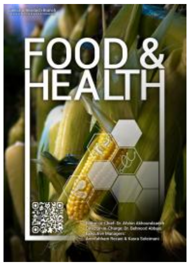
Food & Health 2024, 7(3): 10-18



Food & Health
Journal homepage: sanad.iau.ir/journal/fh
Journal

The Effect of Okra Stem as a By-product on Dough Rheology and Bread Quality

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ARTICLE INFO

Original Article

Article history:
Received 21 October 2024
Revised 11 November 2024
Accepted 03 December 2024
Available online 20 December 2024

Keywords:
Bread
Composite
Okra stem
Waste

ABSTRACT

In this study, Okra stem (OS) flour (OSF), as an agricultural by-product rich in dietary fibers and polysaccharides, was used to formulate new composite wheat bread and improve its functional and rheological properties without having a negative impact on consumer general acceptance. The effect of wheat flour (WF) replacement with OSF (0%, 4%, 8%, and 12%) was investigated on dough rheology, bread quality, and sensory characteristics. There was a positive correlation between the percentage of substitution of WF by OSF and water absorption capacity (WAC), dough consistency, stability time, and degree of softening. Dough development time in OSF-enriched dough was 34.78% shorter than in WF. A significant decrease in the crumb firmness, stretch resistance (BU), tensile strength (mm), and energy (cm^2) was observed with increasing the replacement percentage ($P<0.01$). The volume of bread changed significantly with the percentage of replacement ($P<0.01$) and showed a negative correlation. Including OSF led to a reduction in porosity and an increase in moisture loss. The results demonstrated WF can be replaced by OSF up to 12% without negatively affecting the quality of bread.

1. Introduction

Bread is a very important food product for consumers, especially in developing countries where food patterns have changed (1). It is still popular in many countries and is consumed in various forms daily. Bakery products are constantly expanding new products, often enriched with materials of natural origin. To enrich bread, in order to significantly change the organoleptic properties and health-boosting properties, various additives such as non-cereal grains, herbs, oilseeds, dried fruits, and vegetables are used (2-4). Composite flour technology is the process of mixing two or more flours with or without WF to produce bread with desirable quality and nutritional properties which is called composite bread (2). The Okra (Abelmoschus esculentus L.Moench) is known by many names including ladies' fingers, bamia, bhindi, or gumbo. The nutritional value of Okra is related to the presence of minerals, vitamins, protein, dietary fibers, calcium, bioactive chemicals, phenolic components, and antioxidant activity. Studies have shown that Okra consumption may reduce high blood pressure, cholesterol-binding capacity, anti-diabetic effects, and regulation of glucose and lipid metabolisms. Different research has also shown that Okra can be used as a bone scaffold, serves as a novel immunomodulator, and is consumed as an adjuvant for diabetic nephropathy.

The wastes generated in the food industry are known as co-products that can be recycled because of their high nutritional value. In this context, OS is challenging because it is discarded during Okra cultivation, thus increasing environmental pollution. Including recycled co-products from the food industry in diets has received significant consideration in recent years. OS has been found to possess antioxidant activity and is rich in phenolic compounds mainly because of its composition of catechins, flavonol, and hydroxycinnamic derivatives (5).

These characteristics motivate us to evaluate the effect of using OSF in the formulation of gluten bread. The results of this paper besides reducing Okra cultivation waste and reusing it reduce environmental pollution and prevent the loss of a rich

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source of phenolic compounds with antioxidant properties. Because these wastes are rich in dietary fiber and polysaccharide carbohydrates, the effect of their addition on the rheology, textural, functional, and sensory properties of the resulting dough and bread has been surveyed.

2. Experimental

2.1. Materials

White WF was obtained from Sarcheshmeh Flour Company (Shahroud, Iran) with 14% moisture, 0.5% ash, 25% wet gluten, 9% protein, and pH=6. OS (Basanti cultivar) containing 7.88% dietary fiber, 4.3% reducing sugar, 0.86% starch, 0.9% crude protein, and 0.1% crude fat was collected from an agricultural garden in a village in Iran called Ebrahimabad in the fall of 2019.

2.2. Preparing OSF

After collection OS, the fresh plant was washed under running water (6). OS was cut into 1 mm pieces with a knife. The samples were dried at -53 ˚C for 48 h using a freezing dryer (Christ, Alpha 1-2 LDPlus, GERMANY). The stem was grounded by the mill (TS,2200, IRAN) to turn into flour that passed through a sieve with a mesh of 100. It was stored in polyethylene bags at 28 ˚C until analysis (5).

2.3. Mixing WF and OSF

Three proportions of wheat-OS composite flours (96%:4%, 92%:8%, and 88%:12%) had been prepared according to the results of the Faryngograph, Extensograph, sensory, and textural tests of bread. WF (100%) was used as a blank.

