RHEOLOGICAL PROPERTIES FOR QUALITY CONTROL OF WHEAT AND DRY CAKE MIXES

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Abstract:

Faced with market demand, segments of the industry must be focused on the production of quality food, which is a determining factor in competitiveness in the international market. The quality of raw materials and finished products is important and can be affected by a number of factors. The rheological properties effect on product quality and nutrition have been thoroughly investigated as they relate to the chemical composition and physical characteristics of foods. In the first part of the study, the objective was to evaluate the impact of agricultural management practices (crop succession and nitrogen fertilization) in the quality of grain produced. For the experimental conditions tested, increasing nitrogen applied in broadcast in succession with soybean crop increased protein and reduced starch content in the wheat kernels (wholemeal; $p<0.05$). Those changes in composition consequently affected the rheological properties, increasing the elastic recovery of the gluten and reducing the pasting properties of the paste ($p<0.05$). In the second part, the objective was to determine the best conditions for extraction and analysis of gluten viscoelastic from a set of soft wheat genotypes, and its correlation with standard viscoelastic methods. The best conditions for gluten extraction and analysis were obtained with 4.4 mL of water, 20 s of mixing and 3 min of washing with NaCl solution (2%). The elastic recovery of gluten presented a strong correlation with standard methods used to classify wheat according to its technological quality ($r>0.95$; $p<0.01$). In the third part, the objective was to evaluate the effect of functional ingredients on pasting properties (RVA) of cake mixes (all-purpose flour or gluten-free) and baking performance, texture and sensory characteristics of the baked product. The misformulation of the ingredients (NFDM, salt and baking powder) resulted in significant changes in the pasting properties, presenting similar responses for all-purpose or gluten free cake mix formulation ($p<0.05$). Baking performance, acceptance and texture of the baked products were also affected, with omission (gluten-free) or double dose addition (all-purpose) of baking powder being the less acceptable cake produce from the misformulations tested ($0.40<r<0.90$; $p<0.05$). The pasting properties of the mix presented moderate to strong correlation with baking performance (volume and weight) and texture of the baked products.

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CHAPTER I

INTRODUCTION

Statement of problems

Wheat flour proteins are the source of wheat unique breadmaking quality that make it the most popular cereal for making bread. Two crucial determinants of bread-making quality are the grain protein concentration and protein composition, including the amount and ratio of gluten protein fractions as well as their subunits (Zhang et al., 2009). The chemical composition may vary depending on environmental conditions, cultural practices, among other factors (McKevith, 2004).

The availability of nutrients for the plant is a key factor in agriculture production. Nitrogen is the main limiting nutrient, due its importance in amino acids formation, proteins, chlorophyll and essential enzymes that stimulate the growth and development shoot and root system (Fuertes-Mendizábal et al., 2010; Prando et al., 2012). Cultural practices such as crop succession, management, and proper use of dose and timing of nutrient application are alternatives that can be used to increase nitrogen efficiency, providing nutrients to the plant and consequently affecting its quality (Fornasieri-Filho, 2008). Thus, evaluation of the effect of common agricultural management practices in the rheological properties of kernels can provide a better understanding of its effect in the quality of grain produced.

Several studies are published using a wide range of rheological methods to test dough, which, in combination with various other analytical techniques, seek to uncover the unique functional properties of wheat flour viscoelasticity. However, as stated before, wheat flour proteins are the source of wheat’s unique breadmaking quality, more specifically gluten, and the number of methods available to test the viscoelasticity properties of gluten is limited. The "elasticity" can be observed when gluten is stretched and then released; the gluten is considered elastic if it can quickly return to its original shape. Gluten and dough are typically considered viscoelastic exhibiting properties of solid (cohesion and elasticity) and liquid (viscous or irrecoverable deformation) (Kieffer, 2006).

Rheological measurements are essential in the food industry as a tool for testing the quality of raw materials prior to processing, during manufacturing, and in the final product. There are various methodologies to test quality for breeding programs and baking industry including empirical and rheological tests (small and large deformation) (Tabilo-Munizaga; Barbosa-Cánovas, 2005). Gluten CORE is a rapid axial compression tool designed specifically for testing the elastic recovery of the gluten, and may be used for pasta and other viscoelastic materials. The main advantages of this system are the size of the equipment, small amount of sample, ease of use and time of test (Chapman et al., 2012). However, the method was developed and standardized using American wheat samples making its use limited when analyzing the quality of gluten from wheats with different properties such as Brazilian wheat genotypes, which present soft wheat characteristics. Thus, the development of a method for wheat presenting soft wheat characteristics is important to provide an in-depth view of the behavior of gluten under compression and recovery after deformation and its correlation with standard methods used to test wheat quality.

In other hand, in products such as cake batter, there is no significant formation of the gluten network and the main component of cake structure formation is starch, which is present in traditional formulations using wheat flour (Cauvain, 2017). Food products that do not require practices for processing, and are easy to prepare have attracted the attention of consumers. An example of this type of product is the pre-mixes formulations for cake which allows the cake to be made easily and quickly.

During baking, the combined effect of starch swelling and protein denaturation in presence of other ingredients transformed the liquid batter into a solid foam. These events first increase the viscosity of the batter and finally provide structure of the matrix (Hesso et al., 2015). The quality of the final product is dependent on many factors such as quality and level of ingredients, affecting volume, firmness, color, and weight loss, the main parameters of cake quality (Kahraman et al., 2008).

In the production of bake mixes, common mistakes including omission or double addition of functional ingredients can result in significant economic losses. Viscosity properties, mainly attributed to starch components during heating, cooling and stirring conditions, could reveal mistakes during production (Ahmadi-Abhari et al., 2015). The measurement of the viscosity of the paste under conditions of controlled temperature, could help to detect possible misformulation and predict the quality of the final product.

1.1 Objectives

The overall objective of this study was to evaluate the rheological properties of wholemeal produced from wheat cultivated under different agricultural practices management; to develop a reliable method based on rheological properties of gluten to evaluate the quality of wheat; and to evaluate the effect of functional ingredient misformulation on pasting properties of dry cake mixes and in the quality of the baked product. Specific aims are:

1) To evaluate the impact of crop succession (soybean/wheat or maize/wheat) and nitrogen doses (0, 30, 60, 90 and 120 kg ha^{-1}) on rheological properties of wheat;

2) To determine the best conditions for extraction of gluten from different wheat commercial classes for analysis with a Gluten CORE;

3) To correlate the quality parameters obtained with a Gluten CORE with other common technological quality parameters of wheat;

4) To evaluate the effect of functional ingredients on viscosity profile of all-purpose and gluten-free dry cake mixes;

5) To evaluate the effect of functional ingredients on the baking performance, texture profile and sensory evaluation of all-purpose and gluten-free dry cake mixes;

6) To evaluate the correlation between viscosity profile and final product quality of cakes.

1.2 Null hypotheses and Assumptions

1) Quantitative chemical changes observed in the kernels (protein and starch content) in response to the agricultural practices managements evaluated (crop

succession - soybean/wheat or maize/wheat and nitrogen fertilization, 0, 30, 60, 90 and 120 kg ha^{-1}) do not affect the rheological characteristics of the samples. Crop succession system involving a legume such as soybean and application of high doses of nitrogen fertilizer can result in significant changes in wheat quality. The increase in production and chemical composition changes assist in the improvement of the rheological quality of the flour obtained and its end use.

2) The elastic recovery (%) property of gluten measured by Gluten CORE are not a useful parameter in the classification of commercial wheat flours. The viscoelasticity parameters obtained with glutomatic and compression and recovery test (Gluten CORE) can help classify wheat genotypes according to their technological quality.

3) Misformulation of functional ingredients (nonfat dry milk, salt and baking powder) does not affect the viscosity profile and the quality of the baked cakes. The misformulation of functional ingredients in dry cake mixes affect the viscosity profile and final quality of the cakes (performance, sensory and texture). Thus, the viscosity of the paste can be a reliable tool for quality control in the manufacturing process of dry cake mixes.

CHAPTER II

LITERATURE REVIEW

2.1 Wheat Crop

Due to agronomic adaptability, ease of storage, nutritional characteristics and ability to produce from the flour a variety of tasty, interesting and satisfying food products, wheat is a major component in most diets in the world (Wrigley, 2009). Wheat, monocot belonging to the family of Poaceae (grass), genus *Triticum* and diverse number of species, is one of the oldest crop in the world. *Triticum aestivum* (L.) is the largest species of commercial interest (>90% of world production), being used for baking, production of cake, cookie, pastry and confectionery, while *Triticum durum* (Desf.), with approximately 5% of the production is best suited for pasta for introducing tougher gluten (Gooding, 2009).

From temperate climate, archaeological finds and historical reports indicate the origin of wheat at about 10,000 years BC, in the mountainous regions of Southeast Asia. Furthermore, Egypt is recognized as the place of origin of bread. While Romans, blending yeast with wheat flour were considered responsible for improving its characteristics (Guarienti; Del-Duca, 2002).

Considered the second largest grain crop production in the world (751.07 million metric tons), exceeded in 39.7% by maize, wheat is the first crop in area under cultivation (221.92 million hectares), according to projections for 2016-17 crop season. Among the major cereal producing countries are China (17.2 %), India (11.6%), Russia (9.7%) and United States (8.4%), responsible for producing almost half of the wheat in the world (USDA, 2017a).

2.1.1 Gluten protein composition and properties

As reported, plant-based foods (primarily cereals and legumes) have been a major source of protein for humans for millennia. Currently, plant- and animal-based foods contribute ∼65% and ∼35%, respectively, of protein in human diets on a worldwide basis (Wu et al., 2014).

In the wheat grain, protein content can vary between 8.3 and 19.3% of total dry matter (Pomeranz, 1988). However, the nutritional quality of wheat is limited due to low lysine content, among other essential amino acids. Thus, mixing grain or add other sources of protein such as animal protein or leguminous seeds, is necessary to provide a balance of nutrients (Shewry et al., 2009).

The baking quality of wheat is complex and depends on genetic and environmental factors. The amount of protein and protein composition, which are determinants for wheat quality, are influenced by both genetic and environment (Dupont; Altenbach, 2003).

Wheat proteins are usually divided into metabolic or structural proteins, and storage, which are responsible for the baking properties forming a viscoelastic network (gluten) during dough mixing (Wieser et al., 2008). Albumin and globulin are metabolic proteins, which act in grain development and early germination. The main function is nutritional, since they have reasonably balanced amino acid composition. The albumin and globulin (soluble proteins) account for about 10-15% of the total protein in wheat (endosperm) and occur in compactly folded structures (Camargo et al., 1997).

Gliadin and glutenin represent about 75% of the total protein content and are located mainly in the endosperm of the grain. Considered biologically active, despite lacking enzymatic activity, gliadin and glutenin participate in the dough formation through retention of gases, resulting in spongy baked products (Belderok, 2000).

Gliadins have a molecular weight between 30,000 and 80,000, are single chains and extremely gummy when hydrated, with little or no resistance to extension, and is responsible for cohesiveness, an important rheological characteristic of dough. Glutenin formed by several interconnected chains, has an average molecular weight ranging from 100,000 to several million, and is elastic but cohesive, providing resistance to dough extension (Veraverbeke; Delcour, 2002; Wrigley et al., 2006).

The viscoelastic property of the hydrated gluten is characterized by the plasticizing action of gliadin, proffering extensibility, and glutenin, providing elasticity or resistance to the dough to disruption (Xu et al., 2007). The qualitative and quantitative composition of glutenin and gliadin fractions influence the rheological properties of wheat, more specifically the viscoelastic gluten matrix (Wrigley et al., 2006). The presence of gluten proteins in wheat flour makes it suitable for the production of leavened bread products, due to network formation responsible for retention of carbon dioxide produced during the fermentation process (Al-Dmoor, 2013). Therefore, understanding the mechanical properties of wheat gluten is to understand the processing behavior of wheat products.

2.1.2 Technological quality

The term quality of wheat has different interpretations, depending on the sectors involved: producer, elevator, miller, final consumers, including health workers and consumers of natural products, in addition to cereals scientists (Modenes et al., 2009). Despite the differences between countries, some common standards are used for satisfactorily classify wheat grain according to its quality. However, depending on the point of the production chain (producer, elevator, miller, and final consumer), the parameters used are not the same and differ in importance (Pagani et al., 2014)

Although not all varieties are particularly suited to making bread, hexaploid bread or common wheat (*T. aestivum* subsp. *aestivum*) is the most important wheat type. Much of this variation is genetic and has contributed to varieties being classified based on characteristics relevant to the growing, breeding, marketing of the crop and type of end product, such as seed coat color, endosperm texture, dough strength, and sowing season (Gooding, 2009). Based on growth habit, end use, and physical characteristics, methods are used to classify wheat in the United States according to its characteristics. Thereby, the three most important wheat classification criteria are based in kernel texture (hard or soft kernel), bran color (red or white), and growth habit (spring or winter); hard red winter (46.8%), hard red spring (21.4%), soft red winter (14.9%), white (12.4%), and durum (4.5%), for a production estimated in 62.9 million metric tons in the 2016/17 crop season (USDA, 2017b).

Around the world, wheat producer countries have developed their own wheat class system based on technological characteristics that can include subclasses with specific targeted end-use functional ability in mind, such as “bread wheat class” (Brazil), “noddle wheat class” (Australia), and “pastry wheat class” (India) (Carson; Edwards, 2009). Based on its rheological properties, the commercial classification of wheat in Brazil is carried out according to Normative Instruction No. 38 of November 30, 2010. The normative defines the official classification standard, the requirements for identity and quality, sampling, mode of presentation and marking or labeling aspects, relating to classification of product according to the physical conditions of the product delivered by the producer (MAPA, 2010). According to the regulation, at the reception the grain is classified in Group I and II. The grain classified as Group I is intended directly for food, and the classified as Group II will undergo some processing before being consumed. As shown in Table 1, wheat from Group II is also classified according to technological properties of the flour, based on dough strength (alveograph), dough stability (farinograph) and falling number (MAPA, 2010).

Table 1. Classes of wheat from Group II for milling and other purposes.

Class	Dough strength (10^{-4} J)	Dough Stability (minutes)	Falling Number (seconds)
Improver	300	14	250
Bread	220	10	220
Domestic	160	6	220
Basic	100	3	200
Other Uses	<100	<3	<200

Source: MAPA (2010).

Due to its important role in baking, providing viscoelasticity to the dough for gas expansion and crumb structure, methods have been developed to test the quality of gluten. A very important analysis is used to measure and calculate the gluten content in a particular sample. The principle of the method is to make a dough with wheat flour and water, which must then be washed to remove the starch and water-soluble constituents of gluten, obtaining moist gluten. After this procedure, the gluten is dried and weighed, allowing the determination of the dry gluten content according to AACC International Approved Method 38-12.02. However, even though the determination of gluten content in flour is one of the best tools to know its potentialities, protein quantity in itself does not explain all variation in baking quality.

Several methods can be used to measure rheological properties under conditions of unsteady flow. They are used to assess flour ability to provide dough with good breadmaking performance, to mimic dough stretching and to measure its resistance to rupture. The development of new methods to be used in breadmaking process can be a powerful tool for monitoring the consistency and structural changes in dough and final bread (Kouassi-Koffi et al., 2015). The availability of rheometers, and the associated data processing capabilities, has facilitated fundamental rheological approaches to characterizing flour doughs and gluten.

A rapid axial compression instrument (Gluten CORE, Perten Instruments AB, Huddinge, Sweden) has been specifically designed to test gluten elastic recovery. As described by Chapman et al. (2012), the main advantages of this testing system are the size and instrument's sensitive load cell, and the speed at which a test can be conducted (1 minute). Rather than attached and stretched as in the extensograph test, in the axial compression system the sample only has to be placed into the testing device. The information provided by the method is closely related to the strain gluten undergoes during gas bubble expansion (Chapman et al., 2012).

2.2 Strategies to improve grain quality

The increase in productivity of wheat, during the 70s, is mainly due to genetic improvement, with the launch of modern and more productive cultivars, with a positive response to nitrogen. Although, there are variations in responses to nitrogen rates according to the cultivar, climate, soil and other, most of the results show that the use of this nutrient, even at low doses, always results in higher yields compared to the absence

(Prando et al., 2012). Cultural practices such as crop succession, management, proper use of dose and timing of nutrient application are alternatives that can be used to increase nitrogen efficiency (Fornasieri-Filho, 2008).

The qualitative and quantitative variation of proteins, determined by genetic factors inherent to cultivar (wheat classification), could be influenced directly by environmental and management factors such as temperature, soil fertility and nitrogen fertilization (Daniel; Triboi, 2000). The quantitative chemical composition of the grain is intrinsically defined by genetics. Nevertheless, factors can cause changes in the chemical composition and contamination of grain, and consequently in the quality. Climatic conditions, temperature and rainfall, can influence the filling of the grain, with consequent alteration in the technological quality (Johansson et al., 2013).

2.2.1 Crop succession

The nutrients found in soil, considering nitrogen cycle/dynamics, has its origin based on: nitrogen fertilizer, atmospheric deposition, biological fixation of atmospheric nitrogen (N_2), and nitrogen mineralization of organic soil (Lamb et al., 2014).

The available nitrogen in plants depends among other factors on the amount of organic matter in the soil, characteristic of plant residues, and adopted management. Furthermore, nitrogen concentration and biochemical composition of crop residues are critical factors for mineralization or immobilization of the elements in the soil (Bot, Benites, 2005).

The use of leguminous plants in crop rotation/succession is a strategy to be considered due to the effect on increasing nitrogen content in the soil, and partial substitution of the nitrogen fertilizer for N-fixed symbiotically, which results in gradual release of the element and the reduction of nitrous oxide (N_2O) emission (Roy et al., 2006c). In contrast, corn residue presents high C/N ratio, which promotes greater immobilization of nitrogen in the soil and consequent reduction in the amount available (Veras et al., 2016).

2.2.2 Nitrogen fertilization

Approximately 98% of the nitrogen in the soil is in organic form, the remainder is in inorganic form as ammonium (NH_4+) and/or nitrate (NO_3-), readily available for the plants, originated by the mineralization during crops, and produced through enzymatic hydrolysis by soil microbes (Camargo et al., 1997). However, the inorganic nitrogen is subject to constant losses by volatilization, leaching, immobilization and extraction by crops. The type of loss is determined, among other factors, by the nitrogen source used, by management performed in the culture, and environment to which the fertilizer is exposed.

Direct and indirect negative effects can be caused by the accumulation of excessive amounts of nitrogen in terrestrial and aquatic ecosystems, as well as in the troposphere, generating significant damage to society with respect to environmental quality, ecosystems, biodiversity and human health (Roy et al., 2006b).

The importance of nitrogen in plant development is mainly due to its structural function, as part of organic compounds molecules such as amino acids and proteins, and activator of many enzymes. Nitrogen has an important function as a binder of metal ions, mainly in the form of heterocyclic rings, such as in chlorophyll; participates in the formation of hydrogen bonds, stabilizing and providing the proper conformation of proteins (enzymes) and nucleic acids, and peptide bond formation between amino acid residues, thus, allowing the formation of protein (Mokhele et al., 2012).

Since it is the most absorbed nutrient by plants and the most exported, nitrogen must be frequently replenished in the soil. As reported, the nitrogen requirement in temperate regions is 25 kg, to produce one tone of grain containing 15 percent protein. However, the amount is dependable of soil and climate conditions, management practices and genotype (Roy et al., 2006a).

Protein concentration, determining of wheat quality, is sensitive to environmental conditions, where variations may be observed from 7 to 20% in a single plant variety, depending mainly on the use of fertilizers and growth environment (Carson; Edwards, 2009).

Gutkoski et al. (2002) observed significant correlation ($p<0.05$) between protein content and dough strength ($r=0.75$) and P/L ratio ($r=-0.71$) with the latter one corresponding to the balance between dough strength (P) and extensibility (L). Gutkoski et al. (2011), observed that wheat cultivars had better physical characteristics increasing nitrogen dose, showing an improvement average of about 1.4 times in the flour crude protein content. Cazetta et al. (2008) observed an increasing in dough strength (W), as well as flour protein content and reduction in the P/L ratio, which had a positive influence on the quality of flour for baking.

Saeed et al. (2011) studied the effect of nitrogen (urea) and sulfur (ammonium sulfate) fertilization (both in soil and foliar) in the quality of wheat for bakery in Pakistan and concluded that using these nutrients resulted in increased gluten content and improvements in the rheological quality of the doughs, as well as in the quality of the produced breads.

2.3 Dry cake mixes

The survival of the food industry depends on timing and actions to respond to desires and needs of consumers as well as monitoring changes in consumers' behavior. An average modern consumer is concerned with the issue of quality, in which several aspects are considered, such as ease of preparation, foods with adequate shelf life, product with the least possible amount of additives, food security, products marketed in different servings sizes, and sensory and texture attributes.

Food products that are easily prepared have attracted the attention of consumers. An example of this are cake mixes, which make the elaboration of cakes easier and faster. The consumer of this type of product has the expectation to follow the instructions to get a product with uniformity and quality. This fact does not always occur when the cake is prepared using a homemade recipe, in which problems can occur at different stages of processing, such as measurement errors of ingredients and lack of baking process control, due to lack of experience as well as lack of uniformity of raw material (Conforti, 2014).

The definition of cake varies, but essentially the term refers to products which are characterized by formulations based on wheat flour, sugar, whole eggs, and other liquids in which fat or oil may be added. There is no significant formation of the gluten network in cake doughs and the main component of cake structure formation is starch, which is present in traditional formulations in wheat flour. However, although the amount of protein is low, its quality must be high enough to ensure the formation of films to capture gas in small air bubbles (Cauvain, 2017). The main parameters of cake quality are volume, firmness, color, and weight loss. The cake quality is influenced by several factors like quality and level of ingredients (Kahraman et al., 2008).

Food quality is defined by a complex set of stimulus that starts with visual evaluation. Once the decision is made, the quality is based not only on the magnitude of flavor, taste and texture, but also takes into account temporal coordination of these stimuli (Foegeding et al., 2010). The main parameters of cake quality are volume, firmness, color, and weight loss, and the quality can be influenced by several factors like quality and level of ingredients (Kahraman et al., 2008).

2.3.1 Milk

Dairy proteins are highly functional ingredients and, due to their versatility (solubility) and properties provided (emulsifying and foaming), can be readily incorporated into many food products such as soups, sauces, recombined evaporated milk, confectionery and bakery products. The use of dairy proteins in bakery is due to both nutritional and functional benefits, as their ability to stabilize air-water interface to create a foam (foaming power), flavor and texture enhancement, and storage improvement (Tomas et al., 2004; Mayorga; Gomez, 2014).

The functional properties of milk powders are generally attributed to milk proteins. Lactose influences and enhances the controlled browning of bakery goods, e.g., cakes, breads and morning bake goods. Lactose has unique volatile flavor binding and enhancing properties, particularly useful in products with delicate flavoring and savory flavors (Bennion, Bamford, 1997).

2.3.2 Salt (NaCl)

Sodium chloride (NaCl), commonly known as salt or table salt, provides one of the basic tastes perceived by humans (saltiness) and is widely used in food production as food preservative, enhancing human health by killing or limiting growth of foodborne pathogens and spoilage organisms (Doyle; Glass, 2010). In bakery, salt is also employed to reinforce the plastic properties of the dough, improving strength, and decreasing fermentation activity, favoring the appearance of the product.

Due to the addition of charges, sodium chloride improves molecular interaction affecting the texture of foods. The addition of salt delays the hydration of gluten proteins (glutenin and gliadin) and development of gluten matrix, stabilizing the gluten and prevent stickiness in the dough. This delay leads to the formation of a network with more fibrous protein structure, critical for the development of enough gluten to trap small air bubbles in the dough to produce a high-quality bread (Cauvain, 2017; McCann; Day, 2013).

2.3.3 Leavening agents

Leavening agents are responsible for chemical formation of carbon dioxide, which expands the cells, causing swelling, especially in products such as cakes, giving the desired product structure and taste. During chemical fermentation, one or more acids react with a source of bicarbonate, favoring the release of CO_2 to batter aeration (Conforti, 2014). The gas forms bubbles that are trapped in the batter, which expands during baking to form the holes that are retained in the finished product (Pop, 2007).

Baking powder, one of the most common leaving agents, is responsible for aerating the batter, resulting in lightweight and porous products, which is important and influences on volume, softness and final texture (El-Dash, Germani, 1994). Double-acting baking powder contain a mixture of sodium bicarbonate, a fast-acting leavening acid like monocalcium phosphate monohydrate (MCP) and a slow-acting leavening acid like sodium acid pyrophosphate (SAPP). They react partially at low temperatures and partially at high temperatures to provide uniform leavening throughout processing (Miller, 2016).

The amount of baking powder used depends on the product type, characteristics and quantity of the ingredients used. The method of mixing and the way to manipulate the batter affect the amount of leavening agent employed. Some types of cakes require less or no chemical leavening (sponge cake); in other types, the amount of leavening can range from 0.25 to 5% based on the weight of flour (Moretto; Fett, 1999).

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CHAPTER III

EFFECT OF AGRICULTURAL MANAGEMENT PRACTICES
ON RHEOLOGICAL PROPERTIES OF WHEAT

3.1 Abstract

Wheat (*Triticum aestivum* L.) is one of the most important cereal crop and its technological properties are essential for commercialization and utilization. Gluten, has unique rheological properties that enable the raw material to be used for different industrial uses. To evaluate the impact of agricultural management practices in the quality of grain produced, field trials were conducted with wheat (BRS 220) in the North (Londrina) of Paraná State-Brazil (2010 and 2011). Cultivation system was carried out in succession to maize or soybeans and nitrogen doses (0-120 kg ha^{-1}). The parameters evaluated in the wholemeal were pasting properties, and gluten content and quality (creep-recovery and compression-recovery tests). Application of nitrogen fertilizer (0-120 kg ha^{-1}) increased the protein content observed, when produced in succession with maize correlation (r=-0.429). Wet and dry gluten content increased with nitrogen application. Protein content presented a strong positive correlation with wet (r=0.923) and dry gluten content (r=0.969). Moreover, gluten extracted from wheat grown after maize presented an increase in elastic recovery (+79.8%) and reduction in recovery index (-3.7%), increasing nitrogen application. Wheat grown after maize with limiting nitrogen fertilizer (0 kg ha^{-1}) presented an increase in peak viscosity (+12.0%), trough (+13.4%), breakdown (+10.2%), final viscosity (+12.7%) and setback (+12.0%). Moreover, increasing doses of nitrogen reduced peak (-12.5%) and final viscosity (-5.9%). A moderate positive correlation was observed between starch content and pasting properties. Contrarily, a strong negative correlation was observed with protein content. In addition to breeding, management of soil nitrogen would be recommended as additional procedure to obtain raw materials with desired profile in relation to chemical composition and consequent rheological characteristics. The results demonstrated the importance of studies on appropriate management to maximize productivity and grain quality.

Key words: Crop Succession. Nitrogen Fertilization. Pasting Properties. Gluten Content and Quality

3.2 Introduction

Wheat (*Triticum aestivum* L.), a monocotyledon belonging to the family Poaceae (Grasses), stands out among the world's cereals of higher importance in human food. It occupies the second place in volume of production, first in area of cultivation, with important role in the globalized agribusiness, for the agronomic adaptability of the crop, ease of storage, nutritional quality and enormous diversity of food products developed (USDA, 2017; Wrigley, 2009).

Along with the chemical composition of the kernel (moisture, protein, carbohydrate, lipid and mineral), technological and nutritional characteristics define the quality of wheat flour (Scheuer et al., 2011). Wheat flour proteins are the source of wheat's unique breadmaking quality that make wheat the most popular cereal for making bread. Two crucial determinants of bread-making quality are the grain protein concentration and protein composition, including the amount and ratio of gluten protein fractions as well as their subunits (Zhang et al., 2009). The technological quality of the wheat depends on the quantity and quality of the proteins present in the grain, being glutenins and gliadins considered responsible for the rheological properties of the dough (Franceschi et al., 2009; Rodriguero et al., 2015).

The availability of nutrients to the plant is a determinant variable in production. Nitrogen is a limiting factor for development and productivity of the crop due to its importance in the formation of amino acids, proteins, chlorophyll and essential enzymes that stimulate growth and development of the plant (Fuertes-Mendizabal et al., 2010; Prando et al., 2012).

Saeed et al. (2011) studied the effect of nitrogen (urea) and sulfur (ammonium sulfate) fertilization (both in soil and foliar) in the quality of wheat for bakery in Pakistan and concluded that using these nutrients resulted in increased gluten content and improvements in the rheological quality of the doughs, as well as in the quality of the produced breads.

The qualitative and quantitative variation of proteins, determined by genetic factors inherent to cultivar (wheat classification), could be influenced directly by environmental and management factors such as temperature, soil fertility and nitrogen fertilization (Daniel; Triboi, 2000). The quantitative chemical composition of the grain is intrinsically defined by genetics. Nevertheless, factors can cause changes in the chemical composition and contamination of grain, and consequently in the quality. Climatic conditions, temperature and rainfall, can influence the filling of the grain, with consequent alteration in the technological quality (Johansson et al., 2013).

Several methods can be used to measure rheological properties under conditions of unsteady flow. They are used to assess flour ability to provide dough with good breadmaking performance, to mimic dough stretching and to measure its resistance to rupture. The development of new methods to be used in breadmaking process can be a powerful tool for monitoring the consistency and structural changes in dough and final bread (Kouassi-Koffi et al., 2015). The availability of rheometers, and the associated data processing capabilities, has facilitated fundamental rheological approaches to characterizing flour doughs and gluten.

The objective of this work was to evaluate the impact of agricultural management practices in the chemical composition (protein and starch content) and rheological properties, such as pasting properties, and gluten content and quality of the wheat flour produced. The management was focused on crop succession (after maize or soybean) and increasing nitrogen doses in broadcast (0, 30, 60, 90 and 120 kg ha^{-1}). Thus, we seek a better understanding of the effect that common agricultural practices have on the quality of grain produced.

3.3 Material and Methods

3.3.1 Material and inputs

The BRS 220 cultivar is characterized as average cycle (69 days from emergence to heading), moderately susceptible to scab and belongs to the bread wheat class. According to variety trial conducted in experimental stations in 2009, 2010 and 2011 crop season, the cultivar has the following characteristics for the region of the experiment: 284 10^{-4}J of dough strength (W), 1.0 of tenacity/extensibility ratio (P/L), 37 g of weight of 1000 seed and production of 3879 kg ha^{-1} (Bassoi et al., 2016). In 2011, the experiment was conducted using seed pre-treated with: triadimenol (3 mL kg^{-1} of seed) and imidacloprid (1 mL kg^{-1} of seed).

