermal conductivity and thermal diffusivity. Pure materials have single values; however foods are complex and composed of different constituents. The overall thermal properties are sum of the thermal properties of it is constituents according to their proportionality. Thermal properties of materials are temperature dependent and one key assumption in defining thermal properties of material is that the process occurs at constant pressure.

Specific Heat: Specific heat capacity (C_p) of a material is the quantity of thermal energy required to change temperature by unit degree (1^oC) of a unit mass (1 kg) material without any change in physical state:

$$C_p = \frac{Q}{m\left(T_1 - T_2\right)}$$

where C_p = specific heat ($J/kg.K$), Q = heat quantity (J), m = unit mass (kg) and (T_1-T_2) = temperature difference (K). Water has a high specific heat value and in food systems, it contributes significantly to overall specific heat capacity of material. The heat measured is called sensible heat. When there is a phase change, there is no change in temperature as the material undergoes from one state to another. Here, the heat is called silent or latent heat.

Thermal Conductivity: Thermal conductivity (k) is a property of material that describes its ability to transfer heat energy. It depends on temperature, pressure and other physical properties

of material such as density and composition. The two mechanisms proposed in thermal conductance are through movement of electrons from region of higher temperature to region of lower temperature where they lose their thermal energy and lattice vibrations which increase as thermal energy increases with the former being more efficient in metals than the latter which is more efficient in non-metals (Venkanna 2010). Thermal conductivity is the amount of heat energy that is transferred in a unit time through a unit thickness of material in a given direction. Materials that are poor in thermal conductivity are insulators while those with good thermal conductivity are used as good heat conductors. The inverse of thermal conductivity is thermal resistivity (Singh and Heldman 2001). Materials are classified to good heat conductors and poor heat conductors.

Thermal Diffusivity: Thermal diffusivity (α) is measure of thermal inertia of a material, and it is the ratio of thermal conductivity, density and specific heat (Venkanna 2010; Singh and Heldman 2001).

$$\alpha = \frac{k}{C_p \rho}$$

It measures the rate of heat transfer through a material. The higher the rates of temperature change in the material, the higher the thermal diffusivity. There is a direct relationship between thermal diffusivity and thermal conductivity (Singh and Heldman 2001).

1.4.2. Modes of Heat Transfer

1.4.2.1. Conduction

Temperature gradient drives the flow of heat energy from a region of high to low temperature. Fourier's law of conductance states that the heat flow rate is proportional to the area normal to the direction of the heat flow and the temperature's gradient (Venkanna 2010):

$$q_x = -kA\frac{dT}{dx}$$

where q is heat flux, k thermal conductivity, A is area normal to direction of heat flow, dT/dx is the change in temperature per thickness of material.

Conduction is an indirect form of heat transfer where the food material is in contact with a hot/heated surface. It is widely used to process foods such as in pasteurization of milk, drying of foods. Indirect techniques include use of heat exchangers, tubular heaters and scraped surface

exchangers among others. In indirect heating, the food surface contact of heater must meet food safety standards such as hygienically designed, be stainless steel in addition to having efficient heat conduction.

1.4.2.2. Convection

Convection is a direct mode of heat transfer between fluid and a surface. It involves two mechanisms; macroscopic motion of fluid and molecular motion (Venkanna 2010). Generally the movement of fluid is referred to as advection (Bergman et al. 2011). The media could be forced to move or freely moves (Bergman et al. 2011; Venkanna 2010; Singh and Heldman 2001). Some equipment used includes flash and fluid beds, jet zone and impingement ovens (Bergman et al. 2011; Singh and Heldman 2001). Unlike conduction, convection is a complex type of heat transfer and it is described according to the following equation

$$q = hA\left(T_w - T_f\right)$$

where q is heat flux, A is area, h is convective heat transfer coefficient, T_w is solid surface temperature and T_f is fluid temperature. Unlike in conduction where thermal conductivity is property of material, the heat transfer coefficient in convection depends on several properties such as density, fluid viscosity, geometry, surface properties, specific heat and thermal conductivity (Venkanna 2010; Singh and Heldman 2001).

1.4.2.3. Electromagnetic Radiation

Radiation is the transfer of heat by means of electromagnetic waves. It does not rely upon any contact between the heat source and the heated object as is the case with conduction and convection. The transfer of heat by radiation involves the carrying of energy from an origin to the space surrounding it. The energy is carried by electromagnetic waves and does not involve the movement or the interaction of matter. Thermal radiation can occur through matter or vacuum. All objects radiate energy in the form of electromagnetic waves. The rate at which this energy is released is proportional to the temperature raised to the fourth power (Venkanna 2010). The emissive power is described by the Stefan-Boltzmann law:

$$E_b = \sigma T_s^4$$

where T_s is the absolute temperature (K) of the surface, and σ is the Stefan-Boltzmann constant (σ= 5.67 x 10-8 W/m^2K^4).

The energy radiated from an object is usually a collection or range of wavelengths, which is referred to as an emission spectrum. As the temperature of an object increases, the wavelengths within the spectra of the emitted radiation also decrease. Hotter objects tend to emit shorter wavelength, higher frequency radiation. Thermal radiation is a form of heat transfer because the electromagnetic radiation emitted from the source carries energy away from the source to surrounding objects. This energy is absorbed by those objects, causing the average kinetic energy of their particles to increase and causing the temperatures to rise.

All materials continuously emit and absorb electromagnetic waves, or photons, by changing their internal energy on a molecular level. Strength of emission and absorption of radiative energy depend on the temperature of the material, as well as on the wavelength and frequency. Radiation is an electromagnetic energy emitted by a body at non zero temperature that travels at the speed of light (Venkanna 2010; Singh and Heldman 2001). An increase in temperature of a material causes its internal energy to release this electromagnetic energy which can be transmitted through a vacuum (Venkanna 2010). Gases are theoretically transparent to radiation except in special cases such as ozone (which absorbs ultraviolet ray) and water vapor. Liquids are good absorbers of radiation and solids are opaque and their surface absorption and emission are of significance (Venkanna 2010; Singh and Heldman 2001).

1.5. Thermal Treatment Methods

1.5.1. Infrared

Infrared radiation belongs to electromagnetic spectrum which has wavelengths shorter than microwave but longer than visible light, and lies between 0.075 and 1000 micrometers (Penner 2010). The infrared spectrum is divided into near infrared, mid infrared and far infrared according to wavelength and location in light spectrum. Infrared radiation is produced when molecules have increased internal energy and they vibrate faster releasing some photon energy. Different compounds have different infrared spectra that they can absorb. For example water absorbs infrared in the 2.8-7.0 micrometer range (Penner 2010).

1.5.2. Microwave

Microwave radiation is another common technique used to heat materials. The principle is based on dielectric properties of material. Microwave is a form of electromagnetic energy. Some materials are dipolar meaning that their atoms have partial positive and partial negative charge. In an electromagnetic field, molecules of dipolar compounds oscillate as they try to align themselves to the ever changing electric field. This rapid and constant oscillation results in increased friction which in turn results in heat. Microwave's major advantages are less carbon footprint, rapid, non-thermal gradient and selective heating. However, its heating non-uniform because of uneven distribution of electromagnetic field and it is expensive to generate (Bergman et al. 2011).

1.5.3. Hydrothermal Treatments

Annealing (ANN) and heat-moisture treatments (HMT) are two hydrothermal treatment methods that are widely used to improve functionality of starches and flours without destroying granular structure (Jacobs and Delcour 1998). In annealing, the material is suspended in excess water (40-65% w/w) for a given time and at temperature above glass transition but below gelatinization (Jayakody and Hoover 2008; Jacobs and Delcour 1998). The annealing process affects the crystalline regions, where the weaker crystals disappear and stronger ones become even more perfect because of fusion and re-crystallization (Jayakody and Hoover 2008). Extensive studies deal with the effect of thermal process on physico-chemical properties of various starch sources from corn, barley, wheat, potato and rice (Jacobs 1995). In these studies, ratio of amylose to amylopectin and arrangement of starch chains, biological origin of starch and treatment factors played roles in influencing physico-chemical changes (Jacobs and Delcour 1998).

Heat moisture treatment (HMT) is one of the hydrothermal treatment methods used to improve starch/flour functionality. In this process, the material is hydrated to a moisture content (10-30%) and heated to temperatures between 90-120^{o}C for a short time (~15 min) or long time (~16 h) (Chung et al. 2009: Maache-Rezzoug et al. 2008). The effect of HMT on starch is influenced by the botanical source, amylose content and of course moisture, temperature and time (Jacobs and Delcour 1998). HMT reduced swelling power, solubility, peak viscosity, breakdown viscosity, setback viscosity and relative crystallinity of rice starch, while it increased

pasting time, susceptibility to enzyme. The effects of HTM were more pronounced with increase in amylose content (Zavareze et al. 2010).

1.5.4. Thermo-mechanical Treatments

Thermo-mechanical treatments involve use of twin-screw extrusion techniques. The word extrusion describes the process by which a material is forced through a narrow opening. In conventional food extrusion, the material may be moistened, heated, under pressure and mechanically sheared as it is conveyed. Extrusion is a continuous, versatile, energy efficient, fast, cost effective and robust cooking method (Kim et al. 2006). These numerous advantages food extrusion processes make it attractive and its widely used in manufacturing of breakfast cereal, infant food, animal feed/food, snacks, aquatic feed, precooked flour and grits and pregelatinized starches among others (Singh et al. 2007; Kim et al. 2006). The energy required to cook the same amount of food is therefore lower in comparison to a batch cooking system. Furthermore in comparison to other cooking techniques there is little to no effluent released because little water is used in extrusion cooking and much of that is lost as steam. This makes extrusion cooking an environmentally friendly food processing technique (Riaz 2000). Extrusion cooking is also efficient in inactivating antinutritional elements, denaturing enzymes and sterilizing foods (Singh et al. 2007; Riaz 2000). To ensure complete cooking, material is cooked under high temperature, pressure and shear (Kim et al. 2006; Riaz 2000). The thermal energy is generated by oil, steam or electric heating of the extruder barrel. Steam can also be introduced directly to material. The pressure is influenced by screw configuration, feed rate, screw speed and die size. Having numerous kneading blocks or reverse blocks may slow down conveying rate and increase pressure in barrel. Shear is influenced by screw profile, ingredients, and screw speed (Strahm 2000).

1.6. Effect of Heat Treatment on Food Components

1.6.1 Protein

Proteins are very sensitive molecules, and denatured by heat. Wheat proteins are no exception. Fundamental understanding of the effects of heat on wheat protein is critical. The relationship between the intensity and mode of heat treatment and practical applications (e.g. grain drying, flour heat treatment, extrusion process) and implications of these processes on the

performance of final food products has to be studied in a systematic manner. Functionality of wheat gluten decreases remarkably with heat treatment (Singh and MacRitchie 2004). Some changes which are associated with protein denaturation include increase in viscosity, decrease in solubility and extraction as well as increase in higher molecular weight fractions indicating polymerization due to the formation of covalent bonds (Korablyova and Kasymova 2011; Stathopoulos et al. 2007; Lagrain et al. 2005; Singh and MacRitchie 2004).

Stathopoulos et al. (2007) and Lagrain et al. (2005) reported an increase in the viscosity of heated wheat gluten due to polymerization. The elastic and loss moduli (G′ and G″) increased at treatment temperatures 85^{o}C and above. The RVA viscosity has been reported to increase as a function of the wheat protein fraction, increased holding time and temperature (Lagrain et al. 2008). Solubility and extraction of heated wheat protein decreased with increase in treatment temperature and time. These changes were attributed to polymerization and changes in conformation (Stathopoulos et al. 2007; Singh and MacRitchie 2004). The different types of wheat protein are reported to have different degrees of heat induced polymerization. Shomer et al. (1995) reported that albumins and globulins were more heat stable than HMW glutenin. Lagrain et al. (2008) also reported that gamma and alpha gliadins were more heat liable than omega gliadin and this was attributed to fewer number of cysteine residues in omega gliadins (Singh and MacRitchie 2004).

1.6.2. Starch

1.6.2.1. Granule Morphology

Physical characteristics of starch granules such as morphology, size distributions and surface characteristic influence many of its functional and physical properties (Zavareze and Dias 2011). Many studies show that hydrothermal treatment does not have a great influence on starch morphology (Jacobs and Delcour 1998; Gumaratne and Hoover 2002; Singh et al. 2003). However, a few studies show that hydrothermal treatment caused cracks in maize starch granules and formation of hollows inside starch potato (Kawabate et al. 1994). When rice starches of high and medium amylose content were treated by HMT at 25% moisture, their granules surface became irregular and there was slight agglomeration (Zavareze et al. 2010). The low amylose starch at same moisture treatment (25%) showed a greater loss in physical integrity, probably due to more partial gelatinization (Zavareze et al. 2010). The annealing process was also shown

to cause greater deformation of starch granules of waxy wheat starch than high amylose wheat starch (Kiseleva et al. 2005). Dias et al. (2010) compared effects of annealing on treated high amylose starch and control, where they found that there were more pores on surface of treated starch than control. Annealing corn starch of different amylose contents at 50^{o}C produced increases in starch granule size regardless of amylose content due to swelling (Liu et al. 2009). Pore size of some barley cultivars were shown to increase slightly on annealing treatment where the increases in granule size or pores were attributed to moisture absorption by the amorphous region of starch granule (Waduge et al. 2006).

1.6.2.2. Starch Crystallinity

Starch crystallinity is influenced by crystal size, number of crystalline regions, level of interaction between double helices and their orientation within the crystalline areas. The latter properties are influenced in turn by amylopectin content (Miao et al. 2009). The effect of hydrothermal treatment on starch crystallinity is influenced by starch source (Zavareze and Dias 2011), and treatment conditions (Jacobs and Delcour 1998). B-type starch has been shown to change to starch crystalline type A+B-type polymorph, the latter being more stable in potato starch (Genkima et al. 2004; Gunaratne and Hoover 2002). Stability is attributed to loss of water and movement of a pair of double helices to central channel (Gunaratne and Hoover 2002). Jacobs and Delcour (1998) reported that the effect of HMT depends on the treatment conditions. They did not observe a change in X-ray diffraction pattern of the treated cereals starches. Hoover and Manuel (1996) and Vieira and Sarmento (2008) studied the X-ray diffraction intensities of corn and sweet potato after HMT. Hoover and Vasanthan (1994) reported an increase in X-ray diffraction intensities in treated starch and attributed it to the fact that HMT resulted in a more orderly crystalline matrix. This ordered crystallinity was due to more amylose-amylopectin interactions and less amylose-lipid complexes. The orderly structure was also evidenced by reduced inter-crystalline spacing and helical packing (Gomes et al. 2004). Hormdok and Noomhorm (2007) also suggested that the increased crystallinity of starch granules is as a result of increased mobility of amorphous parts and subsequent re-ordering of helices. This decreases starch swelling power and solubility as well as its volume

However, Vermeylen at al. (2006), Gunaratne and Hoover (2002) and Franco et al. (1995), however, reported a decrease in relative crystallinity of potato, cassava and corn starches. Gomes et al. (2004) reported that all starches they heat treated showed decreased crystallinity

peak intensities. The reduction in X-ray intensities was attributed to reduced crystallinity and/or increase in the amorphous region in semi-crystalline lamellae (Zavareze and Dias 2011). Furthermore, Jenkin and Donald (1995) attributed differences in hydrothermally treated starches to amylose content. They reported that amylose disrupts the packing of amylopectin crystallites, and these changes follow on amylose content; high amylose > normal > waxy.

1.6.2.3. Gel Structure

Hydrothermal treatment of starch also changes its gel hardness. The factors that influence the resulting hardness are type of starch, interactions between continuous and dispersed phase, the volume and formation of resulting granular structure (Choi and Kerr 2003).The native helices of starch granules are stabilized by hydrogen bonds. During gelatinization, these are broken and new hydrogen bonds with water are formed (Lee and Osman 1991). In addition, more junction zones are formed as well as cross linking between chains, amylose specifically (Liu et al. 2000; Hoover and Manuel 1996). This is evident from fact that high amylose starches form harder gels than do amylopectin rich starches. When 27% amylose starch was HMT at 15 and 25% moisture, there was increased hardness. For normal starch treated at 25% moisture, there was no difference in hardness with native starch (Zavareze et al. 2010). Temperature, moisture and time are the treatment parameters that most influence gel hardness. There is a direct relationship between high treatment moisture and temperature and gel hardness (Cham and Suwannaporn 2010). The increased crystallinity of starch granules is as a result of increased mobility of amorphous parts and subsequent re-ordering of helices. This decreases starch swelling power and solubility as well as its volume (Hormdok and Noomhorm 2007).

1.6.2.4. Starch Swelling Power and Solubility

Hydrothermal treatment of starches affects its swelling power and solubility negatively. Several studies on rice starch (Hormdok and Noomhorm 2007), wheat, corn (Chung et al. 2009) and potato starch (Gunarante et al. 2002) showed that their swelling power and solubility decreased after hydrothermal treatment. The gelatinization process increases amylose-amylose, amylose-amylopectin interactions (Oliyinka et al. 2008; Jacobs et al. 1995; Hoover and Vasanthan 1994) and the formation of amylose-lipid complexes (Waduge et al. 2006). These interactions increase the crystalline perfection, reducing swelling power and hydration of the amorphous regions (Hoover and Vasanthan 1994).

1.6.2.5. Impact of Treatment on Starch Pasting

Hydrothermal treatment has been shown to increase paste temperature and decrease peak viscosity, final viscosity and break down viscosity of sorghum starch (Olayinka et al. 2008), rice starch (Hormdok and Noomhorm 2007) and corn starch (Chung et al. 2009). The effect of treatment is more pronounced when higher moisture content and higher temperatures were used (Olayinka et al. 2008). The changes in pasting profile and properties are related to changes in amorphous and crystalline regions of the granule during hydrothermal treatment. During thermal treatment, old hydrogen bonds are replaced with new hydrogen bonds with water as well as more cross linking among starch helices. These bonds are much stronger, thereby requiring more heat energy to disrupt, and form paste, thus higher paste temperature (Adebowale et al. 2005; Gomes et al. 2004). The low break down viscosity indicates that the starch is stable to shear at heating (Olayinka et al. 2008; Hormdok and Noomhorm 2007; Adebowale et al. 2005). Chung et al. (2009) and Lan et al. (2008) have stated that retrogradation is influenced by the degree of amylose leaching, granule size and presence of rigid, non-fragmented swollen granules.

1.6.2.6. Gelatinization Characteristics

Differential scanning calorimetry (DSC) can be used to characterize the gelatinization properties of starch. The four important parameters obtained are the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH). The degree of gelatinization, ratio between the gelatinized starch and total starch, is influenced by starch source, amylose content, its moisture content and process parameters during hydrothermal treatment (Adebowale et al. 2005). Hydrothermal treatment of starch increases onset temperature, peak temperature, conclusion temperature and reduces enthalpy. These changes observed in potato and cassava starch (Gunaratne and Hoover 2002), and cornstarch (Chung et al. 2009; Maache-Rezzoug et al. 2008) are attributed to changes in starch granule structure as a result of amylose-amylose, amylose-lipid interactions and formation of new double helices (Waduge et al. 2006; Hoover and Vasanthan 1994). Hydrothermal treatment reduces amylose interactions, thereby requiring higher temperature for treated starch to swell and to disrupt the crystalline regions.

1.6.2.7. Susceptibility to Acid Hydrolysis

The effects of acid hydrolysis on hydrothermally treated starch vary depending on numerous factors such as starch source, cycles of annealing and annealing temperature. Heat-

moisture treatment (HMT) results in reduced hydrolysis of cereal starches and increased hydrolysis of tuber starches (Hoover and Manuel 1996; Hoover and Vasanthan 1994). For potato starch, Varatharajan et al. (2010) reported a decrease in acid hydrolysis, which was explained to be probably due to the changes from type B starch granule to a complex type (A+B) starch granule, chain interactions and perfection of crystallites. The type A starch granule is more dense in structure and therefore difficult for acid molecules to reach α-1,4 and α-1,6 glycosidic bonds. On the other hand, the easy acid hydrolysis can be explained through crystallite, double helices disruption in amorphous region, thereby exposing glycosidic bonds more accessible for hydrolysis. In addition, for annealed starches, the number of cycles of annealing also influences treated starch behavior (Nakazawa and Wang 2003; Hoover and Vasanthan 1994). The earlier authors reported an increase in acid hydrolysis of multiply annealed wheat, tapioca, potato, normal corn, waxy corn and high amylose corn. The latter authors reported decrease in acid hydrolysis, in wheat; potato and lentil starches that were annealed once.

1.6.2.8. Enzymatic Hydrolysis

Hydrothermal treatment of starch changes the physic-chemical properties of starch granules resulting in lack of accessibility to reaction sites by hydrolytic enzymatic (Chung et al. 2010; Chung et al. 2009; Brumovsky and Thompson 2001; Jacobs and Delcour 1998). Several factors influence these conditions; amylose/amylopectin ratio, crystalline granular structure, the size of particle, storage time, temperature and amount of water in processing (Kutos et al. 2003; Guraya et al. 2001). Some studies show that HMT resulted in higher enzymatic hydrolysis of starches. Vieira and Sarmento (2008) subjected Peruvian carrots, sweet potatoes and ginger starches to HMT at 27% moisture, 100^{o}C for 16 h and digested them for 24 h with α-amylase. They reported increased enzymatic hydrolysis by 25%, 5% and 22% in carrots, sweet potato and ginger starches, respectively. Chung et al. (2010; 2009) reported decrease in levels of slowly digestible starches and an increase in readily digestible starches in annealed corn, pea, lentil and navy bean starches. These increases in digestibility are attributed to formation of pores which permits for easy accessibility by hydrolytic enzymes (O'Brien and Wang 2008; Nakazawa and Wang 2003). However other studies have showed that HMT treatments resulted in increased amounts of slowly digestible starch (SDS) and resistant starch (RS). Shin et al. (2005) found that amount of SDS doubled after HMT of sweet potato starch. Chung et al. (2009) found a decrease in rapidly digestible starch (RDS) (10, 14, and 15%); increase in SDS (2.5, 2.8, and 4.7%); and

increase in RS (7.7, 11 and 10%) for corn, pea and lentil starches, respectively. Similarly, Brumovsky and Thompson (2001) reported more than 50% increase in RS of high-amylose corn starch subjected to HMT. Of the two starch hydrothermal treatment methods, HMT is more effective in creating SDS and or RS (Chung et al. 2009; Brumovsky Shin et al. 2005; Thompson 2001) than is annealing (Dias et al. 2010; Chung et al. 2009; Jayakody and Hoover 2008). This is because as much as both increase crystalline perfection and chain interactions which prevent accessibility to hydrolytic enzymes, annealing promotes formation of porous structure which supports easy hydrolysis (Chung et al. 2010). When sequential HMT was followed by ANN or vice versa treatments, both resulted in higher RS (Chung et al. 2010). Kutos et al. (2003) stated that amount of RS is related to degree of starch gelatinization and starch retrogradation. In annealing process, starch gelatinization is limited and thereby limited retrogradation whereas structural and molecular reorganization occurs in starch crystalline. This explains the differences between the two hydrothermal methods (Zavareze and Dias 2011).

1.6.2.9. Slowly Digestible Starch and Resistant Starch

According to Englyst et al. (1992), starches can be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) according to the rates at which glucose is released during enzymatic digestion. RDS is quickly digested after intake and results in the fastest increase in blood glucose levels. SDS is slowly (20-120 min) and partially digested in in-vitro enzyme hydrolysis resulting in intermediate rates of glucose release (low to medium glycemic index). The starch that is not digested in in-vitro enzymatic hydrolysis after two hours is classified as resistant starch.

SDS and RS can be found in nature or created by chemical modification, extrusion cooking, retrogradation, heating or acid hydrolysis and thermal treatment. RS can further be sub-classified to resistant starches (RS-1, RS-2, RS-3 and RS-4). RS-1 is physically not accessible in grain or seeds that are milled. RS-2 is found in banana, potato or high amylose starch granules. RS-3 is retrograded starch and RS-4 is from chemically modified starch (Fuentes-Zaragoza et al. 2010).

1.6.3. Lipids and Minor Components

Lipid-amylose complexes are formed during starch gelatinization. These complexes form V-type crystallinity. They are not easily digested with amylases. Soluble dietary fiber is reported

to increase in extruded materials. On the other hand, many of the water soluble vitamins are destroyed at processes involving heat.

1.7. Research Objectives

Thermal energy in processes such as heating and cooling has been used to preserve and protect food for a long time. There are several methods used in thermal processing of dry foods including infrared, microwave, hydrothermal treatments such as annealing and heat-moisture treatment, thermomechanical treatments (extrusion), indirect (hot air) and indirect (steam) heating. In its all forms of application, thermal processing has been the most widely used method of (i) preserving and extending the shelf-life (via microbial reduction and enzyme inactivation), and (ii) improving quality (flavor, texture and color), and functionality and performance.

In 2009 the Centers for Disease Control and Prevention (CDC) released a report of an *E.Coli* outbreak resulting from consumers eating raw refrigerated cookie dough which brought attention to heat treatment of flours and powders. Chlorination of wheat flour in the EU countries has been replaced in recent years by heat treated flour which is used to produce high ratio cakes. By applying heat treatment, it is possible to modify the physical and rheological properties.

The primary effect of heat treatment is denaturation of the proteins, partial reduction or inactivation of alpha-amylase and partial gelatinization of the starch. Understanding of relationship between heat transfer, thermal properties of food, heating medium, thermodynamics and the functionality of the resulting heat treated flour is of critical importance for food processor. The overall objective of this study was to investigate the effects of direct, rapid and continuous thermal processing techniques on the functionality of wheat flours. The specific objectives were to characterize the heat treated flours for their mixing, pasting, and baking performance, and explore the potential use of these new products in dough and batter-based food formulations.

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Chapter 2 - Thermo-mechanical Treatment of Low Quality Wheat Flour Fractions

Abstract

Dry milling of wheat generates multiple flour fractions which differ in their composition, therefore, their functionality. The ash content of flour fractions differs and their values are used to grade as well as determine their economic value. Low grade fractions are high in ash and generally not used in baking applications due to their inferior performance. Flour with ash content greater than 0.55% is considered inferior for baking therefore a low economic value. Hydrothermal treatment of low grade fractions could improve their functionality such as gelling and pasting characteristics, and freeze-thaw stability. Thermal treatment of wheat flour by rapid continuous extrusion process is a potential alternative to batch processes. The objectives of this study were to investigate the effect of different in-barrel moisture and temperatures on low, medium and high ash wheat flour fractions, characterize the resulting functional wheat flours for their mixing, pasting, and other performances, and explore the potential use of these new value added products. In this study, 15 flour fractions of hard red winter wheat milled in the Hal Ross Mill (Kansas State University) were grouped into low, medium and high ash fractions. A partial factorial experimental design was employed to conduct nine extrusion runs using a TX 52 pilot scale twin screw extruder. Factors studied were 3 in barrel moisture content (18, 21, 24%), 3 extrusion temperatures (70, 90, 100 °C) and 3 flours fractions with ash contents of 0.47, 0.64 and 1.34%. Process and product characterization were done my measuring the specific mechanical energy (SME), expansion ratio (ER), piece length, bulk and piece densities. Extrudates were dried and ground to reduce their particle size below 240 μm for further analysis including particle size analysis, proximate analysis, water holding capacity and solubility, pasting (RVA), degree of gelatinization (DSC), relative crystallinity (X-ray diffraction). Protein and fiber content of untreated flours decreased from high to low ash fractions (0.57-0.0 and 19.8-13.8%, respectively). Expansion ratio and specific length decreased with increasing feed moisture content and barrel temperature, whereas high ash flour content resulted in higher longitudinal expansion. High ash flour, lower in-barrel moistures and lower extrusion temperatures resulted in smaller particle size distribution after grinding. All of the extrusion process parameters (feed

moisture content, barrel temperature and flour ash content) influenced the pasting properties. Extruded flours were completely gelatinized as indicated by loss of gelatinization peaks and crystallinity peaks. Treated flours developed instant viscosity, and were more stable to shear than the control samples.

2.1. Introduction

Wheat is one of the most important agricultural crops and commodities. It ranks fourth in production largely due to its adaptability to various climatic conditions (Delcour et al. 2012). Conventionally, wheat grain is milled to separate the endosperm (82%), bran (15%), and germ (3%) as cleanly as possible by successive grinding and sifting processes (gradual reduction) (Posner 2009). For millers and consumers, the endosperm is the most important part which is milled to straight grade flour (Barron et al. 2007; Kim and Flores 1999). The bran and germ are separated due to their negative effect on flour quality and functionality such as poor flour color, loaf volume and texture of final products (Robin et al. 2011). The germ and bran are, therefore, considered contaminants and millers use their presence in flour to measure grain milling efficiency (Kim and Flores 1999; Fistes et al. 2013). Composition, functionality and value of flour fractions depend on; at what step they are extracted (break, reduction, low grade, residue). These flours fractions have different starch, protein, ash and crude fiber compositions. After milling, often flour fractions from different milling streams are reconstituted to obtain flours to meet a customer's specifications, the protein and ash content being the major criteria. Protein and ash content of flour affect functional properties and price. Wheat flour with ash content greater than 0.55% is generally considered inferior for baking and therefore of a low economic value.

In 2011 more than 24 million metric tons of US wheat was milled to flour yielding 20.9 million metric tons of flour. About 5.81 million metric tons was used as mill-feed due to low quality and therefore negative effect on baked product quality. Data from Kansas City Board of Trade Wheat market show that it costs ~$378/metric ton to produce milled flour. The market price for bakery flour was $369/metric ton while it was $58/metric ton for byproducts including high as flour fractions. The net profit was approximately $49/metric ton in 2011 (http://www.ers.usda. gov/data-products/wheat-data.aspx). Such low profit margin is challenging to wheat grain millers. Incorporation of low-grade high-ash flour fractions to the straight grade flour deteriorates the overall flour quality dramatically while it does not offer any significant

improvement to the monetary value of the product. Therefore, utilization of high ash flour to value-added products through heat treatment would be good an excellent alternative.

Wheat flour can be physically or chemically modified to improve its functionality, such as pasting performance, cold viscosity, freeze thaw stability, gelling, shear stability. Common chemical modification methods include esterification, oxidation and etherification (Singh et al. 2007). However, increasing numbers of consumers prefer "clean label foods" that have minimal or no chemical added to modify functionality or at processing (Cham and Suwannaporn 2010; Zavareze and Dias 2011). To meet such growing expectations, new processing technologies are required to improve the performance of wheat flour systems with minimum chemical ingredients.

Common physical methods starch modification are annealing, heat moisture treatment and extrusion (Jacobs et al. 1995; Zavarez et al. 2010; Singh et al. 2007). In annealing, starch is hydrated to higher moisture content, generally (35 to 60%) and heat treated at temperatures below its gelatinization temperatures (50 to 60°C). In heat moisture treatment, starch is hydrated to lower moisture, between 15 and 35%, and heat treated at temperatures above its gelatinization temperature (100 to 120^{o}C) (Singh et al. 2007; Hoover and Vasanthan, 1994). Extrusion is a high shear, high temperature, and a shorter thermal process that has been used and is a potential alternative to modify intrinsic properties of native starch (Gropper et al. 2002; Jayakody and Hoover 2008), such as gelling and pasting characteristics faster dispersion, lump-free slurry, increased batter water holding and absorption, freeze/thaw stability, increased and stable viscosity at high speed mixing or prolonged holding and enhanced flavor. Understanding the effects of extrusion on wheat flour fractions would help in adjusting optimum process parameters so that flour with desired functionalities results.

The objectives of this study were to investigate the effect of different in-barrel moisture and extrusion temperatures on performance of low, medium and high ash wheat flour fractions, characterize the resulting functional wheat flours for their mixing, pasting, and explore the potential use of these new value added products.

2.2. Materials and Methods

2.2.1. Flour Fractions Preparation

Locally grown hard red winter (HRW) wheat was milled in Hal Ross pilot mill (24 ton/day capacity) at Kansas State University (Manhattan, KS) to obtain 15 flour fractions. Wheat was tempered to 16.5% moisture content for 20 hours and milled through 5 break and 6 reduction steps. These flour fractions were grouped in to low (0.47%), medium (0.64%) and high (1.34%) ash content.

2.2.2. Extrusion Process

Through ash content analysis of each fraction, low (1/2M, 1SIZ, 2M, 3M, and Q), medium (1/2BK, 2SIZ, 3BK and GR-1) and high (4BK, 5BK, 4M, 5M and GR-F) ash flour groups were identified and blended thoroughly using a ribbon blender (Wenger Manufacturing, Sabetha KS) for 5 min. High ash flour fraction was extruded at three in barrel moistures (18, 21 and 24%) and three in-barrel temperatures (70, 90 and 110^{o}C), whereas low ash and medium ash flour fractions were extruded at medium in-barrel moisture content (21%) and medium temperature (90^{o}C) using a partial factorial design given in (Table 2.1). All samples were extruded using a pilot scale twin screw extruder (TX-52, Wenger Manufacturing, Sabetha, KS) with a six head configuration, screw diameter of 52 mm, L/D ratio of 16:1, and a medium shear screw profile shown (Figure 2.1). A circular die opening of 4.75mm was used. Extrusion runs were conducted at constant extruder screw speed (250 rpm) and raw material feed rate (80 kg/h). Water flow rates in the preconditioner and extruder were adjusted depending on the treatment so as to attain the different moisture contents needed for each treatment. Extruder conditions were allowed to stabilize for ~10 min before sample collection. The product was cut immediately after exit from extruder die with a face mounted rotary cutter rotating at 419 rpm. Extrudates were collected in a trough and using a pneumatic conveyor system transported to the dryer. They were dried at 107.2°C with a double-pass dryer/cooler (4800 Series, Wenger Manufacturing, Inc.) for 10 min (5 min for the top and 5 min for the bottom belt) followed by a 5 min cooling step.

2.2.3. Specific Mechanical Energy

Specific mechanical energy (SME) was calculated using the equation

$$SME\,(kJ/kg) = \frac{\left(\frac{\tau - \tau_0}{100}\right) \times \frac{N}{N_r} \times P_r}{\dot{m}}$$

where τ is the % torque; τ_0 is the no load % torque; N is the extruder screw speed (rpm); N_r is the rated screw speed (508 rpm); P_r is the rated motor power (37.9 kW); and $\dot{m}$ is the mass flow rate or throughput (kg/s).

2.2.4. Extrudate Characterization

2.2.4.1. Expansion Ratio

Expansion ratio (ER) was calculated using the equation below:

$$ER = \frac{D_p^{\,2}}{D_d^{\,2}}$$

where D_p represents diameter of the product and D_d represents diameter of the die.

2.2.4.2. Specific Length

The specific length was calculated by dividing the length of the product per unit mass.

$$Specific\ \ length = \frac{l}{m} \quad (m/kg)$$

where l is average length of the product, and m is unit mass of the product.

2.2.4.3. Bulk and Piece Density

Bulk density (BD) was measured with a one liter steel cup. Piece densities were calculated by dividing the mass of the piece by volume of the same piece:

$$\rho = \frac{m_{piece}}{V_{piece}} \quad (kg/m^3)$$

Extrudates were assumed to be cylindrical, and their measured diameters and lengths were used to calculate volumes. Five samples were selected randomly for piece density, expansion ratio and specific length estimations.

2.2.5. Functionality Testing

2.2.5.1. Sample Preparation / Grinding

Dried extrudates were ground using an experimental roller mill (Ross Machine and Mill Company Inc., Oklahoma City, OK). A set of corrugated rolls were used to break down extrudates to smaller pieces, followed by smooth rolls to obtain fine flour in single pass. Flour was sieved using Rotap (W.S Tyler Corp. Mentor, OH) agitated for 10 min. Amounts of flour retained in each sieve stack were weighed and converted to cumulative percentage yield. Flour passed through 240 μm size opening was used for all analysis, and the rest was discarded. Particle size distribution of flour < 240 μm was determined in duplicates using laser diffraction particle size analyzer (LS 13320, Beckman-Coulter, Inc., Miami, FL) using air as a disperser.

2.2.5.2. Proximate Analysis

All the samples were tested for moisture, protein, crude fiber and ash using standard AOAC Methods (925.05, 992.23, 962.09 and 923.02) for moisture, protein, crude fiber and ash respectively.

2.2.5.3. Differential Scanning Calorimetry

A differential scanning calorimeter (DSC) equipped with a refrigerated cooling system (Q100 TA Instruments, New Castle, DE) was used to study thermal properties of the samples. Approximately 20 mg ground sample was weighed into high-volume indium pans. Distilled water was added using a micropipette to make a suspension ratio of 1:2 (flour to water). Pans were then sealed and equilibrated overnight at 4^{o}C. The instrument was calibrated using an empty pan as reference stainless steel pan. The heating rate was 2°C/min from 20 to 110°C. Initial temperature, (T_o), peak temperature (T_p) and concluding (T_c) temperature of gelatinization, and enthalpy (ΔH) were measured from DSC thermograms using Universal Analysis 2000 software (TA Instruments, New Castle, DE) per unit mass of dry solid. The degree of gelatinization was calculated by the following equation (Marshall et al. 1993).

$$DG = \left(1 - \frac{\Delta H_{treated}}{\Delta H_{raw}}\right) \times 100$$

where DG the degree of gelatinization (%), $\Delta H_{treated}$ is transition enthalpy of hydrothermally-treated sample, and ΔH_{raw} is transition enthalpy of raw sample.

2.2.5.4. X-ray Diffraction

Samples were equilibrated at 100% relative humidity over 48 hour period at room temperature to adjusted moisture content to approximately 28%. Equilibrated samples were examined in a Rigaku X-ray diffractometer (MiniFlex II Rigaku North America Corp., The Woodland, TX). The radiation source was Kα. The voltage was set at 30 kV and a current at 15 mA. The scanning angles were between 3–30° (2θ), the scanning speed was 1.5°C/min in a continuous method. The slit width depth was 1.25 mm and 1°. The crystallinity of samples was obtained according to the procedure of Wakelin et al. (1959).

2.2.5.5. Swelling Power and Solubility Test

15 mg sample was weighed and transferred into a clear dried test tube. 15 cm^3 of distilled water was added to disperse the sample then vortexed. The resultant slurry was heated at the desired temperature (60, 70, 80, 90°C) for 30 min in a water bath while being agitated. At end of each incubation period, the mixture was cooled to room temperature and centrifuged (1,000×g, 15 min). Supernatants were dried to a constant weight at 110°C. The residue obtained after drying the supernatant represented the amount of flour solubilized in water (W_1). Solubility was calculated as g/100 g of sample on a dry weight basis. The weight of sediment after separation of supernatant was determined (W_s).

The water solubility index (WSI) and the swelling power (SP) were calculated as shown below:

$$WSI = \frac{W_1}{Sample\ weight} \times 100$$

$$SP = \frac{W_s}{\left[Sample\ weight \times (100\% - WSI)\right]} \times 100$$

2.2.5.6. Pasting Properties

Pasting properties of wheat flours fractions before and after extrusion were determined using a Rapid Visco Analyser (RVA, Foss North America, Inc. Eden Prairie, MN) following AACC Approved method 76.21-0.1. Pasting curves were analyzed using Thermocline software (Window 3 TCW3 RVA, Foss, North America, Inc. Eden Prairie, MN).

2.2.6. Statistical Analysis

A split factorial design was used for these experiments (Table 2.1). All of the analytical experiments were done at least in duplicates. Data was analyzed for significant difference among studied samples at (alpha = 0.05) using GLM procedure (SAS Institute 2009). Means among treatments were separated using the Bonferroni t-test.

2.3. Results and Discussions

2.3.1. Proximate Analysis

Protein and fiber contents increased from low to high ash flour fractions (Figures 2.2b and 2.2c). This is in agreement with the literature where wheat bran is reported to be rich in protein, minerals as well as vitamins, and the ash content is highly correlated with higher extraction rates in milling process (Hemery et al. 2010; Fistes et al. 2013). Although the aleurone is anatomically classified as part of endosperm, it is separated along with the bran in wheat dry milling. The aleurone is high in protein, vitamins and minerals (Hemery et al. 2010; Fistes et al. 2013).