2.4. Preparing sourdough

The sourdough was prepared by WF-water mixes following the method used by Raccach et al. (7) with some changes. The first 20 g of WF was mixed with 20 g of mineral water and then placed in a glass jar with a lid at room temperature for 8 days. During storage, it was fed every other day. To the weight of the dough, flour, and water in equal proportions were added and mixed thoroughly. After the first 8 days, the sourdough continued to be fed daily for two months in the same manner as described above. Finally, the activity of yeast and lactic acid bacteria was detectable. Gas cells on the surface of the sourdough were formed and the acidic odor caused by the activity of lactic acid bacteria (pH=5) was strongly felt.

2.5. The process of bread-making

Bread preparation formulation was obtained by trial and error on some quality and appearance parameters of the sample such as texture, color, appearance, taste, and general acceptability. The formulation consisted of 1 k WF, 30% sourdough, 2% salt, and 65% water based on the weight of WF (Table 1). All bread was prepared by combining straight-dough and dough-sponge techniques. In the first stage, to prepare the dough 100% flour and 55% water were thoroughly mixed in the mixer (BS 251, IRAN) at a speed of 132 rpm. Then the mixture was rested for 120 min at room temperature. In the second stage, sourdough and 5% water were added to the dough and mixed for 5 min. Subsequently, a mixture of salt and the remaining water (5%) was mixed into the dough for 5 min.

The prepared mixture was aerated twice every 40 min. The dough was rolled at a weight of 500 g and it was placed in a mold. Secondary rest was considered for 15 h at refrigerator temperature. Then it was placed at room temperature for 1 h, and finally, the baking process was carried out at 200 ˚C for 40 min in an electric oven (Alton, V304, IRAN). Then the bread samples were cooled at room temperature (24 ˚C) for 1 h (8) and were placed in polyethylene bags to minimize moisture loss. Physical properties were measured 24 hours after baking (9).

Table.1. Type and amount of ingredients in blank and bread enriched with different levels of Okra stem.

Type of bread sample	WF (g)	OSF (g)	Salt (g)	Sourdough (g)	Water (g)
Blank	1000	0	20	300	650
Enriched with 4% OS	960	40	20	300	650
Enriched with 8% OS	920	80	20	300	650
Enriched with 12% OS	880	120	20	300	650

WF: wheat flour; Blank: 100% WF; OS: Okra stem; OSF: Okra stem flour

2.6. Farinograph test

The Farinograph analysis was carried out in a Farinograph (Brabender, 150-E, GERMANY) using the standard method AACC 54-21A (10). Flour and water were mixed at a speed of 63 rpm to be placed up to the maximum dough consistency in the line of 500 units of Brabender (BU) (6). In this paper, WAC (based on 14% moisture content), development time (min), stability (min), degree of softening (FU), qualitative number of Farinograph, and consistency (FU) were determined.

2.7. Extensograph test

Extensographic characteristics of flour samples were performed using an Extensograph (Brabender, 150-E, GERMANY). Dough extension measurements were performed using the AACC no. 54-10.01 (10). After resting the dough for 45 min, 90 min, and 135 min the stretch resistance (BU), maximum stretch resistance (BU), tensile strength (mm), stretch resistance to tensile strength ratio, maximum stretch resistance to tensile strength ratio, and energy (cm2) of the dough were determined.

2.8. SEM

Morphology and sizes of breadcrumb pores were studied using SEM (Phenom prox, HOLLAND) according to Chisenga et al. (11) with some modifications. Initially, bread samples were stored in the freezer (Samsung, RT79KAEW, KOREA) at a temperature of -80 °C for one week. A freezing dryer was used and then powdered by the mill (12). Bread crumb samples were prepared in approximately 5*5*3 mm and were installed by a two-way adhesive tape on the base of the sample. The coating was done by a thin layer of gold with a thickness of 20 nm. SEM images were analyzed using the imaging system. The images of the control and enriched samples were enlarged with a magnification of 250x, 500x, 1000x, and 2000x.

2.9. Analysis of texture profiles

For tissue analysis, AACC method 74-09 was used with some changes (10). In this way, slices of breadcrumb with a thickness of 2*2*2 cm were prepared. Samples were compressed to 50% of the initial height using a cylindrical prop with a diameter of 50 mm and a speed of 100 mm/s (Hounsfield, H5KS, ENGLAND). The highest force was reported as the stiffness of the breadcrumb.

2.10. Bread staling Analysis

To determine the staling, bread was packed in polypropylene bags and stored at 19-22 °C (4,12) for three days. The texture analysis was examined for bread staling analysis (13) at 0 h, 24 h, and 48 h after baking (12). The stored bread was examined every day for the growth of mold colonies.

2.11. Bread volume and special volume

The volume of bread was determined using the AACC method 10-05 (10) and by Equation 1-4. The loaf-specific volume was calculated as the loaf volume divided by the loaf weight.