3.3.2 Location and characterization of experimental area

Cultivated in two crop seasons (2010 and 2011), field experiments were conducted at the experimental area of Embrapa-CNPSo (Brazilian Agricultural Research Corporation – Soybean Center), located in the district of Warta, Londrina, Parana State, Brazil. The District of Warta (Londrina) is located at 23º11'S, 51º10'W, altitude average of 605 m, and soil characterized as rhodic hapludox. The region, according to Köppen, is

Cfa, or subtropical climate with an average temperature in the coldest month below 18°C (mesothermal) and average temperature in the hottest month above 22°C with hot summers, infrequent frosts and a tendency of concentration of rainfall during the summer, however has no definite dry season. The temperature data (°C) and daily rainfall (mm) during the crop cycle were provided by the meteorological station of Embrapa, located approximately 2 km from the experiment (Fig. 1).

The experimental area was managed under no-tillage system, and wheat grown after maize or soybean (crop succession). Prior to installation of the experiments soil samples were collected from the areas for chemical analysis. Mineral fertilization at sowing was performed with nitrogen, phosphorus and potassium (NPK) based on soil analysis results, according to guidelines from Brazilian Technical Commission Indications for Wheat and Triticale Research in the State of Paraná (Fronza et al., 2008). For this, 250 kg ha^{-1} of the formula 8-28-16 was used, which correspond to 20 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O. Ammonium nitrate (32% N and 3% K_2O) was applied in broadcast, in pre-prescribed doses at the start of tillering (Stage 2 of Feeks Scale; Large, 1954). The amount of K_2O was corrected and all parcels received the same amount of calcium.

3.3.3 Experimental design

The experiments were conducted in a complete randomized block design, with four replications. The factor crop succession was allocated in the block and nitrogen applied in the rows (0, 30, 60, 90 and 120 kg ha^{-1}), and applied at tillering (Stage 2; Feeks

Scale; Larger, 1954). The experimental plot consisted of 13 rows, 8 m in length and 17 cm spacing. The harvested or usable area for each experimental plot was constituted by the seven center rows (excluding 3 rows on each side), ignoring 1.25 m at the ends, totaling 6.54 m^2 of floor area (Fig. 2).

3.3.4 Operational procedure

A mechanical seeder-fertilizer machine for no-tillage system was used in the experiment, obtaining an approximate plant density of 300 per m^2. Cultural practices were carried out according to the technical information from The Brazilian Wheat and Triticale Research Commission (Fronza et al., 2008). The experimental area was monitored weekly and fungicide applied at the onset of symptoms. The weed control was made when necessary if there was occurrence of weeds at critical period for the crop (emergence to heading).

Mechanical harvest of the experimental plots occurred in stage 11.4 of Feeks Scale (Larger, 1954), corresponding to crop maturation with grains presenting moisture of less than 20%. After cleaning, the kernels were ground and stored at -18°C until analysis (MOD FE26, Electrolux®, Manaus, AM, Brazil). The chemical composition analysis (moisture, protein and starch content) of the samples were performed at Londrina State University (Londrina, PR, Brazil), as reported by Souza (2013). Afterwards, the remaining samples were shipped to the Food & Agricultural Products Center, at Oklahoma State University, to be analyzed by rheological methods.

3.3.5 Methods

3.3.5.1 Initial characterization of the samples

The wholemeal samples were obtained by milling the wheat kernels to pass 30 mesh sieve using a hammer mill (MOD MA-090, Marconi®, Piracicaba, SP, Brazil). The protein content was determined by Kjeldahl, using a block digester (Model TE-40/25, Tecnal, Piracicaba, SP, Brazil) and a nitrogen distiller (Model TE-036/1, Tecnal). Thereafter, nitrogen was converted into protein – 5.7 factor (method 979.09; AOAC, 1995). Digestible starch content was determined by enzymatic hydrolysis (Protocol PTF) and analysis in UV-visible measurements (Model British S22, Biochrom, Cambridge, United Kingdom) (Walter et al., 2005).

3.3.5.2 Pasting Properties

The pasting profile of the samples was determined according to AACC International Approved Method 76-21.01, using a Rapid Visco Analyzer (Model RVA 4500, Perten Instruments, Kungens Kurva, Sweden), coupled to a water bath 25°C (Fisher Isotemp 1028P, Pittsburgh, PA, U.S.), and program setting as shown in Table 1. The samples had their weights adjusted to 14% moisture content, previously determined according to method 012/IV (105 ºC per 3 hours; IAL, 2005) in a convection oven (model 52000-55, Cole-Parmer Instrument Company, Chicago, IL, U.S.). The analysis was performed in duplicate.

3.3.5.3 Gluten Preparation

The gluten was extracted from wholemeal (wheat kernels ground to 30 mesh) according to AACCI Approved Method 38-12.02, with modifications. Briefly, the extraction was performed using a Glutomatic System (Model 2200, Perten Instruments, Huddinge, Stockholm, Sweden). Ten g of wholemeal sample and 4.5 mL of 2% NaCl solution (w/v) were mixed for 1 minute in the Glutomatic System. Salt soluble particles were washed with 2% salt solution through a polyester screen (88 µm) for 2 minutes. The polyester screen was changed (840 µm) and the washing process continued for more 3 minutes. The extractions were performed in triplicate. The obtained gluten was analyzed by compression-recovery and creep-recovery tests described below. After completing the compression-recovery test, the gluten sample was dried to estimate the wet and dry gluten content as described by AACCI Approved Method 38-12.02.

3.3.5.4 Compression and Recovery Test

Compression-recovery test was performed to study the elastic recovery of gluten after compression. After extracted, gluten samples were prepared as described by Chapman et al. (2012) with modifications. Briefly, the gluten was centrifuged for 1 min, using a dummy gluten sample to balance the centrifuge, to reduce its moisture and shape the sample into a cylindrical form (Gluten Index Centrifuge 2015, Perten Instruments, Huddinge, Stockholm, Sweden). The obtained samples were placed at the center of the loading plate of the Gluten CORE Analyzer (Perten Instruments AB, Huddinge, Sweden) and analyzed by applying a 5 s compression phase (3 N compression) and a 55 s recovery phase. The gluten height was recorded during both phases at 1 second interval. The analysis was performed in triplicates.

The elastic recovery was calculated according to the equation:

$$\text{Elastic recovery (\%)} = \frac{\text{Height at final recovery}}{\text{Initial height before compression}} x100$$

3.3.5.5 Creep and Recovery Test

Creep-recovery test was used to investigate the rheological properties of gluten using a shear stress, according to Zhao et al. (2010) with modifications. Briefly, the wet gluten extracted was shaped into a ball and relaxed in a setup of 2.5 kg top plate with 2.5 mm space between top and bottom plates for an hour at room temperature. The relaxed gluten was cut with a round metal cutter of 30 mm diameter and gently transferred to the lower plate of the rheometer (Model AR1000N, TA Instruments, New Castle, DE, U.S.). The test applied a constant shear stress of 50 Pa for 100 s followed by 100 s of recovery time, using serrated parallel plates. The analysis was performed in triplicates.

The recovery index was calculated according to equation:

$$\text{Recovery index (\%)} = \frac{\text{Recovery Compliance J(t)}}{\text{Creep Compliance J(t)}} x100$$

3.3.5.6 Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) to assess the effect of crop succession, nitrogen application and interaction of factors, with mean comparison by Tukey test ($p<0.05$), and Pearson's correlation between all the variables evaluated using Statistica 13.2 (Dell™ Statistica™ 13.2, Tulsa, OK, U.S.).

3.4 Results and Discussion

3.4.1 Experimental area characterization

The rainfall precipitation during the crop cycle was low, with cumulative total of 95.9 and 199 mm for 2010 and 2011, respectively, as previously reported by Souza (2013). In a previous study, using another Brazilian wheat cultivar (IAC-24), was observed that the average water consumption by the plant was 347.2 mm (115-day cycle), or 3.02 mm of average daily intake under experimental condition in Piracicaba, Sao Paulo, Brazil (Libardi; Costa, 1997). The low rainfall could have affected soil moisture, exerting negative impact on the final yield of rain fed culture system, reducing photosynthesis, biomass production and plant growth. However, 0.8 and 34.4 mm of accumulated rainfall was observed in 2010 and 2011, respectively, on the first 5 days after nitrogen fertilization was applied in broadcast, which is important to reduce the losses by volatilization by incorporating the fertilizer to the soil (Fig. 1).

Temperature and humidity are important factors in the degradation of plant debris, since they determine the growth rate of microorganisms, and therefore the decomposition of the residue (Duong, 2009). Due to low rainfall in 2010, approximately half of which occurred in 2011 and 1/3 of the amount of water required by the plant, the breakdown of residue by soil microorganisms was hampered and the plant cycle may have been affected.

Flumignan et al. (2013) evaluated the influence of irrigation system, 297 mm (precipitation) plus 318.2 mm (irrigation), on productivity, technological quality of flour and wheat root system in the same area of our study (Londrina). According to the authors, the productivity of the crop increased three times when irrigated. However, increasing the availability of water to the plant reduced the amount of protein in the grain (-13.7%), which could partially explain the reduction observed in dough strength (-22.3%) and extensibility (-38.1%), and increasing the tenacity (+17.5%) of the flour.

Even though 2010 crop year characterize the results that would be obtained in a drought year, based on the results observed and the objective of the study, which is to evaluated the impact of cultural practices on quality of grain produced, with focus on crop succession and application of increasing doses of nitrogen, the remaining of the discussion will focus mainly in 2011 crop year.

3.4.2 Chemical Composition

Immobilization of nitrogen is shown in Figure 3. Wheat kernels produced having soybean as antecedent crop presented higher protein content in 2011, compared to control (0 kg ha^{-1}). Due to the low availability of mineral nitrogen in the soil, it is possible to observe the effect of the residue as a nutrient source for the plant. Using high nitrogen dose (120 kg ha^{-1}), protein content did not differ among soybean/wheat and maize/wheat, once there was no competition between plant and microorganism for nitrogen.

Differences between the presented results is due to availability of nutrients provided by each culture, its incorporation into the soil and subsequent use by the plant. The C/N ratio of maize crop residue is higher, promoting greater immobilization of N in the soil and consequent reduction of its availability to the crop (Regehr et al., 2015).

In 2011, the application of high nitrogen doses in succession with maize leaded to high protein content (+53.6%; 0-120 kg ha^{-1}). In soybean succession, the protein increased +17.6% (0-120 kg ha^{-1}). The nitrogen fertilization showed a more pronounced effect in system under maize succession when compared to soybean, once the deterioration of soybean waste represents a good source of nitrogen. This fact is evident since the protein content in the wheat grown after soybeans is 29.1% higher than the grown after maize, not applying nitrogen (0 kg ha^{-1}).

Prando et al. (2012), evaluating the effect of nitrogen sources (conventional urea, urea with urease inhibitor and slow release urea) and levels (0-120 kg ha^{-1}) in wheat cultivars (BRS 208, BRS Pardela and IWT 04008), having soybean as antecedent crop, noted that the nitrogen content in the grain was not significantly altered by nitrogen doses, however, the nitrogen accumulated increased linearly with increasing nitrogen doses ($y=0.1046x+104.73$; $r^2=0.8314$; $p<0.01$).

According to the results shown in Figure 3, it is evident that the greater the availability of nitrogen to the plant the smallest is the starch content in the kernel. Wheat cultivated after maize in 2011 presented +6.9% more starch than when grown after soybean (120 kg ha^{-1}).

Kindred et al. (2008), evaluating wheat cultivars in response to nitrogen fertilizer (ammonium nitrate; 0-240 kg ha^{-1}), observed an increase of +28.4% and -1.9% reduction in the concentration of protein and starch, respectively, under high nitrogen dose (120 kg ha^{-1}). When a double dose was applied (240 kg ha^{-1}), the difference was greater with an increase of +40.1% in protein content and a reduction of -3.6% in starch, comparing to control (0 kg ha^{-1}).

Studies have shown that nitrogen deficiency usually results in accumulation of nonstructural carbohydrates (Kovacevic et al., 2012). According to Wingler et al. (2006), nitrogen deficiency can lead to sugar accumulation, reducing the use of carbon skeletons for synthesis of amino acids and proteins. Which can be observed in Table 2, protein and starch content in the samples presented a moderate negative correlation in 2011 ($r=-0.429$; $p<0.01$). Changes in starch accumulation correlate negatively with protein concentration, which in turn can correlate positively with gliadin or glutenin accumulation (Johansson et al., 2003).

Depending on the end use of the flour produced the chemical composition requirements are different. In the cake industry, the amount of starch and its pasting properties is more important than protein quantity and quality once there is no significant formation of gluten network in batter, being starch the main component of cake structure formation (Cauvain, 2017). For bread production, the quality and quantity of proteins is important once they are responsible for gluten formation, the major and most crucial component of dough associated directly with bread quality (Marchetti et al., 2012).

3.4.3 Rheological Properties

The antecedent crop (maize or soybean) used not only affected the amount of gluten in the samples but also resulted in alterations in their quality (Table 3). In 2011, wheat cultivated after soybean presented higher concentration of wet (+40.9%) and dry gluten (+56%) when compared to crop succession with maize (0 kg ha^{-1}), by applying low doses of nitrogen fertilizer as shown in Table 3.

Increasing doses of nitrogen applied in broadcast also changed the gluten content in the wheat (Table 3). When cultivated after maize, wet (+67.9%) and dry gluten content (+86.7%) in the wheat samples increased significantly by applying high doses of nitrogen (0-120 kg ha^{-1}). After soybean, similar effect was observed but the increases for wet (+18.40%) and dry gluten content (+19.7%) was a lot lower than the observed having maize as antecedent crop. The gluten concentration was already higher in wheat cultivated after soybean compared to maize, since the residual nitrogen provided by the plants decomposition supplied the nutrient for the plant when the fertilizer was not applied or applied in low doses (0-30 kg ha^{-1}).

The chemical composition of the samples (protein and starch content) presented significant correlation with the rheological properties evaluated in this study (Table 2). The protein content in the samples presented a strong positive correlation with wet ($r=0.923$; $p<0.01$) and dry gluten content ($r=0.969$; $p<0.01$), for 2011 crop season.

Similar results were observed by Rodriguero et al., (2015), evaluating the effect of urea based fertilizers and rates on wheat kernels quality. As reported, the results showed no differences among the three fertilizers but the highest the nitrogen dose applied more protein (+10.8%) and wet gluten (+9.4%) was observed in the wholemeal samples (0-150 kg ha^{-1}), with a strong positive correlation between the variables (r=0.7893; p=0.002).

Practically, the protein fractions and amino acid composition of wheat grains can be deduced directly from the total quantity of nitrogen in the grain (Triboi et al., 2000). Other researchers have reported that the fertilizer dose influenced the total amount of protein and the amount and size distribution of polymeric proteins. Generally, significant positive correlations were found between increased fertilizer rates, increased protein concentration and amount of gliadins and glutenins (Daniel; Triboi, 2000). The increase in protein concentration due to increase in fertilizer dose was reported to increase gliadins and SDS-soluble proteins compared to glutenins and SDS-insoluble proteins, which led to a decreased glutenin/gliadin ratio (Johansson et al., 2001).

The rheological properties of gluten were also affected by crop management in 2011. Gluten extracted from wheat grown after maize increased elastic recovery (+79.8%) however the recovery index reduced by -3.7%, comparing control to the maximum nitrogen dose applied (0-120 kg ha^{-1}; Table 3).

As can be observed in Table 4, protein content presented a moderate but significant positive correlation with elastic recovery (r=0.550; p<0.01) and a negative with recovery to creep deformation (r=-0.370; p<0.01). This results partially explain the observations made by Souza (2013), where dough strength (+15.6%), extensibility (+37.2%) and tenacity (-14.7%) were influenced by the nitrogen fertilization management in 2011 (0 - 120 kg ha^{-1}). Moreover, the author observed that increase in nitrogen dose (0-

120 kg ha^{-1}) provided a +15.2% increase in the elasticity index in 2011. The elasticity index is related to the flour quality, the closer to 100% the greater the elastic recovery of the dough, with a value of more than 50-55% being ideal for industrial baking (Kitissou, 1995).

Fuertes-Mendizábal et al. (2010), evaluating the effect of nitrogen fertilization (ammonium nitrate, 0-180 kg ha^{-1}) on the technological quality and protein composition of wheat grains, observed a positive and significant correlation between extensibility (r=0.953), dough strength (r=0.952) and tenacity (r=0.900), with the nitrogen content in the grain. They also observed a high positive correlation between gliadin (r=0.931) and glutenin (r=0.994), with the nitrogen content in the grain. Confirming that these reserve proteins were responsible for the increase of total protein in the grain in response to fertilization. While the metabolic proteins (albumins and globulins) remained constant, regardless of the applied dose.

The difference observed between the responses to elastic recovery and recovery index was not expected, since both tests measure the gluten's ability to return to its original after suffering deformation, by compression (elastic recovery) or shear (recovery index), as shown in Table 3. Besides the way the deformation is applied by each method, the force used and the period during which this force was applied is quite different. In the case of elastic recovery, a force of 3 N is applied for 5 seconds, and in the recovery index the deformation was more drastic, being applied a force of 50 Pa for 100 seconds. In addition, wholemeal presents a greater fiber content compared to white flour, and the presence of insoluble fibers such as cellulose are believed to disrupt and weaken the gluten matrix (Seetharaman et al., 1997).

In relation to the pasting properties of the samples (wholemeal) was observed that, wheat grown after maize crop under no nitrogen fertilizer dose (0 kg ha^{-1}) presented higher peak viscosity (+12.0%), trough (+13.4%), breakdown (+10.2%), final viscosity (+12.7%) and setback (+12.0%) compared to the grain produced in succession with soybean, as shown in Table 4. Increasing doses of nitrogen applied reduced the peak (-12.5%) and final viscosity (-5.9%) of the samples, under maize crop succession.

As shown in Table 2, a moderate positive correlation was observed between starch content and pasting properties of the wholemeal samples in 2011. Indicating that the higher the starch concentration more elevated is the pasting parameters analyzed. The absence of significant results for wheat grown after soybean crop is due to the higher availability of nitrogen provided by the crop residues, when compared to nitrogen provided by the maize crop. When more nitrogen is available to the plant less carbon skeletons is direct to synthesis of carbohydrates, once they will be used to synthesize amino acids and proteins (Wringler et al., 2006).

A high viscosity of the paste can be desired in certain products. Sanz et al. (2008) suggested that the batter viscosity has an important effect on the bubble incorporation and movement which are considered controlling factors in cake texture, with higher viscosity of the batter contributing to higher bubble stability before and during baking. If the viscosity is too low, the bubbles in the batter can easily rise to the surface and are lost to the atmosphere during baking.

Depending on the composition (amylose/amylopectin ratio) and pasting properties of the starch present in the flour it can have a significant effect in the quality of bread, especially in shelf-life with retrogradation and staling, which is a physical phenomenon that describes deterioration of bread quality during storage and is the main shelf-life limiting factor of bread (Gray; Bemiller, 2003).

3.5 Conclusion

The agricultural management practices evaluated (crop succession and increasing doses of nitrogen fertilizer) resulted in changes in the quality of the wheat (BRS 220), both in chemical composition and rheological properties.

1. Increasing nitrogen doses applied in broadcast in succession with maize (+53.6%; 0-120 kg ha^{-1}) or soybean (+17.6%; 0-120 kg ha^{-1}) increased protein and reduced starch content in the kernels by -8.6% in 2011 (succession with soybean; 0-120 kg ha^{-1}). Protein and starch content presented a moderate negative correlation ($r=-0.429$; $p<0.01$).

2. Wet and dry gluten content increased applying high doses of nitrogen (0-120 kg ha^{-1}), in crop succession with maize or soybean. Protein content presented a strong positive correlation with wet ($r=0.923$; $p<0.01$) and dry gluten content ($r=0.969$; $p<0.01$), in 2011 crop season. Moreover, gluten extracted from wheat grown after maize crop presented an increase in elastic recovery capacity (+79.8%) and reduction in recovery index (-3.7%), when increasing doses of nitrogen fertilization were applied.

3. Wheat grown after maize under limiting nitrogen fertilizer (0 kg ha^{-1}) presented higher peak viscosity (+12.0%), trough (+13.4%), breakdown (+10.2%), final viscosity (+12.7%) and setback (+12.0%), compared to succession with soybean. Moreover, increasing doses of nitrogen applied in broadcast reduced the peak (-12.5%) and final viscosity (-5.9%) of the samples, under maize crop succession. A moderate positive correlation was observed between starch content and pasting properties of the samples in 2011.

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Table 1. Test setting profiles for analyze pasting properties of wheat wholemeal.

Stages	Temperature/speed	Time
1	50°C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 10 sec
4	50°C	1 min, 0 sec
5	95°C	4 min, 42 sec
6	95°C	7 min, 12 sec
7	50°C	11 min, 0 sec
End of test		13 min, 0 sec

Table 2. Protein and starch content of wheat (*Triticum aestivum* L.; BRS 220) in response to interaction of factors (crop succession x nitrogen dose).

N Dose (kg ha^{-1})	2010							
	Protein (%)[a]				Starch (%)[a]			
	Maize	%	Soybean	%	Maize	%	Soybean	%
0	15.2±0.6aC	0.0	14.9±0.5bD	0.0	64.4±3.1aA	0.0	64.7±2.5aA	0.0
30	15.2±0.3aC	0.0	15.3±0.4aC	2.7	63.6±2.2aA	-1.2	64.1±4.4aA	-0.9
60	15.7±0.3aB	3.3	15.6±0.2aBC	4.7	64.8±2.3aA	0.6	64.1±4.8aA	-0.9
90	16.3±0.3aA	7.2	16.1±0.4aA	8.1	59.7±6.3aB	-7.3	61.8±2.3aA	-4.5
120	16.5±0.4aA	8.6	15.8±0.3bAB	6.0	61.8±2.6aAB	-4.0	63.7±3.0aA	-1.5

N Dose (kg ha^{-1})	2011							
	Protein (%)[a]				Starch (%)[a]			
	Maize	%	Soybean	%	Maize	%	Soybean	%
0	11.0±0.4bE	0.0	14.2±0.4aC	0.0	57.7±3.2aA	0.0	57.2±2.0aA	0.0
30	13.0±0.5bD	18.2	15.7±0.5aB	10.6	58.3±1.3aA	1.0	53.1±2.3bB	-7.2
60	14.2±0.2bC	29.1	16.4±0.5aA	15.5	58.3±2.0aA	1.0	54.1±1.2bAB	-5.4
90	16.1±0.4bB	46.4	16.4±0.3aA	15.5	57.4±1.4aA	-0.5	55.6±1.3aAB	-2.8
120	16.9±0.4aA	53.6	16.7±0.3aA	17.6	55.9±2.3aA	-3.1	52.3±4.3bB	-8.6

Expressed on a dry basis; Means (n= 8 ± standard error) followed by the same lowercase letter within a row (crop succession - maize or soybean; p<0.05) and same capital letter within a column are not significantly different (nitrogen doses – 0-120 kg ha^{-1}; p<0.05). Percentage (%) difference when compared to control (0 kg ha^{-1}). Source: Souza (2013).

Table 3. Pearson correlation (r) between all the variables evaluated in this study.

	Year	Protein	Starch	Gluten		Recovery to		Viscosity			
				Wet	Dry	Compression	Creep	Peak	Trough	Breakdown	Final
Starch	2010	-0.509 (p=0.000)									
	2011	-0.429 (p=0.000)									
Wet	2010	0.662 (p=0.000)	-0.291 (p=0.009)								
	2011	0.923 (p=0.000)	-0.405 (p=0.000)								
Dry	2010	0.881 (p=0.000)	-0.412 (p=0.000)	0.809 (p=0.000)							
	2011	0.969 (p=0.000)	-0.401 (p=0.000)	0.971 (p=0.000)							
Compression	2010	-0.081 (p=0.476)	0.052 (p=0.649)	-0.443 (p=0.000)	-0.182 (p=0.106)						
	2011	0.550 (p=0.000)	-0.235 (p=0.036)	0.331 (p=0.003)	0.438 (p=0.000)						
Creep recovery	2010	-0.187 (p=0.098)	0.131 (p=0.245)	-0.209 (p=0.062)	-0.176 (p=0.119)	0.106 (p=0.350)					
	2011	-0.370 (p=0.001)	0.087 (p=0.441)	-0.364 (p=0.001)	-0.391 (p=0.000)	-0.212 (p=0.059)					
Peak	2010	-0.586 (p=0.000)	0.300 (p=0.007)	-0.461 (p=0.000)	-0.630 (p=0.000)	0.120 (p=0.290)	0.110 (p=0.330)				
	2011	-0.756 (p=0.000)	0.435 (p=0.000)	-0.673 (p=0.000)	-0.713 (p=0.000)	-0.479 (p=0.000)	0.277 (p=0.013)				
Trough	2010	-0.419 (p=0.000)	0.178 (p=0.115)	-0.278 (p=0.013)	-0.415 (p=0.000)	0.122 (p=0.283)	0.101 (p=0.372)	0.777 (p=0.000)			
	2011	-0.554 (p=0.000)	0.403 (p=0.000)	-0.483 (p=0.000)	-0.516 (p=0.000)	-0.373 (p=0.001)	0.215 (p=0.055)	0.945 (p=0.000)			
Breakdown	2010	-0.48 (p=0.000)	0.285 (p=0.011)	-0.433 (p=0.000)	-0.554 (p=0.000)	0.061 (p=0.589)	0.067 (p=0.553)	0.755 (p=0.000)	0.174 (p=0.122)		
	2011	-0.896 (p=0.000)	0.397 (p=0.000)	-0.812 (p=0.000)	-0.854 (p=0.000)	-0.537 (p=0.000)	0.312 (p=0.005)	0.887 (p=0.000)	0.687 (p=0.000)		
Final	2010	-0.460 (p=0.000)	0.20 1 (p=0.074)	-0.385 (p=0.000)	-0.444 (p=0.000)	0.097 (p=0.394)	0.237 (p=0.034)	0.778 (p=0.000)	0.746 (p=0.000)	0.441 (p=0.000)	
	2011	-0.544 (p=0.000)	0.425 (p=0.000)	-0.479 (p=0.000)	-0.519 (p=0.000)	-0.386 (p=0.000)	0.264 (p=0.018)	0.929 (p=0.000)	0.975 (p=0.000)	0.688 (p=0.000)	
Setback	2010	-0.255 (p=0.023)	0.117 (p=0.303)	-0.289 (p=0.009)	-0.235 (p=0.036)	0.019 (p=0.868)	0.250 (p=0.025)	0.361 (p=0.001)	0.082 (p=0.471)	0.479 (p=0.000)	0.725 (p=0.000)
	2011	-0.484 (p=0.000)	0.420 (p=0.000)	-0.434 (p=0.000)	-0.481 (p=0.000)	-0.374 (p=0.001)	0.310 (p=0.005)	0.832 (p=0.000)	0.861 (p=0.000)	0.633 (p=0.000)	0.952 (p=0.000)

Table 4. Quantity and quality of gluten from wheat (*Triticum aestivum* L.; BRS 220) in response to interaction of factors (crop succession x nitrogen dose).

N Dose	2010															
	Wet Gluten (14%)[a]				Dry Gluten (%)[b]				Elastic Recovery (%)[c]				Recovery Index (%)[d]			
(kg ha^{-1})	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%
0	46.3±2.7aC	0.0	46.1±3.0aB	0.0	12.8±0.6aC	0.0	12.6±0.6aC	0.0	7.5±1.4aA	0.0	6.3±1.7aA	0.0	73.6±4.1aA	0.0	68.8±1.2bB	0.0
30	46.9±2.4aC	1.3	47.6±3.3aAB	3.3	13.0±0.2aC	1.6	13.1±0.6aBC	4.0	6.4±1.2aA	-14.7	6.6±2.0aA	4.8	72.8±2.2aA	-1.1	72.6±2.4aA	5.5
60	48.0±2.2aBC	3.7	48.6±2.1aAB	5.4	13.6±0.3aB	6.2	13.4±0.4aAB	6.3	7.0±0.8aA	-6.7	6.4±2.2aA	1.6	71.6±2.0aAB	-2.7	71.5±3.0aAB	3.9
90	51.4±2.6aAB	11.0	49.0±2.4aAB	6.3	14.3±0.6aA	11.7	13.9±0.7bA	10.3	5.7±1.8aA	-24.0	6.5±1.2aA	3.2	71.5±2.3aAB	-2.9	69.8±2.5aAB	1.5
120	52.7±2.3aA	13.8	49.5±3.7bA	7.4	14.7±0.6aA	14.8	13.6±0.6bA	7.9	6.7±1.5aA	-10.7	6.9±1.9aA	9.5	68.6±3.2bB	-6.8	72.6±1.0aA	5.5
	2011															
N Dose	Wet Gluten (14%)				Dry Gluten (%)				Elastic Recovery (%)				Recovery Index (%)			
(kg ha^{-1})	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%
0	27.4±2.3bD	0.0	38.6±2.1aC	0.0	7.5±0.8bD	0.0	11.7±0.3aC	0.0	8.9±1.6bC	0.0	15.5±1.1aAB	0.0	76.3±1.8aAB	0.0	73.9±1.2bA	0.0
30	34.9±1.3bC	27.4	42.9±1.8aB	11.1	10.3±0.5bC	37.3	12.9±0.5aB	10.3	14.4±2.6aAB	61.8	13.3±0.8aB	-14.2	77.8±2.4aA	2.0	74.7±1.0bA	1.1
60	41.4±3.6aB	51.1	43.1±1.7aAB	11.7	12.0±0.7bB	60.0	13.5±0.4aAB	15.4	12.0±6.1bBC	34.8	17.8±1.3aA	14.8	74.8±1.4aBC	-2.0	75.6±2.4aA	2.3
90	44.4±1.5aA	62.0	44.2±1.5aAB	14.5	13.7±0.2aA	82.7	13.7±0.2aA	17.1	13.5±3.9aAB	51.7	15.9±2.6aAB	2.6	75.4±2.3aBC	-1.2	73.7±1.4aA	-0.3
120	46.0±1.8aA	67.9	45.7±1.4aA	18.4	14.0±0.6aA	86.7	14.0±0.3aA	19.7	16.0±2.3aA	79.8	15.8±2.4aAB	1.9	73.6±1.4aC	-3.5	74.0±2.0aA	0.1

[a]Percentage corrected to 14% moisture; [b]Expressed on a dry basis. [c]Elastic Recovery measured by applying a constant compression of 3 N for 5 s followed by 55 s of recovery. [d]Recovery Index measured by applying a constant shear stress of 50 Pa for 100 s followed by 100 s of recovery. Means (n= 8 ± standard error) followed by the same lowercase letter within a row (crop succession - maize or soybean; $p<0.05$) and same capital letter within a column are not significantly different (nitrogen doses – 0-120 kg ha^{-1}; $p<0.05$). Percentage (%) difference when compared to control (0 kg ha^{-1}).

Table 5. Pasting properties of wheat (*Triticum aestivum* L.; BRS 220) in response to interaction of factors (crop succession x nitrogen dose).

