



Quality Evaluation of Bread Produced from Wheat Flour (*Triticum aestivum*) Fortified with Date Flour (*Phoenix dactylifera* L.)

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ABSTRACT

Background: This study was carried out to evaluate the quality of bread produced from wheat flour (*Triticum aestivum*) fortified with date flour (*Phoenix dactylifera* L.).

Methods: Bread samples were produced from blends of wheat flour and date flour at varying proportions of (100:0, 90:10, 80:20, 70:30, 60:40 and 50:50) respectively. Proximate composition, physical, functional and pasting properties and sensory response were carried out on the bread samples.

Results: The results showed that the moisture, protein, fat, fibre, ash and carbohydrate content were in the range of 8.54-16.81%, 12.71-14.59%, 1.00-1.90%, 0.98-1.39%, 3.83-4.62% and 61.59-72.32% respectively. The lightness (L*), redness (a*) and yellowness (b*) ranged 24.41-52.26%, 5.59-51.51% and 23.28-55.76%, respectively. The value for peak, trough, breakdown, final viscosity, setback, peak time and pasting temperature ranged from 1090.50-1690.50 RVU, 622.00-1158.50 RVU, 251.50-651.00 RVU, 1494.50-1816.50 RVU, 343.00-985.00 RVU, 4.29-5.68 min and 80.06-90.39°C respectively. The values of taste, aroma, texture, appearance and overall acceptability ranged from 4.37-6.40, 4.47-6.33, 4.07-6.33, 4.20-6.30 and 4.23-7.37 respectively. Supplementation of 40% date flour into wheat flour gave bread with highest sensory value of 7.37 for overall acceptability. The bread sample produced with 60% wheat flour/40% date flour is recommended to consumers due to its high sensory scores.

Key words: Bread, Dates fortification, Quality, Wheat.

INTRODUCTION

Bread is a common staple and baked food product that has received acceptance from children, young and adults. It is made from wheat flour and water to form dough and then baked at high temperature. According to Adeyeye *et al.*, 2019, "Bread is traditionally produced from wheat (*Triticum aestivum*) flour. There has been increase in demand for bread in Nigeria and other African countries in the last three decades. However, most of these African countries are importers of wheat and this has affected their foreign reserves and fragile economies negatively".

Wheat varieties are of two types; these are the soft wheat flour and hard wheat flour. The soft wheat flour contains low gluten content and the hard wheat flour on the other hand contains high gluten content. Hard flour or bread flour is high in gluten with 12% to 14% gluten content and its dough has elastic toughness that holds its shape well once baked (Chu and Michael, 2004). Addition of underutilized crops such as sorghum, millet, chickpea and Dates will improve the nutrition and reduce gluten in the baked products from wheat flour (Erskine *et al.*, 2004; Davana *et al.*, 2021; Goel *et al.*, 2021; Rathore *et al.*, 2021). This will also reduce cost of wheat importation and remove the burden of borrowing by many tropical and developing countries flour (Davana *et al.*, 2021; Goel *et al.*, 2021; Rathore *et al.*, 2021).

Date fruits are considered as ideal foods that contain several essential nutrients that have beneficial effects on

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human health (Erskine *et al.*, 2004). Dates are eaten in different forms. According to Al-Hooti *et al.*, 1997, "30%-40% of Dates are consumed in the fresh form while 60%-70% are eaten in the dried form at semi-ripe or fully ripe stages with little or no processing". Dates fruits contain high amounts of water-insoluble mannan fibers and this could be used to improve the fiber content of certain food products (Gnani *et al.*, 2017).

According to Gnani *et al.*, 2017, "Date fruit is a key food security resource in the arid-lands that requires intensive efforts for valorization. There is a large potential to especially develop healthy food products utilizing the

high value fiber and phenolic antioxidants found in the fruit flesh and seeds.

This study was therefore carried out to evaluate the quality of bread produced from wheat flour (*Triticum aevistivum*) fortified with date flour (*Phoenix dactylifera* L.).

MATERIALS AND METHODS

Materials

The dried date palm fruits were bought from Sabo Market, Ikorodu, Lagos State, Nigeria. Whole wheat, milk, salt, margarine (fats) and baking powder were bought from flour market, Ikorodu, Lagos State, Nigeria. Materials were taken to the Food Processing Laboratory of Department of Food Technology, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria for processing into Date palm flour and bread. The research was carried between December, 2019 and September, 2020.

Preparation of date palm fruit flour

The date fruit flour was made according to Fig 1.

