Okra-gum fortified bread: formulation and quality

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Abstract Freeze-dried okra extract was added to Hard Red Spring (HRS) wheat flour intended for high soluble-fiber bread. Seedless okra pods were blended in 0.05 M NaOH solution and the extract (OE) was freeze-dried at pH7. SE-HPLC of OE showed the presence of covalently bound peptides. Okra extract powder (OE) 4, 7, 10, and 13 % was used to replace wheat flour in preparing four bread formulations. Although Farinograph water absorption was increased up to 4.4 % due to OE addition, the dough mixing Tolerance (MIT) was also increased. In the presence of OE, bread loaf volume was lower and freezable water was higher. Overall, bread firmness was lower at lower storage temperature, but higher OE increased firmness, due to water migration from crumb to crust. Color was darker for both crust and crumb. The bread melting temperature shifted to lower values at higher OE content as shown by Dynamic Mechanical Analysis (DMA). The test indicated that the properties of the blends were similar around the glass transition region. Dynamic rheology of the blends revealed weaker visco-elastic behavior compared to the control. The magnitude of the complex moduli for the 4 % OE was independent of frequency, while the remaining blends were frequency dependent.

Keywords Okra gums · Soluble fiber · Suspension rheology · Bread texture · Freezable water · DMA · DSC

Introduction

Abelmoschus esculentus, commonly known as okra, a native plant from Africa, is known to grow in many other areas of

the world. Okra gums are used as thickeners and flavoring for different foods. Okra polysaccharides are used as egg white substitute (Costandino and Romanchick-Cerpovicz 2004) and fat substitute in chocolate bars or frozen dairy desserts (Romanchick-Cerpovicz et al. 2002). Okra gums are random coil polysaccharides consisting of galactose, rhamnose, galacturonic acid. The repeating unit was reported as α -(1–2)-rhamnose and α -(4)-galacturonic acid residues with disaccharide side chains (Tomada et al. 1980), while the degree of acetylation (DA) was about 58 % (Sengkhampan et al. 2009). Okra polysaccharides are soluble in acidic or alkaline solutions as well as in aqueous alcohol solution. These polysaccharides, when extracted in water, can result in a highly viscose solution with slimy appearance. Sodium borohydride-soluble okra extract exhibited pseudoplastic behavior at pH4–6. The extract was shown to strengthen soft wheat dough (Ramadas and Tharanathan. 1987).

Several approaches were taken to address the effect of freeze-thaw of water in frozen baked products, such as the use of gums, as recommended by (Kobbs 1997). Gums are capable of trapping free water and preventing recrystallization. Locust bean gum, guar gum, carboxymethyl cellulose, and carrageenan are currently used to alleviate the freeze-thaw effect on frozen doughs (Ward and Andon 1993; Schwrzlaff et al. 1996). Sharadanant and Khan (2003a) showed that the use of locust bean gum improved dough mechanical properties indicating better baking quality. The same authors concluded that only carrageenan had a detrimental effect on dough and bread quality.

Generally, the presences of components with high water-binding capacity negatively affect dough rheological properties such as water absorption, dough mixing, and handling. Bread qualities such as loaf volume, crumb texture and color are affected as well (Sharadanant and Khan (2003b); Holas and Tipples (1978); Jelaca and Hynka

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(1971); Kim and D'Appolonia (1977b); McCleary (1986); Michmiewicz et al. (1990); Shelton and D'Appolonia (1985); Mohammed et al. (2012); Kotoki and Deka (2010); Himani Kundu et al. (2012); Barak and Singh (2012).

In a yeast-leavened banana bread study (Mohamed et al. 2010), higher water absorption, lower dough mixing tolerance, and faster bread staling for samples with higher banana powder was reported (Mohamed et al. 2010). Banana powder has high water absorption capacity due to the presence of soluble fiber as well as potassium. Okra polysaccharides effect on baked products is not widely studied topic thus little have been reported in the literature. Since most hydrophilic polysaccharides (gums) have similar characteristics, such as water holding capacity and solubility, the behavior of okra gum can be correlated with other gums. Three types of gums (guar gum, xanthan gum, and methylcellulose) were added to cake formulations so as to study their effect of staling. Microwave-oven baked cake weight loss, firmness; soluble starch and amylose content of the cakes were used as indicators of staling. Variation of staling during storage of cakes followed zero-order kinetics. Use of gums helped to retard staling of microwave-baked cakes. Using gums in combination with emulsifiers gave better moisture retention and softer cakes than using gums alone (Seyhun et al. 2003).

The objectives of this work were to determine the effect of alkaline-soluble okra extract on bread quality, given that, okra extract was shown to strengthen soft wheat dough used for products such as cookies and cakes as shown by previous researchers (Romanchick-Cerpovicz et al. 2002). This statement is true for soft wheat products (cakes and pastries), where gluten strength requirement is much lower than that for bread (hard wheat). For this reason, the positive influence of okra gum on the dough was not enough to meet the gluten strength needed for bread baking, which required the addition of vital gluten to the formulation. The addition of vital gluten helped to maintain bread loaf volume thus consumer acceptability.