N Dose	Peak (cP)				Trough (cP)				Breakdown (cP)				Final Viscosity (cP)				Setback (cP)			
2010																				
(kg ha^{-1})	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%
0	2506.4±89.9bAB	0.0	2698.0±60.7aA	0.0	1773.1±66.9bAB	0.0	1868.8±82.6aA	0.0	733.4±52.0bAB	0.0	829.3±28.8aA	0.0	3706.1±82.9bABC	0.0	3826.0±114.8aA	0.0	1933.1±48.3aAB	0.0	1957.3±45.1aA	0.0
30	2577.0±84.5bA	2.8	2642.5±71.1aA	-2.1	1818.8±54.4aA	2.6	1859.1±74.0aA	-0.5	757.9±38.5aA	3.3	783.4±41.3aAB	-5.5	3810.6±64.7aA	2.8	3766.1±89.2aAB	-1.6	1991.5±34.2aA	3.0	1907.0±56.6bA	-2.6
60	2480.5±77.3bB	-1.0	2651.5±77.0aA	-1.7	1804.5±55.2aAB	1.8	1831.5±57.9aA	-2.0	656.0±72.0bBC	-10.6	820.0±21.4aA	-1.1	3660.3±134.1bBC	-1.2	3751.6±67.8aAB	-1.9	1855.8±138.1aB	-4.0	1920.1±57.4aA	-1.9
90	2520.9±46.2aAB	0.6	2548.4±94.0aB	-5.5	1816.8±34.2aAB	2.5	1789.4±115.7aA	-4.2	704.0±41.8bAB	-4.0	759.0±62.1aB	-8.5	3763.6±107.2aAB	1.6	3693.4±125.1aB	-3.5	1946.9±81.4aAB	0.7	1904.0±61.9aA	-2.7
120	2376.1±49.1bC	-5.2	2610.8±46.7aAB	-3.2	1737.0±74.5bB	-2.0	1828.3±44.5aA	-2.2	639.5±51.1bC	-12.8	782.5±10.2aAB	-5.6	3612.9±78.8bC	-2.5	3761.9±26.9aAB	-1.7	1875.9±38.8aB	-3.0	1933.6±30.7aA	-1.2
2011																				
N Dose	Peak (cP)				Trough (cP)				Breakdown (cP)				Final Viscosity (cP)				Setback (cP)			
(kg ha^{-1})	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%	Maize	%	Soybean	%
0	3310.6±59.9aA	0.0	2955.1±137.6bA	0.0	1908.1±51.1aA	0.0	1682.8±102.9bAB	0.0	1402.5±27.0aA	0.0	1272.4±48.6aB	0.0	3756.1±64.7aA	0.0	3332.1±164.9bAB	0.0	1848.0±31.0aA	0.0	1649.4±65.0bAB	0.0
30	3199.4±96.5aB	-3.4	2896.5±149.1bA	-2.0	1865.4±45.3aAB	-2.2	1662.0±107.7bAB	-1.2	1334.0±60.9aB	-4.9	1234.5±47.0bAB	-3.0	3650.1±84.8aABC	-2.8	3321.3±193.0bAB	-0.3	1784.8±54.9aB	-3.4	1659.3±89.5bAB	0.6
60	3188.6±51.4aB	-3.7	2901.1±143.5bA	-1.8	1881.0±23.7aAB	-1.4	1690.5±111.3bAB	0.5	1307.6±30.9aB	-6.8	1210.6±36.7bB	-4.9	3672.1±50.4aAB	-2.2	3389.6±155.4bA	1.7	1791.1±34.2aB	-3.1	1699.1±55.0bA	3.0
90	3058.8±91.7aC	-7.6	2884.9±116.5bAB	-2.4	1834.9±65.5aBC	-3.8	1719.3±89.7bA	2.2	1223.9±28.0aC	-12.7	1165.6±51.5bC	-8.4	3630.9±101.4aBC	-3.3	3384.3±114.7bAB	1.6	1796.0±37.4aAB	-2.8	1665.0±39.7bAB	0.9
120	2942.9±93.5aD	-11.1	2862.3±130.7bB	-3.1	1771.5±62.6aC	-7.2	1640.6±89.0bB	-2.5	1171.4±37.0aD	-16.5	1161.6±44.7aC	-8.7	3548.0±110.3aC	-5.5	3278.1±119.5bB	-1.6	1776.5±55.2aB	-3.9	1637.5±45.9bB	-0.7

Means (n= 8 ± standard error) followed by the same lowercase letter within a row (crop succession - maize or soybean; $p<0.05$) and same capital letter within a column are not significantly different (nitrogen doses – 0-120 kg ha^{-1}; $p<0.05$). Percentage (%) difference when compared to control (0 kg ha^{-1}).

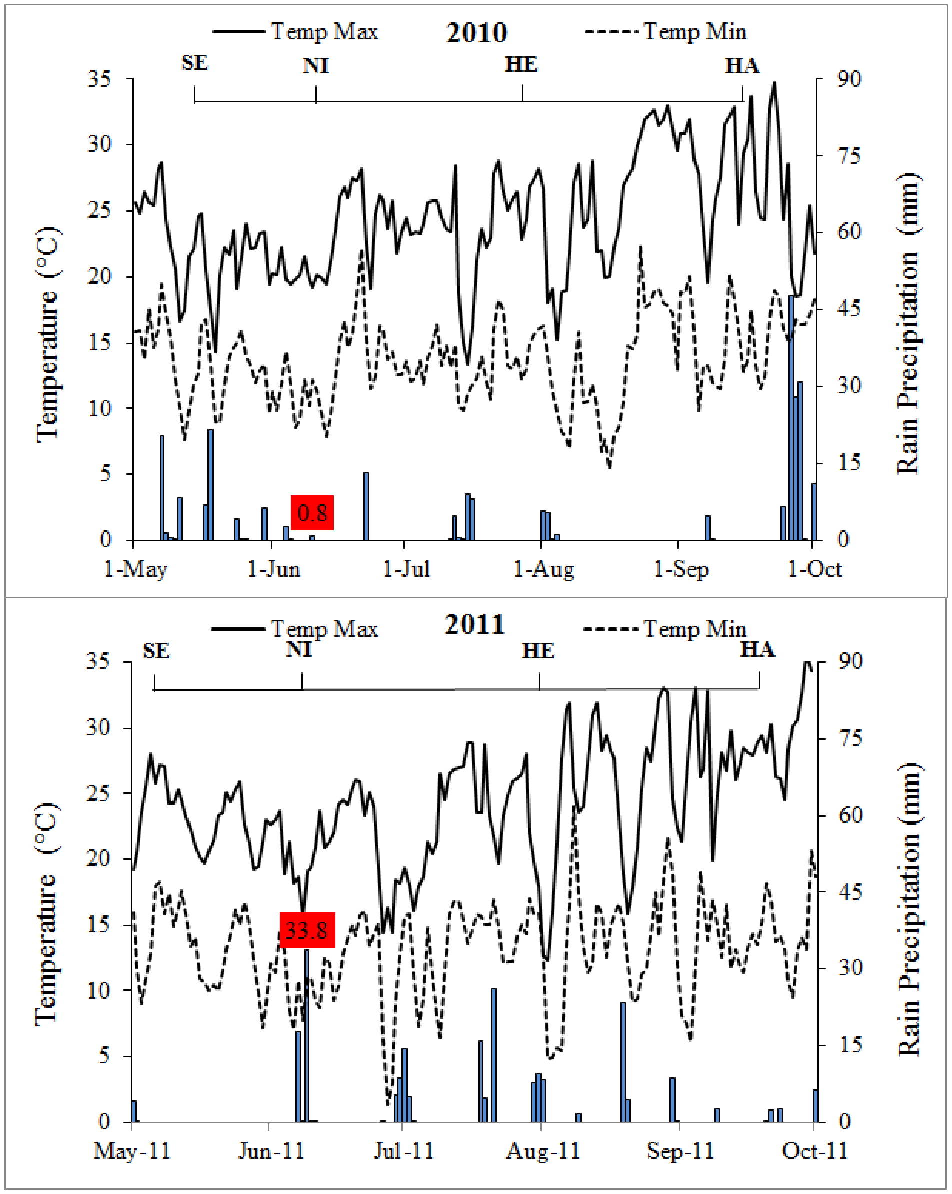

Figure 1. Climate data (temperature and precipitation), Londrina, Parana, Brazil (2010 and 2011). SE: Seeding, NI: Nitrogen fertilization, HE: Heading stage, HA: Harvest. Source: Souza (2013).

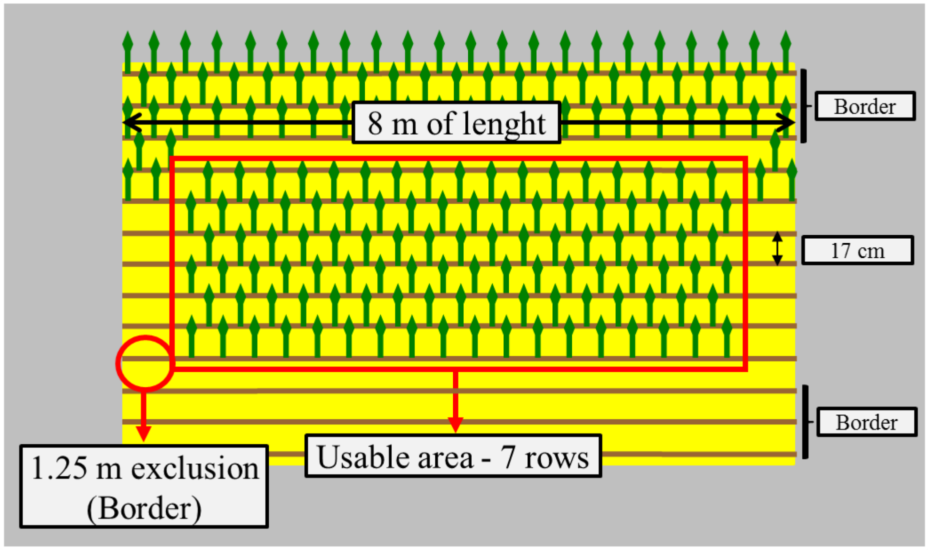

Figure 2. Sketch of the experimental area in Londrina, Paraná, Brazil. Source: Souza (2013).

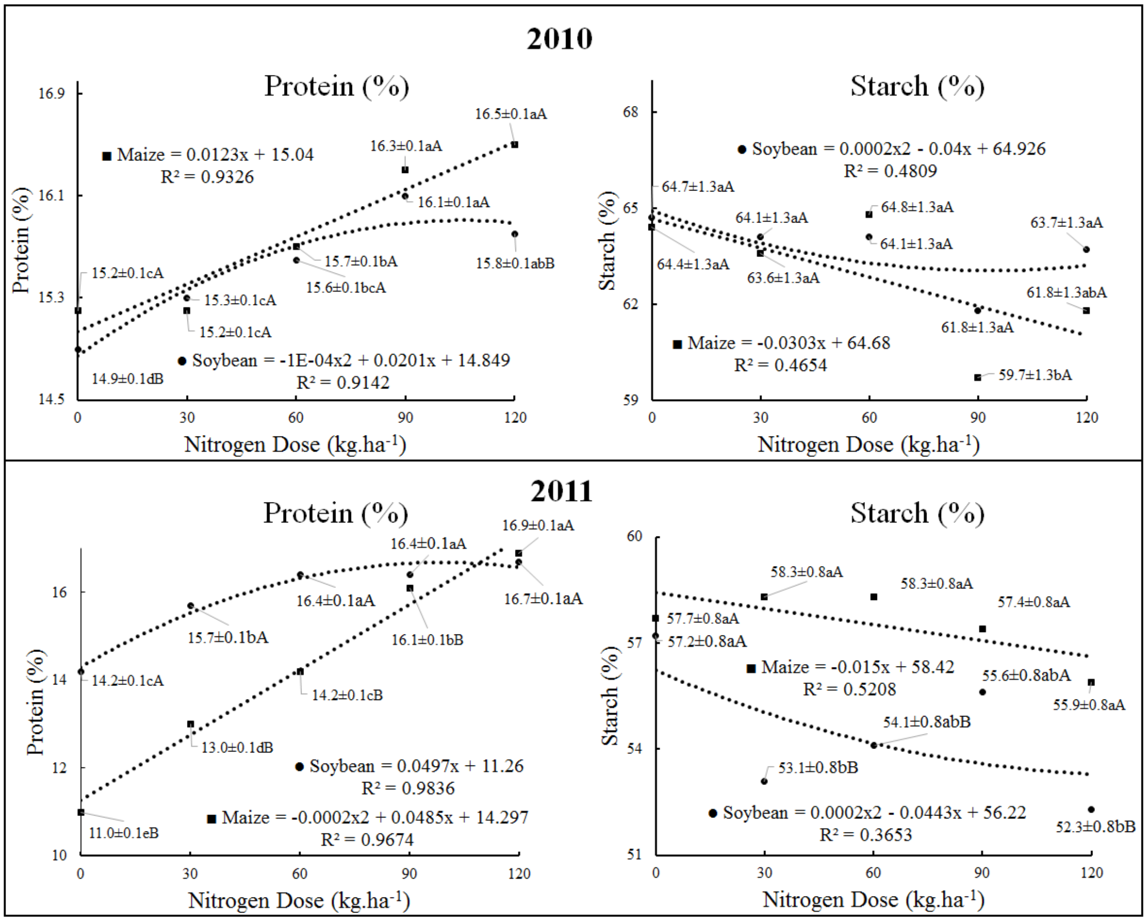

Figure 3. Protein and starch content of wheat (*Triticum aestivum* L.; BRS 220) in response to interaction of factors (crop succession x nitrogen dose). Means (n= 8 ± standard error) followed by the same lowercase letter within the trendline (nitrogen doses – 0-120 kg ha^{-1}; $p<0.05$) and same capital letter between the trendlines for the same nitrogen dose are not significantly different (crop succession - maize or soybean; $p<0.05$). Expressed on a dry basis. Source: Souza (2013).

CHAPTER IV

GLUTEN VISCOELASTICITY: RAPID METHOD FOR

CLASSIFICATION OF SOFT WHEAT GENOTYPES

4.1 Abstract

Standard methods to assess gluten quality are difficult to perform, interpret, time consuming and require a large amount of sample. Gluten CORE is a rapid axial compression instrument based on measuring height during compression and recovery, and specifically designed to test elastic recovery of gluten. The objective of this study was to determine in a set of soft wheat genotypes the separation of gluten viscoelastic properties including extraction and compression-recovery test using Gluten CORE instrument to determine gluten elastic recovery, as well as correlation with standard viscoelastic methods (alveograph, farinograph) and soundness of wheat (falling number). The independent variables evaluated were: 1) cultivars representing three technological quality categories (experimental line, bread and improver), gluten extraction method including 2) water added to the flour for dough formation (4.0, 4.4 and 4.8 mL), 3), mixing time (20 and 30 s), and 4) washing time (3, 4 and 5 min). The experiment was designed in randomized blocks (5 blocks), with factorial structure. The results were submitted to analysis of variance and means compared by protected LSD test ($p<0.05$). The best conditions for gluten extraction and analysis were obtained with 4.4 mL of water, 20 s of mixing and 3 min of washing with NaCl solution (2%). The elastic recovery of gluten showed a strong correlation (r) with standard methods used to classify wheat per its technological quality (dough strength r=0.9885 and dough stability r=0.9632), while no correlation was observed with falling number (r=0.0541). The latter suggests that the samples were sound (no sprout damage) and did not affect the viscoelastic properties of the wheat. Differences in elastic recovery separated the three sample types efficiently. Elastic recovery evaluation may be useful indicator in other application such as breeding programs, import/export scenarios, flour mills, and evaluation of consistent gluten quality in quality control.

Keywords: Rheology, technological properties, flour quality

4.2 Introduction

Defined by several characteristics, the term flour quality has different meanings depending on the designation of use or type of product. The term 'technological quality of wheat’ generally indicates the performance of a wheat cultivar and their suitability in the preparation of a product. Despite the differences between countries established wheat quality standards, some are common for satisfactorily classify wheat grain according to key quality attributes but depending on the point of the production chain (producer, elevator, miller, and final consumer), the parameters used are not the same and differ in importance (Pagani et al., 2014).

There are various methodologies to test quality for breeding programs and baking industry, including empirical and fundamental rheological tests (large and small deformations, respectively). However, the methods are often time consuming, some require a large amount of sample and training for execution (Chapman et al., 2012).

Around the world, wheat producer countries have developed their own wheat class system based on technological characteristics that can include subclasses with specific targeted end-use functional ability in mind, such as “bread wheat class” (Brazil), “noddle wheat class” (Australia), and “pastry wheat class” (India) (Carson; Edwards, 2009).

Inconsistency in the quality of wheat has long been a challenge in the milling and baking industry. Wheat breeders can improve the variation of the overall end-use quality of cultivars through evaluation and selection. Gluten protein is the major and most crucial component of dough associated directly with bread quality (Marchetti et al., 2012).

The dough elasticity in a bakery can be observed when the dough is stretched and then released; a dough is considered elastic if it can quickly return to its original shape. Gluten and dough are typically considered viscoelastic exhibiting properties of solid (cohesion and elasticity) and liquid (viscous or irrecoverable deformation) (Wieser, 2007; Kieffer, 2006).

A rapid axial compression instrument (Gluten CORE, Perten Instruments AB, Huddinge, Sweden) has been specifically designed to test gluten elastic recovery. As described by Chapman et al. (2012), the main advantages of this testing system are the size and instrument's sensitive load cell, and the speed at which a test can be conducted (1 minute). Rather than attached and stretched as in the extensograph test, in the axial compression system the sample only has to be placed into the testing device. The information provided by the method is closely related to the strain gluten undergoes during gas bubble expansion (Chapman et al., 2012).

The development of a time efficient method providing reliable results for the classification of wheat flour is needed. The Gluten CORE has been used with US hard and soft wheat types (Chapman et al. 2012). The instrument software allows the flexibility to adopt to the separation of other wheat types. Brazilian wheat (soft spring) has a viscous and sticky textured gluten and requires a specific method that could separate its gluten characteristics. The method included parameters used in the extraction of gluten and subsequent analysis in the Gluten CORE.

This work presents progress toward the development of a more rapid gluten strength test and provides further insight into the behavior of gluten under compression and recovery after deformation. The objectives of this study were: 1) to determine the best conditions for the extraction of gluten from different Brazilian wheat genotypes of three commercial classes (improver, bread and basic); 2) to determine the axial compression-recovery behavior of gluten (Gluten CORE); and 3) to correlate the results obtained from the compression and recovery test with the standard methods used in Brazil to classify wheat according to its technological quality.

There is a need of efficient evaluation of rheological characteristics of gluten from Brazilian wheat genotypes, which may help to identify wheat genotypes for specific uses. Moreover, the measurement of viscoelasticity properties may be useful in a number of important applications ranging from breeding programs, evaluation during wheat import/export, and flour mills quality control.

4.3 Material and Methods

4.3.1 Location

The three wheat genotypes (BRS Pardela, BRS Gaivota and WT 09021) were grown in experimental plots at EMBRAPA- CNPSO, District of Warta, Londrina, Paraná, Brazil, in 2013. The District of Warta (Londrina) is located 23º11' south latitude, 51º10' west longitude, with an altitude average of 605 m. The soil of the site is characterized as distroferric red latosol. According to the classification of Köppen, the climate is humid subtropical (Cfa); subtropical with average temperature in the coldest month below 18°C (mesothermic) and average temperature in the warmer month above 22°C, with hot summers, frosts infrequent and tendency of rainfall concentration in the summer months, but without defined dry season.

4.3.2 Wheat Samples

The wheat genotypes used in the experiment to determine the best conditions for gluten extraction for analysis by Gluten CORE were: BRS Pardela (cultivar), BRS Gaivota (cultivar), and WT 09021 (experimental line), and classified as improver, bread and basic, respectively, according to the commercial classification of Brazilian wheat (Bassoi et al., 2012; MAPA, 2010). After determine the best conditions for gluten extraction and analysis by Gluten CORE using 3 genotypes (BRS Pardela, BRS Gaivota and WT 09021), 71 samples of wheat flour from 17 genotypes grown in 7 different locations in Brazil (South and Southeast region) were used in a validation test.

For the flour extractions, wheat kernels were first conditioned to 14% moisture for approximately 16 hours, to facilitate extraction and increase yield. After conditioning, the samples were ground in a roller mill (Quadrumat Junior®, Brabender, Duisburg, Germany) according to AACC International Approved Method 26-10.02. The flour was obtained by mixing the break and reduction flours, which was packed in polyethylene bags and stored at -4 ºC until further analysis.

4.3.3 Protein characterization of samples

Protein content in flour was determined by NIR spectroscopy (MOD XDS-Rapid Content Analyzer, FOSS NIRSystems®, Hoganas, Sweden), with double detection system (model 6500 monochromator with measuring range: silicon 400-1100 nm and lead sulphide 1100-2500 nm), and equipped with ISIScanTM software (Infrasoft International LLC, State College, PA, U.S.).

Technological analysis was performed following AACCI Approved Methods for alveograph (54-30.02), farinograph (54-21.02) and falling number (56-81.03). Alveograph and farinograph determined large deformation rheological properties and falling number the soundness of wheat via sprout damage.

For determination of HMW-GS composition 100 mg flour was suspended in 1 ml 0.3 M sodium iodide containing 7.5 % 1-propanol, vortexed for 15 min, centrifuge for at 12,000 x g for 5 min and the supernatant was discarded. The pellet was washed with water for 5 min and centrifuged for 5 min at 12000 x g. Glutenins in the pellet were extracted in 1 mL of 50 % 1-propanol containing 2 % (w/v) sodium dodecyl sulfate (SDS) and 2 % (v/v) 2-mercaptoethanol (BME) by vortex shaking for 30 minutes. Following centrifugation for 5 minutes at 12000 x g, the supernatant was collected and HMW-GS were identified by Lab-on-a-Chip electrophoresis method using an Agilent 2100 Bioanalyzer following the Protein 230 chip kit protocol (Agilent Technologies, Palo Alto, CA, U.S.). Wheat samples of known subunit composition were selected as controls to compare the apparent molecular sizes to facilitate the identification and comparison of HMW-GS. The standards used were cv. Chinese Spring (null, 7+8, 2+12), cv. Karl-92 (1, 7+8, 5+10), and cv. Jagger (1, 17+18, 5+10). Samples with high levels of subunit Glu-B1 7 were confirmed at the genetic level using polymerase chain reaction (PCR). DNA was extracted from flour using the DNeasy Mericon Food Kit (Qiagen, Germantown, MD, U.S.) and PCR was performed using primers and conditions described by Butow et al. (2003). Following amplification, products were analyzed using the Agilent 2100 Bioanalyzer and DNA 1000 kit. Using the Canadian cultivar Glenlea as a positive control samples were assessed for the presence of a 43 bp insertion associated with overexpression. The quality score for glutenin subunits were calculated according to Payne et al. (1997). Varieties with a Glu-1 quality score of between 8 and 10 had three points subtracted, those between 5 and 7 had two points subtracted, and those between 3 and 4 had one point removed, as described by the authors (Appendix I).

The percentage of insoluble polymeric proteins (IPP) was determined as described by Bean et al. (1998). A 100 mg flour sample was suspended in 1 ml of 50% 1-propanol, vortex stirred for 5 min and centrifuged for 5 min at 12,000 x g. The supernatant was discarded and the procedure was repeated twice. The pellet was lyophilized and protein content determined by Nitrogen combustion (LECO analysis). Insoluble polymeric protein percentage (%IPP) is calculated by multiplying nitrogen values by a conversion factor of 5.7 and dividing by total flour protein.

Flour protein compositions were determined quantitatively using the SE-HPLC extraction procedure described by Gupta et al (1993). Briefly, a 20-mg sample was extracted in 1 ml 0.05M Sodium phosphate buffer, pH 6.9, containing 0.5% SDS (w/v) and sonicated for 15 sec at 10 W. Following centrifugation for 5 min at 12,000 x g the supernatant was filtered using a 0.45 µm Spin-X nylon centrifuge tube filter and analyzed using an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA, U.S.). Protein extracts (20 µL) were analyzed using a Phenomenex Biosep-SEC-s4000 column (300 × 7.8 mm, particle size 5 µm, pore size 500 Å) (Phenomenex, Torrance, CA, U.S.). Solvent was 50% acetonitrile in water v/v, containing 0.05% trifluoroacetic acid. The column temperature was 40 °C with a flow rate of 1.0 mL/min with a total runtime of 28 min. A variable wavelength detector set to 214 nm was used and analysis was performed using the Agilent ChemStation software program. The chromatograms were manually integrated into two main peaks. The area of the first peak corresponds to polymeric proteins and the area of the second peak to monomeric proteins. The ratio (TPP/TMP) of total polymeric protein (TPP) to total monomeric protein (TMP) were recorded and calculated as described by Larroque et al. (1997) and Gupta et al. (1993).

Gliadin patterns and HMW/LMW glutenin ratios were determined by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). Briefly, 100 mg of flour was stirred with 1 mL sodium iodate buffer (0.3 M sodium iodate + 7.5% isopropanol) (Fu; Kovacs, 1999) for 15 min and centrifuged for 5 minutes at 17,000 x g. The supernatant was filtered through 0.45 µm filter and analyzed as described by Waga et al. (2013). A Jupiter C18 column (250 × 4.6 mm, Phenomenex, Torrance, California, U.S.) was used. Elution solvents A and B were water containing 0.1% trifluoroacetic (v/v) and acetonitrile containing 0.05% trifluoroacetic (v/v), respectively. The elution linear gradient was 25% B to 50% B for 80 minutes, flow rate 1ml/ minute, column temperature of 70℃, detection at 210 nm and manual integration of peaks using Agilent ChemStation software. Gliadin peaks were classified into four groups with retention times ranged between: 1) 20 - 26 min: ω-gliadins; 2) 26 - 31 min: β-gliadins; 3) 31 - 40 min: α-gliadins; and 4) 40 - 60 min: γ-gliadins.

4.3.4 Gluten preparation and analysis

For gluten extraction, soluble components of flour were washed with 2% NaCl solution (w/v), one sample at a time. The extraction was performed using a Glutomatic System (Model 2200, Perten Instruments, Huddinge, Stockholm, Sweden), following AACCI Approved Method 38-12.02, with modifications. The settings used for the extraction varied according to the experimental design. In the Glutomatic chamber, 10 g of flour sample were placed and 2% NaCl solution was added (4.0, 4.4 or 4.8 mL; w/v) using two pre-mixing times (20 or 30 seconds) to improve the wetting of sample prior to the main mixing period. Then, salt soluble particles were washed with 2% salt solution through a polyester screen (88 µm) for 3, 4, or 5 minutes.

Once the gluten was extracted the next steps were performed as described by Chapman et al. (2012). The gluten was centrifuged for 1 min, using a dummy gluten sample to balance the centrifuge, in order to reduce its moisture and shape the sample into a cylindrical form. The gluten samples were compressed for 5 s with a peak force of 3 N and allowed to recover over 55 s period. The Gluten CORE analyzer recorded the compression distance (height) as a factor of time (Fig. 1). At least five replicates for each cultivar were obtained from independently washed flour samples. The tests were performed applying a light layer of mineral oil to the plate since the gluten stick to them. After completing the compression-recovery test, the gluten sample was dried to estimate the wet and dry gluten content as described by AACCI Approved Method 38-12.02.

4.3.5 Experimental design

In this study the key variables that affect the method for gluten extraction were tested. Independent variables evaluated were: 1) Commercial classification of Brazilian wheat genotypes (improver, bread or basic), 2) Initial water added for extraction (4.0, 4.4 or 4.8 mL), 3) Mixing time of the dough (20 or 30 s) and 4) Washing time of the dough (3, 4, or 5 min). The experiment was designed in randomized blocks (5 blocks), in a factorial structure of 3(genotypes) x 3(water) x 2(mix) x 3(wash) = 54 treatments.

4.3.6 Statistical analysis

On SAS, the data obtained were subjected to analysis of variance (ANOVA) to assess the effect of initial water added, mixing and washing time of the dough, on the extraction of gluten from wheat genotypes from different commercial classes. Mean comparison was performed by protected least significant difference test (LSD) and Pearson’s correlation was performed between the proposed compression and recovery test and the standard methods used in Brazil to classify wheat according to its technological quality (SAS Institute, Cary, North Carolina, U.S.).

4.4 Results and Discussion

4.4.1 Physicochemical and rheological characterization

Traditional rheological properties of three selected Brazilian wheat genotypes describe their distinct characteristics used for selecting them as representatives of the three wheat classes used in Brazil (Table 1).

The protein content of the samples varied from 13.9 to 14.7%, with an average of 14.3% (on dry basis). Therefore, the quality differences among the genotypes studied most likely were due to their genetic background which differences in "protein quality" rather than to the protein content of flours. As described by Williams et al. (1986), the protein content in the flour may be used to classify its quality as very low (≤9.0%), low (9.1-11.5%), medium (11.6‑13.5%), high (13.6-15.5%), very high (15.6-17.5%), and extra high (≥17.6%).

In addition to quantity, the quality of proteins present in the grain is also important for the quality of the wheat genotype. Quantitative characteristics are expressed by a large number of genes, which can be strongly influenced by the environment. Protein content may significantly vary depending on the environmental conditions (climate and soil) in which the grain is cultivated, as well as on genetic diversity (Panozzo; Eagles, 2000).

As in other countries, a normative instruction from the Ministry of Agriculture, Livestock and Food Supply in Brazil, is used as a reference to classify wheat cultivars according to dough strength (alveograph), stability (farinograph) and falling number, into five classes: improver, bread, domestic, basic and other uses (MAPA, 2010).

For example, the wheat to be included in the improver class must meet the minimum values established for dough strength (≥300 W), stability (≥14 min) and falling number (≥250 sec), according to the normative (MAPA, 2010). As observed, the cultivars presented characteristics compatible with the classes to which they belong, even though the cultivar BRS Pardela (Improver) presented dough stability lower than the requirement (8 min<14 min). However, the cultivar classification is done after a series of field trials, being as criterion of classification of cultivars the minimum accumulated relative frequency of 60%.

Because of the influence of several factors, biotic and abiotic, a new quantity of samples has been proposed for commercial classification of wheat cultivars in Rio Grande do Sul and Paraná State, the main wheat-producing states in Brazil, since gluten-strength values have varied in magnitude, which makes it difficult to use the average as a criterion of classification (Castro et al., 2016).

4.4.2 Protein profile

The differences among cultivars evidenced by rheological behaviors presented in Table 1 can be related to their different protein profiles, particularly the different proportions of gliadins and glutenins, as shown in Table 2. HMW glutenin subunit compositions, glutenin/gliadin ratio, and gliadin composition of the wheat genotypes used for this study are shown in Table 2. The samples used in the experiment were analyzed by Lab-on-a-Chip method. Cultivar BRS Gaivota (Bread Class) presented the Bx7 subunit (7^{OE}+8) and rye translocation 1BL/1R, identified by analyzing the products from PCR in Lab-on-a-Chip again, using specific markers and primers, as shown in Figure 2.

As known, the variations in both quantity and quality of glutenin strongly determine the variations in bread making performance. Studies have shown that dough rheological properties are related to the amount and type of HMW glutenin subunit (Veraverbeke; Delcour, 2002; He et al., 2005; Barak et al., 2013; Johansson et al., 2013).