(1) $Density_{seed}(g/cm^3) = m/v$

(2) $W_{seed}\ (g) = W_{total} - (W_{container} - W_{bread})$

(3) $V_{seed}\ (cm^3) = W_{seed}/Density_{seed}$

(4) $V_{bread}\ (cm^3) = V_{container} - V_{seed}$

2.12. Weight loss and Moisture drop

The weight loss was determined based on the Bakare et al. (14) method. Weight loss and moisture drop were defined by Equation 5, and Equation 6, respectively.

(5) $Weight\ loss\ (\%) = (Weight_{dough} - Weight_{bread})/Weight_{dough} \times 100$

(6) $Moisture\ drop\ (g) = Weight_{dough} - Weight_{bread}$

2.13. Sensory evaluation

The sensory evaluation was evaluated according to Protonotariou et al. (15) with slight changes. Sensory evaluation was performed by several consumers and bakers (collectively 20) based on a 5-point Hedonic scale on scale from 1 to 5 (5- excellent, 1- extremely unsatisfactory). The testers evaluated overall quality based on taste, aroma, texture, color, appearance, and general acceptance.

2.14. Statistical analysis

Qualitative experiments were carried out in a completely randomized design with three replications. Sensory evaluation was performed as a factorial in a completely randomized block design with three replications. The variance of the data obtained from the WF-OSF replacement percentage factors was analyzed using SAS software version 9.00. Means Comparison was performed using Duncan's multiple-range method of probability level (P≤0.05). In the obtained results, the means with similar letters were not significantly different from each other in terms of the Duncan test.

3. Results and Discussion

3.1. Farinograph properties

The results showed the addition of OS had a significant effect on the WAC of the dough (P<0.01, Table 2). The WAC of flour mixes showed a positive correlation with the replacement rate of OSF (r=0.99). WAC in 4OS (blend composed of Okra stem and wheat flour at 4:96 ratio), 8OS (blend composed of Okra stem and wheat flour at 8:92 ratio), and 12OS (blend composed of Okra stem and wheat flour at 12:88 ratio) was 8.60%, 16.13%, and 22.76% higher than control, respectively (Fig. 1a). There was little positive correlation between WAC, and fiber because the high fiber in WF played a meaningful role in the absorption of water (16-17). Struck et al. (16) observed the addition of berry pomace fiber had increased the WAC of WF. According to Linina et al. (18), WAC in poor flour was less than 55%, in average flour was 54-60%, and in strong flour was over 58%. WAC in WF-OSF was in the range of strong flours. WAC and flour consistency had a strong positive correlation (r=0.97).

A significant difference in development time was observed between dough samples by the addition of OS (P<0.01, Table 2). A shorter dough development time was observed by replacing the OS. Dough development time in composite flours was 34.78% less than control (Fig. 1b). This could be because of the high WAC of composite flours. Higher WAC increased gluten hydration and therefore helped the dough to develop faster. Torbica et al. (19) stated dough development time was inversely related to WAC. Jafari et al. (20)

investigated that the high dough expansion time was related to a decrease in gluten hydration. The dough development time of control differed significantly from 4OS, 8OS, and 12OS (P<0.01), while there was no statistically meaningful difference between the dough development time in composite flours (Fig. 1b).

Table.2. Analysis of variance of Farinograph characteristics of flour enriched with different levels of Okra stem.

Source of changes	Degree of freedom	Mean squares	F value	Prob
Water absorption based on 500 FU and 14% moisture (%)	3	89.77	2244.19	<0.0001**
Development time (min)	3	0.48	12	0.0025**
Stability time (min)	3	0.05	5	0.0306*
Degree of softening (10 min after starting) (FU)	3	2034.75	508.69	<0.0001**
Degree of softening (12 min after maximum) (FU)	3	1604.75	401.19	<0.0001**
Consistency (FU)	3	1523	380.75	<0.0001**
Farinograph quality number	3	0.75	0.75	0.5523ns

**: Significant at the level of 1%, *: Significant at the level of 5%, ns: meaningless

No significant differences in dough stability time were observed between the control and composite samples (p≥0.05, Table 2). The dough stability of 4OS, 8OS, and 12OS was 6.8%, 3.45%, and 10.34% higher than the control, respectively (Fig. 1c). The values of dough stability were an indicator of dough strength and quality during kneading (21). Flour with a stability time of over 10 min was resistant to mechanical stress and was classified as high-quality flour. Flour with a resistance time lower than 3 min had poor quality (18). The stability time of the dough showed a slight positive correlation with the replacement percentage (r=0.8). The highest stability time of the dough was related to the replacement rate of 12% (Fig. 1c). Although Xu et al. (21) reported that dough development time was also an indicator of dough quality and was highly correlated with dough stability.