Extrusion process did not change total ash and protein content (Figure 2.2a and 2.2b), as expected. However, there was a decrease in crude fiber content of extruded high ash flour except when extrusion was done at highest in-barrel moisture content (24%) and high temperature (110°C) conditions (Figure 2.2c). Extrusion at combinations of 18-21% moisture and 70-90°C caused up to 50% decrease in the fiber content. These low temperature low moisture content combinations create high shear condition in the barrel due to restricted flow of the material. High SME and shearing action during extrusion resulted in destructions of some of the cellulosic material more soluble and thus make fiber difficult to detect. Martin-Cabrejas et al. (1999) reported a decrease in insoluble fiber when beans were extruded at lower moisture content. However, Robin et al. (2011) reported no change into total, insoluble and soluble fiber after extrusion of wheat flour with added bran extruded in which the and SME about 650 kJ/kg (highest) and 260 kJ/kg (lowest).

2.3.2 Specific Mechanical Energy

The basic concept behind specific mechanical energy (SME) is to measure the energy going into the extrusion system per unit mass. This energy that is put into the extrudate through

viscous dissipation is primarily converted to heat in the extruder. The energy input is responsible for chemical reactions such as gelatinization of starch, denaturation of protein. The independent and dependent extrusion (process) variables affect these physical parameters. In extrusion process, the critical parameters such as thermal, mechanical energies and retention time are better predicators of the properties of the extrudates (Huber 2000).

At feed moisture content of 18% and barrel temperature of 90°C, specific mechanical energy increased with decrease in ash content (Table 2.2). SME increased with increase in extrusion temperature. This further increases the melt viscosity resulting in longer residence times leading to even higher SME (Huber 2000). When extrusion temperature (thermal energy input) is increased, the level of starch degradation is increased. A more degraded starch is more viscous due to starch swelling. Higher viscosity leads to higher SME, causing even further starch gelatinization (Gropper et al. 2002; Politz et al. 1994). Starch is the dominant component and it is responsible for higher viscosity and therefore higher SME. As starch content decreased (fiber, protein and ash increased), the viscosity and SME decreased as well. Zhu et al. (2010) also noted decrease in SME when added soy protein content was increased, which was attributed to both lipids and fiber. High ash flour extruded at temperature of 90°C had a decrease then an increase in SME as in-barrel moisture was varied from 18 to 21 then to 24%. Extrusion at the lowest (18%) in barrel moisture resulted in greater shear, viscosity and therefore higher SME. At medium moisture (21%), there was a decrease in both shear and viscosity.

2.3.3 Physical Properties of Extrudates

The expansion ratio, specific length, and piece density results are show in (Table 2.2.). There was a decrease in expansion ratio with increase in flour ash content and increase in extrusion temperature. The high ash flour fractions had more protein and fiber compared to low ash samples. Protein has been reported to be a dilutent thereby interfering with starch expansion by preventing formation of continuous starch matrix (Robin et al. 2011; Allen et al. 2007; Anderson and Ng 2003; Moraru and Kokini 2003). Robin et al. (2011), who studied the influence of bran on protein and starch matrices, attributed the decrease in expansion to the ability of fiber to disrupt continuous starch matrices. Carrying out extrusion at the highest in-barrel temperature might have caused greater degree of starch degradation that were not able to sustain pressure

generated from moisture which caused gas cells to collapse (Camire 2000; Blanche and Sun 2004).

Expansion ratio has been reported to decrease with increase in feed moisture in extrusion cooking. Expansion of extrudates is associated with pressure differential between exit and atmosphere (Chang and Ng 2011). Samples extruded at higher moisture were subjected to lower shear, had lower viscosity and shorter residence time. Therefore samples treated at the highest hydration (24%) probably had lower pressure differential, lesser flashing off than those extruded at lower moisture. It is also possible that extrusion at higher moisture leads to greater shrinkage. Sample treated at highest in-barrel moisture content was probably subjected to lowest shear and due to lower viscosity as well as lower degree of starch dextrinization/gelatinization in the extrusion process which explains the lowest specific length. As expected, the piece density results were the reverse of expansion ratio results. Piece density has been reported to increase with increase in barrel temperature. This is attributed to a decrease in melt viscosity (Chang and Ng 2011; Robin et al. 2011). High moisture leads to lower SME, and lesser starch gelatinization, therefore a less expanded product characterized by less radial and longitudinal expansion and increased piece density (Anderson and Ng 2003). There was a decrease in piece density with increase in ash content as well as decrease in piece density with increase in in-barrel moisture.

2.3.4 Particle size Analysis and Grinding Pattern

Unextruded low and medium ash flour fractions had narrow particle size distribution with a mean diameter of 68 μm whereas high ash flour had wider particle size distribution (Figure 2.4a). The high ash flour fractions were mostly obtained from last stages of milling process. Bran is reported to be elastic and difficult to mill which explains the wider particle size distribution for high ash flour fractions with larger particle being attributed to bran (Hemery et al. 2010).

The extrudate mechanical properties directly influence deformation behavior during grinding. Both independent and intermediate extrusion process parameters directly influenced extrudate mechanical properties which in turn influenced their grinding efficacy, thus ground products particle size distribution. Extrudates made from low and medium ash flours were harder to grind and had similar particle size distribution of the ground stock (Figure 2.3a). Extrusion of high ash flour at the highest temperature and the highest in-barrel moisture resulted in extrudates that were harder to grind (Figure 2.3b and 2.3c). As the starch content, treatment temperature and

extrusion in barrel moisture were increased, particle size distribution of flour of extrudates increased. Starch is responsible for retrogradation therefore hardness of extrudates. Santillan-Moreno et al. (2011) reported that corn starch-whey blend without fiber had a higher penetration force compared to blends with added agave fiber. According to Xanthos (2005), fiber influences mechanical properties of extrudates whereby applied shear stress causes rupture at the fiber-starch interface. High ash samples were easy to grind because ash, fiber and protein prevent and disrupted continuous starch matrix (Xanthos 2005; Robin et al. 2011 and Santillan-Moreno et al. 2011). High temperature, high in-barrel moisture combinations resulted in dense glassy extrudates that were harder to grind. High thermal energy input meant greater SME, therefore greater starch degradation which resulted in greater extrudate expansion and shrinkage (Blanche and Sun 2004). Such extrudates are dense, difficult to grind leading to larger particles size. Extrusion at the lowest (18%) in barrel moisture resulted in greater higher viscosity, greater shear and longer residence time. The resulting extrudates had greater degree of gelatinization and high expansion ratio, thus they were easy to mill and therefore created smaller particles compared to those extruded at the highest (24%) in barrel moisture (Figure 2.3c).

2.3.4. Differential Scanning Calorimetry

Control (unextruded) samples gelatinization enthalpy decreased with increase in ash content. Low, medium and high ash flour fractions had 1.313, 1.136 and 0.569 J/g of gelatinization enthalpies, respectively (Figure 2.5a). The decrease could be attributed to low starch content of low ash flour fractions because enthalpy change is related to energy needed to gelatinize the starch. Also it is possible that there was greater starch damage in high ash flour compared to low and medium ash flour fractions since former is obtained at later stages of milling. This means that the high ash flour was subjected to greater mechanical damage for a longer time.

There was no difference in the initial, peak, final gelatinization temperatures and enthalpies of extruded flours (Figure 2.5b, 2.5c and 2.5d). The extruded samples had complete gelatinization as indicated by absence of peaks and zero enthalpy values indicating nonexistence of ordered starch structure. Several authors have also reported complete starch gelatinization after extrusion (Blanche and Sun 2004; Chang and Ng 2011; Robin et al. 2011).

2.3.5. X-ray Diffraction

The X-ray diffraction pattern of control (untreated) and extruded flour fractions are presented in Figure 2.6. The typical A-pattern that is normally observed for native cereal starches can be observed for the untreated flour fractions. This is due to the present of a semi-crystalline structure of amylopectin inside the starch granules (Zobel, 1988). The control flour samples exhibited A-type crystallinity peaks at 2θ of 15, 17.9, 19.8, 23, and 26.7° (Figure 2.6a). However, the A-pattern was devastated by the extrusion process. After extrusion, there was only V-type crystallinity at 2θ around 13 and 19° (Figures 2.6b, 2.6c and 2.6d). The V-type is attributed to formation of amylose-lipid complexes due to extrusion process (Hoover 2001). The high ash and medium ash flour fraction seem to retain the peak intensity at 2θ ~13° although this was absent in low ash flour fraction. The degree of crystallinity of treated flours was significantly much less than determined for the control samples.

2.3.6. Swelling Power and Solubility Indices

Swelling power and solubility are used to assess the degree of starch damage due to gelatinization and fragmentation of starch during high temperature, high shear treatment. The swelling power (i.e. water holding capacity) measures the amount of water held by the sample after its dispersion in excess water for a given time at a given temperature. The solubility index measures the amount of soluble components.

The swelling power of unextruded flour fractions decreased with increasing ash content. Swelling power of low, medium and high ash flours were found to be 4.7, 4.5 and 4.2 (g/g), respectively (Figure 2.7b). There was an inverse relationship between the solubility of unextruded flour fractions and their ash contents. The solubility values increased from 6.2 to 9.2 % as the ash content increased from 0.47 to 1.34%.These results can be attributed to compositional differences. As explained before, gradual reduction process in wheat flour milling creates a flour fraction at the end of each grinding-sifting step. Since these fractions are extracted from different locations in wheat kernel and they experience varying length and intensity of process history, the resulting flour fractions exhibit varying compositions of starch, damaged starch, protein, ash and other minor constituents. Irrespective of their type, the swelling power and solubility of unextruded flour samples increased with increase in incubation temperature, as

expected. The swelling power increase rate was high between 60 and 80°C whereas solubility was high between 80 and 90°C.

Table 2.3 shows that extruded flour fractions had significantly higher water solubility and absorption indices compared to the untreated flour fractions. This can be attributed to the destruction of starch granules, reduction of the degree of crystallinity, and degradation of starch molecules during pre-gelatinization. The porous structure of treated starches has been reported to readily absorb more water compared to the native starches (Srichuwong et al. 2005). Slaughter et al. (2001) reported higher water solubility and swelling for fully gelatinized wheat, maize, and rice starches. Higher solubility and water absorption values were also reported for pre-gelatinized banana starch by Waliszewski et al. (2003).

Extrusion process caused a greater increase in swelling and solubility of flour samples. The swelling power and solubility for extruded samples decreased with increase in ash content, extrusion temperature and increase in in-barrel moisture (Figure 2.7). Observed decrease in swelling power with decrease in starch content is expected because starch is the main component contributing to swelling (Robin et al. 2011). Other constituents such as lipids, protein influence gelatinization through formation of complexes thereby reducing starch's swelling ability (Belitz et al. 2009).

2.3.7. Pasting Characteristics

The pasting curve obtained from a RVA is a measure of the viscosity of starch or cereal suspension during the heating cycle, which reflects the molecular events occurring in the starch granules. Therefore, the integrity of starch granules and hydration properties resulting from the starch native properties, or from the inter- or intra-molecular interactions during thermo-mechanical can be easily investigated by measuring the pasting curves before and after modification.

Figure 2.8 shows the RVA patterns of the extruded and untreated flour fractions. The pasting curve of high ash flour before extrusion was significantly different than that of medium and low as flour samples (Figure 2.8a). There was maximum starch swelling of unextruded flour samples with peak viscosities of 2426, 2369 and 1600 cP at 95°C for low, medium and high ash fractions, respectively. Final viscosities untreated low, medium and high ash flour fractions were around 3300, 3200 and 2200 cP, respectively. High ash flour experienced slight delay in the

gelatinization; however it had earlier peak time compared to low and medium ash flours. These differences could be attributed both composition and the milling history of these flour fractions. High ash flour fractions not only contain less starch but also exhibit higher intensities of mechanical damage (i.e. higher starch damage). As mentioned before, dry milling is a continuous gradual reduction process, where high ash flour fractions are collected at the later end of the grinding-sifting steps.

Extrusion process caused a dramatic change in the pasting profiles (Figures 2.8b, 2.8c and 2.8d). None of the extruded samples exhibited a peak viscosity upon elevated pasting temperatures. Instead, they developed an instant viscosity ranging from 400 to 1000 cP at low temperature. No peak was seen for the untreated samples at this temperature. Nakorn et al. (2009) studied pasting properties of pre-gelatinized rice starch using RVA. They also found that a cold peak viscosity can be obtained for pre-gelatinized rice starch of high amylose content. The results indicate that the extruded samples had the ability to increase the viscosity at temperatures below gelatinization temperature of native starch. However, if extruded flour fractions are heated and then cooled down, they produce much lower final viscosity (250-350 cP) than untreated flours (2200-3300 cP).

Chang and Ng (2011) and Blanche and Sun (2004) also reported that extrusion cooking caused starch degradation which resulted in lower pasting peak viscosity. Extrusion caused dramatic decrease in peak viscosity as a result of starch gelatinization as evidenced by viscosity development within the first 2 min (versus 6 min) of mixing (Figures 2.8a, 2.8b).This indicates the ability of extruded flour to form cold pastes. The differences in pasting behavior of unextruded samples can be attributed to their composition of starch, high protein and fiber contents. Robin et al. (2011) reported high pasting viscosities in extruded samples without bran compared to those with bran inclusions. Highest in-barrel temperature (110°C) decreased paste viscosity compared to products extruded at 70 or 90°C. Samples extruded at the highest in-barrel moisture (24%) exhibited lowest peak viscosity and had a distinct secondary pasting viscosity. Starch gelatinizes faster in higher moisture (Robin et al. 2011). The observed secondary peak could be from partially gelatinized starch granules. Although starch gelatinization is faster at lower moisture (Robin et al. 2011), highest in-barrel moisture caused shorter residence time and low shear viscosity. This might have countered this higher moisture effect in fastening starch gelatinization resulting in high quantities partially gelatinized starch granules. Blanche and Sun

(2004) also reported that corn starch extruded at (30 vs 24%), low screw speed (300 vs 400 rpm), medium shear screw configuration had lower starch degradation. The remaining un-gelatinized starch after extrusion would increase in hot paste viscosity

2.4. Conclusion

Extrusion process imparted a number of morphological and textural characteristics to the extruded flour fractions, reflecting the intensity of the treatment. Differences in flour ash content influenced both process (e.g. SME) and product parameters. High ash flour fractions required less SME to cook, they were easy to grind and more stable under shear. Therefore thermomechanical treatment of low grade high ash flour fractions through extrusion process could change their functionality such as pasting characteristics and shear stability, and thus add value. Such flour can be "clean labeled" as natural functional wheat products that be used in batter and dough-based food systems.

In this study, extruded products had the following properties: coarser granular structure, low crystalline structure, high cold water viscosity, and high water solubility and absorption. The results support the notion that the integrity of starch granules has a great contribution to the rheology of starch pastes. Treated flours produced under different extrusion conditions and with varying ash content had dramatically different rheological properties that should be considered before application. Based on these properties, such treated flours can be used mainly as a thickening and gelling agent in refrigerated and instant foods or heat sensitive products such as cold desserts, salad dressing, cake and bakery mixes, and baby foods. Treated flours have ability to develop viscosity in the solutions or mixes in which it is being used without any heat treatment.

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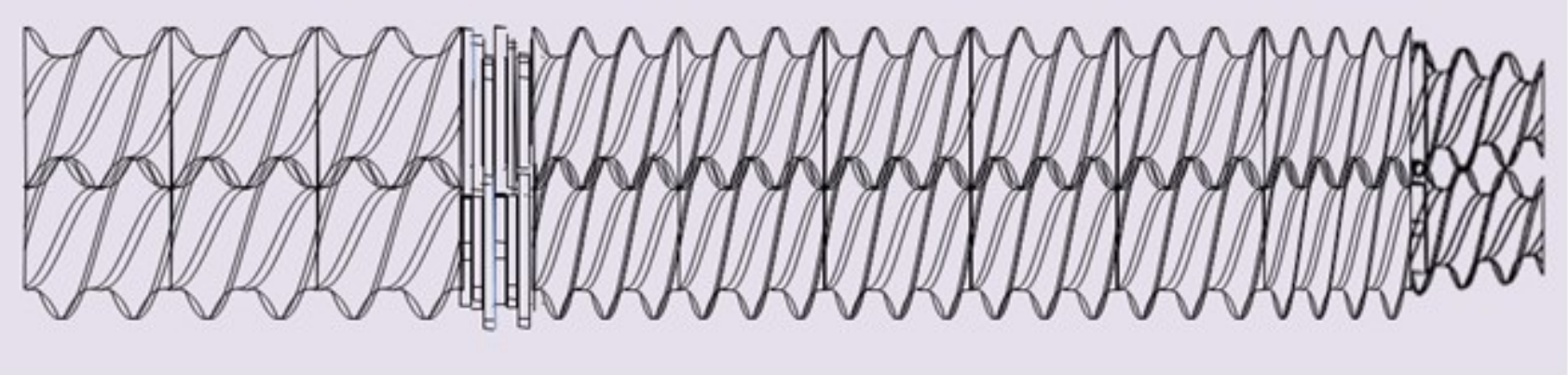
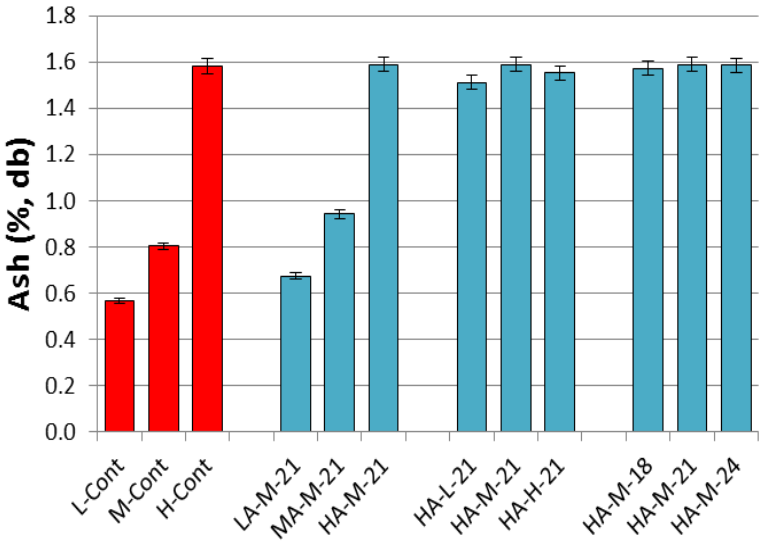
Head Number	1	2	3	4	5	6
Barrel Temperature (°C)	40 50 60	40 50 60	50 70 80	60 80 90	60 80 90	70 90 110



FPS, **78mm**	FPS, 78mm	FPS, 78mm	6FKB, 8.7mm (total 52.2 mm)	3/4th pitch, 78mm	3/4th pitch, 78mm	3/4th pitch, 78mm	3/4th pitch, 78mm	3/4th pitch, 78mm	½ pitch, 52mm	3/4th pitch, cone, 78mm
FSE: forward conveying screw element (all double flighted, intermeshing) FKB: forward kneading block										

Figure 2.1. Screw configuration and temperature profile. All elements double flighted, except for first two elements on right shaft (single flight)



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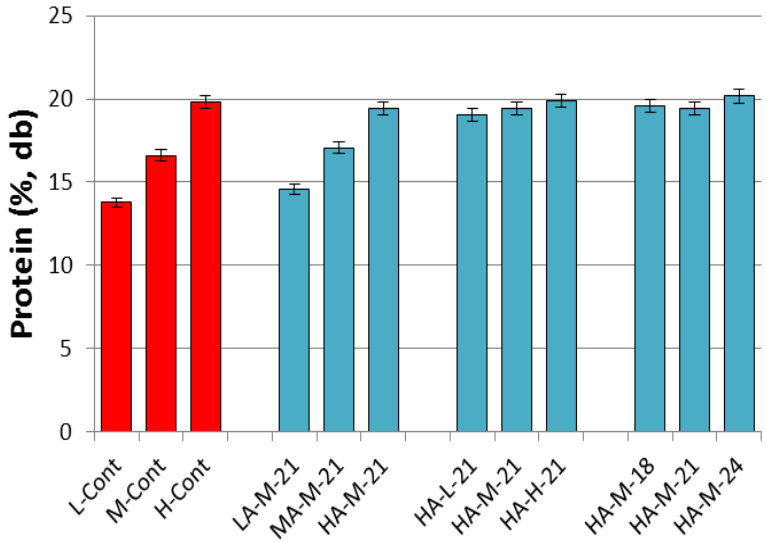

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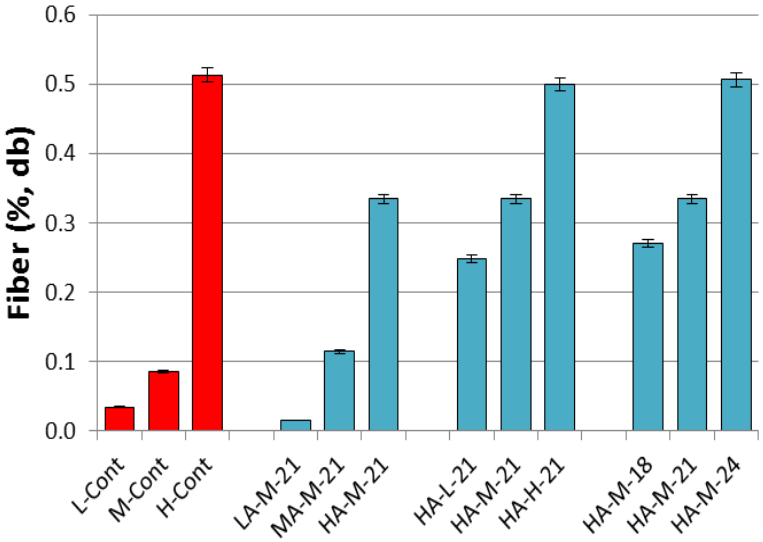
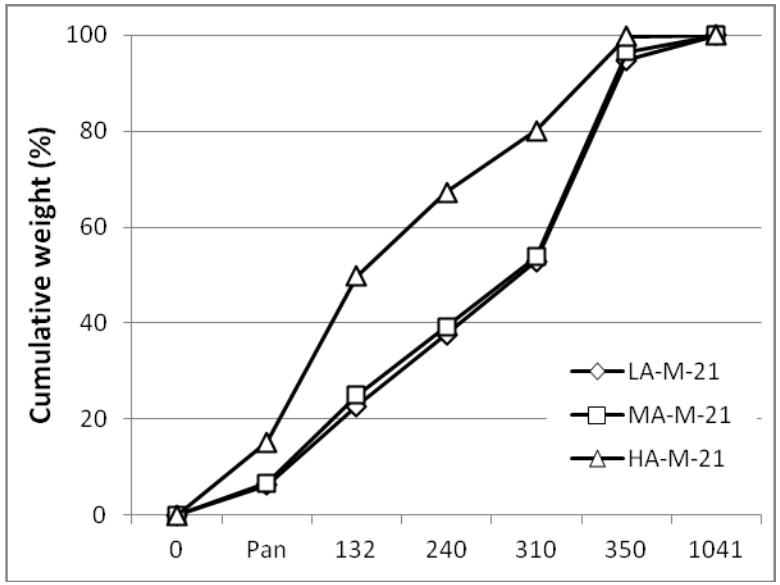
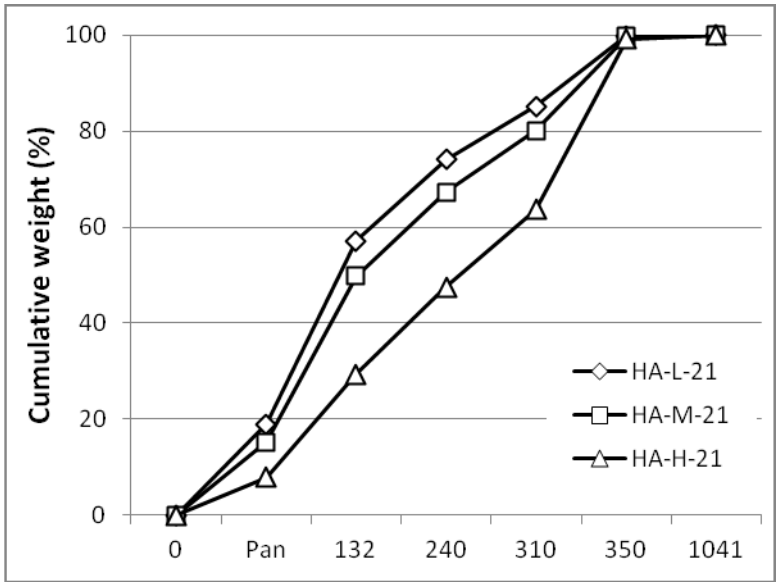


(c)

Figure 2.2. Composition of flour fractions before and after extrusion (a) ash content, (b) Protein content, (c) fiber content.



(a)



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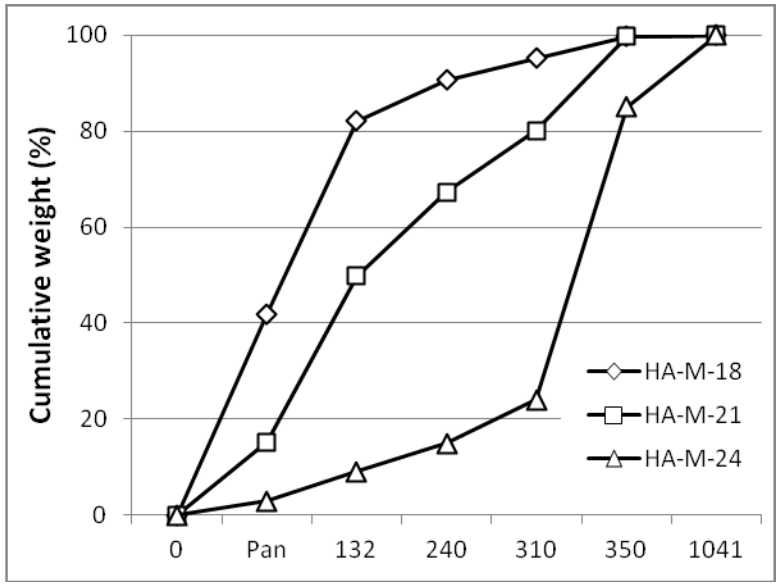


(c)

Figure 2.3. Grinding yield of extruded flours with respect to (a) flour ash content, (b) extrusion temperature, (c) feed moisture content.

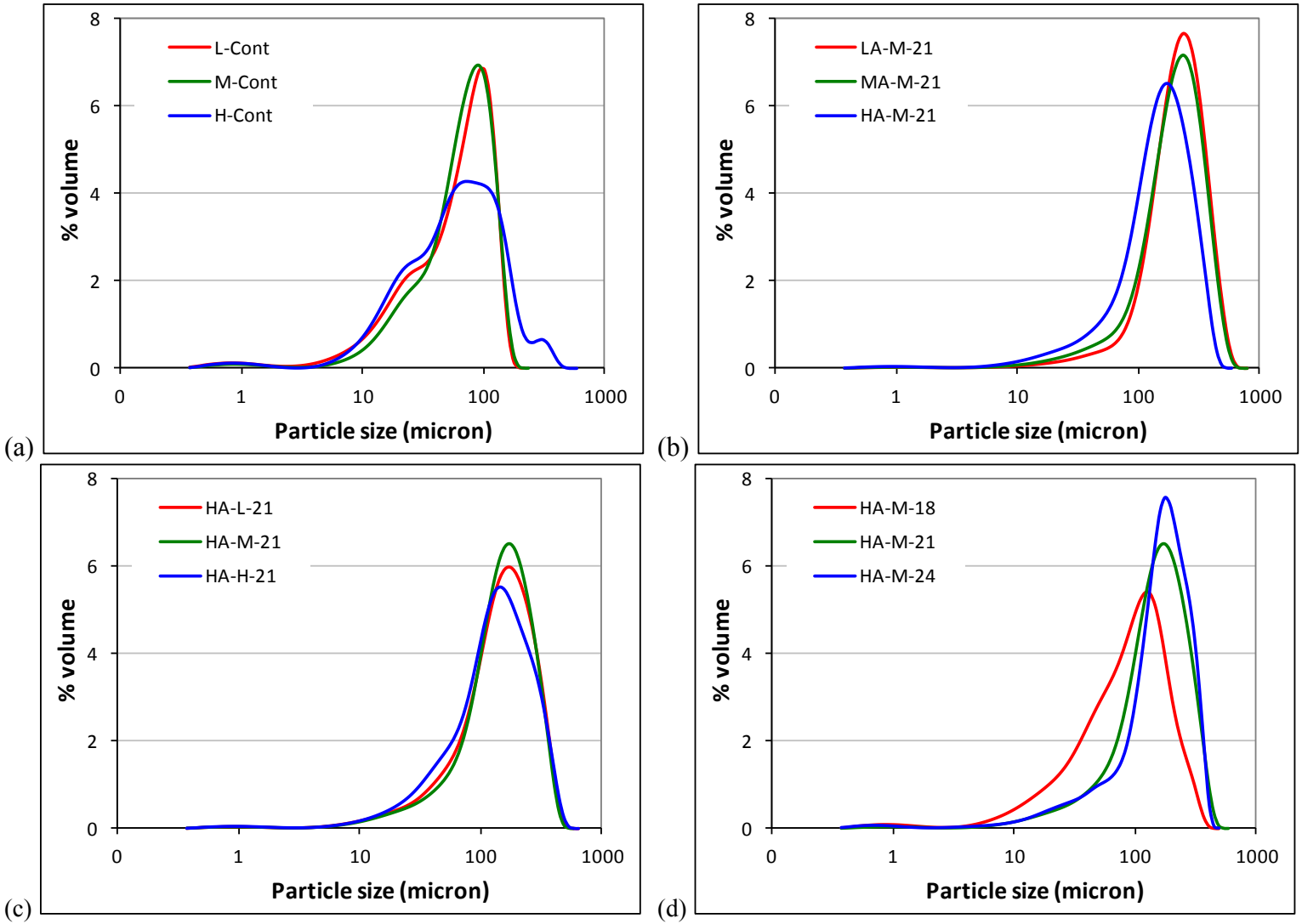


Figure 2.4. Particle size distribution of (a) control samples, and extruded flours with respect to (b) flour ash content, (c) extrusion temperature, (d) feed moisture content.

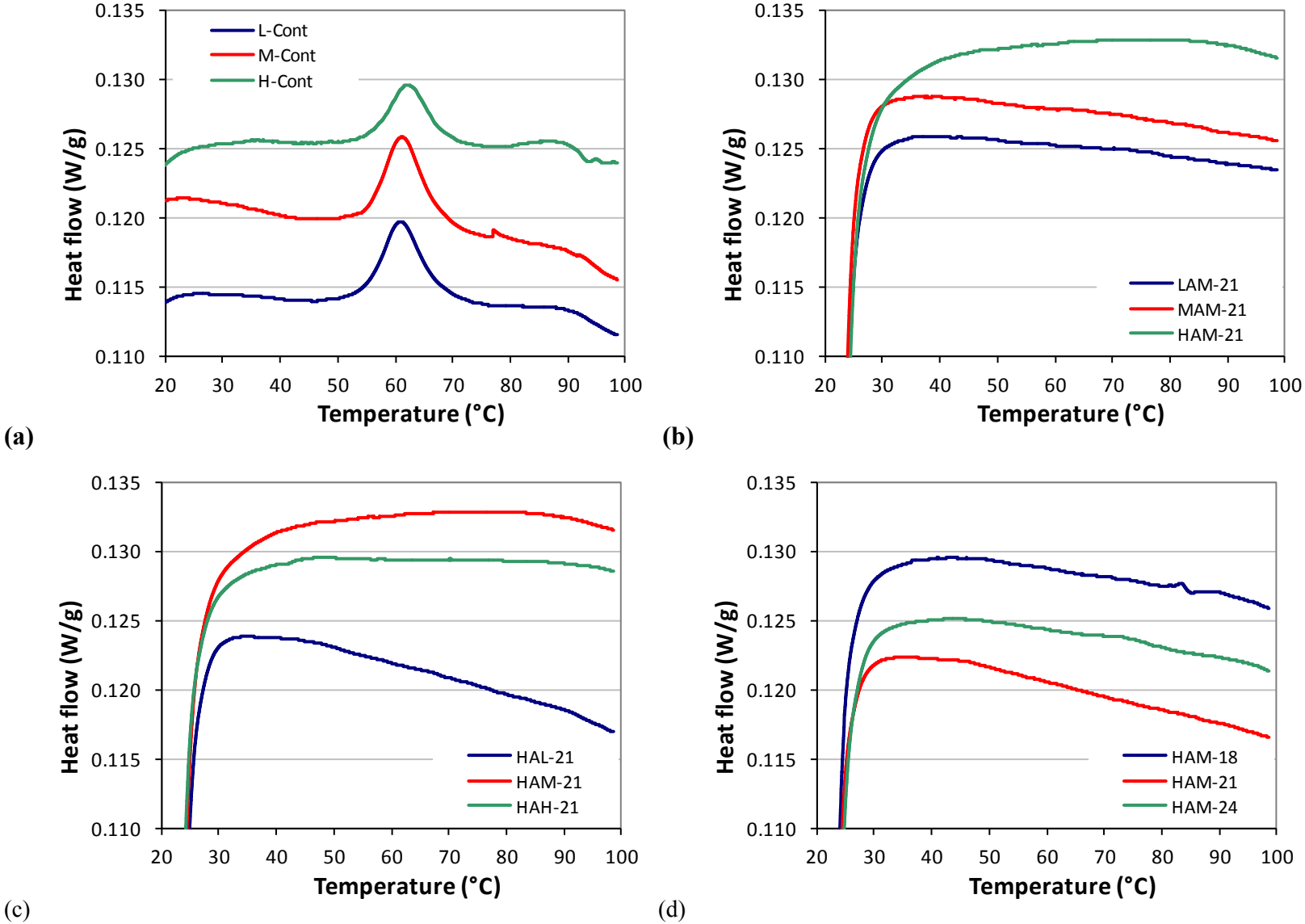


Figure 2.5. DSC thermograms of control samples, and extruded flours with respect to flour ash content, extrusion temperature, (d) feed moisture content.

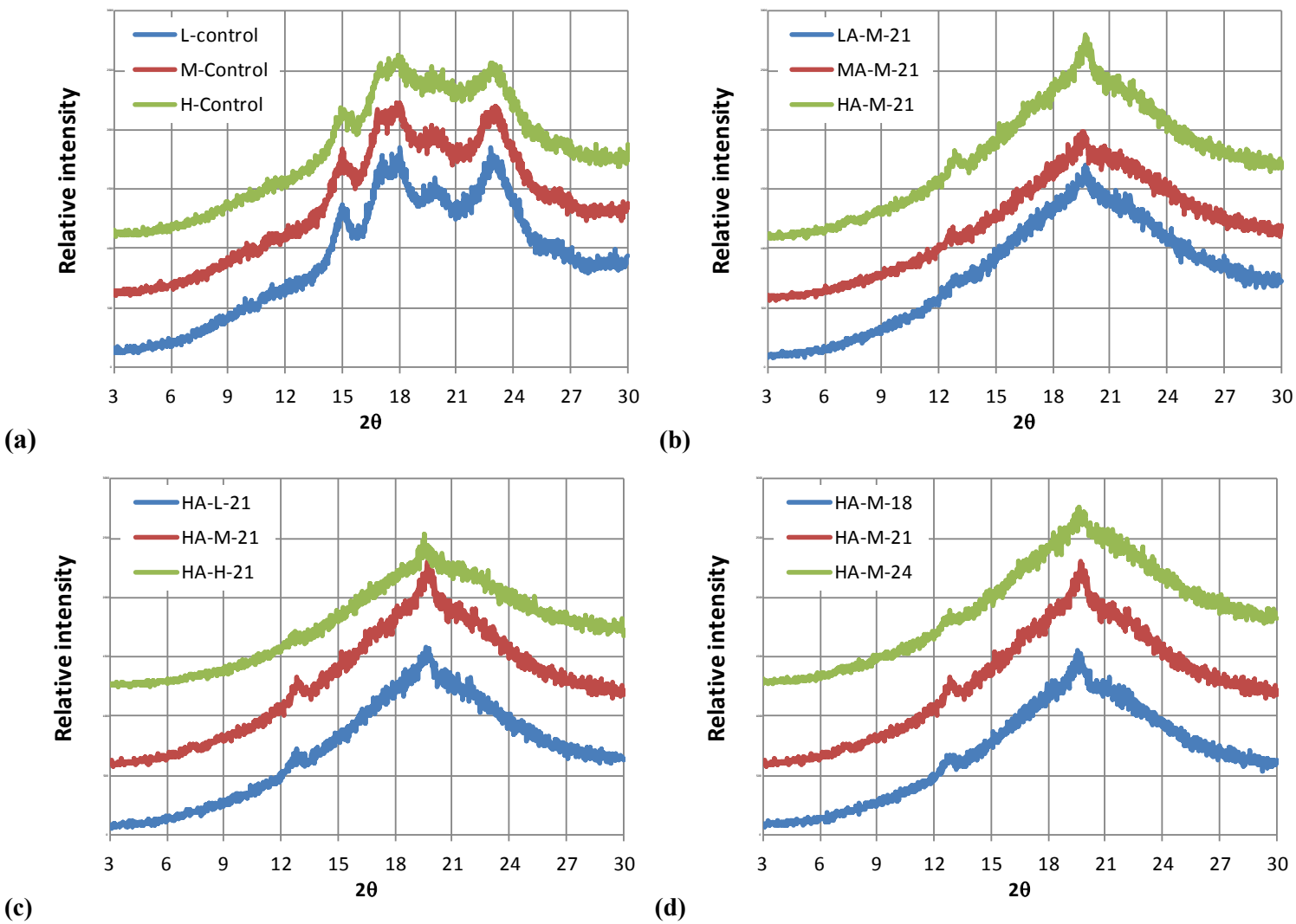
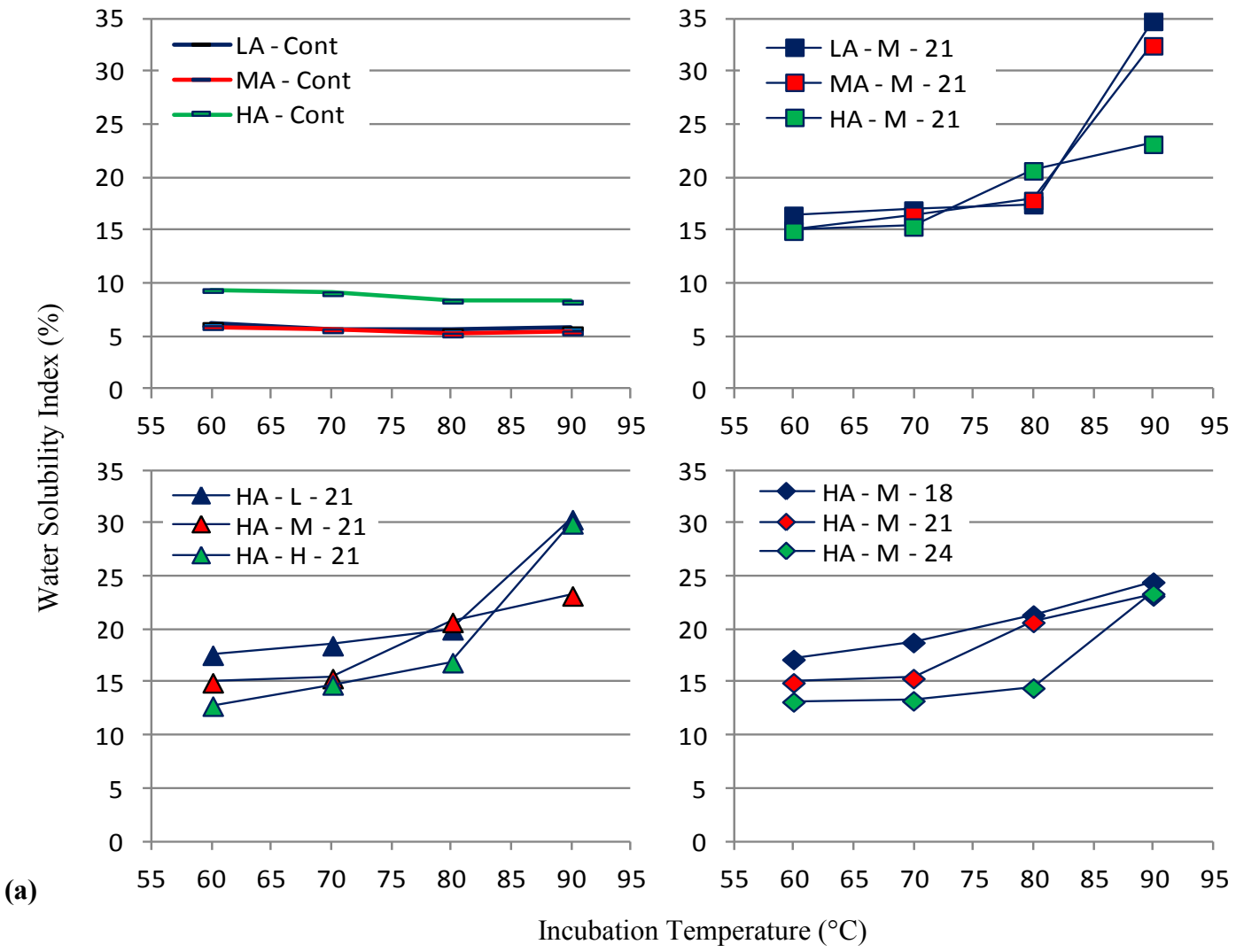
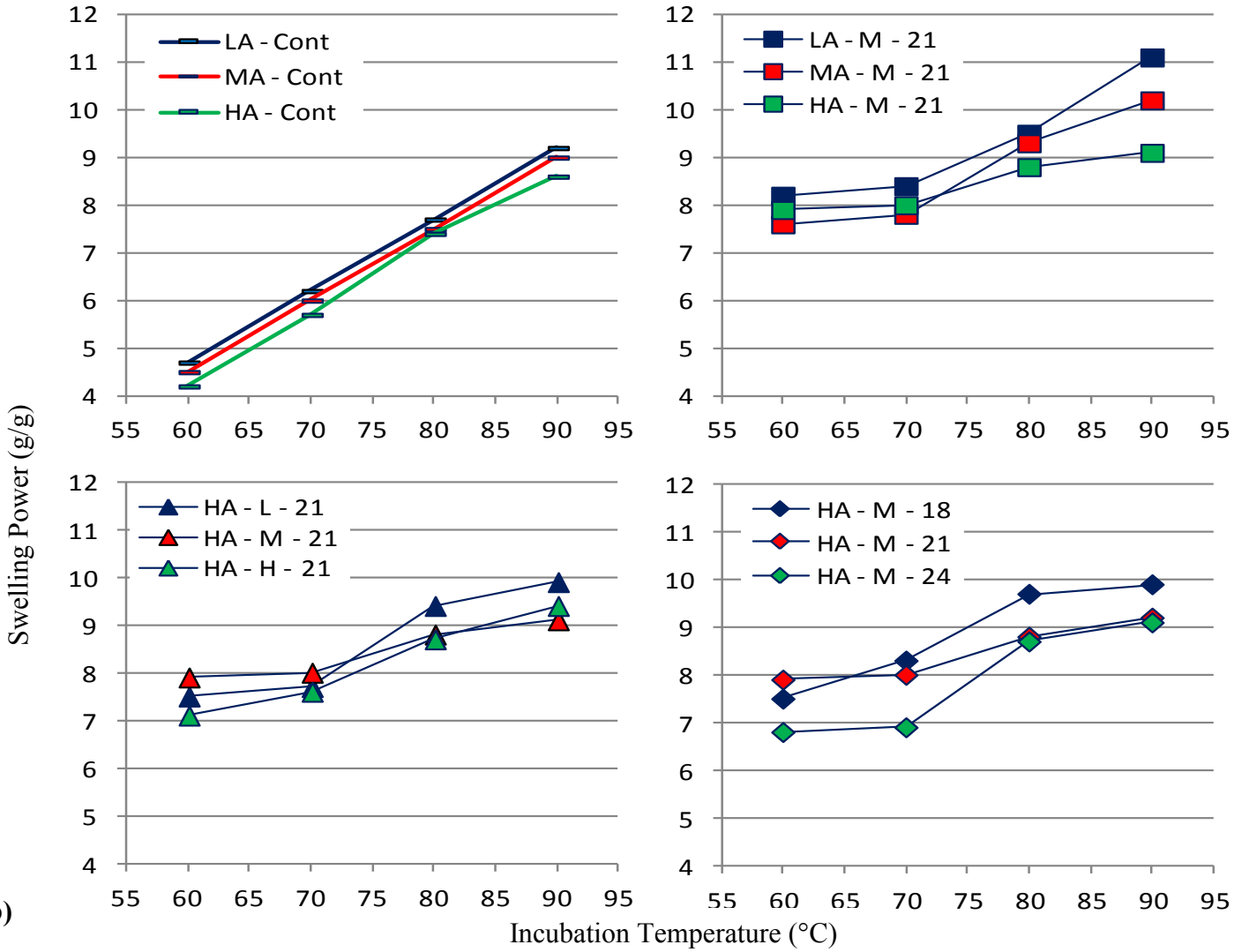


Figure 2.6. X-ray diffractograms (a) control samples, and extruded flours with respect to (b) flour ash content, (c) extrusion temperature, (d) feed moisture content.


