UTILIZATION OF MODIFIED WHEAT AND TAPIOCA STARCHES AS FAT REPLACEMENTS IN BREAD FORMULATION

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ABSTRACT

Using fat in bread production is expensive, and from the diet point of view, it counts as high caloric food. Since obesity is a significant problem in the USA and many other countries, food industries are turning to the fat replacers in food. This research investigated the effectiveness of octenyl succinate anhydride (OSA) modified starches, from two sources (wheat and tapioca), as fat replacers in bread formulation. Sample for control was 2% shortening, 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 control sample with 2% shortening had better or the same characteristics.

PRACTICAL APPLICATIONS

Modification of starch with octenyl succinic anhydride (OSA) alters the starch properties in many ways that are beneficial to the food industry. Because of the dual, hydrophobic/hydrophilic, nature of OSA starches they have applications as emulsifier or fat-replacement ingredients in bakery products. Modification of starch with OSA also changes the levels of slowly digestible starch, which is an added benefit for formulation of low calorie breads using OSA starches as fat-replacers. It is important to carefully investigate the use of OSA starches as fat-replacers to determine their specific functionality and to develop a formula with the same characteristics as bread containing fat.

INTRODUCTION

Starches have always been essential to human nutrition, as they are the only polysaccharide carbohydrate digestible by humans (Miao *et al.* 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 *et al.* 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 (Hadnađev *et al.* 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 *et al.* 2015; Krstonošić *et al.* 2015; Miao *et al.* 2015; Wu and McClements 2015).

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
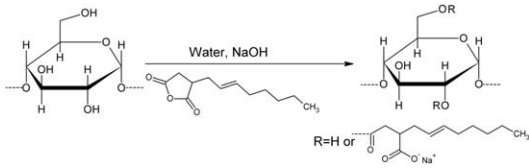
FIG. 1. STRUCTURE OF OCTENYL SUCCINIC ANHYDRIDE-MODIFIED STARCHES [ADAPTED FROM: (SWEEDMAN *ET AL.* 2013)]

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 *et al.* 2010).

The modification of starch through esterification with dicarboxylic acids was patented in 1953 by Caldwell and Wurzburg (Xie *et al.* 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. Structure of modified OSA starch is presented in Fig. 1. OSA modified starch can be used to produce slowly digestible and resistant starches (RS). 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 *et al.* 2007).OSA starches have also 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. Also, OSA modified starches can be used for films and coatings as well as for gel production and in food products (Sweedman *et al.* 2013).

OSA starches have several food uses, including use as emulsifiers, stabilizers, texturizers and many other applications. OSA starches are able to improve water retention, texture, thickness, freeze-thaw stability and other properties of baked products (Kittipongpatana *et al.* 2007). Previous studies have shown that OSA starches have increased swelling volume and ability to alter the gel firmness (Bao *et al.* 2003) as well as the ability to prevent retrogradation of amylopectin (Thirathumthavorn and Charoenrein 2006). These properties may be useful in various bakery and bread applications, to alter the texture and staling properties of the bakery products. The rheological characteristics of dough is also an important factor in production of bread. Addition of OSA starches has been found to reinforce the dough system because of the introduction of hydrophilic and hydrophobic bonds between the components of the dough system (Hadnađev *et al.* 2013). The emulsifying properties of OSA starches also affect the end product quality of breads by improving loaf volume, crumb color and crumb softness (Hadnađev *et al.* 2014). Because of the potential properties of OSA starches, the aim of this research was to study the effect of 2% and 4% OSA modified wheat and tapioca starches as fat replacers in bread formulation.

MATERIAL AND METHODS

Raw Material

Ingredients used in making the bread for this research were: tap water, hard spring wheat patent flour (North Dakota Mill, Grand Forks, ND), native wheat starch (Sigma S5127, St. Louis, MO), native tapioca starch (Ingredion, Bridgewater, NJ), Instafirm yeast (Lallemand Inc., Montreal, Canada), Doh-tone α-amylase (Caravan ingredients, Lenexa, KS), the octenyl succinic anhydride (Dixie Chemical Company, Pasadena, TX), and all other chemicals and reagents were supplied by Sigma and of at least ACS grade. The flour had a protein content of 13% determined by NIR (AACC-I method 39-10.01) and an ash content of 0.48% determined gravimetrically (AACC-I method 08-01.01) (AACC-I 2009).

