UTILIZATION OF MODIFIED STARCH AS A FAT REPLACEMENT IN BREAD

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ABSTRACT

Since industrial revolution, scientists started to develop new bread formulations in order to improve bread quality and shelf life. This research investigated the effectiveness of octenyl succinate anhydride (OSA) modified starches, from two sources (wheat and tapioca), as fat replacers in bread formulation. Samples for control were with different levels of shortening (0% and 2%), and for test samples 2% and 4% OSA modified starch and tapioca were used as fat replacers. Tests were performed on dough and baked product (bread). Results showed that samples with 4% OSA modified wheat and tapioca starch can be used as fat replacers in bread production. Dough and bread properties in comparison with controls sample with 2% shortening had better or the same characteristics.
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LIST OF ABBREVIATIONS

APE .................................................................................................................. Acylphosphatidylethanolamine
DAG ................................................................................................................... Diacidgliceride
DGDG ............................................................................................................... Digalactosyldiglycerols
GRAS ............................................................................................................... Generally Recognized as Safe
HMF ................................................................................................................ Hydroxymethylfurfural
HPMC .............................................................................................................. Hydroxypropylmethycellulose
LPC ..................................................................................................................... Lysophosphatidylcholine
LPE ..................................................................................................................... Lysophosphatidylethanolamine
MAG ................................................................................................................... Monoacidgliceride
MGDG ................................................................................................................ Monogalactosyldiglycerols
OSA ..................................................................................................................... Octenyl Succinic Anhydride
PC ....................................................................................................................... Phosphatidylcholine
RVA ..................................................................................................................... Rapid Visco Analyzer
TAG ..................................................................................................................... Triacidgliceride
1. INTRODUCTION

Bread making is a handicraft, with a long tradition. Bread is also a staple food for many people. In countries with established economies, nutrition-related diseases such as obesity, heart disease, diabetes and others are increasing due to high caloric intake. These public health concerns have caused the food industry to develop products with less fat and more complex carbohydrates. Proteins, lipids, and carbohydrates, are the three major food constituents, and fat (lipid) has the highest caloric value (Varela & Fiszman, 2013). Fat replacement is one approach which the food industry may use to produce reduced calorie and low fat foods that are desired by the public. In response to dietary guidelines and health goals, the food industry has introduced a variety of innovative food products designed to help consumers lower their fat intake (Peterson & Sigman-Grant, 1997).

Bread usually contains 2% fat based on flour weight. Fat in bread is usually in the form of shortening, which assists in the entrapment of air bubbles into the dough during mixing and assists in leavening, tenderizes the crumb, contributes moistness, and enhances mouth feel (Pyler & Gorton, 1973).

For the baking industry, optimization of dough properties and the quality of the finished product are high priority. For the consumer, the taste and mouth feel of the finished product are the most important (Jackson, 2009). After baking, bread starts the process of staling. The losses for the baking industry due to bread staling are of great economic significance. Therefore, it is a challenge to improve dough properties and to understand and retard staling to keep bread quality high as long as possible (Curti, Carini, Tribuzio, & Vittadini, 2014).

Producing bread without fat generally reduces the quality aspects of the bread such as loaf volume, soft texture and staling. Therefore, it is of great importance to find some fat
replacers that would maintain or improve bread quality. According to literature, when speaking of fat replacers for bread making, inulin (Morris & Morris, 2012), Simplesse™ (Oganean, Darie, & Oganean, 2006), HPMC (hydroxypropyl metilcellulose) (Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014) and modified starches (Tavakolipour, Vahid-moghadam, & Jamdar, 2014) have shown promising results. These fat replacers have shown excellent results as fat replacers in a variety of food products such as, milk, meat and baked goods.

Modified starches are often used as emulsifiers. Emulsifiers are substances possessing both lipophilic and hydrophilic properties and are referred to as amphiphilic. Emulsifiers can act to make dough stronger. However, the mechanism of dough strengthening due to emulsifiers is not fully understood. Emulsifiers may form liquid films at the interface between gluten and starch, which results in gas retention and improved dough and bread volume (Stampfli & Nersten, 1995).

Adding hydrophobic side chains to hydrophilic starch molecules, starch may adsorb to the interface of water and oil, stabilizing the emulsion. An example of this type of modification is esterification of starch with Ocetyl Succinic Anhydride (Shogren, Viswanathan, Felker, & Gross, 2000; Song, He, Ruan, & Chen, 2006). In this research, OSA modified wheat and tapioca starch were used as fat replacers for bread.
2. LITERATURE REVIEW

2.1. Bread

Bread, in all its countless forms, is the most widely consumed food in the world. It is an important source of carbohydrate, fiber, protein and some minerals (magnesium, phosphorus, iron) (Dewettinck, Van Bockstaele, Kühne, Van de Walle, Courtens, & Gellynck, 2008). Bread has been an essential part of our diet for thousands of years (Scanlon & Zghal, 2001). During the long history of bread, different baking technologies have been developed (Decock & Cappelle, 2005), in order to respond to the demands of a growing market.

Historically, many studies have been conducted to determine the mechanism of bread staling and to study various preventative measures. Many early studies have been conducted on the role of starch on bread staling (Bice & Geddes, 1953; Fuller, 1938; Kim & D'appolonia, 1977; Schoch & French, 1947). In addition to the effects of starch on staling, Katz, (1928) conducted a study on the relationships between bread staling and the processes of starch gelatinization and retrogradation. There had been also significant investigations into effects of fermentation variables on staling (Freilich, 1948) and use of frozen storage for retarding staling in bread (Cathcart & Luber, 1939). The use of polyoxyethylene stearate has shown positive influence on preventing hardening of bread in two ways. First, a very efficient “shortening” action which softens the bread crumb. While the second was a depression of the swelling and swelling power of starch gels which, in effect, causes the starch to be partially stale, the bread therefore does not change as rapidly with age(Volz & Ramstad, 1951). These studies are just a small sampling of the many studies conducted on bread staling since the early 1900’s.

After determining that wheat protein lacks lysine in its composition, Flodin et al., (1993) attempted to introduce lysine by the addition of larger quantities of milk solids or dry yeast to a
bread formulation. Although this was not found to be successful, because the taste or texture of the bread is adversely affected by such additions. In the nineteen-sixties Calhoun et al., (1960) performed a feeding study using white rats with 2 basal diets alone, or supplemented with different amounts of lysine, or with wheat, flour, bread or gluten. They found that the availability of lysine in the products tested in wheat, flour and bread was highest in bread for both diets.

Khatkar et al., (1995) conducted research on the dynamic rheological properties of glutens and gluten fractions (gliadin and glutenin) from different cultivars and how they affect bread quality. These studies are just a few examples of extensive research conducted on evaluation and improvement of bread quality.

Currently, in order to improve the taste and texture of the bread, Flander et al., (2007) developed a new baking technology with high whole meal oat content. Alamir et al.,(2013) studied improvement of energy efficiency through combinations of energy sources in order to decrease the cost of bread making, by developing a mechanistic model of heat and mass transfer in bread. Recently there has also been continued research on bread staling conducted by many researchers. For example a study has been carried out using proton nuclear magnetic resonance ($^1$H NMR) techniques to study water mobility in bread during staling (Curti, Bubici, Carini, Baroni, & Vittadini, 2011). There also have been additional studies on the effects of various ingredients (Gomes-Ruffi, da Cunha, Almeida, Chang, & Steel, 2012; Kerch, Zicans, & Meri, 2010; Purhagen, Sjöö, & Eliasson, 2011), storage temperatures (Aguirre, Osella, Carrara, Sánchez, & Buera, 2011) and baking temperature (Alain Le-Bail, Agrane, & Queueau, 2012) on bread staling. Throughout the history of bread production, scientists have always tried to improve bread properties such as flavor, texture, nutritional value, remove allergens and prevent staling.
2.2. Bread ingredients and manufacturing

Bread, as a leavened product, is produced by fermentation of sugars provided by wheat starch and added sugar. ‘Baker’s percentage’ represents the ratio between ingredients needed when baking bread. The mass of the flour is expressed as a 100% and so the total percentage of all ingredients will be greater than 100%. Therefore, bread is generally made up of 100% flour and approximately 60% water. The remaining ingredients, such as yeast, sugar, salt and fat are added in much smaller quantities of between 1 and 4% (Pyler & Gorton, 1973).