As observed in Table 2, two of the cultivars (basic and bread classes) presented glutenin subunits 2+12, and 5+10 in the improver class. Torres et al. (2008), analyzing the HMW-GS profile of 83 wheat genotypes from the Brazilian breeding program observed similar composition with the following proportions: Glu-A1 = 2* (74.7%) > 1 (16.9%) > N (8.4%); Glu-B1 = 7+9 (47.0%) > 7+8 (26.5%) > 17+18 (16.9%) > 13+16 (8.4%) > 7 (1.2%); Glu-D1 = 5+10 (55.4%) > 2+12 (44.6%). Furthermore, Costa et al. (2013), evaluating 16 Brazilian wheat cultivars observed that 81.3 and 18.7% of the samples presented the subunit 5+10 and 2+12, respectively, in the allele Glu-D1.

Although two of the three cultivars (basic and bread) used in this study presented similar protein content (Table 1) and allelic composition in Glu-D1 (2+12), a variation was observed in overall dough strength (Table 1). Several HMW-GS are found to be associated with bread making quality, and its combination affects the quality of the flour. As observed by Barak et al. (2013), subunit 2* and 5+10 subunits, the same as improver class cultivar, contributed significantly to improve bread quality of wheat. He et al. (2005) studying the effects of the subunits of each locus on gluten quality for breadmaking also observed that the combinations 1, 7+8, 5+10 contribute to an improved quality of wheat genotypes. Further it can be concluded that subunit 5+10 is more closely associated with better bread making quality whereas 2+12 with poor bread making properties.

In wheat, HMW-GS proteins genes are located at the orthologous loci Glu-A1, Glu-B1 and Glu-D1. Through genetic, breeding and transgenic studies, it has been found that the overexpression of certain subunits (i.e., 1Dx5, 1Ax1, 1Bx7OE) can be correlated with superior bread-making quality (Dong et al., 2010).

Although the bread class cultivar presented subunits 2+12, similar to the basic class, it also presented the overexpression of allele 7 ($Bx7^{OE}$), as can be seen in Figure 2. Specific selections of these subunits in conventional and molecular breeding programs have contributed to the genetic improvement of breadmaking quality in worldwide wheat production. When Glu-B1al is present, the Bx7 subunit is overexpressed (7^{OE}+8) as a percentage of the total amount of HMW glutenin present, and this overexpression is associated with greater dough strength (Eagles et al., 2002). However, in the same cultivar (bread class) it was detected the wheat-rye translocation (1BL/1RS), known to have detrimental effects for producing a sticky dough, with inferior dough-mixing properties, and low SDS sedimentation volumes (Carver; Rayburn, 1995). These undesirable dough properties are considered to be partially caused by the presence of ω-secalins, which are a family of small monomeric proteins (Hussain; Lukow, 1994). Li et al. (2016) observed that the presence of 1RS arm increased protein, wet gluten and dry gluten content and water absorption, but it decreased gluten index and stability time of the dough, trending to a greater dough stickiness. Costa et al. (2013), observed that from the 16 Brazilian wheat cultivars evaluated 4 genotypes (25%) presented rye translocation.

The Glu-1 quality score presented in Table 2, can partially help to explain the observations made for the technological properties of the cultivars used in this study. However, the calculation proposed by Payne et al. (1987) does not consider the overexpression of proteins. Thus, it is possible to observed the superiority of cultivar improver over basic class, but the cultivar bread presented the lowest score due to reduction in the score (-3 point) due to the presence of rye translocation and not consideration of the overexpression of allele 7 ($Bx7^{OE}$). The Glu-1 quality score is an important approach to estimate the quality of a genotype based on its allelic composition, however, for our study using this specific set of samples, it does not match the technological quality observed.

Apart from the amount of protein in flour, probably the most important characteristic of gluten that determines the mixing time of dough is the size distribution of the gluten proteins. This consideration involves the ratio of the monomeric-to-polymeric proteins, and specially the size distribution of the polymeric proteins. The ratio polymeric to monomeric proteins, i.e. glutenin to gliadin ratio, observed in the samples indicated a higher concentration of monomeric proteins. As known, monomeric gliadins is mostly responsible for the cohesiveness and extensibility characters in the system, whereas polymeric glutenin are the elasticity and strength of gluten. Thus, the ratio of glutenins to gliadins controls the dough strength and extensibility (Khatkar et al., 2002; Wrigley; Békés; Bushuk, 2006).

Meanwhile, the composition of both the glutenin and gliadin proteins must be taken into account because, for example, at the same glutenin-to-gliadin ratio, the balance of HMW-to-LMW glutenin subunits in the polymeric fraction can significantly alter dough strength and extensibility (Wrigley; Békés; Bushuk, 2006)

Several researchers use glutenin to gliadin ratio to predict breadmaking quality and found it to be accurate and reliable. It is desirable to have a stable ratio of glutenin to gliadin for the purpose of good breadmaking quality. However, glutenin to gliadin ratio is affected by environmental factors such as heat stress, soil fertility (Zhu; Khan, 2001).

More than 45% of the gliadin presented in the flours was from the class γ-gliadin, presenting the following concentration order γ-gli (average – 47.7%) > α/β-gli (average – 34.6%) > ω-gli (average – 17.6%), as shown in Table 2. These observations are in agreement with previous reports finding that α/β- and γ-gliadins are major components, with ω-gliadins occurring in much lower proportions (Wieser; Kieffer, 2001).

According to Khatkar et al. (2002), the addition of gliadin and its subgroups to flour changes the mixograph properties increasing the resistance breakdown of the dough (RBD). Within gliadin subgroups, RBD values increased in the sequence ω- < γ- < α- < β-gliadins. They also pointed out that addition of gliadin subgroups (α-, β-, and γ -gliadins) increased the peak dough resistance of the base flour to a greater extent than gluten and ω-gliadin addition.

4.4.3 Gluten extraction

Even with the standardized method as published by Chapman et al. (2012), it was necessary to adapt the existing method for the characteristics found in Brazilian wheat cultivars. Compared to American wheats, gluten from Brazilian wheat has a higher concentration of gliadin, which makes the wheat soft, presenting a sticky gluten behavior (Table 2). Thereby, the method for gluten extraction needed to be improved, since the Brazilian samples became sticky and clogged the glutomatic sieves, preventing the passage of water and consequently the washing of the sample for gluten extraction.

The best method/settings from the experiment performed in order to obtain the best conditions for gluten extraction are shown in Table 3. Among the 54 treatments (3 (genotypes) x 3 (water) x 2 (mix) x 3 (wash)), results were analyzed for selecting those in which the Gluten CORE equipment could significantly differentiate the genotypes (improver> bread> basic), where high elastic recovery was observed for the improver and the low for basic class wheat genotype (Table 3). Seven treatments showed satisfactory results, however, due to limited amount of sample available for validation tests, treatment two was selected to continue with the experiments for presenting the lowest CV% for all the three genotypes (Improver class – CV =5.8%; Bread class - CV= 8.8%; Experimental line – CV=7.0%).

During the execution of the experiment it was observed that: 1) the addition of higher amount of water (4.8 mL) made difficult the gluten formation, as well as lower amount of water (4.0 mL); 2) time of mixing (water+flour) greater than 30 seconds resulted in a sticky dough which adhered to the sieve of the chamber, preventing the passage of the salt solution to wash the sample; 3) washing time greater than 3 minutes de-structuring the gluten, resulting in a gluten sticky and difficult to wash.

The amount of water is essential for hydration and gluten development. Glutenin and gliadin absorb about twice their weight in water. Less water results in less gluten development by reducing protein mobility, but too much water reduces gluten development by diluting the proteins and affecting their interaction. Water absorption is important for changing flow regimes in batters or flour suspensions, affecting the final product quality (Barrera et al., 2013).

The wet gluten for use in Gluten CORE test can be obtained easily and quickly, directly from flour, using the glutomatic system (3:20 minutes). Figure 1 shows one compression and recovery curve or observation for a gluten extracted from each cultivar evaluated (improver=BRS Pardela; Bread=BRS Gaivota; Basic=WT 09021) using treatment two (4.4 mL of water, 20 s of mixing and 3 min of washing). After the 5 s hold period with a compression force of 3 N, each gluten sample showed difference of elastic recovery. Elastic recovery is defined as the ratio of the height at final recovery over initial height before compression, times 100 to convert to percentage. As presented in Table 3, the degree of the recovery ranged from a low of 3.1% for the experimental line (WT 09210) to 36.8% for improver cultivar (BRS Pardela).

The cultivar containing the 5+10 subunit presented the highest recovery capacity (Improver>Bread>Basic), which was also observed by several authors (Hernandez-Estrada et. al., 2017; Hernandez-Estrada et. al., 2014; Barak et al., 2013; Hernandez-Estrada et. al., 2017; Zhao et al., 2010; He et al., 2005). The basic and bread wheat classes presented the same allelic in Glu-D1 (2+12), however they have different alleles of Glu-B1 (17+18 and 7^{OE}+8, respectively). The overexpression of subunit 7 (7^{OE}) is known for improve the rheological characteristics of the cultivar, which explains the better elastic recovery of bread class over basic (Dong et al., 2010; Eagles et al., 2002). However, the bread class also present the wheat-rye translocation (1BL/1RS), which is detrimental to the strength of the gluten and may have ended up balancing with the overexpression of the protein.

Our results are in agreement with previous research, gluten samples presented HMW-GS Glu-1 1 and 2* has shown similar viscoelasticity behavior (Hernandez-Estrada et. al., 2017; Hernandez-Estrada et. al., 2014). According to the authors' observations, loaf volume was higher with Glu-B1 17+18 than 7+8 and Glu-D1 5+10 than 2+12. In the Glu-D1 locus, good quality is specially associated with the 5+10 pair of subunits compared with the poor quality allelic subunits 2+12. The subunits 5+10 also presented lower wet gluten content but higher dough stability to mixing when compared to 2+12, in agreement with the observations in the present study (Table 1 and Table 3).

The amount of wet gluten extracted from the samples was lower in the cultivar BRS Pardela (Improver Class), as well as the dry gluten content obtained, as presented in Table 3. That can be due to the lower protein content compared to the other two cultivars, which results in a reduction in water absorption (Table 1).

4.4.4 Compression and recovery test (%) vs rheological standard methods

The results of Gluten CORE (Table 4), gluten extracted by treatment 2 (initial water = 4.4 mL, mixing time = 20 sec and washing time = 3 min) showed a strong correlation with official methods used in Brazil to classify wheat flour according to their technological quality, except for falling number test (r=0.0541).

The rheological properties of dough are critical in food manufacturing. The results of dough rheological characteristics are depicted in Table 1. The good correlation between dough strength and degree of recovery measured with the Gluten CORE analyzer is understandable, since dough strength is directly related to the degree of elasticity measured by the texture analysis, i.e., more elastic gluten would have a greater snap back, or degree of recovery during a axial compression and relaxation test (Chapman et al., 2012).

Observing a satisfactory correlation within the proposed method (Gluten CORE) and the official methodologies used for commercial classification of wheat, 71 wheat samples, 18 cultivars grown in 5 locations in Brazil were used for an initial validation of the method. A Pearson (r) correlation of 0.64 was observed between Gluten CORE and dough strength of the samples (data not shown), due to a great variation among the samples evaluated. The extraction and quality of the gluten depends on a number of factors such as mixing, which directly affects the interaction between the molecules and consequently formation of gluten. Therefore, more studies are required using a larger number of samples with different rheological characteristics.

4.5 Conclusion

The best conditions for gluten extraction and analysis of the particular set of samples (WT 090210, BRS Gaivota, BRS Pardela) were obtained by adding 4.4 mL of water for dough formation, 20 seconds of mixing and 3 minutes of washing with NaCl solution (2%). These conditions showed a strong correlation (r) with standard methods used to classify wheat according to its technological quality (dough strength =0.9885 and dough stability=0.9632), while no correlation was observed with falling number (r=0.0541).

The preliminary test of validation using 71 samples, with different rheological characteristics and coming from different regions of Brazil (south and southeast), presented satisfactory results when correlated (r) with dough strength (r = 0.64). However, more studies are necessary using a larger number of samples with different characteristics for a better understanding of class separation in a satisfactory and efficient way.

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Table 1. Protein and traditional rheological properties of Brazilian wheat genotypes representative of basic, bread and improver wheat class.

		WT 09021 (Basic Class)	BRS Gaivota (Bread Class)	BRS Pardela (Improver Class)
	Protein content (%)*	14.2±0.1b	14.7±0.2a	13.9±0.1b
Alveograph	**Dough strength (W; 10^{-4}J)**	154.0±0.0c	257.0±2.0b	332.0±0.0a
	Tenacity (P, mm)	54.0±1.0c	67.0±0.0b	84.0±1.0a
	Extensibility (L; mm)	180.0±1.0a	150.0±0.0b	167.0±1.0c
	Elasticity index (%)	34.9±0.4b	50.1±0.9a	50.5±0.2a
Farinograph	**Peak time (min)**	4.1±0.1b	6.7±0.9a	6.4±0.1a
	Stability (min)	2.70±0.1b	5.2±0.9b	8.0±0.7a
	Mixing tolerance index (UF)	71.0±9.0a	42.0±11.0ab	23.0±2.0b
	Falling number (sec)	431.0±8.0b	566.0±3.0a	399.0±1.0c

*Dry basis; Means ± standard deviations within a row followed by the same letter are not significantly different ($p>0.05$).

Table 2. Protein characterization of three Brazilian genotypes analyzed for high molecular weight glutenin subunits composition of the locus *Glu-A1*, *Glu-B1* and *Glu-D1*, gliadin type composition content, total polymeric and monomeric protein ratio, insoluble polymeric protein and ratio of low vs. high molecular weight glutenin subunits.

		WT 09021 (Basic Class)	**BRS Gaivota** (Bread Class)	**BRS Pardela** (Improver Class)
Glutenin Subunits	**Glu-A1**	1	2*	2*
	Glu-B1	17+18	7^{OE}+8	17+18
	Glu-D1	2+12	2+12	5+10
	Glu-1 Quality Score	8	5	10
	TPP / TMP	0.84±0.0a	0.66±0.0c	0.78±0.0b
	IPP (%)	39.6±1.9b	43.3±1.3ab	45.9±3.3a
	LMW-GS / HMW-GS	2.6±0.0a	1.6±0.1c	2.1±0.0b
Gliadin classes (%)	**ω-gli**	16.6±0.3b	19.2±0.3a	17.0±0.4b
	α/β-gli	35.3±0.2a	34.9±0.4a	33.7±0.1b
	γ-gli	48.2±0.5a	45.8±0.1b	49.3±0.6a

Means ± standard deviations within a row followed by the same letter are not significantly different (p>0.05).

Table 3. Treatment variables used in the analysis of gluten elastic recovery (%), wet and dry gluten, and coefficient of variation (%) for elastic recovery.

Water (mL)	**Mixing (s)**	**Washing (min)**	**Genotype**	**Elastic recovery (%)**	**Gluten**		**CV%**
					Wet (14%)	**Total Dry (%)**	
4.0	20	5	BRS Pardela	22.1±1.5a	34.3±0.4c	11.0±0.0b	14.9
			BRS Gaivota	15.8±0.4b	40.9±0.1b	12.9±0.1a	6.0
			WT 09021	4.7±1.0c	42.1±0.4a	13.2±0.1a	49.0
4.4	20	3	BRS Pardela	36.8±1.0a	35.0±0.2c	11.5±0.1b	5.8
			BRS Gaivota	27.5±1.1b	41.8±0.3b	13.6±0.1a	8.8
			WT 09021	4.6±0.1c	43.2±0.3a	13.7±0.1a	7.0
4.4	20	4	BRS Pardela	31.7±1.9a	34.2±0.4c	11.2±0.1b	13.3
			BRS Gaivota	24.3±1.3b	40.7±0.5b	13.1±0.1a	12.1
			WT 09021	5.3±0.9c	42.1±0.4a	13.3±0.3a	36.8
4.4	20	5	BRS Pardela	23.8±1.4a	34.2±0.4b	11.0±0.1b	13.6
			BRS Gaivota	12.3±0.8b	40.4±0.6a	13.0±0.2a	15.4
			WT 09021	3.1±0.2c	40.7±0.5b	12.5±0.2a	12.4
4.8	20	3	BRS Pardela	34.4±1.6a	35.1±0.3c	11.5±0.1b	10.4
			BRS Gaivota	24.3±1.1b	41.4±0.2b	13.4±0.1a	9.8
			WT 09021	4.8±0.5c	43.0±0.7a	13.5±0.2a	20.8
4.8	20	4	BRS Pardela	30.7±1.2a	33.9±0.2b	11.1±0.1c	9.1
			BRS Gaivota	15.9±0.8b	40.8±0.1a	13.1±0.1a	11.3
			WT 09021	3.8±0.4c	40.9±0.4a	12.7±0.1b	25.8
4.8	20	5	BRS Pardela	22.6±1.0a	33.5±0.4b	11.0±0.2b	10.2
			BRS Gaivota	13.1±0.6b	39.9±0.6a	12.7±0.1a	10.2
			WT 09021	3.1±0.5c	40.4±0.6a	12.3±0.2a	46.2

* Means (n=25 ± standard error) within a column, within the same settings, followed by the same letter are not significantly different ($p>0.05$). CV%, coefficient of variation for the Elastic Recovery analysis.

Table 4. Pearson correlation (r) between elastic recovery (Gluten CORE, treatment 2) and the standard rheological properties used in Brazil for commercial classification of wheat.

Method		**Pearson Correlation (r)**
Alveograph	**Dough strength (W; 10^{-4}J)**	0.9885
	Tenacity (P, mm)	0.9562
	Extensibility (L; mm)	-0.6482
Farinograph	**Peak time (min)**	0.9241
	Stability (min)	0.9632
	Mixing tolerance index (UF)	-0.9914
	Falling number (sec)	0.0541

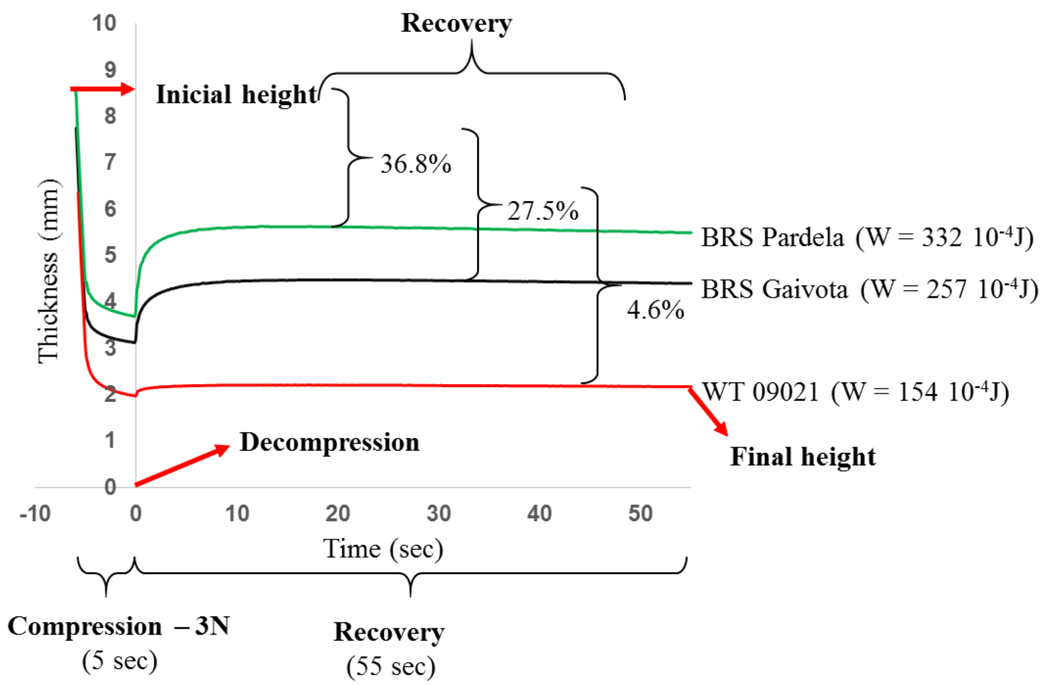

Figure 1. Recovery curves of gluten (extracted with treatment 2 = 4.4 mL of salt solution 2%, 20 seconds of mixing and 3 minutes of washing) from different commercial classes (improver, bread and basic) obtained after a compression and recovery test with Gluten CORE.

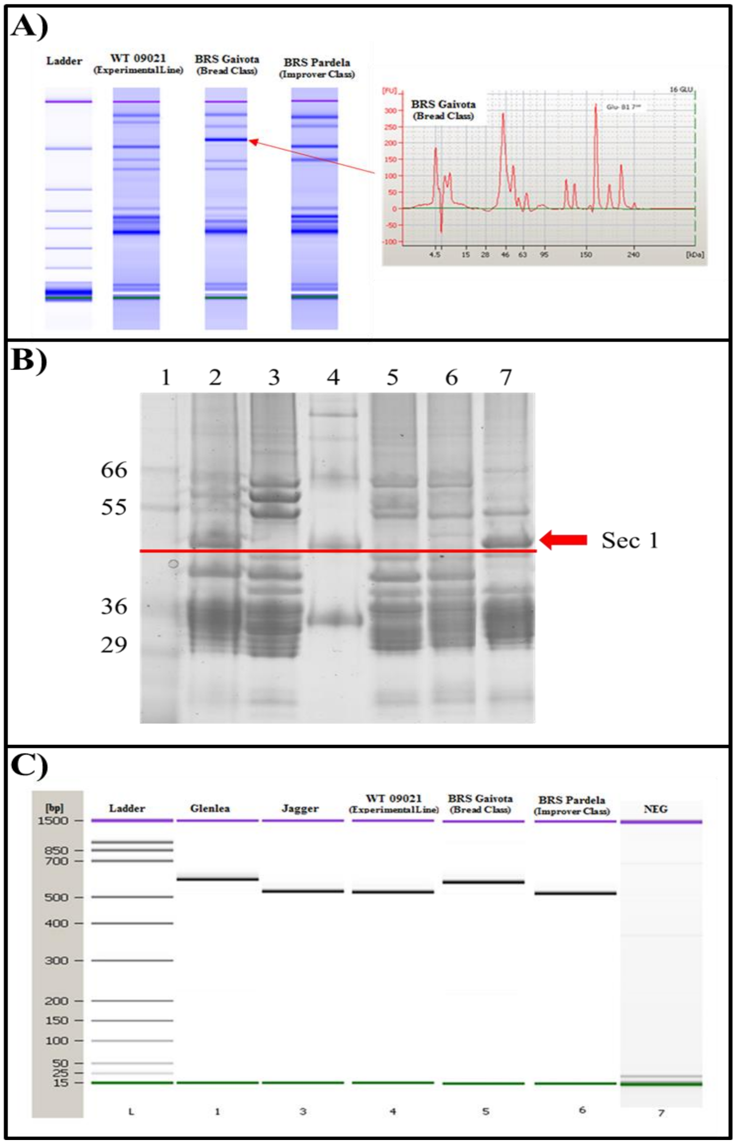

Figure 2. A) Identification of high molecular weight glutenin subunits (HMW–GS); B) SDS-PAGE analysis for Bx7 subunit overexpression (7^{OE}+8): 1) Molecular weight markers, 2) Tam 107 (positive control), 3) Chinese Spring (negative control), 4) Rye flour (positive control), 5) WT 09021, 6) BRS Pardela, 7) BRS Gaivota; C) Lab on a Chip separation of PCR products using primer set for overexpression of subunit Bx7 (7^{OE}+8), with Glenlea as positive and Jagger cultivar as negative control.

CHAPTER V

MISFORMULATION OF DRY CAKE MIXES

ON PASTING PROPERTIES AND QUALITY OF FINAL PRODUCT

5.1 Abstract

Common mistakes during production of bake mixes such as omission or double addition of functional ingredients can result in significant economic losses and affect the quality of final products. The objective of this study was to evaluate the effect of functional ingredients on pasting properties (RVA) of cake mixes and baking performance, texture and sensory characteristics of the final product using a randomized complete block with 3 blocks. Three ingredients (nonfat dry milk-NFDM, salt, and baking powder) were evaluated in 3 different concentrations (omission, normal dose, double dose). Analysis of variance and mean separation by protected LSD test (<0.05) were conducted. When compared to control formulation: 1) NFDM - Double addition reduced the peak (-8.1%) and final viscosity (-6.1%) of the paste. Cakes with lower weight (-1.1%) were obtained omitting the ingredient, due to reduction in height (-5.3%) and volume (-7.7%). Lighter crust and darker crumb colors were observed with omission or double dose addition, respectively. Omission affected texture after 4 days of storage reducing resilience (-13.4%) and cohesion (-11.6%), and reducing springiness after 6 days (-2.6%). 2) Salt - Omission of the ingredient reduced peak viscosity (-13.8%), and double dose affected height (-7.2%) and increased off flavors perception. Omission reduced resilience (-13.6%) and cohesion (-12.1%), and double dose reduced the springiness of cakes after day 6 (-3.2%). 3) Baking powder - Omission reduced peak viscosity of the paste (-29.8%), and height (-36.9%) and volume (-32.4%) of the cakes. Double dose also reduced cake volume (-16.0%). Omission or double dose reduced moistness (-11.7 and 16.3%) and softness perception (-29.2 and -12.5 %), respectively. In addition, darker crust and less uniform cakes were observed with double dose. Omission of the ingredient affected the texture increasing hardness (+51.0%) and chewiness (+42.2%) at day 4 of storage. The misformulation scenarios studied serve as a tool to relate information of dry mixes quality control with batter viscosity and final product quality.

Keywords: Functional ingredient, nonfat dry milk, salt, baking powder, baking quality

5.2 Introduction

Food products that do not require practices for processing, and are easy to prepare have attracted the attention of consumers. An example of this type of product is the pre-mixes formulations for cake mix which allows the cake to be made easily and quickly.

According to a survey, three types of cakes from 3 brand owned more than 60% (311.34 million consumers) of the market share of dry cakes mixes in the U.S. in 2016 (Statista, 2017).

The consumer of this type of product has the expectation, when following the instructions, to obtain a uniform and quality product. This fact does not always occur when the cake is prepared using a homemade recipe, in which problems can occur in the various stages of processing. These problems are due in general to the lack of experience and practice of the modern consumer in the preparation of cakes, in addition to the non-uniformity of the raw material. One of the advantages of using cake mixes is the guarantee of product standardization, since the responsibility for the quality control of the product is transferred from the consumer to the industry.

During baking, the combined effect of starch swelling and protein denaturation in presence of other ingredients transformed the liquid batter into a solid foam. These events first tremendously increase the viscosity of the batter and finally provide structure of the matrix (Hesso et al., 2015).

Cakes are defined as being aerated, chemically leavened bakery products, containing many ingredients, such as wheat flour, sugar, egg, fat, leavening agents, salt, nonfat dry milk solids and water. The main parameters of cake quality are volume, firmness, color, and weight loss. The cake quality is influenced by several factors like quality and level of ingredients (Kahraman et al., 2008).

The dairy-based powders are not only used for recombination or reconstitution, but they can be exploited for their intrinsic functional properties for application as a food ingredient in several “value-added foods” such as confectionery, bakery, and meat products (Sharma et al., 2012). The incorporation of dairy ingredients is long established in the baking industry. Dairy proteins are highly functional ingredients, and can be readily incorporated into many food products. They may be used in baking for both nutritional and functional benefits including flavor and texture enhancement, and storage improvement (Gallagher et al., 2003b).

Salt (sodium chloride) has a number of different functions in the manufacture of bakery products, some of which are product specific. The most immediately recognized one is to contribute to the flavor profile of the product. Salt has its own characteristics and is considered as one of the five basic tastes. In addition to its own distinctive flavor salt plays a significant role in enhancing other, often subtler, flavors (Belz et al., 2012). Salt slows down water imbibition and swelling of flour proteins; reduces dough extensibility and improves gas retention, bread crumb and slicing properties (Ngemakwe et al., 2014).

Chemical leaveners are used to give cookies, cakes, and other baked goods their characteristic textures. They produce gas from the reaction that takes place when a carbon dioxide source and an acid are mixed together and come into contact with water. The gas forms bubbles that are trapped in the batter or dough and then expand during baking to form the holes that are retained in the finished product (Pop, 2007). Double-acting baking powder contain a mixture of sodium bicarbonate, a fast-acting leavening acid like monocalcium phosphate monohydrate (MCP) and a slow-acting leavening acid like sodium acid pyrophosphate (SAPP). They react partially at low temperatures and partially at high temperatures to provide uniform leavening throughout processing (Miller, 2016). Sodium bicarbonate increases the pH, which leads to more intensive browning and alkaline taste (Hesso et al., 2015). Sodium pyrophosphate reacts with sodium bicarbonate, and when in excess can give a metallic taste (Miller, 2016).

Food quality is defined by a complex set of stimuli that starts with visual evaluation. Once the decision is made to consume food, the quality is based not only on the magnitude of flavor, taste and texture, but also takes into account temporal coordination of these stimuli. Historically, food ingredients have been viewed from three perspectives regarding how they function in foods: 1) formation of structures, 2) stabilization of structures and 3) contribution to sensory quality (Foegeding et al., 2010).

The purpose of this study was to evaluate the effect of functional ingredients on pasting properties (RVA) of cake mixes and baking performance, texture and sensory characteristics of the final product. Thus, we seek a better understanding of the effect that the misformulation of functional ingredients has on the quality of the final product.

5.3 Materials and Methods

5.3.1 Materials

The ingredients used in the experiment were purchased at local retail stores: Sugar (Imperial Sugar®, Imperial Sugar Company, Sugar Land, TX, U.S.), nonfat dry milk or NFDM (Great Value®, Wal-Mart, Bentonville, AR, U.S.), Salt (Special Purity®, North American Salt Company, Overland Park, KS, U.S.), fresh eggs (Large Grade A, Great

Value®, Wal-Mart, Bentonville, AR, U.S.), and shortening (Crisco®, The JM Smucker Co., Orrville, OH, U.S.).

5.3.2 Experimental design

The experimental design for this study was a randomized complete block (3 blocks) with seven treatments. The formulation of the treatments is detailed in Table 2. The treatments were randomized and baked accordingly during 3 days, considering each individual day a block.

5.3.3 Methods

The effect of nonfat dry milk, salt (sodium chloride) and baking powder (Table 1), added in 3 different concentrations (omission, normal and double dose) were evaluated in dry cake mixes formulated with all-purpose (Pillsbury best®, M Smucker Co, Orrville, OH, U.S.).

Particle size of salt and sugar was reduced using a coffee grinder (Model 80335, Hamilton Beach, NC, U.S.) for 1 min followed by sieving to pass through 80 mesh or 0.177 mm sieve (VWR Scientific, Westchester, PA, U.S.), using a RoTap (RoTap RX-29, WS Tyler, Mentor, OH, U.S.), to minimize differences due to particle size during analysis.