The softening index of the control dough during mixing differed significantly from the 4OS, 8OS, and 12OS (P<0.01, Table 2). Replacement of WF by OSF led to the formation of a weaker protein network, which led to higher values of softening of the Farinograph. The softening rate after 10 min from the beginning of mixing 4OS, 8OS, and 12OS was 18.11%, 31.16%, and 44.20% higher than the control, respectively Fig. 1d). The softening rate after 12 min from the maximum in 4OS, 8OS, and 12OS was 11.36%, 21.02%, and 30.68% higher than the control dough, respectively (Fig. 1e). This index showed softening of the dough during one mixing step (22). The less this index the better the dough quality. Liniņa et al. (18) reported if this index was less than 70 FU, the quality of dough mixing was considered satisfactory. In dough with a value higher than 110 FU, the quality of dough was considered weak and had problems in mechanical processing during dough preparation. Because of this issue, even the softening of the control dough was in the weak dough range. Dietary fiber, depending on its amount and composition, could have a debilitating effect on gluten proteins (23).

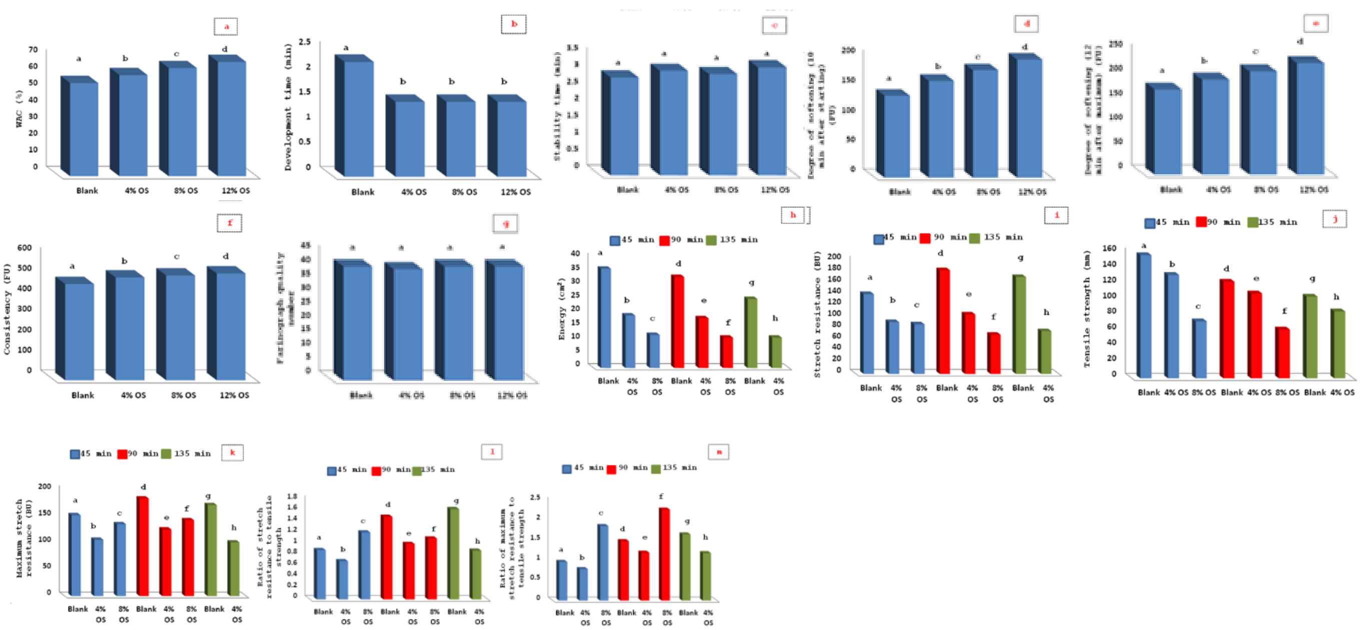

Fig. 1. Comparisons of mean Farinograph and Extensograph characteristics between control and fortified flour samples: (a) water absorption capacity (WAC); (b) Development time; (c) Stability time; (d) Degree of softening (10 min after starting); (e) Degree of softening (12 min after maximum); (f) Consistency; (g) Farinograph quality number; (h) Energy; (i) Stretch resistance; (j) Tensile

strength; (k) Maximum stretch resistance; (l) Ratio of stretch resistance to tensile strength; (m) Ratio of maximum stretch resistance to tensile strength.

The percentage of WF substitution by OSF displayed a meaningful effect on dough consistency between the control and composite samples ($P<0.01$, Table 2). The consistency of 4OS, 8OS, and 12OS was 6.79%, 8.92%, and 11.04% higher than the control, respectively (Fig. 1f). The consistency of 12OS was higher than other composite and control dough. These showed that OSF had increased this property of the dough. This property of the dough had a positive correlation with the WAC of flour and the percentage of replacement. There was no statistically significant difference between the quality numbers of the Farinograph in control, 4OS, 8OS, and 12OS ($p\geq0.05$, Table 2, and Fig. 1g). Farinographic parameters could be used to predict the quality of bread only if WF was replaced by up to 12% of OS. In amounts higher than 12% of substitution, the structure of wheat dough was severely disrupted.