Esterification of Starches With Octenyl Succinic Anhydride. Commercial samples of native wheat and tapioca starches were esterified according to the method of Han and BeMiller (Han and BeMiller 2007). The starches (100 g, db) were dispersed in water (225 mL) with continuous stirring. The slurry (at $\sim$ 25C) was adjusted to pH of 8.5$\sim$9.0 with 1M NaOH. Octenyl succinic anhydride (3g [3 mL], 3% of the weight of the starch) was added at room temperature ($\sim$ 25C), 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 $\times$ 15 min). The residues were washed three times with water and once with acetone, and then air-dried (40C, 24 h).

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_2O) three times to remove excess water. The starches were dissolved in 1 mL D_2O by stirring while heating at 80C for 2 h before lyophilization. Before analysis, the samples were dissolved a final time in 650 μL of D_2O, 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 25C 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 and Daigle 2003). Integration and analysis of the spectra was conducted with Topspin software version 3.2. The equatorial proton of the anhydroglucose unit of starch (Oluwasina *et al*. 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 × 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) (You and Lim 2000; Simsek *et al*. 2013).

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 50C for 1 minute, and then heated to 95C at about 12.3 degrees per minute. The temperature was held at 95C for about 2.5 minutes before cooling at approximately 12.3 degrees per minute to 50C with a hold time of 2 minutes at 50C (Derycke *et al*. 2005).

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 4C 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 *et al*. 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 4C for 7 days.

Dough Stickiness

The dough stickiness was measured using a texture analyzer (Texture Technologies, Hamilton, MA, US) 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 and Hoseney 1995).

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 *et al*. 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.

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 *et al*. 1992).

Baking

Samples were baked according to AACC approved method 10-09.01 (Sedlacek and 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 h fermentation schedule, rather than 3 h 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).

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 h after baking and placed in plastic zip top bags prior to imaging.

TABLE 1. PASTING PROPERTIES OF SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)

	Peak viscosity RVU	Hot paste viscosity RVU	Breakdown RVU	Cold paste viscosity RVU	Setback RVU	Peak time Min
2% Shortening	214.47[e]	121.78[d]	92.69[c]	228.17[d]	106.39[d]	6.07[a]
2% OSA Wheat	220.72[d]	120.61d	100.11[b]	234.50[c]	113.89[c]	5.98[a]
4% OSA Wheat	244.97[b]	136.31b	108.67[a]	260.75[a]	124.44[a]	6.03[a]
2% OSA Tapioca	228.47[c]	126.14c	102.33[b]	241.20[b]	115.05[c]	6.00[a]
4% OSA Tapioca	252.61[a]	142.61a	110.00[a]	260.64[a]	118.03[b]	6.07[a]

*RVU = Rapid visco units, OSA = Octenyl succinic anhydride.

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

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 two slices lying on top of each other to measure force in grams. The firmness of the bread was measured at four time points during a total storage period of 7 days. A separate loaf of bread was used for each measurement.

Statistical Analysis

Analysis of variance (Ramirez-Jimenez *et al.*) for completely randomized design and a cell-means model were performed in SAS 9.4. All tables include averages of three replicates per treatment. Significant difference for pairwise comparisons between treatments was defined at $P < 0.05$ based on Tukey's test.

RESULTS AND DISCUSSION

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 (Zhou *et al.* 2009). The Rapid Visco Analyzer (RVA) parameters of peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback, and peak time are presented in Table 1. 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 and 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 et al. 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 sample. Samples with 2% OSA modified starches also had significantly higher values than control sample. 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 *et al.* 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
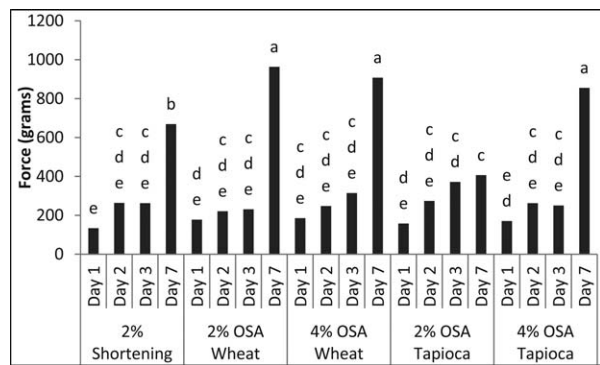
FIG. 2. BREAD FIRMNESS OF SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)
*Columns with the same letter are not significantly different ($P < 0.05$), OSA = Octenyl succinic anhydride.