Since the addition of soluble fiber to any food increases its nutritional value, this work intended to use okra polysaccharides as a source of soluble fiber and determine its effect on bread quality. It is common knowledge that soluble fiber reduces wheat-gluten development thus negatively affects bread quality and reduces consumer acceptability of the bread. So that to maintain dough properties, bread quality, and consumer acceptability, the author included additional vital wheat gluten to the suggested bread formulation. The additional vital gluten was decided by testing different levels and found that 50 % vital gluten of the added amount of okra gum will maintain bread quality.

Materials and methods

Materials

Fresh okra was purchased from a local supermarket. Okra pods were cut, seeds were removed, and 100 g of seedless okra were blended in 500 ml 0.05 M NaOH for 5 min at high speed using an Ultra Turrax blender (Janke & Kunkel KG, Germany). After adjusting the pH to 7, the suspension was centrifuged at $1 \times 2,000$ g. The extraction was repeated twice and the combined supernatant was freeze-dried and stored at 4 °C for further use. The freeze-dried material will refer to as okra extract (OE). Hard Red Spring Wheat Flour 89 (HRSW) (Miller's Choice) was obtained from North Dakota State Mill (Fargo, ND). Vital wheat gluten was supplied by Midwest Grain (Pekin, IL). Ascorbic acid was obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA), α -amylase (Doh-tone) was obtained from American Ingredients (St. Louis, MO), and instant dry yeast was obtained from Fleischmann's Yeast (Chesterfield, MO). Xylanase, Pentopan Mono BG (2500 FXU-W/g) was supplied by Novozymes (Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark). Trifluoromethanesulfonate (TFMS) was obtained from Sigma-Aldrich.Co.

Methods

Characterization of okra extract

Okra extract (OE) extract was tested for the presence of peptides covalently bound to the polysaccharide chain as in the case of other types of gums, such as Arabic gum and pentosans. The protein content in the freeze-dried supernatant was done using LECO CHN-2000 instrument (Feuerwerkerstr, 39 Ch-3602 Thun, Switzerland), which measures the total nitrogen multiplied by 6.25 factor. The OE characterization was done by trifluoromethanesulfonate (TFMS) digestion, which is otherwise known as deglycosylation. Freeze-dried OE (60 mg) and TFMS were placed on ice to cool for several minutes followed by slowly adding 10 g TFMS to the OE powder (Patel et al. 1993). The mixture was maintained cool and gently mixed until complete OE dissolution. Pyridine (60 %) solution and digested sample were placed in dry ice/methanol bath. Slowly, pyridine was added drop wise to digested OE (TFMS/okra mixture) until neutralized (checked with pH test strips). Once neutralized, the solution was transferred into dialysis tube (MWCO 3500) and dialyzed against water overnight and freeze-dried. Following freeze-drying, the yield of digested sample was 9 mg.

SE-HPLC of treated okra extracts

SE-HPLC was conducted using HP 1100 system (Hewlett Packard) with automatic injector. Deglycosylated OE was dissolved in sodium nitrate and samples were injected (50 μ L) on a BioSep-SEC-S4000 column and detected at 254 nm. The eluting solvent was 0.2 M sodium nitrate, pH 7, at a flow rate of 0.5 ml/min for 90 min.

Farinograph tests

The baking experiment was designed as a flour replacement study, where four different levels of okra supernatant extract (OE) 4, 7, 10, and 13 % were added to replace wheat flour in the formulation. In each blend, vital gluten was added at 50 % of the OE powder level i.e., 2, 3.5, 5, and 6.5 % for the 4, 7, 10, and 13 % levels, respectively, based on wheat flour weight. The control flour (hard red spring wheat flour) and the four blends (flour + okra powder + vital gluten) were tested according to Farinograph method No. 54-21 and moisture content as determined by method No. 39-06 (AACCI Approved methods 2000). The 10 g mixing bowl was used under standard conditions (60 % absorption, 14 % moisture, 500 FU consistency, and 20 min run time). The dough water absorption, stability profiles, and mixing tolerance index (MTI) were determined.

Bread baking

The baking procedure was based on the modified AACCI method (10-09) (AACCI Approved methods 2000). The ingredients used for making the 35 g loaf were a mix of solutions and dry material. Two solutions were prepared; 350 mg ascorbic acid in 100 ml water (American Ingredients (Saint Louis, MO), and 105 mg α -amylase (Doh-tone) in 100 ml water (American Ingredients, Saint Louis, MO). The dry ingredients were prepared by combining the following: 35 g Flour (control) + OE + vital gluten blends as follows; 94 % flour + 4 % OE + 2 % vital gluten; 89.5 % flour + 7 % OE + 3.5 % vital gluten; 85 % flour + 10 % OE, + 5 % vital gluten; 80.5 % flour + 13 % OE + 6.5 % vital gluten. To each dry blend the following was added: 0.875 g (2.5 %) instant dry yeast (Lallem Company, Derry, NH); 2.1 g (6 %) shortening; 1.4 g (4 %) non-fat dry milk (from the local supermarket). To the dry ingredients; 5 ml ascorbic acid solution (500 ppm) and 1 ml α -amylase solution were added and the blend was mixed in a Micro mixer (National Manufacturing, Lincoln, NE).