Wheat flour contains two major components needed for production of bread. These are gluten-forming proteins, and starch. Gluten properties have a big influence on the viscoelasticity of dough. The gluten network is formed by two types of proteins found in wheat flour, polymeric glutenins and monomeric gliadins. Based on their quality, quantity, structure, conformation and physical properties it’s possible to define rheological properties of the gluten network (Attenburrow, Barnes, Davies, & Ingman, 1990). Glutenin proteins form a disulfide cross-linked protein polymeric network, while gliadins don’t contribute to the protein matrix formation, but they interact with gluten in structures through non covalent bonds and affect viscous properties of dough. (P. Shewry & Tatham, 1997). Existence of cross-linked bonds in the gluten network explains resistance to extension, but not elasticity of the dough. Elasticity of the dough is explained through formation of hydrogen bonds between glutamine, glycine and proline residues in the protein chain. (Belton, 1999; P. R. Shewry, Popineau, Lafiandra, & Belton, 2000; Tatham & Shewry, 2000).

The gluten matrix entraps other flour components and results in the viscoelastic and related properties that allow the dough to retain gas. However, it is widely recognized that starch also plays an important role in the rheological characteristics of dough (Primo-Martin, Van
Nieuwenhuijzen, Hamer, & Van Vliet, 2007). Starch granules, initially entrapped simply as filler in the gluten network, strengthen this network as they began to swell upon heating. The addition of water plays a large role in the formation of dough and the final properties of bread (Zeleznak & Hoseney, 1986).

The main function of water is hydration and dispersion of dry materials (Letang, Piau, & Verdier, 1999; Primo-Martín, Sözer, Hamer, & Van Vliet, 2009). The texture of the bread after baking will be dependent on the amount water used in the formula during dough mixing. Based on research that Le-Bail (2011) conducted, the size of holes in bread after baking depends on stickiness of the dough, and the stickiness depends partly on the amount of water added during dough mixing. In addition to water, bread dough requires the assistance of yeast for gas production (Papasidero, Manenti, Corbetta, & Rossi, 2014).

Yeast is responsible for flavors in the bread and its porous texture through fermentation. Sugar is often added for initiation of fermentation (Hidalgo & Brandolini, 2011). During the fermentation of wheat starch, by baker’s yeast (*Saccharomyces cerevisae*), CO₂, water and other compounds are produced. Carbon dioxide inflates gas cells and dough volume depends on the amount of CO₂ formed and the ability of the dough to hold the gas (Salvador, Varela, Fiszman, & Gómez, 2006). Razei et al., (2014) reported that beside the CO₂ there are other products formed during fermentation, which are responsible for bread flavor and rheology. Some of those products are ethanol, succinic acid and hydrogen peroxide. The flavor accrued by yeast is enhanced with the addition of salt, but salt also contributes to the functionality of the dough (Zhao, Kinner, Wismer, & Gaenzle, 2014).

Salt influences gluten strength and decreases yeast activity in the dough, thus retarding gas production. Salt also enhances bread flavor and influences hydrophobic interactions as they
induce conformational changes of the biopolymers in the dough (Simsek & Martinez, 2015). It is generally agreed that salt increases dough development time, resistance to extension and extensibility, gelatinization temperature and maximum hot paste viscosity of wheat dough (Linko, Härkönen, & Linko, 1984).

2.3. Fat and it’s function in bread manufacturing

Lipids are important in bread making because they provide mouth feel, longer shelf life and texture of the bread. In bread they originate from three sources: wheat flour, shortenings and emulsifiers.

2.3.1. Wheat flour lipids

In wheat kernels, lipids are distributed in bio-membranes of cells and organelles in oil rich tissues such as scutellum, embryonic axis and aleurone layer of the wheat kernel (Douliez, Michon, Elmorjani, & Marion, 2000). Therefore, the terminology of wheat or flour lipids is greatly dependent on the extraction conditions, including the extractants (solvents), extracting temperature, moisture contents, or extraction and quantification methodology. In addition, because lipids are unevenly distributed in wheat structural parts, lipid content and composition are also affected by milling practice, i.e., flour extraction rate, various milled streams, etc. Furthermore, the growing environments as well as the genetic backgrounds of the wheat result in variations in lipid content and composition. Many abbreviated terms are used in order to simplify a complex subject. Besides the three sources of lipids that were previously mentioned, there are lipids class (phospholipids) which form starch lipid complexes (Pasini, Riciputi, Verardo, & Caboni), and they are more saturated than other lipid classes. Examining at the total lipid amount in wheat, it has been found that the two most prevalent fatty acids are linoleic acid (18:2, 55-60%) and Palmitic acid (16:0, 17-24%) (Morrison, Mann, & Coventry, 1975).
According to Edwards et al., (2010) lipids are found in small amounts (2.5-3.3%) in the wheat caryopsis. Among the wheat lipids in caryopsis tissues, 30-36% are located in the germ, 25-29% in the aleurone, and 35-45% in the endosperm. The composition of wheat endosperm, regarding lipids, significantly differs from germ and aleurone. It has been found that wheat endosperm contains galactolipids (mostly monogalactosyldiglycerols [MGDG], digalactosyldiglycerols [DGDG]) and phospholipids (mainly lysophosphatidylcholine [LPC], phosphatidylcholine [PC], lysophosphatidylethanolamine [LPE], and acylphosphatidylethanolamine [APE]) (Finnie, Jeannotte, Morris, & Faubion, 2010). Germ and aleurone lipids are mostly nonpolar, and they include free fatty acids, MAG, DAG and TAG (Marti, Torri, Casiraghi, Franzetti, Limbo, Morandin, et al., 2014).

2.3.2. Shortenings

Lipid shortenings, used mostly for bread manufacturing, usually represent a mixture of oils and fats that are partially or fully hydrogenated, and sometimes have the addition of emulsifiers and other additives. Soybean oil, palm oil and rapeseed oil are the main vegetable oils used in industrial shortenings (Braipson-Danthine & Deroanne, 2004). Shortenings used in bread baking have a wide range in level of plasticity at room temperature (Rogers, Zeleznak, Lai, & Hoseney, 1988). Typical plastic shortening contains partially hydrogenated blend of soybean (85%) and cottonseed (15%) oils, representing 93.5% of the shortening. The remainder of the shortening components include monoacylglyceride of vegetable oil, which contains fatty acid group, 4.2%, propylene glycol monostearate 1.8% and polyoxyethylene, sorbitan monostearate (pH 7.0) 0.5% (Ghotra, Dyal, & Narine, 2002). The purpose of shortenings in bread production is to plasticize and lubricate dough, as well as increase the rise of the dough, oven spring and loaf
volume. The shortening also affects crumb structure, tenderizes the baked bread and extends the shelf life (Le Bail, Monteau, Margerie, Lucas, Chargelegue, & Reverdy, 2005).

In bread formulation, bakers usually add 2-5% of shortening according to “Bakers percent” (Mondal & Datta, 2008). Determination of the correct amount of shortening to add in the bread formula is based on the ratio of solid to liquid phase, depending on the amount shortenings that transitions to the liquid phase, which will determine the plasticity of shortening (Hallberg & Chinachoti, 1992). The next factor that defines the amount of shortening added is based on the crystal structure of shortening. The crystalline structure of the shortening will determine the level of supercoiling and shear rates during bread production (Yi, 2008). Besides these two factors, the level of autoxidation of the shortening, which is affected by unsaturation of the shortening lipids, plays a very important influence of how much shortening is added to the bread formulation (Matuda, Parra, Lugao, & Tadini, 2005).

2.3.3. Surfactants

Surfactants or emulsifiers are a class of compounds that are amphiphilic, which means that they contain both hydrophilic and hydrophobic characteristics. These compounds are surface-active agents, meaning that they have the ability to decrease interfacial tension between two different phases such as water and oil (Gomes-Ruffi, da Cunha, Almeida, Chang, & Steel, 2012). The use of emulsifiers is expected to improve dough strength, water absorption, tolerance to resting time, shock and fermentation, improve crumb structure, slicing characteristic, crust thickness, symmetry, gas retention, oven spring, proofing, loaf volume, shelf-life of bread and most important reduce the amount of shortening (Eduardo, Svanberg, & Ahrné, 2014). In bread making, surfactants are usually divided into the two categories of dough strengtheners and crumb softeners. Strengtheners interact with gluten network, while crumb softeners react with starch.
The most popular crumb softeners are monoacylglycerol (MAG) and diacylglycerol (DAG), while the best dough strengtheners are derived from acylglycerol (diacetyl tartaric acid esters) (Azizi, Rajabzadeh, & Riahi, 2003).