Water Solubility Index (%)
LA - Cont
MA - Cont
HA - Cont
LA - M - 21
MA - M - 21
HA - M - 21
HA - L - 21
HA - M - 21
HA - H - 21
HA - M - 18
HA - M - 21
HA - M - 24
Incubation Temperature (°C)

(a)



(b)

Figure 2.7. Swelling power (a) and solubility (b) of control samples, and extruded flours with respect to flour ash content, extrusion temperature and feed moisture content.

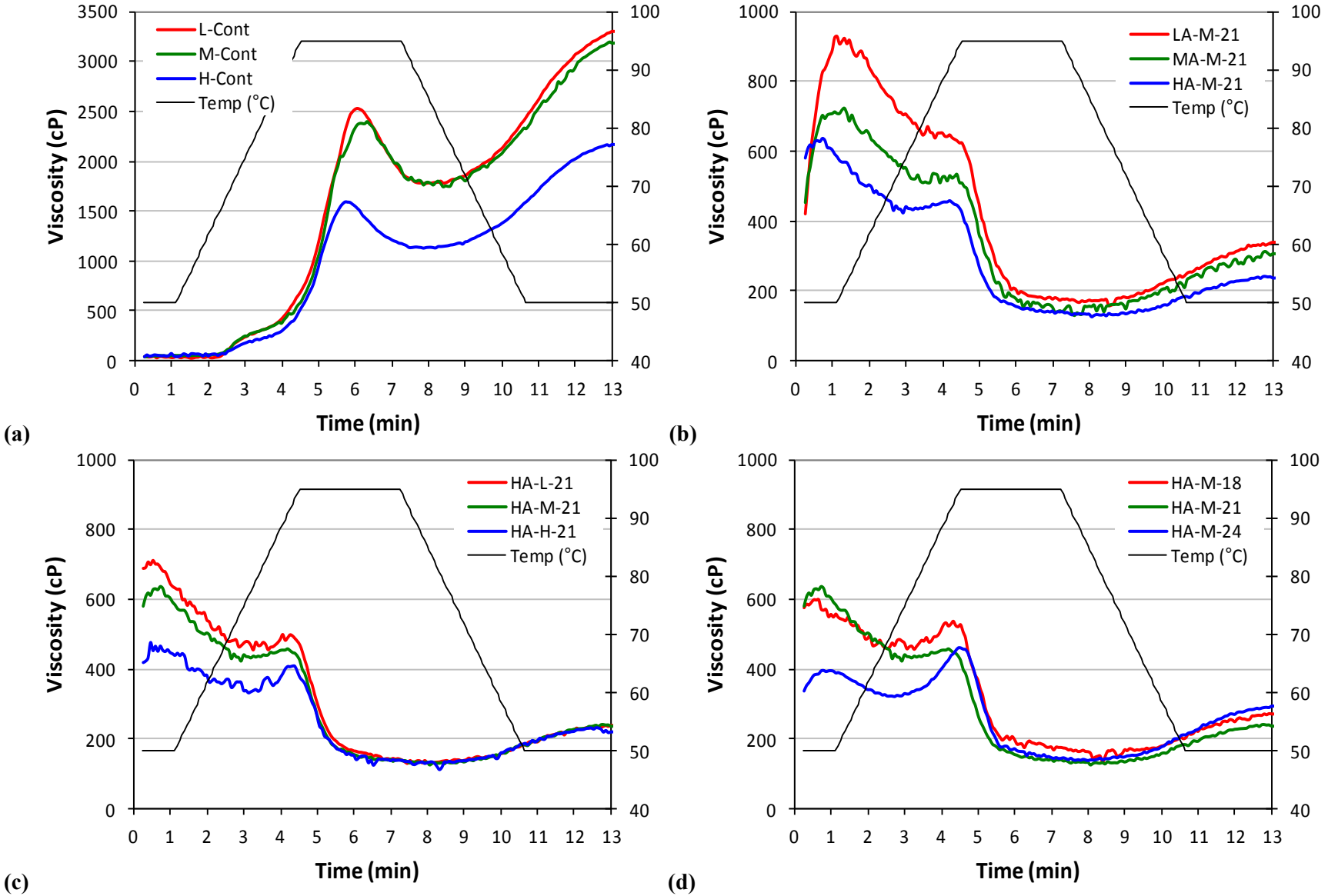


Figure 2.8. Pasting properties of (a) control samples, and extruded flours with respect to (b) flour ash content, (c) extrusion temperature, (d) feed moisture content.

Table 2.1. Experimental design

Treatment ID	**Ash content (%)**		**Extrusion temperature (°C)**		**Feed moisture content (%)**	
LA - M - 21	**LA - Low**	**(0.47%)**	M – Medium	(90°C)	Medium	(21%)
MA - M - 21	**MA - Medium**	**(0.64%)**	M – Medium	(90°C)	Medium	(21%)
HA - M - 21	**HA - High**	**(1.34%)**	M – Medium	(90°C)	Medium	(21%)
HA - **L** - 21	HA - High	(1.34%)	**L – Low**	**(70°C)**	Medium	(21%)
HA - **M** - 21	HA - High	(1.34%)	**M – Medium**	**(90°C)**	Medium	(21%)
HA - **H** - 21	HA - High	(1.34%)	**H – High**	**(110°C)**	Medium	(21%)
HA - M - **18**	HA - High	(1.34%)	M – Medium	(90°C)	**Low**	**(18%)**
HA - M - **21**	HA - High	(1.34%)	M – Medium	(90°C)	**Medium**	**(21%)**
HA - M - **24**	HA - High	(1.34%)	M – Medium	(90°C)	**High**	**(24%)**

Table 2.2. Extrusion process and product parameters

Treatment ID	SME (kJ/kg)	Expansion ratio (-)	Piece density (g/cm^3)	Specific length (cm/g)
LA - M - 21	242	8.28±0.091^{a}	0.38±0.015^{bc}	2.24±0.196^{a}
MA - M - 21	233	5.97±0.063^{b}	0.40±0.025^{ba}	2.23±0.071^{ba}
HA - M - 21	203	7.08±0.067^{b}	0.34±0.030^{c}	2.35±0.109^{a}
HA - **L -** 21	195	7.92±0.075^{ba}	0.33±0.032^{c}	2.49±0.160^{ba}
HA - **M -** 21	203	7.08±0.067^{b}	0.34±0.030^{c}	2.35±0.109^{a}
HA - **H -** 21	224	6.58±0.022^{b}	0.33±0.019^{c}	2.49±0.173^{ba}
HA - M - **18**	222	6.62±0.069^{b}	0.33±0.008^{c}	2.94±0.070^{a}
HA - M - **21**	203	7.08±0.067^{b}	0.34±0.030^{c}	2.35±0.109^{a}
HA - M - **24**	220	5.69±0.060^{b}	0.44±0.034^{a}	2.15±0.095^{b}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 2.3. Swelling and solubility of control and extruded samples at 60-90°C

Treatment ID	60°C		70°C		80°C		90°C	
	Swelling	**Solubility**	**Swelling**	**Solubility**	**Swelling**	**Solubility**	**Swelling**	**Solubility**
L - Control	4.7±0.1^{e}	6.2±0.1^{d}	6.2±0.0^{bdc}	5.7±0.3^{e}	7.7±0.0^{ed}	5.6±0.0^{i}	9.0±0.6^{bdc}	5.8±0.0^{f}
M - Control	4.5±0.1^{e}	5.9±0.1^{d}	6.0±0.1^{dc}	5.6±0.3^{e}	7.5±0.1^{c}	5.2±0.0^{i}	8.5±0.2^{d}	5.4±0.2^{f}
H - Control	4.2±0.1^{e}	9.4±0.1^{c}	5.7±0.1^{c}	9.1±0.1^{ed}	7.4±0.1^{c}	8.4±0.1^{h}	9.0±0.2^{bdc}	8.3±0.4^{e}
LA - M - 21	8.2±0.1^{a}	16.5±0.9^{a}	8.4±0.2^{a}	17.6±0.9^{a}	9.5±0.1^{bac}	17.5±0.4^{bc}	11.1±0.1^{a}	34.8±1.4^{a}
MA - M - 21	7.6±0.1^{bac}	15.1±0.6^{ba}	7.8±0.3^{bac}	17.9±0.8^{a}	9.3±0.0^{bac}	16.5±0.4^{dc}	10.2±0.0^{bac}	32.5±2.1^{ba}
HA - M - 21	8.0±0.2^{ba}	15.0±0.4^{ba}	8.0±0.1^{ba}	15.4±0.4^{bac}	8.8±0.0^{bdc}	20.7±0.6^{a}	9.1±0.1^{bdc}	23.2±0.9^{dc}
HA - **L -** 21	7.5±0.1^{bdac}	17.6±0.8^{a}	7.7±0.9^{bac}	18.5±0.3^{a}	9.4±0.0^{bac}	20.0±0.1^{ba}	9.9±0.0^{bdac}	30.4±1.5^{bac}
HA - **M -** 21	8.0±0.2^{ba}	15.0±0.4^{ba}	8.0±0.1^{ba}	15.4±0.4^{bac}	8.8±0.0^{bdc}	20.7±0.6^{a}	9.1±0.1^{bdc}	23.2±0.9^{dc}
HA - **H -** 21	7.6±0.1^{bdac}	16.9±0.0^{a}	7.1±0.3^{bdac}	14.8±0.4^{bac}	8.7±0.3^{bdc}	12.8±0.7^{gfe}	9.4±0.0^{bdc}	30.0±1.9^{bac}
HA - M **- 18**	7.5±0.1^{bdac}	17.2±0.0^{a}	8.3±0.1^{a}	18.8±0.8^{a}	9.9±0.0^{a}	21.4±0.2^{a}	9.7±0.3^{bdac}	24.5±1.4^{bc}
HA - M **- 21**	8.0±0.2^{ba}	15.0±0.4^{ba}	8.0±0.1^{ba}	15.4±0.4^{bac}	8.8±0.0^{bdc}	20.7±0.6^{a}	9.1±0.1^{bdc}	23.2±0.9^{dc}
HA - M **- 24**	6.8±0.0^{d}	13.8±0.6^{b}	6.9±0.1^{bdac}	13.2±0.1^{bdc}	8.7±0.5^{bdc}	14.5±0.3^{dfe}	9.1±0.1^{bdc}	23.4±0.9^{bdc}

Means in columns with same letters are not significantly different ($P > 0.05$)

Chapter 3 - Indirect Continuous Heat Treatment of Wheat Grain and Whole Wheat Flour

Abstract

Thermal energy has been used in processes like heating and cooling to preserve and protect food for a long time. High temperature applications are mostly used to kill pathogens, spoilage microbes, inactivate enzymes. Other beneficial functions of heating include improvement in flavor, texture and color. The primary effect of heat treatment is denaturation of the proteins, partial reduction or inactivation of alpha-amylase and partial gelatinization of the starch. Understanding of relationship between heat transfer, thermal properties of food, heating medium, thermodynamics and the functionality of the resulting heat treated flour is of critical importance for the food processor. The *overall objective* of this study was to investigate the effects of direct, rapid and continuous thermal processing techniques on the functionality of wheat flours. The *specific objectives* were to characterize the heat treated flours for their mixing, pasting, and baking performance; and explore the potential use of these new products in dough and batter-based food formulations. Whole wheat flour or whole wheat grain hydrated to12, 16 and 20% moisture was subjected to indirect heat treatment at 75, 85 and 95°C for 0.5, 1.0 and 1.5 min via a steam jacket thermal heat processing unit. Treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF) samples were analyzed for their mixing and pasting profiles using the MixoLab for protein molecular size distribution by size exclusion-HPLC. Treated flours were subjected range of characterization tests including particle size analysis, proximate analysis, water holding capacity and solubility, pasting (RVA), degree of gelatinization (DSC), relative crystallinity (X-ray diffraction). Treated samples had lower pasting viscosity, lower crystallinity, and higher solvent retention capacity than control (untreated) flours. Treated flour was not able to develop into viscoelastic dough. Mixing time decreased with increase in treatment moisture and temperature. There was decrease in total extractable proteins compared to control. Adverse effects increased with increase in thermal and mechanical energy inputs, both of which were influenced by treatment temperature, residence time and moisture. Moisture and temperature were the most significant factors that influencing properties of starch in treated flours. These effects were more pronounced in treated whole wheat flour compared to treated whole wheat grain.

3.1. Introduction

Cereals are good sources of nutrients, specifically starch for energy and industrial applications, protein, vitamins, minerals and bioactive compounds. Wheat storage protein (gluten) is well known for its unique functional properties. It can form continuous, cohesive, viscoelastic protein networks which set to form the desirable texture characteristic of baked products (Delcour et al. 2012; Wieser, 2007; Singh and MacRitchie 2001). The viscoelastic property of wheat flour dough enables it to keep carbon dioxide generated by yeast during sugar fermentation, from leavening agent or air that is incorporated at mixing (Dobraszczyk et al. 2003; Shewry et al. 2002).

There are various sources of starch; cereal, root and tubers. Botanical source, variety and growth conditions can influence physico-chemical properties of the starch. Consistency in functional properties, their wider application in food and other industries functions necessitates starch to be modified to meet specific applications (Jacobs and Delcour 1998; Burrell 2003; Varatharajan et al. 2010). Chemical, physical and biological or their combination modification techniques methods have been applied to change inherent starch properties. Chemical modification techniques include cross linking, acetylation, acid hydrolysis, oxidation, substitution among others (BeMiller 1997). However increasing number of consumer desire that their foods have none or minimal chemical processing or chemical additives.

Physical methods of starch modification are gaining popularity due to their safety. Annealing and heat-moisture treatment and high pressure are among commonly used techniques to physically modify starch to improve functionality (Jacob and Delcour 1998; Maache-Rezzoug et al. 2008). Annealing and heat moisture treatments are the most common techniques used. These processes ensure that physico-chemical properties of starch are changed with no destruction to granular structure of the starch (Jacob and Delcour 1998). However, such processes are batch and require a longer time. Continuous and rapid techniques are needed to make heat treatment of whole grain products attractive to food processors.

Direct and indirect continuous heat treatments have been used in processes to preserve and protect food for a long time. High temperature applications are mostly used to kill pathogens, spoilage microbes, inactivate enzymes. Other beneficial functions of heating include improvement in flavor, texture and color. However, heat treatment can create adverse effects on the food material. The primary effect of heat treatment is denaturation of the proteins, partial

reduction or inactivation of alpha-amylase and partial gelatinization of the starch. Understanding of relationship between heat transfer, thermal properties of food, heating medium, thermodynamics and the functionality of the resulting heat treated flour is of critical importance for food processor.

Wheat flour can be physically or chemically modified to improve its functionality, such as pasting performance, cold viscosity, freeze thaw stability, gelling, shear stability. Native starch has poor resistance to shear, low thermal stability and has higher potential to retrograde (Hoover 2001). Functional applications of thermally treated starch include modifying food texture by acting as a gelling agent, thickener, colloidal stabilizer, bulking agent, and water retention. Other industrial application include pharmaceutical, paper, oil and adhesive (Copeland et al. 2009). Common chemical modification methods include esterification, oxidation and etherification (Singh et al. 2007). However, increasing numbers of consumers prefer "clean label foods" that have minimal or no chemical added to modify functionality or at processing (Cham and Suwannaporn 2010; Zavareze and Dias 2011). To meet such growing expectations, new processing technologies are required to improve the performance of wheat flour systems with minimum chemical ingredients.

There is an increasing demand for specialty flours with targeted quality and end-use. Increased consumer interest in clean label naturally functional products created a need for (i) developing continuous and rapid techniques for treating of whole grain products and (ii) providing equipment and process guidelines for the grain industry. Our objective is to conduct a systematic study on cause and effect relationships, and to identify relevant process parameters to serve as predictors of desired quality for specific end-use. Understanding the heat induced changes in starch and protein functionality can be used as a powerful design tool in developing thermal processes to modify intrinsic properties of native wheat flours for targeted end-use.

Our ultimate aim is to investigate the effects of rapid and continuous thermal processing techniques on the functionality of whole wheat flours. The main objective of this study was to develop an indirect, rapid and continuous process for treating whole wheat flour and whole grain to reduce microbial load while preserving or improving the flour functionality in targeted applications. The specific objectives were (i) to characterize the heat treated flours for their mixing, pasting, and baking performance; and (ii) to explore the potential use of these new products in dough and batter-based food formulations.

3.2. Materials and Methods

3.2.1. Material

About 5 tons of hard red winter wheat (HRW) grown in 2013 with 12.76% protein content and good for bread-making quality was purchased from a local farmer in Manhattan KS. It was cleaned at the cleaning house of the Hal Ross Mill, Manhattan, KS. Cleaned wheat was divided in two portions to perform two heat treatments routes (Figure 3.1):

(i) First milled to whole wheat flour, then heat treated: Referred as "*treated whole wheat flour*" or TWWF.

(ii) First heat treated, then milled to whole wheat flour: Referred as "*treated grain whole wheat flour"* or TGWWF.

3.2.2. Milling

3.2.2.1. Before Heat Treatment

About 2.5 tons cleaned hard red winter wheat was milled to whole wheat flour before heat treatment at Hal Ross pilot scale flour mill using a short-flow specifically design for the production of whole wheat flour.

3.2.2.2. After Heat Treatment

The other half of the wheat was first heat treated following the experimental design (Table 3.1). Treated wheat kernels were milled to whole wheat flour using combination of two mills; a Buhler experimental mill (Buhler 202, Uztwil Switzerland) and Comminutor FitzMill® (Fitzpatrick Company Elmhurst, IL). For the Buhler mill, the AACC-International procedure (Approved Method 26-21.02) was followed to obtain straight grade flour. The bran and shorts were further milled with a Comminutor FitzMill® below 215 μm size. These constituents were then blended back to obtain whole wheat flour using a cross flow blender (Patter-Kelly Company, East Stroudsburg, PA).

3.2.3. Heat Treatment

The whole wheat flour and whole wheat grain were shipped to Bepex International (Bepex International, LLC Minneapolis, MN). Both whole grain and whole wheat flour samples

were subjected to an indirect, short time and continuous heat treatment at various combinations of moisture content, temperature and time as outlined in the experimental design (Table 3.1).

The process unit was composed of feeder, turbulizer, heater, dryer, and cooler as shown in (Figure 3.2). The material was gravimetrically metered using a feeder (Brabender Technologie Inc., Mississauga Ontario, Canada). Calculated amount of water was added via a small pump at the first portion of the Turbulizer (Bepex International, LLC Minneapolis, MN). The mixture was dropped via gravity to the screw conveyor of the Turbulizer. The conveyor screw speed was reduced to 1.3 rpm when treating wheat grain in order to increase residence time to 4 minutes. The mixture was then conveyed to the Solidaire Model TCJS-8 (Bepex International, LLC Minneapolis, MN) heating unit. Three paddle settings were used in the Solidaire to adjust residence times. Treated material from Solidaire was dropped to a twin screw feeder and then to a PCX dryer (Bepex International, Minneapolis, MN). The PCX was turned off when heat treating the wheat grain. After drying, the materials entered a cyclone system via a rotary valve where further cooling occurred. It was then conveyed via a sanitary pneumatic conveyor system to a bag-house. About 15 kg of final product was collected in double plastic lined drums and sealed until further analysis. It was shipped to Grain Science and Industry Department at Kansas State University (Manhattan, KS) for analysis. Temperature, power, mass flow data were collected during each treatment run for energy and mass calculations (Figure 3.2).

3.2.4. Product Characterization

3.2.4.1. Kernel Morphology

Kernel hardness index, weight, diameter and moisture were determined following the standard procedure of AACC International (Approved Method 55-31.01) using a Perten 4100 Single Kernel Characterization System (SKCS) (Perten Instruments North America Inc., Springfield, IL). This was done for treated grain and untreated grain. For every run, about 300 individual kernels were run to obtain a frequency distribution for the tested parameters and their means and standard deviations (Martin et al. 1993).

3.2.4.2. Microbial Load

Aerobic plate counts (APC) were carried out at Medallion Laboratory (Minneapolis, MN) using the procedure outlined in Compendium Methods the Microbiological Examination of Foods. These tests were also done for total yeast, mold and coliform (cfu/g).

3.2.4.3. Physicochemical Properties

3.2.4.3.1. Color Measurements

The color of treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF) was evaluated using a Minolta color meter (Minolta CR-310, Tokyo Japan). The three measured parameters of L-value, a-value and b-values. L-value measures the lightness and its value ranges for black to white (0 to 100). The a-value measures green to red (60 to -60) and b-value measures blue to yellow (60 to -60) (Papadakis et al. 2000).

3.2.4.3.2. Particle Size Analysis

Flour particle size distribution was determined by Light Scattering (LS 13 320) single wavelength laser diffraction particle size analyzer using the Tornado dry powder system (Beckman-Coulter, Inc., Miami, FL). The sample holder was filled to about ¾, loaded and system started. The sample was dispersed in air as particle size measured. Software was used to calculate particle size and volume percentage, and statistics on mean, mode, median, d10, d50 and d90.

3.2.4.3.3. Proximate Analysis

The protein, moisture and ash content were determined using Near-Infrared Reflectance (NIR) method for determining protein in wheat flour following AACC Approved Method (39-11.01) using NIR FOSS DS2500 (FOSS, Eden Prairie, MN).

3.2.4.4. Functional Properties

3.2.4.4.1. Mixing and Pasting Behavior (excess water)

Pasting properties of wheat flours fractions before and after extrusion were determined using a Rapid Visco Analyser (RVA, Foss North America, Inc. Eden Prairie, MN) following AACC Approved method 76.21-0.1. Pasting curves were analyzed using Thermocline software (Window 3 TCW3 RVA, Foss, North America, Inc. Eden Prairie, MN).

3.2.4.4.2. Mixing and Pasting Behavior (limited water)

Rheological properties of treated flour were studied using a MixoLab (Chopin Technologies, France) following the AACC standard procedure (Approved Method 54.60-01). Mixing tests were carried out at a constant water absorption (98% db.) mixing speed (80 rpm) and temperature (30°C) using MixoLab Chopin + protocol.

3.2.4.4.3. Solvent Retention Capacity

The AACC International protocol (Approved Method 56-11.02) with slight modification was followed to determine solvent retention capacity (SRC) of treated and control flours. The modification involved using a 15 ml cap tube and 1.0 g of sample used instead of 5.0 and 50 ml cap tube.

3.2.4.4.4. Swelling Power and Solubility

15 mg sample was weighed and transferred into a clear dried test tube. 15 cm^3 of distilled water was added to disperse the sample then vortexed. The resultant slurry was heated at the desired temperature (60, 70, 80, 90°C) for 30 min in a water bath while being agitated. At end of each incubation period, the mixture was cooled to room temperature and centrifuged (1,000×g, 15 min). Supernatants were dried to a constant weight at 110°C. The residue obtained after drying the supernatant represented the amount of flour solubilized in water (W_1). Solubility was calculated as g/100 g of sample on a dry weight basis. The weight of sediment after separation of supernatant was determined (W_s).

The water solubility index (WSI) and the swelling power (SP) were calculated as shown below:

$$WSI = \frac{W_1}{Sample\ weight} \times 100$$

$$SP = \frac{W_s}{\left[Sample\ weight \times \left(100\% - WSI\right)\right]} \times 100$$

3.2.4.5. Differential Scanning Calorimetry

A differential scanning calorimeter (DSC) equipped with a refrigerated cooling system (Q100 TA Instruments, New Castle, DE) was used to study thermal properties of the samples. Approximately 20 mg ground sample was weighed into high-volume indium pans. Distilled water was added using a micropipette to make a suspension ratio of 1:2 (flour to water). Pans

were then sealed and equilibrated overnight at 4°C. The instrument was calibrated using an empty pan as reference stainless steel pan. The heating rate was 2°C/min from 20 to 110°C. Initial temperature, (T_o), peak temperature (T_p) and concluding (T_c) temperature of gelatinization, and enthalpy (ΔH) were measured from DSC thermograms using Universal Analysis 2000 software (TA Instruments, New Castle, DE) per unit mass of dry solid. The degree of gelatinization was calculated by the following equation (Marshall et al. 1993).

$$DG = \left(1 - \frac{\Delta H_{treated}}{\Delta H_{raw}}\right) \times 100$$

where DG the degree of gelatinization (%), $\Delta H_{treated}$ is transition enthalpy of hydrothermally-treated sample, and ΔH_{raw} is transition enthalpy of raw sample.

3.2.4.6. X-ray Diffraction

Samples were equilibrated at 100% relative humidity over 48 hour period at room temperature to adjusted moisture content to approximately 28%. Equilibrated samples were examined in a Rigaku X-ray diffractometer (MiniFlex II Rigaku North America Corp., The Woodland, TX). The radiation source was Kα. The voltage was set at 30 kV and a current at 15 mA. The scanning angles were between 3–30° (2θ), the scanning speed was 1.5°C/min in a continuous method. The slit width depth was 1.25 mm and 1°. The crystallinity of samples was obtained according to the procedure of Wakelin et al. (1959).

3.2.4.7. Size Exclusion – HPLC

The protein extractability in treated flour was characterized by size exclusion chromatography to determine their molecular weight profiles. The procedure used was according to the method used by Bean and Lookhart (2001). All regents were purchased from Fisher (Fisher brand- Thermo Fisher, Waltham, MA). Flour samples (100 mg) were weighted into 2.0 ml micro centrifuge tube (Fisher brand- Thermo Fisher, Waltham, MA). “Soluble protein” (SP) was extracted from pellet by vortexing 2×5 min with 1 mL of 50 mM Na-phos pH 7.0 /1% SDS, was then centrifuged and 500 uL of supernatant transferred to clean 2.0 ml micro centrifuge tube. This step was repeated. Total extract which was heated at 80°C for 2 minutes to deactivate “soluble protein” supernatant. The “insoluble protein” (IP) was extracted from the pellet first by using sonication (30 sec at 10W) with 1 mL 50 mM Na-phos pH 7.0/1% SDS in an ice bath, was then centrifuged and 500 uL of supernatant transferred to clean 2.0 ml micro centrifuge tube,

heated to deactivate any enzymes supernatant at 80°C for 2 min. Each pooled extracted is then transferred to vial (C4011-1 National Scientific, Thermo Scientific, Rockwood, TN). SE-HPLC analysis of soluble and insoluble protein was conducted using an Agilent 1100 HPLC system with a 300×7.8 mm BioSep-SEC-S3000 column (Phenomenex, Torrance, CA) using 50mM Na-phos pH7.0 /1% SDS as mobile phase with BioSep SEC-4000 column, at 40°C and flow rate 1 mL/min with 15uL injection volume. Proteins were detected by measuring UV absorbance at 214 nm. Measure total peak areas, adjust for SP extract being pooled by multiplying by a dilution factor of 2.

3.2.4.8. FT-IR Spectroscopy

A Perkin Elmer® Spectrum™ 100 FT-IR spectrometer (Thermo-Nicolet Corp., Madison, WI) equipped with single reflection diamond attenuated total reflectance (ATR) cell with liquid nitrogen cooled detector Mercury/Cadmium/Telluride/ and The OMNIC (Thermo-Nicolet Corp., Madison, WI) was used to scan the samples at room temperature between 4000-600 cm-1 at rate of 4 cm^{-1} with 64 scans.

3.2.5. Experimental Design and Statistical Analysis

A Box Behnken experimental design (Table 3.1) with 3 hydration levels (12, 16 and 20%), 3 temperatures (75, 85 and 95°C) and 3 residence times (30, 60 and 90 sec.) was employed.

$$\text{Response} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

where b_0 is constant, b_1, b_2, b_3, b_{11}, b_{22}, b_{33}, b_{12}, b_{13}, b_{23} are regression coefficients, X_1, X_2 and X_3 are parameters for hydration level, residence time and temperature, respectively.

For every characterization test conducted at least two replicate measurements were done per treatment, unless otherwise specified. The results were analyzed using analysis of variance (ANOVA) with the general linear model procedure (SAS version 9.1, SAS Institute, Cary, NC). The Bonferroni multiple-range tests indicated by ANOVA were applied at a significance level of $p < 0.05$. Pearson's coefficient of correlation (r) and their significances were determined for all parameters. Responses were subjected to regression analysis. A full quadratic model for the dependent variables was established to fit experimental data for each response and was analyzed by using statistical analysis system, Minitab® software V. 16 (Minitab Inc., College Station, PA, USA).

3.3. Results and Discussions

3.3.1. Thermal and Mechanical Energy Input

The thermal, mechanical and total energies imparted on the material during treatment were calculated from the temperature and mass flow rate data that was collected (Figure 3.3). Mechanical energy required for running the Solidaire heating unit, PCX drying unit and cooling units was calculated for each run (Tables 3.2 and 3.3). Mechanical energies of the cyclone and bag house were constant for all runs. In addition, during the heat treatment of the grain, the PCX was turned off to minimize any damage to grain because the spokes were known to damage grain kernels.

Much of the energy that was used to treat the samples was thermal (Figure 3.4). Treated wheat grain required greater amount of mechanical energy to convey it when compared to flour treated at same moisture, residence time and temperature combinations. This is because of wheat grain's higher density, larger size and thus poor flow properties compared to flow. The flour received greater thermal energy than the grain when samples were subjected to same moisture, residence time and temperature. The flour has larger surface area; therefore the heat energy easily penetrated the samples. The thermal energy increased with increase in treatment temperature. The impact of hydration level on thermal energy was greater in treated flour when compared to treated grain at same residence time and temperature. While increasing either moisture (at constant temperature and residence time) or temperature (at constant same moisture content and residence time) in treated whole wheat flour led to an increase in thermal energy, it led to a decrease in thermal energy in treated grain.

3.3.2. Microbial Load Reduction

Although microbial reduction was not the main focus of this study, microbial load reduction during the heat treatment was monitored. As mentioned earlier, this study was done in collaboration with Bepex International LLC. Bepex designs custom thermal processing systems for a range of heating applications to deliver effective pasteurization and sterilization functions needed by its customers in the food industry.

The microbial load (aerobic plate count) of feed flour was found to be one-log higher than that of wheat grain (Table 3.4). Heat treatment only reduced the microbial load of wheat flour by one log. However, for wheat grain, the results were mixed; either there was more than

two log reduction or almost no change in aerobic plate counts. Overall flour and milled flour products have lower microbial load and therefore microbial safe partly due to lower water activity of flour. Berghofer et al. (2003) examined microbial analysis of Australian wheat and flour milling. They reported that although microbial levels were generally lower, they were very prevalent. They also reported that distribution of microbes varied among the grain anatomical structures as well as among milling stream products. Aerobic mesophilic, coliform, mold and yeast were present in 95, 93, 100 and 100% respectively of wheat received. Their population ranged from 10-10^6, 10-10^3, 10^2-10^5 and 10^2-10^6 cfu/g for aerobic mesophilic, coliform, mold and yeast respectively. In conclusion, these results were not very reliable especially for coliform, yeast and mold because it took more than the two days to ship samples to Medallion Laboratory for analysis, which beyond the acceptable time period for aerobic plate count microbial analysis.

3.3.3. Physical Properties

Protein and ash content remained fairly constant (Tables 3.5 and 3.6), as expected. The moisture content was lower and varied significantly in treated flours than treated grain and treated grain whole wheat flour due to their smaller surface area of treated grain and the limitation in ability of moisture to penetrate of grain. The untreated flour had a moisture content of (~12%) and they were hydrated to 16 and 20% prior to heat treatment. Treated flour samples had moisture content between 6.83% -12.3% after drying and cooling indicating 5% to 8% moisture loss due to evaporation during heat treatments. The moisture of whole wheat grain feed material was 11.6%. Treated grain had moisture between 11.0 and 16.3% (i.e. 1% to 4% moisture loss due to evaporation).

There was a slight decrease in whiteness of treated whole wheat flour. L-value of untreated whole wheat flour was 81.5; it dropped to 76.3-79.8 after treatment indicating a slight darkening effect of heat treatment (Table 3.5). There was a slight improvement in whiteness of treated grain whole wheat flours. L-values of untreated control flour were around 77.2 while that of treated samples ranged between 77.6 and 79.3. This can be explained with the abrasion of outer layer of wheat kernels as they pass through the paddles and screws conveyors in the processing unit. About 4% of bran was observed to peel off from wheat grain. Peeling level increased with increase in hydration level. L-values of treated whole wheat flours correlated

fairly (r=0.625) with the thermal energy input. Higher the thermal energy input, lower the L-value. The trend was opposite for the treated grain whole wheat flours (r= 0.514).

The loose bulk density of untreated whole wheat flour and whole grain were 450 kg/m^3 and 813 kg/m^3, respectively. After treatment, the loose bulk densities of treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF) were between 433 and 454 kg/m^3. The loose bulk densities of treated whole wheat grain samples were 607-830 kg/m^3.

The single kernel characterization system (SKCS) was developed primarily to objectively classify wheat kernel according to their hardness (Osborne and Andreessen 2003). Wheat hardness influences milling efficiency and energy used. SKCS data indicated that there was no significant difference ($F = 0.45$, $p = 0.943$; $F = 0.05$, $p = 1.00$; $F = 0.06$, $p = 1.00$) in hardness index (55-75), kernel weight (25.1-28.9 mg) or diameter (2.43-2.58 mm) between the heat treatment runs. These were within recommended quality target for hard red winter wheat grown in 2013 season by the Wheat Quality Council (http://wheatquality council.org/). There were significant differences ($F = 15.26$, $p < 0.0001$) in moisture content of treated grain. This could be attributed to treatment moisture hydration level that the wheat grains were subjected to, and also to the time-temperature combinations used in the experimental design. There was a negative correlation between mechanical energy input and hardness index (r=0781). High thermal energy input caused softening of wheat kernel. Kernel hardness was significantly influenced by hydration level and residence time (Table 3.7).

3.3.4. Milling Performance

The first step in wheat milling (after cleaning) is tempering. Wheat is tempered to toughen the bran to prevent it from fragmenting to small pieces at crushing since the major objective is to separate the anatomical parts (endosperm, bran and germ) as clean as possible (Dexter and Sarkar 2003; Posner 2009; Delcour and Hoseney 2010). However, we did not temper any of our milled grain since our major objective was to obtain whole wheat flour. Besides, tempering would have resulted in larger bran particles. Such large particles would have been even much difficult to mill to finer bran. The straight grade flour extraction rates were between 69.4 and 74.0percent, which was within the expected range for normal performance of Buhler 202 Mill. Enough time was given between successive runs to which avoided mixing up of flours from different runs and also minimized any possible error in estimating milling

performance. Total extraction rates were between 94.8 and 98.6%, indicating 1.4-5.2% physical loss. Much of the losses occurred in milling bran using the Fitzmill.

3.3.5. Particle Size Analysis

Flour particle size is a very important physical property that affects water absorption, solvent retention, dough stability at mixing and final product (Posner 2009). Flour of different particle size distribution could vary in their composition and starch damage. Kent (1994) reported that there were three groups of flour particle classes; under 17µm (mostly wedge protein), 17-40µm (primarily starch) and greater than 40µm (starch and protein). Overall, the particle size distribution is influenced by grain type, mill type, milling condition, grain moisture (Posner 2009).