The amount of needed water was determined by mixing and feeling the dough: Control (100 % hard red spring wheat flour)=8 ml; 4 % OE=16 ml; 7 % OE=15 ml; 10 % OE=14 ml; 13 % OE=13 ml of water. In addition to the water, 5 ml ascorbic acid and 1 ml amylase solutions were added to all

samples equally. Mixing times were 8 min for the control, 10 min for the 4 % OE, 12 min for the 7 % OE, 13 min for the 10 % OE, and 15 min for the 13 % OE. During initial testing, it was determined that the addition of sugar/salt solution (as instructed by AACC method) to the blends containing OE caused little or no dough development (no gluten development). Therefore, sugar and salt were not added to samples containing OE. The method was further modified to maximize loaf volume as follows: After dough-mixing, the different formulations were proofed for 105 min, punched, placed in the baking pan, and proofed for an additional 105 min. Loaves were baked at 218 °C for 20 min.

Bread loaf volumes, color, and firmness

Baking performance was done in triplicate on pup loaves, while loaf volume was determined according to AACCI (2000) method 10-05 (AACCI Approved methods 2000). After baking, bread samples were cooled and stored at room temperature in double zipper, Ziploc® Brand (SC Johnson, Racine, WI), for color measurement on the same day. The L* (Lightness), a* (Redness), and b* (Yellowness) values of the crust and the crumb were measured in triplicate using a Hunter Lab to LabScan XE Spectrophotometer with a D65 light source (Hunter Associates Laboratory, Inc., Reston, VA).

Bread firmness testing was performed on bread loaves after being stored for 1, 3, and 6 days at 25, 4, and -20 °C using a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a 4-inch diameter disc probe and a 5 kg load cell. The purpose of storing bread at 4 °C and 2 °C was to replicate possible storage conditions that might be used at supermarkets or by consumers. Bread samples were analyzed using a modified AACCI (2000) method (74-09) (AACCI Approved methods 2000) by cutting 2×2 cm piece from the bread. The bread macro available in the applications software of the texture analyzer was used without modification, where testing was performed in triplicate, and the force recorded in grams.

Differential scanning calorimetry (DSC) and freezable water

Bread samples were analyzed using a Q2000 DSC (TA Instruments, New Castle, DE). Bread samples (12–16 mg) were hermetically-sealed in aluminum pans, placed in DSC cell, and cooled to -80 °C using the refrigeration system connected to the DSC. The samples were then heated from -80 to 110 °C at 5 °C/min. The onset and peak temperatures were determined by the tangent method utilized by the instrument software, which minimizes error committed by the operator. The DSC data was used to determine the amount of freezable water according to the method of (Vittadini and Vodovotz 2003).

Dynamic mechanical analysis (DMA)

Samples were weighed in 25.4×50.8×3 mm stainless steel windows and compressed in a carver press at room temperature for 10 min at 2,000 lbs force (8896.5 N). The samples were removed and cut into strips and placed in torsion rectangular fixtures as the samples were prepared for testing on the TA ARES LS2 controlled strain rheometer. Each sample had different dimensions, which were recorded before testing. The samples were clamped in the fixtures using a torque wrench set at 20 cN.m. The bread samples were cooled to −60°C and measured to as high a temperature as possible before samples slipped out of grips due to change in dimensions. The temperature ramps were 2 °C/min, 0.1 % strain and 1 rad/s. Storage modulus, loss modulus, and $\tan \delta$ were determined, and the storage modulus was fitted into the Fermi Equation.

Rheological measurements

Samples of Hard Red Spring (HRS) wheat flour and the four blends mentioned above in the Farinograph section were used for rheological experiments. Samples, HRS wheat flour or blends (HRS, OE, and vital gluten), were suspended in 0.05 M sodium phosphate buffer (pH 7.0 at 25 °C) at a concentration of 20 % by weight in duplicate (Xu et al. 2001). The samples were well dispersed and stored at 4 °C and used within 2 days after preparation.

The rheological analysis was done according to Xu et al. (Xu et al. 2001, 2007). A strain-controlled Rheometric ARES rheometer (TA Instruments, New Castle, DE) was used to perform the rheology analysis. A cone-plate geometry with 50-mm diameter was used at 25 °C±0.1 °C controlled by a water circulation system. To ensure that all the measurements were made within the linear viscoelastic range, a strain-sweep experiment was conducted initially. An applied shear strain in the linear range was adopted for the other viscoelastic property measurements for the same material; new samples were used for each experiment. Linear viscoelastic behavior indicates that the measured parameters are independent of applied shear strain. The linear range for all measured suspensions was less than 1 % of strain. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1–500 rad/s, yielding the oscillatory shear storage (G') and loss (G'') moduli. At low frequencies (0.1–1 rad/s), the measurement time was between 2 and 15 min, while at relatively high frequencies (1–500 rad/s), the measurement time was between few seconds to 2 min. The storage modulus represents the non-dissipative component of mechanical properties. Elastic or “rubber-like” behavior is suggested if the G' spectrum is independent of frequency and greater than the loss modulus over a certain range of frequency. The loss

modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The complex modulus is defined as $G^* = G' + iG''$. Therefore, $|G^*| = (G'^2 + G''^2)^{1/2}$. The phase shift or phase angle (δ) is defined by $\delta = \tan^{-1}(G''/G')$, and indicates whether a material is solid with perfect elasticity ($\delta=0$), or liquid with pure viscosity ($\delta=90^\circ$), or rather in between. Non-linear rheological studies were conducted at the same ARES instrument with the same geometry as described above. The steady shear measurements were conducted with the shear rate increased stepwise in the range of shear rate of 0.1 s^{−1}–500 s^{−1}, where the delay time was 10 s.

