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Research Article

Sweet Orange (*Citrus Sinensis*) Seed Oil as a Functional Ingredient for Bread-Antioxidant, Total Phenolic, Flavonoid, Carotenoid Content and Sensory Evaluation

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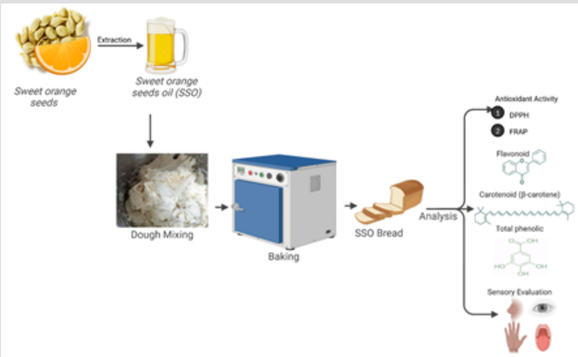
Graphical Abstract



Abstract

This study assessed the substitution of margarine with 10, 20, 30, 40 and 50% Sweet Orange (Citrus Sinensis) Seed Oil (SSO) to enhance their total phenolic, total flavonoid and total carotenoid contents. In addition, phytochemical composition, antioxidant activity, instrumental color, physical properties, and sensory attributes of the bread were investigated. The sensory scores for the bread attributes decreased as the level of SSO increased in the breads. However, the bread with 10% substitution showed no significant difference (p<0.05) in sensory attributes compared to the control. The bread with 10% SSO substitution exhibited the following values for height, loaf weight, oven spring, loaf volume, specific volume, and proofing ability: 19.50, 335.3, 20.30, 956, 2.90, and 24.20%, respectively. In contrast, the bread made with 100% margarine had values of 19.20, 313.75, 19.30, 735.5, 2.30, and 32.40% for the same attributes. Interestingly, the 10% substitution led to an increase in loaf volume, oven spring, and specific volume, but a decrease in oven spring. Moreover, the bread with 10% SSO substitution contained higher carotenoid contents and antioxidant activity, but lower phenol contents than the control bread. In terms of color, the bread with 10% SSO had a higher redness, yellowness, chrome, and hue angle values compared to the control. In conclusion, substituting 10% of margarine with SSO improved the overall quality of the bread.

Keywords: Bread; Margarine; Oil; Quality; Substitution

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Introduction

Bread, a widely accepted baked product, has gained rapid interest due to its ease of processing and flexibility in incorporating different ingredients [1,2]. Valorizing underutilized ingredients, by-products or waste presents an opportunity to create wholesome bread products with enhanced nutrient composition [2]. Margarine, a key ingredient in bread formulation alongside wheat flour, yeast, water, salt, and sugar, is used in substantial amounts [3,4]. The production of margarine involves a hydrogenation process, which increases saturated fatty acid content and produces trans fats as a side product. Trans fats have been strongly associated with an increased risk of heart disease, hay fever in children, and atopy disease which are no longer considered safe [5,6]. Their consumption through margarine-containing bread can pose health hazards such as increased cancer risk, lowered quality of breast milk, and decreased immune response [7].

The growing demand for healthy foods with reduced margarine content has prompted researchers to explore the formulation of wholesome food products enriched with functional components that enhance health and nutritional composition [8]. In this context, various margarine substitutes, including complex carbohydrates, gums, gels, and whole food matrices, have been studied for use in baked food products. Each fat substitute possesses unique qualities that can impact the quality of the final food product. Citrus seed oil, with its health benefits, can serve as a vegetable oil substitute for margarine in bread production [9]. Utilizing citrus seed oil instead of margarine in bread can help reduce bad cholesterol and saturated fat levels [10]. Additionally, citrus seed oil acts as an antioxidant, reducing the risk of cardiovascular disease and preventing cancer [9]. It also contains vitamin E, which has can be applied in bread baking and extends its shelf life [11,12]. While orange seeds are typically discarded as waste during citrus fruit processing, their oil has found use in the pharmaceutical, food, textile, and chemical industries for soap, detergent, perfume, candle, and insecticidal production [13-15]. Consequently, the impact of citrus seed oil on bread quality remains to be investigated. Therefore, this study aimed to evaluate the effect of substituting margarine with SSO on bread quality.