Preparation of whole wheat flour

Whole wheat was prepared according to Fig 2.

Production of bread from wheat flour and date flour

Whole wheat flour and date flour were measured according to the recipe in Table 1. Bread samples were made by using Straight dough method according to the method of Olu Malomo, *et al.*, 2013 as reported by Adeyeye, *et al.*, 2019 (Fig 3)".

Proximate analysis

Determination of moisture, protein, fat, crude fibre and ash contents

The moisture, protein, fat, crude fibre and ash contents were determined by using standard methods described by (AOAC, 2005).

Determination of carbohydrate content

The carbohydrate contents of each sample were determined by method described by (AOAC, 2005). Carbohydrate was determined by the difference using the formula below:

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude fibre} + \% \text{ crude protein} + \% \text{ fat}).$$

Determination of physical properties of bread

The measurement analyses on the fresh products are loaf volume, specific loaf volume, loaf weight, oven spring, texture and colour were determined by using method described by (Onwuka, 2005).

Determination of functional properties of the flour

Bulk density

Bulk density was determined by the method described by Elavaveniya and Jayamuthunagai (2014).

Determination of pasting properties of flour blends

This was determined by the Rapid Visco Analyser (RVA TECMASTER, Perten Instrument) method as described by Newport Scientific (1998).

Foam capacity and stability

Foam capacity and stability of the flour samples were determined according to the AOAC, (2006) method.

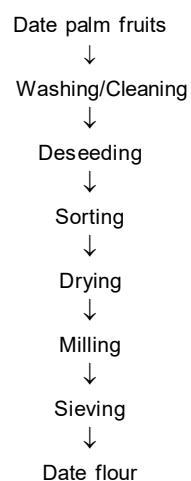


Fig 1: Flow diagram for the production of date palm flour.

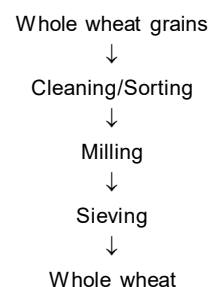


Fig 2: Flow diagram for the production of whole wheat flour.

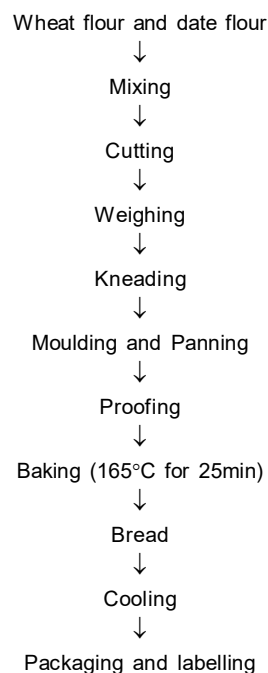


Fig 3: Flow diagram for the production of bread from wheat - date flour.

Water/oil absorption capacity

Water/oil absorption capacities were determined by AOAC, (2006) method.

Swelling index determination

This was determined by the method of Elaveniya and Jayamuthunagai (2014).

Sensory evaluation of bread samples

Bread samples made from wheat-dates composite flour were evaluated for consumer acceptance. The sensory evaluation of the bread including the control were done for texture, taste, aroma, crust colour, crumb colour and general acceptability of the product by a twenty man panel on a 9 point hedonic scale (1 = extremely dislike, 9 = extremely like).

Statistical analysis

Data were obtained in triplicates and one way Analysis of variance (ANOVA) with random model using IBM SPSS 19.0 version 2011 software. Mean differences were separated and compared using turkeys test ($p \leq 0.05$) to study the difference among means.

RESULTS AND DISCUSSION**Proximate composition of bread produced from blend of wheat flour and date flour**

Proximate composition of the bread was presented in Table 2 above. The value of protein ranged from 12.71-14.59. The highest value was found in sample STE (50% wheat flour/50% date flour) while the lowest value was found in sample EST (100% wheat flour) and ETS (90% wheat flour/10% date flour). The values in this study were similar to the values of (3.51-24.51) reported by Makinde and Adeyemi (2018) for biscuits. The value of fat ranged from 1.00-1.90. Sample

ETS (90% wheat flour/10% date flour) has the highest value of fat content while sample STE (50% wheat flour/50% date flour) has the lowest value. The value of fat reduced with increase in date flour.