Statistics

The statistical analysis of the bread samples data (done on triplicate) was carried out using PROC GLM in SAS Version 8.2 for PC Windows. A Completely Random Design (CRD) was used to compare bread texture data of the control and the blends stored for 1, 3 and 6 days at 25, 4, and −20 °C. The same test was performed for the freezable water and the loaf volume. From the ANOVA, an F-test value was obtained and a multiple comparison test was performed on the means, using Duncan's Multiple Range Test at 0.05 levels.

Results and discussion

Characterization of okra extract

The protein content of OE after extraction and freeze drying was 8.3 % as determined by LECO, the total nitrogen content method. Tomada et al. (1980) and Sengkhamparn et al. (2009) reported the molecular structure of OE polysaccharides (gums), but no mention for covalently bound peptides was included in the report. In general, gums contain covalently-bound peptides. In order to verify whether the 8.3 % proteins are just trapped or covalently bound, TFMS (super acid) was used, which is specifically used for deglycosylation. This acid releases the peptides by cleaving the bond between proteins and carbohydrates. The TFMS digested samples were analyzed by HPLC and compared to undigested samples. The profile showed that the gum contained covalently bound peptide (Fig. 1), because the molecular weight was decreased after digestion with TFMS and detection at 245 nm. Therefore, this conclusion was reached because the undigested sample eluted earlier due to higher molecular weight caused by the attached carbohydrates. The digested sample contained the free peptides (lower MW) eluted at later time, which constitutes proof for the presence of covalently-bound peptides. The untreated sample started eluting after 16 min, sonicated

Fig. 1 Size-Exclusion HPLC of deglycosylated okra-extract using trifluoromethanesulfonate (TFMS). In the graph, a TFMS profile was shown as, a native sample, sonicated sample, as well as sonicated TFMS treated sample were shown for comparison

after 17 min, and treated after 22 min. Since this is Size Exclusion HPLC, higher MW elutes earlier. In addition, the untreated sample exhibited 5 peaks (including shoulders), sonicated 8 peaks, and treated 3 peaks signifying the released proteins after TEMS treatment were three different MW (Fig. 1). Finally, the first peak of the untreated sample eluted after 19 min, sonicated after 20 min, and treated after 23 min. The role of the bound peptides on the behavior of OE needs to be further investigated for their molecular structure and effect on the overall functionality of OE.

Farinograph tests

A Farinograph was used to determine the effect of OE on flour water-absorption and dough mechanical properties. Although the amount of water required for dough formation in the Farinograph was different from that used for bread baking, Farinograph is nonetheless an appropriate tool to test the effect of OE on flour rheology and dough handling. The presence of OE increased the Farinograph water absorption of the control by 2.8, 3.3, 3.5, and 4.4 %, for the 4, 7, 10, and 13 % OE blends respectively. The extra water was needed to bring the Farinograph curve to the 500 Brabender

units (BU) as a starting point, which is required by the AACCI method (Table 1). It is worth mentioning that the 3 % increment on the OE did not result in linear increase in Farinograph water absorption. Mixing Tolerance Index (MTI) is the difference in BU value of the top of the curve at peak time and the value at the top of the curve 5 min after the peak. The dough mixing tolerance index (MTI) had increased in the presence of OE, where MTI for the 4, 7, 10, and 13 % OE was 46, 80, 82, and 81 Farinograph Units (FU) (Fig. 2). This data is different from what was reported before (Mohamed et al. 2010) on the effect of banana powder on the same type of flour, where MTI was decreased as the banana powder increased (Table 1). This is an example of how the composition of the added fiber has different influence on the dough flowing properties. It indicates the degree of softening during mixing. Therefore, larger difference in FU unites indicates greater OE influence on the control. The addition of OE has increased the MTI of the control, which signifies stiffer dough. The stiff dough is caused by the competition for water between gluten and OE, resulting in less available water for gluten-development as it competes with OE. Further increase in OE content caused little change in MTI of the control.

Table 1 Farinograph data of the control flour and the blends including water absorption, mixing tolerance index, and dough stability

% OE ¹	% Water absorption	Mixing tolerance index (MTI) (BU) ²	Dough stability (min)
0	60.1±0.91a ³	43±1.62d	22.1±1.86a
4	61.8 ±1.23a	46±1.16d	5.5±1.57c
7	62.1±0.89a	80±3.12c	5.2±2.35c
10	62.2±2.1a	82±5.14a	8.5±1.01bc
13	62.8±1.73a	81±1.96b	12.4±4.23b

¹ OE okra extract, ² BU brabender units, ³ Means ($n=3$) with the same letter within the same column are not significantly different, $p \leq 0.5$.