Materials and Methods

Ripe sweet orange fruits (*Citrus sinensis*) were purchased from new market in the Donga Local Government Area of Taraba State. The orange seeds were removed from the fruits, washed, sun dried to constant and used for this work. All the reagents and solvents employed in the analysis were of the analytical grade. They were obtained from the chemical store of the Food Science and Technology Department, Federal University Wukari.

Extraction of the Oil

Oil was extracted from the seeds of sweet orange fruits using a Soxhlet apparatus with n-hexane following the method described by Reazai et al. [16]. The oil was recovered from the combination after the extraction process was completed by evaporating the remaining extracting solvent at 50°C in an oven. The oil was stored in a bottle undisturbed for three days. The extraction yield of sweet orange seeds oil was calculated as:

Determination of Phytochemical Composition

Total Flavonoids Content

Total flavonoids contents of the samples were determined by spectrophotometric method using aluminum chloride and rutin as standards Popova et al. [17]. The sample extract (1ml) was diluted with 4ml of water in 10ml volumetric flask and 5%w/v of $NaNO_2$ solution (0.3ml) was added to sample flask. After 5minutes, 10%w/v of aluminum chloride (0.3ml) was added and after 6minutes, 1.0M NaOH solution (2ml) was equally added Distilled water (2.4ml) was added to the reaction flask and mixed thoroughly.

Total Phenolic Content

Total phenols contents were determined following the Folin-Ciocalteu's method using gallic acid as standard as described by Phuyal et al. [18]. Folin-Ciocalteu's reagent (12.5ul) along with 7% sodium carbonate (12.5ul) was added to the flour extracts. Samples were then incubated for 90 minutes at room temperature. The absorbance was measured at 750nm using micro plate reader (Synergy HT, Bio Tek Instruments, and Winooski VT USA).

$$Phenolic\ content\ \left(mg\frac{GAE}{g}\right) = c\frac{\mathrm{V}}{\mathrm{M}} \qquad enq\ 1$$

Where C=total phenolic content mg GAE/g dry extract, c=concentration of gallic acid obtained from calibration curve in mg/mL, V=volume of extract in mL, and m=mass of extract in gram.

Carotenoids Content

Carotenoid's content was determined according to the method described. The Sample (2g) was homogenized in methanol using a laboratory blender. A 1:10 (1%) mixture was used. The homogenate was filtered to obtain the initial crude extract, and 20ml of ether was added to the filtrate and mixed well and then, treated with 20ml of distilled water in a separating funnel. The ether layer was recovered and evaporated to dryness at low temperature (50°C) in a vacuum desiccator. The dry extract was then saponified with 20ml of ethanoic potassium hydroxide and left over in a dark cupboard. The next day, the carotenoid was taken up in 20ml of ether and then washed with two portions of 20ml distilled water. The carotenoid extract (ether layer) was dried in a desiccator and then allowed to stand overnight in a freezer (-10°C). The precipitated steroid was removed by centrifugation after 12 hours and the carotenoid extract was evaporated to dryness in a weighed evaporation dish cooled in a desiccator and weighed. The weight of carotenoid was determined and expressed as percentage of the weight as:

$$\%\ Carotenoid\ content = \frac{\text{Weight}}{\text{Weight of sample taken}}\ x\ 100 \qquad eqn\ 2$$

Antioxidant Activities by DPPA of Oil and Bread

The antioxidant activity of the oil was measured by the DPPH radical scavenging ability as modified method described by Hejna et al. [19]. Five different concentrations of the extract were tested. The results were expressed as radical scavenging activity. 3.9mL of 0.2mM DPPH solution was added to 0.1mL of extracted sample. The mixture was vortexed rigorously and stored in the dark for 30mins. The absorbance of the mixture (A_1) was measured at 517nm with the help of a UV-Vis spectrophotometer (AU-2701, Systronics, In- dia). A blend of 3.9mL of DPPH solution and 0.1mL methanol was considered as control (A_0), and methanol (100%) was used as blank. The scavenging activity of the SSO samples were computed by applying the following formula:

$$DPPH\ \% = \frac{A_0 - A_1}{A_0}\ x\ 100 \qquad eqn\ 3$$

Physical Characteristic of Bread

The loaf volume was measured using the rapeseed displacement method as modified by Abdelghafor et al. [20]. The height of bread was measured with a metal rule. The oven spring was determined as the difference in height of dough before and after baking. Loaf weight was measured 30 minutes after the loaves were removed from the oven using electronic balance and the specific loaf volume was determined as described. All determinations were carried out in 4 replicates.