The values of fiber ranged from 0.96-1.39. The highest value of fibre content was found in sample STE (50% wheat flour/50% date flour) while the lowest value was found in sample EST (100% wheat flour). The values obtained for ash in this study were higher than the value of between (1.09-1.99) reported by Bibiana *et al.* (2019) who worked on the quality evaluation of composite bread produced from wheat, water yam and brown hamburger bean flours. The values for carbohydrate ranged from 61.59-72.32. Sample EST (100% wheat flour) has the highest value of carbohydrate while sample STE (50% wheat flour/50% date flour) has the lowest value. There was reduction in the value of carbohydrate as the level of date flour increases. There was also significant difference ($p < 0.05$) between all the samples. This result was similar to that reported by Abdalla (2003) who studied the total carbohydrate content of Indian wheat flour (75.39%).

Functional properties of wheat flour and date flour

The bulk density, water absorption capacity, oil absorption capacity, swelling capacity, solubility, least gelation concentration, dispersibility, foam capability and foam stability are shown in Table 3. The value of bulk density ranged from 0.63-0.73. The highest value of bulk density was found in sample STE (50% wheat flour/50% date flour) while the lowest value was observed in sample EST (100% wheat flour). There was significant difference ($p < 0.05$) in bulk density between the samples. From the results, the value of bulk density increase with increase in levels of date flour. The values obtained for bulk density in this study were lower than the values of (0.89-0.95) reported by Alake *et al.* (2016) who worked on physico-chemical properties and sensory attributes of cassava-enriched custard powder.

The values of water absorption capacity ranged from 82.77-120.74. The highest value of water absorption capacity was found in sample EST (100% wheat flour) while the lowest value was found in sample STE (50% wheat flour/50% date flour). There was significant difference ($p < 0.05$) between the samples. The value of water absorption in this study increases as the level of date flour increases from

Table 1: Experimental Design

Samples	Wheat flour	Date Flour
EST	100% (300 g)	-
ETS	90% (270 g)	10% (30 g)
ESE	80% (240 g)	20% (60 g)
TES	70% (210 g)	30% (90 g)
SET	60% (180 g)	40% (120 g)
STE	50% (150 g)	50% (150 g)

Table 2: Proximate composition of bread produced from blend of wheat flour and date flour.

Sample	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
EST	8.54±0.07 ^e	12.71±0.08 ^d	1.65±0.08 ^b	0.96±0.01 ^e	3.83±0.01 ^e	72.32±0.13 ^a
ETS	11.29±0.02 ^d	12.71±0.21 ^d	1.90±0.03 ^a	1.02±0.01 ^d	3.86±0.01 ^{de}	69.23±0.18 ^b
ESE	14.36±0.01 ^c	13.05±0.10 ^c	1.62±0.08 ^b	1.09±0.03 ^c	3.93±0.00 ^{cd}	65.95±0.01 ^c
TES	15.81±0.23 ^b	13.54±0.17 ^b	1.31±0.04 ^c	1.15±0.01 ^b	4.02±0.02 ^c	64.19±0.04 ^d
SET	16.70±0.03 ^a	13.82±0.04 ^b	1.21±0.03 ^c	1.36±0.02 ^a	4.31±0.03 ^b	62.61±0.01 ^e
STE	16.81±0.08 ^a	14.59±0.09 ^a	1.00±0.14 ^d	1.39±0.01 ^a	4.62±0.08 ^a	61.59±0.22 ^f

*Mean±standard deviation with same superscripts along the column are not significantly different at ($p > 0.05$).

Key: EST: 100% Wheat flour; ETS: 90% Wheat flour; 10% Date flour; ESE: 80% Wheat flour; 20% Date flour; TES: 70% Wheat flour; 30% Date flour ; SET: 60% Wheat flour; 40% Date flour; STE: 50% Wheat flour; 50% Date flour.

Table 3: Functional and Pasting Properties of the wheat flour and date flour.