In comparison to untested flour, the particle size distribution of treated whole wheat flour (TWWF) was shifted towards the higher end of the scale (Figure 3.5), due to aggregates formed during heat moisture treatment. The level of aggregation was influenced mostly by moisture content and temperature (Table 3.5). Effect of treatment moisture on particle size distribution depended on treatment temperature and it followed nonlinear trend (Table 3.21). Based on the regression analysis, flour particle size distribution increased quadraticaly as moisture content increased, and particle size was smaller at lower temperature till reaching a minimum value then increased at higher temperature. Probably at higher temperature, the level of aggregation decreases due to drying effect of higher temperatures. The flour size distribution of treated grain whole wheat flour (TGWWF) was also influenced mainly by hydration level which depended on treatment temperature (Figure 3.7). Higher hydration level resulted in higher final moisture contents, which in a sense created an effect similar to tempering (Table 3.22). As explained earlier, tempering toughens the bran and softens endosperm facilitating easy milling of endosperm and therefore creation of smaller flour particles (Posner 2009; Delcour and Hoseney 2010).

TWWF and TGWWF had different particle sizes at their respective mean, d_{10}, d_{50} and d_{90} values (Table 3.5 and 3.6). Untreated whole wheat flour obtained through Buhler+Fitzmill (i.e. control of TGWWF) had higher average particle size, and higher percentage of large particles as compared to the untreated whole wheat flour obtained through Hal Ross mill (i.e. control of

TWFF). These differences could be attributed to different milling history (type and number of grinding-sifting steps) and type of equipment involved in each milling practice.

3.3.6. Differential Scanning Calorimetry

The DSC data showed slight change of the thermal properties of the flour after heat treatment (Tables 3.8 and 3.9). Starch gelatinization is a phenomenon dependent on the amount of water available. Since the moisture content of the treated samples was relatively low (12-20%), the starch was not affected significantly. None of the treated samples underwent complete gelatinization as indicated by clear peaks around 65-68°C (Figures 3.8 and 3.9). Treated and untreated samples (both TWWF and TGWWF) showed similar onset temperature (ranged from 57.4-59.7°C) and peak temperature (ranged 65.6-67.5°C) to their control samples (Table 3.8 and 3.9). Gelatinization enthalpy varied from 7.12 to 10.4 J/g, while higher values observed for the treatments of low moisture, low temperature and low residence time combinations.

Other studies have showed that hydrothermally treated starches are characterized by broader gelatinization temperature range, their gelatinization endotherms shift to higher temperatures and reduced enthalpy (Chung et al. 2009; Maache-Rezzoug et al. 2008; Gunaratne and Hoover 2002). According to Chung et al. (2009), the changes to gelatinization endotherm are related to changes within the starch crystalline region. The gelatinization process melts the starch crystalline and the double helices followed by changes such as amylose-amylose, amylose-lipid interactions, and formation of new double helices (Zavareze and Dias 2001; Waduge et al. 2006; Hoover and Vasanthan 1994). Such hydrothermally treated starch requires higher temperature for it to swell and to disrupt the crystalline region. Partial gelatinization explains the decrease in gelatinization enthalpy. In comparison to our procedure, it's important to state that, most of these hydrothermal treatment were either at higher pressure and temperature (Maache-Rezzoug et al. 2008) or annealing for a long time and high moisture (Chung et al. 2009). Therefore probably treatment effects were more significant than in ours. This might explain the lack of conclusive results.

3.3.7. X-ray Diffraction

The X-ray diffraction patterns and control and treated samples are presented in (Figures 3.10 and 3.11). All starches displayed the characteristic A-type crystalline pattern as depicted by the peaks centered at2θ of ~ 15, 17, 18 and 24°. Relative crystallinity (RC) decreased from

23.9% to 13.9-17.4% in TWWF, and from 20.3% to 12.1-15.5% in TGWWF samples (Tables 3.8 and 3.9). Decrease in RC on heat treatment could be attributed to disruption of amylopectin crystallites, which was evidenced by a decrease in gelatinization enthalpy since gelatinization enthalpy reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin. The reduction in RC was lower in TGWWF samples, which was in agreement with trend of gelatinization enthalpy. RC of untreated WWF was significantly higher than that of untreated GWWF, which could be attributed to the milling history of these samples. Hal Ross mill (used for WWF production) creates less mechanical damage to starch granules compared to experimental mills used for the production of GWWF. Overall, TWWF flours had higher relatively crystallinity in comparison to TGWWF at their respective treatment level. This could be explained by the RC of the untreated controls of each set. Heat treatment of whole grain caused 24-40% reduction in relative crystallinity, while it caused 27-42% reduction in the relative crystallinity of the treated flour. This is expected and can be explained by the protective nature of intact kernels due to less surface area exposed to treatment environment. Hydration levels and treatment temperatures had the highest effect on relative crystallinity of both TWWF and TGWWF (Tables 3.21 and 3.22).

The magnitude of the effect of heat moisture treatment on starch crystallinity is characteristics of starch source (Zavareze and Dias 2011). Starch type B has been showed to change to starch crystalline type A+B-type polymorph, the latter being more stable in potato starch (Genkima et al. 2004; and Gunaratne and Hoover 2002). The stability is attributed to loss of water and movement of a pair of double helices to central channel (Gunaratne and Hoover 2002). Researchers studied the X-ray diffraction intensities of corn (Hoover and Manuel 1996) and sweet potato (Vieira and Sarmento 2008) after hydrothermal treatments. Vermeylen at al. (2006), Gunaratne and Hoover (2002), and Franco et al. (1995) reported decrease in relative crystallinity after hydrothermal treatment of for potato, cassava and corn starches, respectively. The reduction in X-ray intensities was attributed to reduced crystallinity and/or increase in the amorphous region in semi-crystalline lamellae (Zavareze and Dias 2011). Jacobs and Delcour (1998) explained that annealing of wheat and potato starches as a hydrothermal treatment technique led to low water absorption in inter crystalline amorphous lamellae which led to decrease in X-ray diffraction intensities.

3.3.8. Swelling and Solubility

The swelling power and solubility of treated and untreated samples the temperature range 60–90°C are presented in Tables 3.10 and 3.11. There was decrease in swelling power and solubilities of heat TWWF and TGWWF when compared to their controls. The swelling power and solubility of TWWF and TGWWF increased with increase in incubation (60-90°C) temperature. These increases in swelling power and solubility were rapid when incubation temperature was between 60 and 70°C, and then between 70 and 80°C. However, samples incubated at 80°C and above either remained fairly constant or experienced a slight decrease. Swelling power and solubility influenced by hydration level, and to lesser extent by treatment temperature (Tables 3.21 and 3.22). The swelling power and solubilities of TGWWF was not significantly affected for samples incubated at 70°C and below. This could be related to amount of ungelatinized starch that was gelatinized since 60-70°C is the temperature range for wheat starch gelatinization (Delcour and Hoseney 2010). In comparison to their respective controls, TGWWF had minimal changes in their swelling power and solubilities compared to TWWF when treatment factors were kept constant. .

Chung et al. (2009), Olayinka et al. (2008), Adebowale and Lawal (2002) also reported decreases in the swelling power and solubility of hydrothermally treated starches. The reduction in swelling power and solubility of starch after hydrothermal treatment is related to changes to starch granule crystalline. Many researchers have shown a reduction in granular swelling and amylose leaching on annealing and heat moisture treatment (Hoover and Vasanthan 1994; Waduge et al. 2006). Crystalline perfection, interactions involving amylose chains, V-type amylose lipid complex formation, increased interactions between amylose and amylopectin, strengthening of intra-molecular bonds have been shown to be factors influencing the reduction in swelling power and solubility (Jacobs et al. 1995). In this study, unchanged X-ray pattern suggest that the main causative factor influencing the decreased swelling power is crystalline perfection. These changes limit water penetration to the crystalline and therefore starch swelling as well as limited leaching of amylose and therefore solubility.

3.3.9. Solvent Retention Capacity

Solvent retention capacity (SRC) is a physical-chemical method used to weight amount of solvent as percentage of flour weight (14%) that is retained by flour after centrifugation. The

four solvents are independently used to produce four SRC values: water SRC, 50% sucrose SRC, 5% sodium carbonate SRC, and 5% lactic acid SRC. The combined pattern of the four SRC values establishes a practical flour quality/functionality profile useful for predicting baking performance and specification conformance. The ability of treated flour to retain solvents of these regents has the following main principles: Lactic acid solution retention is related to glutenin, sucrose solution retention is related to pentosans and sodium carbonate retention is related to starch damage (van Steertegem 2013).

The solvent retention capacity of control and treated flours are presented in (Table 3.12). Untreated WWF flour has significantly lower Eater SRC, sucrose SRC and Na-bicarbonate SRC compare the retention capacities observed for untreated GWWF. All three retention parameters are related to starch and non-starch polysaccharides components of wheat flour which can be affected by the process history and the milling practice. Higher values observed in Na-bicarbonate SRC supports the idea of having higher starch damage in experimental Buhler and Fitz mill compared to commercial milling process the Hal Ross mill offers. The lactic acid SRC values for untreated WWF and untested GWWF were found to be similar which suggest that type and intensity milling process do affect the protein component, as they affect the starch.

Heat treatment increased the solvent retention capacity of all solvents (Table 3.12). Based on the regression results, treated whole wheat flour retention capacities of the four solvent were influenced to varying degree by treatment factors (Tables 3.21 and 3.22). The effect of moisture content on sucrose SRC depended on residence time whereas the temperature effect on Na-bicarbonate SRC depended on time. The lactic acid SRC was also influenced by treatment temperature. Increase in lactic acid SRC can be considered as an indication of improvement in gluten functionality which improved the dough stability as it will be discussed later in section 3.3.11. All the SRC increased with increase in hydration levels.

There were weak positive correlations between thermal and mechanical energies and water retention capacities of treated whole wheat flour ($r = 0.247$), and treated grain whole wheat flour ($r = 0.454$). The flour particle size values for TGWWF were negatively correlated with all four SRC; water ($r = -0.611$, $p < 0.000$), sucrose ($r = -0.774$, $p < 0.000$), Na-bicarbonate ($r = -0.579$, $p = 0.001$) and lactic acid ($r = -0.601$, $p < 0.000$). The d_{10} and d_{50} flour particle size values for TWWF were negatively correlated with Na-bicarbonate SRC ($r = -0.411$, $p = 0.024$). Smaller flour particles have greater surface area and therefore greater solvent retention for all of

these solvents. Furthermore as mentioned earlier, smaller particle have might experienced greater starch damage as well as have higher protein content (Kent 1994).

3.3.10. Size Exclusion-HPLC

Size-exclusion high performance liquid chromatography (SE-HPLC) has been widely used to characterize the wheat proteins and to study the functional effects of gluten components that differ in their degree of polymerization (Schober et al. 2006). Several studies focused on correlating SE-HPLC data with dough rheology through empirical and fundamental tests, as well as the breadmaking tests as an attempt to predict end-product quality (Delcour and Hoseney 2010; Shewry et al. 2002; Shewry and Halford 2002).

Composition of soluble polymeric proteins (SPP), insoluble polymeric proteins (IPP), gliadins (Gli), and albumins and globulins (AG) analyzed by SE-HPLC is given in Table 3.13 and 3.14. For calculation the relative distribution of these extractable proteins the method reported by Schober et al. (2006) was used. The weight of insoluble polymeric protein (IPP) was calculated from the weight and protein content of the freeze dried pellet, extractable protein (EP) was calculated from the difference between flour protein and protein in the pellet (IPP). The protein size groups (soluble polymeric proteins, SPP; gliadins, Gli; and albumins and globulins, AG) were quantified as the percentage of the respective areas relative to the total HPLC area multiplied by EP. The size groups were calculated as absolute values, corresponding to percent protein on a flour weight basis. In addition, the percentage of each size group was calculated on the basis of percent flour protein. To test for correlations between protein properties and the fundamental rheological and quality parameters, additional relevant sums and ratios between the protein size classes were calculated. A complete description of all individual classes and sums and ratios is given as follows (Schober et al. 2006):

Individual classes, sums and ratios	**Description**
IPP	Insoluble glutenin polymers of the highest Mw having a greater HMW/LMW subunit ratio than SPP
EP	All proteins soluble in 50% 1-propanol (SPP, Gli, AG)
SPP	Soluble glutenin polymers with a continuous range of molecular sizes and a lower average Mw than IPP, having also a lower HMW/LMW subunit ratio
Gli	Monomers of lower Mw than SPP
AG	Metabolic proteins (non-gluten proteins) of lower Mw than Gli

IPP/EP	Ratio of insoluble (highest Mw polymers) to 50% 1-propanol soluble proteins (SPP, Gli, AG)
IPP/SPP	Ratio of insoluble (large) to soluble (smaller) glutenin polymers
IPP/Gli and SPP/Gli	Ratio of large and smaller glutenin polymers, respectively, to monomers
(IPP+SPP)/Gli	Ratio of glutenin (polymers of all sizes) to gliadin
IPP/(SPP+Gli)	Ratio of insoluble to soluble gluten proteins (AG excluded)
IPP [%]+SPP [%]	Percentage of glutenin in flour protein
SPP [%]+Gli [%]	Percentage of soluble (lower Mw) gluten proteins in flour protein

Although the extractable protein profile of the untreated WWF and GWWF were expected to be identical there were slight differences in their Gli (61.4 *vs* 59.3%) and IPP concentrations (22.6 *vs* 24.6%) (Tables 3.13 and 3.14). After heat treatment both set of flours experienced decrease in SPP, and A/G, and increase in Gli and IPP. Among the heat treated whole wheat flours, those treated at high moisture-high residence time (20% MC, 90 sec) and high moisture-high temperature (20% MC, 110°C) and combinations resulted in the highest IPP (32.27 and 35.54%, respectively), which were 9.6-12.9% higher than that in the untreated flour. These two conditions correspond to the lowest SPP (6.2-6.7% less than the control) and the lowest Gli (2.3-4.8% less than the control), while A/G percentages remained fairly constant in the range of 2.26 to 3.00. Extractable SPP and A/G amounts were higher in control flours than their respective treated whole wheat flour and treated grain whole wheat flour. Gliadin and IPP extraction percentages for both treated whole wheat flour and treated grain whole wheat flour did not show any clear pattern (Tables 3.13 and 3.14). In addition, amount of extractable SPP were higher in treated grain whole wheat flour in comparison to treated whole wheat flour at their respective treatment level. The amount of extractible proteins in treated whole wheat flour were influenced by the following interaction factors in a decreasing order; moisture by temperature, moisture by time and time by temperature (Table 3.21). Treated grain whole wheat flour extraction proteins were influenced by the following interaction factors in decreasing order; moisture by time moisture by temperature, and time by temperature (Table 3.22).

Heat treatment of flour has been reported by several authors to cause molecular and conformation changes to protein by inducing sulfhydryl-disulfides inter change reactions and formation of new bonds (Guerrieri et al. 1996; Lagrain et al. 2005, 2006, 2008 and 2010; Lagrain et al. 2008; Weegels et al. 1996; Singh and McRitchie 2004; Korablyova and Kasymova 2011).

Shomer et al. (1995) reported that nonfunctional wheat protein of albumen and globulins coagulate as a result of heat treatment. Shomer et al. (1995) and Weegels et al. (1996) reported that solubility and extraction of protein substances reduced during heat treatment of moist gluten. Korablyova and Kasymova (2011) subjected hydrated gluten incubated in thermostat for 10 min at 40, 50, 60, 70 and 80°C. They reported a decrease of about 16% of extractable protein and subsequent increase of insoluble sediment. The decrease in extractable protein was attributed to oxidation and formation of new intermolecular disulfide bond followed by the aggregation of proteins and gluten strengthening. Their reverse phase HPLC data showed that different wheat protein types and their sub-fractions responded differently to heat treatment as influenced by heating temperature and time. Gelinas and McKinnon (2004), heated wheat gluten at 80°C for 15 min in a water bath and fractionated it. They reported that although it was easy to extract the gluten, it was slacker and broke down easily, however it improved dough mixing stability.

3.3.11. FT-IR Spectroscopy

FT-IR is a vibrational spectroscopic technique that can be used to study secondary molecular structures and conformations proteins and polysaccharides of heterogeneous foods. It is a non-destructive and technique that requires small quantities of test samples. FT-IR technique operational principle is based on the dipole moment of molecules (Sivam et al. 2013).

Shifts in frequencies of amide I, II or III band in FTIR spectrum correspond to the changes in the secondary structure of proteins. Figure 3.12 shows the original infrared spectra in wavenumbers from 600 to 4000 cm^{-1}, while Figure 3.13 shows the amide I and amide II regions of treated wheat flours in comparison to the control samples. Intensities of amide I and amide II bands revealed a decrease in the spectra of heat treated flours. This decrease indicates an important change in the secondary structure of wheat proteins that occurred as a result of heating. The decrease of signal intensity was presumably attributed to the thermal dissociation of molecular polymer formed by two typical components in wheat gluten (gliadin and glutenin).

Further analysis is needed to investigate if there were any changes to protein's secondary structure. The amide I band of proteins consists of many overlapping component bands that represent different structural elements such as α-helices, β-sheets, turns and unordered or irregular structures (Zhang et al. 2012). In order to quantitatively estimate the content of secondary structure segments (α-helices, β-sheets or β-turns), the amide I band of the spectra has

to be deconvoluted (Goormaghtigh et al. 2009 and Elangovan et al. 2007). Comparison of the secondary structures of control flours and untreated flours will reveal the changes in the amounts of α-helices, β-sheets or β-turns, and extended structures such as α-sheets.

3.3.12. Mixing and Pasting (excess water)

The rapid visco-analyzer measures the viscosity of sample as results of programmed heating and cooling cycles. Slurry is heated from 50°C to 95, held for few minutes then cooled to 50°C. As the sample temperature increases, viscosity increases due to starch gelatinization. Wheat starch, gelatinization temperature is between 50 and 57°C. The increase in viscosity beyond gelatinization temperature is described as pasting. As the sample is held at constant temperature, it is viscosity decreases due to shear-thinning. At the cooling stage, the viscosity decreases phenomena known as setback (Delcour and Hoseney 2010).

Pasting properties of control and heat treated flours are summarized in Tables 3.15 and 3.16. Pasting parameters for untreated whole wheat flour (WWF) were significantly higher than that of untreated grain whole wheat flour (GWWF). WFF developed much higher peak, through and final viscosities compared to GWFF (Figure 3.14). These differences could be attributed to different milling history (type and number of grinding-sifting steps) and type of equipment involved in each milling practice, as discussed earlier.

Significant changes were observed in pasting parameters for both treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF) relative to native, untreated samples. Heat treatment of WWF caused 33-48% drop in peak viscosities (from 2610 cP to 1353-1756 cP). The time to reach peak viscosity only varied by up to 0.4 min. Treatment temperature was found to be the most important factor affecting the peak viscosity followed by the hydration moisture content. Residence time had a minor effect (Tables 3.21 and 3.22).

There were negative correlations between RVA peak viscosity of both treated whole wheat flour and treated grain whole wheat flour and water SRC ($r = -0.577$, $p = 0.001$; $r = -0.548$, $p = 0.002$) and Na-bicarbonate SRC ($r = -0.625$, $p < 0.001$; $r = -0.798$, $p < 0.000$). Peak time was negatively correlated with Na-bicarbonate SRC ($r = -0.363$, $p = 0.049$).

Heat moisture treatment causes partial gelatinization which promotes amylose-amylose, amylose-amylopectin interactions (Oliyinka et al. 2008; Jacobs et al. 1995; Hoover and Vasanthan 1994) and formation of amylose-lipid complexes (Waduge et al. 2006). Studies on

various cereal starches have shown that hydrothermal treatment decreases peak viscosity, final viscosity and break down viscosity and increases pasting temperature of sorghum starch (Olayinka et al. 2008), rice starch (Hormdok and Noomhorm 2007), and corn starch (Chung et al. 2009). A plausible explanation for these decreases in viscosities is that heat treatment causes changes to crystalline structure of starch whereby old hydrogen bonds are broken and new ones formed after heat treatment. In addition, there is increased starch crosslinking after heat-moisture treatment. These new hydrogen bonds and crosslinks are stronger, requiring greater heat energy to break (Adebowale et al. 2004; Gomes et al. 2004). Chung et al. (2009) have stated that retrogradation is influenced by degree of amylose leaching, granule size and presence of rigid, non-fragmented swollen granule. This reduces starch swelling power and hydration of the amorphous region (Hoover and Vasanthan 1994). This explains lower pasting values in comparison to control. Also the lower breakdown values indicate that heat-moisture treated starch is more stable to shear than control (Olayinka et al. 2004; Hormdok and Noohorm 2007; Adebowale et al. 2005). Setback is commonly used to describe the increase in viscosity that occurs on cooling a pasted starch (Fisher and Thompson 1997; Ward et al. 1994). Higher setback values observed at higher hydration moisture contents and residence times indicated that there was much higher re-aggregation of starch granules after heat treatment, suggesting a re-association of amylopectin branch chains in the heat-treated flour.

3.3.13. Mixing and Pasting (limited water)

A MixoLab is a relatively new dough measurement system developed by Chopin Technologies that is used to access quality of protein and starch in limited water dough systems such as dough strength and stability. Some of the important parameters include C5 time (dough development time) and C1-C5 torques, alpha, beta, gamma, stability, amplitude, absorption, amylases, viscosity gluten, mixing, retrogradation indices (Koksel et al., 2007). C1 torque is used to determine the level water absorption required for developing dough at 1.1 Nm torque which corresponds to 500 BU in Farinograph mixing. We used constant water absorption protocol based on optimum absorption for control flour. The C2 torque measures the weakening of the protein under thermal and mechanical energy. A C3 torque measures starch gelatinization while C4 torque measures the hot paste gel stability under cooking due to amylytic activity. C5 torque measures the starch setback in the cooling phase (Koksel et al. 2007). Alpha measures the rate of

protein weakening. Beta measures rate of starch gelatinization, Gamma measures the speed of enzyme degradation speed (Dubat 2013).

Tables 3.17 through 3.20 present MixoLab parameters measured for treated whole wheat flour and treated grain whole wheat flour and their respective untreated control samples. Heat treatment affected the mixing and pasting profiles of WFF significantly while only a major change was observed in that of GWFF. In generally speaking TWFF had higher C1 torques than its respective untreated control indicating an increase in the water absorption values (Figure 3.15). Those samples also had longer stability, less protein softening, and slightly early pasting times and thus lower pasting temperatures. In general, treated whole wheat flours and treated grain whole wheat flours had lower C1and C2 and higher C3, and C4 torques than their control (Figure 3.15). However the pasting section of the profile followed that of the control samples. Regression analysis indicated that time and temperature combinations had highest effect on the mixing behavior and water absorption, compared to the effect of hydration temperature on the same.

The differences among treated grain whole wheat flour C5 torque values were not of clear trend. Also the magnitude in C1-C5 torque differences between treated grain whole wheat flours and their control were minimal. Only C2 torque values were noticeable higher. Treated whole wheat flour and treated grain whole wheat flour had higher alpha and beta, but lower gamma values than control. The higher C1 torque implied that more water was needed to fully hydrate the flour. Heat treatment might have caused some partial starch gelatinization which takes up more water readily than non-gelatinized starch (Delcour and Hoseney 2010). The higher C2 torque coupled with higher alpha values of treated flours showed that such flour is able to resist/stronger mechanical and thermal energy (Koksel et al. 2009). The higher C3 torque, beta values and C4 torque values of control flour in comparison to treated whole wheat flour indicated partial gelatinization of starch due to heat treatment. Treated flours were able to resist shear better than control. Level of starch gelatinization increased with increase in treatment moisture and residence time, especially for both treated whole wheat flour.

There was also a moderate correlations observed between mixing stability and thermal energy input for treated whole wheat flour ($r=0.719$), but no relationship with the treated grain whole wheat flour ($r=0.141$). SPP correlated negatively with C2 torque of treated whole wheat flour ($r=-0.519$, $p=0.00$) and treated grain whole wheat flour ($r=-0.607$, $p<0.000$). Also treated

whole wheat flour SPP and A/G were negatively correlated with mixing stability (r=-0.545, p=0.002; r=-0.401, p=0.028), and gluten index (r=-0.507, p=0.004; r=-0.498, p=0.005), respectively. Gli was positively correlated with C2 torque (r=0.517, p=0.003) of treated whole wheat flour and negatively correlated with that of treated grain whole wheat flour(r=-0.758, p<0.000). Extractable Gli content was negatively correlated with gluten index (r=-0.667, p<0.000) of treated whole wheat flour and that of treated grain whole wheat flour (r=-0.657, p<0.000). Heat treatment reduced extraction rate of SPP for treated whole wheat flour and treated grain whole wheat flour.

3.4. Conclusions

C2 torque, mixing stability and gluten index are the most commonly used MixoLab parameters that characterize wheat protein (gluten) quality. Heat treatment increased C2 torque, stability and gluten indices for treated whole wheat flour and treated grain whole wheat flour showed that heat treatment. Theoretically flour with good stability, gluten index and C2 torque has superior qualities as it is able to resist both mechanical and thermal energies during dough development and bread-making. Heat treatment reduced extraction rate of SPP and A/G. The higher C2 torque and stability values coupled with decrease in extractable soluble polymeric protein indicate that heat treatment resulted in polymerization of the protein. Larger molecular weight proteins therefore were able to resist both thermal and mechanical energies and had lower extractability. There were strong negative relationships between Gli, SPP and thermal energy input, and strong positive relationship between IPP and thermal energy input for treated whole wheat flour. Similar relationships, but much weaker, were observed for the treated grain whole wheat flour.

Thermal energy input is a good predictor change in the percentage distribution of protein fractions. Soluble proteins (SPP, A/G and gliadins) decreased while insoluble proteins (IPP) increased with the intensity of thermal energy input. The mathematical models relating input parameters to the response functions will be useful in predicting the end product quality at a given process condition. Solidaire heating unit is very effective in processing whole wheat flour and wheat grain. Process parameters can easily be manipulated in a controllable manner to achieve targeted end-quality and functionality.

3.5. References

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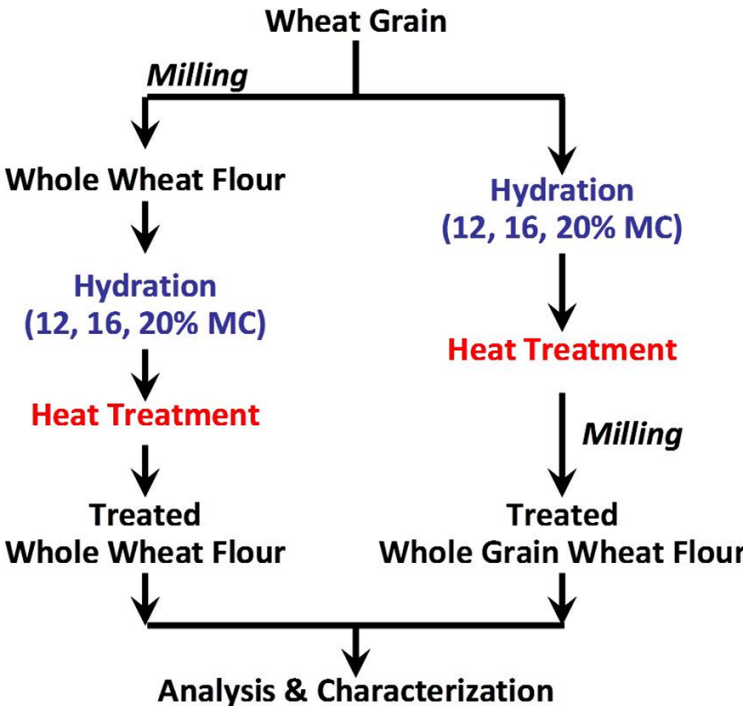

Figure 3.1. Schematic of experiment procedure

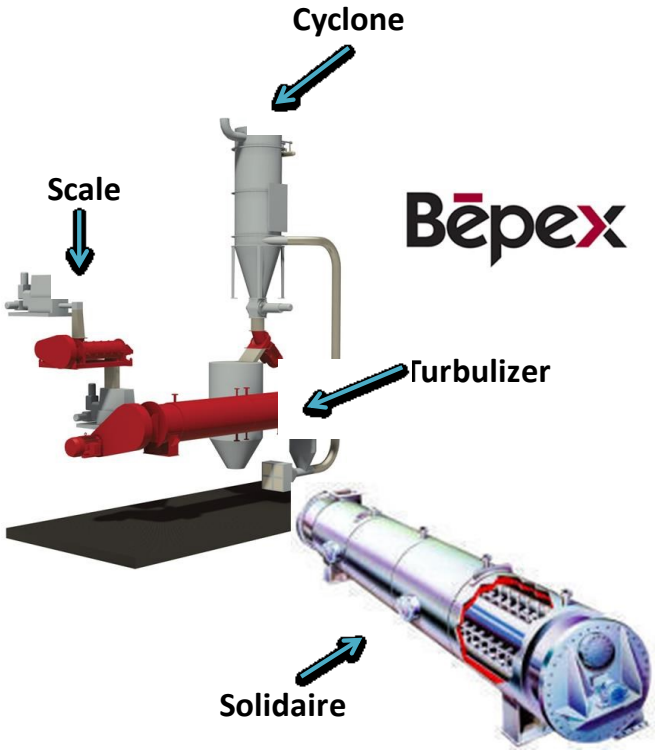

Figure 3.2. Processing units

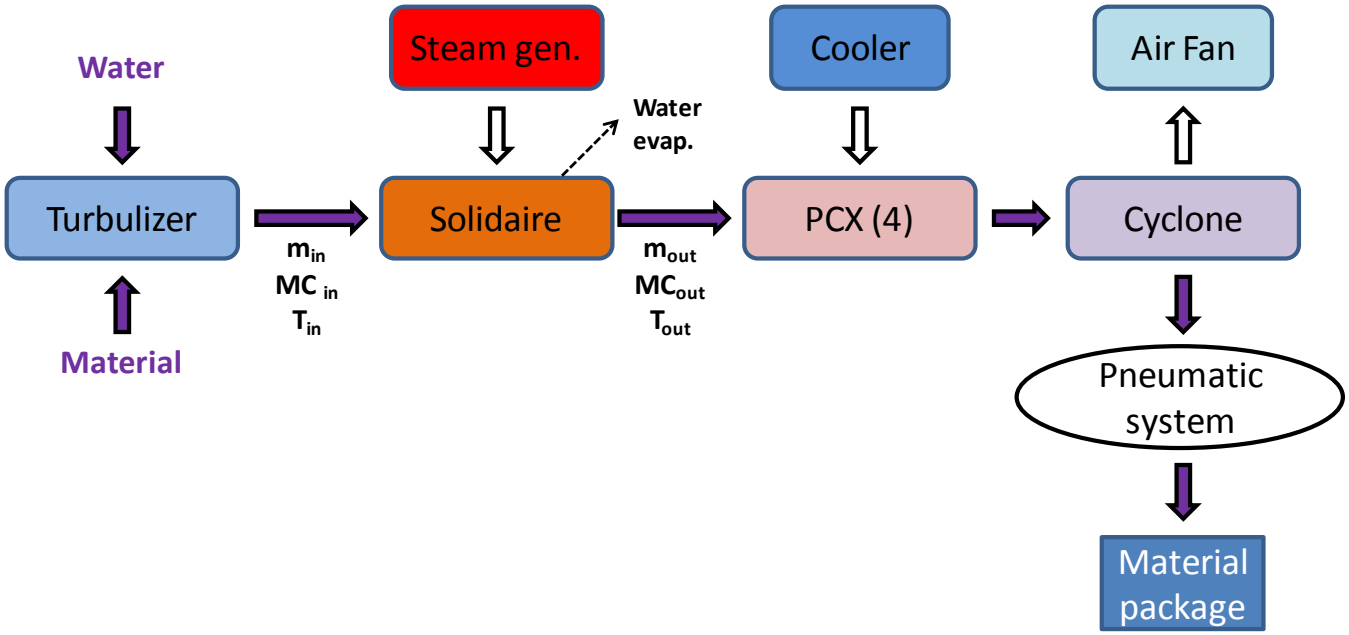

Figure 3.3. Schematic of indirect heat treatment processing units

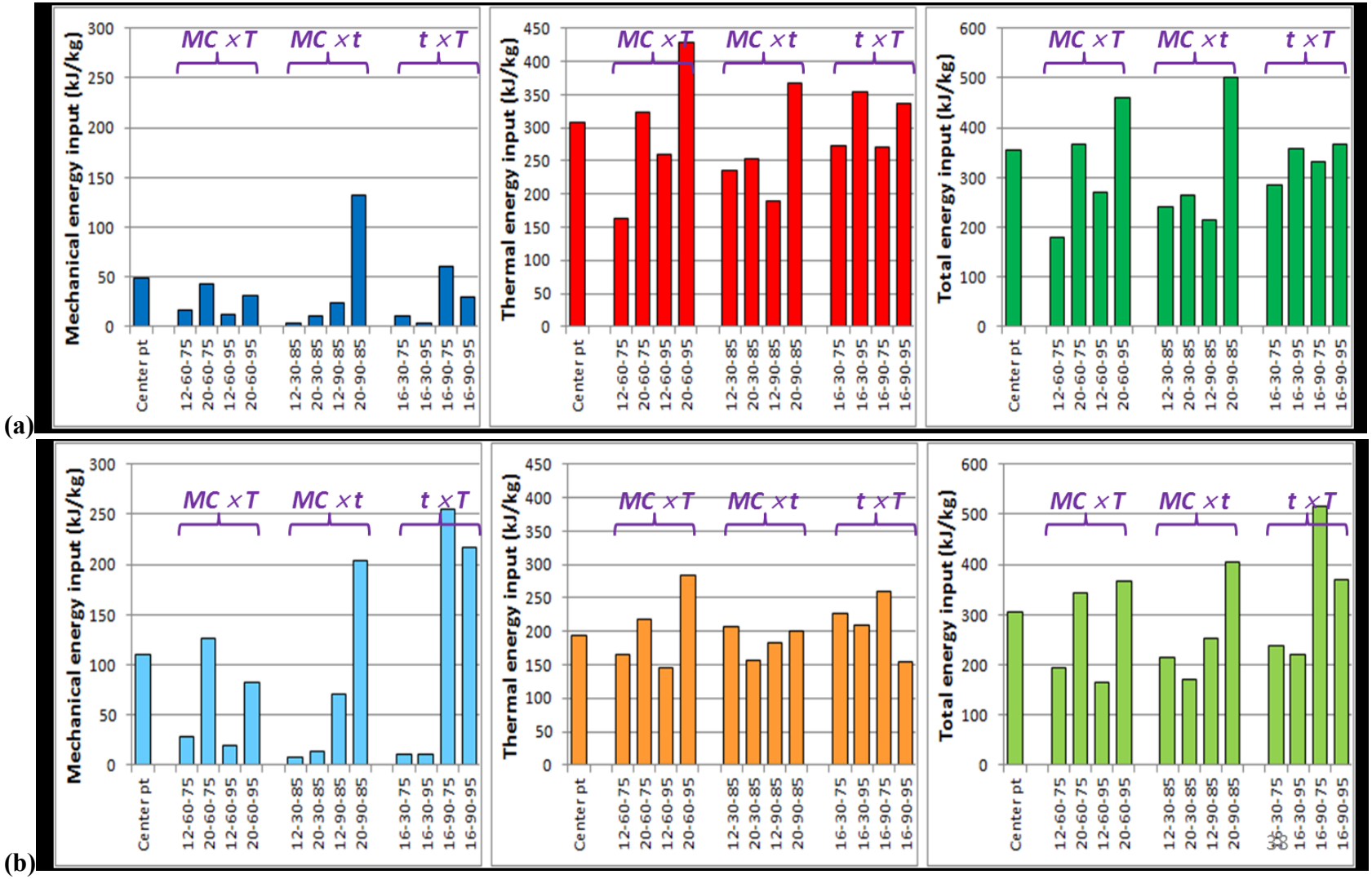

Figure 3.4. Mechanical, thermal and total energy inputs during treatment of (a) whole wheat flour, (b) whole wheat kernels.

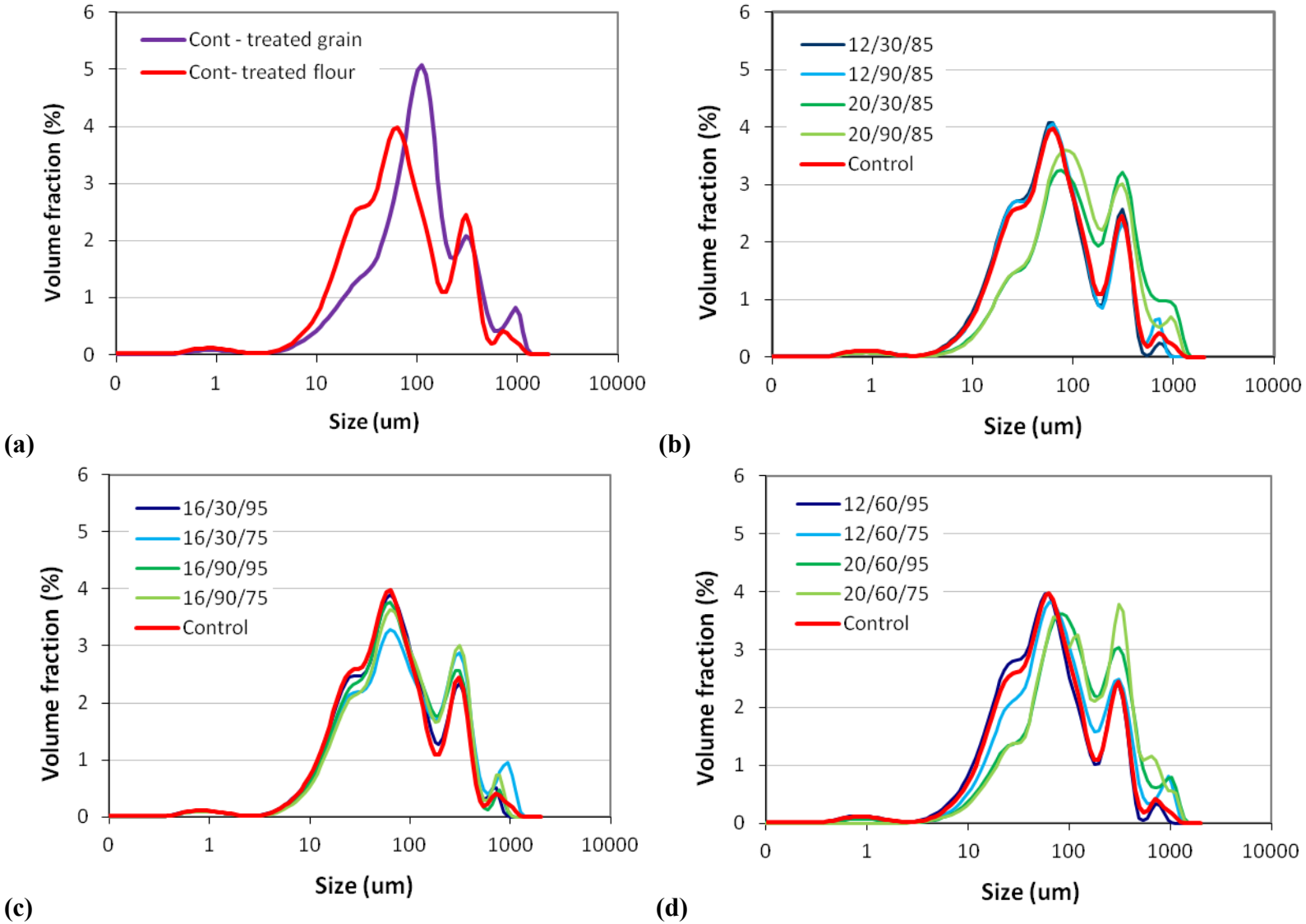

Figure 3.5. Particle size distribution of treated whole wheat flour (TWWF) (a) Control and center point treatment, (b) moisture content × residence time effect, (c) temperature × residence time effect, (d) moisture content × temperature effect.