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 *et al*. 2015). Peak time values weren't significantly different ($P < 0.05$).

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 (Zhou *et al*. 2015).

The changes in the hardness of starch gels are reported in Fig. 2. 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 do not show significantly different results ($P < 0.05$).

The hardness of starch gels after seven days 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 not significantly higher ($P < 0.05$) for all samples. 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 *et al*. 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 *et al*. 1959). Starches that exhibit harder gels tend to have higher amylose content and longer amylopectin chains (Singh *et al*. 2002).

The stickiness values of gels after 1 day were not significantly different ($P < 0.05$). The sample with 2% OSA modified wheat starch did not show any difference from the control, all other samples were slightly but not significantly ($P < 0.05$) different from the control.

Stickiness of the Dough

Stickiness of dough is an important quality parameter in bakery production because it could be used as a processing parameter for dough. Dough can stick to proofing baskets and conveyor belts and thus, create problems in automated bakeries (Cauvain and Young 2009). Therefore, the effect of addition of OSA starches vs. shortening in wheat flour on stickiness, work of adhesion and cohesiveness were determined (Table 3).

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 ass 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

TABLE 2. DOUGH STICKINESS AND MICROEXTENSIBILITY OF SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)

	Stickiness g	Work of adhesion g.sec	Dough strength/ cohesiveness mm	Resistance to extension g	Extensibility mm
2% Shortening	28.53[a]	2.54[ab]	3.02[a]	27.37[d]	36.73[a]
2% OSA Wheat	26.42[b]	2.47[b]	2.03[c]	33.14[b]	28.41[c]
4% OSA Wheat	26.30[b]	1.78[c]	2.33[b]	40.31[a]	28.42[c]
2% OSA Tapioca	24.17[c]	2.71[a]	1.65[d]	32.08[bc]	27.43[d]
4% OSA Tapioca	21.92[d]	1.41[d]	1.49[d]	30.38[c]	31.93[b]

*OSA = Octenyl succinic anhydride.

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

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 sample.

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 (Skaf *et al.* 2009; Domingues *et al.* 2014). The dough strength and extensibility of samples with shortening or OSA starches can be seen in Table 2. 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. Previously, the addition of other functional carbohydrates (xylitol) was determined to effect the extensibility and elasticity of wheat flour doughs (Sun *et al.* 2014). Similar to the study by Sun *et al.* (2014), the addition of the OSA starches increased the resistance to extension, in some cases significantly ($P < 0.05$), compared to dough with shortening.

Farinograph

Protein and polypeptide composition of a bread flour play a significant role in the water absorption rheological characteristics (Janković *et al.* 2015). Specifically, Janković *et al.* (2015) found that the amount of free sulfhydryl groups in wheat flour affect the water absorption and dough softening. However, these dough characteristics can change greatly due to the addition of other ingredients in the dough system. Dough rheology has been shown to change with the

addition of dietary fiber addition (Marti *et al.* 2014). 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 3. 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 and Christopher 2015), and is a result of additional hydroxyl groups in modified starch, that allow more water interactions with hydrogen bonding (Mali *et al.* 2005).

Addition of OSA starches altered some of the rheological characteristics of the doughs. 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.

End Product Quality

Baking is the last but most important step in the bread making procedure. A dramatic change in physical and chemical properties of dough takes place during baking. The end-

TABLE 3. FARINOGRAPH OF SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)

	Absorption 14% MB	Peak time Minutes	Stability Minutes
2% Shortening	58.40[c]	8.90[a]	17.37[a]
2% OSA Wheat	60.60[b]	6.90[b]	15.03[ab]
4% OSA Wheat	62.03[a]	6.83[b]	14.57[a]
2% OSA Tapioca	60.77[b]	6.77[b]	14.67[ab]
4% OSA Tapioca	61.73[a]	7.20[ab]	14.73[a]

*MB = Moisture basis, OSA = Octenyl succinic anhydride.