Fig. 2 Farinograph profile of Hard Red Spring wheat flour and 4, 7, 10, and 13 % okra extract blends

Dough Stability is the difference in time (min) between arrival time at the 500 FU line and departure time. This indicates the time the dough maintains maximum consistency and is a good indication of dough strength. Dough stability was reduced by 73.9 %, 75.3 %, 59.7 %, and 41.2 % (control 21.1 min minus 5.5, 5.2, 8.5, and 12.4 min) in the presence of 4, 7, 10, and 13 % OE. Higher OE content in the blends maintained its influence on the dough stability, but 13 % OE kept the stability above the 500 BU after 30 min run. Mixing time has increased from 1.7 min to 23 min for the 13 % OE. The effect of OE on the Farinograph profile of the control could be attributed to the competition between OE and flour components for water. The competition is due to the ability of OE to absorb water faster than flour components (starch and protein). This competition prevented gluten development, consequently precludes viscoelastic dough formation. Although banana powder contains soluble fiber, the presence of sugars and electrolytes inhibited gluten development in an opposite way (dough became too soft instead of stiff) (Mohamed et al. 2010). The purpose of adding vital gluten to the blends was to manage gluten inability to fully develop in the presence of OE, thus permitting appropriate dough viscoelastic properties needed to maintain good bread quality. The arrival time is also one of the quality parameters obtained from the Farinograph testing. It is the time needed for the curve to reach the 500 BU mark, where longer arrival time indicates slower gluten development during mixing. Consequently, this will increase dough mixing time and delay bread production. The arrival time for the control flour was 1 min while the 10 % OE needed 9 min and the 13 % blend required 3 min to arrive.

Freezable water and bread loaf volume

A typical DSC profile of bread, scan (−80 to 120 °C), includes a major endothermic transition around 0 °C attributed mainly to ice melting (Baik and Chinachoti (2003); Mudgi and Barak (2011); Kang and Choi (1997)). The peak temperature of ice melting shifted to lower temperatures at higher OE content due to increase in the water-solubles. The percent freezable water (FW) was calculated according to Vittadini and Vodovotz (2003). The method is based on DSC scan of bread, where the peak enthalpy of the bread was divided by the latent heat of fusion of ice. The %FW has changed as a function of bread moisture content (Fig. 3a). The amounts of the added water was 14, 22, 21, 20, and 19 ml for the 0, 4, 7, 10, 13 % OE, respectively, while the bread moisture content was 17.8, 15.3, 14.5, 16.1, and 18.8 %, correspondingly. Although bread moisture is usually between 30 and 45 %, the moisture of the bread reported here is much lower because the moisture content was measured few days after baking. That could mean moisture loss or water migration from crumb to crust. The amount of OE powder in blends, as expected, influenced the moisture content of the bread and consequently the %FW of the bread. The %FW of the control was similar to that of the 13 % OE blend despite the lower amounts of water added during dough mixing, i.e. 14 ml for the 0 % OE (bread moisture content was 17.8 %) and 19 ml for the 13 % OE (bread moisture content was 18.8 %) (Fig. 3a). It is clear that OE trapped and prevented water freezing. A gradual decrease in FW was apparent at higher OE despite (almost) the same amount of added water in the course of dough mixing (Fig. 3a). The 0, 4, 7, 10, and 13 % OE blends exhibited 10, 14.5, 13.7, 12.8, and 10 % FW, respectively.

Fig. 3 Freeze-able water and loaf volume of control bread and okra extract blends

The 4 % OE exhibited the highest FW, while the 13 % OE was the lowest. It is interesting to observe how one extra ml of water added to the 7 % OE blend during dough mixing can decrease the %FW of the 4, 7, and 10 % OE blends by 1 %. Although, the initial excess amounts of water added to the blends compared to the control managed to increase %FW, the increase in the amounts of OE brought it down (Fig. 3a).

The peak temperature data of ice melting gathered from the DSC profile reflected a decreased (lower) temperature as OE increased indicating stronger water binding by OE, where the 4 %, 7, 10, and 13 % OE blend exhibited ice melting temperatures around -6.4 ± 1.1 , -7.6 ± 0.9 , -10.0 ± 0.8 , and -12.7 ± 1.4 °C, respectively. This trend was not apparent in the moisture content, but was reflected in the %FW.

Loaf volume is one of the indicators of wheat flour quality and can be used to reveal the effect of added

ingredients on the base flour and on the final bread quality attributes. Mudgi and Barak (2011) and Kang and Choi (1997) showed that gums types like locust bean gum, guar gum, xanthan, and carrageenan gums results in acceptable baked and other food product [Li et al. (1998); Vodovotz et al. (1996)]. Although, the addition of vital gluten to the blends will increase cost, it was necessary to maintain bread quality. Due to their structure, okra-gum polysaccharides absorb water, therefore affects gluten development during dough mixing, which has adverse effect on dough formation, mixing time, and bread quality. The 4, 7, 10, and 13 % blends exhibited significantly ($p \leq 0.05$) lower loaf volume compared to the control, whereas the 10 % blend loaf volume was the lowest of all (Fig. 3b). Take into account that the quantity of gluten added to the blends was 50 % of the OE added in each blend. Consequently, the lower loaf volume is exclusively due to the OE and the difference among the blends is caused by the difference in OE level. An alternative approach could have been to fix the amount of vital gluten added and maintain same loaf volume for all blends as in Mohamed et al. (2010), but it would have been more challenging to determine the effect of OE. It is worth mentioning here that the presence of salt and sugar as part of bread formulation as recommended by AACCI (2000) method (10-09) did not allow gluten development, lead to their removal from formulation. Possibly, sugar and salt, in addition to OE have competed for water and prevented gluten development.