Sample	Bulk density	Water absorption capacity	Oil absorption capacity	Swelling capacity	Solubility	Least gelation concentration	Dispersibility	Foam capability	Foam stability
EST	0.63±0.00 ^e	120.74±0.94 ^a	98.38±0.37 ^a	676.25±0.64 ^a	8.38±0.05 ^f	10.85±0.07 ^f	65.35±0.21 ^d	18.00±0.00 ^a	13.43±0.14 ^e
ETS	0.71±0.00 ^d	111.03±0.09 ^b	91.08±0.07 ^b	664.46±0.13 ^b	11.63±0.01 ^e	12.40±0.14 ^e	67.00±0.00 ^c	12.00±0.00 ^f	16.62±0.07 ^a
ESE	0.71±0.00 ^d	101.77±0.04 ^c	88.05±0.01 ^c	610.97±0.18 ^c	15.16±0.23 ^d	12.85±0.07 ^d	69.25±0.35 ^b	12.50±0.00 ^e	15.82±0.05 ^b
TES	0.72±0.00 ^c	93.63±0.25 ^d	87.18±0.01 ^d	602.43±0.62 ^d	15.75±0.40 ^c	13.88±0.04 ^c	71.00±0.00 ^a	14.64±0.08 ^d	14.31±0.03 ^c
SET	0.72±0.00 ^b	88.67±0.25 ^e	86.05±0.26 ^e	568.58±0.06 ^e	22.31±0.05 ^b	14.85±0.07 ^b	71.00±0.00 ^a	15.21±0.29 ^c	14.03±0.09 ^d
STE	0.73±0.00 ^a	82.77±0.47 ^f	74.44±0.13 ^f	523.85±0.33 ^f	25.63±0.11 ^a	15.10±0.14 ^a	71.25±0.35 ^a	16.41±0.04 ^d	12.72±0.03 ^f

Sample	Peak	Trough	Breakdown	Final viscosity	Setback	Peak time	Pasting temperature
EST	1480.00±1.41 ^b	812.00±16.97 ^d	651.00±8.49 ^a	1816.50±3.54 ^a	985.00±7.07 ^a	5.68±0.07 ^a	87.57±0.45 ^b
ETS	1690.50±13.44 ^c	1158.50±3.54 ^b	489.50±43.13 ^c	1686.50±3.54 ^d	525.00±4.24 ^d	4.29±0.04 ^c	80.06±0.08 ^c
ESE	1366.50±2.12 ^a	991.00±1.41 ^a	374.00±2.83 ^b	1432.00±.83 ^b	444.50±3.54 ^b	4.42±0.02 ^e	80.73±0.03 ^d
TES	1098.50±0.71 ^d	843.00±4.24 ^c	251.50±2.12 ^d	1192.00±0.00 ^e	343.00±4.24 ^e	4.35±0.02 ^{cd}	80.77±0.04 ^c
SET	1093.50±7.78 ^d	843.00±4.24 ^c	252.50±0.71 ^d	1190.50±2.12 ^e	345.00±1.41 ^e	4.33±0.00 ^{cd}	80.80±0.00 ^c
STE	1090.50±0.71 ^d	622.00±2.83 ^e	466.00±1.41 ^b	1494.50±2.12 ^c	870.50±2.12 ^c	5.57±0.05 ^b	90.39±0.09 ^a

*Mean±standard deviation with same superscripts along the column are not significantly different at (p>0.05).

KEY: EST: 100% Wheat flour; ETS: 90% Wheat flour; 10% Date flour; ESE: 80% Wheat flour; 20% Date flour; TES: 70% Wheat flour; 30% Date flour

SET: 60% Wheat flour; 40% Date flour; STE: 50% Wheat flour; 50% Date flour.

10%-50%. The values gotten in this sample were higher compared to the values reported by Otegbayo *et al.* (2013) who worked on functional properties of soy-enriched tapioca.

The values for oil absorption capacity ranged from 74.44-98.38. The highest value of oil absorption capacity was found in sample EST (100% wheat flour) while the lowest value was found in sample STE (50% wheat flour/ 50% date flour). The values obtained for oil absorption capacity in this study was lower than the values of (106.00-160.00) reported by Ajani *et al.* (2016) on functional properties of composite flour made from wheat and breadfruit.

The values of swelling capacity the flour ranged from 523.85-676.25. The highest value was found in sample ETS (90% wheat flour/10% date flour) while the lowest value was found in sample STE (50% wheat flour/50% date flour). The value of solubility of the flour ranged from 8.38-25.63. The highest value of solubility was found in STE (50% wheat flour/50% date flour) while the lowest value was found in EST (100% wheat flour).

Dispersibility values ranged 65.35-71.25. There was significant difference (p<0.05) in least gelation concentration between sample EST (100% wheat flour), ETS (90% wheat flour/10% date flour) and ESE (80% wheat flour/20% date flour).

For foam capability, the value ranged from 12.00-18.00. Sample EST (100% wheat flour) has the highest value of foam capability while sample ETS (90% wheat flour/10% date flour) has the lowest value. The foam stability value ranged from 12.72-16.62. Sample ETS (90% wheat flour/10% date flour) has the highest value of foam stability while sample STE (50% wheat flour/50% date flour) has the lowest value.