3.2. Extensograph analysis

The results showed the addition of OS had a significant effect on all Extensograph characteristics of flour samples ($P<0.01$, Table 3). Energy and tensile strength in composite dough decreased significantly compared to the control dough ($P<0.05$, Fig. 1h, and 1j). Higher energy showed higher elasticity of the dough (21). Energy in 4OS was reduced by 47.22%, 45.45%, and 56% at rest times of 45 min, 90 min, and 135 min, respectively, compared to the control. Energy in 8OS was reduced by 66.66% in both 45-minute and 90-minute rest periods compared to the control (Fig. 1h). The results agreed with Mis et al. (24) who reported adding oat fiber reduced the energy of bread dough. Therefore, in the dough's enrichment with OS, the elasticity of the dough and the resistance to deformation were reduced.

Adding the OS decreased the ability to stretch from 157±2.5 mm in control to 73±2.5 mm in 8OS at a rest time of 45 min. It was reduced from 124±3.0 mm in control to 62±2.5 mm in 8OS at 90 min rest. The ability to stretch reduced from 104±2.5 mm in the control to 85±2.5 mm in 4OS at a rest time of 135 min. It could be concluded that gluten protein played an important role in the rheological properties of the dough. So by decreasing the amount of this protein and increasing the percentage of OS replacement, all properties measurable by Extensograph had decreased. Our results were in line with the study of Mis et al. (24) who reported adding carob fiber reduced the ability to stretch in bread dough. However, the Extensograph properties of the 8OS at rest time of 135 min and 12OS at all rest time couldn't be measured. As a result, adding over 8OS weakened the dough in terms of Extensograph characteristics. Stretch resistance and maximum stretch resistance of the dough decreased significantly with increasing replacement level ($P<0.01$, Fig. 1i, and 1k). The highest stretch resistance of 141±1.5 BU, 184±3.5 BU, and 170±3.2 BU at rest times of 45 min, 90 min, and 135 min, respectively, were related to the control. The lowest stretch resistance of 70±3.0 BU at a rest time of 90 min was related to the 8OS. Xu et al. (21) reported that the higher the ratio of stretch resistance to tensile strength, the firmer the dough would be. Based on the ratio of maximum stretch resistance to tensile strength, it could be concluded that adding over 4OS caused the dough to be hardened compared to the control (Fig. 1m).

Table.3. Analysis of variance of Extensograph characteristics of flour enriched with different levels of OS.

Source of changes	Fermentation time (min)	Degree of freedom	Mean squares	F value	Prob
Energy (cm2)	45	2	457	114.25	<0.0001**
	90	2	379	66.88	<0.0001**
	135	1	294	73.50	0.0010**
Stretch resistance (BU)	45	2	2681.44	618.79	<0.0001**
	90	2	10252.11	654.39	0.0001**
	135	1	13728.17	748.81	<0.0001**
Tensile strength (mm)	45	2	5665.33	1019.76	<0.0001**
	90	2	3031	363.72	<0.0001**
	135	1	541.50	85.50	<0.0008**
Maximum stretch resistance (BU)	45	2	1648.11	90.45	<0.0001**
	90	2	2692.11	183.55	<0.0001**
	135	1	7420.17	967.85	<0.0001**
Ratio of stretch resistance to tensile strength	45	2	0.20	483.50	<0.0001**
	90	2	0.21	26.57	0.0010**
	135	1	0.84	236.54	0.0001**
Ratio of maximum stretch resistance to tensile strength	45	2	0.99	171.23	<0.0001**
	90	2	0.95	222.80	<0.0001**
	135	1	0.33	90.78	0.0007**

**: Significant at the level of 1%, *: Significant at the level of 5%, ns: meaningless

3.3. SEM analysis

In the control sample, the starch granules were distributed throughout the protein strands. There was a continuous gluten system that stored the starch, so the starch granules were not visible compared to the other samples (Fig. 2a). While swollen starch granules were seen in the images of 4OS, 8OS, and 12OS with 2000 magnification (Fig. 2b, 2c, and 2d). The results agreed with the study of Sardabi et al. (25) who reported in control bread starch granules were completely gelatinized however in fortified bread with Moringa peregrina seed peel starch granules were seen. Because OS proteins did not make gluten like wheat, OS bread was granular rather than porous. The size of starch granules in OS bread had increased with increasing replacement percentage, which indicated an increase in their WAC in the Farinograph test. The results of SEM and the rheological properties of the dough tests were consistent. Photographs of control and enriched bread by OS displayed the enriched samples lacked filament-like structures. They mostly had a spherical starch structure (Fig. 2b, 2c, and 2d).