Bread color

Considering the extra gluten added to the blends, darker bread than the control was expected (lower L^* value), because dark color is mainly an outcome of Maillard reaction between reducing sugars and proteins (Table 2). Likewise, samples with more OE powder should be darker than the control. The bread crust data in Table 2 showed just that, where the L^* value decreased significantly for the 10 and 13 % OE blends. The same trend was noticed for the a^* and b^* values. The crumb showed similar pattern with no significant differences between blends or the control. The crumb of the 13 % OE blend exhibited significantly the highest redness (a^*), while the remaining samples including the control were similar (Table 2). The yellowness (b^*) of the crumb was significantly higher based on OE levels, where the 13 % had the highest value and the control the lowest. This indicates that blends were darker but without major color contrast compared to the control.

Table 2 Bread firmness, after storage for 1, 3, and 6 days at 25, 4, and –20 °C

Sample/days % OE ¹			
	1	3	6
25 °C			
0	528.4±301.52b ²	1099.5±314.0bc	1720.3±1025.51b
4	516.5±287.42b	985.4±470.30c	1729.2±778.90b
7	702.2±303.30ab	1479.5±447.6abc	2428.7±888.80ab
10	607.7±81.00ab	1613.8±86.60ab	3109.2±812.80a
13	863.3±269.00a	1881.8±113.0a	3027.1±1035.81a
4 °C			
0	334.5±166.00c	477.1±204.11b	426.3±170.80b
4	486.7±50.70abc	707.4±114.12a	689.1±113.90a
7	599.6±145.00a	794.7±104.80a	782.9±38.40a
10	372.5±81.30bc	615.5±71.80ab	750.0±143.92a
13	553.9±127.64ab	700.9±100.76ab	776.9±171.95a
–20 °C			
0	136.7±39.70b	140.7±42.42b	136.7±42.90c
4	155.9±38.23b	160.5±37.42ab	157.4±44.11bc
7	234.7±4.10a	216.7±17.73a	229.5±21.15a
10	196.2±19.05ab	196.5±38.57ab	200.8±32.44abc
13	228.8±79.81a	218.1±69.15a	215.0±74.31ab

¹ OE okra extract, ² mean values ($n=3$) followed by the same letter within temperature are not significantly different based on overlap of the 95 % confidence intervals

Firmness

One of the most important physicochemical changes that occur in stored bread is “Bread-staling”, where baked products become firmer in addition to change in aroma causing customer rejection. Therefore, bread texture can be correlated to bread staling. Amylose and amylopectin retrogradation believed to be the main cause of staling. Monoglycerides complexation with amylose effectively reduced staling (Willhoft (1971). Thus, any added materials that prevent lipid-amylose complex

formation, such as chitosan oligosaccharides, will contribute to firmer bread during storage (Kerch et al. (2008). In order to determine the effect of time and temperature on stored bread, firmness tests were done at 25, 4.0, and –20 °C for 1, 3, and 6 days (Table 3). The time and temperature were selected to mimic supermarkets and consumers storage conditions. The control (0 % OE) exhibited higher firmness with respect to time at 25 °C, but at 4.0 °C it was higher only between 1 and 3 days storage and slightly changed at –20 °C (Table 3). In addition to the increase in firmness with time, the lower temperature reduced bread firmness at all storage time. The data in Table 3 also showed that higher OE content increased bread firmness due to water migration from crumb to crust. These results are consistent with reports in the literature, where bread stored with the crust exhibited firmer crumb (Willhoft (1971); Kerch et al. (2008). In addition, non-starch polysaccharides are proven to increase bread firmness during storage (Mudgi and Barak (2011). Soluble-fiber enriched bread is one of the popular products in the baking industry. Unlike other polysaccharides, products high in soluble fiber and protein content exhibited lower firmness [17]. The mechanism of this action was not examined, but preventing water migrating from crumb to crust could be considered as a cause.

Dynamic mechanical analysis (DMA)

The dynamic mechanical analysis (DMA) profile of the 4 % okra extract (OE) bread samples is shown in Fig. 4, where the profiles of the remaining samples were discussed but not shown. The glass transition (T_g) can be observed as a drop in the storage modulus when viewed on a log scale against a linear temperature scale. The T_g temperature (T_g) for the control (HRS wheat) was -15.1 ± 0.1 °C and the blends as follows: -8.1 ± 0.5 °C for the 4 % OE, -9.1 ± 0.5 °C for the 7 % OE, -12.1 ± 0.2 °C for the 10 % OE and -15.0 ± 0.7 °C for the 13 % OE. The T_g of all blends were higher than the glass transition temperature of the control, while the glass transition of the 13 %

Table 3 Bread color of the control and the blends

% OE ¹	Color					
	Crumb			Crust		
	L^{*1}	a^{*2}	b^{*3}	L^{*}	a^{*}	b^{*}
0	72.1±2.13a ⁴	2.2±0.55ba	23.4±1.63d	44.8±3.22a	17.1±0.40a	28.7±3.62a
4	69.4±1.97a	1.8±0.27ba	25.9±0.54c	43.4±1.96a	15.9±0.81a	26.1±2.64a
7	70.3±1.45a	2.0±0.47ba	28.8±0.80b	43.1±2.43a	16.0±0.25a	26.8±0.52a
10	71.2±2.21a	1.6±0.29b	29.0±0.26b	35.1±3.11b	12.6±1.84b	17.5±4.60b
13	68.3±2.73a	2.4±0.50a	31.9±1.64a	31.9±1.12b	11.9±1.90b	14.9±2.33b

¹ OE okra extract, ² L lightness, higher values indicate lighter color;

³ a =redness; ⁴ b =yellowness; higher color intensity is indicated by higher values

⁵ mean values ($n=3$) followed by the same letter within a column are not significantly different based on overlap of the 95 % confidence intervals.