### 2.4. Lipid interaction in bread making

The main task of lipids occurs in the mixing stage of bread processing. During mixing of all ingredients for bread production, after their hydration, gluten matrix is formed and wheat flour lipids become bound or trapped in that matrix. Entrapment of wheat flour lipids in the gluten matrix allows them the ability to increase gas cell’s ability throughout the bread making process (Gerits, Pareyt, & Delcour, 2015). The role of shortening is to plasticize the dough during the mixing stage so that lipids from shortening will coat the components of the gluten-starch matrix, and so reduce the amount of water needed to reach the desired dough consistency (Mehta, Scanlon, Sapirstein, & Page, 2009). During the mixing stage, shortenings also lubricate spaces in the gluten network, resulting in a reduction of elasticity (Sroan, Bean, & MacRitchie, 2009). Next, the role of shortening during mixing is incorporation of air that allows the CO$_2$ that is formed during the fermentation stage to diffuse into these air pockets in the dough (Lucas, Doursat, Grenier, Wagner, Trystram, & Flick, 2015). While shortenings allow air entrapment in the dough matrix, emulsifiers can increase air entrapment capacity and form smaller cells during the mixing process, that provide lower surface tension. By lowering surface tension, emulsifiers provide stabilization of gas cells in bread dough (Singh, GUJRAL, & SINGH, 2002).

After dough mixing, the next steps are fermentation and proofing. During these steps carbon dioxide and ethanol are formed, and gas diffuses into previously formed and stabilized air pockets in the dough (Sroan, Bean, & MacRitchie, 2009). Fermentation causes a noticeable change in dough volume and in order to evenly distribute gas cells in dough, it is necessary to
punch the dough. The purpose of having even gas cell distribution is to achieve a more uniform crumb with smaller cells (Pauly, Pareyt, Fierens, & Delcour, 2014). During fermentation, the interaction between the gluten matrix and lipids is very important. The gluten network retains gas, while lipids provide viscosity of dough and stabilization of gluten film formed between gas cells, and ultimately increase gas retention (Brooker, 1996).

2.5. Fat replacers

Fat is a very important ingredient in bread, which gives many positive characteristics to bread properties. Recently, because of high fat content in foods, which is associated with health concerns, consumers have demanded low fat products. However, consumers expect that these low-fat products have not lost any of their texture or quality (Zanoli, François, Midmore, O’Doherty-Jensen, & Ritson, 2007). During the last years, many researchers were dealing with finding appropriate fat replacers in different food systems.

Many types of fat replacers are used to lower the fat content of food and the type of fat replacer used affects the appearance and taste of the food. The three main categories of fat replacers are based on a carbohydrate, protein or lipid (Jones & Jonnalagadda, 2005; Ognean, Darie, & Ognean, 2006; Oreopoulou, 2006). Fat replacers in baked products should be stable at high temperatures and be classified as Generally Recognized as Safe (GRAS) (Dreher, Leveille, Auerbach, Callen, Klemann, & Jones, 1998). O’Brien et al. (2003) studied the performance of inulin gel in wheat bread compared with normal shortening and found that bread containing 2.5% inulin gel was similar in qualitative characteristics to the control. But Morris and Morris, (2012) found that addition of inulin to bread generally resulted in smaller loaves with a harder crumb and darker color. So, there is still a great deal of work to be done to establish whether bread prepared with inulin will be able to reach a significant activity and be manufactured
without compromising consumer acceptance. Esteller et al., (2004) compared different hamburger bun formulas containing sucrose and fat replacers such as polydextrose, sucralose and Benefat©. The tests were based on measuring the texture of the buns during a 10 day period, and comparing those results to a control sample. These ingredients showed improvement of the texture of buns and reduced staling when used separately or in combination. Guarda et al., (2004) studied the use of several hydrocolloids with different chemical structure and from diverse origin in the bread making process. The hydrocolloids studied were sodium alginate, xanthan, κ-carrageenan and hydroxypropylmethycellulose (HPMC). HPMC showed positive influence on fresh bread quality and bread staling. All hydrocolloids were also able to reduce the loss of moisture content during bread storage, reducing the crumb dehydration rate. In addition, alginate and HPMC showed an anti-staling effect, retarding the crumb hardening.

Zoulias, (2002) and his team worked with five different fat replacers in fat-reduced cookies and tested textural characteristics. Five types of fat replacers used in their research were an improved polydextrose, a maltodextrin with low dextrose equivalent (Dairytrim™), an oat derived product rich in β-glucans, an oligofructose (inulin) and a blend of microparticulated whey proteins and emulsifiers (Simplesse®). According to this research, depending on the type of fat replacer added, an increase in polydextrose content resulted in harder cookies, while an increase in maltodextrin, Simplesse or inulin content had the opposite effect.

Recent research has been done on the influence of fat replacers, such as HPMC (hydroxypropyl methylcellulose) and inulin, on the quality of biscuits (Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014). The instrumental analysis of biscuit texture showed that use of inulin and HPMC resulted in harder biscuits. However, the consumer study revealed that fat replacement of up to a certain level of inulin or HPMC provided acceptable biscuits, but higher
replacement did not show good quality in biscuits. These results show that the level of fat replacement is very important for end product quality (Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014).

2.6. OSA modified starch

Regarding modified starches as fat replacers, recently there have been studies that showed satisfying results using octenyl succinic anhydride (OSA) esterified starches. Some of these studies have investigated changes in dough rheology or bread quality when using OSA starches as fat replacers (Dapčević-Hadnađev, Dokić, Pojić, Hadnađev, Torbica, & Rakita, 2014; Hadnađev-Dapčević, Dokić, Hadnađev, Pojić, Rakita, & Torbica, 2013; T. D. Hadnađev, Pajić-Lijković, Hadnađev, Mastilović, Torbica, & Bugarski, 2013; T. R. D. Hadnađev, Dokić, Hadnađev, Pojić, & Torbica, 2014; Sarneel, Peremans, & Jonckers, 2006). In all of these studies the main goal was to satisfy consumers and their needs, which would be low fat products with better or similar characteristics to full fat products.

Starches have always been essential to human nutrition, as they are the only polysaccharide carbohydrate digestible by humans (Miao, Jiang, Jiang, Zhang, & Li, 2015). The wheat grain can be roughly divided into three parts: (1) germ, 2%; (2) endosperm, 85% and (3) husk, 13% (De Brier, Hemdane, Dornez, Gomand, Delcour, & Courtin, 2015). The wheat endosperm has a cellular structure and each cell is filled with starch granules. Size of granules vary from 1 to 40 microns. Between these starch granules is a material which contains, proteins, the minerals and enzyme of the endosperm. The entire area between starch granules in the endosperm is filled with wheat protein. Water-soluble proteins are confined to locations immediately surrounding the granules and this area is capable of rapid swelling upon hydration. There is evidence of residues of the original amyloplast membranes (Tomlinson & Denyer,
2003), rich in lipids separating the starch and protein, as well as those of endoplasmic reticulum, existing around the starch granule. The soluble proteins associated with starch granules form a complex, known as starch-gluten complex. The total water-soluble material appears to play the role of a cementing substance between starch granules and storage protein (Schiraldi & Fessas, 2012). Wheat starch contains about 30 % amylose and 70 % amyllopectin.

Tapioca starch shows a wide variation in the chemical composition, ash (0.03–0.29%), protein (0.06–0.75%), lipid (0.01–1.2%), phosphorous (0.0029–0.0095%), and fiber (0.11–1.9%). Compared with cereal starches, tapioca starch contains much less amount of lipids. The amylose content is a major quality attribute of starch and determines diverse properties of starch and eventually the end-use purposes, which has been subjected to a great deal of investigation. The amylose content ranged from 15.2 to 26.5% in tapioca genotypes from a world collection. Genotypes with higher amylose content are sometimes desirable for specific applications (Zhu, 2015).