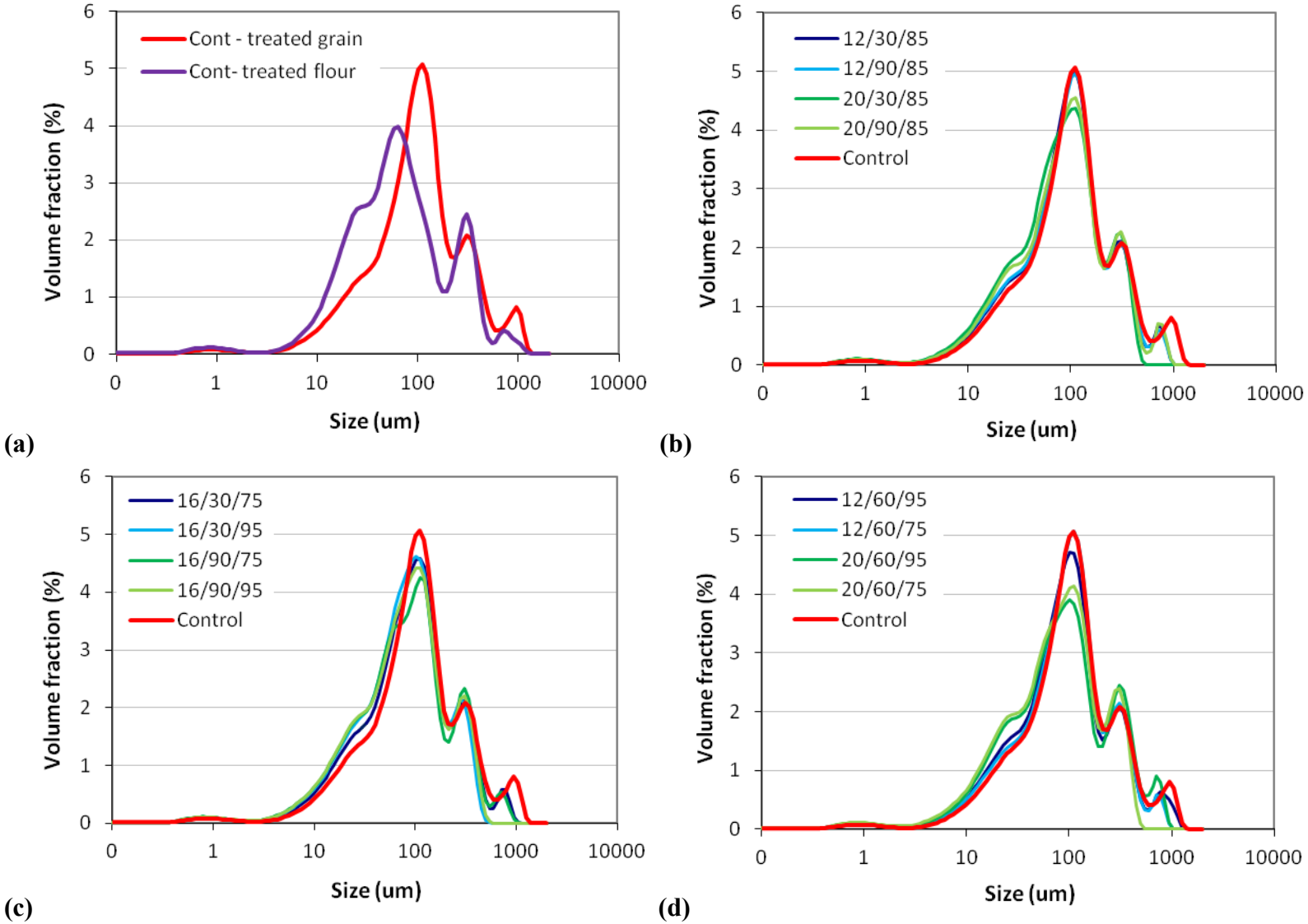

Figure 3.6. Particle size distribution of treated whole wheat flour (TGWWF) (a) Control and center point treatment, (b) moisture content × residence time effect, (c) temperature × residence time effect, (d) moisture content × temperature effect.

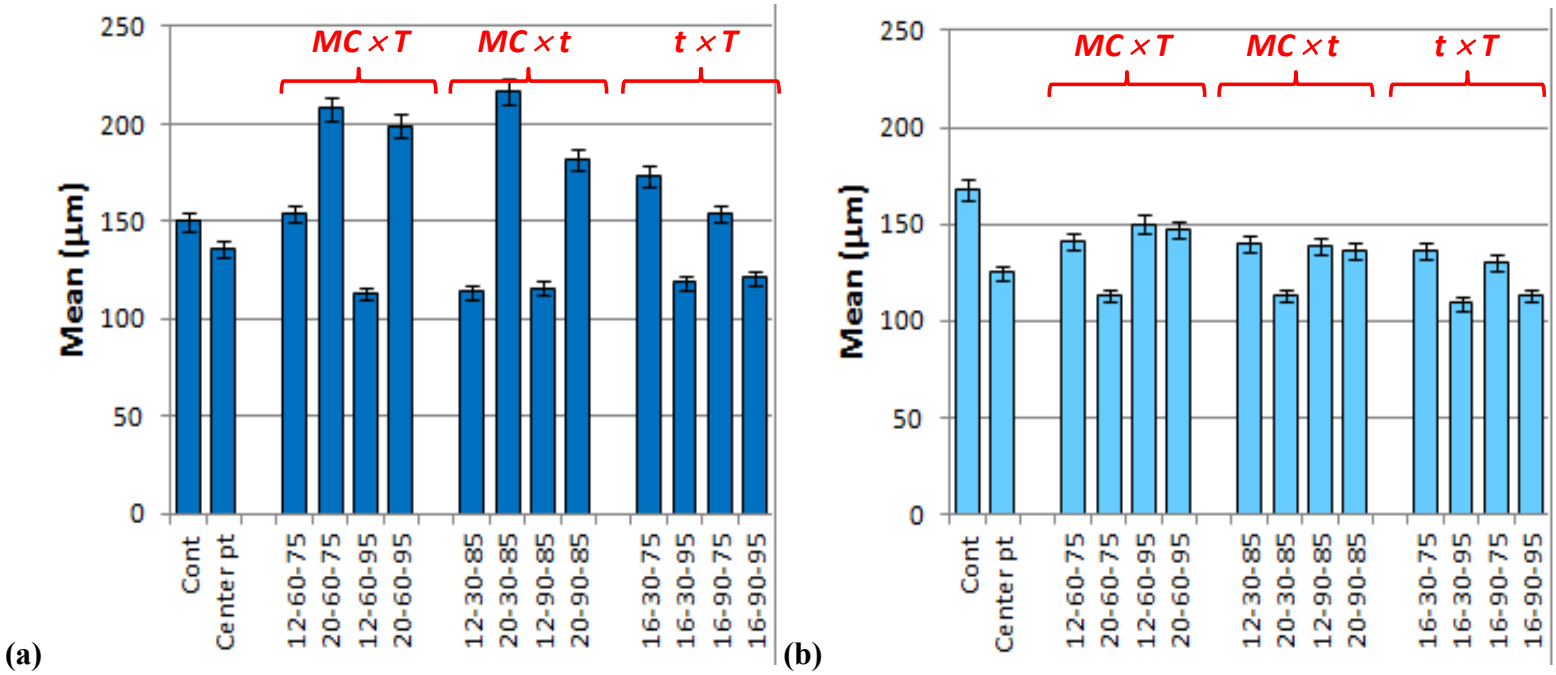

Figure 3.7. Comparison of the mean particle sizes (a) treated whole wheat flour (b) treated grain whole wheat flour.

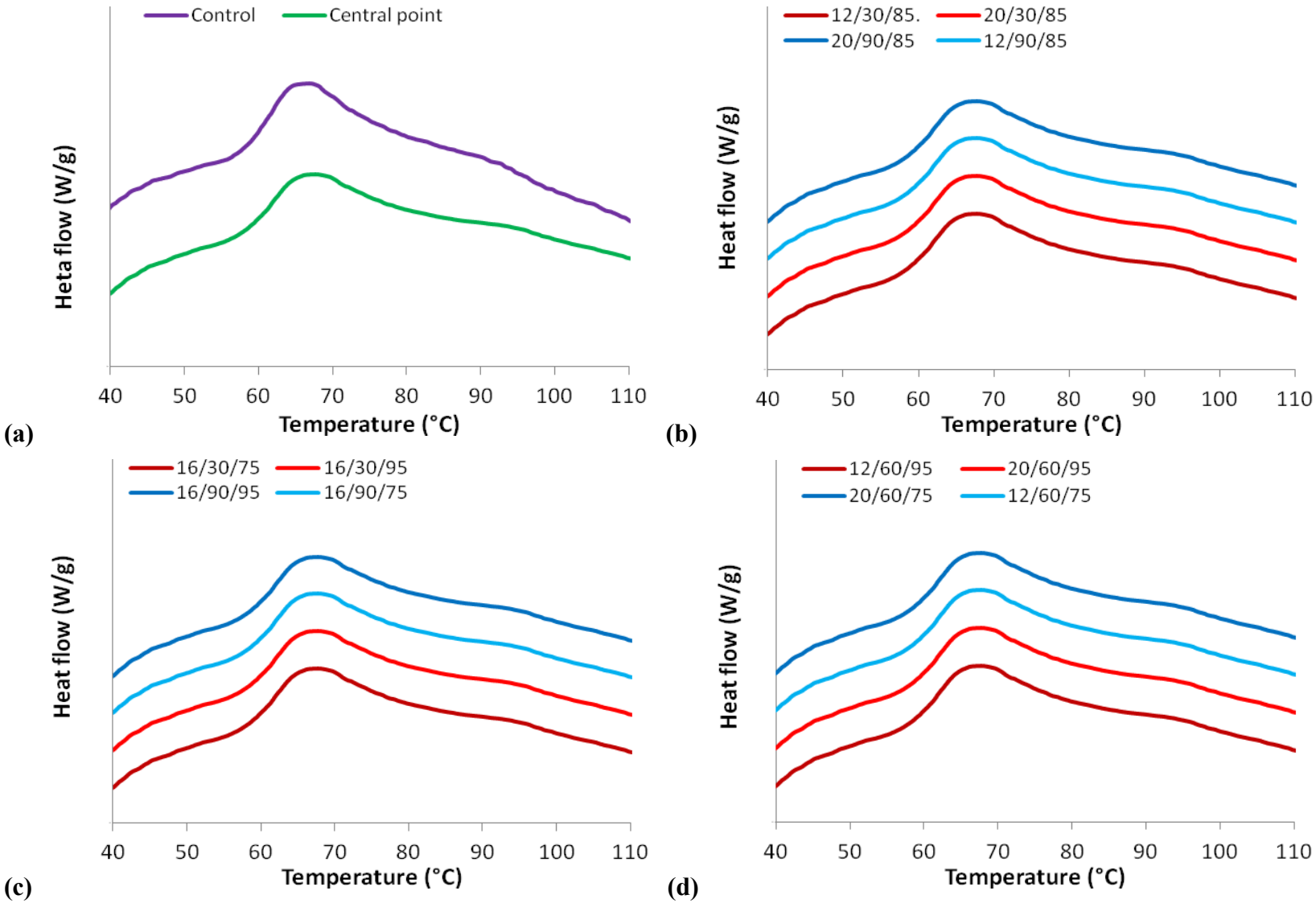

Figure 3.8. DSC thermograms of treated whole wheat flours (TWWF) (a) Control and center point treatment, (b) moisture content × residence time effect, (c) temperature × residence time effect, (d) moisture content × temperature effect.

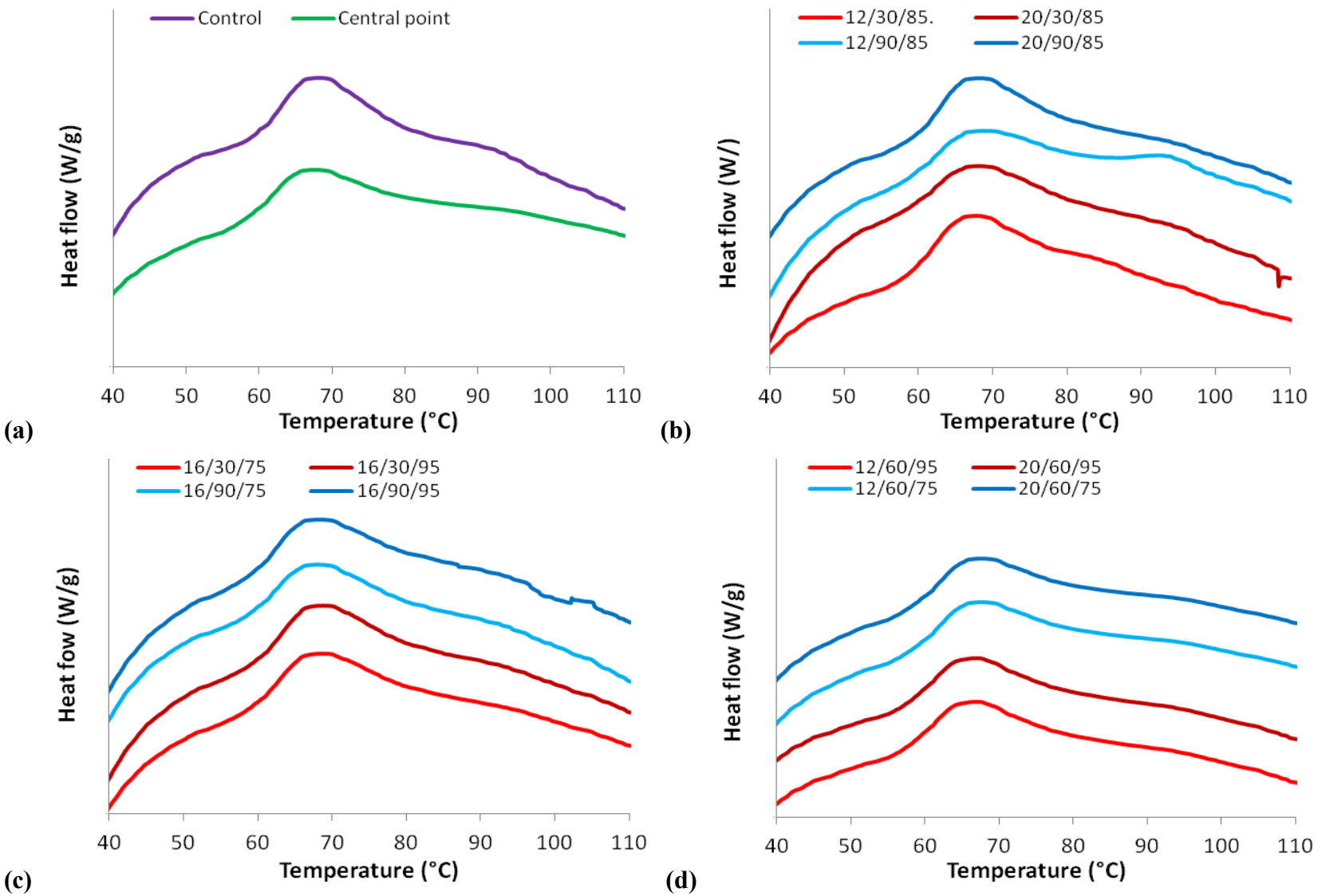

Figure 3.9. DSC thermograms of treated grain whole wheat flours (TGWWF) (a) control and center point treatment, (b) moisture content × residence time effect, (c) temperature × residence time effect, (d) moisture content × temperature effect.

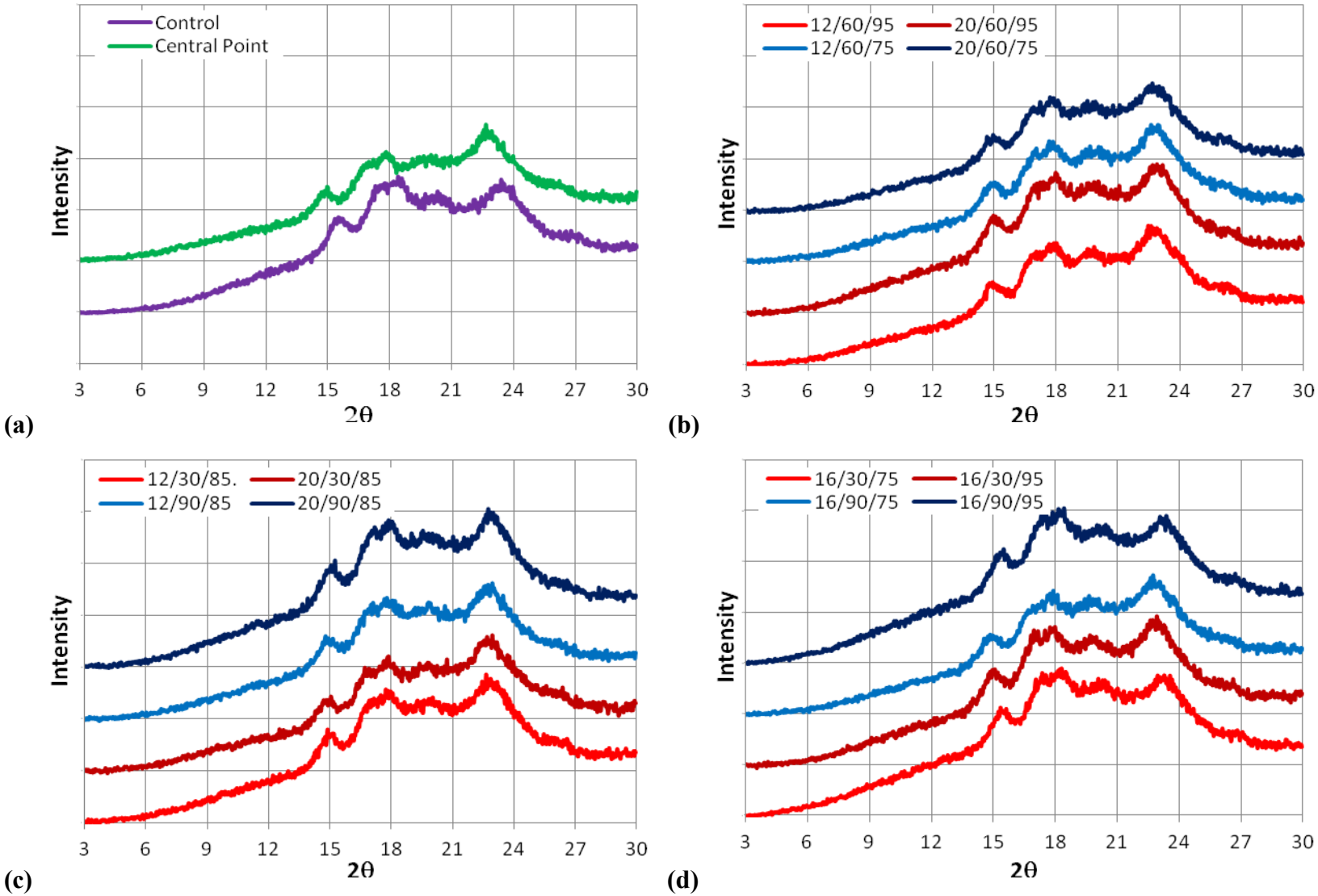

Figure 3.10. X-ray diffractograms of treated whole wheat flours (TWWF) (a) control and center point treatment, (b) moisture content × residence time effect, (c) temperature × residence time effect, (d) moisture content × temperature effect.

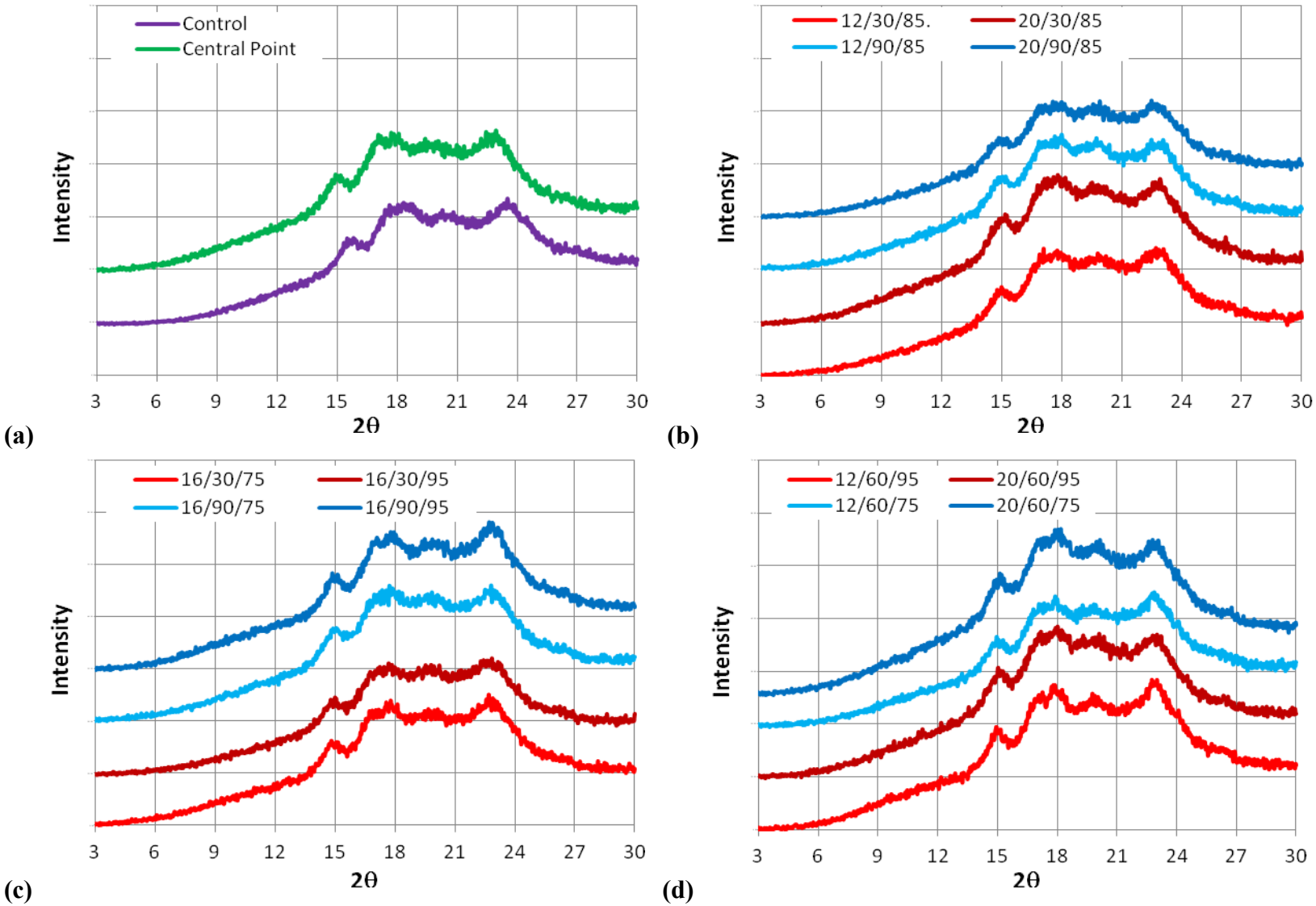

Figure 3.11. X-ray diffractograms of treated grain whole wheat flours (TGWWF) (a) control and center point treatment, (b) moisture content × residence time effect, (c) temperature × residence time effect, (d) moisture content × temperature effect.

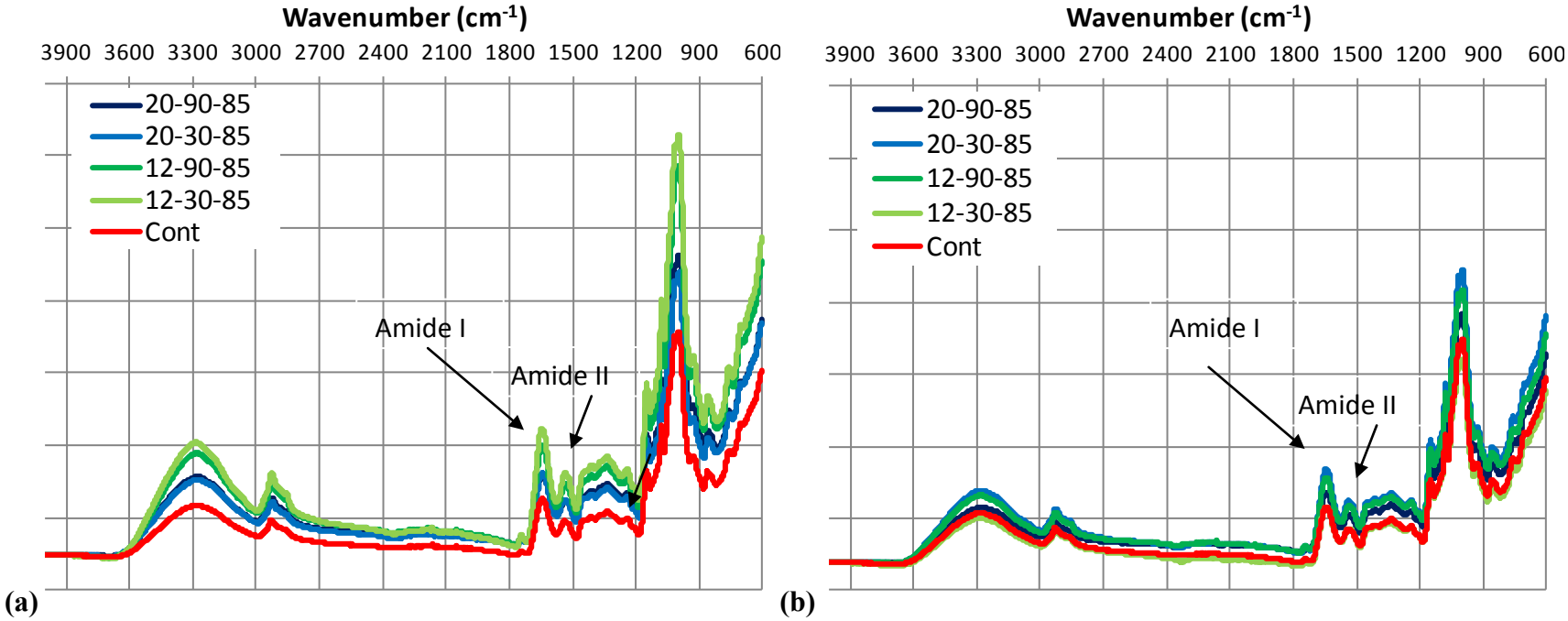

Figure 3.12. FT-IR spectra for select samples of (a) treated whole wheat flours (TWWF), and (b) treated grain whole wheat flours (TGWWF)

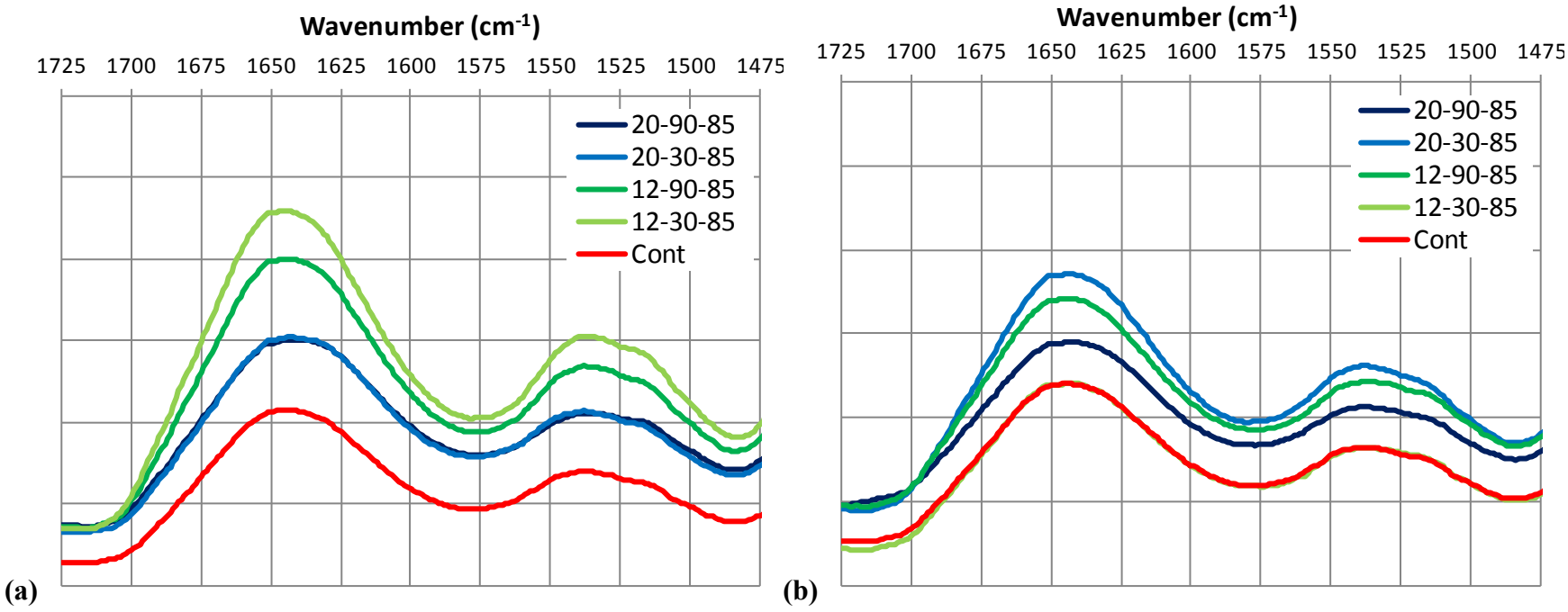

Figure 3.13. FT-IR spectra of amide I and amide II for selected samples of (a) treated whole wheat flours (TWWF), and (b) treated grain whole wheat flours (TGWWF)

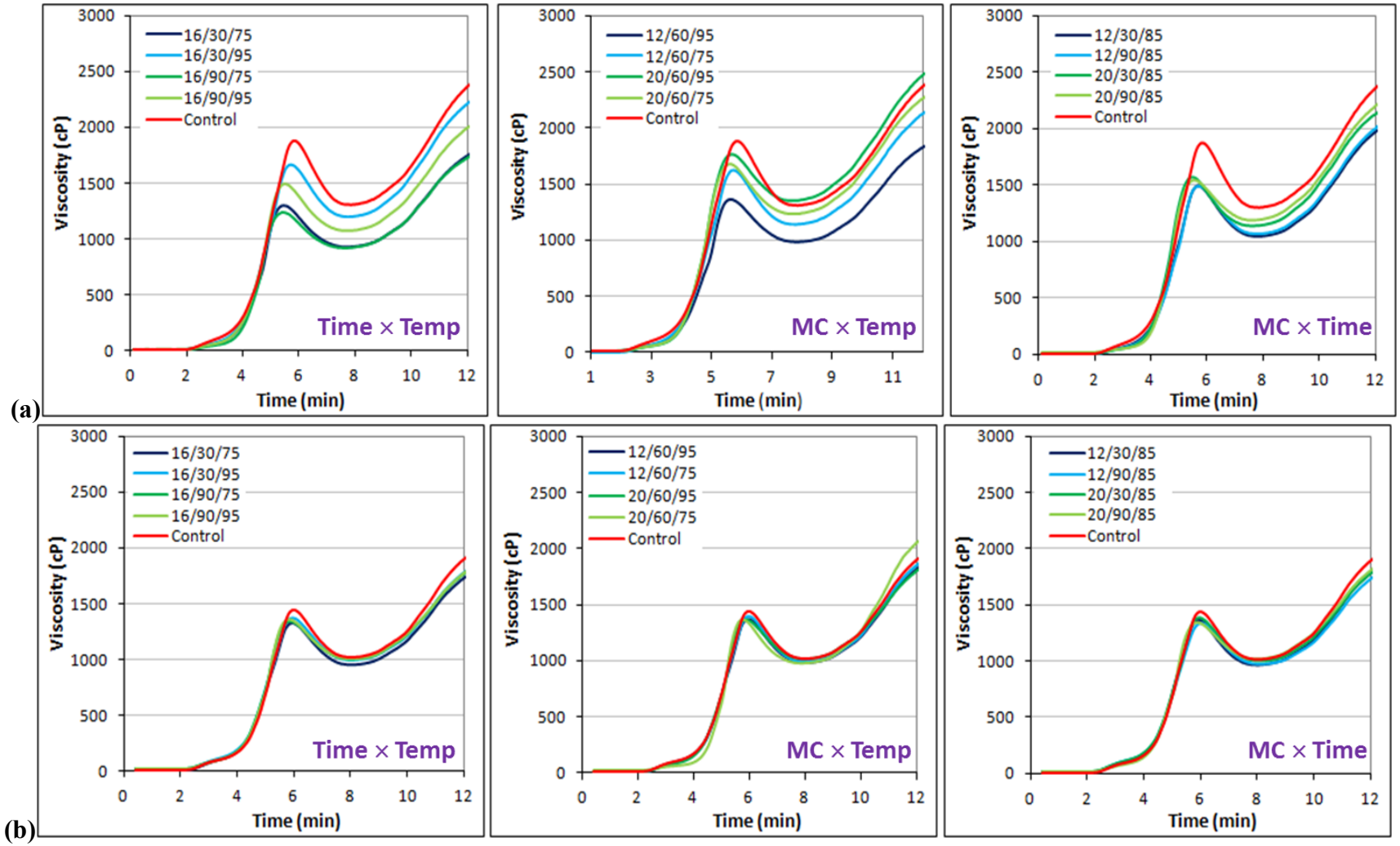

Figure 3.14. RVA pasting profiles of (a) treated whole wheat flours (TWWF), and (b) treated grain whole wheat flours (TGWWF)

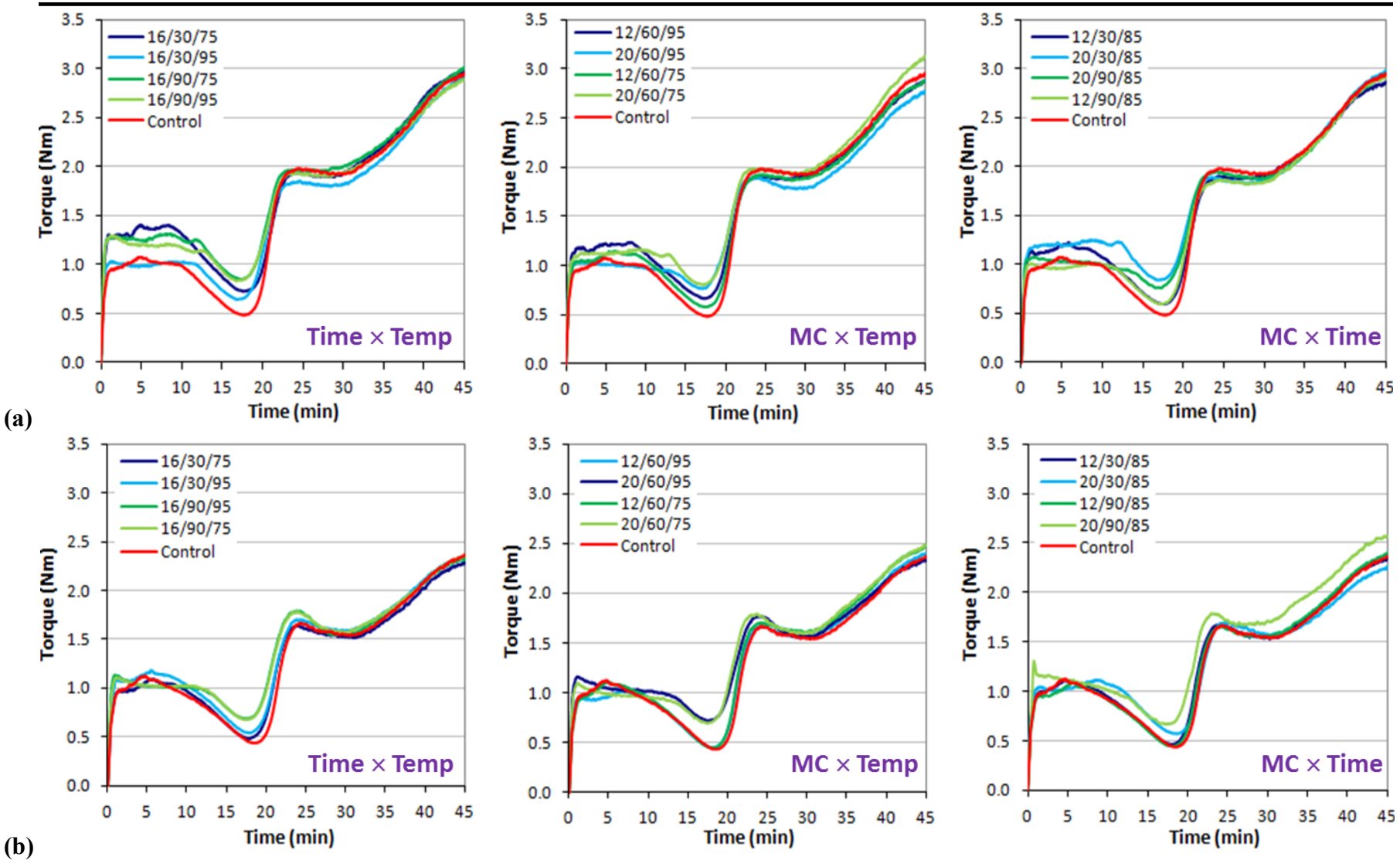

Figure 3.15. MixoLab profiles of (a) treated whole wheat flours (TWWF), and (b) treated grain whole wheat flours (TGWWF)

Table 3.1 Box-Behnken 3-parameters 3-levels experimental design

Treatment ID	Coded			Uncoded		
	X_1	X_2	X_3	Moisture (%)	Time (s)	Temperature (°C)
1	-1	0	-1	12	60	75
2	1	0	-1	20	60	75
3	-1	0	1	12	60	95
4	1	0	1	20	60	95
5	-1	-1	0	12	30	85
6	1	-1	0	20	30	85
7	-1	1	0	12	90	85
8	1	1	0	20	90	85
9	0	-1	-1	16	30	75
10	0	-1	1	16	30	95
11	0	1	-1	16	90	75
12	0	1	1	16	90	95
13	0	0	0	16	60	85
14	0	0	0	16	60	85
15	0	0	0	16	60	85

Table 3.2 Energy balance calculations for treated whole wheat flour (TWWF)

Treatment ID	X_1	X_2	X_3	Mechanical energy (kJ)			Thermal energy (kJ)			LMTD[g] (°C)	U[h] (kJ/m^2.°C)	System energy[i] (kJ)			Product energy[j] (kJ/kg)		
				Sol[a]	PCX[b]	Other[c]	Sol[d]	Sol[e]	Sol[f]			ΣME	ΣTE	Total	ME	TE	Total
1	-1	0	-1	1440	5400	140400	2746	7719	4404	45	441	147240	14869	162109	16	163	179
2	1	0	-1	4320	6120	140400	5116	7641	19745	48	904	150840	32501	183341	43	324	367
3	-1	0	1	1080	5400	140400	2998	8990	11626	51	621	146880	23614	170494	12	259	271
4	1	0	1	3240	6120	140400	6351	9254	27659	51	1133	149760	43265	193025	32	429	461
5	-1	-1	0	360	5400	140400	2902	8222	10388	51	573	146160	21512	167672	4	236	240
6	1	-1	0	1080	6480	140400	5836	8340	11555	48	726	147960	25730	173690	11	254	265
7	-1	1	0	2160	5400	140400	3109	8546	5654	48	489	147960	17309	165269	24	190	214
8	1	1	0	13320	5400	140400	5795	8471	22735	48	1036	159120	37002	196122	132	368	500
9	0	-1	-1	1080	6120	140400	3520	6851	15914	46	777	147600	26285	173885	11	273	284
10	0	-1	1	360	6480	140400	5017	9162	19973	51	909	147240	34151	181391	4	354	358
11	0	1	-1	5760	6120	140400	3942	7582	14512	49	718	152280	26036	178316	60	271	331
12	0	1	1	2880	5040	140400	4607	8866	18915	51	855	148320	32388	180708	30	337	367
13	0	0	0	5400	5400	140400	3970	7937	18024	48	834	151200	29930	181130	56	311	367
14	0	0	0	5400	6480	140400	3980	7868	18325	48	843	152280	30173	182453	56	315	371
15	0	0	0	3240	5400	140400	4570	8376	15661	47	815	149040	28606	177646	34	297	331