Fig. 4 Dynamic mechanical analysis (DMA) profile of bread made from 7 % OE blend; G' (black circle), G'' (white circle), $\tan(\delta)$ (black square)

OE was nearly identical to that of the control. The similarity in T_g value between the 13 % blend and the control was observed in other parameters such as %FW and moisture content and Farinograph data. The storage moduli (G') obtained from DMA measurements for the control and the blends were fitted in the glass transition model as proposed by Peleg (Kerch et al. (2010); Peleg (1993)). The model equation can be written as:

$$R(T) = 1 / \{1 + \exp[(T - T_c)/a]\}$$

Where, $R(T)$ is stiffness ratio, T is temperature, T_c is temperature level which characterizes the transition region and is the inflection point of the stiffness, and a is indicator of the steepness of the curve of $R(T)$ vs. T . In our case, $R(T)$ can be expressed as $R(T) = G'/G''(-30^\circ\text{C})$. The results of data fitting are shown in Table 4. The R^2 of all fitting results were above

Table 4 Storage moduli obtained from DMA of the control bread and blends

Bread	T_c ($^\circ\text{C}$) ¹	α ($^\circ\text{C}$) ²	R^2
Control (100 % HRS)	$-25.9 \pm 0.33e^4$	$5.1 \pm 0.31a$	0.90
4 % OE ³	$-16.1 \pm 0.24a$	$3.2 \pm 0.17b$	0.95
7 % OE	$-17.2 \pm 0.20b$	$3.2 \pm 0.10b$	0.95
10 % OE	$-18.9 \pm 0.31c$	$3.2 \pm 0.13b$	0.95
13 % OE	$-20.1 \pm 0.26d$	$3.2 \pm 0.14b$	0.95

¹ T_c is the temperature level which characterizes the transition region and it is the inflection point of the stiffness

² α is the indicator of the steepness of the curve $R(T)$ vs T ; $R(T)$ is the stiffness ratio; T is the temperature; the data is based on the model proposed by Peleg (1993)

³ OE okra extract

⁴ Means values ($n=3$) with the same letter within the same letter are not significantly different, $p \leq 0.5$.

0.90. For the control bread, T_c was a slightly lower than those for the blends, which indicated a slight shift in the transition region to a lower temperature. The greater value of the control bread sample, which reflected a gradual drop in G' , might suggest that the control (100 % HRS) sample had slightly greater heterogeneity compared to the blends. Overall, the differences in stiffness for all breads, control or blends, were quite small according to the model fit, which suggested that the properties for these breads were similar around the glass transitions region.

Rheological measurements

Both linear and non-linear rheological properties were investigated for HRS flours (control) and the four blends. In Fig. 5, the results of linear dynamic frequency sweep of complex-moduli magnitude at 25 $^\circ\text{C}$ for the 20 % (wt. %) suspensions of HRS flours and the blends are displayed. For the HRS flour suspension, the magnitude of complex moduli was almost independent of frequency and the shape of the complex moduli values was essentially flat. The plateau value of the complex modulus for the control suspension was about 63 Pa (Fig. 5). The phase shifts for HRS flour suspension were in the range of 15–30 $^\circ$ at 25 $^\circ\text{C}$. These results indicated that the control suspension exhibited viscoelastic solid behavior. The suspensions of the blends showed weaker viscoelastic behaviors compared to HRS

Fig. 5 The linear viscoelastic properties for the 20 % (wt. %) suspensions of HRS flour and four blends at 25 $^\circ\text{C}$. (black circle): $|G^*|$ for 20 % HRS flour suspension. (white circle): $|G^*|$ for the 4 % okra supernatant (OE) suspension. (black square): $|G^*|$ for the 7 % OE suspension. (black up-pointing triangle): $|G^*|$ for the 10 % OE suspension. (black down-pointing triangle): $|G^*|$ for the 13 % OE suspension