Pasting properties of the blend of wheat and date flour

The results of pasting properties of the flour were shown in Table 3. The values for peak viscosity ranged from 1090.50-1690.50. The highest value of peak viscosity was found in sample ETS (90% wheat flour/10% date flour) while the lowest value was found in sample STE (50% wheat flour/ 50% date flour). According to Abioye *et al.*, 2011, "The relatively low peak viscosity in the higher level of date flour in composite flour indicates that the flour may be suited for products requiring low gel strength and elasticity".

The results in this study were lower than the values of (2311.67-4423.00) reported by Elisa *et al.* (2015) for composite flour from cassava, rice, potato, soybean and xanthum gum as an alternative of wheat flour. The values for trough ranged from 622.00-1158.50. Sample ETS (90% wheat flour/10% date fruit) has the maximum value while sample STE (50% wheat flour/50% date flour) has the minimum value. The results obtained in this study were higher than the results of (39.60RVU-59.19RVU) reported by Blessing (2014) who worked on chemical, functional and pasting properties of wheat-walnut flour. The values for breakdown ranged from 251.50-651.00. The highest value was found in sample EST (100% wheat flour) while the

lowest value was found in sample TES (70% wheat flour/30% date flour).

The values for breakdown viscosity in this study were higher than the values of (56.64-69.30) reported by Ojo *et al.* (2017) for functional and pasting properties of cassava starch and mushroom flour blends. Final viscosity values ranged from 1190.50-1816.50. The values obtained for final viscosity in this study were lower than those reported by Otegbayo *et al.* (2013) who worked on functional properties of soy-enriched tapioca. For setback, the value ranged from 343.00-985.00. The highest value for setback was found in sample EST (100% wheat flour) while the lowest value was found in sample TES (70% wheat flour/30% date flour). The values of setback in this study were higher than the values of (115.37-139.49) reported by Ojo *et al.* (2017).

Table 4: Colour attributes and Physical properties of the bread produced from wheat flour and date flour.

Sample	L*	a*	b*
EST	24.41±0.01 ^e	51.51±1.16 ^a	24.41±0.01 ^e
ETS	34.23±0.63 ^d	37.54±0.33 ^b	28.00±0.00 ^d
ESE	52.26±0.37 ^a	23.12±0.32 ^d	23.28±0.06 ^e
TES	44.28±0.01 ^b	5.59±0.09 ^e	49.78±0.03 ^b
SET	37.64±0.66 ^c	6.38±0.26 ^e	55.75±0.01 ^a
STE	36.69±0.13 ^c	27.06±0.71 ^c	35.56±1.41 ^c

Sample	Loaf weight	Loaf volume	Specific Loaf volume
EST	271.00±4.24 ^a	346.00±0.71 ^b	1.28±0.02 ^b
ETS	254.00±2.83 ^{ab}	405.25±1.06 ^a	1.60±0.01 ^a
ESE	247.00±9.89 ^{ab}	329.95±0.07 ^c	1.34±0.06 ^b
TES	226.00±31.11 ^b	236.32±0.97 ^f	1.06±0.15 ^c
SET	235.00±21.21 ^{ab}	325.25±0.35 ^d	1.39±0.13 ^b
STE	244.00±5.66 ^{ab}	305.00±0.71 ^e	1.25±0.03 ^{bc}

*Mean±standard deviation with same superscripts along the column are not significantly different at ($p>0.05$).

Foot Note: L*: Lightness; a*: Redness; b*: Yellowness

Key: EST: 100% Wheat flour; ETS: 90% Wheat flour; 10% Date flour; ESE: 80% Wheat flour; 20% Date flour; TES: 70% Wheat flour; 30% Date flour; SET: 60% Wheat flour; 40% Date flour; STE: 50% Wheat flour; 50% Date flour.

Table 5: Results of the sensory scores of the samples.

Sample	Taste	Aroma	Texture	Appearance	Overall Acceptability
EST	5.47±0.82 ^{bc}	5.60±1.00 ^{bc}	5.80±1.57 ^{abc}	5.90±0.92 ^a	6.70±0.88 ^b
ETS	4.37±1.03 ^e	4.47±1.00 ^e	4.07±1.14 ^e	4.20±1.16 ^d	4.23±0.97 ^d
ESE	4.87±0.89 ^{de}	5.13±0.97 ^{cd}	5.30±1.15 ^{cd}	4.90±0.88 ^b	6.00±0.91 ^c
TES	4.77±0.98 ^{de}	4.97±1.16 ^d	4.87±1.14 ^d	4.70±1.18 ^{bc}	5.93±0.87 ^c
SET	6.40±1.19 ^a	6.33±0.99 ^a	6.33±1.21 ^a	6.30±1.12 ^a	7.37±1.16 ^a
STE	5.80±1.03 ^b	5.77±1.17 ^b	6.17±1.05 ^{ab}	6.00±1.17 ^a	6.95±1.07 ^{ab}

*Mean±standard deviation with same superscripts along the column are not significantly different at ($p>0.05$).