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

TABLE 4. BAKING DATA OF SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)

Sample	Bake absorption %	Mix time Min	Dough 1 to 10	Volume cc	Symmetry 1 to 10	Crust color 1 to 10	Crumb texture 1 to 10	Crumb color 1 to 10
2% Shortening	64.10[c]	4.42[a]	9.0[a]	930.00[a]	6.7[bc]	8.3[ab]	7.0[a]	6.3[a]
2% OSA Wheat	65.77[b]	4.00[b]	9.0[a]	823.33[b]	6.5[bc]	7.7[b]	5.3[a]	5.7[a]
4% OSA Wheat	67.10[a]	4.08[b]	9.7[a]	773.33[bc]	6.3[c]	8.0[b]	5.7[a]	6.3[a]
2% OSA Tapioca	65.77[b]	4.00[b]	9.0[a]	771.67[bc]	7.7[ab]	9.0[a]	5.7[a]	6.0[a]
4% OSA Tapioca	66.77[ab]	4.00[b]	9.0[a]	746.67[c]	8.0[a]	9.0[a]	6.0[a]	6.0[a]

*OSA = Octenyl succinic anhydride.

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

product quality evaluation of pan bread baked with OSA modified starches is presented in Table 4. 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 sample. Absorption % for samples with 2% OSA what and tapioca starches had statistically similar values among themselves, while sample with 4% OSA wheat modified starch, had significantly higher value for absorption %.

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 *et al.* 2001). All samples with OSA modified starch had significantly ($P < 0.05$) lower mixing time (4.00–4.08 min) than the mix time of control samples without and with shortening (4.42–4.50 min). Hence, the result shows that the samples with substituted fat are more favorable from the economical perspective of bread production (Fravolini *et al.* 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 control, but significantly higher than samples with 4% OSA tapioca starch. 2% OSA Tapioca modified starch and 4% OSA wheat starch at $P < 0.05$ didn't show significant difference from 2% wheat starch. 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 2 % OSA wheat starch have the same symmetry as control sample. Samples with OSA tapioca starch had significantly different ($P < 0.05$) symmetry in comparison with control sample,

and samples with OSA modified wheat starch. Bread with OSA tapioca starch had break and shred, but it did not have dumb-belling 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 with one being the worst and ten being the best. 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 a dark golden brown, which is highly desirable.

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. Crumb texture was not significantly ($P < 0.05$) changed by replacing fat with OSA modified starch, there was a lower score (5.3-6.0) compared with control.

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 and Hoseney 1990). The changes in crumb firmness determined after 1, 2, 3, and 7 days of storage are shown in Fig. 2. 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 one reason for bread higher firmness. After the first day of baking all samples had different, but not significantly different, results than the control. Samples with modified starches showed higher firmness than the sample with 2% shortening. It was noticed that on the second and third days of

TABLE 5. C-CELL OF SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)

| Sample	Slice area mm^2	Wrapper length mm	Slice brightness	Number of cells	Number of holes	Cell wall thickness μm
2% Shortening	33.45[a]	2229.7[a]	119.1[a]	4622[a]	2[a]	2.95[a]
2% OSA Wheat	29.55[b]	2037.7[b]	106.6[b]	3948[b]	3[a]	2.99[a]
4% OSA Wheat	30.13[b]	2065.0[b]	113.1[ab]	4179[b]	4[a]	2.98[a]
2% OSA Tapioca	30.07[b]	2071.7[b]	109.8[ab]	4163[b]	2[a]	2.95[a]
4% OSA Tapioca	28.69[b]	2022.7[b]	106.3[b]	3896[b]	2[a]	2.96[a]

*OSA = Octenyl succinic anhydride.
**Values in the same column with the same letter are not significantly different ($P < 0.05$).

observation, all samples and control didn't show any significant difference in bread firmness results. Addition of functional ingredients have been shown to effect bread end-product characteristics, such as bread firmness (Sun *et al.* 2014; Kurek *et al.* 2015; Mastromatteo *et al.* 2015). A study by Kurek *et al.* (2015) showed that the addition of β-glucan

in bread rolls had a significant influence on the bread firmness level. Also, the addition of pea flour to bread prepared with semolina was found to decrease bread firmness (Mastromatteo *et al.* 2015).