Being a very important nutritional compound, generally starch can be also used for a variety of purposes, including thickening, gelling, increasing the process of stability and replacing or extending costly ingredients. Native starches have relative low solubility in water and a limited functionality. Native starches are inherently unsuitable for most industrial applications (van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002) and are often being designed by chemical or physical modification in order to develop desirable functional properties, such as solubility, pasting properties, dispersion or digestibility. Modification of starches with octenyl succinic anhydride can suppress undesirable properties of native starches (T. R. D. Hadnadev, Dokić, Hadnadev, Pojić, & Torbica, 2014). Modification of starch with OSA results in the creation of amphiphilic molecules of starch, which possesses
hydrophobic characteristics without modifications. This means that starch can adsorb to the interface of water and oil, and therefore stabilize the emulsion. Application of OSA modified starch, due to its amphiphilic characteristics, is very wide (Domian, Brynda-Kopytowska, & Oleksza, 2015; Krstonošić, Dokić, Nikolić, & Milanović, 2015; Miao, Jiang, Jiang, Zhang, & Li, 2015; Wu & McClements, 2015).

Synthesis of OSA modified starches is done under alkaline conditions by the reaction of esterification. The modification of starch through esterification with dicarboxylic acids was patented in 1953 by Caldwell and Wurzburg (Xie, Liu, & Cui, 2005). A common chemical modification of starch in order to achieve amphiphilic properties is esterification of the starch and anhydrous octenyl succinic acid under alkaline conditions. The reaction is performed under heterogeneous alkaline conditions, and it is expected that the substituent distribution be unevenly distributed between granules and the octenyl succinate groups to be favorably connected at the surface of the granules (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). Substitution with OSA can occur at carbon 2, 3 and 6 in the glucose molecule (Dapčević-Hadnađev, Dokić, Pojić, Hadnađev, Torbica, & Rakita, 2014). Structure of modified OSA starch is presented in Figure 1.

![Figure 1. Structure of OSA-modified starches (Adapted from: (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013))](image)

OSA starches have been used successfully for encapsulation. Emulsification of bioactive molecules has been shown to be an effective way of increasing the bioavailability of drugs and
nutrients. Cheuk et al., (2015) showed that octenyl succinic anhydride modified starch can be used to encapsulate coenzyme Q10 (CoQ10). During encapsulation OSA modified starch shows good protection against oxidation. Another use of OSA modified starches is in beverage emulsions. OSA modified starches are colorless, tasteless substances in solution that gives the high consistency of quality to a beverage (Piorkowski & McClements, 2014).

Using OSA starches in cheese from Brazil, showed that by binding extra water, starch creates a sense of lubricity similar to that of full-fat products. But it did not replace the non-polar functional properties of fat, such as palatability, creaminess and flavor-carrying capacity (Diamantino, Beraldo, Sunakozawa, & Penna, 2014). Culinary products like low fat spreads provide some advantages; e.g., starches are cholesterol-free unlike egg yolk. Since starches increase the viscosity of the continuous phase (Tesch, Gerhards, & Schubert, 2002), it is possible to reduce the cost for the final product, because emulsifying starches may act as a combination of surfactant and stabilizer (Dar & Embuscado, 2014).

Also, OSA modified starches can be used for films and coatings as well as for gel production (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). OSA starches are able to improve water retention, texture, thickness, freeze-thaw stability and other properties of baked products (Kittipongpatana, Chaitep, Kittipongpatana, Laenger, & Siroth, 2007). Certain publications claim that OSA modified starch can be used to produce resistant starch (RS). Resistant starch (RS) is defined as a portion of the starch that survives digestion in the small intestine of healthy humans. There are five types of RS, (Landon, 2007) and OSA starch is classified as type four (RS4), chemically-modified starch (Wang, Jin, & Yuan, 2007).

It is of great importance to understand the mechanism of emulsion formation and its effects. The emulsification process includes two steps (Walstra, 1993). The first one includes
deformation and disruption of droplets, while the second one refers to a stabilization of the newly formed interfaces. Emulsifiers adsorb on surfaces because of theirs amphiphilic characteristics. When dissolved in water, such macromolecules preferentially migrate to the air/water interface, forming a boundary layer, whereby hydrophobic groups are oriented towards the air and starch extending into the water. The quality of an emulsion depends on the droplet size of the dispersed phase. Usually, the goal of emulsification is to produce droplets as small as possible (McClements, 2002). Droplets tend to merge together, which explains emulsions of thermodynamic instability. This could be accomplished by controlling merging frequency (Hung, Lin, & Lee, 2010).

2.7. Bread staling

Staling of bread indicates decreasing of consumer acceptance of the product. Changes occur in bread crumb. Changes that occur in bread crumb are increase in bread crumb hardness and crumb opacity, changes in bread taste, crumbliness and starch crystallization, decrease in β-amylase susceptibility if the starch and in soluble starch content (Gray & Bemiller, 2003). Also how staling progresses with time moisture loss occurs. This changes occur in certain order, so first is hardening and toughening of the crumb, then crumbliness starts to appear, next phase is moisture loss by evaporation (Baik & Chinachoti, 2000).

Processes that result in crumb firming are mostly connected with starch retrogradation. When starch retrogrades, it reverts in part from an amorphous state to a less hydrated crystalline state, with simultaneous release of water that is presumably absorbed by the gluten proteins (Morgan, Gerrard, Every, Ross, & Gilpin, 1997). During baking, because of restricted amount of water in dough, starch granules undergo just a partial gelatinization. During swelling linear fraction of starch, amylose, begins to dissolve and diffuse into the surrounding aqueous medium,
and form a concentrated solution after some time. By the time loaf is cooled after baking, this concentrated solution sets up into an insoluble gel structure. This gel structure doesn’t undergo any change in fresh bread, so amylose doesn’t affect staling of a bread (S Hug-Iten, Escher, & Conde-Petit, 2003).

The firming of the bread crumb structure during staling is completely dependent on branched fraction of starch, amylopectin (Susanna Hug-Iten, Handschin, Conde-Petit, & Escher, 1999). In dependence how much free water bread loaf has, amylopectin molecules will be diluted in certain level (Zeleznak & Hoseney, 1986). In solution like that, branched parts of amylopectin molecules gradually undergo aggregation by aligning and associating with each other through different type of bonds. System like this has less firm structure and dissolves much easier than firm gel that amylose molecules form, what brings to a slow stiffening of the internal structure of the swollen starch granule (Hallberg & Chinachoti, 2002). This transformation is most accounted for bread staling, by transforming soft, extensible, swollen starch granules from fresh bread into firm, rigid granules found in same structure in stale bread.
3. OBJECTIVES AND NEED STATEMENT

3.1. Research objectives

Objective 1: To evaluate properties of dough using modified OSA wheat and tapioca starches as fat replacers.

Objective 2: To evaluate properties of end product quality of bread made with modified OSA wheat and tapioca starches as fat replacers; and

Objective 3: To determine which one of the modified starches is more preferable in bread formulation.

3.2. Need statement

Using fat in bread production is expensive, and from the diet point of view, it counts as high caloric food. As obesity is a significant problem in the USA and many other countries, many industries are turning to the fat replacers in food. Fat replacers are present in the food industry, but there is a lack of information regarding OSA modified starches as fat replacers. This study will investigate properties of modified OSA wheat and tapioca starch as fat replacers, and compare these two modifications of starch, which one gives better properties to bread. By examining the properties of bread loaf after replacing fat with OSA wheat or tapioca starch, some conclusions may be drawn as in difference of influence of these two modified starches. It will be of significant advantage to determine if, when using OSA starches as fat replacers, the dough and bread quality remain the same, are improved or are undesirable in comparison with loaves made with 0 or 2% fat.
4. MATERIALS AND METHODS

4.1. Materials

Hard spring wheat patent flour was obtained from North Dakota Mill (Grand Forks, ND). The flour had a protein content of 13% and an ash content of 0.48% (14% moisture basis).

Native wheat starch was purchased from Sigma (S5127, St. Louis, MO) and the native tapioca starch was a gift from Ingredion (Bridgewater, NJ). Instafirm yeast used for baking was purchased from Lallemand Inc. (Montreal, Canada) and Doh-tone α-amylase was supplied by Caravan ingredients (Lenexa, KS). The octenyl succinic anhydride (Espinosa-Ramírez et al.) was purchased from Dixie Chemical Company (Pasadena, TX). All other chemicals and reagents were supplied by Sigma and of at least ACS grade.