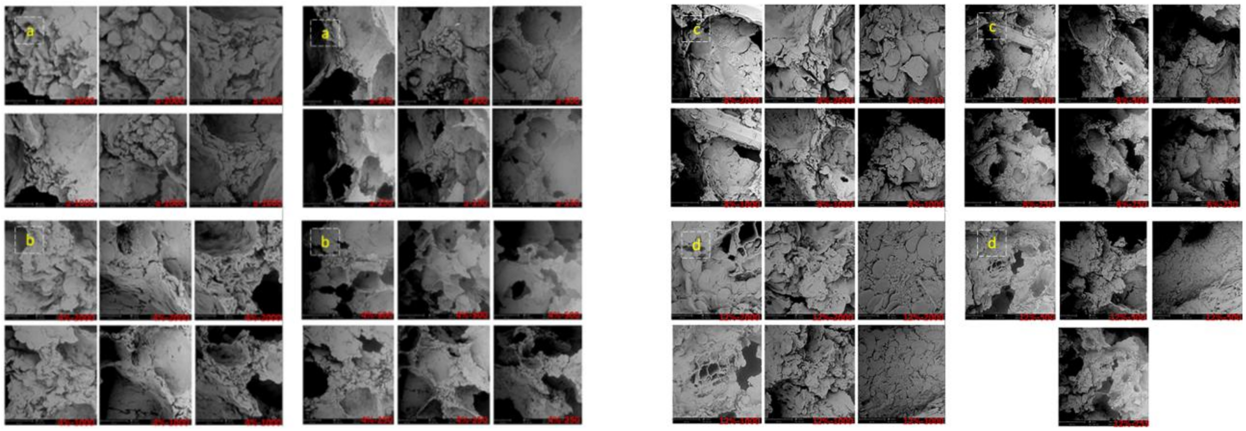

Fig. 2. Scanning electron microscopy (SEM) pictures of bread crumb samples with magnifications of 2000 x, 1000 x, 500 x, and 250 x: (a) Control; (b) Enriched with 4% Okra stem (OS); (c) Enriched with 8% OS; (d) Enriched with 12% OS.

The results indicated the control bread had a more open structure and larger cavities compared to other treatments. The diameter and thickness of the cells in the control were larger than the enriched samples. The microstructure of the control gas cell wall showed it had a higher number of gas cells compared to other treatments. It seemed there was a fusion of gas cells in the control, which was shown by the larger diameter and thickness of the cell layers. The results demonstrated the addition of OS to the formulation led to the creation of products with a more continuous (dense) network than the product free of this plant. OS weakened the structure of gluten and inhibited the proliferation of gas cells. This formed a dense crumb structure (fewer small bubbles) at the end of the oven spring. These results were consistent with tissue results because harder tissue was observed in OS bread compared to control (Fig. 2b, 2c, 2d, and 3a). The deep and longitudinal cracks in the control were minimized in OS bread. The depth of the cracks decreased with increasing replacement percentage.