flour. Among the four blends, the 4 % OE blend was expected to be closer to the control flour due to the low OE added to the blend. The values of the complex moduli for the 4 % OE suspension were lower than those for the HRS flour suspension and the plateau value of the complex modulus for the 4 % OE suspension was about 2.2 Pa which was less than one order of magnitude of the value for the control flour (Fig. 5). The magnitude of the complex moduli was almost independent of the frequency, which was similar to the control flour suspension. The phase shifts of the 4 % OE suspension were in the range of 15–36° at 25 °C, which were similar to those for the suspension of the control, but indicated a slightly higher fluid-like behavior than the control with somewhat larger phase shifts. These results suggested that a small amount of OE could affect the viscoelastic properties of the flour and cause the material to exhibit weaker viscoelastic behavior. Adding small amounts of gluten seemed to have little effect on reproducing the original viscoelastic behavior of the control. The viscoelastic effect of OE and vital gluten added has increased for both the 7 and 10 % OE, whereas their complex moduli were similar to those for the 4 % OE. However, the magnitudes of the complex moduli were more dependent on the frequency than the 4 % OE, and no plateau was observed. The shapes of the moduli curves for the 7 and 10 % OE suspensions exhibited steeper slope than that of the 4 % OE (Fig. 5). Phase shifts for the 7 and 10 % OE blends at 25 °C were in the range of 19–49° and 22–50° respectively. These results indicated that 7 and 10 % OE exhibited more fluid-like properties than the 4 % OE and the control. The 13 % OE blend had the highest amount of okra and gluten among the blends. The values of the complex moduli for the 13 % OE suspension were highest among the blends, which justified stronger viscoelastic properties among four blends (Fig. 5). The magnitude of the complex moduli for the 13 % OE was more dependent on the frequency than the 4 % OE, and no plateau was observed (Fig. 4). The phase shifts for the 13 % OE suspension at 25 °C were in the range of 19–42°, which implied that the 13 % OE blend displayed more fluid-like properties than the 4 % OE and the control. The smaller phase angles of the 13 % OE compared to those for the 4 and 7 % OE suggested that its viscoelastic properties started to shift into a little more solid-like behavior than 7 and 10 % OE blends due to the higher quantity of gluten in the 13 % OE blend. It is also noticed that the high-frequency behaviors of the moduli for the 4 and 13 % OE blends were different from the other two blends. The high-frequency moduli behavior was determined by the log-log complex moduli $|G^*|$ ($|G^*| = G'^2 + G''^2$)^{1/2} curve slope at frequencies higher than 50 rad/sec. High-frequency moduli curve for the 4 % OE was proportional to $\omega^{1/2}$ (Fig. 5) and indicative of the presence of flexible coil-like polymer (Peleg (1994). However, high-frequency moduli curve for the 13 % OE

blend was proportional to $\omega^{3/4}$ (Fig. 5), indicating material with semi-flexible behavior (Ferry (1980); Morse (1998a, b, c). The high-frequency moduli curves for the 7 and 10 % OE blends were in between, but closer to that of the 13 % OE and was proportional to $\omega^{3/4}$ (Fig. 5). The above hypothesis of semi-flexible or flexible behavior was based on the Doi-Edwards' theory, which is applicable to mono-disperse solutions (Doi and Edwards (1986). This model may not be directly applied to the current study. However, information of high-frequency moduli difference implies the presence of difference in polymer chains behavior. Thus, the observed high-frequency moduli behavior shift suggested structure change in the blends due to the different amount of okra and gluten added.

To better understand the processing behavior of the suspensions, non-linear steady shear viscoelastic properties experiment for the control flour and the blends were designed. Figure (6) displays the shear viscosity versus the shear rate for the 20 % (wt. %) suspensions of the control and the blends at 25 °C. They all showed shear-thinning behavior over the entire measured shear rates (Fig. 6). The control exhibited stronger non-Newtonian behavior than the blends. In the range of low shear rate, the control suspension had higher viscosities than the four blends, whereas in the range of high shear rate it exhibited lower viscosities than the other four blends (Fig. 6). Over all, the viscosity curves for the blends showed steeper slopes compared to the control, which indicated that the blends suspensions exhibited

Fig. 6 The non-linear steady shear viscoelastic properties for the 20 % (wt. %) suspensions of HRS flour and four blends at 25 °C. (black circle): viscosity for the 20 % HRS flour suspension. (white circle): viscosity for the 4 % okra supernatant (OE) suspension. (black square): viscosity for the 7 % OE suspension. (black up-pointing triangle): viscosity for the 10 % OE suspension. (black down-pointing triangle): viscosity for the 13 % OE suspension

weaker non-Newtonian behavior (Fig. 6). The 13 % OE blend displayed the highest viscosity among the four blends around the low and the high shear rate. In the range of 1 s^{-1} – 100 s^{-1} , which is within most industrial processing shearing range, the 13 % OE blend viscosities were $0.1 \text{ Pa}\cdot\text{s}$ – $1.2 \text{ Pa}\cdot\text{s}$, while the viscosities of the control were $0.02 \text{ Pa}\cdot\text{s}$ – $0.6 \text{ Pa}\cdot\text{s}$ (Fig. 6). These results suggest that okra should impart more viscous properties to the blends causing the blends to deviate further from non-Newtonian into less non-Newtonian behaviors.

Conclusion

Okra gum is a NaOH-soluble polysaccharide with a peptide covalently-bound to it.

In the presence of okra gum, Farinograph water absorption, dough mixing tolerance, and bread freezable water were higher. Bread loaf volume was lower, color was darker, and higher okra increased bread firmness. Dynamic Mechanical Analysis of bread showed melting shift to lower temperature, but overall the tested breads were all similar. Weaker viscoelastic behavior was noticed in the presence of okra and most samples showed frequency-dependent behavior. The control showed higher viscosities around the low shear rate and lower viscosity at higher shear rate. The 13 % OE blend exhibited higher viscosities at high and low shear rate.

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