4.2. Methods

4.2.1. Esterification of starches with octenyl succinic anhydride

OSA starches were characterized by the Wheat Quality and Carbohydrate Chemistry group in the Department of Plant Science at North Dakota State University. Commercial samples of native wheat and tapioca starches were esterified according to the method of Han and BeMiller (Han & BeMiller, 2007). The starches (100 g, db) were dispersed in water (225 mL) with continuous stirring. The slurry (at ~ 25 ºC) was adjusted to pH of 8.5~9.0 with 1M NaOH. Octenyl succinic anhydride (3g [3 mL], 3% of the weight of the starch) was added at room temperature (~ 25 ºC), while continuously stirring and maintaining pH at approximately 8.5. After 6 h of stirring and maintaining the pH at 8.5, the starch slurries were neutralized to pH 7.0 with 1M HCl. The modified starches were centrifuged (2.5 krpm x 15 min). The residues were washed three times with water and once with acetone, and then air-dried (40ºC, 24 h).
Table 1. Composition of flour samples analyzed with no fat, shortening and octenyl succinic anhydride esterified starches as fat replacers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Shortening</td>
<td>Commercial patent flour No fat</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>Commercial patent flour 2% Vegetable shortening</td>
</tr>
<tr>
<td>2% OSA Wheat</td>
<td>Commercial patent flour 2% OSA-wheat starch</td>
</tr>
<tr>
<td>4% OSA Wheat</td>
<td>Commercial patent flour 4% OSA-wheat starch</td>
</tr>
<tr>
<td>2% OSA Tapioca</td>
<td>Commercial patent flour 2% OSA-tapioca starch</td>
</tr>
<tr>
<td>4% OSA Tapioca</td>
<td>Commercial patent flour 4% OSA-tapioca starch</td>
</tr>
</tbody>
</table>

4.2.2. Determination of degree of substitution

The degree substitution (the average number of hydroxyl groups substituted per glucose unit) was determined by $^1$H nuclear magnetic resonance (NMR). The OSA modified starch samples were purged with deuterium oxide (D$_2$O) 3 times to remove excess water. The starches were dissolved in 1ml D$_2$O by stirring while heating at 80°C for 2 hours before lyophilization. Before analysis, the samples were dissolved a final time in 650μl of D$_2$O, as previously described and transferred to NMR tubes (Wilmad NMR tubes, 528-PP-8).

The $^1$H NMR spectra were obtained using a Bruker 400 MHz instrument (Billerica, USA). The analysis was conducted at 25°C for 64 scans with a delay time of 1 s. The degree of substitution (Edwards) was calculated according to the method of Shih and Daigle {Shih, 2003}. 

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Integration and analysis of the spectra was conducted with Topspin software version 3.2. The equatorial proton of the anhydroglucose unit of starch (Oluwasina, Lajide, & Owolabi, 2014) (δ 5.2-5.4 ppm) was considered to be the internal standard. The extent of OSA substitution was determined by integration of the methyl protons of the OSA (δ 0.8-0.9 ppm). The degree of substitution was calculated as such, DS = A0.8-0.9/ (3 x A5.10-5.26), where A is the integral value of the peak assigned.

The amylopectin and amylose contents and molecular weights were determined by high performance size exclusion chromatography with multiple angle light scattering detection (HPSEC-MALS) (Simsek, Whitney, & Ohm, 2013; You & Lim, 2000).

Table 2. Chemical characterization of OSA starches used as fat replacers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Degree of Substitution</th>
<th>Amylopectin %</th>
<th>Amylose %</th>
<th>Amylopectin (Mₖ, Da) (10⁷)</th>
<th>Amylose (Mₖ, Da) (10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSA Tapioca</td>
<td>0.018</td>
<td>75.53</td>
<td>24.47</td>
<td>1.22</td>
<td>8.63</td>
</tr>
<tr>
<td>OSA Wheat</td>
<td>0.018</td>
<td>74.88</td>
<td>25.12</td>
<td>1.43</td>
<td>13.20</td>
</tr>
</tbody>
</table>

4.2.3. Pasting properties

The pasting properties of the samples were measured with a Rapid Visco analyzer (RVA, Perten instruments, Springfield, IL). The test was conducted according to AACC-I approved method 76-21.01. The samples (3.5g) were weighed on a 14% moisture basis and added to the shortening or OSA starch along with 25g of deionized water in an aluminum test can. Standard 1 heating profile was used while stirring at 160 rpm. The sample was held at 50°C for 1 minute,
and then heated to 95°C at about 12.3 degrees per minute. The temperature was held at 95°C for about 2.5 minutes before cooling at approximately 12.3 degrees per minute to 50°C with a hold time of 2 minutes at 50°C (Derycke, Veraverbeke, Vandeputte, De Man, Hoseney, & Delcour, 2005).

4.2.4. Gel texture

The flour paste from the RVA was used to measure texture profile analysis (TPA) with a texture analyzer (TA-XT2i, Texture Technologies). The paste was stored at 4 ºC for 24 h. Samples were penetrated with a TA-53 cylinder probe (3 mm, stainless steel) to a distance of 15 mm, following the conditions used by Chávez-Murillo, Wang, quintero-Gutierrez, & Bello Pérez (Chávez - Murillo, Wang, & Bello - Pérez, 2008). The peak force of the penetration was reported as hardness (g-force) and the negative peak during retraction of the probe was reported as stickiness (g-force). The same analysis was done in samples stored at 4 ºC for 7 days.

4.2.5. Dough stickiness

The dough stickiness was measured using a texture analyzer (Texture Technologies, Hamilton, MA) with a Chen-Hoseney dough stickiness rig. The dough was mixed in a 25g pin mixer until optimum consistency was reached. Optimum consistency was determined with mixograph in previous experiments conducted in the lab. The dough was rested for 10 minutes in a plastic zip top bag before a piece (approximately 2g) was cut from the dough mass and placed in the extrusion apparatus. A small amount of the dough was extruded through the extrusion apparatus and carefully cut off. The dough was then extruded to a length of 1mm, covered with plastic and rested for 30 seconds. The dough was then compressed with a 25mm acrylic cylinder probe to conduct the adhesive test (Chen & Hoseney, 1995).
4.2.6. Microextensibility

Dough strength was measured by determining the resistance to extension using a texture analyzer with a Kieffer microextension rig according to the method of Kieffer et al (Kieffer, Wieser, Henderson, & Graveland, 1998). The dough was mixed in a 25g pin mixer until optimum consistency was reached. Then, the dough pieces (10 g) were placed into the mold and rested for 40 minutes. The mold pressed the dough into several strips which were approximately 4 mm in width by 50 mm length. Dough strips were placed into the microextension rig and stretched vertically. The resistance to extension was measured as force against the hook in grams.

4.2.7. Farinograph

The water absorption and dough strength were measured using a Farinograph (C.W. Brabender Instruments Inc., Hackensack, NJ) according to AACC approved method 54-21.02 applying the constant flour weight method (Slaughter, Norris, & Hruschka, 1992).

4.2.8. Test baking

Samples were baked according to AACC approved method 10-09.01 (Sedlacek & Horcicka, 2011) with the following modifications; fungal α-amylase instead of malt dry powder, instant yeast (1.0%) instead of compressed yeast and the addition of 5ppm ammonium phosphate to improve yeast function. The bread was prepared using a 2 hour fermentation schedule, rather than 3 hour fermentation to avoid over fermentation and dough was punched once during fermentation. After baking and cooling, bread loafs were stored in dark cabinet in zip log bags. Bread loaf volume was measured according to AACC approved method 10-05.01(Narbad, 1983).
4.2.9. **Bread crumb image analysis (C-Cell)**

A C-Cell imaging system and software (Calibre Control Intl. Ltd., UK) was used for image analysis of sliced bread. The bread was sliced (2 cm thickness) approximately 18 hours after baking and placed in plastic zip top bags prior to imaging.

4.2.10. **Bread firmness**

Bread firmness was determined according to AACC approved method 74-09.01 (AACC-I, 2009) using a texture analyzer (Texture Technologies, Hamilton, MA) with a 25 mm acrylic cylinder probe with rounded edges. The bread was compressed in the center of the crumb of 2 slices lying on top of each other to measure force in grams. The firmness of the bread was measured at 4 time points during a total storage period of 7 days. A separate loaf of bread was used for each measurement.