5.3.3.1 Rapid visco analyzer (RVA)

The pasting properties of the dry cake mixes was determined using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Kungens Kurva, Sweden), coupled to a water bath 25°C (Fisher Isotemp 1028P, Pittsburgh, PA, U.S.), following AACC International Approved Method 76-21.01, with modifications in the settings (Table 3). The samples had their weights adjusted to 14% moisture content, previously determined according to method 012/IV (105 ºC per 3 hours; IAL, 2005) in a convection oven (model 52000-55, Cole-Parmer Instrument Company, Chicago, IL, U.S.). Around five grams of dry mix were added to 25 mL of distilled water, instead of the 3.5 grams of flour or starch used in the standard method. The samples had their weights corrected to flour basis so all the treatment would contain the same amount of starch (2.5 g of flour; Appendix II). The analysis was done in duplicates.

5.3.3.2 Baking procedure

The dry cake mixes formulated were baked following the AACCI Approved Method 10-90.01 for testing baking quality of cake flour, with modifications in the formulation as described in Table 2. The amount of dry mix used was calculated on flour weight basis (170 g flour, Appendix II), as well as water (65%, tap water), fresh egg whites (25%) and shortening (15%). The dry mix was transferred to a mixing bowl and added of egg whites, shortening and 60% of water. The ingredients were mixed at speed 2 in a kitchen mixer (Model KSM90, KitchenAid Inc., St. Joseph, MO, U.S.) for 30 seconds. Dry ingredients and cake batter from the side walls of mixing bowl were scraped off with a rubber spatula and continue mixing at speed 4 for 2 min. The remaining water was added, mixed at speed 2 for 30 seconds, the walls scraped off, and mixed at speed 4 for 2 min. Round cake pans (10.16 x 5.08 cm/4 x 2 inches, Fat Daddio's, Spokane, WA, U.S.) were lightly greased with shortening and bottom lined with parchment paper. Three cakes of 150 g of batter were prepared from each batter batch and baked at 160°C (325°F) for 30 minutes in a rotating Rack Oven (Model OV300E, Baxter Manufacturing, Orting, WA, U.S.). Three batter batches were obtained for each treatment, one in each block (day), with 3 cakes from each batch (9 cakes total for each treatment). Another small batch for each treatment was prepared to have the pH measured (electrode 4M KCl saturated with AgCl, pH meter AR5, Fischer Scientific, Pittsburg, PA, U.S.).

5.3.3.3 Cake evaluation (performance)

After cool down for 30 minutes, the cakes were removed from the pans and allowed to continue cooling for approximately one hour. The cakes were analyzed for crust color using CIELAB scale with illuminant D65 and angle 10 ° (Model MiniScan XE Plus, Hunter Associates Laboratory, Inc., Reston, VA, U.S.). In this system, the chromaticity coordinate a* indicates the intensity of the red (+a*) or green (-a*), the chromaticity coordinate b* the yellow intensity (+b*) or blue (-b*) and L* the brightness of white (L*=100) to black (L *=0). From the values of L*, a* and b*, the angle chromatic hue (h°) and browning index (BI) were calculated, where h = 0° (pure red), h = 90° (pure yellow), h =180° (pure green) and h = 270° (pure blue), using the formula h°

$= \text{arctn} \times (\frac{\text{b}*}{\text{a}*})$ and BI=$\frac{100\ (x-0.31)}{0.172}$, where x=$\frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$ (Maskan, 2001). The cakes were also evaluated for weight (Model AR5120, OHAUS, Parsippany, NJ, U.S.), height (benchtop micrometer, Mitutoyo Corp., Kawasaki, Kanagawa, Japan), and volume by rapeseed displacement with a loaf volumeter (1000 cc, National MFG, Lincoln, NE, U.S.) following AACCI Approved Method 10-05.01. Moisture loss was calculated by different between weight before (150 g) and after baking. Cold cakes were stored in Ziploc bags until the next day. One day after baking, the cakes were sliced (25 mm, Mini-Supreme Bread Slicer Model 709, Oliver Products Company, Grand Rapids, MI, U.S.), color of the crumb was measured and the slices were placed back in Ziploc bags. For each treatment, two of the 3 cakes baked on each day (block) was used for texture analysis and one was used for sensory evaluation.

5.3.3.4 Sensory evaluation

Sensory analysis was carried out the next day after baking. A panel of 22 panelists received training for scoring cake internal structure using a nine-point hedonic scale, according to AACCI Approved Method 10-90.01 (Appendix III). The panel was formed by 14 female and 8 male participants with percentage age distribution of 59% younger than 25 years old, 32% 25-35 years old, 4.5% of both 36-45 and older than 45 years old. The samples were identified with three random digit numbers. In each evaluation session, seven samples cut into strips of approximately 3 cm long were presented to the judges. The sensory analysis was performed in 3 sessions (3 blocks) in different weeks so the judges would not be fatigued by the number of samples. The sensory evaluation was approved by Institutional Review Board (IRB) at Oklahoma State University (Appendix III).

5.3.3.5 Texture analysis

Texture analysis were performed in duplicate (2 slices per cake), measuring 3 points per slice, using a texture profile analysis (TPA) test and a TA-XT2i Texture Analyzer (Model Plus-Upgrade, Stable Micro Systems, Surrey, UK) equipped with Texture Expert software, at day one, three, five and seven after baking. Cake crumb texture was determined with a 25 mm diameter cylindrical acrylic probe, double compression test to penetrate to 25% of the height, test speed of 2 mm/s and 5 s delay between the first and second compressions. Hardness, adhesiveness, resilience, cohesion, springiness and chewiness were calculated from the TPA graphs. Two slices were selected randomly (25 mm thickness) from each cake (two cakes per treatment) in each day (1, 3, 5 and 7 days after baking).

5.3.3.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) to assess the effect of ingredient (NFDM, salt and baking powder) and level of misformulation (omission, normal and double), with means comparison by least significant difference test (protected LSD; $p<0.05$), using SAS (SAS Institute, Cary, NC, U.S.). Pearson's correlation was performed between the pasting properties and the parameters evaluated in the baked cakes (baking performance, sensory and texture), using SAS statistical software and STATISTICA 13.2 program for Windows (StatSoft Inc., Tulsa, OK, U.S.).

5.4 Results and Discussion

5.4.1 Pasting properties

Pasting properties of the batter from the dry cake mixes are reported in Table 4. The misformulation of nonfat dry milk (NFDM) in dry cake mix resulted in an inverse effect in the pasting properties (peak viscosity, trough and final viscosity) compared to the effect observed with salt and baking powder. The double addition of NFDM resulted in reduction (-8.1%) of the peak viscosity, while the double addition of salt and baking powder increased the peak viscosity by +10.0 and +86.6%, respectively (Table 4). Reduction of peak viscosity of the dry cake mix resulted by the omission of salt (-12.2%) and baking powder (-22.9%) (Table 4).

Similar effect for NFDM, salt and baking powder was observed in the trough variable, i.e., the lowest point the viscosity reaches when the samples start cooling to 50ºC after the heating process at 90ºC. The breakdown of pasting, which is the difference between peak viscosity and trough shows that the baking powder misformulation resulted in the largest differences in the sample viscosity during heat and cooling process, with a reduction of -17.1% and increase of +134.8%, when the ingredient was omitted or added twice to the formulation, in comparison to the normal dose (Table 4).

When heated in water, the starch granules absorb and bind more water as they swell, reducing the available water and causing physical interactions (collisions) between granules. It is these interactions that are observed as the phenomenon of "pasting", that is, the sudden increase in viscosity see during the heating phase of the test (Batey, 2007). The double addition of baking powder to the dry mix contribute to those interactions resulting in higher peak viscosity. These changes are collectively referred to as starch gelatinization and are accompanied by the loss of characteristic birefringence of intact granules (Wang et al., 2015). Once the paste reaches the maximum peak the decrease in viscosity happens due to granules breaking down with the sheer field of the instrument. The shear not only solubilizes but also shears the amylopectin molecules, which causes a large drop in molecular weight of amylopectin and leads to a subsequent viscosity drop (Batey, 2007). The increase in breakdown observed adding double dose of baking powder is related to the increase observed for maximum viscosity of the paste.

Final viscosity of the treatments presented similar trend as that observed for peak viscosity. The omission of NFDM resulted in an increase (+7.1%) in final viscosity, and a reduction (-6.1%) when the ingredient was added twice to the formulation. As shown in the graphs, a distinct separation of the doses (omission, normal or double dose) for salt and baking powder can be observed in the final viscosity of the samples (Table 4). For both ingredients, omission and double dose addition resulted in reduction and increase of the final viscosity, respectively.

Baking powder misformulation was the functional ingredient evaluated that resulted in major changes in the pasting properties of the paste, presenting a large instability of the viscosity when double dose of the ingredient was added (Table 4). The instability observed for peak viscosity when added double dose of the ingredient is related to the production of carbon dioxide. Although, the double addition increase the viscosity observed, when compared to control, the large amount of gas produced by the leavening agent increase the amount of air present in the paste, compromising the interactions between starch granules.

Misformulation of NFDM affected the viscosity of the cake mix differently than salt and baking powder. Wheat flour is the major component of the dry cake mixes (50.4%), containing between 10-12% of protein and more than 60% of starch, which is the major responsible for viscosity of the paste when heated. A dilution effect of the starch content in the mix can explain in part some of the results, once omission, normal and double dose presented 0%, 12% and 24% of NFDM calculated in flour basis, respectively. Therefore, omitting the ingredient from the mix would result in a higher concentration of starch (flour) when compared to normal and double dose of NFDM, resulting in higher peak and final viscosity.

The addition of higher doses of salt and baking powder to the formulation increase molecular interaction due to the addition of charges to the mix, influencing starch/starch and starch/protein interactions. Besides, the increase in the water absorption capacity of ingredients reduces the amount of free water available to facilitate the movement of particles in batters and consequently gives high viscosity (McCann; Day, 2013; Vetter, 2003).

Wyatt and Liberatore (2010) evaluating the effect of salts anion size on xanthan viscosity observed that the viscosity of the solution increases with the size of the added salt ion (i.e., the larger the salt ions produce larger viscosity increases), when compared to the salt-free solution (LiCL <NaCl <KCl <CsCl). However, the increase in viscosity appeared to be more dependent on ion valency than on ion size, since the addition of similar size ions with different valency (Mg^{2+} and Li^{+}) resulted in different viscosity, with divalent ion producing a larger increase in viscosity in every case.

The addition of baking powder would affect more the viscosity of the paste, since it contains more ions present in different size and valency, as evidenced in Table 4 for peak and final viscosity (salt and baking powder>no salt>no baking powder). Moreover, baking powder contain in its composition 29% of corn starch, which could affect the viscosity of the paste when added in higher concentrations (7%).

5.4.2 Baking performance

As presented in Table 5, cakes with lower weight were obtained when nonfat dry milk (NFDM) was omitted from the formulation (-1.1%), as well as reduced height (-5.3%) and volume (-7.7%). The double dose of NDFM resulted in heavier cakes (+0.7%) with lower height (-4.2%) than the normal formulation, with no change in volume. Light crust (+6.3%) and dark crumb colors (-2.0%) were observed when NFDM was omitted or added twice to the formulation (Table 6), respectively, which was also detected by the sensory panelists (Table 7).

Gallagher et al. (2003b) observed similar result evaluating the effect of adding milk protein isolate (3%) and novel rice starch (3%) on gluten-free breads. In their study, the addition of milk protein and rice starch resulted in an increase in loaf volume. Moreover, lower crust L* values where observed when the ingredients evaluated were added to bread, resulting in a darker crust when compared to control. According to the authors, this result was expected as dairy powder contained a small amount of lactose, which can be involved in Maillard browning and caramelization reactions, caused by the reaction between reducing sugar and amino acids.

Double addition of salt reduced the height of the cakes (-7.2%). Sodium chloride, is commonly added to cereal based food not only to enhance sensory properties, but also for its impact on the functional properties of cereal constituents. Evaluating the effect of sodium chloride on gluten network formation. Tuhumury et al. (2014) observed that samples prepared in the presence of NaCl presented fibrous structure in contract to those without NaCl for which large aggregates of gluten were seen. These observations suggest that the gluten proteins form more ordered alignments resulting in fibrous structure in the presence of NaCl. Moreover, salt is known for increase the dough strength (resistance to extension) with increasing levels of sodium chloride, which also resulted in lower load volume because of the strong interaction between polymers cross-linking, which led to an increase in the resistance (Simsek; Martinez, 2016; McCann; Day, 2013; Lynch et al., 2009). However, there is no significant formation of the gluten network in cake batter and the main component of cake structure formation is starch, which is present in traditional formulations in wheat flour (Cauvain, 2017). Therefore, the effect of salt addition observed can be attributed to increase in molecular interaction due addition of charges to the mix, influencing starch/starch and starch/protein interactions (McCann; Day, 2013).

As expected, omission of baking powder reduced the cake height (-36.9%) and volume (-32.4%). However, double addition of baking powder also reduced cake height in -16.0% when compared to the correct or normal formulation, with no effect on the volume.

The production of large amount of gas by the leavening agent can lead the batter to collapse during baking, since the structure is not strong sufficient to hold the volume of carbon dioxide produced. Book and Brill (2015), evaluating the effect of chemical leavening on yellow cake properties, observed that the lowest amount of soda produced the cake with the lowest volume. When the soda level was increased further to 4.6%, the volume decreased, suggesting that cakes made with higher levels of soda decreased in volume because there was too much gas for the system to retain it all.

In addition to our results, darker crust was observed when double dose of baking powder was added. As expected, the browning index also presented the same results once it considers the lightness values, as well as a* and b*, for its calculation.

A few baked products are improved by a slightly alkaline or slightly acid final pH. For example, the color and flavor of chocolate products are enhanced by higher pH, which can be manipulated easily by adjusting the acid/soda balance (Vetter, 2003). Even though sodium acid pyrophosphate (36% of the leavening agent) is known for its buffer effect, i.e. it’s supposed not to affect the pH when used, it was observed an increase (+2.3%) in the pH by adding double dose of the ingredient (Fig. 1B). That is because the heat of baking can cause baking soda (30% of the baking powder formulation) to decompose, giving of carbon dioxide leavening gas without reacting with a leavening acid. The sodium carbonate formed by the decomposition of baking soda is very alkaline and will tend to give a baked product a high pH (Vetter, 2003). The lower the pH, the less color development in terms of brown notes will be observed. The higher the pH the more browning will occur because of its effect on Maillard reaction (Oliete et al., 2010).

Previous research has observed similar results, where higher levels of baking powder produced cakes with a darker crust color, and pointed out a possible correlation between cake's pH and color of the crust and flavor (Book; Brill, 2015; Pop, 2007).

5.4.3 Sensory evaluation

Misformulation of baking powder was the functional ingredient that resulted in most detectable changes by the panelists (Table 7). Double addition of nonfat dry milk (NFDM) resulted in lower scores (-15.7%) for uniformity of the cake crumb, as can be seen in the pictures in Table 6, when compared to scores of normal or control formulation. The cake crumb developed uneven distribution of air cells (Table 7). Double dose addition of nonfat dry milk resulted in cakes with darker crumb color, with acceptance scores 7.3% lower than the control. Similar result was detected by CIELAB (Table 6), with double dose of NFDM resulting in darker crumb color (-2.0%), when compared to control.

In previous study, the addition of a mix containing dairy and rice powder resulted in loaves with a more open structure, presenting a lower uniformity when compared to the typical cake-like tight structure/appearance. The authors attributed this effect to the strong water absorptive properties presented by dairy proteins, which may, in turn, lead to finer, denser, crumb structures in the baked products (Gallagher et al., 2003a). In our study, double dose of NFDM reduced the moisture loss of the cakes (-5.4%), when compared to control formulation. In addition, the omission of the ingredient increased the moisture loss in +8.4% (Fig. 1A).

Sodium chloride influences many quality characteristics in food which are important for consumer acceptance and industrial suitability. These include salty taste in foods as well as the enhancing effect salt has on other flavor constituents (Belz et al., 2012). The panelists perceived other flavors in the cakes when double addition of salt was used in the formulation. These flavor-enhancing effects seems to be related to the effect of salt on water activity. The water restriction (reduction of water activity) by salt results in the concentration of flavor molecules in solution according to the ratio of free available and bound water and affects their volatility (Costa-Corredor et al., 2009).

Omission or double addition of baking powder resulted in reduction of moistness (-11.7 and -16.3%) and softness scores (-29.2 and -12.5 %), respectively, indicating drier and firmer cakes. Moreover, double addition also resulted in lower scores for uniformity of the cake crumb (-18.3%), compared to control formulation. As shown in Figure 1A, omission of baking powder from the mix increased the moisture loss by the cakes (-5.4%), however, the double dose did not present significance difference from the control ($p>0.05$).

As observed in the total score for the sensory evaluation, the double addition of any of the tree ingredients tested (NFDM, salt or baking powder) resulted in lower scores than the control, i.e., the cakes did not present the characteristics desired by the judges when compared to the control formulation.

5.4.4 Texture analysis

Texture is a critical factor for consumer acceptance, which can be evaluated in several ways. In this study was used trained panelists (Table 7) and texture analyzer (Table 8) to evaluate the quality of cakes and texture during storage.

As shown in Figure 2, the interaction of ingredient vs days of storage was significant with omission of NFDM affecting texture of the cakes after 4 days of storage in resilience (-13.4%) and, cohesion (-11.6%) and springiness (-2.6%) at 6 days (Table 8). However, for the control formulation the reduction in resilience (-16.9%) and cohesion (-10.9%) was only observed after 6 of storage. Indicating that the omission of NFDM from the dry mix could resulted in a final product with a shorter shelf-life.

Moreover, the addition of NFDM to the dry mix formulation increase the protein content of the cakes which will lead to a higher water redemption. As shown in Figure 1A, NFDM presented the highest (18.1%) and lowest (15.8%) moisture loss before and after baking when omitted or added twice to the formulation, respectively.

The functional properties of milk powder are important when the powders are used for recombination or in the manufacture of various food products. These functional properties include emulsification, foaming, water absorption, viscosity, gelation, and heat stability, which are essentially the manifestations of the physical and chemical properties of the milk (Sharma et al., 2012). Studying gluten-free bread, Gallagher et al. (2003a) observed that breads formulated with higher protein-content powder tended to have the firmest (least soft) crumb compared to control.

Omission of salt reduced resilience (-13.6%) and cohesion (-12.1%) after 4 days of storage, and double dose reduced springiness of cakes after day 6 (-3.2%). However, for the control formulation the reduction in resilience (-16.9%) and cohesion (-10.9%) were observed after 6 days of storage (Fig. 2 and Table 8). Which could indicate that the omission of salt from the dry mix could reduce the shelf-life of the cakes.

When baked, omission of baking powder from the dry mix formulation affected the texture of the cakes, increasing hardness (+309.1%), cohesion (+14.1%) and chewiness (+361.4%), compared to control formulation (one day after baked). During storage, the omission of the ingredient from the mix increased hardness (+51.0%) and chewiness (+42.2%) at day 4 (Fig. 2 and Table 8).

Cake volume is a measure of cake size and reflects the amount of air initially entrapped during mixing and the air, moisture, and CO_2 entrapped and expanded during baking. These gases may be dispersed in small cells throughout a fine crumb or in larger cells throughout a coarser crumb. Although high volumes do not always indicate a desirable cake, low volumes generally indicate a heavy, less desirable crumb, like the one observed in this study (Fig. 2) and not acceptable by the sensory panelists (Table 7).

5.4.5 Pasting properties vs quality of final product

As observed in Table 8, pasting properties changes presented significant correlations with the quality of the cakes. Peak viscosity of the paste showed a moderate negative correlation with cohesion of the cake crumb (r^2=-0.4800; $p<0.05$). Remembering that, double dose of baking powder increased peak viscosity (+86.6%), besides resulted in a harsh to coarse texture of the cakes perception according to the sensory evaluation (Table 7).

The baking powder most often used is double-acting, like the one for this study, which contains sodium bicarbonate (30%) and two leavening acids, sodium acid pyrophosphate-SAPP-28 (36%) and monocalcium phosphate monohydrate-MCP (5%). One leavening acid reacts at room temperature (fast-acting) to help nucleate the batter during mixing (MCP), while the other reacts in the oven (slow-acting) to expand the air cells during baking (SAPP). As observed by adding double dose of baking powder, if the leavening acid reacts too early before the batter viscosity is high enough, the air cells will diffuse out of the batter, resulting in a low volume and a coarse crumb grain (Miller, 2016). However, double dose of baking powder did not affect the volume cake but reduced its height when compared to control (-15.9%; Table 5).

Trough and final viscosity of the paste presented similar correlation with the final cake quality. For baking performance, the volume of the cakes presented a moderate to strong positive correlation with trough (r^2=0.6041; $p<0.01$) and final viscosity (r^2=0.615; $p<0.01$) of the paste. Indicating that, a higher and more stable batter viscosity during baking could result in a cake with a bigger volume. A higher trough and final viscosity could also result in lower hardness, resilience, cohesion, and chewiness, and a higher adhesiveness in the cakes.

A higher trough and final viscosity could indicate a high viscosity of the batter during baking. Sanz et al. (2008) suggested that the batter viscosity has an important effect on the bubble incorporation and movement which are considered controlling factors in cake texture, with higher viscosity of the batter contributing to higher bubble stability before and during baking. If the viscosity is too low, the bubbles in the batter can easily rise to the surface and are lost to the atmosphere during baking.

Setback, which is the difference between trough and final viscosity, presented a positive correlation with springiness of the cakes (r^2=0.4440; $p<0.05$). The cooling stage, referred as 'set-back', gives a second rise in viscosity because the amylose and amylopectin begin to reassociate. Typically, starch presenting higher setback values has a greater tendency for retrogradation (Zaidul et al., 2007). Springiness is a perception of gel "rubberiness" in the mouth, and is a measure of how much the gel structure is broken down by the initial compression. High springiness appears when the gel structure is broken into few large pieces during the first TPA compression, whereas low springiness results from the gel breaking into many small pieces (Sang et al., 2015). A higher retrogradation process leads to the formation of a more dense/compact structure which explain the correlation between the parameters.

5.5 Conclusion

Omission of NFDM from the dry mix increased the trough and final viscosity of the paste, producing small cakes (weight, height and volume) with lighter crust color and changes in the texture during storage (resilience, cohesion and springiness). Double dose addition of NFDM affected the viscosity of the paste, reducing peak viscosity, trough and final viscosity, producing slightly firm cakes with dark crumb color. Omission of salt (sodium chloride) reduced viscosity of the paste and affect the texture of the cakes after 4 days of storage reducing resilience and cohesion. Double dose of salt reduced the height of the cakes and increased off flavors. The omission or double dose addition of baking powder affected the pasting properties, increasing all the parameters evaluated (peak viscosity, trough, breakdown, final viscosity, setback and peak time) when more ingredient was added to the mix. The misformulation of baking powder (omission or double dose) produced small cakes (volume) characterized as slightly firm and dry. Besides, omission of the ingredient changed the texture of cakes during storage, increasing hardness and chewiness. Cakes containing double dose of baking powder presented a dark crust and a less uniform crumb. Peak viscosity of the paste presented a moderate negative correlation with cohesion of the crumb (r^2=-0.4800; $p<0.05$). The volume of the cakes presented a moderate to strong positive correlation with trough (r^2=0.6041; $p<0.01$) and final viscosity (r^2=0.615; $p<0.01$) of the paste. Setback presented a moderate positive correlation with springiness of the crumb (r^2=0.4440; $p<0.05$).

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Table 1. Leavening agent formulation (baking powder).

Ingredient	Percentage (%)
Sodium acid pyrophosphate (SAPP-28) (ICL Performance Products LP; ST Louis; Missouri, U.S.)	36
Sodium bicarbonate (Arm & Hammer®, Church & Dwight CO. INC., Princeton, New Jersey, U.S.)	30
Corn starch (Great Value®, Wal-Mart, Bentonville, Arkansas, U.S.)	29
Monocalcium phosphate monohydrate (MCP-M) (Regent 12XX®, Innophos, Cranbury, New Jersey, U.S.)	5

Table 2. Dry cake mixes formulation (%).

Ingredients*	Omission			Normal	Double		
Flour	100	100	100	100	100	100	100
Sugar	80	80	80	80	80	80	80
Nonfat dry Milk	0	12	12	12	24	12	12
Salt	3	0	3	3	3	6	3
Baking Powder	3.5	3.5	0	3.5	3.5	3.5	7

*All ingredients are expressed as percentage based on flour weight (100 g flour).

Table 3. Test setting profiles used for analysis of pasting properties of dry cake mixes.

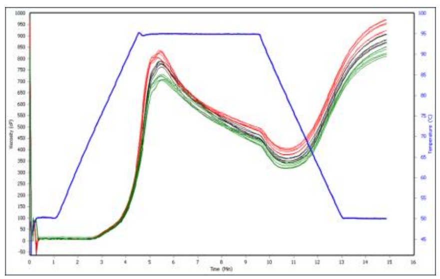
Stages	Temperature/speed	Time
1	50°C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 10 sec
4	50°C	1 min, 0 sec
5	95°C	4 min, 30 sec
6	95°C	9 min, 30 sec
7	50°C	13 min, 0 sec
End of test		15 min, 0 sec

Table 4. Pasting properties of dry cake mix under misformulation (omission-red, normal-black, double-green) of functional ingredients (nonfat dry milk, salt and baking powder)*.

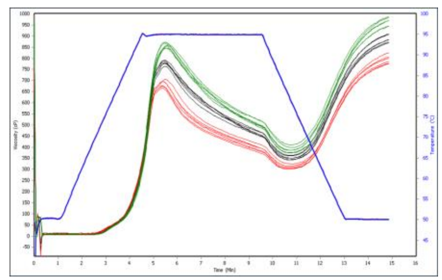
	Peak Viscosity (cP)			**Trough (cP)**			**Breakdown (cP)**		
	Omission[a]	Normal[b]	Double[c]	Omission	Normal	Double	Omission	Normal	Double
NFDM	821.8±5.5aA	779.8±4.0aA	717.0±4.2bC	391.8±4.7aA	351.7±3.5bA	327.5±4.6cC	430.0±2.3aA	428.2±5.4aA	389.5±7.6aC
Salt	685.0±6.4cB	779.8±4.0bA	858.2±4.6aB	309.5±3.4cB	351.7±3.5bA	388.5±5.6aB	375.5±3.5bAB	428.2±5.4abA	469.7±8.3aB
Baking Powder	601.0±5.1cC	779.8±4.0bA	1455.3±30.9aA	246.0±1.9cC	351.7±3.5bA	450.0±3.8aA	355.0±5.0cB	428.2±5.4bA	1005.3±33.3aA

	Final Viscosity (cP)			**Setback (cP)**			**Peak Time (min)**		
	Omission	Normal	Double	Omission	Normal	Double	Omission	Normal	Double
NFDM	952.8±8.9aA	889.8±6.6bA	835.5±7.2cC	561.0±4.3aA	538.2±3.1aA	558.0±51.3aB	5.37±0.04aAB	5.46±0.01aA	5.49±0.01aB
Salt	796.5±8.0cB	889.8±6.6bA	969.2±7.5aB	487.0±4.7bB	538.2±3.1abA	580.7±2.8aAB	5.38±0.02aA	5.46±0.01aA	5.52±0.03aB
Baking Powder	676.7±4.1cC	889.8±6.6bA	1071.3±7.1aA	430.7±2.8cC	538.2±3.1bA	621.3±3.7aA	5.17±0.02cB	5.46±0.01bA	6.07±0.11aA

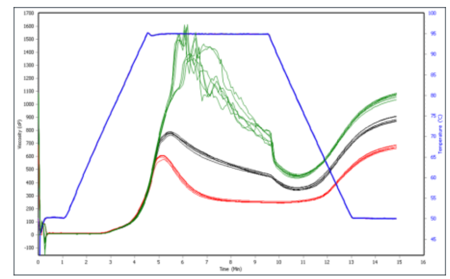
MILK



SALT



BAKING POWDER



*Means (n= 6 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$). [a]Omission of the ingredient = 0 g per 100 g flour (NFDM or salt or baking powder). [b]Normal dose = 12.0 g of NFDM or 3.0 g of salt or 3.5 g of baking powder per 100 g flour. [c]Double dose = 24.0 g of NFDM or 6.0 g of salt or 7.0 g of baking powder per 100 g flour.

Table 5. Baking performance of cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder)*.

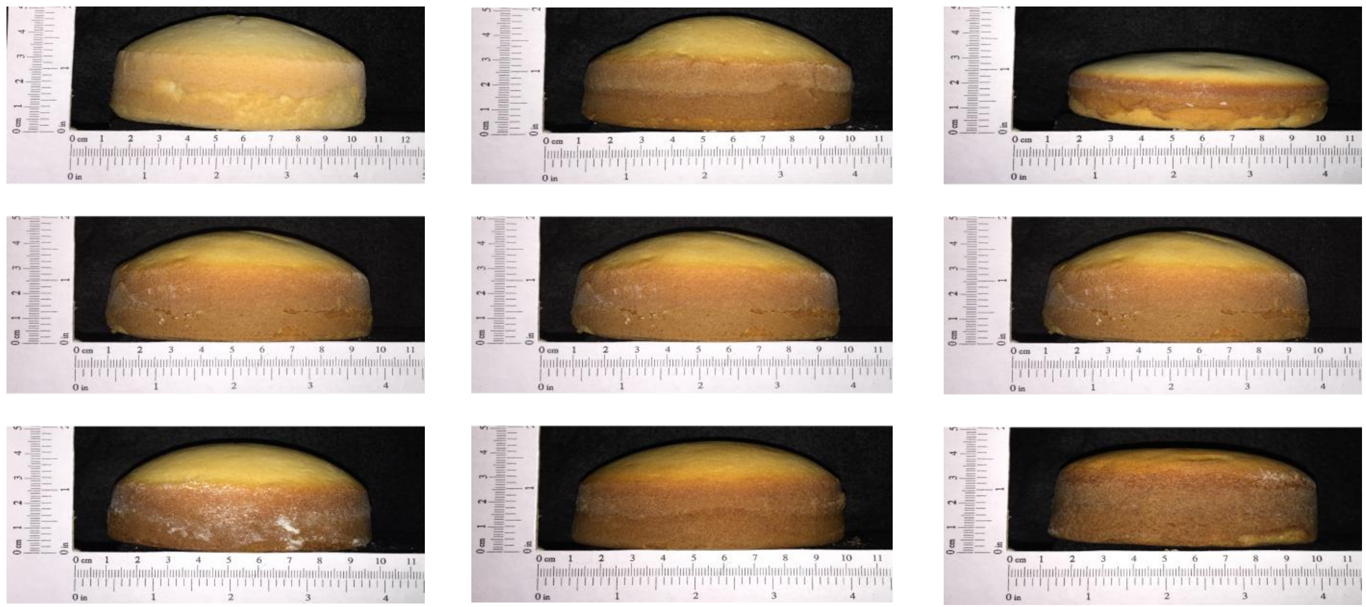
	Weight (g)			Height (mm)			Volume (cc)		
	Omission	Normal	Double	Omission	Normal	Double	Omission	Normal	Double
NFDM	131.9±0.3cB	133.3±0.5bA	134.2±0.5aA	51.2±0.6bB	54.0±0.2aA	51.7±1.0bA	308.9±4.2bB	334.7±3.1aA	328.8±6.8aA
Salt	133.2±0.3aA	133.3±0.5aA	133.8±0.4aA	55.5±0.4aA	54.0±0.2aA	50.1±0.5bB	331.6±4.8aA	334.7±3.1aA	330.5±11.9aA
Baking Powder	132.4±0.4bAB	133.3±0.5abA	133.8±0.5aA	34.1±0.4cC	54.0± 0.2aA	45.4±0.5bC	226.2±9.1bC	334.7±3.1aA	331.6±10.6aA

NFDM **SALT** **BAKING POWDER**

Omission

Normal

Double



*Means (n= 9 ± standard error) followed by the same lowercase letter within a row (dose; p<0.05) and same capital letter within a column are not significantly different (ingredient; p<0.05).