[a, b] mechanical energy for running Solidaire and PCX,
[c] mechanical energy consumption for turbulizer, cyclone and bag house (constant at 7200, 100800 and 32400 respectively).
[d, e and f] thermal energy in Solidaire for grain, moisture and steam evaporated respectively,
[g] log mean temperature difference,
[h] overall heat transfer coefficient
[i] mechanical, thermal and total energy done on the system,
[j] mechanical, thermal and total energy done on the product per unit mass

Table 3.3 Energy balance calculations for treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	Mechanical energy (kJ)		Thermal energy (kJ)			LMTD[g] (°C)	U[h] (kJ/m^2.°C)	System energy[i] (kJ)			Product energy[j] (kJ/kg)		
				Sol[a]	Other[c]	Sol[d]	Sol[e]	Sol[f]			ΣME	ΣTE	Total	ME	TE	Total
1	-1	0	-1	2520	140400	2529	7319	5197	47	433	142920	15045	157965	28	165	193
2	1	0	-1	12600	140400	6327	8428	7739	50	607	153000	22495	175495	126	218	344
3	-1	0	1	1800	140400	3143	9096	1076	51	352	142200	13314	155514	20	146	166
4	1	0	1	8280	140400	6986	9307	13064	50	792	148680	29357	178037	82	285	367
5	-1	-1	0	720	140400	2357	6822	9720	45	567	141120	18899	160019	7.9	208	216
6	1	-1	0	1440	140400	6456	8499	1156	47	457	141840	16111	157951	14	156	170
7	-1	1	0	6480	140400	2848	8243	5445	49	458	146880	16536	163416	71	182	253
8	1	1	0	20520	140400	6699	8924	5001	50	557	160920	20624	181544	204	200	404
9	0	-1	-1	1080	140400	4167	7083	11050	45	667	141480	22299	163779	11	227	238
10	0	-1	1	1080	140400	4614	7862	8090	47	588	141480	20565	162045	11	210	221
11	0	1	-1	24480	140400	4851	8287	12370	49	703	164880	25508	190388	255	260	515
12	0	1	1	20880	140400	5335	9137	601	51	397	161280	15072	176352	217	154	371
13	0	0	0	10080	140400	4880	8358	6871	48	560	150480	20109	170589	105	205	310
14	0	0	0	10800	140400	4880	8358	4724	48	507	151200	17961	169161	113	183	296
15	0	0	0	10800	140400	4883	8428	5885	48	539	151200	19196	170396	112	196	308

[a] mechanical energy for running Solidaire and PCX,
[c] mechanical energy consumption for turbulizer, cyclone and bag house (constant at 7200, 100800 and 32400 respectively).
[d, e and f] thermal energy in Solidaire for grain, moisture and steam evaporated respectively,
[g] log mean temperature difference,
[h] overall heat transfer coefficient
[i] mechanical, thermal and total energy done on the system,
[j] mechanical, thermal and total energy done on the product per unit mass

Table 3.4. Microbial load of treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF)

				Treated Flour				Treated Grain			
Treatment				APC	Coliform	Yeast	Mold	APC	Coliform	Yeast	Mold
ID	X_1	X_2	X_3	CFU/g				CFU/g			
Control				6400	<10	<10	<10	940	<10	<10	10
1	-1	0	-1	800				300			
2	1	0	-1	300				25000	<10	<10	22000
3	-1	0	1	700	<10	<10	<10	100	<10	<10	<10
4	1	0	1	400	<10	<10	<10	<100	<10	<10	7300
5	-1	-1	0	700				100			
6	1	-1	0	100				<100			
7	-1	1	0	500				500			
8	1	1	0	400				<100			
9	0	-1	-1	700				<100			
10	0	-1	1	100	<10	<10	<10	200			
11	0	1	-1	200				200			
12	0	1	1	5600	<10	<10	<10	200	<10	<10	<10
13	0	0	0	---[a]				<100			
14	0	0	0	800				<100			
15	0	0	0	6200				<100			

[a] This sample had outlier data and was excluded

Table 3.5. Particle size, color and composition of treated whole wheat flour (TWWF)

Treatment				Particle size				Color			Composition (%)		
ID	X_1	X_2	X_3	Mean	d10	d50	d90	L-value	a-value	b-value	Moisture	Ash	Protein
Control	-	-	-	150±0d^{ef}	17±0^{d}	82±0^{d}	401±3^{dc}	81.46±0.04^{a}	0.97±0.00^{g}	8.62±0.01^{g}	10.31±0.07^{b}	2.09±0.04^{edc}	13.94±0.06^{dfe}
1	-1	0	-1	154±2de	20±0^{c}	79±0^{edf}	368±5de	79.44±0.03^{c}	1.31±0.00^{e}	9.6±0.00^{e}	9.29±0.05^{c}	2.00±0.02^{edf}	13.90±0.03^{fe}
2	1	0	-1	208±0^{a}	26±0^{a}	120±0^{a}	473±0^{b}	76.16±0.02^{k}	1.97±0.01^{a}	10.81±0.01^{a}	10.07±0.08^{b}	1.91±0.07^{egf}	13.72±0.08^{fe}
3	-1	0	1	113±7^{h}	15±0^{f}	59±0^{i}	307±13^{g}	79.25±0.01^{d}	1.35±0.01^{e}	10.00±0.01^{d}	6.05±0.14^{h}	2.41±0.07^{a}	15.27±0.14^{a}
4	1	0	1	199±1^{ba}	25±0^{b}	111±0^{cb}	453±7^{b}	78.17±0.02^{f}	1.46±0.01^{d}	10.31±0.01^{c}	8.75±0.17^{dc}	1.83±0.03^{gf}	13.68±0.06^{fe}
5	-1	-1	0	114±9^{h}	16±0^{f}	61±2^{ih}	312±14^{fg}	79.37±0.03^{dc}	1.33±0.01^{e}	9.29±0.00^{f}	6.87±0.17^{g}	2.14±0.02^{dc}	14.90±0.11^{ba}
6	1	-1	0	217±2^{a}	24±0^{b}	115±1^{b}	523±8^{a}	76.65±0.02^{i}	1.93±0.01^{a}	10.78±0.00^{a}	12.00±0.04^{a}	1.96±0.04^{edf}	13.09±0.05^{g}
7	-1	1	0	116±1^{h}	16±0^{e}	63±0^{ih}	317±1^{fg}	79.22±0.01^{d}	1.35±0.00^{e}	9.62±0.00^{c}	8.57±0.07^{de}	1.94±0.02^{egf}	14.01±0.14^{dfe}
8	1	1	0	182±1^{bc}	25±0^{b}	107±1^{c}	413±2^{c}	77.39±0.01^{h}	1.67±0.00^{c}	10.31±0.00^{e}	10.19±0.22^{b}	1.77±0.02^{g}	13.61±0.05^{f}
9	0	-1	-1	173±2^{dc}	18±0^{d}	82±1^{d}	405±4^{dc}	76.61±0.01^{i}	1.97±0.02^{a}	10.43±0.00^{b}	8.94±0.09^{dc}	2.21±0.01^{c}	14.50±0.03^{bc}
10	0	-1	1	119±0^{gh}	16±0^{e}	66±1^{h}	318±1^{fg}	79.83±0.01^{b}	1.20±0.01^{f}	9.64±0.00^{e}	7.91±0.14^{f}	1.94±0.02^{egf}	14.08±0.16^{dfce}
11	0	1	-1	154±9de	19±0^{c}	81±0^{ed}	371±12de	76.31±0.01^{j}	1.92±0.01^{a}	10.78±0.01^{a}	8.94±0.10^{dc}	2.27±0.04^{bac}	14.39±0.03^{dc}
12	0	1	1	121±6^{gh}	18±0^{d}	71±1^{g}	313±10^{fg}	77.63±0.11^{g}	1.78±0.04^{b}	10.40±0.09^{cb}	7.01±0.01^{g}	2.23±0.04^{bc}	14.97±0.13^{a}
13	0	0	0	129±10^{ghf}	19±0^{c}	75±3^{gf}	325±14^{fg}	75.76±0.01^{l}	1.98±0.01^{a}	10.86±0.00^{a}	7.90±0.15^{f}	1.87±0.02^{gf}	14.13±0.11^{dce}
14	0	0	0	136±2^{ghef}	19±0^{c}	75±0^{gf}	339±2^{feg}	76.51±0.01^{i}	1.77±0.00^{b}	10.44±0.01^{b}	8.11±0.16^{fe}	2.39±0.02^{ba}	15.05±0.08^{a}
15	0	0	0	142±0^{gef}	19±0^{c}	77±0^{ef}	351±2^{fe}	78.52±0.01^{e}	1.43±0.00^{d}	9.92±0.01^{d}	8.51±0.12^{de}	1.99±0.05^{edf}	13.98±0.05^{dfe}

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.6. Particle size, color and composition of treated grain whole wheat flour (TGWWF)

Treatment				Particle size				Color			Composition (%)		
ID	X_1	X_2	X_3	Mean	d10	d50	d90	L-value	a-value	b-value	Moisture	Ash	Protein
Control	-	-	-	168±1^{a}	23±0^{a}	105±0^{a}	375±0^{a}	77.20±0.03^{h}	1.87±0.01^{a}	10.98±0.01^{a}	9.84±0.03^{bdc}	1.93±0.05^{bdac}	13.48±0.06^{cd}
1	-1	0	-1	141±0^{b}	21±0^{b}	99±1^{b}	328±3^{b}	77.65±0.01^{gf}	1.76±0.01^{ba}	10.78±0.01^{d}	9.11±0.01^{g}	2.01±0.02^{bdac}	14.16±0.07^{a}
2	1	0	-1	113±0^{dc}	16±0^{g}	81±1^{i}	283±1^{c}	78.61±0.02^{b}	1.70±0.01^{ba}	10.36±0.00^{j}	10.25±0.03^{a}	1.97±0.04^{bdac}	13.52±0.10^{d}
3	-1	0	1	150±11^{ba}	20±0^{cb}	95±1^{dc}	346±16^{ba}	77.72±0.00^{ef}	1.74±0.01^{ba}	10.75±0.00^{d}	9.26±0.01^{gf}	1.97±0.03^{bdac}	14.01±0.02^{ba}
4	1	0	1	147±1^{b}	18±0^{ef}	87±0^{gf}	367±0^{a}	79.37±0.01^{a}	1.34±0.15^{c}	9.81±0.00l^{k}	9.30±0.06^{egf}	1.90±0.04^{bdc}	13.60±0.03^{cd}
5	-1	-1	0	140±2^{b}	20±0^{b}	97±1^{cb}	323±3^{b}	77.49±0.12^{g}	1.75±0.01^{ba}	10.68±0.01^{e}	9.24±0.04^{gf}	2.04±0.01^{bac}	14.01±0.06^{ba}
6	1	-1	0	113±1^{dc}	17±0^{efg}	84±0^{gih}	275±3^{c}	77.93±0.03^{d}	1.80±0.01^{ba}	10.42±0.01^{i}	9.78±0.04^{dc}	1.91±0.03^{bdc}	13.54±0.03^{cd}
7	-1	1	0	139±1^{b}	20±0^{c}	97±0^{cb}	323±0^{b}	77.18±0.08^{h}	1.86±0.01^{a}	10.99±0.01^{a}	10.05±0.06^{bac}	2.08±0.03^{a}	14.24±0.04^{a}
8	1	1	0	136±6^{b}	17±0^{fg}	87±0^{gfh}	329±10^{b}	77.96±0.02^{d}	1.81±0.01^{ba}	10.70±0.02^{e}	9.76±0.02^{dc}	2.01±0.01^{bdac}	13.55±0.05^{cd}
9	0	-1	-1	136±1^{b}	20±0^{cb}	93±0^{ed}	320±0^{b}	78.30±0.01^{c}	1.70±0.01^{ba}	10.56±0.00^{i}	10.14±0.09^{ba}	1.87±0.03^{d}	13.75±0.06^{bcd}
10	0	-1	1	109±0^{d}	18±0^{ed}	83±0^{ih}	259±1^{c}	77.96±0.05^{d}	1.85±0.02^{ba}	10.87±0.02^{b}	9.64±0.19^{ed}	1.89±0.04^{dc}	13.66±0.03^{cd}
11	0	1	-1	130±9^{bx}	17±0^{efg}	85±2^{gih}	320±15^{b}	77.88±0.06^{ed}	1.73±0.01^{ba}	10.46±0.00^{h}	9.58±0.06^{edf}	2.05±0.03^{ba}	13.79±0.08^{bc}
12	0	1	1	113±0^{dc}	18±0^{efg}	84±1^{gih}	278±0^{c}	77.92±0.02^{d}	1.68±0.01^{ba}	10.41±0.00^{i}	9.57±0.05^{edf}	1.93±0.02^{bdac}	13.68±0.04^{cd}
13	0	0	0	150±2^{ba}	19±0^{d}	90±0^{ef}	370±1^{a}	78.25±0.01^{c}	1.65±0.01^{b}	10.20±0.00^{k}	9.50±0.04^{edf}	1.95±0.02^{bdac}	13.68±0.05^{cd}
14	0	0	0	111±0^{dc}	17±0^{fg}	81±0^{i}	274±0^{c}	78.28±0.00^{c}	1.72±0.01^{ba}	10.51±0.01^{g}	9.63±0.05^{ed}	1.99±0.04^{bdac}	13.74±0.03^{bcd}
15	0	0	0	114±1^{dc}	18±0^{ef}	85±0^{gih}	279±1^{c}	77.55±0.03^{gf}	1.85±0.01^{ba}	10.81±0.00^{c}	9.73±0.08^{dc}	1.95±0.03^{bdac}	13.78±0.04^{bc}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.7. Single kernel characteristic for treated whole grain

Treatment ID	X_1	X_2	X_3	Hardness index	Weight (mg)	Moisture (%)	Diameter (mm)
Control	-	-	-	72±8	26.1±2.4	12.2±0.4^{ed}	2.51±0.22
1	-1	0	-1	73±10	25.1±4.8	11.3±0.2^{e}	2.43±0.23
2	1	0	-1	55±9	28.0±5.5	16.3±0.2^{a}	2.52±0.21
3	-1	0	1	65±10	25.9±4.4	12.3±0.8^{ecd}	2.45±0.20
4	1	0	1	60±9	27.3±5.1	13.7±1.0^{bcd}	2.49±0.21
5	-1	-1	0	72±10	27.5±5.0	11.8±0.3^{e}	2.54±0.19
6	1	-1	0	61±8	27.5±4.6	15.5±0.2^{ba}	2.52±0.21
7	-1	1	0	75±9	26.8±4.8	12.0±0.2^{ed}	2.50±0.18
8	1	1	0	55±9	28.9±4.5	16.2±0.3^{a}	2.57±0.18
9	0	-1	-1	69±9	28.3±5.4	13.9±0.2^{bc}	2.58±0.20
10	0	-1	1	69±9	27.7±4.2	13.8±0.2^{bcd}	2.56±0.20
11	0	1	-1	62±10	27.3±4.9	14.3±0.2^{b}	2.51±0.17
12	0	1	1	62±10	26.4±4.9	13.7±0.2^{bcd}	2.48±0.17
13	0	0	0	62±1	26.6±4.9	14.2±0.2^{b}	2.51±0.20
14	0	0	0	62±10	25.5±4.8	14.3±0.2^{b}	2.43±0.20
15	0	0	0	61±9	25.4±4.8	14.4±0.2^{b}	2.45±0.20

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.8. Gelatinization temperature, gelatinization enthalpy and relative crystallinity of treated whole wheat flour (TWWF)

Treatment ID	X_1	X_2	X_3	Initial temp (°C)	Peak temp (°C)	Final temp (°C)	Enthalpy (J/g)	Relative crystallinity (%)
Control	-	-	-	58.3±0.4	65.6±0.9	77.5±1.4b	8.03±0.07	23.9±0.1[a]
1	-1	0	-1	58.1±0.2	66.2±0.1	81.7±0.3b[a]	9.96±0.35	15.5±0.1[ed]
2	1	0	-1	58.3±0.3	66.3±0.3	82.3±0.8[a]	9.38±1.27	14.1±0.0[f]
3	-1	0	1	58.2±0.0	65.9±0.7	82.5±1.1[a]	9.26±0.72	15.5±0.2[ed]
4	1	0	1	58.1±0.1	65.8±0.7	81.7±1.0b[a]	10.09±0.32	15.7±0.0[ed]
5	-1	-1	0	58.3±0.2	66.3±0.1	81.1±1.0b[a]	8.59±0.17	15.9±0.2[d]
6	1	-1	0	58.3±0.3	66.3±0.2	82.3±0.7[a]	10.02±0.35	13.9±0.0[f]
7	-1	1	0	58.3±0.4	66.5±0.1	82.4±0.3[a]	10.18±0.01	17.4±0.4[b]
8	1	1	0	58.1±0.0	66.4±0.0	81.4±0.6[ba]	8.92±0.35	15.2±0.0[ed]
9	0	-1	-1	58.1±0.1	66.7±0.2	83.2±0.5[a]	10.39±0.26	16.1±0.2[cd]
10	0	-1	1	58.1±0.1	65.9±0.7	82.4±1.5[a]	9.74±0.79	17.0±0.2[cb]
11	0	1	-1	58.2±0.2	66.5±0.1	83.2±0.4[a]	9.87±0.89	14.8±0.5[ef]
12	0	1	1	57.8±0.4	65.9±0.7	81.6±0.7[ba]	9.59±0.29	17.8±0.0[b]
13	0	0	0	57.5±0.0	66.6±0.1	80.9±0.5[ba]	7.97±0.04	15.4±0.1[ed]
14	0	0	0	58.4±0.2	66.4±0.1	81.4±1.4[ba]	10.33±0.33	14.1±0.1[f]
15	0	0	0	58.0±0.0	66.4±0.0	81.8±0.3[ba]	9.80±0.25	16.1±0.0[cd]

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.9. Gelatinization temperature, gelatinization enthalpy and relative crystallinity of treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	Initial temp (°C)	Peak temp (°C)	Final temp (°C)	Enthalpy (J/g)	Relative crystallinity (%)
Control	-	-	-	59.7±0.1^{a}	67.5±0.5	80.4±0.4	7.26±0.24^{e}	20.3±0.0^{a}
1	-1	0	-1	57.8±1.1^{ba}	66.4±0.1	80.8±0.6	9.00±0.05^{bdac}	14.4±0.1^{c}
2	1	0	-1	57.4±0.1^{b}	67.2±0.1	81.3±1.0	10.4±0.48^{a}	14.1±0.0^{c}
3	-1	0	1	58.6±0.2^{ba}	66.4±0.2	80.3±0.2	8.15±0.41^{de}	15.5±0.0^{b}
4	1	0	1	57.8±0.3^{ba}	66.4±0.2	80.3±0.7	7.90±0.52^{de}	13.3±0.1^{dfe}
5	-1	-1	0	58.4±0.3^{ba}	66.3±0.1	81.1±0.5	7.13±0.15^{e}	13.0±0.0^{gfe}
6	1	-1	0	58.9±0.5^{ba}	66.8±0.4	80.8±0.4	7.12±0.16^{e}	13.7±0.1^{dce}
7	-1	1	0	58.8±0.4^{ba}	66.4±0.0	80.9±0.3	8.48±0.20^{dec}	12.1±0.1^{g}
8	1	1	0	58.8±0.1^{ba}	66.6±0.3	81.6±0.5	9.51±0.09^{bac}	13.0±0.6^{gfe}
9	0	-1	-1	59.3±0.2^{ba}	66.8±0.1	80.4±0.5	8.78±0.29^{bdc}	13.0±0.0^{gfe}
10	0	-1	1	59.0±0.0^{ba}	67.2±0.0	80.8±0.4	7.89±0.22^{de}	12.2±0.1^{gh}
11	0	1	-1	58.4±0.4^{ba}	66.8±0.4	82.7±0.4	9.97±0.20^{ba}	13.1±0.1^{fe}
12	0	1	1	58.4±0.3^{ba}	66.7±0.4	80.5±0.7	9.55±0.23^{bac}	14.0±0.0^{dc}
13	0	0	0	58.3±0.2^{ba}	66.3±0.1	80.8±0.7	8.00±0.07^{de}	12.8±0.0^{ghfe}
14	0	0	0	58.6±0.0^{ba}	66.5±0.0	79.8±0.5	7.77±0.09^{de}	13.3±0.1^{dfe}
15	0	0	0	58.5±0.2^{ba}	66.6±0.2	82.0±0.7	9.97±0.20^{ba}	12.7±0.1^{ghf}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.10. Swelling and solubility of treated whole wheat flour (TWWF)

Treatment ID	X_1	X_2	X_3	Swelling (g/g)				Solubility (%)			
				60°C	70°C	80°C	90°C	60°C	70°C	80°C	90°C
Control	-	-	-	5.12±0.00^{g}	18.45±0.03^{ebdc}	25.85±0.39^{a}	27.76±0.07^{ba}	10.62±0.21^{a}	10.92±0.13^{a}	10.28±0.04^{a}	11.29±0.13^{a}
1	-1	0	-1	5.56±0.05^{dc}	18.49±0.12^{ebdc}	23.17±0.13^{d}	26.89±0.10^{bc}	7.07±0.01^{fed}	7.42±0.03^{d}	7.19±0.07^{f}	9.28±0.09^{dce}
2	1	0	-1	5.55±0.01^{dc}	18.21±0.09^{ef}	22.74±0.01^{d}	26.88±0.09^{bc}	6.19±0.29^{g}	7.35±0.08^{d}	6.70±0.11^{g}	7.98±0.05^{gf}
3	-1	0	1	5.70±0.01^{a}	18.63±0.06^{bac}	24.17±0.14^{c}	28.11±0.15^{a}	8.46±0.23^{b}	7.97±0.01^{c}	7.25±0.03^{ef}	9.98±0.47^{bc}
4	1	0	1	5.45±0.02^{de}	18.76±0.03^{bac}	24.21±0.02^{c}	26.46±0.36^{dc}	6.31±0.20^{fg}	7.98±0.02^{c}	7.59±0.11^{ed}	8.53±0.07^{dgfe}
5	-1	-1	0	5.60±0.04^{bc}	18.88±0.06^{a}	23.33±0.08^{d}	27.77±0.22^{ba}	7.78±0.03^{cbd}	7.26±0.04^{d}	7.46±0.08^{ef}	10.14±0.17^{bc}
6	1	-1	0	5.45±0.01^{de}	18.61±0.04^{bdac}	23.07±0.06^{d}	26.50±0.06^{dc}	6.31±0.01^{fg}	6.89±0.06^{e}	7.93±0.03^{cd}	7.69±0.01^{g}
7	-1	1	0	5.35±0.04^{f}	18.56±0.13^{ebdac}	25.19±0.06^{ba}	28.01±0.21^{a}	7.20±0.03^{ced}	7.35±0.01^{d}	6.63±0.10^{g}	9.20±0.07^{dce}
8	1	1	0	5.46±0.01^{de}	18.26±0.02^{edf}	23.02±0.08^{d}	25.82±0.33^{d}	6.48±0.06^{feg}	6.73±0.01^{e}	6.50±0.02^{g}	8.96±0.40^{dfe}
9	0	-1	-1	5.39±0.01^{fe}	18.55±0.06^{ebdac}	24.48±0.01^{bc}	24.36±0.32^{e}	8.35±0.07^{b}	8.53±0.04^{b}	8.12±0.10^{c}	10.64±0.13^{ba}
10	0	-1	1	5.55±0.02^{dc}	18.42±0.01^{edfc}	24.12±0.01^{c}	27.43±0.09^{bac}	7.36±0.23^{cd}	7.25±0.01^{d}	6.44±0.14^{g}	8.33±0.13^{gfe}
11	0	1	-1	5.56±0.03^{dc}	18.10±0.02^{f}	23.20±0.13^{d}	25.51±0.08^{d}	7.51±0.02^{cd}	7.25±0.01^{d}	6.57±0.01^{g}	9.37±0.17^{dc}
12	0	1	1	5.68±0.02^{ba}	18.86±0.12^{a}	24.26±0.23^{c}	27.78±0.27^{ba}	7.02±0.12^{fed}	8.40±0.02^{b}	6.38±0.05^{g}	9.15±0.20^{dce}
13	0	0	0	5.48±0.01^{de}	18.54±0.06^{ebdac}	24.85±0.11^{bc}	26.91±0.26^{bc}	7.83±0.01^{cb}	8.38±0.03^{b}	8.28±0.12^{cb}	10.60±0.15^{ba}
14	0	0	0	5.54±0.01^{dc}	18.80±0.04^{ba}	24.58±0.30^{bc}	25.72±0.02^{d}	6.60±0.09$_{feg}$	8.42±0.05^{b}	8.22±0.07^{cb}	8.71±0.02^{dgfe}
15	0	0	0	5.31±0.01^{f}	18.86±0.02^{a}	24.47±0.14^{bc}	27.30±0.02^{bac}	6.54±0.19^{feg}	8.36±0.09^{b}	8.52±0.03^{b}	8.29±0.21^{gfe}

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.11. Swelling and solubility of treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	Swelling (g/g)				Solubility (%)			
				60°C	70°C	80°C	90°C	60°C	70°C	80°C	90°C
Control	-	-	-	5.53±0.02^{ebdc}	19.93±0.09^{a}	26.41±0.25^{a}	27.91±0.08^{ba}	7.33±0.08^{a}	8.02±0.11^{bdec}	8.41±0.24^{a}	10.49±0.03^{a}
1	-1	0	-1	5.46±0.04^{edc}	20.39±0.04^{a}	25.76±0.39^{ba}	28.24±0.34^{ba}	6.83±0.07^{b}	8.30±0.01^{ba}	8.18±0.03^{ba}	7.14±0.01^{ed}
2	1	0	-1	5.33±0.05^{e}	19.40±0.09^{ba}	25.14±0.00^{ebdc}	28.11±0.37^{ba}	5.77±0.13^{ed}	7.92±0.05^{dec}	7.93±0.08^{bac}	6.71±0.13^{f}
3	-1	0	1	5.72±0.02^{bac}	20.33±0.05^{a}	25.92±0.07^{ba}	24.00±0.15^{de}	6.65±0.05^{cb}	8.21±0.04^{bac}	7.38±0.09^{bc}	7.17±0.02^{ed}
4	1	0	1	5.75±0.02^{bac}	18.44±0.04^{c}	23.98±0.23^{e}	25.45±0.33^{c}	6.24±0.03^{cd}	8.40±0.12^{a}	7.07±0.44^{c}	7.29±0.11^{ed}
5	-1	-1	0	5.86±0.04^{a}	18.70±0.17^{bc}	25.42±0.12^{bac}	24.50±0.40^{dce}	7.51±0.19^{a}	7.45±0.03^{f}	7.18±0.01^{c}	8.92±0.12^{cb}
6	1	-1	0	5.45±0.03^{edc}	20.20±0.08^{a}	25.57±0.24^{bac}	27.08±0.40^{b}	6.22±0.12^{cd}	7.82±0.01^{de}	7.08±0.16^{c}	6.89±0.29^{ed}
7	-1	1	0	5.71±0.03^{bdac}	19.39±0.19^{ba}	25.97±0.01^{ba}	23.38±0.15^{e}	6.54±0.16^{cb}	7.66±0.08^{fe}	7.77±0.20^{bac}	8.39±0.01^{cd}
8	1	1	0	5.56±0.17^{ebdc}	19.45±0.46^{ba}	24.09±0.24^{ed}	23.56±0.21^{e}	5.38±0.03^{e}	7.95±0.09^{bdec}	7.12±0.0^{6c}	9.17±0.12^{b}
9	0	-1	-1	5.42±0.07^{ed}	20.15±0.21^{a}	25.16±0.21^{ebdc}	29.03±0.41^{a}	6.49±0.15^{cb}	7.67±0.12^{fe}	7.55±0.20^{bac}	6.63±0.33^{f}
10	0	-1	1	5.51±0.03^{ebdc}	20.40±0.18^{a}	25.12±0.10^{ebdc}	28.47±0.14^{ba}	5.30±0.02^{e}	7.82±0.01^{de}	7.21±0.27^{bc}	7.71±0.23^{ed}
11	0	1	-1	5.77±0.05^{ba}	20.13±0.12^{a}	24.75±0.36^{ebdc}	24.16±0.30^{dce}	6.32±0.20^{cb}	8.08±0.01^{bdac}	7.95±0.06^{bac}	9.51±0.04^{b}
12	0	1	1	5.78±0.02^{ba}	19.88±0.13^{a}	25.27±0.32b^{dac}	23.67±0.15^{de}	6.66±0.04^{cb}	7.68±0.06^{fe}	7.27±0.09^{bc}	9.29±0.26^{b}
13	0	0	0	5.77±0.01^{ba}	20.01±0.33^{a}	25.18±0.15^{ebdac}	24.51±0.28^{dce}	6.65±0.07^{cb}	8.12±0.05^{bdac}	7.02±0.21^{c}	7.15±0.04^{ed}
14	0	0	0	5.59±0.02^{ebdac}	18.61±0.04^{bc}	24.35±0.29^{edc}	25.12±0.18^{dc}	6.65±0.05^{cb}	8.14±0.08^{bdac}	7.23±0.03^{bc}	7.14±0.09^{ed}
15	0	0	0	5.53±0.07^{ebdc}	19.76±0.12^{a}	24.12±0.24^{ed}	24.79±0.05^{dce}	6.45±0.21^{cb}	7.97±0.03^{bdec}	7.66±0.12^{bac}	7.12±0.12^{ed}

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.12. Solvent retention capacity of treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	Treated Flour				Treated Grain			
				Water (g/g)	Sucrose (g/g)	Na-carbonate (g/g)	Lactic acid (g/g)	Water (g/g)	Sucrose (g/g)	Na-carbonate (g/g	Lactic acid (g/g)
Control	-	-	-	77 ± 0^{e}	105 ± 0^{f}	86 ± 1^{f}	91 ± 3^{ebdac}	87 ± 0^{d}	122 ± 0^{h}	103 ± 1^{f}	92 ± 3^{e}
1	-1	0	-1	86 ± 1^{d}	120 ± 0^{ebdc}	101 ± 1^{edc}	90 ± 0^{edc}	88 ± 0^{d}	124 ± 1^{g}	105 ± 2^{e}	92 ± 1^{e}
2	1	0	-1	91 ± 1^{bdac}	117 ± 0^{e}	97 ± 0^{e}	87 ± 0^{e}	$96\pm1b^{c}$	134 ± 2^{be}	110 ± 0^{ed}	95 ± 1^{dec}
3	-1	0	1	90 ± 1^{bdac}	123 ± 0^{ebdac}	109 ± 1^{a}	93 ± 1^{bdac}	95 ± 2^{bc}	123 ± 1^{g}	106 ± 0^{e}	92 ± 1^{e}
4	1	0	1	91 ± 0^{bdac}	119 ± 0^{edc}	104 ± 2^{bdac}	92 ± 0^{ebdac}	103 ± 0^{a}	135 ± 1^{bcd}	121 ± 1^{a}	105 ± 1^{a}
5	-1	-1	0	86 ± 1^{dc}	109 ± 0^{f}	103 ± 0^{bdac}	90 ± 1^{ebdc}	88 ± 1^{d}	126 ± 0^{fg}	109 ± 1^{ed}	93 ± 0^{e}
6	1	-1	0	92 ± 0^{bac}	125 ± 4^{bdac}	103 ± 1^{ebdac}	94 ± 1^{bac}	93 ± 1^{bdc}	133 ± 2^{becd}	114 ± 2^{bcd}	98 ± 2^{bdec}
7	-1	1	0	89 ± 1^{bdc}	118 ± 1^{ed}	100 ± 1^{ed}	89 ± 0^{ed}	90 ± 1^{dc}	127 ± 0^{feg}	109 ± 1^{ed}	94 ± 1^{de}
8	1	1	0	89 ± 2^{bdc}	119 ± 2^{edc}	99 ± 0^{ed}	88 ± 2^{ed}	94 ± 1^{bdc}	142 ± 1^{a}	116 ± 0^{ba}	101 ± 0^{bac}
9	0	-1	-1	92 ± 1^{bdac}	122 ± 0^{ebdac}	107 ± 0^{ba}	94 ± 2^{bac}	92 ± 2^{bdc}	129 ± 0^{fegd}	114 ± 1^{bc}	96 ± 0^{bdec}
10	0	-1	1	89 ± 2^{bdc}	118 ± 0^{edc}	102 ± 0^{ebdc}	92 ± 0^{ebdac}	95 ± 2^{bc}	131 ± 1^{fecd}	117 ± 1^{ba}	98 ± 0^{bdec}
11	0	1	-1	90 ± 2^{bdc}	122 ± 1^{ebdac}	103 ± 0^{ebdac}	91 ± 0^{ebdc}	96 ± 1^{bc}	138 ± 3^{ba}	115 ± 2^{bc}	102 ± 1^{bac}
12	0	1	1	90 ± 1^{bdc}	127 ± 1^{a}	107 ± 1^{ba}	96 ± 0^{ba}	96 ± 1^{bac}	136 ± 0^{bc}	119 ± 0^{ba}	100 ± 0^{bdac}
13	0	0	0	96 ± 1^{a}	126 ± 2^{ba}	106 ± 2^{bac}	96 ± 0^{a}	99 ± 3^{ba}	130 ± 2^{fecd}	122 ± 1^{a}	102 ± 2^{ba}
14	0	0	0	94 ± 1^{ba}	125 ± 1^{bac}	105 ± 1^{bac}	95 ± 1^{bac}	99 ± 1^{ba}	135 ± 0^{bcd}	119 ± 1^{ba}	101 ± 1^{bdac}
15	0	0	0	87 ± 1^{dc}	124 ± 2^{ebdac}	101 ± 2^{edc}	89 ± 1^{edc}	95 ± 0^{bc}	131 ± 1^{fecd}	116 ± 1^{ba}	97 ± 1^{bdec}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.13. Protein extraction profile of treated whole wheat flour (TWWF)

Treatment ID	X_1	X_2	X_3	SPP (%)	Gliadin (%)	Albumen/Globulin (%)	IPP (%)
Control	-	-	-	12.30±0.00^{a}	61.40±0.30^{fe}	3.65±0.04^{a}	22.65±0.15^{d}
1	-1	0	-1	7.18±0.11^{e}	61.88±0.36^{dfe}	2.48±0.02^{f}	28.47±0.50^{c}
2	1	0	-1	7.97±0.05^{d}	63.81±0.28^{dce}	2.67±0.02^{dce}	25.56±0.34^{dc}
3	-1	0	1	9.45±0.07^{c}	70.10±0.20^{a}	2.77±0.02^{dc}	17.70±0.12^{e}
4	1	0	1	5.58±0.22^{f}	56.63±1.20^{g}	2.26±0.06^{g}	35.54±1.48^{a}
5	-1	-1	0	10.05±0.01^{b}	70.95±0.52^{a}	2.82±0.03^{c}	16.19±0.56^{e}
6	1	-1	0	8.42±0.01^{d}	63.60±0.60^{dce}	2.61±0.03^{dfe}	25.38±0.62^{dc}
7	-1	1	0	10.24±0.05^{b}	68.56±0.05^{ba}	2.62±0.00^{dfe}	18.59±0.10^{e}
8	1	1	0	6.11±0.13^{f}	59.14±0.08^{gf}	2.50±0.00^{fe}	32.27±0.05^{b}
9	0	-1	-1	9.42±0.09^{c}	70.52±0.58^{a}	3.00±0.00^{b}	17.07±0.70^{e}
10	0	-1	1	9.23±0.20^{c}	66.07±0.99^{bc}	2.46±0.04^{f}	22.25±1.22^{e}
11	0	1	-1	8.49±0.20^{d}	65.74±0.95^{bc}	2.80±0.03^{c}	22.98±1.18^{d}
12	0	1	1	6.95±0.01^{e}	63.20±0.35^{dce}	2.66±0.01^{dce}	27.20±0.36^{c}
13	0	0	0	7.03±0.29^{e}	64.73±0.62^{dc}	2.99±0.06^{b}	25.25±0.84^{dc}
14	0	0	0	6.99±0.10^{e}	64.23±0.13^{dce}	2.83±0.03^{c}	25.96±0.19^{dc}
15	0	0	0	7.36±0.03^{e}	62.41±0.62^{de}	2.52±0.04^{fe}	27.72±0.63^{c}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.14. Protein extraction profile of treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	SPP (%)	Gliadin (%)	Albumen/Globulin (%)	IPP (%)
Control	-	-	-	12.20±0.20^{a}	59.30±0.60^{dec}	3.90±0.00^{a}	24.60±0.80^{ef}
1	-1	0	-1	9.94±0.21^{b}	65.85±0.56^{a}	2.73±0.06^{b}	21.49±0.71^{f}
2	1	0	-1	6.55±0.20^{f}	54.31±0.45^{f}	2.52±0.01^{ced}	36.63±0.66^{b}
3	-1	0	1	9.95±0.29^{b}	65.33±0.26^{a}	2.73±0.01^{b}	22.01±0.56^{f}
4	1	0	1	7.90±0.05^{ed}	56.68±0.47^{fe}	2.51±0.01^{ced}	32.92±0.43^{cb}
5	-1	-1	0	9.49±0.26^{cb}	64.06±1.25^{ba}	2.70±0.05^{cb}	23.77±1.04^{ef}
6	1	-1	0	10.12±0.02^{b}	64.94±0.31^{ba}	2.52±0.01^{ced}	21.92±0.79^{f}
7	-1	1	0	9.60±0.33^{cb}	63.68±0.42^{bac}	2.54±0.03^{cebd}	24.18±0.78^{ef}
8	1	1	0	5.59±0.07^{g}	48.34±0.49^{g}	2.54±0.01^{cebd}	44.54±0.57^{a}
9	0	-1	-1	10.15±0.11^{b}	65.13±0.09^{a}	2.59±0.01^{cbd}	22.15±0.18^{f}
10	0	-1	1	10.01±0.18^{b}	63.62±2.80^{bac}	2.54±0.08^{cebd}	23.83±3.06^{ef}
11	0	1	-1	8.31±0.25^{ed}	57.70±0.28^{fde}	2.37±0.01^{e}	31.64±0.54^{cd}
12	0	1	1	7.58±0.15^{e}	56.80±0.62^{fe}	2.36±0.05^{e}	33.27±0.81^{cb}
13	0	0	0	9.55±0.04^{cb}	60.53±0.30^{bdec}	2.39±0.02^{ed}	27.54±0.28^{ed}
14	0	0	0	8.68±0.26^{cd}	58.03±0.31^{fde}	2.36±0.08^{e}	30.93±0.14^{cd}
15	0	0	0	9.43±0.20^{cb}	61.35±0.35^{bdac}	2.48±0.01^{ed}	26.75±0.56^{edf}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.15. RVA pasting profile parameters of treated whole wheat flour (TWWF)