Key: EST: 100% Wheat flour; ETS: 90% Wheat flour; 10% Date flour; ESE: 80% Wheat flour; 20% Date flour; TES: 70% Wheat flour; 30% Date flour; SET: 60% Wheat flour; 40% Date flour; STE: 50% Wheat flour; 50% Date flour.

For peak time, the value ranged from 4.29-5.68. The values for pasting temperature ranged from 80.06-90.39. The highest value of pasting temperature was found in sample STE (50% wheat flour/50% date flour) while the lowest value was found in sample ETS (90% wheat flour/10% date flour). The values obtained for pasting temperature in this study were lower to the values of 91.47°C-94.56°C reported by Blessing (2014) for functional and pasting properties of wheat-walnut flour.

Colour attributes of bread produced from blend of wheat flour and date flour

The lightness (L*), redness (a*) and yellowness (b*) values of the bread samples were presented in Table 4. The colour attributes of bread varies and this can be attributed to the addition of date flour. The value for lightness (L*) ranged from 24.41-52.26. The value of redness (a*) ranged 5.59-51.51. Sample EST (100% wheat flour) has the highest value for redness while TES (50% wheat flour/50% date flour). The value of yellowness (b*) ranged 23.28-55.75. Sample ESE (80% wheat flour/20% date flour) has the highest value of yellowness while sample SET (60% wheat flour/40% date flour).

Physical properties of the bread

The physical properties of the bread were presented in Table 4. The values of weight vary from 226.00-271.00 with sample EST having the highest value while sample TES has the lowest value. The values for loaf volume content ranged from 236.32-405.25 with sample ETS (90% wheat flour/10% date flour) having the highest value while sample TES (70% wheat flour/30% date flour) has the lowest value. The values for specific loaf volume ranged from 1.06-1.09. The highest value was found in sample ETS (90% wheat flour/10% date flour) while the lowest value was found in sample TES (70% wheat flour/30% date flour). The values for specific loaf volume in this study were lower than the value (5.04-5.93) reported by Ranasalava and Visvanathan (2014).

Sensory evaluation of the bread produced from blend of wheat flour and date flour

The results of sensory evaluation were presented in Table 5 above. The value of taste ranged 4.37-6.40. The highest value was found in sample SET (60% wheat flour/40% wheat

flour) while the lowest value was found in sample ETS (90% wheat flour/10% date flour). The aroma value ranged 4.47-6.33. The highest value was found in sample SET (60% wheat flour/40% date flour) while the lowest value was found in sample ETS (90% wheat flour/10% date flour). The values of texture ranged from 4.07-6.33. The highest value was found in sample SET (60% wheat flour/40% date flour) while the lowest value was found in sample ETS (90% wheat flour/10% date flour). The value for appearance ranged 4.20-6.30. The highest value was found in sample SET (60% wheat flour/40% date flour) while the lowest value was found in sample ETS (90% wheat flour/10% date flour). Statistically, the value of overall acceptability ranged from 4.23-7.37. Sample SET (60% wheat flour/40% date flour) is the most preferable samples by panelists based on 9-point hedonic scale.

CONCLUSION

In conclusion, the production of bread, an important baked product prepared mainly from wheat flour could be prepared with date flour as a replace for refined sugar which will be beneficial to the body. Generally, the water absorption capacity, oil absorption capacity and swelling capacity decreased with increasing levels of date flour addition. Supplementation of 40% date flour into wheat flour gave bread with highest sensory value of 7.37 for overall acceptability. The bread sample coded with SET (60% wheat flour/40% date flour) can be recommended for consumers but further research work should be done on this product to focus on the storage stability and textural analysis of the bread.

Author's contributions

B.O.T, A.S.A.O and A.M.E. designed the study; B.O.T and A.M.E. conducted the experimental work; A.M.E. analyzed the data; A.S.A.O wrote the manuscript.

Conflict of interest: None.

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