4.3. **Statistical Analysis**

All analysis was conducted in triplicate. The statistical analysis was done using SAS version 9.3. Analysis of variance (Ramirez-Jimenez, Guerra-Hernández, & García-Villanova) was determined for all data using completely random design (CRD). Least significant difference (LSD) was determined for mean separation.
5. RESULTS AND DISCUSSION

5.1. Pasting properties

Gelatinization is a critical physical phenomenon that occurs in starch-based foods during processing. It is a process that disrupts the native molecular order of starch granules in the presence of water during thermal processing. During the gelatinization process, granular swelling, loss of molecular order, loss of birefringence and crystallinity, water uptake, increase in viscosity, and the starch solubilization have occurred (J. Zhou, Ren, Tong, & Ma, 2009). Pasting profiles of flour slurries are shown in Figure 2.

![Pasting profiles of flour slurries](image)

Figure 2. Pasting properties of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

The Rapid Visco Analyzer (RVA) parameters of peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback, and peak time are presented in Table 2. The addition of OSA modified wheat and tapioca starches affected the pasting properties of starch slurry. Peak
 viscosity increased significantly (P<0.05) with the addition of 4% (OSA) tapioca starch and 4% OSA wheat starch. Peak viscosity also increased significantly (P<0.05) by adding 2% modified starches, but not as much as with 4% of modified OSA wheat or tapioca starch. Bao et al., (2003) reported that OSA modified wheat starch increased the peak viscosity. Further, Thirathumthavorn et al., (2006) reported similar results with the addition of OSA modified tapioca starch, with respect to peak viscosity.

These findings indicate that the 4% OSA tapioca starch sample has the highest swelling power. The high swelling power allows it to reach peak viscosity more quickly, but the starch paste will break down more easily because of weak intermolecular forces among starch molecules and because of increased sensitivity to shear forces as temperature increases (Bagley & Christianson, 1982). This indicates that starch granules could be expected to be easily broken by shear force and starch will swell faster (Zheng & Wang, 1994). Based on RVA results, the sample with 4% OSA tapioca starch will have a less dense structure or higher crystallinity than other samples with different starch and a different level of modification (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013).

The breakdown values (the difference between peak viscosity and cold paste viscosity) were higher for larger amounts of modified starch added to flour samples, therefore samples with 4% modified starch have significantly higher (P<0.05) breakdown values than samples with 2% or in control samples. Breakdown value represents paste stability or degree of disintegration of starch granules. At breakdown, amylose molecules will leach out from granules into the solution. Samples with 4% OSA modified starches do not have significantly different (P<0.05) values from each other, but substantially higher breakdown value than other treatments; it shows that 4% samples have lower resistance to high temperature and shear force.
The final viscosity-holding strength or setback value indicates the value that resulted from gelling ability or retrogradation tendency of the amylose (Purhagen, Sjöö, & Eliasson, 2011). The highest setback was observed in the sample with 4% OSA wheat starch, suggesting that the highest amylose retrogradation occurred in this sample. It is noted that during baking, amylose partially swells and part of it is dissolved and diffused into the surrounding aqueous medium, where it forms a concentrated solution, which during cooling of the bread becomes an insoluble gel. Fresh bread ends up containing swollen elastic starch granules in a firm gel, which does not undergo any other change, so amylose does not play any role in bread staling at earlier stages of storing (Izadi Najafabadi, Hamdami, Le-Bail, Monteau, & Keramat, 2015).

5.2. Gel firmness

Starch granules are semi-crystalline particles containing a small quantity of lipids, phosphate monoesters, and enzymes, 20–30% amylose and 70–80% amylopectin. During the heating of starch in an excess of water to higher temperatures the granules will swell, after which amylose leaches from the granule. Then, during cooling the gel becomes more rigid. The last step is retrogradation. In this step, there is development of synergies in two stages: the first stage is recognized as conformational ordering of amylose and it is completed within a few hours of storage, while the second stage represents the successive reordering and crystallization of amylopectin, which requires a few days (Y. Zhou, Zhao, Winkworth-Smith, Foster, Nirasawa, Tatsumi, et al., 2015).

The changes in the hardness of starch gels are reported in Table 3. The texture analysis of gel samples was performed after one day to allow the retrogradation of amylose, and after seven days to allow the retrogradation (recrystallization) of amylopectin. After one day, results for gel hardness showed that samples with 2% and 4% OSA modified tapioca starch (25.20 and 25.35
g(force), respectively) do not show significantly different results (P<0.05), while these values are different from the sample with 2% wheat modified starch 26.75 g(force) and significantly different from the sample with 4% OSA wheat modified starch (P<0.05), which showed the highest hardness at 28.79g(force). Also, the control samples had higher hardness values than OSA tapioca starch samples. Stickiness of gels after 1 day were not significantly different (P<0.05).

The hardness after seven days of starch gels is compared to one-day old gels. The highest value for hardness was for the sample with 4% wheat modified starch at 31.58 g (force), but compared to control samples this value is lower than a sample with 2% shortening (32.88 g (force). The highest value isn’t significantly different (P<0.05) from the sample with 4% tapioca modified starch (31.25 g (force). After comparison with results for one-day old gels, hardness was significantly higher (P<0.05) for all samples, as was expected (Singh et al. 2007). The gel firmness is mainly caused by a retrogradation of starch gels, which is associated with the syneresis of water and crystallization of amylopectin, leading to harder gels (Majzoobi, Kaveh, Farahnaky, & Blanchard, 2015).

Amylopectin, as a branched polymer of glucose, forms aggregates with each other through different types of bonds that result in intermolecular associations that are less firm than retrogradation. This association dissolves easily during heating which causes the rigid form of the structure of swollen starch granules (Leach, McCowen, & Schoch, 1959). Starches that exhibit harder gels tend to have higher amylose content and longer amylopectin chains (Singh, GUJRAL, & SINGH, 2002).
Table 3. Pasting properties of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peak Viscosity (RVU)</th>
<th>Hot Paste Viscosity (RVU)</th>
<th>Breakdown (RVU)</th>
<th>Cold Paste Viscosity (RVU)</th>
<th>Setback (RVU)</th>
<th>Peak Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Shortening</td>
<td>213.70e</td>
<td>116.72e</td>
<td>96.97d</td>
<td>225.33e</td>
<td>108.61d</td>
<td>5.96e</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>214.47c</td>
<td>121.78d</td>
<td>92.69e</td>
<td>228.17d</td>
<td>106.39e</td>
<td>6.07a</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>220.72d</td>
<td>120.61d</td>
<td>100.11c</td>
<td>234.50c</td>
<td>113.89c</td>
<td>5.98bc</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>244.97b</td>
<td>136.31b</td>
<td>108.67a</td>
<td>260.75a</td>
<td>124.44a</td>
<td>6.03ab</td>
</tr>
<tr>
<td>2% OSA tapioca Starch</td>
<td>228.47c</td>
<td>126.14c</td>
<td>102.33b</td>
<td>241.20b</td>
<td>115.05c</td>
<td>6.00bc</td>
</tr>
<tr>
<td>4% OSA tapioca Starch</td>
<td>252.61a</td>
<td>142.61a</td>
<td>110.00a</td>
<td>260.64a</td>
<td>118.03b</td>
<td>6.07a</td>
</tr>
</tbody>
</table>

*RVU = Rapid visco units, Values in the same column with the same letter are not significantly different (P<0.05)
5.3. Stickiness of the dough

Stickiness of dough is an important quality parameter in bakery production because it could be used as a processability parameter for dough. Dough can stick to proofing baskets and conveyor belts and thus, create problems in automated bakeries (Cauvain & Young, 2009). Therefore, the effect of addition of OSA starches vs shortening on wheat flour on stickiness, work of adhesion and cohesiveness were determined (Table 4).

Dough stickiness is mainly a consequence of the degree of hydration of gluten molecules. This will promote the gluten molecules’ migration to the upper dough layers due to a higher mobility. Furthermore, the significant increase of cohesiveness is related to the plasticizing effect of water. The more water there is in the dough; the more intermolecular space is exhibited between the structural elements (mainly protein phase, starch granules). Therefore, the cohesiveness and the extensibility increases until the adhesion forces of the plunger are too weak or the dough itself disrupts. In summary, the measurement of dough stickiness can be considered as a variation of dough rheology measurements (Dobraszczyk, 1997). Results showed that the sample with 4% OSA tapioca starch had significantly lower (P<0.05) values for stickiness and adhesion, while 4% OSA tapioca had the lowest value of cohesiveness, which is not significantly (P<0.05) lower than for the sample with 2% OSA tapioca starch.