Treatment ID	X_1	X_2	X_3	Viscosity (cP) Peak	Trough	Breakdown	Final	Setback	Peak time (min)	Peak temp (°C)
Control	-	-	-	2610±1^{a}	1696±4^{a}	919±2^{a}	3357±6^{a}	1667±4^{a}	6.0±0.0^{a}	67.3±0.4
1	-1	0	-1	1718±10^{cb}	1223±15^{cbd}	495±5^{b}	2315±12^{c}	1092±3^{cb}	5.8±0.1^{fbdec}	50.1±0.0
2	1	0	-1	1548±4^{fe}	1142±7^{f}	406±3^{gef}	2144±13^{e}	1002±6^{ed}	5.6±0.0^{g}	85.9±0.4
3	-1	0	1	1501±5^{fe}	1080±4^{g}	421±1^{gefd}	2006±11^{gf}	926±7^{ef}	5.9±0.1^{bac}	70.2±1.7
4	1	0	1	1679±8^{c}	1275±18^{b}	404±26^{gef}	2413±14^{b}	1138±32^{b}	5.8±0.0^{fgdec}	63.2±3.7
5	-1	-1	0	1527±1^{fe}	1077±13^{g}	451±12^{cebd}	2049±0^{f}	973±13^{edf}	5.9±0.0^{bdec}	82.8±2.0
6	1	-1	0	1615±4^{d}	1155±7^{fe}	461±3^{cbd}	2279±26^{dc}	1125±33^{b}	5.7±0.0^{fg}	66.0±8.3
7	-1	1	0	1689±3^{c}	1201±1^{fed}	488±2^{cb}	2293±18^{dc}	1092±17^{cb}	6.0±0.0^{ba}	86.0±1.3
8	1	1	0	1561±3^{e}	1176±11^{fed}	385±14^{g}	2218±6^{d}	1042±17^{cd}	5.7±0.0^{fgde}	87.2±0
9	0	-1	-1	1419±34^{g}	974±29^{h}	446±6^{cebd}	1949±49^{g}	975±20^{edf}	5.7±0.0^{fge}	77.1±7.7
10	0	-1	1	1756±8^{b}	1268±11^{cb}	488±3^{cb}	2389±9^{b}	1121±2^{cb}	5.9±0.0^{bdac}	65.9±1
11	0	1	-1	1516±0^{fe}	1070±5^{g}	446±5^{cebd}	2049±2^{f}	979±4^{edf}	5.7±0.0^{fgde}	57.5±7
12	0	1	1	1499±24^{f}	1080±13^{g}	419±11^{gefd}	2035±16^{f}	955±3^{ef}	5.7±0.0^{fge}	62.4±1
13	0	0	0	1353±5^{h}	963±3^{h}	390±2^{gf}	1871±6^{h}	909±9^{f}	5.6±0.0^{fg}	68.0±1
14	0	0	0	1488±14^{f}	1049±14^{g}	438±1^{cefd}	2007±8^{gf}	957±23^{ef}	5.6±0.0^{g}	78.4±1
15	0	0	0	1697±7^{c}	1211±8^{ced}	486±15^{cb}	2301±1^{c}	1090±9^{cb}	5.8±0.0^{fgdec}	77.9±6.9

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.16. RVA pasting profile parameters of treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	Viscosity (cP)					Peak time (min)	Peak temp (°C)
				Peak	Trough	Breakdown	Final	Setback		
Control	-	-	-	1460±14^{a}	1032±14^{ba}	429±1^{ba}	2023±14^{cb}	992±1^{dc}	6.0±0.0^{ba}	88.8±0.9
1	-1	0	-1	1468±2^{a}	1004±14^{bac}	464±13^{a}	2028±13^{b}	1024±2^{c}	5.9±0.1^{bac}	85.2±5.2
2	1	0	-1	1430±12b^{a}	992±4^{bc}	438±8^{ba}	2289±27^{a}	1298±23^{b}	5.7±0.0^{dc}	87.9±0.7
3	-1	0	1	1425±112^{ba}	1011±7^{bac}	415±6^{bc}	1944±1^{cbd}	934±8^{e}	6.0±0.1^{a}	69.5±0.1
4	1	0	1	1422±16^{ba}	1046±11^{a}	376±5^{fecd}	1902±22^{ced}	856±11^{gfh}	5.9±0.1^{bdac}	80.3±6.8
5	-1	-1	0	1410±12^{bc}	977±5^{c}	433±17^{ba}	1924±7^{cebd}	947±12de	5.9±0.0^{bac}	77.8±10.1
6	1	-1	0	1389±3^{becd}	1005±9^{bac}	384±7^{fecd}	1853±1^{ed}	848±10^{gfh}	5.9±0.0^{bac}	69.0±1.2
7	-1	1	0	1397±5^{bcd}	979±20^{c}	418±16^{bc}	1915±14^{cebd}	936±7^{e}	5.9±0.0^{bdac}	53.8±1.0
8	1	1	0	1372±10^{fecd}	975±1^{c}	397±9^{becd}	2379±3^{a}	1435±27^{a}	5.7±0.0^{d}	80.7±11.3
9	0	-1	-1	1375±3^{fecd}	978±5^{c}	397±7^{becd}	1857±6^{ed}	879±10^{f}	6.0±0.0^{ba}	85.1±2.1
10	0	-1	1	1429±9^{ba}	1020±7^{bac}	409±2^{bcd}	1893±5^{ed}	873±2^{gf}	6.0±0.0^{ba}	73.5±2.5
11	0	1	-1	1339±5^{f}	985±5^{bc}	354±0^{fe}	1797±3^{e}	812±2^{h}	5.9±0.0^{bdc}	72.9±14.3
12	0	1	1	1363±6^{fd}	1021±4^{bac}	342±10^{f}	1838±2^{ed}	817±3^{gh}	5.8±0.0^{bdac}	78.7±5.2
13	0	0	0	1349±9^{fe}	1006±8^{bac}	343±1^{f}	1823±4^{ed}	817±4^{gh}	5.8±0.0^{bdac}	60.7±7.0
14	0	0	0	1340±5^{f}	972±3^{c}	368±2^{fed}	1891±84^{ed}	829±9^{gfh}	5.8±0.0^{bdc}	69.3±14.7
15	0	0	0	1339±7^{f}	983±11^{bc}	357±3^{fe}	1812±11^{e}	829±0^{gfh}	5.8±0.0^{bdc}	72.4±14.8

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.17. MixoLab mixing and pasting properties of treated whole wheat flour (TWWF)

Treatment				Mixing time(min)	Torque(N.m)				
ID	X_1	X_2	X_3	C1	C1	C2	C3	C4	C5
Control	-	-	-	4.8±0.2^{d}	1.10±0.02^{e}	0.50±0.01^{f}	2.01±0.02^{a}	1.94±0.02^{b}	2.94±0.03^{bac}
1	-1	0	-1	1.64±0.0^{c}	1.04±0.01^{d}	0.58±0.01^{e}	1.92±0.01^{bdc}	1.87±0.01^{cd}	2.91±0.02^{bc}
2	1	0	-1	1.25±0.0^{c}	1.10±0.00^{d}	0.82±0.02^{b}	1.98±0.00^{ba}	1.93±0.00^{cb}	3.12±0.02^{a}
3	-1	0	1	1.50±0.0^{c}	1.20±0.01^{d}	0.67±0.01^{d}	1.87±0.00^{ed}	1.93±0.00^{cb}	2.93±0.03^{bc}
4	1	0	1	2.1±0.1^{e}	1.05±0.02^{fg}	0.77±0.01^{c}	1.90±0.3^{edc}	1.77±0.00^{e}	2.81±0.0^{4c}
5	-1	-1	0	1.30±0.1^{c}	1.12±0.01^{d}	0.62±0.02^{e}	1.87±0.04^{ed}	1.88±0.02^{cbd}	2.86±0.0^{1c}
6	1	-1	0	1.40±0.3^{c}	1.18±0.01^{d}	0.84±0.08^{b}	1.90±0.00^{edc}	1.83±0.01^{ed}	2.97±0.03^{bac}
7	-1	1	0	1.2±0.0^{f}	1.00±0.01^{h}	0.60±0.40^{e}	1.87±0.0^{ed}	1.82±0.01^{ed}	2.90±0.02^{bc}
8	1	1	0	1.4±0.0^{f}	1.09±0.01^{fe}	0.77±0.01^{c}	1.96±0.01^{bac}	1.87±0.01^{cd}	2.96±0.01^{bac}
9	0	-1	-1	1.29±0.2^{c}	1.29±0.01^{b}	0.74±0.01^{c}	1.95±0.0^{bac}	1.90±0.00^{cb}	2.88±0.10^{bc}
10	0	-1	1	1.3±0.0f	1.03±0.00^{hg}	0.64±0.00^{d}	1.85±0.00^{e}	1.79±0.01^{e}	2.92±0.02^{bc}
11	0	1	-1	1.42±0.3c	1.29±0.00^{b}	0.85±0.01^{b}	1.94±0.00^{bac}	2.00±0.00^{a}	3.07±0.06^{ba}
12	0	1	1	1.3±0.0c	1.32±0.02^{b}	0.84±0.01^{b}	1.96±0.02^{bac}	1.92±0.02^{cb}	2.94±0.05^{bac}
13	0	0	0	1.9±0.1f	1.38±0.01^{a}	0.90±0.00^{a}	1.93±0.01^{bdc}	1.87±0.00^{cd}	2.86±0.01^{c}
14	0	0	0	1.4±0.1c	1.33±0.02^{b}	0.89±0.01^{a}	1.93±0.02^{bdc}	1.87±0.02^{cd}	2.87±0.03^{c}
15	0	0	0	1.9±0.0f	1.16±0.01^{d}	0.77±0.01^{c}	1.95±0.03^{bac}	1.94±0.02^{b}	3.12±0.01^{a}

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.18. MixoLab mixing and pasting properties of treated grain whole wheat flour (TGWWF)

Treatment				Mixing time(min)	Torque(N.m)				
ID	X_1	X_2	X_3	C1	C1	C2	C3	C4	C5
Control	-	-	-	4.8±0.1d	1.12±0.00^{efdc}	0.43±0.01^{h}	1.67±0.01^{egf}	1.52±0.03^{e}	2.33±0.04^{ed}
1	-1	0	-1	5.0±0.2d	1.11±0.01^{efd}	0.44±0.01^{gh}	1.70±0.02^{ed}	1.58±0.02^{cbd}	2.48±0.01^{bc}
2	1	0	-1	1.0±0.0e	1.10±0.01^{efg}	0.70±0.00^{ba}	1.80±0.00^{a}	1.60±0.01^{b}	2.51±0.02^{b}
3	-1	0	1	1.5±0.1e	0.96±0.02^{hg}	0.46±0.00^{gfh}	1.68±0.01^{edf}	1.56±0.01^{cebd}	2.43±0.02^{bcd}
4	1	0	1	1.0±0.0e	1.15±0.01^{bac}	0.72±0.01^{a}	1.77±0.00^{bac}	1.56±0.01^{cebd}	2.35±0.02^{ed}
5	-1	-1	0	4.6±0.0d	1.13±0.01^{efdc}	0.47±0.01^{gf}	1.69±0.01^{edf}	1.54±0.01^{ced}	2.40±0.05^{ecd}
6	1	-1	0	1.6±0.1e	0.98±0.01^{hg}	0.58±0.01^{d}	1.70±0.00^{d}	1.56±0.00^{cebd}	2.29±0.02^{e}
7	-1	1	0	5.8±0.2c	1.09±0.01^{fg}	0.45±0.01^{gh}	1.66±0.01^{gf}	1.55±0.01^{cebd}	2.40±0.01^{ecd}
8	1	1	0	0.9±0.1e	1.18±0.01^{ba}	0.66±0.00^{c}	1.78±0.01^{ba}	1.66±0.00^{a}	2.60±0.03^{a}
9	0	-1	-1	1.95±0.1c	1.11±0.02^{efdc}	0.48±0.00^{f}	1.64±0.01^{g}	1.51±0.01^{e}	2.29±0.01^{e}
10	0	-1	1	5.5±0.1c	0.98±0.01^{hg}	0.54±0.00^{e}	1.70±0.01^{d}	1.58±0.01^{cbd}	2.37±0.02^{ed}
11	0	1	-1	1.0±0.0e	1.12±0.01^{efdc}	0.67±0.01^{bc}	1.78±0.01^{ba}	1.56±0.01^{cebd}	2.36±0.00^{ed}
12	0	1	1	1.0±0.1e	1.14±0.01^{edc}	0.68±0.00^{bc}	1.80±0.01^{a}	1.53±0.01^{ed}	2.33±0.00^{ed}
13	0	0	0	1.4±0.0e	1.07±0.01^{hg}	0.68±0.02^{bc}	1.77±0.01^{bac}	1.56±0.02^{cebd}	2.33±0.00^{ed}
14	0	0	0	1.4±0.1e	1.14±0.00^{bdc}	0.68±0.00^{bc}	1.74±0.00^{c}	1.56±0.01^{cebd}	2.36±0.01^{ed}
15	0	0	0	1.4±0.0e	1.12±0.00^{efdc}	0.67±0.01^{c}	1.76±0.01^{bc}	1.59±0.00^{cb}	2.35±0.02^{ed}

Means in columns with same letters are not significantly different (P > 0.05); n=2

Table 3.19. MixoLab secondary mixing and pasting properties of treated whole wheat flour (TWWF)

Treatment ID	X_1	X_2	X_3	Alpha (-)	Beta (-)	Gamma (-)	Amplitude (N.m)	Stability (min)
Control	-	-	-	-0.064±0.002	0.532±0.100^{a}	-0.041±0.003^{f}	0.085±0.005^{egdf}	9.9±0.1^{f}
1	-1	0	-1	-0.047±0.001	0.413±0.021^{b}	-0.011±0.001^{a}	0.085±0.005^{egdf}	10.3±0.1^{f}
2	1	0	-1	-0.035±0.001	0.354±0.000^{cebd}	-0.025±0.001^{dec}	0.105±0.005^{ebdac}	13.7±0.1^{ba}
3	-1	0	1	-0.078±0.002	0.373±0.007^{cebd}	-0.012±0.002^{ba}	0.007±0.000^{g}	10.0±0.1^{f}
4	1	0	1	-0.010±0.000	0.377±0.001^{cebd}	-0.026±0.002^{de}	0.075±0.005^{gf}	13.8±0.3^{a}
5	-1	-1	0	-0.066±0.004	0.396±0.006^{cb}	-0.023±0.003^{dec}	0.080±0.010^{egf}	9.7±0.1^{f}
6	1	-1	0	-0.065±0.005	0.336±0.002^{ced}	-0.024±0.002^{dec}	0.110±0.000^{bdac}	12.9±0.1^{bdc}
7	-1	1	0	-0.085±0.001	0.396±0.014^{cb}	-0.017±0.001^{bdac}	0.075±0.010^{gf}	12.3±0.1^{ed}
8	1	1	0	-0.009±0.001	0.405±0.029^{cb}	-0.022±0.002^{bdec}	0.090±0.010^{ebdfc}	13.2±0.0^{bac}
9	0	-1	-1	-0.059±0.005	0.373±0.001^{cebd}	-0.015±0.001^{bac}	0.125±0.005^{ba}	10.5±0.1^{f}
10	0	-1	1	-0.083±0.004	0.383±0.005^{cbd}	-0.031±0.001^{e}	0.090±0.000^{ebdfc}	12.7±0.1^{edc}
11	0	1	-1	-0.058±0.004	0.343±0.015^{cebd}	-0.024±0.002^{dec}	0.130±0.010^{a}	12.6±0.1^{edc}
12	0	1	1	-0.002±0.000	0.319±0.017^{efd}	-0.020±0.000^{bdac}	0.115±0.005^{bac}	12.0±0.3^{e}
13	0	0	0	-0.060±0.005	0.265±0.007^{f}	-0.022±0.002^{bdec}	0.130±0.000^{a}	12.6±0.1^{edc}
14	0	0	0	-0.060±0.003	0.306±0.010^{ef}	-0.012±0.002^{ba}	0.110±0.000^{bdac}	12.8±0.03^{edc}
15	0	0	0	-0.031±0.003	0.372±0.024^{cebd}	-0.019±0.001^{bdac}	0.100±0.010^{ebdfc}	12.9±0.2^{bdc}

Means in columns with same letters are not significantly different (P > 0.05)

Table 3.20. MixoLab secondary mixing and pasting properties of treated grain whole wheat flour (TGWWF)

Treatment ID	X_1	X_2	X_3	Alpha (-)	Beta (-)	Gamma (-)	Amplitude (N.m)	Stability (min)
Control	-	-	-	-0.050±0.002^{edf}	0.493±0.009^{a}	-0.013±0.003^{ba}	0.075±0.015	7.7±0.2^{h}
1	-1	0	-1	-0.059±0.003^{egf}	0.483±0.021^{a}	-0.037±0.005^{bdac}	0.070±0.000	8.7±0.2^{fg}
2	1	0	-1	-0.040±0.002^{dc}	0.360±0.012^{cb}	-0.047±0.025^{bdc}	0.095±0.005	11.6±0.4^{c}
3	-1	0	1	-0.078±0.002^{h}	0.485±0.013^{a}	-0.041±0.003^{bdac}	0.080±0.010	10.4±0.0^{d}
4	1	0	1	-0.025±0.003^{bac}	0.293±0.005^{d}	-0.051±0.005^{bdc}	0.085±0.005	11.5±0.2^{c}
5	-1	-1	0	-0.057±0.003^{egf}	0.468±0.014^{a}	-0.025±0.003^{bdac}	0.095±0.005	8.5±0.2^{g}
6	1	-1	0	-0.047±0.003^{ed}	0.397±0.019^{b}	-0.045±0.001^{bdc}	0.090±0.010	11.5±0.2^{c}
7	-1	1	0	-0.066±0.002^{hgf}	0.470±0.000^{a}	-0.038±0.004^{bdac}	0.075±0.005	8.9±0.1^{feg}
8	1	1	0	-0.023±0.003^{ba}	0.319±0.009^{cd}	-0.060±0.002^{d}	0.095±0.005	9.6±0.2^{e}
9	0	-1	-1	-0.068±0.005^{hg}	0.409±0.007^{b}	-0.018±0.003^{bac}	0.105±0.015	9.4±0.1^{fe}
10	0	-1	1	-0.074±0.008^{hg}	0.399±0.025^{b}	-0.006±0.000^{a}	0.085±0.005	9.4±0.1^{fe}
11	0	1	-1	-0.037±0.006^{bdc}	0.377±0.001^{cb}	-0.029±0.001^{bdac}	0.085±0.005	12.2±0.0^{bc}
12	0	1	1	-0.016±0.002^{a}	0.347±0.007^{cbd}	-0.031±0.003^{bdac}	0.095±0.005	11.6±0.1^{c}
13	0	0	0	-0.017±0.001^{a}	0.347±0.001^{cbd}	-0.060±0.006^{d}	0.085±0.015	13.0±0.1^{a}
14	0	0	0	-0.014±0.002^{a}	0.355±0.003^{cb}	-0.054±0.002^{dc}	0.095±0.005	12.5±0.0^{ba}
15	0	0	0	-0.013±0.001^{a}	0.362±0.008^{cb}	-0.052±0.004^{bdc}	0.090±0.000	12.5±0.1^{ba}

Means in columns with same letters are not significantly different ($P > 0.05$)

Table 3.21. Regression coefficients of the responses tested for treated whole wheat flour (TWWF)

		Linear			Quadratic			Interactions			S	R^2
	Constant	X_1	X_2	X_3	X_1^2	X_2^2	X_3^2	$X_1 x X_2$	$X_1 x X_3$	$X_2 x X_3$		
Particle size(µm)												
Mean	136	39***	-6**	-17***	24***	-3	9**	-9***	8**	5	8.83	0.96
Median	75	24***	0	-7***	14***	-3***	3***	-2***	3***	2**	1.88	0.99
d_{10}	19	4***	0.5***	-1.3***	2.5***	-1.4***	0.1	0.1	0.4*	0.2	0.55	0.98
d_{50}	75	24***	0	-7***	14***	-3***	3***	-2***	3***	2**	1.88	0.99
d_{90}	338	70***	-18***	-28***	51***	2	11*	-29***	10	7	16.67	0.96
BD (kg/m^3)	509	2.8	-1.2	-2	-7	18.3	-33.3*	-3.3	-23.3	-0.9	25.54	0.71
MixoLab parameters												
C1Time	1.74	0.27	-1.05**	-2.20***	2.25***	0.32	1.89**	-0.73	-1.72**	-0.9	1.87	0.73
C2	17.26	-0.20***	-0.07	-0.24***	-0.06	0.04	0.08	0.1	0.05	0.15*	0.24	0.64
C3	1.94	0.03***	0.02***	-0.03***	-0.02***	-0.01*	0	0.01**	-0.01	0.03***	0.02	0.86
C4	1.89	-0.01	0.03**	-0.04***	-0.04***	-0.01	0.02	0.03*	-0.05***	0.01	0.04	0.72
C5	45.04	0	0	0	0	0	0	-0.01	-0.01	0	0.01	0.41
C1Torque	1.29	-0.01	-0.03	-0.05*	-0.13***	-0.01	-0.01	0.02	-0.06	0.09**	0.1	0.58
C2	0.85	0.09***	0.03	-0.01	-0.10***	-0.04	-0.04*	-0.02	-0.04	0.02	0.06	0.8
C3	1.94	0.03***	0.02***	-0.03***	-0.02***	-0.01*	0	0.01**	-0.01***	0.03	0.02	0.86
C4	1.89	-0.01	0.03**	-0.04***	-0.04**	-0.01	0.02	0.03	-0.05***	0.01	0.04	0.72
C5	2.95	0.03	0.03	-0.05**	-0.02	-0.01	0.01	-0.01	-0.08**	-0.04	0.09	0.48
Alpha	-0.14	0.02	0.01	0	0.05	0.04	0.05	0.02	0.01	0.02	0.1	0.22
Beta	0.31	-0.01	0	0	0.05***	0.02*	0.02	0.02	0.02	-0.01	0.03	0.54
Gamma	-0.02	0.00***	0	0.00*	0	0.00**	0	0	0	0.01***	0	0.66
Amplitude	0.11	0.01***	0	-0.01***	-0.03***	0	0	0	0	0.01	0.01	0.79
Stability	12.78	1.40***	0.55***	0.17	-0.36**	-0.36**	-0.47**	-0.57***	0.09	-0.69***	0.46	0.92
RVA Parameters												
Breakdown	438	-25***	-13*	-8	-5	13	-1	-28***	18*	-18*	26.96	0.65
Setback	985	28*	-16	12	52**	20	2	-51**	76***	-43**	54.34	0.67
Final	2060	49	-9	48	132***	18	27	-76*	145***	-114**	123.61	0.63

Peak time	5.65	-0.10***	0	0.06***	0.08	0.07***	0.03**	-0.01	0.01	-0.06**	0.07	0.76
Peak temp	74.74	1.64	2.68	-2.37	4.94	0.79	-12.31	4.51	-10.71	9.01	14.65	0.44
XRD (RC%)	15.19	-0.69***	0.28	0.67***	-0.4	0.81***	0.42	-0.04	0.39	0.53*	0.75	0.7
Swelling at °C												
60	5.44	-0.04	0.01	0.04*	0.02	0	0.10***	0.07*	-0.06*	-0.01	0.09	0.53
70	18.73	-0.09**	-0.08**	0.16***	-0.06	-0.1	-0.15**	-0.01	0.10*	0.22***	0.15	0.76
80	24.63	-0.35***	0.08	0.39***	-0.71***	-0.27	-0.35*	-0.48**	0.12	0.35*	0.48	0.72
90	26.64	-0.65***	0.14	0.77***	0.61**	-0.2	-0.17	-0.25	-0.42	-0.2	0.74	0.67
Solubility at °C												
60	6.99	-0.65***	-0.2	0.01	-0.3	0.25	0.32	0.19	-0.31	0.13	0.51	0.67
70	8.39	-0.13***	-0.03	0.13**	-0.75***	-0.58***	0.05	-0.06	0.02	0.61***	0.19	0.93
80	8.34	0.02	-0.49***	-0.12	-0.45***	-0.76***	-0.71***	-0.15	0.21	0.37***	0.35	0.85
90	9.2	-0.68***	-0.01	-0.16	-0.32	0.11	0.06	0.55**	-0.04	0.52*	0.74	0.55
Solvent retention capacity												
Water	92.55	1.47**	-0.35	0.27	-2.04*	-1.59	-1.06	-1.45	-1.04	0.81	2.66	0.46
Sucrose	124.92	1.15	1.46*	0.67	-5.14***	-2.30*	-0.15	-3.74***	-0.48	2.31*	3.18	0.69
Carbonate Sodium	104.23	-1.21	-0.85	1.56**	-2.62**	-0.42	1.05	-0.12	-0.38	2.41**	2.85	0.53
Lactic	93.45	-0.08	-0.99	1.36**	-3.01***	-0.08	-0.26	-1.05	0.57	1.74*	2.35	0.57
SE-HPLC												
SPP	7.12	-1.11***	-0.67***	-0.23**	0.30*	1.28***	0.12	-0.63***	-1.16***	-0.34**	0.41	0.94
Gliadin	63.79	-3.54***	-1.81***	-0.74**	-0.75	2.52***	0.07	-0.52	-3.85***	0.47	1.41	0.92
A/G	2.78	-0.08**	-0.04	-0.10***	-0.16***	0.02	-0.07	0.02	-0.18***	0.10**	0.14	0.7
IPP	26.31	4.73***	2.52***	1.08**	0.62	-3.82***	-0.11	1.12*	5.19***	-0.24	1.8	0.93

*** Significant at $p<0.01$
** Significant at $p<0.05$
* Significant at $p<0.10$

Table 3.22. Regression coefficients of the responses tested for treated grain whole wheat flour (TGWWF)

		Linear			Quadratic			Interactions			S	R^2
	Constant	X_1	X_2	X_3	X_1^2	X_2^2	X_3^2	$X_1 x X_2$	$X_1 x X_3$	$X_2 x X_3$		
Particle size(µm)												
Mean	125	-8*	2	0	11*	-4	1	6	6	2	4.94	0.39
Median	85	-6***	0	-1	5***	1	0	1	3**	2**	2.77	0.85
d_{10}	18	-1.4****	-0.5***	0.1	0.6***	0.3	0.2	0.2	0.5**	0.5**	0.59	0.87
d_{50}	85	-6***	0.5	-1.2	5.1***	0.7	0.1	0.8	2.5**	2.0**	2.77	0.85
d_{90}	308	-8	9	0	21	-16	2	14	17	4	34.59	0.33
BD (kg/m^3)	624	-16.5	-19.5	40.2	6.7	-12	98.5**	20.7	42.6	25.8	68.63	0.74
SKCS values												
Hardness index	63.33	-0.75	1.13**	2.88	-7.42**	-3.17	9.33**	5.25*	3.25	-1.5	4.79	0.88
Weight (mg)	27.33	0.5	0.63	-0.38	0.21	-0.04	-0.54	-1.00*	1	0.25	0.89	0.8
Diameter (mm)	2.53	0.03*	0.02	-0.01	-0.02	0.01	-0.03	-0.03	0.02	0.01	0.04	0.7
Moisture (%)	13.97	0.19	0.16	-0.55	0.94	0.69	-1.88**	-0.25	-0.98	0.33	0.97	0.85
MixoLab parameters												
C1Time	1.4	-1.37***	-1.98***	0.23	1.94***	1.71***	0.22	-2.25***	-0.54	0.08	1.13	0.88
C2	17.59	-0.37***	-0.06	0.07	0.30***	0.16	-0.08	-0.48***	0.08	0.08	0.25	0.81
C3	23.75	-0.24***	-0.04	0.07	0.03	0.13	0.13	-0.32***	0.1	-0.02	0.31	0.53
C4	29.43	-0.56***	-0.48**	-0.12	-0.08	0.12	0.44*	-0.57**	0.03	0.4	0.69	0.6
C5	45.04	0	0	0	0	0	0	0	0	0.01	0.01	0.22
C1Torque	1.11	0.02***	0	0.01*	-0.01	0.03***	0	0.02**	0.03***	-0.02*	0.02	0.72
C2	0.67	0.11***	0.05***	0.01	-0.08***	-0.06***	-0.02	0.03	0	-0.01	0.04	0.91
C3	1.76	0.04***	0.04***	0	-0.02	-0.03**	0	0.03**	0	-0.01	0.03	0.8
C4	1.57	0.02***	0.02**	0	0.02*	-0.01	-0.01	0.02**	-0.01	-0.03**	0.03	0.65
C5	2.35	0	0.04***	-0.02	0.09***	-0.02	0.01	0.08***	-0.03	-0.03	0.05	0.76
Alpha	-0.01	0.02***	0.01***	0	-0.02***	-0.02***	-0.02***	0.01**	0.01**	0.01*	0.01	0.88
Beta	0.35	-0.07***	-0.02***	-0.01**	0.04***	0.02**	0.01	-0.02***	-0.02**	-0.01	0.02	0.94
Gamma	-0.06	-0.01***	-0.01***	0	0	0.02***	0.02***	0	0	0	0.01	0.78
Amplitude	0.09	0.01**	0	0	-0.01	0	0	0.01*	-0.01	0.01**	0.01	0.52
Stability	12.65	0.94***	0.44**	0.14	-1.55***	-1.46***	-0.56*	-0.59*	-0.46	-0.15	0.81	0.81

RVA Parameters												
Pasting peak	1342	-11**	-17***	3	55***	-5	39***	-1	9	-8	19.5	0.84
Trough	987	6	-3	18***	5	-8	22***	-8	12**	-2	14	0.75
Breakdown	356	-17***	-14**	-14**	50***	2	17**	7	-3	-6	20.9	0.78
Setback	825	75***	56**	-67**	199***	17	3	150***	-88**	3	101.6	0.78
Final	1842	77***	50*	-49*	185***	-9	14	134***	-76**	1	101.9	0.75
Peak time	5.79	-0.06***	-0.07***	0.03**	0.02	0.02	0.02***	-0.05**	0.02	0.01	0.05	0.77
Peak temperature	67	4	-2	-4	3	0	10**	9**	2	4	10.7	0.49
XRD (RC%)	12.92	-0.1	0.04	0.04	0.61**	-0.60**	0.77***	0.07	-0.47**	0.41*	0.64	0.63
Swelling at °C												
60	5.63	-0.08**	0.07*	0.10**	-0.02	0.03	-0.04	0.06	0.04	-0.02	0.1	0.52
70	19.46	-0.17	-0.08	-0.13	-0.26	0.24	0.44*	-0.36	-0.22	-0.12	0.7	0.36
80	24.59	-0.54***	-0.43***	-0.1	0.12	0.55***	0.49***	-0.51***	-0.33*	0.21	0.5	0.78
90	24.8	0.51**	-1.79***	-0.99***	-0.03	-0.15	1.68***	-0.60*	0.39	0.02	1	0.84
Solubility at °C												
60	6.58	-0.49***	-0.08	-0.07	0	-0.18	-0.21	0.03	0.16	0.38**	0.4	0.65
70	8.07	0.06	0.07*	0.02	0.02	-0.38***	0.11*	-0.02	0.14**	-0.13**	0.2	0.79
80	7.3	-0.13*	0.14*	-0.40***	0.13	-0.15	0.33***	-0.14	-0.07	-0.08	0.3	0.72
90	7.14	-0.19*	0.78***	0.18*	0	1.21***	-0.06	0.70***	0.14	-0.32**	0.4	0.9
Solvent retention capacity												
Water	97.87	3.09***	1.01	2.27***	-3.13***	-3.38***	0.53	-0.09	-0.07	-0.66	2.4	0.78
Sucrose	132.12	5.40***	3.23***	0.24	-2.69***	2.32***	-0.88	2.14***	0.62	-1.2	2	0.91
Sodium	119.03	4.04***	0.61	2.37***	-6.26***	-0.72	-2.02**	0.49	2.55	0.35***	2.2	0.89
Lactic	99.86	3.49***	1.73***	1.26**	-3.04***	-0.2	-0.52	0.55	2.54***	-0.84	2.3	0.79
SE-HPLC												
SPP	9.22	-1.23***	-1.21***	0.06	-0.60***	-0.17	-0.04	-1.41***	0.34**	-0.15	0.4	0.95
Gliadin	59.97	-4.33***	-3.90***	-0.07	0.01	0.27	0.56	-4.06***	0.72	0.15	1.6	0.93
A/G	2.41	-0.08***	-0.07***	-0.01	0.16***	0	0.05*	0.04*	0	0.01	0.07	0.81
IPP	28.41	5.63***	5.18***	0.02	0.43	-0.11	-0.58	5.43***	-1.06	-0.01	1.93	0.94

*** Significant at p<0.01
** Significant at p<0.05
* Significant at p<0.10

Chapter 4 - Mixing Behavior of Gluten Fractions in Composite Dough Systems at Varying Temperature and Mixing Speed

Abstract

Wheat gluten is a complex mixture of two different proteins, glutenin and gliadin, that vary in their biochemical, functional properties in influencing dough development. The glutenin-gliadin ratios relates directly to the balance of dough strength and extensibility. Properly developed dough is also affected by mixing intensity, work (energy) and temperature imparted to it. Dough is developed as a result of rearrangement of cysteine thiol and disulfide covalent bonds as well as non-covalent bonds. Hofmeister salts can influence changes to the secondary and tertiary protein structure by promoting or disrupting hydrophobic interactions. The objective of this research study the effect of gluten fractions, mixing speed and temperature in presence and absence of Hofmeister salts series. Commercial wheat gluten isolates (Arise®5000, Arise®6000 and Arise®8000) and wheat starch were used to form composite flour doughs that were used to further investigate the relationships between protein composition and rheological properties. Different mixing profiles were obtained when the commercial protein isolates were mixed at different ratios, temperature, mixing speed and two Hofmeister salt solutions. A unique synergistic effect was seen when starch, Arise®8000 and Arise®6000 were mixed at 85:10:5 ratio, displaying an increased torque at prolonged mixing times. Salt type and concentration influenced amount of extractable soluble proteins. No difference in extracted soluble protein with sodium thiocynate at two concentration and control was observed, whereas in sodium sulfate, there was an increase. Arise®8000 had higher surface hydrophobicity than Arise®6000.

4.1. Introduction

The major objective of mixing in dough preparation is to disperse and incorporate the ingredients, so as to have a homogenous, continuous, cohesive, viscoelastic dough which sets to form aerated baked product with desirable texture (Delcour et al. 2012; Angioloni and Rosa 2005; Goesaert et al. 2005). The dough properties are influenced by several factors such as level of hydration, mixing time, energy, ingredients, and protein quantity/quality which eventually have a major effect on baking performance and quality of final product (Dobraszczyk and Morgenstern 2003; Angioloni and Rosa 2005).

Gluten is a polydisperse system of wheat functional proteins found in wheat grain endosperm. Constituents of gluten are differentiated to gliadin and glutenin. Glutenin is responsible for dough elasticity whereas gliadin is responsible for dough viscous properties. A number of models have been proposed to describe gluten network, however most do not get greater validity as the model proposed by Graveland et al. (1985) which states that HMW gluten subunit make up the backbone and the LMW are lateral side branches. Linday and Skierritt (1999) concurred with is model, and suggested that in addition to LMW, HMW subunits are also included to lateral side branches which provide strength to the structure. Gluten structure model has also been hypothesized to be loop and train model where long chains of glutenin form intermolecular linkages in form of a train by hydrophobic bonds. This model has been suggested to describe formation of gluten dough and to explain its viscoelastic property during hydration. It states that HMW glutenin subunits are linked through inter and intra hydrogen bonds. During hydration, inter and intra chain are broken forming loops. At same time hydrogen bonds are formed between glutamine of one peptide, water and glutamine of second peptide. On further hydration, more loops are formed. However, beyond optimum hydration level, the gluten elasticity weakens because hydrogen bonds are no longer strong enough (Delcour et al. 2012; Belton 1999).

The rheological properties of dough can be used to describe its physical characteristics, to understand molecular, structural organization, and composition of biopolymers (Dobraszczyk 2004). The quantity, quality and proportionality of gliadin to glutenin have critical influence on their functionality in bread-making (Veraverbeke and Delcour 2002; Dobraszczyk 2004; Wieser et al. 2007; Shewry et al. 2009). Currently available methods to study molecular size of gluten and relate to functionality such as electrophoresis, SE-HPLC to greater extend require that gluten structure is first disrupted for better analytical results because of gluten's large molecular weight and low solubility (Shewry et al. 2003; Dobraszczyk 2004; Delcour al., 2012). However, combination of these methods with rheology studies can give a better understanding (Dobraszczyk and Morgenstern 2003).

The dough network is held by covalent and non-covalent bonds that are formed when wheat flour is optimally hydrated, and kneaded over time. The rearrangement of cysteine sulfhydryl (SH) and disulfide (SS) are two most common ways to generate covalent bond in dough (Bruun et al. 2007). Although not a major contributor to dough network, structure and

strength, the significance of non-covalent bonds cannot be underestimated. They are formed during dough hydration and provide flexibility to the dough. The hydration process is greatly influenced by hydrophobic properties of gluten (Kinsella and Hale 1984; Belton 1999; Wieser, 2007). However, due to their high diversity and large size, the relative surface hydrophobicity of gluten proteins has not yet been determined. The hydrophobic characteristic of gluten is attributed to its high content proline, glutamine amino acid residues content which favor folding (Delcour and Hoseney 2010).

Protein folding/unfolding is accompanied by changes in the transfer free energies and free-energy changes which influence hydrophobicity (Melander and Horvath 1977; Auton and Bolan 2005). The Hofmeister series is arrangement of salts in sequence according to their ability to salt-out or salt-in protein (Baldwin 1996). The salts are reported to directly interact with the macromolecules and change their physical properties by interfering with their hydration shell by altering their free energy. To the left of the Hofmeister salt series are the komotropes (salting-out) and to the right are the chaotropes (salting-in). Kosmotropes precipitate proteins by deterring them from unfolding thereby inducing physical changes through two means; hydrating shell molecules and changes in surface tension. On the other hand, chaotropes promote protein solubility and denaturation by altering ion strength and dipole. Cation are less effective than anions, accordingly, the anions are ranked in following salting-out decreasing order; sulphate, hydroxyl, fluoride, chloride, bromide, nitrite, chlorate, iodine, thiocynate. Sulfate is most kosmotropic, and thiocynate most chaotropic and chloride neutral anion between two extremes (Zhang and Cremer 2010). Starch and protein, the two major flour constituents and added salt compete for water, decreasing hydration capacity of gluten at dough preparations thereby affecting dough development time and extensibility. The HMW glutenin in particular is reported to be more sensitive perhaps due to tertiary structure and its amino acid composition (Preston 1989; Butow et al. 2002; Angioloni and Rosa 2005).

In the industry, sometimes commercial wheat gluten isolates are added to wheat flour to maximize some desired flour/dough functionality. Composite flour therefore can be used to further investigate the relationships between protein composition and rheological properties of wheat flour. The objective of this research was to investigate the types of interactions that occurred between the protein isolates during mixing to explain the unique synergistic effect found in various gluten fractions mixed at different ratio, temperature and mixing speed using a

MixoLab in absence and presence of Hofmeister salts and analysis using SE-HPLC, hydrophobicity.

4.2. Materials and Methods

4.2.1. Materials

Three specialty wheat proteins and vital gluten were used. Wheat protein isolate, Arise®6000, Arise®8000, Arise®5000, vital wheat gluten and starch were supplied by MGP Ingredients Inc. (Atchison, KS). Arise®5000 and Arise®6000 were reported to be treated by sulfate to different degrees to create protein fractions that vary in their protein content and rheological properties.

(1) Wheat protein isolate, Arise®5000
Purity > 90% protein (N×6.25, d.b.)
More extensible, less elastic (gliadin-like)
Hydrated pH is ~ 4
Sulfite-treated (residual sulfite ~ 45 ppm)

(2) Wheat protein isolate, Arise®6000
Purity > 85% protein (N×6.25, d.b.)
More elastic, less extensible compared to Arise 5000
Hydrated pH is ~ neutral
Sulfite-treated (residual sulfite ~ 55 ppm)

(3) Wheat protein isolate, Arise®8000
Purity > 90% protein (N×6.25, d.b.)
High elasticity, less extensible

(4) Vital gluten
Purity > 75% protein (N×6.25, d.b.)

4.2.2. Solvent Retention Capacity and Solubility Index

Characteristics of gluten of gluten fractions with and without starch were evaluated for their ability to retain water and lactic acid, and their ability to dissolve in these two solutions. Lactic acid solution retention is related to glutenin. The AACC Approved Method 56-11.02 with slight modification was followed to determine solvent retention capacity and solubility of composite flour and protein fractions alone.

4.2.3. Mixing Profile

Three sets of mixing studies were conducted:

4.2.3.1. Mixing Performance of Individual Protein Isolates

Composite flours containing 85-92% wheat starch and 8-15 % of specialty wheat protein isolates were prepared. Mixing tests were carried out at the constant water absorption (98% db.), constant mixing speed (80 rpm) and temperature (30°C) using MixoLab Chopin + protocol (Chopin Technologies, France). The resulting mixing curves were analyzed to determine mixing time, dough consistency and mixing stability.