Table 6. Crust and crumb lightness (L*), hue color (h°) and browning index (BI) of cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder)*.

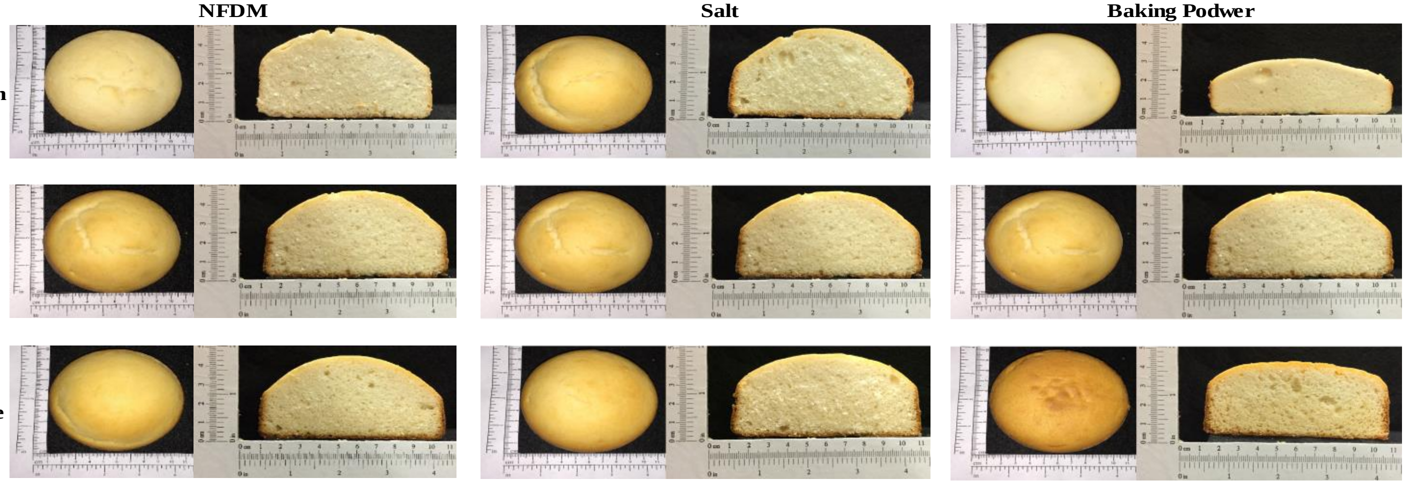
Ingredients	Lightness (L*)			Hue (h*)			Browning Index (BI)		
	Omission	Normal	Double	Omission	Normal	Double	Omission	Normal	Double
				Crust					
NFDM	81.1±0.5aB	76.3±0.3bA	74.1±1.5bA	1.41±0.01aB	1.36±0.01bA	1.33±0.01bA	49.1±2.6cB	80.3±2.9bA	93.5±7.3aB
Salt	76.3±0.7aC	76.3±0.3aA	74.2±1.2aA	1.37±0.01aC	1.36±0.01aA	1.34±0.02aA	80.0±3.8aA	80.3±2.9aA	87.3±6.6aB
Baking Powder	84.3±0.4aA	76.3±0.3bA	66.1±0.7cB	1.49±0.01aA	1.36±0.01bA	1.24±0.01cB	35.0±1.0cC	80.3±2.9bA	114.2±3.5aA
				Crumb					
NFDM	86.8±0.2aA	85.5±0.23aA	83.8±0.2bA	1.53±0.00aA	1.52±0.00bA	1.52±0.00bA	22.2±0.1cB	26.6±0.0bA	30.8±0.0aB
Salt	85.7±0.3aB	85.5±0.23aA	84.4±0.2aA	1.52±0.00aB	1.52±0.00aA	1.52±0.00aA	26.0±0.1bA	26.6±0.0bA	28.6±0.0aC
Baking Powder	86.0±0.2aB	85.5±0.23aA	80.8±0.5bB	1.51±0.00bC	1.52±0.00aA	1.50±0.00cb	25.3±0.7cA	26.6±0.0bA	34.2±0.4aA

NFDM **Salt** **Baking Podwer**

Omission

Normal

Double



*Means (n= 9 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$).

Table 7. Sensory evaluation (acceptance test) of cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder)*.

	Uniformity of the Crumb			**Tenderness**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	8.2±0.5aA	8.14 ± 0.37aA	6.86 ± 0.55bA	12.8±0.4aA	12.2±0.7aA	12.0±0.4aA
Salt	7.9±0.5aA	8.14 ± 0.37aA	7.52 ± 0.47aA	12.5±0.3aA	12.2±0.7aA	11.9±0.5aA
Baking Powder	8.4±0.5aA	8.14 ± 0.37aA	6.65 ± 0.50bA	11.4±0.6aA	12.2±0.7aA	11.0±0.6aA
	Size of the Cells			**Softness**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	7.9±0.4aB	7.71 ± 0.35aA	7.62 ± 0.30aA	9.6±0.2aA	9.5±0.2aA	8.4±0.3bA
Salt	8.0±0.4aB	7.71 ± 0.35aA	7.19 ± 0.38aAB	8.6±0.4aB	9.5±0.2aA	8.7±0.3aA
Baking Powder	9.0±0.3aA	7.71 ± 0.35bA	6.65 ± 0.38cB	6.7±0.5cC	9.5±0.2aA	8.3±0.3bA
	Thickness of Cell Wall			**Color of Crumb**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	7.8±0.5aA	7.5±0.5aA	7.14 ± 0.42aA	8.9±0.2aA	8.5±0.2aA	7.9±0.3bB
Salt	7.5±0.5aA	7.5±0.5aA	6.62 ± 0.65aA	8.6±0.2aA	8.5±0.2aA	8.5±0.2aA
Baking Powder	7.4±0.7aA	7.5±0.5aA	6.90 ± 0.50aA	9.1±0.3aA	8.5±0.2bA	7.1±0.3cC
	Grain			**Flavor**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	13.5±0.7aA	12.7±0.7abA	11.6±0.7bAB	8.7±0.6aA	9.2±0.5aA	8.7±0.5aA
Salt	12.7±0.6aA	12.7±0.6aA	12.5±0.7aA	8.3±0.6abA	9.2±0.5aA	7.2±0.8bA
Baking Powder	13.4±0.7aA	12.7±0.6aA	10.2±0.7bB	8.0±0.7aA	9.2±0.5aA	8.5±0.5aA
	Moistness			**Total Score**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	8.3±0.4aA	8.9±0.3aA	8.0±0.4aA	85.2±2.6aA	83.9±2.7aA	77.7±2.0bA
Salt	8.2±0.4aA	8.9±0.3aA	7.9±0.4aA	82.0±2.4aA	83.9±2.7aA	78.0±2.9bA
Baking Powder	7.9±0.4bA	8.9±0.3aA	7.5±0.4bA	80.8±2.1aA	83.9±2.7aA	72.6±2.4bB

*Means (n= 22 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$).

Table 8. Shelf-life of cakes under misformulation (omission, normal, double) of functional ingredients (nonfat dry milk, salt and baking powder)*.

	NFDM						Salt						Baking Powder					
	Resilience						Resilience						Hardness					
	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%
Day 1	35.6±0.5aA	0.0	32.0±0.6aA	0.0	34.0±0.8aA	0.0	31.4±1.1aA	0.0	32.0±0.6aA	0.0	34.7±0.5aA	0.0	762.2± 21.9aC	0.0	186.3±0.5bA	0.0	256.0±4.2bA	0.0
Day 2	35.2±0.8aA	-1.1	31.8±1.0aA	-0.5	34.0±1.4aA	0.0	30.0±1.6bAB	-4.6	31.8±1.0bA	-0.5	35.8±1.5aA	3.2	942.7±34.4aBC	23.7	223.9±7.7bA	20.2	226.1±28.1bA	-11.7
Day 4	30.8± 1.1aB	-13.4	31.0±0.3aA	-3.1	33.2±0.4aA	-2.4	27.7±0.5bB	-11.9	31.0±0.3abA	-3.1	33.1±2.1aA	-4.6	1151.2±30.8aAB	51.0	295.9±12.5bA	58.9	344.8±31.9bA	34.7
Day 6	28.6±1.8aB	-19.7	26.6±1.0aB	-16.7	30.0±1.6aA	-11.7	27.2±1.4bB	-13.6	26.6±1.0bB	-16.7	33.1±0.9aA	-4.5	1265.6±51.5aA	66.1	323.5±21.1bA	73.6	391.9±18.4bA	53.1
	Cohesion						Cohesion						Cohesion					
	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%
Day 1	0.69±0.01aA	0.0	0.64±0.01aA	0.0	0.68±0.02aA	0.0	0.66±0.02aA	0.0	0.64±0.01aA	0.0	0.68±0.01aA	0.0	0.73±0.01aA	0.0	0.64±0.01bA	0.0	0.64±0.02bA	0.0
Day 2	0.67±0.01aA	-2.9	0.64±0.01aA	0.0	0.66±0.01aA	-2.9	0.63±0.02aAB	-4.5	0.64±0.01aA	0.0	0.67±0.02aA	-1.5	0.72±0.00aA	-1.4	0.64±0.01bA	0.0	0.59±0.04cB	-7.8
Day 4	0.61±0.01aB	-11.6	0.62±0.00aA	-3.1	0.65±0.00aAB	-4.4	0.59±0.01aBC	-10.6	0.62±0.00aA	-3.1	0.64±0.02aA	-5.9	0.69±0.01aA	-5.5	0.62±0.00bA	-3.1	0.60±0.01bAB	-6.3
Day 6	0.59±0.02aB	-14.5	0.57±0.01aB	-10.9	0.61±0.02aB	-10.3	0.58±0.02bC	-12.1	0.57±0.01bB	-10.9	0.64±0.01aA	-5.9	0.68±0.01aA	-6.8	0.57±0.01bB	-10.9	0.58±0.01bB	-9.4
	Springiness						Springiness						Chewiness					
	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%
Day 1	93.4±0.4aAB	0.0	92.3±0.2aB	0.0	94.4±0.5aA	0.0	93.4±0.5aA	0.0	92.3± 0.2aB	0.0	94.0±0.7aA	0.0	510.0±6.5aB	0.0	110.5±1.8bA	0.0	151.5±7.2bA	0.0
Day 2	94.4±0.5aA	1.1	94.3±1.1aA	2.1	94.3± 0.8aA	-0.1	92.8±0.5aA	-0.6	94.3±1.1aA	2.1	94.7±1.1aA	0.7	618.5±24.8aAB	21.3	135.3±7.3bA	22.4	127.7±23.9bA	-15.7
Day 4	92.6±0.3aBC	-0.9	92.1±0.4aB	-0.3	93.5±0.4aA	-0.9	91.9±0.5aA	-1.6	92.1 ±0.4aB	-0.3	93.0±0.1aA	-1.0	725.4±22.6aA	42.2	168.9±8.4bA	52.8	189.5±21.3bA	25.1
Day 6	91.0±1.0abC	-2.6	89.6±1.4bC	-3.0	92.4±0.6aA	-2.2	91.0±0.5aA	-2.5	89.6±1.4aC	-3.0	91.1±1.4aB	-3.1	778.6±39.5aA	52.7	163.7±5.0bA	48.1	209.1±14.2bA	38.1

*Means (n= 12 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (days of storage; $p<0.05$). Percentage (%) – Difference from the day 1 of storage.

Table 9. Pearson correlation (r) between pasting properties of the dry cake mixes and the final product.

Variables		Peak Viscosity	Trough	Breakdown	Final Viscosity	Setback	Peak Time	Pasting Temperature
Baking Performance	Weight	0.2027 (p=0.378)	0.2664 (*p*=0.243)	0.2065 (*p*=0.369)	0.2728 (*p*=0.232)	0.3186 (*p*=0.159)	**0.5639 (p=0.008)**	**0.7379 (p=0.000)**
	Height	-0.0349 (p=0.881)	0.3493 (p=0.121)	-0.132 (p=0.568)	0.3518 (p=0.118)	0.3959 (p=0.076)	0.1408 (p=0.543)	-0.0079 (p=0.973)
	Volume	0.3574 (p=0.112)	**0.6041 (p=0.004)**	0.2938 (p=0.196)	**0.6115 (p=0.003)**	**0.5966 (p=0.004)**	**0.4756 (p=0.029)**	0.1057 (p=0.648)
Crust	Lightness	**-0.7775 (p=0.000)**	**-0.6972 (p=0.000)**	**-0.7760 (p=0.000)**	**-0.7035 (p=0.000)**	**-0.6352 (p=0.002)**	**-0.8466 (p=0.000)**	-0.2212 (p=0.335)
	Hue*	**-0.7585 (p=0.000)**	**-0.7310 (p=0.000)**	**-0.7407 (p=0.000)**	**-0.7322 (p=0.000)**	**-0.6644 (p=0.001)**	**-0.8176 (p=0.000)**	-0.1697 (p=0.462)
	Browning Index	**0.6365 (p=0.002)**	**0.5982 (p=0.004)**	**0.6346 (p=0.002)**	**0.6104 (p=0.003)**	**0.5871 (p=0.005)**	**0.7499 (p=0.000)**	0.2395 (p=0.296)
Crumb	Lightness	**-0.8235 (p=0.000)**	**-0.5644 (p=0.008)**	**-0.8579 (p=0.000)**	**-0.5733 (p=0.007)**	**-0.6066 (p=0.004)**	**-0.7730 (p=0.000)**	-0.2766 (p=0.225)
	Hue*	**-0.5799 (p=0.006)**	-0.1147 (p=0.621)	**-0.6707 (p=0.001)**	-0.1267 (p=0.584)	-0.1834 (p=0.426)	-0.4029 (p=0.070)	-0.1970 (p=0.392)
	Browning Index	**0.6798 (p=0.001)**	**0.4353 (p=0.049)**	**0.7230 (p=0.000)**	**0.4478 (p=0.042)**	**0.5189 (p=0.016)**	**0.7314 (p=0.000)**	0.3893 (p=0.081)
Texture	Hardness	-0.3214 (p=0.155)	**-0.7037 (p=0.000)**	-0.2178 (p=0.343)	**-0.7048 (p=0.000)**	**-0.6427 (p=0.002)**	**-0.4345 (p=0.049)**	-0.0075 (p=0.974)
	Adhesiveness	0.3300 (p=0.144)	**0.5723 (p=0.007)**	0.2661 (p=0.244)	**0.5719 (p=0.007)**	**0.5175 (p=0.016)**	**0.4359 (p=0.048)**	0.1966 (p=0.393)
	Resilience	-0.3937 (p=0.077)	-0.4321 (p=0.050)	-0.3449 (p=0.126)	**-0.4335 (p=0.050)**	**-0.4676 (p=0.033)**	**-0.5290 (p=0.014)**	-0.2098 (p=0.361)
	Cohesion	**-0.4800 (p=0.028)**	**-0.5752 (p=0.006)**	-0.4192 (p=0.059)	**-0.5818 (p=0.006)**	**-0.6240 (p=0.003)**	**-0.6105 (p=0.003)**	-0.2978 (p=0.190)
	Springiness	-0.0094 (p=0.968)	0.2418 (p=0.291)	-0.0377 (p=0.871)	0.2545 (p=0.266)	**0.4440 (p=0.044)**	0.0725 (p=0.755)	-0.1590 (p=0.491)
	Chewiness	-0.3457 (p=0.125)	**-0.7161 (p=0.000)**	-0.2424 (p=0.290)	**-0.7173 (p=0.000)**	**-0.6611 (p=0.001)**	**-0.4626 (p=0.035)**	-0.0282 (p=0.904)

*Values in bold are significant (p<0.05).

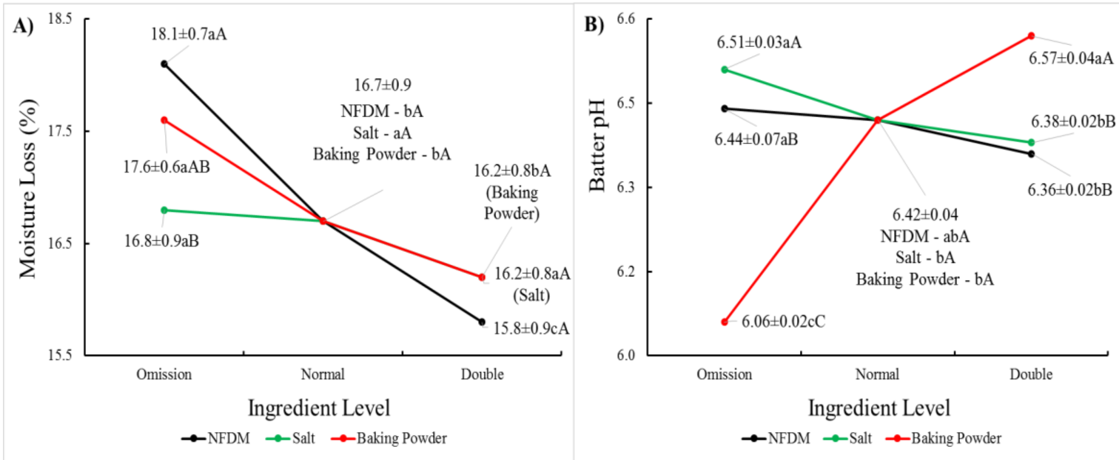


Figure 1. Moisture loss (A) during baking and pH of the batter (B) of cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder). *Means (n= 9 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$).

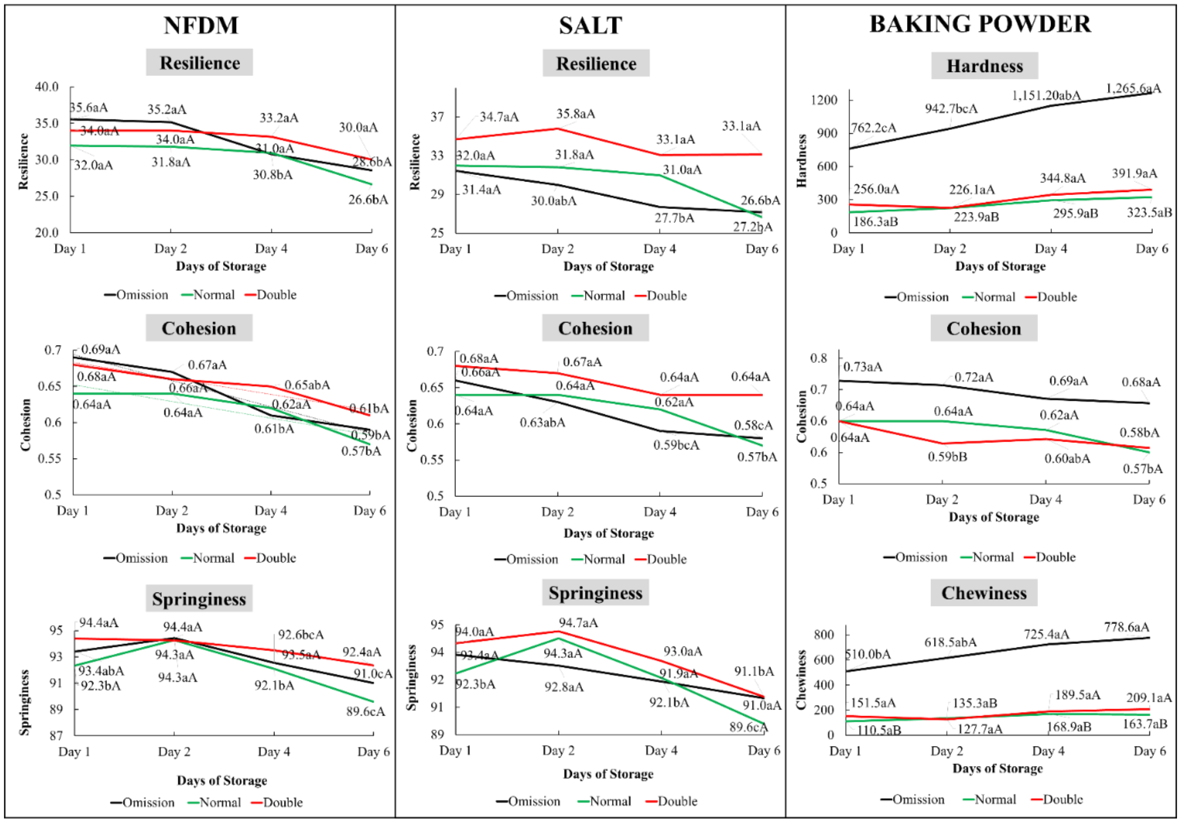


Figure 2. Shelf-life of cakes under misformulation (omission-black, normal-green, double-red) of functional ingredients (nonfat dry milk, salt and baking powder). Means (n= 12 ± standard error) followed by the same lowercase letter within a row (days of storage; p<0.05) and same capital letter within a column are not significantly different (ingredient; p<0.05).

CHAPTER VI

MISFORMULATION OF GLUTEN-FREE DRY CAKE MIXES

ON PASTING PROPERTIES AND QUALITY OF FINAL PRODUCT

6.1 Abstract

Mistakes on formulation can occur during production of bake mixes such as omission or double dose addition of functional ingredients, resulting in significant economic losses and compromising the quality of final products. The objective of this study was to evaluate the effect of functional ingredients on pasting properties (RVA) of gluten-free cake mixes and cake baking performance, texture and sensory characteristics using a randomized complete block with 3 blocks. Three ingredients (nonfat dry milk-NFDM, salt, baking powder) were evaluated in 3 different concentrations (omission, normal and double dose). Analysis of variance and mean separation by protected LSD test (<0.05) were conducted. Double dose of NFDM increased peak viscosity (+4.3%), as well as salt (+6.3%) and baking powder (+3.0%). Omission of salt (-5.1%), and omission (-2.9%) or double addition (-8.2%) of baking powder reduced final viscosity. Time to reach maximum viscosity of the paste was increased when salt was omitted (+19 sec),

and decreased adding double dose of baking powder (-18 sec). Omitting NFDM decreased weight (-1.5%), height (-13.9%) and volume (-19.8%) of cakes while omitting salt increased height (+6.2%). Omission of baking powder reduced cake height (-23.8%) and volume (-23.8%). However, double dose of baking powder increased volume (+6.4%). Lighter crust color was observed omitting NFDM, salt or baking powder. In addition, darker crust and darker crumb were observed when baking powder was added twice or omitted, respectively. Omission of NFDM and baking powder affected the cakes texture perception of panelists who determined increased firmness and chewiness and varying from slightly dry to gummy. Double dose of salt increased resilience (+10.2%), cohesion (+7.2%) and springiness (+2.7%). Peak and breakdown viscosity during pasting showed negative correlation with hardness (r^2=-0.5707, $p<0.01$; r^2=-0.5728, $p<0.01$) and chewiness (r^2=-0.5494, $p<0.05$; r^2=-0.5593, $p<0.01$), respectively. Peak viscosity also presented a moderate positive correlation with volume (r=0.4422; $p<0.05$). The misformulation scenarios studied serve as a tool to relate information of cake dry mixes quality control with viscosity and cake quality.

Keywords: Functional ingredient, nonfat dry milk, salt, baking powder, cake quality

6.2 Introduction

Gluten is the main protein present in wheat flour and plays an important role in their functionality in baking, providing viscoelasticity, gas expansion and crumb structure

(Al-Dmoor, 2013). Cakes are chemically leavened batter-based products. The definition of cake varies, but essentially the term refers to products which are characterized by formulations based on flour, sugar, whole eggs, and other liquids in which fat or oil may be added (Gallagher, 2008).

In recent years, significant increase in research has been conducted in developing gluten-free products using ingredients such as starches, dairy products, gums and hydrocolloids, probiotics and other combinations as alternatives to gluten in order to improve the structure, taste, acceptability and shelf life of products (Kelly et al., 2008). Dairy proteins are significant functional ingredients and due to their versatility can be readily incorporated into many food products.

They are used in bakery products for both nutritional and functional benefits including flavor and texture enhancement, and storage improvement (Ngemakwe et al., 2014). Dairy products may be used in gluten-free bread formulas to increase water absorption and, therefore, enhance the handling properties of the batter (Gallagher et al., 2004). Seven dairy powders were applied to a gluten-free bread formulation by Gallagher et al. (2003a). In general, the powders with a high protein/low lactose content (sodium caseinate, milk protein isolate) gave breads with an improved overall shape and volume, and a firmer crumb texture.

Salt (sodium chloride) has several different functions in the manufacture of bakery products, some of which are product specific. Salt is added at about 1.5% of gluten-free flour weight for taste and to improve dough handling. Salt slows down water imbibition and swelling of flour proteins, reduces dough extensibility and improves gas retention, bread crumb and slicing properties (Ngemakwe et al., 2014).

Chemical leaveners are used to give cookies, cakes, and other baked goods their characteristic texture. They produce gas from the reaction that takes place when a carbon dioxide source and an acid are mixed together and come into contact with water. The gas forms bubbles that are trapped in the batter or dough and then expand during baking to form the holes that are retained in the finished product (Pop, 2007). Double-acting baking powder contain a mixture of sodium bicarbonate, a fast-acting leavening acid like monocalcium phosphate monohydrate (MCP) and a slow-acting leavening acid like sodium acid pyrophosphate (SAPP). They react partially at low temperatures and partially at high temperatures to provide uniform leavening throughout processing (Miller, 2016). Sodium bicarbonate increases the pH, which leads to more intensive browning and alkaline taste (Hesso et al., 2015). Sodium pyrophosphate reacts with sodium bicarbonate, and when in excess can give a metallic taste (Miller, 2016).

Food quality is defined by a complex set of stimulus that starts with visual evaluation. Once the decision is made, the quality is based not only on the magnitude of flavor, taste and texture, but also takes into account temporal coordination of these stimuli (Foegeding et al., 2010). The main parameters of cake quality are volume, firmness, color, and weight loss, and the quality can be influenced by several factors like quality and level of ingredients (Kahraman et al., 2008).

The aeration of the batter, and consequently the quality of the baked product, is highly dependent on the viscosity. A low viscosity batter will fall short to hold the air cells in the structure resulting in a low volume cake. If the batter is thick, it would be difficult for the air bubbles to get away from cake, which would result in a high volume cake (Al-Dmoor, 2013). During baking, the combined effect of starch swelling and protein denaturation in presence of other ingredients transformed the liquid batter into a solid foam. These events first tremendously increase the viscosity of the batter and finally provide structure to the matrix (Hesso et al., 2015).

The purpose of this study was to evaluate the effect of functional ingredients on pasting properties (RVA) of gluten-free cake mixes and baking performance, texture and sensory characteristics of the baked product. Thus, we seek a better understanding of the effect that the misformulation of functional ingredients has on the viscosity of the paste, quality of the baked product and their acceptance by the consumers.

6.3 Materials and Methods

6.3.1 Materials

All the ingredients used in the experiment were purchased at local retail stores: sugar (Imperial Sugar®, Imperial Sugar Company, Sugar Land, TX, U.S.), NFDM (Great Value®, Wal-Mart, Bentonville, AR, U.S.), alt (Special Purity®, North American Salt Company, Overland Park, KS, U.S.), fresh eggs (Large Grade A, Great Value®, Wal-Mart, Bentonville, AR, U.S.), and shortening (Crisco®, The JM Smucker Co., Orrville, OH, U.S.).

6.3.2 Experimental design

The experimental design for this study was a randomized complete block (3 blocks) with seven treatments. The formulation of the treatments is detailed in Table 3. The treatments were randomized and baked accordingly during 3 days, considering each individual day a block.

6.3.3 Methods

The effect of nonfat dry milk (NFDM), salt (sodium chloride) and baking powder (Table 1), added in 3 different concentrations (omission, normal and double dose) were evaluated in dry cake mixes formulated with gluten-free flour (Table 2). For the gluten-free flour, the ingredients were weighted and blended (Patterson-Kelley Co., Stroudsburg, PA, U.S.) for 20 minutes to achieve homogeneity within the batch. From the prepared batch, each treatment was individual weighed at the beginning of each repetition or block (3 block).

Particle size of salt and sugar was reduced using a coffee grinder (Model 80335, Hamilton Beach, NC, U.S.) for 1 min followed by sieving to pass through 80 mesh or 0.177 mm sieve (VWR Scientific, Westchester, PA, U.S.), using a RoTap (RoTap RX-29, WS Tyler, Mentor, OH, U.S.), to minimize differences due to particle size during analysis.

6.3.3.1 Rapid Visco Analyzer (RVA)

The pasting properties of the dry cake mixes was determined using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Kungens Kurva, Sweden), coupled to a water bath 25°C (Fisher Isotemp 1028P, Pittsburgh, Pennsylvania, U.S.), following AACC International Approved Method 76-21.01, with modifications in the settings (Table 4). The samples had their weights adjusted to 14% moisture content, previously determined according to method 012/IV (105 ºC per 3 hours; IAL, 2005) in a convection oven (model 52000-55, Cole-Parmer Instrument Company, Chicago, IL, U.S.). Around five grams of dry mix were added to 25 mL of distilled water, instead of the 3.5 grams of flour or starch used in the standard method. The samples had their weights corrected to flour basis so all the treatment would contain the same amount of starch (2.5 g of flour; Appendix II). The analysis was done in duplicates.

6.3.3.2 Baking procedure

The dry cake mixes formulated were baked following the AACCI Approved Method 10-90.01 for testing baking quality of cake flour, with modifications in the formulation as described in Table 3. The amount of dry mix used was calculated on flour weight basis (170 g flour, Appendix II), as well as water (65%, tap water), fresh egg whites (25%) and shortening (15%). The dry mix was transferred to a mixing bowl and added of egg whites, shortening and 60% of water. The ingredients were mixed at speed 2 in a kitchen mixer (Model KSM90, KitchenAid Inc., St. Joseph, MO, U.S.) for 30 seconds. Dry ingredients and cake batter from the side walls of mixing bowl were scraped off with a rubber spatula and mixed at speed 4 for 2 min. The remaining water was added, mixed at speed 2 for 30 seconds, the walls scraped off, and mixed at speed 4 for 2 min. Round cake pans (10.16 x 5.08 cm/4 x 2 inches, Fat Daddio's, Spokane, WA, U.S.) were lightly greased with shortening and bottom lined with parchment paper. Three cakes of 150 g of batter were prepared from each batter batch and baked at 160°C (325°F) for 30 minutes in a rotating Rack Oven (Model OV300E, Baxter Manufacturing, Orting, WA, U.S.). Three batter batches were obtained for each treatment, one in each block (day), with 3 cakes from each batch (9 cakes total for each treatment). Another small batch for each treatment was prepared to measure the pH (electrode 4M KCl saturated with AgCl, pH meter AR5, Fischer Scientific, Pittsburg, PA, U.S.).