Samples with OSA wheat starch showed significantly lower (P<0.05) values for all three parameters than the sample with 2% shortening, but still substantially higher than samples with modified tapioca starch, making these samples less desirable than ones with OSA tapioca starch, for industrial baking. However, OSA wheat starch samples are still better than control samples with shortening.
Table 4. Gel firmness of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hardness [g (force)]</th>
<th>Stickiness [g (force)]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Shortening</td>
<td>28.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.11&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>26.60&lt;sup&gt;de&lt;/sup&gt;</td>
<td>13.67&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>26.75&lt;sup&gt;de&lt;/sup&gt;</td>
<td>13.71&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>28.79&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>13.17&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Tapioca Starch</td>
<td>25.20&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Tapioca Starch</td>
<td>25.35&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.26&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Shortening</td>
<td>31.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.94&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>32.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>30.49&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>20.76&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>31.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.28&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Tapioca Starch</td>
<td>26.78&lt;sup&gt;de&lt;/sup&gt;</td>
<td>18.86&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Tapioca Starch</td>
<td>31.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.95&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values in the same column with the same letter are not significantly different (P<0.05)
Table 5. Stickiness of the dough of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stickiness</th>
<th>Work of Adhesion</th>
<th>Dough Strength/Cohesiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g)</td>
<td>(g*sec)</td>
</tr>
<tr>
<td>0% Shortening</td>
<td>23.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.77&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>28.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>26.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>26.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.78&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Tapioca Starch</td>
<td>24.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Tapioca Starch</td>
<td>21.92&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.41&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.49&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values in the same column with the same letter are not significantly different (P<0.05)

5.4. Dough strength and extensibility

While dough stretches, it also rebounds when released from stretching. This elastic behavior is an integral feature of doughs. Dough elasticity can be related to dough strength. In bread making, it is preferred to have higher extensibility. During the fermentation stage, dough will extend, so in order for a free extension, greater extensibility is needed so gas cells can extend without rupture. However, this has to be in good correlation with resistance to extension, so gas cells do not collapse under the weight of the dough (Domingues, Kackman, Kirk, Nagy, Tostenson, & Lorence, 2014; Skaf, Nassar, Lefebvre, & Nongaillard, 2009). The dough strength
and extensibility of samples with shortening or OSA starches can be seen in Table 5. The dough strength (resistance to extension) ranged from 25.60 to 10.31 g and the extensibility of the doughs ranged from 27.43 to 31.90 mm. There were significant (P<0.05) differences among treatments for resistance to extension and dough extensibility.

5.5. Farinograph

Farinograph information on flour mixtures without shortening and with 2% of shortening, 2 and 4% of OSA modified wheat and tapioca starches is presented in Table 6. Water absorption increased with the addition of modified starches. Significantly (P<0.05) higher absorption was obtained with 4% OSA modified starches (P<0.05). This effect was reported previously (Falade & Christopher, 2015), and is a result of additional hydroxyl groups in modified starch, that allow more water interactions with hydrogen bonding (Mali, Sakanaka, Yamashita, & Grossmann, 2005).

All samples did not have significantly different (P<0.05) values for peak time, except for sample with 2% shortening (8.90 min), which had significantly different value for peak time than other samples. This shows that modified OSA wheat and tapioca starches did not affect mixing time. This implies that bakers do not need more energy for dough mixing, and use of OSA starch is cost-effective in this case. Dough tolerance to over-mixing is described with stability and MTI. Stability is, again, not significantly different (P<0.05) among the samples, except the sample with 2% shortening (17.37 min), which is more desirable, because dough can maintain its strength during mixing. The MTI value shows significant difference (P<0.05) between the sample with 2% shortening and the sample with 2% OSA tapioca starch, as well as samples with 4% OSA modified wheat and tapioca starches. The MTI for sample with 2% OSA wheat
starch was not significantly different (P<0.05). This shows that the MTI index does not changed much with replacement of shortening with OSA modified starches.

Table 6. Dough strength and extensibility of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Resistance to Extension</th>
<th>Extensibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g)</td>
<td>(mm)</td>
</tr>
<tr>
<td>0% Shortening</td>
<td>25.60\textsuperscript{c}</td>
<td>29.64\textsuperscript{c}</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>27.37\textsuperscript{d}</td>
<td>36.73\textsuperscript{a}</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>33.14\textsuperscript{b}</td>
<td>28.41\textsuperscript{d}</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>40.31\textsuperscript{a}</td>
<td>28.42\textsuperscript{d}</td>
</tr>
<tr>
<td>2% OSA Tapioca Starch</td>
<td>32.08\textsuperscript{b}</td>
<td>27.43\textsuperscript{e}</td>
</tr>
<tr>
<td>4% OSA Tapioca Starch</td>
<td>30.38\textsuperscript{c}</td>
<td>31.93\textsuperscript{b}</td>
</tr>
</tbody>
</table>

*Values in the same column with the same letter are not significantly different (P<0.05)

5.6. End product quality

Baking is the last but most important step in the bread making procedure. A dramatic change of physical and chemical properties of dough take place during baking. In Table 7, some of the most important baked bread qualities such as bake absorption, mix time, volume, symmetry, crust color, crumb texture and crumb color are shown. The bake absorption followed a similar trend as the absorption determined with farinograph. The samples with 4% OSA wheat and tapioca had the significantly higher (P<0.05) absorption values than control samples or samples with 2% OSA wheat and tapioca modified starches. Absorption % for samples with 2%
OSA what and tapioca starches had statistically similar values as sample without shortening (P<0.05).

Table 7. Dough quality measured by farinograph of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absorption (%) (14% MB)</th>
<th>Peak Time (min)</th>
<th>Stability (min)</th>
<th>MTI (BU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Shortening</td>
<td>59.27(^c)</td>
<td>7.37(^b)</td>
<td>15.53(^b)</td>
<td>15.67(^{ab})</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>58.40(^d)</td>
<td>8.90(^a)</td>
<td>17.37(^a)</td>
<td>12.33(^b)</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>60.60(^b)</td>
<td>6.90(^b)</td>
<td>15.03(^b)</td>
<td>16.67(^{ab})</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>62.03(^a)</td>
<td>6.83(^b)</td>
<td>14.57(^b)</td>
<td>19.67(^a)</td>
</tr>
<tr>
<td>2% OSA Tapioca Starch</td>
<td>60.77(^b)</td>
<td>6.77(^b)</td>
<td>14.67(^b)</td>
<td>17.33(^a)</td>
</tr>
<tr>
<td>4% OSA Tapioca Starch</td>
<td>61.73(^a)</td>
<td>7.20(^b)</td>
<td>14.73(^b)</td>
<td>19.33(^a)</td>
</tr>
</tbody>
</table>

\*MB = moisture basis, MTI = Mixing tolerance index, BU = Braebender Units, Values in the same column with the same letter are not significantly different (P<0.05)

Mixing time is an important factor for bakers in the industry. Bakers prefer a mixing time that is not too short, as to be easily overdone or too long which takes valuable time and energy (Alava, Millar, & Salmon, 2001). All samples with OSA modified starch had significantly (P<0.05) lower mixing time (4.00-4.08min) than the mix time of control samples without and with shortening (4.42 - 4.50min). Hence, the result shows that the samples with substituted fat are more favorable from the economical perspective of bread production (Fravolini, Ficola, & La Cava, 2003)
The loaf volume of the bread was determined after bread was baked and then cooled. It was expected, according to O’Brien et al., (2003) that samples with fat replacers would have lower volume because fat influences bread volume. The control with 2% shortening had a significantly higher (P<0.05) volume. The sample with 2% OSA wheat starch had a significantly lower (P<0.05) loaf volume than samples with or without shortening but significantly higher than samples with 4% OSA wheat starch and 2% and 4% OSA tapioca starch at P<0.05. These results do not compare with results of Hadnadev et al., (2014), who reported bread loaves with larger volume by adding OSA wheat modified starch. In that study, researchers used higher amounts (5%) of modified starch, which could be a reason for the different results.

Results from this study showed that loaves with OSA wheat starch have the same symmetry as control samples. Samples with OSA tapioca starch had significantly different (P<0.05) symmetry. Bread with OSA tapioca starch had break and shred, but it did not have dumbbelling appearance caused by unaffordable pressure during molding.