4.2.3.2. Synergy between Protein Isolates

Two top performing protein isolates were selected for this study. Composite flours containing 85% wheat starch and 15 % of specialty wheat protein isolates (Arise®8000 and Arise®6000) were prepared at 0:15, 5:10, 10:5 and 15:0 ratios. Mixing tests were carried out at the constant water absorption (98% db.), constant mixing speed (80 rpm) and temperature (30°C) using MixoLab Chopin + protocol (Chopin Technologies, France). The resulting mixing curves were analyzed to determine mixing time, dough consistency and mixing stability.

4.2.3.3. Effect of Mixing Temperature and Mixing Speed

Composite flours containing 85% wheat starch and 15 % of specialty wheat protein isolates (Arise®8000 and Arise®6000) were prepared at 0:15, 5:10, 10:5 and 15:0 ratios. Mixing tests were carried out at the constant water absorption (98% db), three levels of mixing speed (80, 100 and 120 rpm) and three temperatures (30, 40, 50°C) using MixoLab (Chopin Technologies, France). The resulting mixing curves were analyzed to determine mixing time, dough consistency and mixing stability.

4.2.4. Quantification of Protein-Protein Interactions

To investigate the role of non-covalent interactions between the protein fractions, composite flours containing 85% wheat starch and 15% of specialty wheat protein isolates were prepared in the presence of kosmotrope and chaostrope salts. A 85:10:5 proportion of wheat starch:Arise®8000:Arise®6000mixture was prepared. Mixing tests were carried out at the constant water absorption (98% db.), constant mixing speed (80 rpm) and temperature (30°C) using MixoLab Chopin + protocol (Chopin Technologies, France). Samples were mixed in the

presence of 0 (control), 0.1 or 0.5 M kosmotrope (sodium sulfate, Na_2SO_4) or chaostrope (sodium thiocynate, NaSCN) salts purchased from Sigma Aldrich (Sigma Aldrich Inc., St. Louis, MO). Dough samples were collected at the end of 7, 14 and 20 min of mixing time and were immediately frozen at -82°C, and kept for further analysis.

4.2.4.1 Size Exclusion-HPLC Analysis

The protein extractability in treated flour was characterized by size exclusion chromatography to determine their molecular weight profiles. The procedure used was according to the method used by Bean and Lookhart (2001). All regents were purchased from Fisher (Fisher brand- Thermo Fisher, Waltham, MA). Flour samples (100 mg) were weighted into 2.0 ml micro centrifuge tube (Fisher brand- Thermo Fisher, Waltham, MA). "Soluble protein" (SP) was extracted from pellet by vortexing 2×5 min with 1 mL of 50 mM Na-phos pH 7.0 /1% SDS, was then centrifuged and 500 uL of supernatant transferred to clean 2.0 ml micro centrifuge tube. This step was repeated. Total extract which was heated at 80°C for 2 minutes to deactivate "soluble protein" supernatant. The "insoluble protein" (IP) was extracted from the pellet first by using sonication (30 sec at 10W) with 1 mL 50 mM Na-phos pH 7.0/1% SDS in an ice bath, was then centrifuged and 500 uL of supernatant transferred to clean 2.0 ml micro centrifuge tube, heated to deactivate any enzymes supernatant at 80°C for 2 min. Each pooled extracted is then transferred to vial (C4011-1 National Scientific, Thermo Scientific, Rockwood, TN). SE-HPLC analysis of soluble and insoluble protein was conducted using an Agilent 1100 HPLC system with a 300×7.8 mm BioSep-SEC-S3000 column (Phenomenex, Torrance, CA) using 50mM Na-phos pH7.0 /1% SDS as mobile phase with BioSep SEC-4000 column, at 40°C and flow rate 1 mL/min with 15uL injection volume. Proteins were detected by measuring UV absorbance at 214 nm. Measure total peak areas, adjust for SP extract being pooled by multiplying by a dilution factor of 2.

4.2.4.2. Hydrophobicity Test

The assay was derived from method reported by Chelh et al. (2006). It's based on the preferential binding of Bromophenol blue to hydrophobic groups on the surface of proteins. Mixture of Arise®8000 and Arise®6000 in ratio of 2:1 were prepared with of 0.0, 0.1 or 0.5 M sodium sulfate (Na_2SO_4) or sodium thiocynate (NaSCN) solutions. A 20 mM Na-phos buffer at pH 6.0 was prepared in following steps. First, 1.0 M Na-phos buffer was prepared by dissolving

5.64 g of Na-phos monobasic and 1.139 g of Trisodium phosophate, dodecahydrate in 50 mL of deionized water. Secondly it was diluted to prepare the 200 mL 20 mM solution.

The Bromophenol solution was prepared by dissolving 50 mg of solid in 50 mL of deionized water. Then the 0.1 M and 0.5 M conc. of Na_2SO_4 or NaSCN were prepared in 20 mM Na-phos buffer at pH 6.0. Then 20 mL of 20 mM Na-phos pH 6.0 was added to each tube and vortex for 15min at 40°C. This allows for more efficient mixing. The dissolved solutions was transferred to a 50mL graduated cylinder and brought to volume with the 20 mM Na-phos pH6.0.

The protein content of samples was determined using LECO method and found to be 76.52% and 79.22% for Arise®6000 and Arise®8000, respectively. This was used to calculate mass of each gluten fraction to prepare bulk composite flour sample in 2:1 ratio. Gluten fraction composite were weighed out to give 5 mg/mL protein concentration for 1mL total in 20 mM Na-Phos buffer pH6.0, plus desired salt concentration. It was briefly vortex to mix, and then 200 µL of 1 mg/mL BPB in deionized water was added. The suspension was vortexed (including control) for 10 min at room temperature. It was then centrifuged for 15 min at 2,000 rcf. Supernatant (100 µL) was transferred to clean micro-tube and 900 µL of 20 mM Na-phos buffer pH 6.0 was added to make 1/10 dilution. It was briefly vortex to mix and the absorbance read using a spectrophotometer at 595 nm as well as that of a blank with 20 mM Na-phos buffer pH 6.0 only. Surface hydrophobicity was calculated using the equation:

BPB bound (µg) = 200 µg (Abs control – Abs sample) / Abs control

4.3. Results and Discussions

4.3.1. Solvent Retention Capacity and Solubility Index

There were no differences in water retention capacity by the composite flour or protein alone (Figure 4.1). In presence of starch, amount of water retained was almost half than in absence. This shows that it is the protein that retained much of the water. Lactic acid retention of the composite flour decreased with decrease in Arise®8000 addition. Lactic acid SRC is used to assess gluten quality. Higher SRC implies better gluten quality. These results show that Arise®8000 had better gluten functionality quality than Arise®6000. Protein mixture alone was less soluble in water in the composite. Solubility in both water and lactic acid increased with increase in Arise®6000 proportion only that the solubility in lactic acid was 4-10 times than in

water at their respective ratios. These results show that Arise®6000 was more soluble in both water and lactic acid than Arise®6000.

4.3.2. Effect of Composition

Dough development, consistency and stability highly depend on the type of protein and their proportion in the mixture (Figure 4.2). Composite flours made of wheat starch-vital gluten and starch-Arise®8000 had comparable dough consistency, stability and mixing behavior to that of bread-making quality wheat flour, except that they had short mixing time and stability. Arise®6000and Arise®5000 composite flour exhibited dramatic loss in dough consistency although they were more stable under prolonged mixing conditions. Distinctly different mixing curves were observed at 10:5 ratio of Arise®8000: Arise®6000 wheat protein isolates included in composite flour at total of 15% protein. A second mixing peak was observed after 13-14 minutes of mixing which was higher in consistency than the first mixing (arrival) peak. Mixing curves for composite flours at Arise®8000:Arise®6000 (5:10 ratio) indicated an average of individual proteins behavior. Unlike gliadin which is monomeric, glutenin is polymeric, and different polypeptides are linked through inter-sulfide bond. Glutenin is responsibility for elasticity of dough and therefore resistance to extension whereas gliadin is responsible for viscosity and therefore extensibility of dough (Shewry 2009; Barak et al. 2013). Greater number of these covalent bonds perhaps explains differences in their rheological properties. Treating Arise®5000 and Arise®6000 with sulfite may have resulted in cleavage of some of these disulfide bonds which explains their low resistance at mixing. Uthayakumaran et al. (1999) reported that increasing protein content at fixed glutenin:gliadin ratio or increasing glutenin:gliadin ratio at fixed protein content resulted in increased mixing time, mixograph peak resistance, maximum resistance to extension, but decreased resistance breakdown, and extensibility. Similarly, Khatkar et al. (2013) observed that addition of gliadin to base flour resulted in a decrease in dough stability and development time. The chemical methods used to treat Arise®6000 and Arise®5000 may be detrimental to glutenin fraction of gluten protein, which explains loss of resistance at mixing when Arise®6000 is used or at high proportion.

4.3.3. Effect of Temperature and Mixing Speed

The mixing profiles indicated that the effect of mixing temperature was more significant than the mixing speed (Figure 4.3). Dough consistency decreased dramatically with increase in

temperature (30-50°C). At constant temperature, dough consistency increased slightly with increasing mixing speed (80-120 rpm). Softening effect of temperature was more significant at low mixing speeds. Gluten fraction-wheat starch composite dough with varying ratios of high and low molecular weight wheat protein isolates displayed different degree of sensitivity to varying mixing speeds and temperatures. When the mixing temperatures was increased from 30 to 40°C, those peaks developed even earlier (around 4-8 min), and they disappeared at 50°C. Arise®8000 and Arise®6000 (10:5 ratio) showed similar responses to change in mixing temperature and speed, although the arrival times did not change significantly. There was a dramatic shift in mixing times at which the second mixing peak appeared. Such peaks were observed much earlier when the mixing speed was increased from 80 rpm to 120 rpm. Other studies have also showed that mechanical shear and thermal energy (temperatures <60°C) caused gluten polymerization as observed by increase in RVA viscosity, dough elasticity and decrease in extractable gluten. These heating effects were reported to have more pronounced on glutenin than gliadin (Stathopoulos et al. 2008; Lagrain et al. 2006).

4.3.4. Mixing Behavior in the Presence of Salts

The result of mixing Arise®8000 and Arise®6000in 2:1 ratio in presence of salt is shown in Figure 4.4. Mixing time was not a significant factor ($p=0.242$) whereas concentration ($F=88.93$), salt type ($F=177.93$) and their interactions ($F=128.23$) were significant factors. Control, treatments with 0.1 and 0.5 M Na_2SO_4 were significantly different ($t=12.21$ and $t=14.68$). Control and treatment with 0.1 M NaSCN or 0.1 M Na_2SO_4 were similar ($t=1.34$ and $t=0.168$) respectively. Strong mixing performance of Arise®8000 is an indication that it forms stronger dough. The unique increase in torque seen in Arise®8000:Arise®6000 (10:5 ratio) blend was not seen when the samples were mixed in the presence of salts. This suggests non-covalent interactions may be responsible for the synergistic behavior seen in the 10:5 Arise®8000:Arise®6000 blend. These observations could be explained as follows; at low salt (>0.3M) concentration, the anions are tightly adsorbed to protein due to non-electrostatic ions. However at concentration greater than 0.5M, hydrophobic influence starts to be effective (Preston 1984). The kosmotropic salt (Na_2SO_4) had the largest impact on mixing properties, substantially increasing torque during mixing. Kosmotrope do this by promoting firmness and elastic dough appearance. Chaotropes causes dough to be viscous and sticky, and influence

dough properties by preventing hydrophobic interactions (Preston 1984; Bruun et al. 2007; Melnyk et al. 2011).

4.3.5. Size Exclusion-HPLC

Size exclusion chromatography (SEC) was conducted to determine if there was any changes to the molecular weight distribution of the protein blends during mixing and in the presence of the salts (Figure 4.5). In the control run, samples were mixed in presence of 0.1M Na_2SO_4 and 0.5M NaSCN, the amounts of extractable soluble proteins increased over the course of mixing. The amount of extractable soluble protein in control and almost all treatments had similar amounts of extractable soluble protein, except those mixed in presence of 0.5M kosmotropic (sodium sulfate) where there was an increase in amounts of extracted proteins (~23 vs 74%). This suggests that the unique synergistic increase in mixing strength in the Arise®8000:Arise®6000 10:5 blends was not due to the formation of large polymeric protein complexes via disulfide bonds. The increase in extractable soluble protein in presence of kosmotropes could be attributed to the salts ability to promote hydrophobic interactions, which kept protein intact therefore making them available for extraction (Preston 1981; Kinsella and Hale 1984; Bean and Lookhart 2001). Preston (1981, 1984) has stated that the effects of Hofmeister salt series is not observed at salts concentrations less than 0.3M due to electrostatic interactions/shielding. Although we did not observe any major change (control vs 0.5M NaSCN treated) in amount of extracted protein using SEC, several authors have reported that Chaotropes (NaSCN) in general promote gluten extraction because it prevents hydrophobic interactions among polypeptides through the formation of hydrogen bonds (Preston 1981; Preston 1984; Lagrain et al. 2006; Stathopoulos et al. 2008).

4.3.6. Surface Hydrophobicity

Arise®6000 has slightly lower surface hydrophobicity compared to Arise®8000 in control (Figure 4.6). The mixture of Arise®8000 and Arise®6000 had a similar surface hydrophobicity as Arise®8000 and drastic differences in surface hydrophobicity were not noted for the mixture. An unexpectedly, exposure of fractions and their mixture to Na_2SO_4 increased surface hydrophobicity while exposure to NaSCN substantially decreased surface hydrophobicity. Treating Arise®6000 with sulfite may have led to increase in charges of polar amino acid residues which reduced hydrophobicity therefore explains low hydrophobicity in

buffer (control). Since the fractions have similar hydrophobicities, the resulting mixture had also same hydrophobicity. Kosmotropes promote hydrophobicity by forcing protein to fold thereby having fewer hydrophobic sites to which BPB can bind, while chaotropes prevent hydrophobic availing more sites for BPB to bind (Zhang and Cremer 2010). These results are opposite of what is expected according analysis principle in which more BPB is supposed to bind to less hydrophobic compound. However, this unique observation could be related to higher solubility of Arise®6000 in both water and lactic acid solution.

4.4. References

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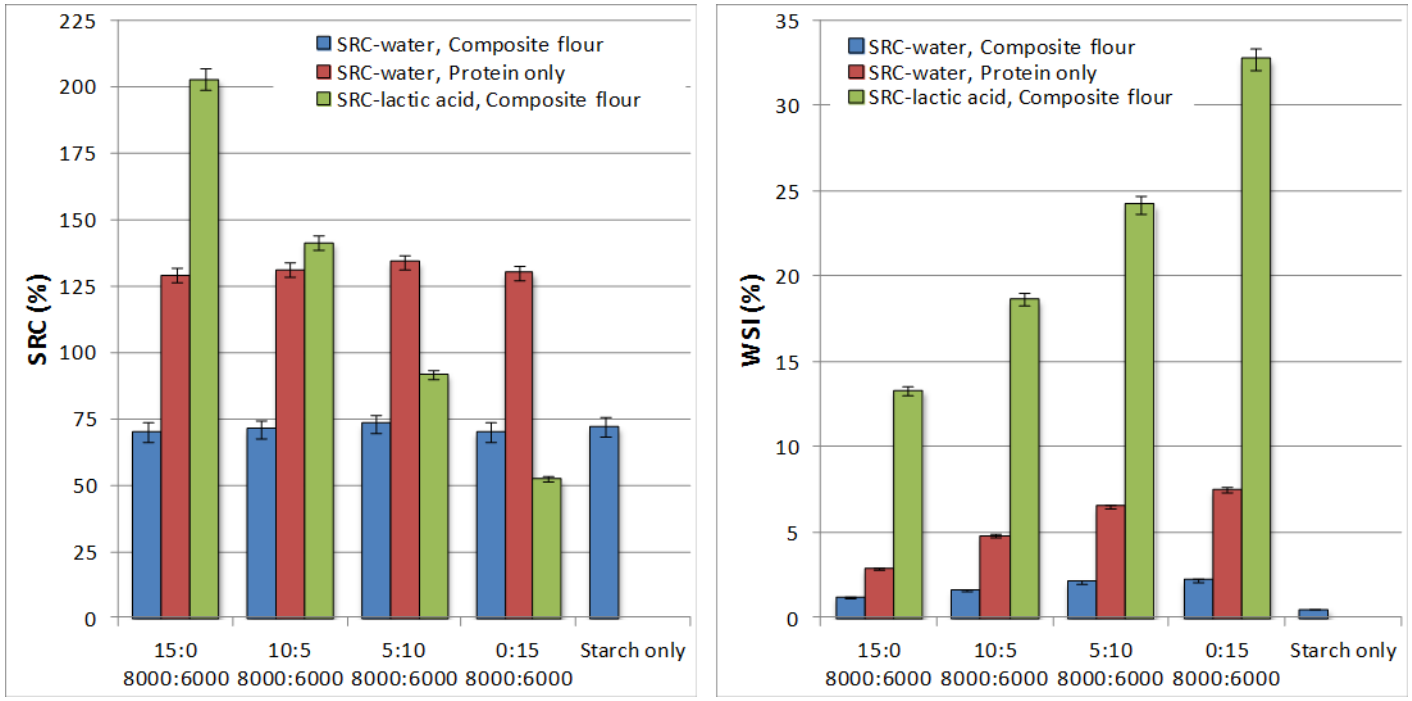

Figure 4.1 Solvent retention capacity (SRC) and solubility of gluten fractions (WSI)

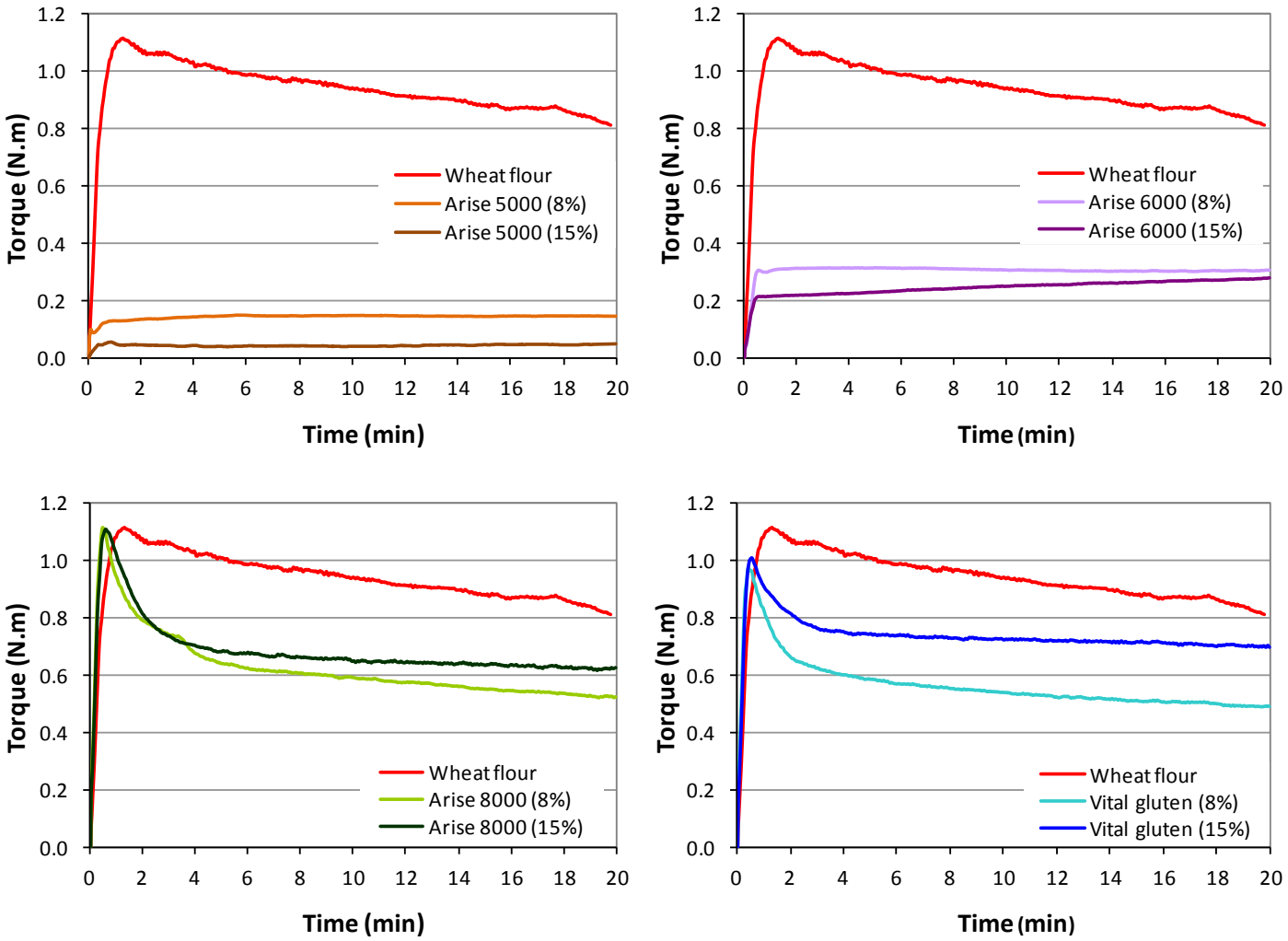

Figure 4.2. MixoLab mixing profiles of composite flours in the presence of (a) vital gluten, (b) Arise®8000, (c) Arise®6000 and (d) Arise®5000 added at 8 and 15%.

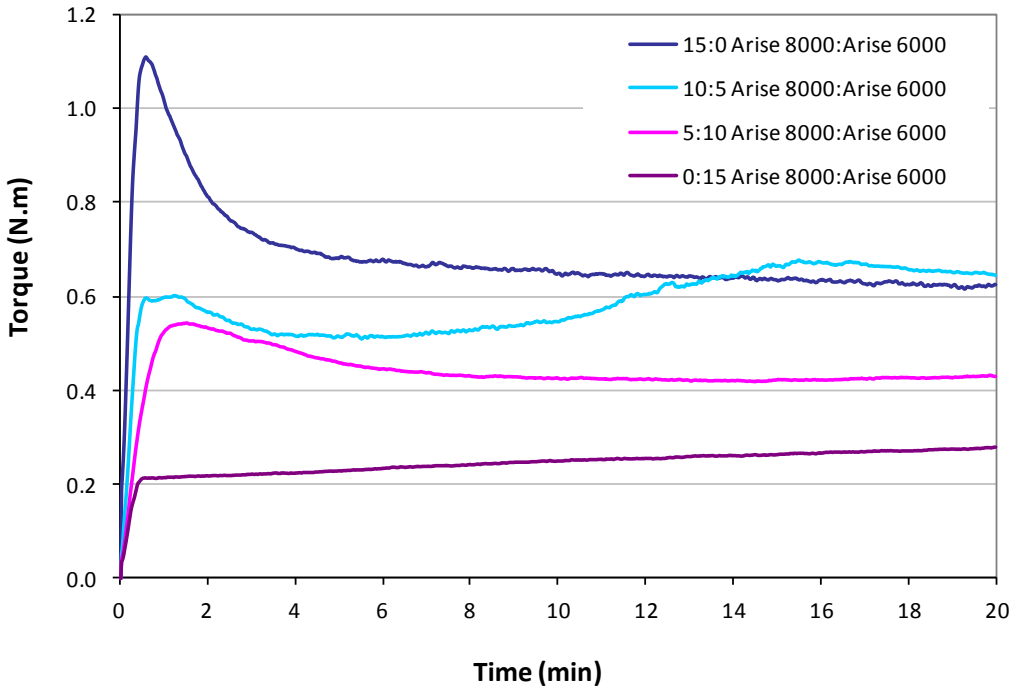

Figure 4.3. Synergy between Arise®8000 and Arise®6000

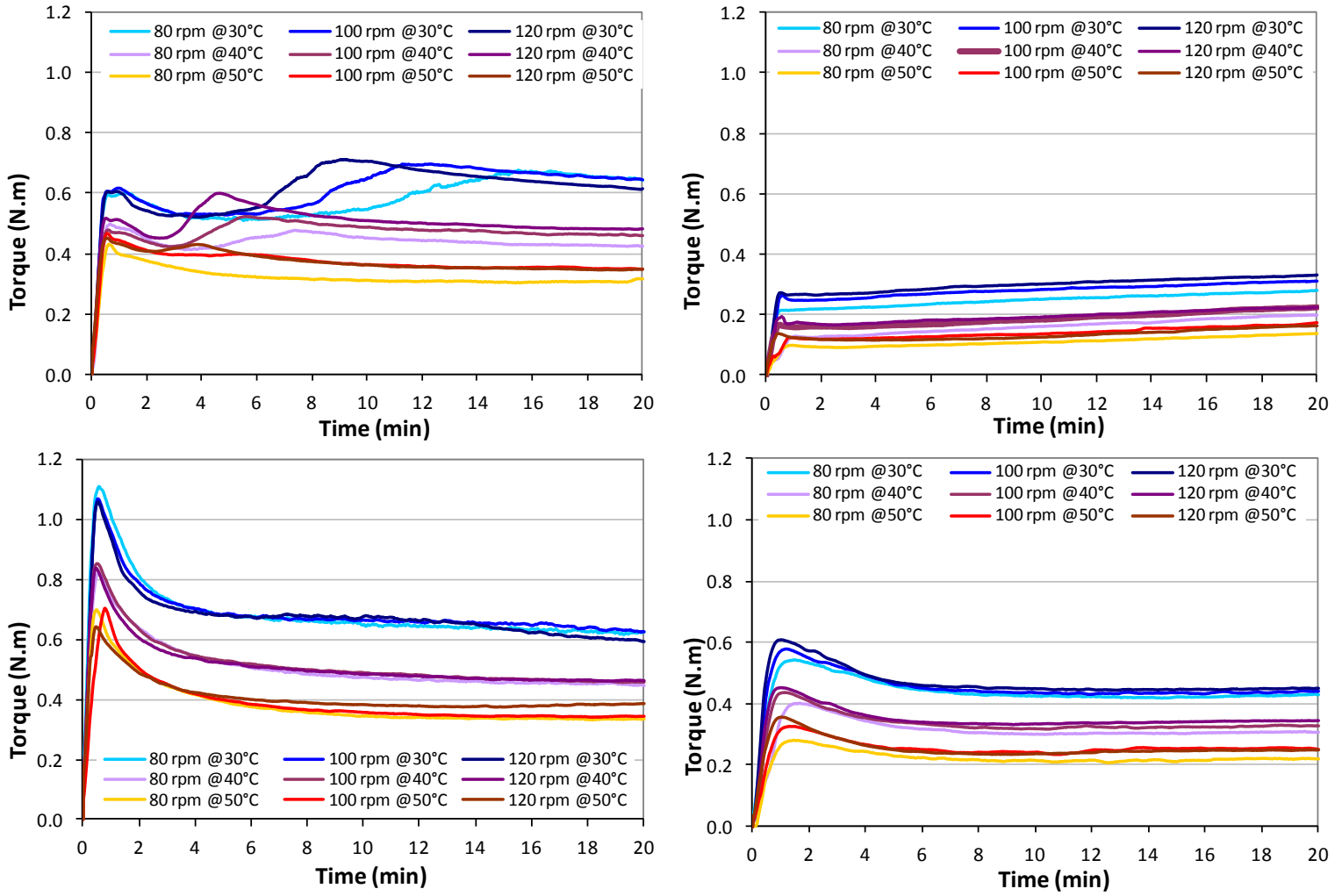

Figure 4.4. MixoLab mixing profiles of Arise®8000 and Arise®6000 added composite flours at ratios of (a) 15:0, (b) 10:5, (c) 5:10, and (d) 0:10 at three levels of mixing speed (80, 100 and 120 rpm) and three temperatures (30, 40, 50°C).

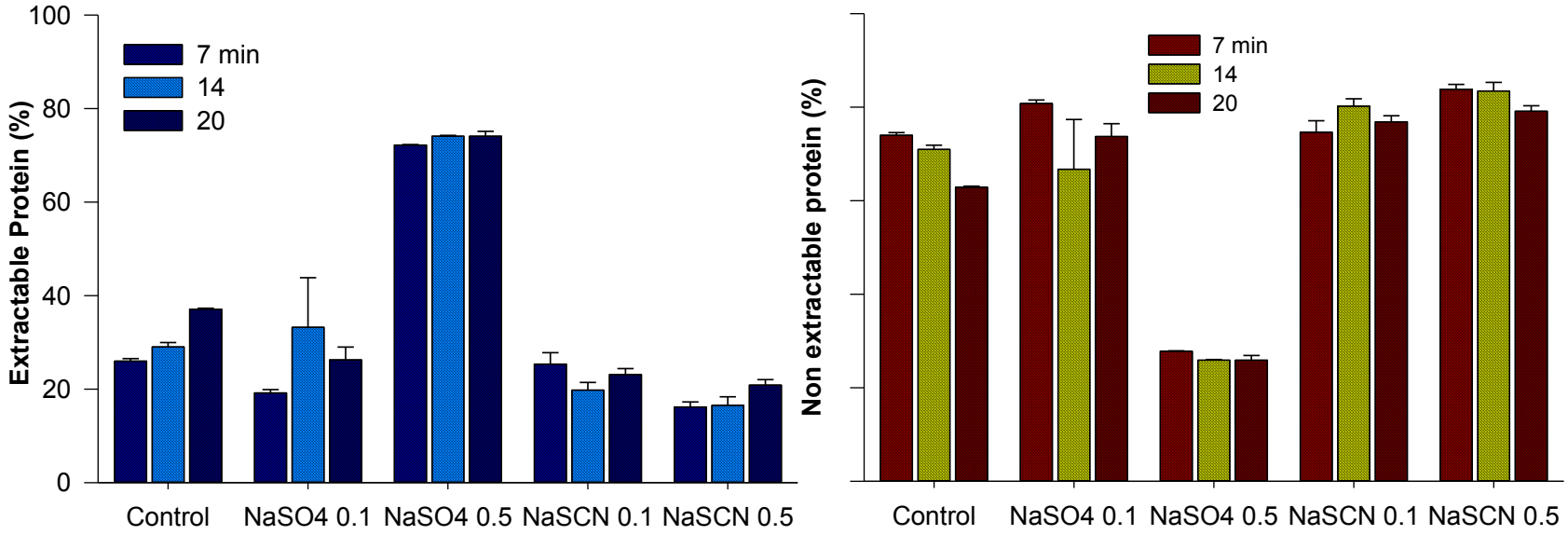

Figure 4.5. SE-HPLC extractable and non-extractable protein from dough mixed in absence and presence of the two salt solutions

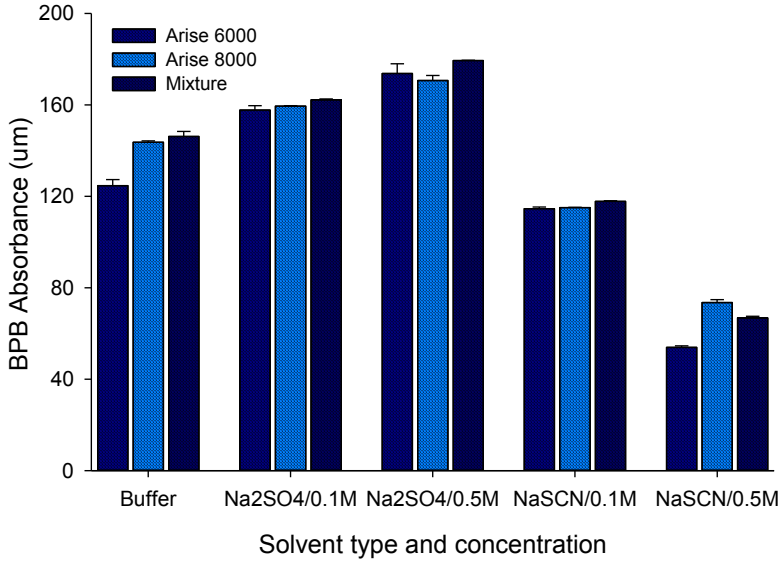

Figure 4.6. BPB absorbance in presence or absence of the two salt solutions without starch in dough

Chapter 5 - Conclusion and Future Research

5.1. Research Summary

Dough conditioners, additives, modified starches are often added to cereal products to compensate for some of the lacking functional attributes of flour such as water absorption, freeze-thaw stability, gelling and consistency. Increasing numbers of consumers are demanding for whole, natural, clean label food products which are processed with little or no chemicals. Thus, there is need for newer processing technologies that can improve the performance of wheat flour in general with minimum or no addition functional ingredients.

The concept of thermal processing of foodstuff has been used extensively since 1920s when the first scientific basis for safe sterilization process was developed. There are several methods used in thermal processing of dry foods: Infrared, microwave, annealing and heat-moisture treatment, thermo-mechanical treatments, indirect and indirect heating. In its all forms of application, thermal processing has been the most widely used method of preserving and extending the shelf-life (via microbial reduction and enzyme inactivation), and improving quality and functionality. By applying heat treatment, it is possible to modify the physical and rheological properties of cereal flours. Primary effect of heat treatment is range of macromolecular changes in starch and proteins. Understanding of relationship between heat transfer, thermal properties of food, heating medium, thermodynamics and the resulting functionality is of critical importance.

The first study (*Chapter-2*) focused on developing a thermo-mechanical treatment (extrusion) for improving the functionality of low quality (ash > 1.3%) wheat flour. Although the crude protein ash content did not change, the crude fiber content of extruded high ash flour decreased dramatically except when the extrusion was done at highest in barrel temperature and moisture conditions. Process conditions favoring higher Send high shearing actions resulted in destructions of some fiber making it more soluble or difficult to detect. Specific mechanical energy increased with decrease in flour ash content, and increased with increase in extrusion temperature. Starch is the dominant component and it is responsible for higher viscosity and therefore higher SME. There was a decrease in expansion ratio with increase in ash content and an increase in extrusion temperature. Low and medium ash flours were harder to grind and had

similar particle size distributions. Control (unextruded) samples gelatinization enthalpy decreased with increase in ash content, which was attributed to the decrease in starch content because enthalpy change is related to energy needed to gelatinize the starch. Control flour samples showed typical A-type of crystallinity. However, after extrusion, there was only V-type of crystallinity. There was maximum starch swelling of unextruded samples with peak viscosities of (2426, 2369 and 1600 cP) for low, medium and high ash flours. High ash flour had earlier peak time compared to low and medium ash flours. Extrusion caused dramatic decrease in peak viscosity as a result of starch gelatinization as evidenced by viscosity development within the first 2 min (versus 6 min) of mixing. Treated flours produced under different extrusion conditions and with varying ash content had dramatically different rheological properties, indicating their ability to form cold pastes. Based on these properties, such treated flours can be used mainly as a thickening and gelling agent in refrigerated and instant foods or heat sensitive products such as cold desserts, salad dressing, cake and bakery mixes, and baby foods. Treated flours have ability to develop viscosity in the solutions or mixes in which it is being used without any heat treatment. Such flour can be "clean labeled" as natural functional wheat products that be used in batter and dough-based food systems.

In the second study (*Chapter-3*) whole wheat flour and whole wheat grain were subjected to an indirect, rapid and continuous thermal processing technique for treating whole wheat flour and whole grain to reduce microbial load while preserving or improving the flour functionality in targeted applications. The treated whole wheat flour (TWWF) and treated grain whole wheat flour (TGWWF) were characterized by examining their functional properties such as solubility, high swelling power, instant viscosity development (cold pasting) at low temperatures. The concept was to explore the potential use of this new value added products and their applications as specialty flours with targeted quality and end-use. Treated flours were concluded to have a great potential to be utilized as thickener for soups and sauces, used as ideal coating for adhering batter and breading to foods.

We also examined systematic cause and effect relationships; identify relevant process parameters to serve as predictors of desired quality for specific end-use. Residence time influenced mechanical energy input of Solidaire and therefore overall mechanical energy on the product. Hydration level and treatment temperature had greater impact on thermal energy, while less extent mechanical energy. As expected, higher treatment temperatures resulted in higher

thermal energy input, but a slight decrease in mechanical energy input. The particle size of TWWF was influenced mostly by hydration moisture and temperature. The level of aggregation increased with increased water hydration level. The control flours had higher relative crystallinity (RC) in comparison to TWWF and TGWWF. In addition, TWWF had higher RC in comparison to TGWWF. Treated flour solvent retention capacities (water, sucrose, Na-bicarbonate and lactic acid SRC) were influenced to varying degrees by the treatment factors. The effect of hydration moisture on sucrose SRC depended on residence time whereas temperature effect on Na-bicarbonate SRC depended on the residence time. The lactic acid SRC was also influenced by treatment temperature. SE-HPLC studies indicated that extractable SPP and A/G were higher in control flours than their respective TWWF and TGWWF. The amounts of extractable SPP, gliadin, A/G and IPP of TWWF were influenced by all treatment factors individually and in an interactive way. Heat treatment caused molecular and conformation changes to protein by inducing sulfhydryl-disulfides inter change reactions and formation of new bond.

The third study (*Chapter-4*) focused on the performance of commercial protein isolates (Arise®5000, Arise®6000, Arise®8000 and vital gluten) in development of composite flour doughs, and the mixing behavior of these gluten fractions in composite dough systems at varying temperature and mixing speed combinations. Lactic acid SRC of the composite flour increased with increase in Arise®8000 addition indicating its superior gluten functionality compared to other protein isolates. Composite flours made of wheat starch-vital gluten and starch-Arise® 8000 had comparable dough consistency and development to that of bread-making quality wheat flour, except for their shorter mixing time and stability. A synergy Arise®6000 and Arise®8000 was observed as indicated by a second mixing peak observed after 13-14 minutes of mixing which was higher in consistency than the first mixing (arrival) peak. Mixing curves for composite flours at 5:10 ratio of Arise®8000:Arise®6000 indicated an average behavior of individual proteins. Dough consistency decreased dramatically with increase in temperature (30-50°C), while at constant temperature, dough consistency increased slightly with increasing mixing speed (80-120 rpm). Softening effect of temperature was more significant at low mixing speeds. The unique increase in torque seen in Arise®8000:Arise®6000 10:5 ratio blend was not seen when the samples were mixed in the presence of salts, which suggested that non-covalent interactions may be responsible for the synergistic behavior between two proteins. The increase

in extractable soluble protein in presence of kosmotropes could be attributed to the salts ability to promote hydrophobic interactions, which kept protein intact therefore making them available for extraction.

5.2. Future Work

Further investigations can be done in two areas: (i) Heat treatment process, (ii) Product characterization and evaluation.

Heat treatment process:

Future research is needed to provide equipment and process guidelines for grain industry. Research is needed to design and explore direct continuous heat treatment process using steam instead of indirect heating protocols used in this study. Use of steam will provide an intensive and controllable energy input in very short residence time without the need of hydration step prior to heat treatment. Steam application can also be done under vacuum which will help to deliver effective heat treatment without the need for high temperatures. Such low temperature short time direct treatment is expected to deliver effective reduction in microbial loads with minimal detrimental changes to protein functionality and viscoelasticity. Mathematical models need to be validated under the theoretical optimal conditions so as to be used as reliable predictive models.

Product characterization and evaluation:

Although massive amount of characterization test have been performed in this study, there is still room to do more by conducting additional techniques such as SDS-PAGE to explore the changes in protein molecular weight distributions, and gel permeation chromatography (GPC) to investigate the changes in starch molecular weight. Fundamental rheological tests both at small and large deformation rates are needed for through understanding of the viscoelastic nature of heat treated dough in comparison to their respective untreated flour doughs. Uni- and biaxial deformation tests (using Kieffer rig and dough inflation system) can be used to test the elasticity and resistance of heat treated dough as well as strain hardening effect of heat treatment. Frequency and temperature sweeps, creep and stress relaxation tests would offer a deeper understanding in viscoelasticity and rheological behavior of dough under imposed temperature, deformation and stress conditions. Evaluating the performance of heat treated samples in a range of model food systems is another area needs to be explored thoroughly. Various batter- and

dough-based systems can be used to assess the performance of the newly created functional flours, and market them as specialty flours with targeted applications.