6.3.3.3 Cake evaluation (performance)

After cooled down for 30 minutes, the cakes were removed from the pans and allowed to continue cooling for approximately one hour. Cakes were analyzed for crust color using CIELAB scale with illuminant D65 and angle 10° (Model MiniScan XE Plus, Hunter Associates Laboratory, Inc., Reston, VA, U.S.). In this system, the chromaticity coordinate a* indicates the intensity of the red (+a*) or green (-a*), the chromaticity coordinate b* the yellow intensity (+b*) or blue (-b*) and L* the brightness of white (L*=100) to black (L *=0). From the values of L*, a* and b*, the angle chromatic hue (h°) and browning index (BI) were calculated, where h = 0° (pure red), h = 90° (pure yellow), h =180° (pure green) and h = 270° (pure blue), using the formula $h^\circ = \text{arctn} \times (\frac{b*}{a*})$ and BI = $\frac{100\ (x-0.31)}{0.172}$, where $x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$ (Maskan, 2001). The cakes were also evaluated for weight (Model AR5120, OHAUS, Parsippany, NJ, U.S.), height (benchtop micrometer, Mitutoyo Corp., Kawasaki, Kanagawa, Japan), and volume by rapeseed displacement with a loaf volumeter (1000cc, National MFG, Lincoln, NE, U.S.) following AACCI Approved Method 10-05.01. Moisture loss was calculated by difference between weight before (150 g) and after baking. Cold cakes were stored in Ziploc bags until the next day. One day after baking, the cakes were sliced (25 mm, Mini-Supreme Bread Slicer Model 709, Oliver Products Company, Grand Rapids, MI, U.S.), color of the crumb was measured and the slices were placed back in Ziploc bags. For each treatment, two of the 3 cakes baked on each day (block) was used for texture analysis and one was used for sensory evaluation.

6.3.3.4 Sensory evaluation

Sensory analysis was carried out the next day after baking. A panel of 22 panelists received training for scoring cake internal structure using a nine-point hedonic scale, according to AACCI Approved Method 10-90.01 (Appendix III). The panelists were students and staff at Oklahoma State University. The panel was formed by 14 female and 8 male participants with percentage age distribution of 59% younger than 25 years old, 32% 25-35 years old, 4.5% of both 36-45 and older than 45 years old. The samples were identified with three random digit numbers. In each evaluation session, seven samples cut into strips of approximately 3 cm long were presented to the judges. The sensory analysis was performed in 3 sessions (3 blocks) in different weeks so the judges would not be fatigued by the number of samples. The sensory evaluation was approved by Institutional Review Board (IRB) at Oklahoma State University (Appendix III).

6.3.3.5 Texture analysis

Texture analysis was performed in duplicate (2 slices per cake), measuring 3 points per slice, using a texture profile analysis (TPA) test and a TA-XT2i Texture Analyzer (Model Plus-Upgrade, Stable Micro Systems, Surrey, UK) equipped with Texture Expert software, at day one, three, five and seven days after baking. Cake crumb texture was determined with a 25 mm diameter cylindrical acrylic probe, double compression test to penetrate to 25% of the height, test speed of 2 mm/s and 5 s delay between the first and second compressions. Hardness, adhesiveness, resilience, cohesion, springiness and chewiness were calculated from the TPA graphs. Two slices were selected randomly (25 mm thickness) from each cake (two cakes per treatment) from each day (1, 3, 5 and 7 days after baking).

6.3.3.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) to assess the effect of ingredient (NFDM, salt and baking powder) and level of misformulation (omission, normal and double), with means comparison by least significant difference test (protected LSD; $p<0.05$), using SAS (SAS Institute, Cary, NC, U.S.). Pearson's correlation was performed between the pasting properties and the parameters evaluated in the baked cakes (baking performance, sensory and texture), using SAS statistical software and STATISTICA 13.2 program for Windows (StatSoft Inc., Tulsa, OK, U.S.).

6.4 Results and Discussion

6.4.1 Pasting Properties

Double addition of nonfat dry milk (NFDM) increased the peak viscosity of the paste by +4.3%, as well as the double addition of salt (+6.3%) and baking powder (+3.0%), comparing to control formulation (Table 5). However, omission of salt (-13.0%) and baking powder (-9.8%) reduced the viscosity of the paste. The increase in viscosities could be explained by the fact that cations reduced intermolecular repulsion and promoted network formation.

Dairy proteins possess functional properties similar to gluten, as they are able to form networks and good swelling properties (Gallagher, 2009). Proteins are typically added to gluten-free applications to increase elastic modulus by crosslinking, improving structure with gelation and to aid in foaming. A whey protein particle system can provide elastic and strain hardening properties to the gluten-free dough when mixed with starch, resulting in a more stable particle system than gluten, probably due to a too high degree of internal crosslinking (Van Riemsdijk et al., 2011).

Changes in the viscoelastic properties of the systems could be attributed to the limitation of starch swelling and gelatinization by sodium caseinate or casein (Bertolini et al., 2005). The addition of higher doses of salt and baking powder to the formulation increase molecular interaction due to the addition of charges to the mix, influencing starch/starch and starch/protein interactions. Besides, the increase in the water absorption capacity of ingredients reduces the amount of free water available to facilitate the movement of particles in batters and consequently gives high viscosity (McCann; Day, 2013; Vetter, 2003).

The omission of NFDM (+13.1%) and baking powder (-14.1%) resulted in different effect in the trough viscosity, i.e. the lowest point the viscosity of the batter reaches when the samples starts cooling to 50ºC after the heating process at 90ºC. Once the paste reaches the maximum peak the drop in viscosity is due to granules breaking down with the sheer stress during the test. The shear not only solubilizes but also shears the amylopectin molecules, which causes a large drop in molecular weight of amylopectin and leads to a subsequent viscosity drop (Batey, 2007).

The breakdown viscosity, which is the difference between peak viscosity and trough viscosity shows that misformulation of NFDM (+8.7%), salt (+17.6%) and baking powder (+11.1%) affects the viscosity of the paste during heat and cooling down process when the ingredients are added twice to the formulation (Table 5).

The misformulation of NFDM had no effect on the final viscosity of the paste ($p>0.05$). The omission of salt (-5.1%), and omission (-2.9%) or double addition (-8.2%) of baking powder from the formulation resulted in a reduction of final viscosity (Table 5).

Omission of NFDM (+30.0%) and double dose of salt (+16.0%) increased the setback viscosity of the paste. The cooling stage, referred as ‘set-back’ and defined as the difference between final viscosity and trough viscosity, reflect the retrogradation of amylose and amylopectin in a starch paste. Typically, starch presenting higher setback values has a greater retrogradation, which can affect the quality and self-life of the product (Zaidul et al., 2007).

The paste took a longer time to reach the maximum viscosity before the onset of cooling (peak viscosity) when salt was omitted from the formulation (+19 sec), when compared to control. Contrarily, double dose of baking powder reduced the time to reach the maximum viscosity (-18 sec), as shown in Table 5. Due to higher polarization, the ions present in the paste can break the hydrogen bonds between the starch molecules to a certain degree. The starch granules can absorb more water and be more easily dissolved. Therefore, the starch mixed with structure-breaking ions or salting-in ions had higher swelling power and solubility (Wang et al., 2017).

6.4.2 Baking performance

Cakes with lower weight were obtained when nonfat dry milk (NFDM) was omitted from the formulation (-1.5%), presenting also a reduction in height (-13.9%) and volume (-19.8%), comparing to control (Table 6).

Due its functional properties, especially water absorption and the ability to form a network similar to gluten, dairy proteins are frequently added to gluten-free formulations (Gallagher, 2009; Gallagher et al., 2003a). The presence of dairy proteins in the batter retain more water inside the crumb during baking, i.e., less water is loss to the surface. In addition, the increase in batter viscosity provided by the milk proteins helps to trap the carbon dioxide produced by the leavening agent, resulting in a higher cake when compared to the misformulation omitting the ingredient. As shown in Figure 1A, omission of NFDM from the formulation resulted in a moisture loss +16.3% higher when compared to control (Fig. 1A).

Gallagher et al. (2003b), evaluating the effect of adding milk protein isolate (3%) and novel rice starch (3%) on gluten-free breads, observed that the addition of milk protein and rice starch resulted in an increase in loaf volume.

Omission of salt increased the height of the cakes (+6.2%), comparing to control formulation. Sodium chloride, is commonly added to cereal based food not only to enhance sensory properties, but also for its impact on the functional properties of cereal constituents. The addition of charges to the mix influence starch/starch and starch/protein interactions, resulting also in larger protein network with more a uniform orientation, which makes the batter more resistant to expand (McCann; Day, 2013; Lynch et al., 2009).

Omission of baking powder reduced the cake height (-23.8%) and volume (-23.8%) while double addition increased volume (+6.4%) but reduced weight (-0.65%), comparing to control formulation. Baking powder is a mixture of chemical substances, fast-acting leavening acid like MCP (monocalcium phosphate monohydrate) and a slow-acting leavening acid like SAPP (sodium acid pyrophosphate), that, by the influence of heat and/or humidity, produce gas to expand batters elaborated with flours, starches and other ingredients, increasing the crumb porosity. Book and Brill (2015), evaluating the effect of chemical leavening on yellow cake properties observed that the formulation containing the lowest amount of soda produced the cake with the lowest volume.

Lighter crust color was observed when any of the three functional ingredients tested (NFDM, salt or baking powder) were omitted from the mix formulation (Table 7). In addition, darker crust and darker crumb were observed when baking powder was added twice or omitted from the formulation, respectively. As for NFDM, this result was expected as dairy powder contain lactose, which can be involved in Maillard browning and caramelization reactions, caused by the reaction between reducing sugar and amino acids. Per 100 grams of NFDM the addition of protein and sugar to the mix is 36.2 and 52.0g, respectively (USDA, 2016). However, crumb color is not significantly affected by dairy product, once high temperature is required for the Maillard or caramelization reactions to occur (Gallagher et al., 2003a).

Previous research has observed similar results, where higher levels of baking powder produced cakes with a darker crust color, and pointed out a possible correlation between cake's pH and color of the crust and flavor (Book; Brill, 2015; Pop, 2007). Even though sodium acid pyrophosphate (36% of the leavening agent) is known for its buffering effect, i.e. it's supposed not to affect the pH when used, it was observed an increase (+1.7%) in the pH by adding double dose of the baking powder (Fig. 1B).

The heat of baking can cause baking soda (30% of the baking powder formulation) to decompose, giving of carbon dioxide leavening gas without reacting with a leavening acid. The sodium carbonate formed by the decomposition of baking soda is very alkaline and will tend to give a baked product a high pH (Vetter, 2003). The lower the pH, the less color development in terms of brown notes will be observed. The higher the pH the more browning will occur because of its effect on Maillard reaction (Oliete et al., 2010). However, the darker crumb color observed when baking powder was omitted from the formulation cannot be explained by the pH results. Omission of the ingredient reduced the pH of the batter (-3.8%), which in theory should have resulted in less color development (Miller, 2016).

6.4.3 Sensory evaluation

Omission of nonfat dry milk (NFDM) from the mix resulted in firm cakes with moistness perception varying from slight dry to gummy (Table 8). However, those changes did not affect the total score observed for acceptability of the cakes. The reduction in moistness perception detected by the panelists is related the moisture loss observed when NFDM was omitted from the mix (+16.3%), as shown in Figure 1A. It is evident that the addition of NFDM to the gluten-free cake mix formulation increase the water holding capacity of the crumb.

Baking powder was the functional ingredient with more detectable changes in the cakes as reported by the judges. Omission of baking powder resulted in cakes slightly tough to tough, firm, and moistness perception varying from slight dry to gummy (Table 8).

Baking powder lightens the texture of cakes by enlarging air bubbles within the batter. The correct use of baking powder makes the difference between a light and fluffy cake and a denser texture. Cake volume is a measure of cake size and reflects the amount of air initially entrapped during mixing and the air, moisture, and CO_2 entrapped and expanded during baking. These gases may be dispersed in small cells throughout a fine crumb or in larger cells throughout a coarser crumb (Miller, 2016). Although high volumes do not always indicate a desirable cake, low volumes generally indicate a heavy, less desirable crumb. As observed in this study, omission of baking powder from the cake mix increased the hardness of the cakes (+264.0% at day 1, Table 9, Fig. 2) and resulted in total acceptance scores given by the judges -10.2% lower than the control formulation (Table 8).

6.4.4 Texture analysis

Texture is a critical factor for consumer acceptance, which can be evaluated in a number of ways. In this study trained panelists (Table 8) and texture profile analysis (Fig. 2 and Table 9) were used to evaluate the quality of cakes and changes in texture during storage. Omission of nonfat dry milk (NFDM) from the formulation increased the hardness of the cakes, compared to control (+137.1% at day 1) (Fig. 2). In addition, significant changes in hardness was detected after 4 days of storage (+39.8%), compared to control formulation (Table 9). Omission of NFDM reduced springiness of cakes (-3.2% at day 1), when compared to control, but did not affect the texture during storage. Increase in chewiness was observed when NFDM (+131.0% at day 1) or baking powder (+281.1% at day 1) were omitted from the formulation, compared to control. For both ingredients (NFDM and baking powder), similar effect on texture during storage was observed, where omission increased cake chewiness after 4 days of storage (Table 9).

Springiness is associated with a fresh, aerated and elastic product, thus high springiness is desirable in this type of products. Low springiness value is indicative of brittleness and this reflects the tendency of the product to crumble when sliced. Krupa-Kozak et al. (2013), evaluating the effect of dairy proteins on gluten-free bread, observed that dairy proteins incorporated at a 12% level decreased the hardness of the baked product.

The addition of NFDM to a gluten free formulation increase the amount of protein in the product, 36.2 g of protein in 100 g of NFDM, increasing the viscosity of the batter and provide the ability to build up a structure capable of trapping the carbon dioxide produced by the leavening agent (USDA, 2016). A higher volume will decrease the hardness of the product and increase its springiness, due to increase in elasticity provided by the milk proteins. In addition, not adding baking powder to the mix formulation will result in a heavy, denser crumb, once there is not gas production to the structure to form.

Salt misformulation affected significantly resilience, springiness and cohesiveness, which indicates how well the cake crumb holds together or conversely, falls apart. Double dose addition of salt increased resilience (+10.2% at day 1), cohesion (+7.2% at day 1) and springiness (+2.7% at day 1) of the cakes (Fig.1), when compared to control. Cakes in which salt was omitted from the formulation presented a reduction for all the three characteristics (resilience, cohesion and springiness) after 4 days of storage, as presented in Table 9. The addition of salt increase the water holding capacity of the cakes (Fig. 1A), playing a significant role in retard the rate of starch retrogradation and consequently from staling too quickly (Wang et al., 2015).

Non-addition of baking powder in the dry mix formulation increase the hardness of the cakes (+264.0% at day 1) (Table 9, Fig. 2), which was also detected by the sensory panelists as previously reported (Table 8). After 4 days of storage, hardness of the cakes increased (+40.2%), when baking powder was omitted. Similar effect was observed for adhesiveness, which increased in the second day of storage (+54.8%), while there were no changes in the cake with control formulation (Table 9).

6.4.5 Pasting properties vs quality of final product

Pasting properties changes presented significant correlations with the quality of the cakes (Table 10). Peak viscosity of the paste showed a moderate negative correlation with hardness (r^2=-0.5707; $p<0.01$) and chewiness (r^2=-0.5494; $p<0.05$) of the cakes crumb. High viscosity of the batter results in a high capacity of the system to trap the gas produced by the leavening agent, resulting in high porosity of the crumb and reducing the hardness and chewiness of the cake.

Trough of the paste presented a strong positive correlation with weight (r^2=0.603; $p<0.01$) of the baked cakes (Table 10). Indicating that, a higher and more stable batter viscosity during baking could result in heavier cakes. The breakdown viscosity of the paste, which is the difference between peak viscosity and trough variable, presented a moderate positive correlation with volume of the cakes (r^2=0.4422; $p<0.05$), and negative with hardness (r^2=-0.5728; $p<0.01$) and chewiness of the crumb (r^2=-0.5593; $p<0.01$).

According to Sanz et al. (2008), batter viscosity has an important effect on the bubble incorporation and movement which are considered controlling factors in cake texture, with higher viscosity of the batter contributing to higher bubble stability before and during baking. If the viscosity is too low, the bubbles in the batter can easily rise to the surface and be lost during baking. Hard and chewy are characteristics usually attributed to cakes with a dense and/or compact crumb, caused by insufficient rising/expansion during baking. As the temperature of the batter rises in the oven, the trapped gas bubbles expand and eventually, just as the mass of batter is setting, they burst into one another to form the porous structure of cake crumb (Al-Dmoor, 2013).

6.5 Conclusions

The misformulation (omission or double dose addition) of any of the three ingredients evaluated affected the pasting properties of the dry mix, such as peak, trough, and final viscosity as well as time to reach the maximum viscosity during the heating phase. The omission of the ingredient from the formulation resulted in small cakes (height and volume reduction – NFDM and baking powder) or increased the height of the cakes (salt), presenting lighter crust color (NFDM, salt or baking powder), when compared to control formulation. Double dose of baking powder produced cakes with a dark crust color and more volume, but reduced weight. Omission of NFDM and baking powder from the mix produced firm cakes with moistness perception varying from slight dry to gummy. Hard and chewy cakes were observed when omitting NFDM or baking powder from the mix, and affected the shelf-life. Double dose of salt increased resilience, cohesion and springiness of the cakes. Peak viscosity and breakdown properties showed a moderate negative correlation with hardness and chewiness of the cakes. Peak viscosity also presented a moderate positive correlation with volume ($r=0.4422$; $p<0.05$).

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Table 1. Leavening agent formulation (baking powder).

Ingredient	Percentage (%)
Sodium acid pyrophosphate (SAPP-28) (ICL Performance Products LP; ST Louis; Missouri, U.S.)	36
Sodium bicarbonate (Arm & Hammer®, Church & Dwight CO. INC., Princeton, New Jersey, U.S.)	30
Corn starch (Great Value®, Wal-Mart, Bentonville, Arkansas, U.S.)	29
Monocalcium phosphate monohydrate (MCP-M) (Regent 12XX®, Innophos, Cranbury, New Jersey, U.S.)	5

Table 2. Gluten-free flour formulation.

Ingredient	Percentage (%)
White rice flour (Bob's Red Mill Natural Foods, Milwaukie, OR, U.S.)	49
Potato Starch (Bob's Red Mill Natural Foods, Milwaukie, OR, U.S.)	19
Tapioca starch (Bob's Red Mill Natural Foods, Milwaukie, OR, U.S.)	19
Garbanzo Bean Flour (Bob's Red Mill Natural Foods, Milwaukie, OR, U.S.)	5
Corn starch (Great Value, Walmart, Bentonville, AR, U.S.)	5
Xanthan gum (Bob's Red Mill Natural Foods, Milwaukie, OR, U.S.)	3

*Commercially milled gluten-free flours.

Table 3. Gluten-free dry cake mixes formulation (%).

Ingredients*	Omission			Normal	Double		
Flour	100	100	100	100	100	100	100
Sugar	80	80	80	80	80	80	80
Nonfat dry Milk	0	12	12	12	24	12	12
Salt	3	0	3	3	3	6	3
Baking Powder	3.5	3.5	0	3.5	3.5	3.5	7

*All ingredients are expressed as percentage in flour basis.

Table 4. Test setting profiles for analyze pasting properties of gluten-free dry cake mixes.

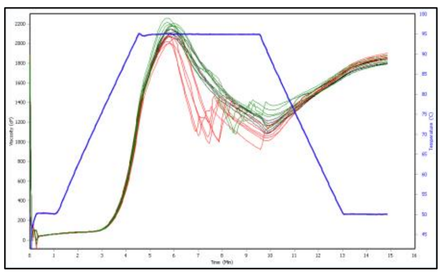
Stages	Temperature/speed	Time
1	50°C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 10 sec
4	50°C	1 min, 0 sec
5	95°C	4 min, 30 sec
6	95°C	9 min, 30 sec
7	50°C	13 min, 0 sec
End of test		15 min, 0 sec

Table 5. Pasting properties of gluten-free dry cake mix under misformulation (omission-red; normal-black; double-green) of functional ingredients (nonfat dry milk, salt and baking powder)*.

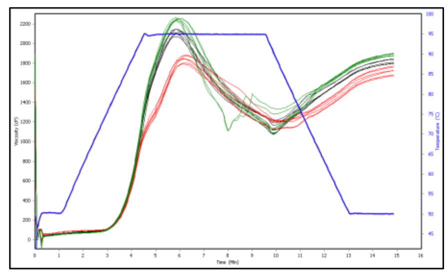
	Peak Viscosity (cP)			Trough (cP)			Breakdown (cP)		
	Omission[a]	Normal[b]	Double[c]	Omission	Normal	Double	Omission	Normal	Double
NFDM	2053.0±14.8bA	2114.5±11.7bA	2206.2±11.8aAB	993.0±15.6bC	1142.7±22.7aA	1149.8±25.2aA	1060.5±14.0aA	971.8±25.4bA	1056.3±34.5aB
Salt	1839.2±15.0cC	2114.5±11.7bA	2248.2±4.9aA	1178.0±17.1aB	1142.7±22.7abA	1105.7±10.7bAB	661.2±5.9cB	971.8±25.4bA	1142.5±10.1aA
Baking Powder	1907.3±14.8cB	2114.5±11.7bA	2178.8±24.4aB	1304.0±13.5aA	1142.7±22.7bA	1099.7±11.3bB	603.3±4.5cB	971.8±25.4bA	1079.2±17.7aAB

	Final Viscosity (cP)			Setback (cP)			Peak Time (min)		
	Omission	Normal	Double	Omission	Normal	Double	Omission	Normal	Double
NFDM	1868.8±9.6aA	1816.7±8.9bA	1831.2±14.4abB	875.8±14.9aA	674.0±16.9bA	681.3±38.4bB	5.86±0.06aB	5.86±0.04aA	5.86±0.06aA
Salt	1723.7±16.6cB	1816.7±8.9bA	1887.5±4.2aA	545.7±8.4cB	674.0±16.9bA	781.8±7.4aA	6.18±0.03aA	5.86±0.04bA	5.90±0.03bA
Baking Powder	1764.7±7.9bB	1816.7±8.9aA	1666.8±10.9cC	460.7±6.5cC	674.0±16.9aA	567.2±10.9bC	5.70±0.04bC	5.86±0.04aA	5.57±0.05cB

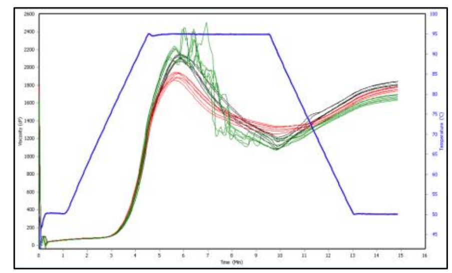
NFDM



SALT



BAKING POWDER



*Means (n= 6 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$). [a]Omission of the ingredient = 0 g per 100 g flour (NFDM or salt or baking powder). [b]Normal dose = 12.0 g of NFDM or 3.0 g of salt or 3.5 g of baking powder per 100 g flour. [c]Double dose = 24.0 g of NFDM or 6.0 g of salt or 7.0 g of baking powder per 100 g flour.

Table 6. Baking performance of gluten-free cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder)*.

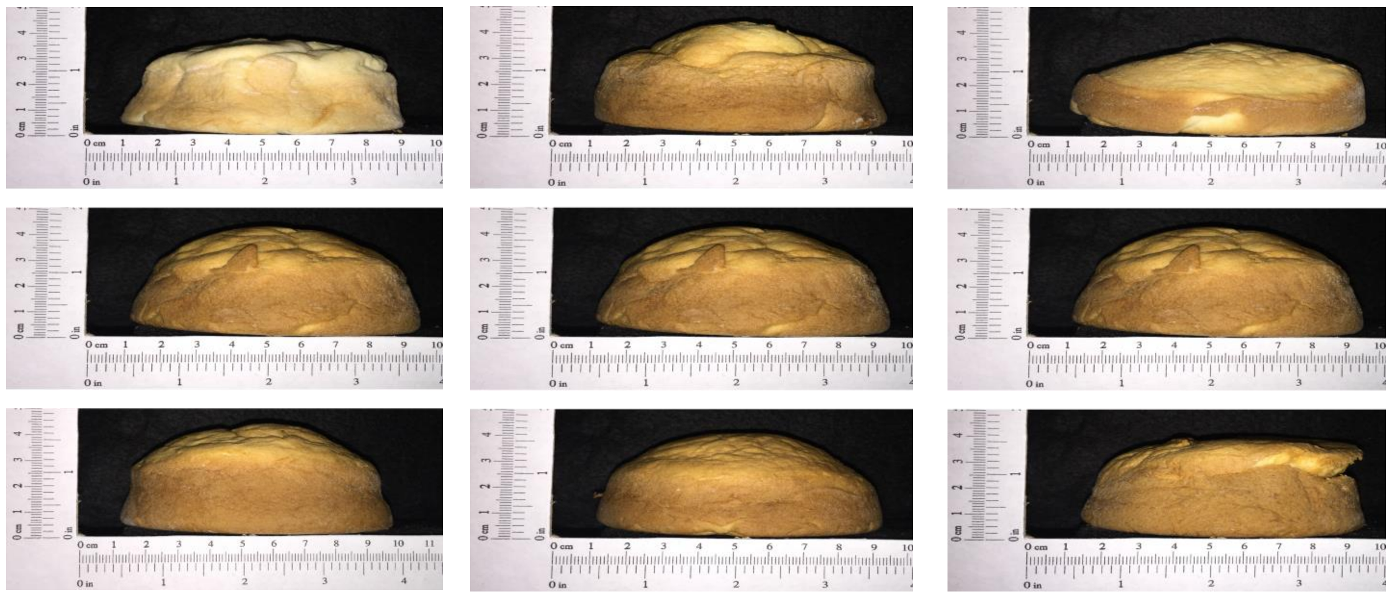
	Weight (g)			Height (mm)			Volume (cc)		
	Omission	Normal	Double	Omission	Normal	Double	Omission	Normal	Double
NFDM	135.0±0.7bC	137.1±0.1aA	137.4±0.3aA	39.1±1.0bB	45.4±0.7aA	46.7±0.9aA	213.2±7.4cB	265.8±12.4bA	286.2±12.8aA
Salt	136.5±0.2aB	137.1±0.1aA	137.5±0.1aA	48.2±0.1aA	45.4±0.7bA	44.4±0.4bB	265.8±18.5aA	265.8±12.4aA	267.9±11.4aB
Baking Powder	137.8±0.1aA	137.1±0.1aA	136.2±0.5bB	34.6±1.1bC	45.4±0.7aA	44.1±0.1aB	202.7±10.3cB	265.8±12.4bA	282.9±12.0aA

NFDM **SALT** **BAKING POWDER**

Omission

Normal

Double



*Means (n= 9 ± standard error) followed by the same lowercase letter within a row (dose; p<0.05) and same capital letter within a column are not significantly different (ingredient; p<0.05).

Table 7. Crust and crumb lightness (L*), hue color (h°) and browning index (BI) of gluten-free cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder)*.

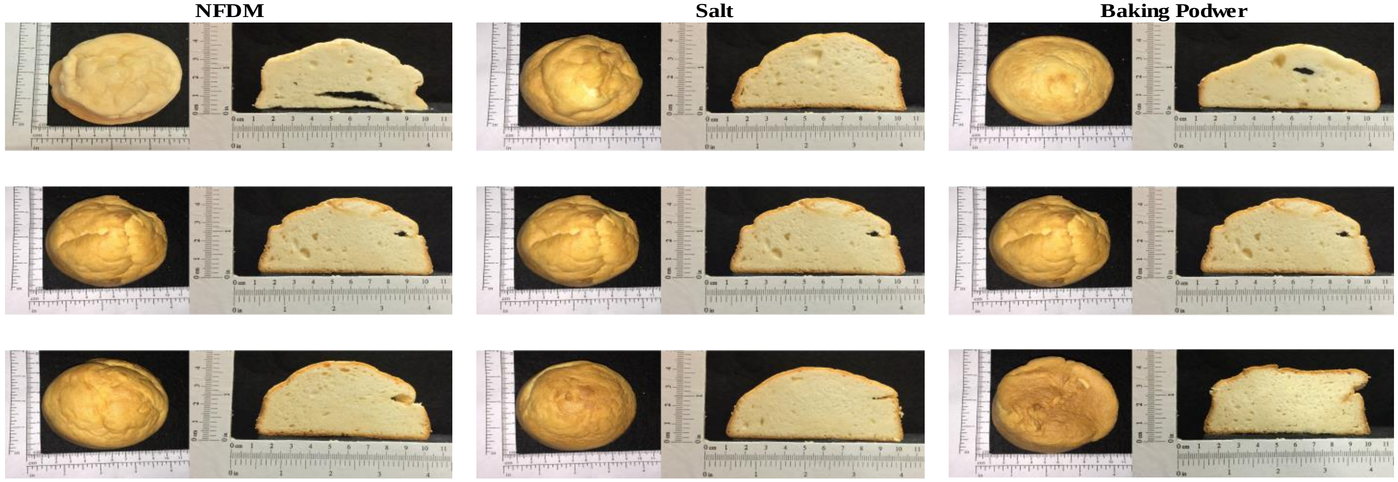
Ingredients	Lightness (L*)			Hue (h*)			Browning Index (BI)		
	Omission	Normal	Double	Omission	Normal	Double	Omission	Normal	Double
					Crust				
NFDM	80.9±0.1aA	65.5±0.6bA	65.8±1.6bA	1.34±0.00aA	1.21±0.01bA	1.22±0.01bA	48.6±0.8bC	95.3±4.2aA	97.9±5.4aB
Salt	69.9±1.2aC	65.5±0.6bA	63.5±0.9bA	1.25±0.01aC	1.21±0.01bA	1.18±0.00cB	87.8±3.3bA	95.3±4.2aA	95.8±2.5aB
Baking Powder	74.3±1.0aB	65.5±0.6bA	56.7±0.8cB	1.27±0.01aB	1.21±0.01bA	1.16±0.00cB	76.5±2.6cB	95.3±4.2bA	105.9±1.5aA
					Crumb				
NFDM	86.7±0.3aAB	88.2±0.7aA	88.1±0.7aA	1.53±0.00aA	1.54±0.00aA	1.53±0.00aA	21.4±0.3aB	22.5±0.3aA	24.0±0.9aA
Salt	88.5±0.0aA	88.2±0.7aA	88.4±0.8aA	1.53±0.00aA	1.54±0.00aA	1.54±0.00aA	24.0±2aAB	22.5±0.3aA	22.2±0.2aA
Baking Powder	84.5±1.3bB	88.2±0.7aA	88.1±0.7aA	1.50±0.02aA	1.54±0.00aA	1.54±0.00aA	26.6±2.3aA	22.5±0.3bA	23.3±0.6bA

NFDM **Salt** **Baking Podwer**

Omission

Normal

Double



*Means (n= 9 ± standard error) followed by the same lowercase letter within a row (dose; p<0.05) and same capital letter within a column are not significantly different (ingredient; p<0.05).