The bread with 2% OSA wheat starch had significantly higher ($P < 0.05$) firmness after 7 days of storage than the
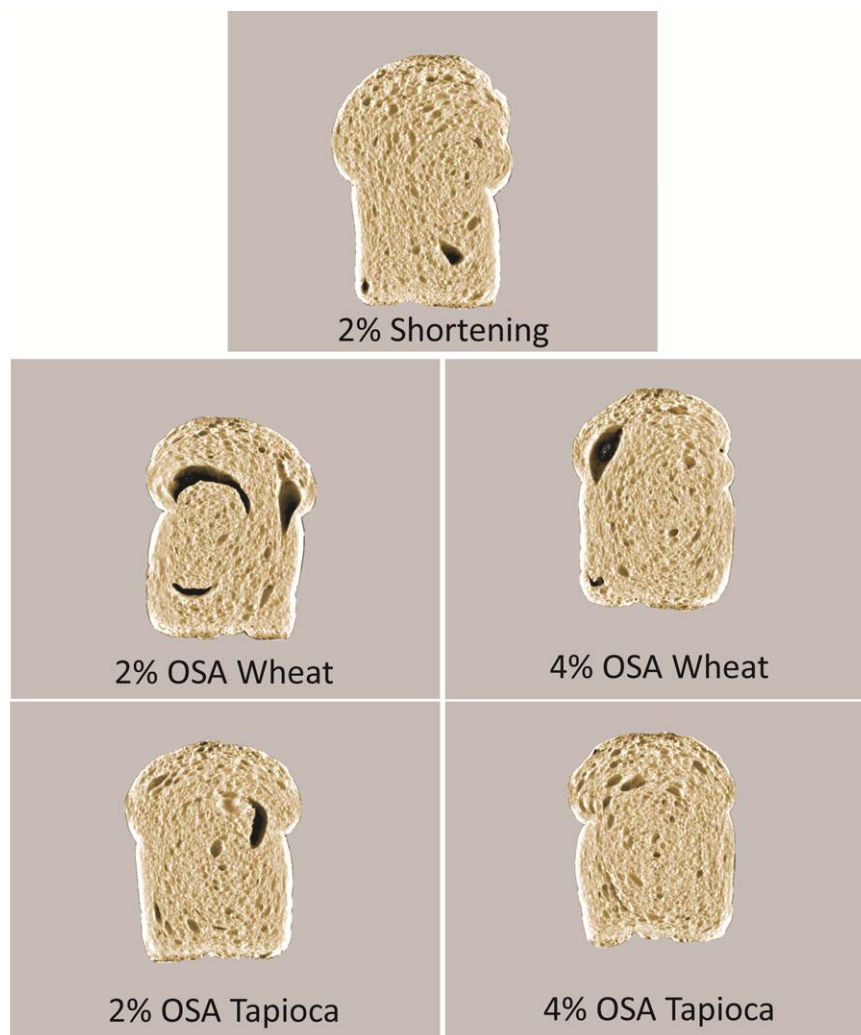
FIG. 3. IMAGES OF SLICED BREAD SAMPLES WITH SHORTENING (2%) AND SAMPLES WITH OCTENYL SUCCINIC ANHYDRIDE MODIFIED WHEAT OR TAPIOCA STARCH (2 AND 4%)
*OSA = Octenyl succinic anhydride.

other samples, while samples with 4% OSA wheat and tapioca starches had different but not significantly ($P < 0.05$) different results on 7th day. 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 (Hadnaev-Dapčević *et al.* 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 (CO_2) present in gas cells. This can give nice texture to final product and softer bread crumb (Hug-Iten *et al.* 1999).

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 5, 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 3 represents the image analysis applied on slices of breads with 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 significantly higher ($P < 0.05$) for control sample, while values for 2% OSA wheat and 4% OSA tapioca modified starches had significantly lower values than other samples and control. 2% OSA tapioca and 4% OSA wheat had different, but not significantly different values than control.

The number of cells was significantly higher in the control with 2% shortening, but samples with modified starch didn't show any difference among the samples. A lower number of cells indicates that the bread is more firm. Thus, it can be said that samples with modified starches, have firmer texture, what is not desirable.

CONCLUSION

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, there were significant difference found 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.

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NOMENCLATURE

NMR	Nuclear magnetic resonance
OSA	Octenyl succinate anhydride
RS	Resistant starche
RVA	Rapid Visco analyzer
TPA	Texture profile analysis

AUTHOR CONTRIBUTIONS

Senay Simsek designed and supervised the study. Ratko Balic collected the test data, interpreted the results, and drafted the manuscript. Bahri Ozsisli helped with data interpretation and preliminary studies. Tatjana Miljkovic coordinated and performed the statistical analysis.

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