Bread surface color together with its texture and flavor are the main features considering consumer preference. Crust color is a degree of color darkness in the crust ranging from pale to dark brown. Samples were evaluated using a 10-point scale. Highest score for crust color were given to samples with OSA tapioca modified starch, 9.0. Moreover, this is significantly greater (P<0.05) than other samples and control. This high grade of crust color indicates that the color was more close to dark brown color, which is not highly desirable. The most desirable is golden brown crust color. One reason for dark the brown color may be the amount of hydroxymethylfurfural (HMF) produced during Maillard and caramelisation reactions. These processes are non-enzymatic processes of browning in food. Mainly, the formation of HMF in bread surface is responsible for a browning development (Ramirez-Jimenez, Guerra-Hernández,
& García-Villanova, 2000). Existence of the correlation between HMF and temperature during baking has been reported previously (Purlis & Salvadori, 2009). Thus, this overly dark color can be avoided by modifying baking process.

Crumb quality is also a very important factor in loaf bread. Crumb color is a degree of color darkness in the crumb ranging from creamy to white. There was no significant difference (P<0.05) between samples with OSA modified starches, but there was significantly lower (P<0.05) score of crumb color for the control with no shortening. Lower loaf volume, resulted in a lower surface area and therefore a dense and darker crumb appearance. Crumb texture was significantly (P<0.05) changed by replacing fat with OSA modified starch, there was a lower score (5.3-6.0) compared with control (2% shortening).

5.7. Bread firmness

Firmness of the bread crumb is an important bread characteristic because it directly affects the consumer preference. Firming of bread crumb is associated with bread staling (He & Hoseney, 1990). The changes in crumb firmness determined after 1, 2, 3 and 7 days of storage are shown in Figure 3. The firmness after 1 day of storage was different, but not significantly (P<0.05) different among the samples.

Crumb firmness gradually increased for all samples on the second and third days, respectively, with the same difference as the first day. This was expected as first day bread contained the highest moisture (decreasing moisture is reason for bread higher firmness.) The bread with 2% OSA wheat starch had significantly higher (P<0.05) firmness after 7 days of storage than the other samples, while samples with 4% OSA wheat and tapioca starches had different but not significantly (P<0.05) different results. The sample with 2% OSA tapioca modified starch had significantly (P<0.05) lower firmness than the other samples after the 7th
day of storage. This could be because of fine emulsification properties (Hadnađev-Dapčević, Dokić, Hadnadev, Pojić, Rakita, & Torbica, 2013) of 2% OSA tapioca modified starch, which would give strength and elasticity of gluten-starch matrix surrounding gas cells in dough, which affected the higher retention rate of carbon dioxide (CO₂) present in gas cells. This can give nice texture to final product and softer bread crumb (Susanna Hug-Iten, Handschin, Conde-Petit, & Escher, 1999).
Table 8. End product quality of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bake Absorption (%)</th>
<th>Mix Time (min)</th>
<th>Volume (cc)</th>
<th>Symmetry (1 to 10)</th>
<th>Crust Color (1 to 10)</th>
<th>Crumb Texture (1 to 10)</th>
<th>Crumb Color (1 to 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Shortening</td>
<td>65.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>741.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>64.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>930.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Wheat</td>
<td>65.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>823.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Wheat</td>
<td>67.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>773.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% OSA Tapioca</td>
<td>65.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>771.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% OSA Tapioca</td>
<td>66.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>746.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values in the same column with the same letter are not significantly different (P<0.05)
Figure 3. Bread firmness of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

*Column with the same letter are not significantly different (P<0.05)

5.8. Image analysis

A number of characteristics were obtained from the C-Cell analyzer, such as slice area, slice brightness, number of cells, number of holes and cell wall thickness. Results presented in Table 8, which showed that replacement of fat with OSA modified starches did not significantly (P<0.05) affect the number of holes (2-4) or cell wall thickness (2.96-3.02mm). Figure 4 represents the image analysis applied on slices of breads with no shortening, 2% shortening, 2 and 4% OSA wheat modified starch and 2 and 4% OSA tapioca modified starches.

Wrapper length and slice area showed significantly lower (P<0.05) values for samples with fat replacement. These results for slice area do not correlate with results for bread volume, where the sample with 2% OSA wheat starch had significantly higher volume than samples with fat replacers. Slice brightness was different for all samples; the samples showed more similar
results with control sample without shortening, except the sample with 4% OSA wheat starch, that had different brightness but sample baked with 2% shortening (P<0.05).

The number of cells was significantly higher in the control with 2% shortening, but samples with 4% OSA wheat starch and 2% tapioca starch showed significantly higher (P<0.05) number of cells than the control without shortening and 2% OSA wheat starch and 4% OSA tapioca starch. A great number of cells indicates that the bread is less firm. Thus, it can be said that beside control with 2% shortening samples with 4% OSA wheat modified starch and 2% OSA tapioca modified starch have a soft texture, which is more desirable. Cell diameter was not significantly different between samples and controls.
Table 9. C-cell of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slice Area ((10^4))</th>
<th>Wrapper Length ((10^2))</th>
<th>Slice Brightness</th>
<th>Number of Cells ((10^3))</th>
<th>Number of Holes</th>
<th>Cell Wall Thickness ((\text{mm}))</th>
<th>Cell Diameter ((\text{mm}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Shortening</td>
<td>28.58(^b)</td>
<td>20.20(^b)</td>
<td>105.20(^c)</td>
<td>3.80(^c)</td>
<td>3.00(^a)</td>
<td>3.02(^a)</td>
<td>14.88(^a)</td>
</tr>
<tr>
<td>2% Shortening</td>
<td>33.46(^a)</td>
<td>22.30(^a)</td>
<td>119.10(^a)</td>
<td>4.62(^a)</td>
<td>2.00(^a)</td>
<td>2.95(^a)</td>
<td>14.08(^a)</td>
</tr>
<tr>
<td>2% OSA Wheat Starch</td>
<td>29.56(^b)</td>
<td>20.36(^b)</td>
<td>106.60(^bc)</td>
<td>3.95(^bc)</td>
<td>3.00(^a)</td>
<td>2.99(^a)</td>
<td>13.84(^a)</td>
</tr>
<tr>
<td>4% OSA Wheat Starch</td>
<td>30.14(^b)</td>
<td>20.65(^b)</td>
<td>113.10(^ab)</td>
<td>4.18(^b)</td>
<td>4.00(^a)</td>
<td>2.98(^a)</td>
<td>14.21(^a)</td>
</tr>
<tr>
<td>2% OSA Tapioca Starch</td>
<td>30.07(^b)</td>
<td>20.71(^b)</td>
<td>109.80(^bc)</td>
<td>4.16(^b)</td>
<td>2.00(^a)</td>
<td>2.95(^a)</td>
<td>13.77(^a)</td>
</tr>
<tr>
<td>4% OSA Tapioca Starch</td>
<td>28.69(^b)</td>
<td>20.22(^b)</td>
<td>106.30(^c)</td>
<td>3.90(^c)</td>
<td>2.00(^a)</td>
<td>2.96(^a)</td>
<td>13.97(^a)</td>
</tr>
</tbody>
</table>

*Values in the same column with the same letter are not significantly different (P<0.05)*
Figure 4. C-cell images for bread slices of samples with different level of shortening (0 and 2%) and samples with OSA modified wheat or tapioca starch (2 and 4%)
6. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

When examining the quality of dough and bread as a final product with different levels of OSA modified starch from two sources, wheat and tapioca, several interesting conclusions can be made. In reference to Objective 1 and 2 of this research, there were significant difference in the quality of dough and end-product quality. Primarily, the pasting properties, gel firmness, stickiness mixing properties and strength of the dough was improved by adding of 4% OSA modified starch to the formulation instead of shortening, while 2% OSA modified starches did not result in substantial changes to the quality of the dough. The use of 4% OSA modified wheat and tapioca starch, improved the overall characteristics of dough and bread quality, but we can say that 4% OSA modified tapioca starch had more significant influence on dough and bread quality than samples with 4% OSA modified wheat starch (Objective 3).

The results of this study present some interesting research questions and additional opportunities for further investigation, which are listed below,

1. Analysis of a larger set of OSA starches with different botanical sources, such as rice, corn or potato. By applying these samples and conducting same set of experiments another benefits could be found.

2. Determination of bread quality with addition of modified starches, using different flour sources (ex. whole wheat, rice) would allow for investigation on OSA modified starch contributes to quality of different bread products.
7. REFERENCE


physicochemical properties of Minas fresh cheese. *LWT-Food Science and Technology, 56*(2), 356-362.