Table 8. Sensory evaluation (acceptance test) of gluten-free cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder)*.

	Uniformity of the Crumb			Tenderness		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	6.2±0.7aB	6.5±0.5aA	6.0±0.4aB	10.9±0.6aA	12.2±0.5aA	12.0±0.6aA
Salt	7.5±0.5abA	6.5±0.5bA	7.8±0.5aA	12.0±0.4aA	12.2±0.5aA	12.9±0.3aA
Baking Powder	7.3±0.5aAB	6.5±0.5aA	7.8±0.4aA	8.8±0.8bB	12.2±0.5aA	12.0±0.5aA
	Size of the Cells			**Softness**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	8.1±0.4aA	7.2±0.4aA	7.4±0.3aA	6.7±0.5bB	7.8±0.4aA	8.3±0.3aA
Salt	7.9±0.3aA	7.2±0.4aA	8.2±0.4aA	8.3±0.4aA	7.8±0.4aA	8.9±0.3aA
Baking Powder	7.8±0.4aA	7.2±0.4aA	8.0±0.4aA	5.8±0.5cC	7.8±0.4bA	8.7±0.2aA
	Thichness of Cell Wall			**Color of Crumb**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	6.7±0.6aA	6.7±0.6aA	6.8±0.5aA	8.5±0.3aA	8.1±0.3aA	8.3±0.2aA
Salt	7.4±0.4aA	6.7±0.6aA	8.0±0.4aA	8.3±0.2aA	8.1±0.3aA	8.5±0.3aA
Baking Powder	6.9±0.6aA	6.7±0.6aA	7.4±0.4aA	7.4±0.3bB	8.1±0.3aA	8.1±0.3aA
	Grain			**Flavor**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	11.7±0.8aA	12.7±0.7aA	12.9±0.6aA	7.5±0.8aA	8.1±0.7aA	9.0±0.6aA
Salt	12.8±0.7aA	12.7±0.7aA	12.7±0.6aA	8.5±0.6aA	8.1±0.7aA	7.2±0.8aA
Baking Powder	12.0±0.7aA	12.7±0.7aA	12.1±0.8aA	6.7±0.9bA	8.1±0.7abA	9.2±0.5aA
	Moistness			**Total Score**		
	Omission	Normal	Double	Omission	Normal	Double
NFDM	7.6±0.4bAB	8.8±0.3aA	8.5±0.3aA	73.6±3.0aB	77.6±2.4aA	78.7±2.5aA
Salt	8.2±0.3aA	8.8±0.3aA	8.4±0.3aA	80.5±2.0aA	77.6±2.4aA	82.1±2.3aA
Baking Powder	7.2±0.3bB	8.8±0.3aA	8.1±0.3aA	69.7±3.0bB	77.6±2.4aA	80.9±2.3aA

* Means (n= 22 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$).

Table 9. Shelf-life of gluten-free cakes under misformulation (omission, normal, double) of functional ingredients (nonfat dry milk, salt and baking powder)*.

	NFDM						Salt						Baking Powder					
	Hardness						Resilience						Hardness					
	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%
Day 1	963.4±172.0aB	0.0	406.3±9.4bA	0.0	409.0±13.6bB	0.0	36.2±1.5bA	0.0	36.4±0.6bA	0.0	40.0±2.7aA	0.0	1478.8±249.0aB	0.0	406.3±9.4bA	0.0	367.8±13.1bA	0.0
Day 2	954.6±103.8aB	-0.9	473.2±22.1bA	16.5	470.6±28.5bB	15.1	34.2±1.5bAB	-5.7	37.2±1.4abA	2.4	40.2±1.5aA	0.5	1588.6±291.1aB	7.4	473.2±22.1bA	16.5	448.2±36.1bA	21.8
Day 4	1346.5±369.1aA	39.8	588.5±5.5bA	44.8	594.8±10.3bAB	45.4	30.7±0.7cC	-15.4	34.8±0.3bA	-4.4	38.8±1.7aA	-3.1	2073.0±229.2aA	40.2	588.5±5.5bA	44.8	633.9±42.0bA	72.3
Day 6	1345.1±193.1aA	39.6	713.1±34.9bA	75.5	761.9±82.9bA	86.3	31.7±1.5bBC	-12.7	36.6±1.7aA	0.7	39.8±1.2aA	-0.7	2219.0±208.1aA	50.1	713.1±34.9bA	75.5	391.9±18.4bA	6.6
	Springiness						Cohesion						Adhesiveness					
	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%
Day 1	87.0±0.7cA	0.0	89.9±0.3bA	0.0	93.5±0.9aA	0.0	0.67±0.02bA	0.0	0.69±0.01bA	0.0	0.74±0.03aA	0.0	-3.14±1.83bC	0.0	-0.69±0.06aA	0.0	-0.36±0.16aA	0.0
Day 2	87.6±0.7bA	0.6	91.6±1.0aA	1.9	93.2±0.5aA	-0.4	0.64±0.02bA	-4.5	0.69±0.02aA	0.0	0.73±0.02aA	-1.4	-1.42±0.83bB	54.8	-0.17±0.05aA	75.4	-0.11±0.01aA	69.4
Day 4	88.3±1.0bA	1.4	91.5±0.4aA	1.7	92.0± 0.5aA	-1.7	0.59±0.01cB	-11.9	0.65±0.00bA	-5.8	0.71±0.02aA	-4.1	-0.20±0.13aA	93.6	-0.04±0.05aA	94.2	0.03±0.02aA	108.3
Day 6	88.0±0.5bA	1.1	91.2±0.3aA	1.4	92.3±0.1aA	-1.3	0.59±0.02cB	-11.9	0.67±0.02bA	-2.9	0.71±0.01aA	-4.1	-0.13±0.04aA	95.9	0.02±0.02aA	102.9	0.06±0.01aA	116.7
	Chewiness						Springiness						Chewiness					
	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%	Omission	%	Normal	%	Double	%
Day 1	582.6±135.9aB	0.0	252.2±8.9bA	0.0	269.9±11.7bA	0.0	90.3±0.9bA	0.0	89.9±0.3bA	0.0	92.4±1.0aA	0.0	961.2±144.8aB	0.0	252.2±8.9bA	0.0	236.0±46.5bA	0.0
Day 2	577.5±77.3aB	-0.9	300.6±19.7bA	19.2	302.9±27.0bA	12.2	89.9±0.2bA	-0.5	91.6±1.0abA	1.9	92.8±0.8aA	0.4	1026.0±202.5aB	6.7	300.6±19.7bA	19.2	233.3±14.2bA	-1.2
Day 4	819.3±252.3aA	40.6	350.8±2.9bA	39.1	352.6±15.6bA	30.6	87.6±1.2bB	-3.0	91.5±0.4aA	1.7	92.5±0.9aA	0.1	1304.1±140.4aA	35.7	350.8±2.9bA	39.1	274.8±23.3bA	16.4
Day 6	757.3±92.9aA	30.0	435.8±32.8bA	72.8	473.3±55.8bA	75.4	88.5±0.4cAB	-1.9	91.2±0.3bA	1.4	93.0±0.3aA	0.6	1319.4±173.6aA	37.3	436.0±32.8bA	72.8	379.0±30.3bA	60.6

*Means (n= 12 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (days of storage; $p<0.05$). Percentage (%) – Difference from the day 1 of storage.

Table 10. Pearson correlation (r) between pasting properties of gluten-free dry cake mixes and final product.

Variables		Peak Viscosity	Trough	Breakdown	Final Viscosity	Setback	Peak Time	Pasting Temperature
Baking Performance	Weight	0.1029 (p=0.657)	**0.6033 (p=0.004)**	-0.2047 (p=0.373)	0.0620 (p=0.790)	-0.3820 (p=0.087)	0.0554 (p=0.811)	-0.1164 (p=0.615)
	Height	0.2573 (p=0.260)	-0.2008 (p=0.383)	0.2758 (p=0.226)	-0.1076 (p=0.643)	0.0784 (p=0.736)	**0.4443 (p=0.044)**	0.2986 (p=0.189)
	Volume	0.4245 (p=0.055)	-0.3035 (p=0.181)	**0.4422 (p=0.045)**	-0.2384 (p=0.298)	0.0761 (p=0.743)	-0.0651 (p=0.779)	-0.1969 (p=0.392)
Crust	Lightness	**-0.5555 (p=0.009)**	-0.0039 (p=0.987)	-0.3939 (p=0.077)	0.3447 (p=0.126)	0.1958 (p=0.395)	0.2469 (p=0.281)	0.1102 (p=0.634)
	Hue*	**-0.5390 (p=0.012)**	-0.0721 (p=0.756)	-0.3507 (p=0.119)	0.2560 (p=0.263)	0.1933 (p=0.401)	0.1910 (p=0.407)	0.1489 (p=0.520)
	Browning Index	0.4039 (p=0.069)	0.1603 (p=0.488)	0.2138 (p=0.352)	-0.3556 (p=0.114)	-0.3100 (p=0.171)	-0.1636 (p=0.479)	-0.1279 (p=0.581)
Crumb	Lightness	0.3548 (p=0.114)	-0.2936 (p=0.196)	0.3880 (p=0.082)	0.0224 (p=0.923)	0.2154 (p=0.348)	0.3841 (p=0.086)	0.2212 (p=0.335)
	Hue*	0.4112 (p=0.064)	**-0.5269 (p=0.014)**	**0.5357 (p=0.012)**	0.1878 (p=0.415)	**0.4691 (p=0.032)**	0.1996 (p=0.386)	-0.0405 (p=0.862)
	Browning Index	-0.3456 (p=0.125)	**0.7667 (p=0.000)**	**-0.5995 (p=0.004)**	-0.3102 (p=0.171)	**-0.7034 (p=0.000)**	0.0307 (p=0.395)	0.3649 (p=0.104)
Texture	Hardness	**-0.5707 (p=0.007)**	0.3608 (p=0.108)	**-0.5728 (p=0.007)**	-0.0091 (p=0.969)	-0.2543 (p=0.266)	-0.1401 (p=0.545)	-0.0437 (p=0.851)
	Adhesiveness	0.3510 (p=0.119)	-0.3639 (p=0.105)	0.4177 (p=0.060)	-0.0769 (p=0.740)	0.2083 (p=0.365)	-0.0726 (p=0.755)	-0.2477 (p=0.279)
	Resilience	0.0390 (p=0.867)	-0.1478 (p=0.523)	0.0959 (p=0.679)	-0.0493 (p=0.832)	0.0745 (p=0.748)	**-0.4741 (p=0.030)**	**-0.6673 (p=0.001)**
	Cohesion	0.2396 (p=0.295)	-0.1760 (p=0.445)	0.2518 (p=0.271)	-0.0628 (p=0.787)	0.0864 (p=0.710)	**-0.5356 (p=0.012)**	**-0.7345 (p=0.000)**
	Springiness	0.2729 (p=0.231)	0.2821 (p=0.215)	0.0643 (p=0.782)	-0.2179 (p=0.343)	-0.3170 (p=0.162)	-0.3302 (p=0.144)	**-0.4391 (p=0.046)**
	Chewiness	**-0.5494 (p=0.010)**	0.3646 (p=0.104)	**-0.5593 (p=0.008)**	-0.0320 (p=0.891)	-0.2698 (p=0.237)	-0.2003 (p=0.384)	-0.1293 (p=0.576)

*Values in bold are significant at 0.05.

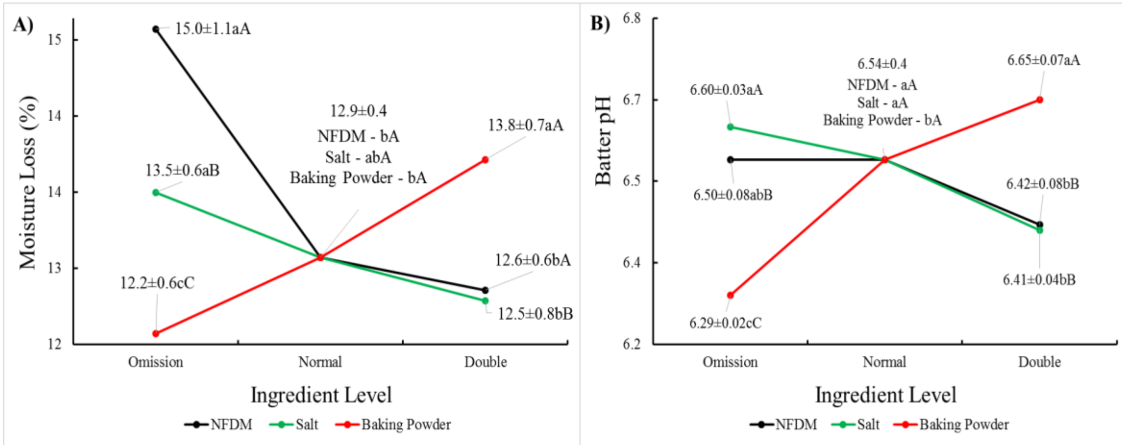


Figure 1. Moisture loss (A) during baking and pH of the batter (B) of gluten-free cakes under misformulation (omission, normal, or double dose) of functional ingredients (nonfat dry milk, salt and baking powder). *Means (n= 9 ± standard error) followed by the same lowercase letter within a row (dose; $p<0.05$) and same capital letter within a column are not significantly different (ingredient; $p<0.05$).

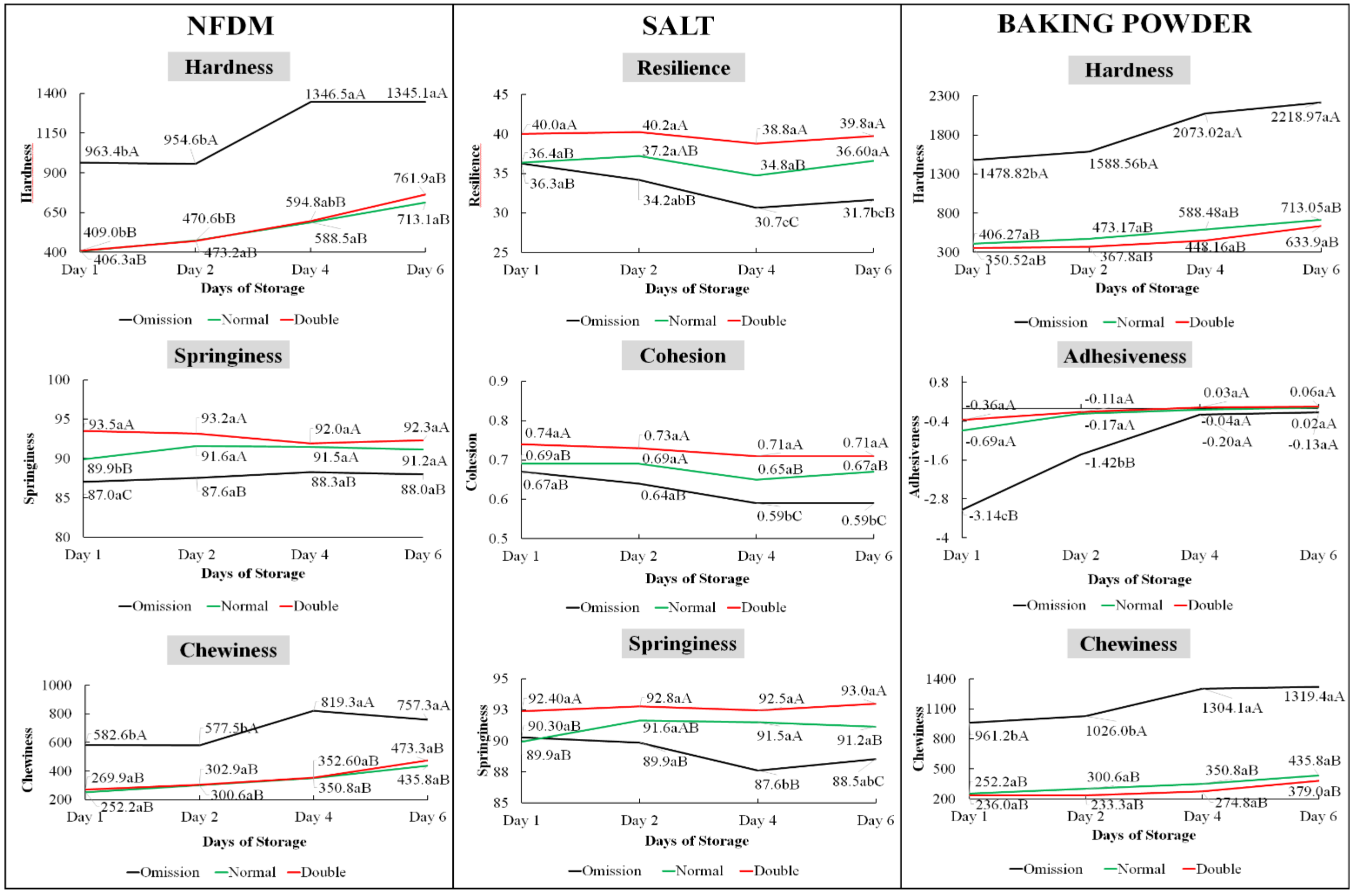


Figure 2. Shelf-life of gluten-free cakes under misformulation (omission-black; normal-green; double-red) of functional ingredients (nonfat dry milk, salt and baking powder). Means followed by the same lowercase letter within a row (days of storage; p<0.05) and followed by the same capital letter within a column are not significantly different (ingredient; p<0.05).

CHAPTER VII

CONCLUSIONS AND FUTURE STUDIES

7.1 Conclusions

The agricultural management practices evaluated (crop succession and increasing doses of nitrogen fertilizer) resulted in changes in the quality of the wheat (BRS 220). Increasing nitrogen doses applied in succession with maize or soybean crop increased total proteins and gluten, and reduced starch content in the kernels, which consequently affected the rheological properties (gluten elasticity and pasting properties). In addition to breeding, management of soil nitrogen would be recommended as additional procedure to obtain raw materials with desired profile in relation to chemical composition and rheological properties. The results demonstrated the importance of studies on appropriate management to maximize productivity and grain quality.

The best conditions for gluten extraction and analysis by compression and recovery test (Gluten CORE) of the particular set of samples (WT 090210, BRS Gaivota, BRS Pardela) were obtained by adding 4.4 mL of water for dough formation, 20 seconds of mixing and 3 minutes of washing with NaCl solution (2%). The gluten extracted using these conditions presented a strong correlation between the elastic recovery properties (%) and the standard methods used to classify wheat according to its technological quality (dough strength and stability), while no correlation was observed with falling number. According to the results, Gluten CORE showed to be an efficient equipment to evaluate the rheological characteristics of wheat gluten, which can help to characterize wheat genotypes for specific uses, as well as to predict the baking process. The next step will be the validation of the method using a larger quantity of wheat genotypes, with different characteristics of technological quality and cultivation sites.

Misformulation (omission or double dose addition) of functional ingredients (non-fat dry milk, salt or baking powder) affected the pasting properties of the dry cake mixes (all-purpose or gluten-free flour). Depending on the ingredient, changes were observed in weight, height, volume, and color of crumb and crust of the baked cakes, as well as in texture and shelf-life. Misformulation of baking powder, omission (gluten-free flour) or double dose addition (all-purpose flour), produced the less acceptable cake by the panelist. The misformulations scenarios studied serve as a tool to relate information at the dry mix level with the final product quality.

7.2 Future Studies

This dissertation mainly focused on develop and apply new rheological methods to test the quality of wheat and dry cake mixes formulations. The measurement of viscoelasticity properties of gluten may be useful in a number of important applications ranging from breeding programs, evaluation during wheat import/export, and flour mills quality control. The misformulation scenarios studied for the dry cake mixes (all-purpose and gluten-free) serve as a tool to relate information of dry mixes quality control with batter viscosity and final product quality, which may be useful in the industry reducing economic losses.

Study I aimed to evaluate the impact of agricultural management practices in the chemical composition (protein and starch content) and rheological properties, such as pasting properties, and gluten content and quality of the wheat flour produced. Due to the low rainfall observed during the field trial in 2010, approximately half of which occurred in 2011 and 1/3 of the amount of water required by the plant, I recommend to continue the study to increase the number of years to increase the power to assess the effect of the treatments and reduce environmental effects. Depending on the region, other legumes crops can be use in succession with wheat such as canola and peanut, for example.

Study II aimed to develop a method to test the quality of gluten extracted from soft wheat genotypes, providing further insight into the behavior of gluten under compression and recovery. Regarding the correlation observed between the elastic recovery % (compression and recovery test) and the standard methods currently used in Brazil to classify the wheat, I recommend to include other cultivars with different technological quality characteristics, especially weaker cultivars since they presented the highest elastic recovery (%) variation. By doing this we will include a wider range of variability and reduce the coefficient of variation (%) in the method.

Study III aimed to evaluate the effect of three functional ingredients (nonfat dry milk, salt and baking powder) on pasting properties (RVA) of cake dry mixes (all-purpose and gluten-free) and baking performance, texture and sensory characteristics of the final product. Further studies including intermediate levels of the ingredients tested would be the ideal, such as 0.5 and 1.5 times the normal dose of the ingredient, to access the effect of the ingredients in a wider range and reduce the coefficient of variation (%) in the method. Other ingredients could also be included in the study such as sugar, shortening and emulsifier, etc. The next step in this study will be the development of a method based on pasting properties to detect misformulation of ingredients in dry cake mixes. For this, an experiment similar to the one used in study III will be executed but instead of the amount of sample to be used in the RVA being corrected to flour basis (%) it will be measured in weight basis. The reason is because, when the dry cake mix industry try to use the method to detect the misformulation of ingredients they may not know the amount of flour or starch in the mix. For this dissertation, the test in RVA was performed measuring the sample in flour basis (%) because we wanted to exclude the effect of flour (starch), once all the treatments had the same amount of flour, and focus on the effect of the ingredients in the pasting properties (Appendix II). For the new study, the samples will be measured in weight basis so the results will be a mix of flour and ingredient effects on the pasting properties.

APPENDIX I

Genotypes containing wheat-rye translocation (1BL/1RS) were corrected as described by Payne et al. (1997): Varieties with a Glu-1 quality score of between 8 and 10 had three points subtracted, those between 5 and 7 had two points subtracted, and those between 3 and 4 had one point removed.

Table 1. Quality score assigned to individual or pairs of HMW glutenin subunits.

Score	**Chromosome**		
	1A	**1B**	**1D**
4	-	-	5+10
3	1	17+18	-
3	2*	7+8	-
2	-	7+9	2+12
2	-	-	3+12
1	Null	7	4+12
1	-	6+8	-

Payne, P. I., Nightingale, M. A., Krattiger, A. F., & Holt, L. M. (1987). The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. Journal of the Science of Food and Agriculture, 40(1), 51-65.

APPENDIX II

For the pasting properties analysis (2.5 g of flour; RVA) and baking of the dry cake mixes (170 g of flour) the samples had their weight corrected to flour basis (all-purpose or gluten-free flour) so all the treatments would contain the same amount of flour, according to equation:

$$Amount\ of\ Dry\ Mix\ (g) = \frac{(Amount\ of\ flour\ desired\ x\ Total\ amout\ of\ dry\ mix\ containing\ 100\ g\ of\ flour)}{100\ g\ of flour}$$

Table 1. Dry cake mixes formulation and amount of dry mix used in the analysis (g).

Ingredients*	Omission			Normal	Double		
Flour	100	100	100	100	100	100	100
Sugar	80	80	80	80	80	80	80
Nonfat dry Milk	0	12	12	12	24	12	12
Salt	3	0	3	3	3	6	3
Baking Powder	3.5	3.5	0	3.5	3.5	3.5	7
Total	186.50	195.50	195.00	198.50	210.50	201.50	202.00
	Amount of Dry Cake Mix (g) – All purpose and Gluten-free						
2.5 g of flour (RVA)	4.66	4.89	4.88	4.96	5.26	5.04	5.05
170 g of flour (Baking)	317.05	332.35	331.50	337.45	357.85	342.55	343.40

*All ingredients are expressed as percentage based on flour weight (100 g flour).

APPENDIX III

Sensory ballot sheet used for bread flavor profile evaluation.

Trained Panelist Test – Cakes

Date____ Gender: Male____ Female ____

Age group: 25 yr or younger____, 26-35____, 36 -45 ____, over 46 yr ____

Instructions:

1. FOOD ALLERGEN WARNING: contains wheat, gluten and nonfat dry milk.
2. Use the score that better describe the sample that you are evaluating.

Response (Physical attributes)	**Sample Number**						
A. CELLS							
Uniformity (Even/Normal-10; Slightly uneven-6; Uneven-2)							
Size (Dense/Normal-10; Close-8; Slightly open-6; Open-4)							
Thickness of Walls (Thin/Normal-10; Slightly thick-6; Thick-2)							
B. GRAIN							
Silky/Normal-16; Harsh-10; Coarse/Corn bread-8 Harsh							
C. TEXTURE							
Moistness (Moist-Normal-10; Slightly dry-8; Gummy-6; Dry-4)							
Tenderness (Very tender- Normal-14; Tender-12; Slightly tough-10; Tough-4)							
Softness (Soft-Normal-10; Slightly firm-8; Firm-4)							
D. CRUMB COLOR							
Bright white-Normal-10; White-8; Slightly dull-8; Slightly creamy (yellow)-8; Creamy (yellow)-6; Slightly dull and slightly creamy-4							
E. FLAVOR							
Normal (no off-flavor)-10; Foreign-0. What kind of off-flavor?							

Comments:

IRB approval

Oklahoma State University Institutional Review Board

Date:	Thursday, August 27, 2015	Protocol Expires:	7/2/2016
IRB Application No:	AG1335		
Proposal Title:	Natural Replacers of Sodium Chloride in Bread and other Baked Goods		

Reviewed and Processed as: Exempt

Modification

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s):

Patricia Rayas Duarte 107 FAPC Stillwater, OK 74078	Sabitri Sharma Gautam 1719 S Jackson Ave #23G Tulsa, OK 74107	Sudhir Kumar Pasupuleti 75 S University Place #5 Stillwater, OK 74075

The requested modification to this IRB protocol has been approved. Please note that the original expiration date of the protocol has not changed. The IRB office MUST be notified in writing when a project is complete. All approved projects are subject to monitoring by the IRB.

- [x] The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

The reviewer(s) had these comments:

Mod to add "Wheat Flour" to the title in consent form and to add "or wheat flour substitution" to the consent form.

Signature :

Hugh Crethar, Chair, Institutional Review Board

Thursday, August 27, 2015
Date

ADULT CONSENT FORM
OKLAHOMA STATE UNIVERSITY

PROJECT TITLE: **Natural Replacers of Flour and Sodium Chloride in Bread and Other Baked Goods**

INVESTIGATORS: Thiago Montagner Souza thiagom@okstate.edu, graduate student assistant
Rayas-Duarte, Patricia pat.rayas_duarte@okstate.edu, principal investigator
Rm 123 Food and Ag Products Center (FAPC)

PURPOSE:
This study will examine the sensory perception of bread and other baked products containing reduction of sodium chloride or wheat flour substitutions.

PROCEDURES
You will complete a questionnaire asking for your perception of the flavor and sensory attributes of bread or other baked products. This study is designed to last approximately 10 minutes.

RISKS OF PARTICIPATION:
The bread or other baked products contain dairy products and **GLUTEN**. If you have allergies to dairy and gluten products you cannot participate in the study.

Other than the allergens gluten and dairy products, there are no known risks associated with this project which are greater than those ordinarily encountered in daily life.

BENEFITS OF PARTICIPATION:
Your participation will help to assess the sensory attributes of the products along with potential consumer acceptability. The society at large will benefit by having novel bread or baked fermented products with different flavor profile in the future.

CONFIDENTIALITY:

You will not be identified individually; we will be looking at the group as a whole. Research records will be stored on a password protected computer in a locked office and only researchers and individuals responsible for research oversight will have access to the records.

COMPENSATION:
You will receive either candy bar or cash inventive(s) for your participation when you complete the study. Candy bar will be for completion of studies that are one session in length and cash compensation of $5 - $15 would be offered for studies of two or more sessions.

CONTACTS:

You may contact any of the researchers at the following addresses and phone numbers, should you desire to discuss your participation in the study and/or request information about the results of the study: Patricia Rayas-Duarte, Ph.D., 123 FAPC, Oklahoma State University, Stillwater, OK 74078, (405) 744-6468. If you have questions about your rights as a research volunteer, you may contact the IRB Office at 219 Cordell North, Stillwater, OK 74078, (405) 744-3377 or irb@okstate.edu

PARTICIPANT RIGHTS:

I understand that my participation is voluntary, that there is no penalty for refusal to participate, and that I am free to withdraw my consent and participation in this project at any time, without penalty.

CONSENT DOCUMENTATION:

I have been fully informed about the procedures listed here. I am aware of what I will be asked to do and of the benefits of my participation. I also understand the following statements:
I affirm that I am 18 years of age or older.
Preface the signature lines with the following statement (expand if appropriate):
I have read and fully understand this consent form. I sign it freely and voluntarily. A copy of this form will be given to me. I hereby give permission for my participation in this study.

______________________________ __________________

Signature of Participant Date

I certify that I have personally explained this document before requesting that the participant sign it.

______________________________ __________________

Signature of Researcher Date

VITA

Thiago Montagner Souza

Candidate for the Degree of

Doctor of Philosophy

Thesis: RHEOLOGICAL PROPERTIES FOR QUALITY CONTROL OF WHEAT AND DRY CAKE MIXES

Major Field: Food Science

Biographical:

Education:

- Completed the requirements for the Doctor of Philosophy in Food Science Oklahoma State University, Stillwater, Oklahoma in /May, 2017.
- Completed the requirements for the Master of Science in Food Science Londrina State University, Londrina, Parana/Brazil in 2013.
- Completed the requirements for the Bachelor of Science in Agronomy Londrina State University, Londrina, Parana, Brazil, 2011.

Experience:

Graduate research assistant in the Food Science Program at Oklahoma State University, Stillwater, OK. May 2013 – April 2017.
Teaching assistant in the Department of Animal Science at Oklahoma State University, Stillwater, OK. January-May 2016.
Teaching assistant in the Food Science Program at Oklahoma State University, Stillwater, OK. January-May 2015.
Graduate research assistant at Londrina State University, Londrina, Parana, Brazil. March 2011 – February 2013.

Professional Memberships:
Institute of Food Technologists (IFT); American Association of Cereal Chemists (AACC); Honor Society - Phi Beta Delta and Phi Kappa Phi.