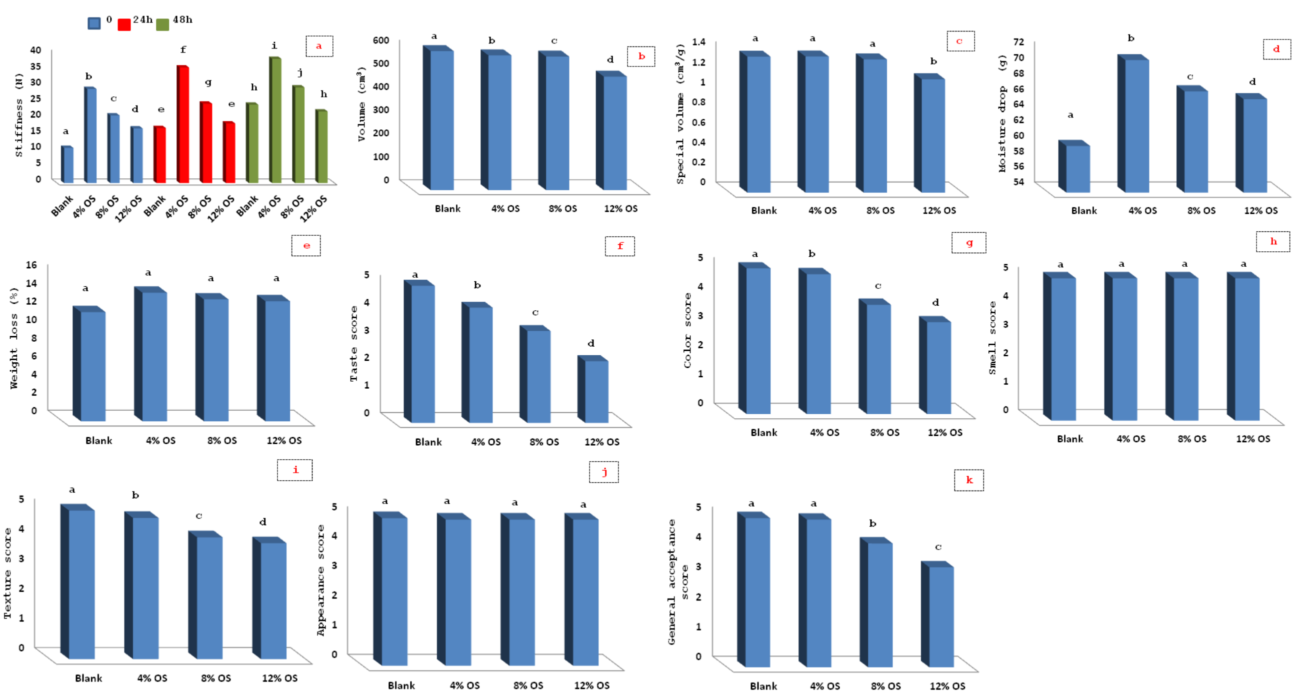

Fig. 3. Comparisons of mean (a) texture during storage (0h, 24h, and 48h); (b) volume; (c) special volume; (d) moisture drop; and (e) weight loss; (f) Taste score; (g) Color score; (h) Smell score; (i) Texture score; (j) Appearance score; (k) General acceptance score between control and fortified bread samples.

3.4. Texture profile and bread staling analysis

Statistical analysis showed that the interaction effect of the percentage of replacement WF by OSF and storage time on the texture of bread had a meaningful difference (P<0.01). As shown in Fig. 3a, the rigidity of the bread increased with the addition of OS, and this rate is lower with increasing replacement percentage. The results of the comparison of the rigidity average between the control and 4OS (P<0.01), 8OS, and 12OS (P<0.05) displayed a meaningful statistical difference. On the first day of maintenance, the highest firmness at 29.2±1 N was related to 4OS. On the same day, the lowest number at 10.93±1 N was related to control (Fig. 3a). The reason was a reduction in gas retention by the gluten network in samples containing OS, which led to a dense bread structure. The results of the texture were consistent with the results of SEM. In SEM, reducing retention of gases was observed by adding OS. The addition of the OS weakened the elasticity of the gluten structure and caused less elasticity and compact (dense) microstructure in the crumb. The results were in line with the results of Gonzalez et al. (26) who reported the use of 4% activated carbon increased the rigidity of bread. The texture results were consistent with the results of loose dough in the evaluation of the Farinograph. In the Farinograph test, with an increasing percentage of replacement of WF by OSF, loose grade increased.

The staling process led to a change in the tissue and reduced the characteristics of fresh bread, resulting in a decrease in the duration (4). During storage (maximum 3 days), an increase in the bread's crumb firmness was observed for all bread (Fig. 3a). The highest increase in the bread's crumb firmness of 122.69% compared to the first day of storage was related to control bread. The lowest (31.15%) was for 12OS. The results obtained of texture during storage were consistent with the results of Mikulec et al. (4). These researchers reported all samples displayed the lowest stiffness on the first day of storage compared to the second to fifth days. In this paper, the highest stiffness was related to 3 days of storage (Fig. 3a). The firmness of 12OS was not significantly different from the control on the second and third days of storage (P≥0.05, Fig. 3a).

3.5. Bread volume and special volume

Statistical analysis showed the effect of WF-OSF substitution on the volume, and the special volume of bread had a meaningful difference (P<0.01). There was a statistically significant difference between the volume of control and 4OS, 8OS, and 12OS (P<0.01, Fig. 3b). A significant difference in special volume was observed between the control and 12OS (P<0.01, Fig. 3c). The volume of 4OS, 8OS, and 12OS was 3.05%, 3.89%, and 18.31% lower than the control, respectively (Fig. 3b). The special volume of 12OS was 16.66% lower than the control (Fig. 3c). Replacing part of WF by OSF reduced the volume of bread due to the weakening of wheat gluten performance.

Bread crumb porosity was positively correlated with volume and gluten content. Higher gluten increased gas retention and pores formation. As a result, the volume of bread increased. The results of the volume test were consistent with the results of the SEM analysis. In SEM analysis, it was displayed the diameter and thickness of gas cells in the control were higher than the samples enriched with OS. Espinosa-Ramírez et al. (27) and Sun et al. (28) accounted for the high preservation of CO2 during fermentation increasing the bread volume. Higher rates of wheat substitution led to a reduction in the bread volume also a decrease in the bread's porosity crumb. The results demonstrated the higher values of the Farinographic parameters (WAC, stability time, and degree of softening) were related to a low volume drop.

3.6. Moisture drop and weight loss

Statistical analysis showed the effect of WF-OSF replacement percentage had a significant difference in the moisture drop (P<0.01). There was a statistically significant difference between the moisture drop of control and 4OS (P<0.01), 8OS, and 12OS (P<0.05, Fig. 3d). Moisture in the samples enriched with OS had a greater drop compared to the control (Fig. 3d). The moisture drop of prepared bread with 4OS, 8OS, and 12OS was 18.33%, 11.66%, and 10% higher than the control, respectively. The results of moisture loss were consistent with the results of stiffness. The highest moisture loss and the highest stiffness were related to 4OS. The lowest moisture content and the lowest stiffness were related to control (Fig. 3a, and 3d). Our results were in line with the results of Simsek (12) who reported water migration was the main reason why bread hardened. The results of comparing the moisture drop mean between the enriched samples displayed the lowest values of the mentioned Farinograph parameters (WAC, and softening) were associated with high moisture drop.

Fig. 3E shows additional OS does not cause a significant difference in weight loss (P≥0.05). No significant difference in weight loss was observed between the control and composite samples (p≥0.05). The weight loss of control and 4OS, 8OS, and 12OS were 12±2.5%, 14.13±2.4%, 13.4±2.5%, and 13.2±2.5%, respectively. In the Vouris et al. (29) study, bread weight loss was between 16-18%, which was partly more than the values in this article. Evaporation of water and ethanol caused bread weight loss within the baking process (14, 30) (Fig. 3e).

3.7. Sensory analysis

The time of occurrence of the first organoleptic changes determined the shelf life of the product (2). From the point of view of mold growth the shelf life of 4OS, 8OS, and 12OS at room temperature were 8 days, 10 days, and 11 days, respectively while the shelf life of control was only 6 days. The reason for this could be related to flavonols with the group of galates in the OS that improved the microbiological quality of the tested bread. These results were consistent with the results of Mikulec et al. (4), who reported that shelf life had been increased from 3 days in control to 5 days in fortified bread by Cistus.

The effect of WF-OSF replacement percentage on the taste, color, texture, and general acceptance had a statistically significant difference ($P<0.01$). There was no statistically significant difference between the smell and appearance in the control and all composite samples ($P\geq0.05$, Fig. 3h, and 3j). The taste and color score in 12OS was significantly lower than other breads ($P<0.01$, Fig. 3f, and 3g). In this fortified bread, OS particles were quite noticeable in the mouth, while in other bread this issue was not observed. In addition, the bitter taste, which was related to the high amount of phenolic compounds in the formulation of this bread, was noticeable. It was important to note the taste of this bread was not protested by the panelists. The darker crust color of bread fortified with OS compared to control was related to green OS powders. A meaningful statistical difference was observed in texture scores between control and 4OS ($P<0.05$), 8OS, and 12OS ($P<0.01$, Fig. 3i). Bread samples containing OS were firmer that was matched the results of the tissue test.

While the width was the same in all bread (10 cm), the different heights of fortified bread compared to the control had caused a statistically significant difference between the widths-to-height ratio in bread samples ($P<0.01$). The results of mean comparisons showed the highest and lowest heights were related to control and 12OS, respectively. It was important to note this had not led to negative scores for bread by evaluators. Also, there was no meaningful statistical difference in general acceptance scores between control and 4OS (Fig. 3k).

Bakare et al. (14), Bolarinwa et al. (2), Chisenga et al. (11), Dube et al. (1), and Torbica et al. (19) reported that bread flour permissible substitution levels are 5-10%, 5%, 10%, 20-30%, and 10%, respectively.

4. Conclusion

The highest WAC, stability time, and consistency were related to 12OS. The results showed the OSF reduced the development time of the dough by 34.78% compared to the control. Using 12% OSF produced enriched bread with the lowest moisture drop, crumb firmness even during storage, and highest storage time. The highest energy, stretch resistance, tensile strength, and volume were related to 4OS. Also, the lowest degree of softening was related to 4OS. There was no statistically significant difference in appearance and weight loss between the control and bread fortified with OS. Also, there was no meaningful statistical difference in special volume and general acceptance score between control and 4OS. In OS bread the deep and longitudinal cracks were minimized and the depth of the cracks decreased with increasing replacement percentage. Overall, OSF could be a suitable strategy for producing enriched bread to consume by-products rich in dietary fibers and polysaccharides.

5. Acknowledgement

The authors thank the Sabzevar Department of Food Science and Technology for their support..

6. Authors' contributions

S. Amirabbasi Conceptualized, Investigated, Conducted research, Wrote-Original Draft; A. Elhamirad Conceptualized, Investigated, Designed research, Supervised; M. Saeediasl and M. Armin analyzed data; SH. Ziaolhagh Wrote-Review & Edited. All the authors read and approved the final manuscript.

7. Conflict of interest

The authors declared no competing interests.

8. Funding information

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

9. Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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