Proofing Ability and Baking Loss

The dough was fermented (proofed) at initial a final fermentation time of 15 and 28min, respectively and at ambient condition of 28±2°C temperature and 85±12% relative humidity. The Baking loss of the bread was determined as described by Oyeyinka & Bassey [21]; Waziiroh et al. [22].

$$Baking\ loss\ \% = \frac{W_d - W_b}{W_d}\ x\ 100 \qquad eqn\ 4$$

Where is W_d the mass of the dough and W_b is the mass of the baked bread after cooling.

Sensory Evaluation of Bread

The breads including the control were assessed by 20 trained panelists who were randomly selected from students and staff of Federal university Wukari, based on their familiarity with quality attributes of bread. All the bread samples were evaluated on a 9-point Hedonic scale (1=disliked extremely and 9=liked extremely) for color, texture, taste, flavor and overall acceptability as described by Oyeyinka and Bassey [21].

Results and Discussion

Physical Properties of Breads

Figure 1 shows the physical characteristics of the loaves in which sweet SSO (SSO) substituted margarine. The height of the bread containing 100% margarine was 19.20cm, whereas that of the bread containing SSO ranged from 18.60 to 20.95cm. The heights of the breads were not significantly ($P<0.05$) affected by the seed oil addition. The difference in the loaves' heights may be the result of weakening of margarine with increase in the amount of SSO in the bread. Similar findings for bread height were recorded by Kaur et al. [23], who substituted bakery shortening with rice bran oil. While the weight of the breads containing SSO ranged from 304.4g to 379.4g, the weight of the breads containing 100 percent margarine was 313.75g. Some of the breads with SSO had heavier weights than the control, which was most likely caused by an increase in the amount of SSO in the bread [24]. Margarine was blamed for the lightness of the weight of wheat flour bread [25]. Although, the flour absorbed water, and the water is lost during baking. The amount of moisture and carbon dioxide that diffuses out of the loaf while it bakes determines the loaf weight [26,27].

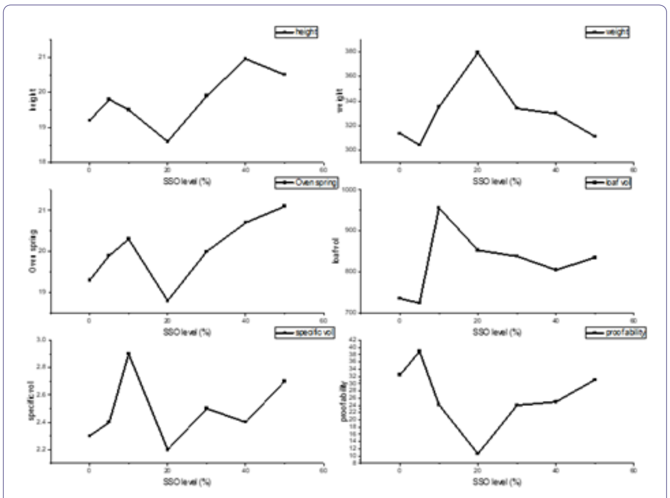

Figure 1: Physical properties of breads in which SSO substituted margarine.

In general, the bread containing 50% SSO had an oven spring of 21.10cm³ than that of the control 19.30cm³. With a higher concentration of SSO, the oven spring increased. The addition of SSO to wheat flour could suggest a decrease in free sulfhydryl groups of the gluten, thereby enhancing polymerization of the gluten via disulfide bond [28]. Similar findings was reported by Jia et al. [29], who investigated the effect of oil content on gluten network. Oven spring is a crucial baking characteristic. It assesses the dough's strength to changes brought on by heat in the oven.

The bread with 10% SSO had the largest loaf volume, measuring 956.00cm³, while the loaf volume increased from 735.5 to 838cm³. Study on the manufacture of bread using pumpkin seed oil, loaf volume rose with an increase in the amount of SSO. Loaf volume assesses the dough's strength to changes brought by heat in the oven. Since it provides a qualitative measurement of baking performance. Loaf volume is recognized as one of the most significant properties of baked goods [21]. The quantity and quality of protein and fat in the flour has an impact on loaf volume [30]. The addition of SSO at 10% significantly increase the loaf volume of the bread. This could be because of SSO improving the gluten network of the dough [21]. The size of the loaf is a crucial factor in determining the price and quality of the bread.

The bread's specific volume was 2.30cm³/g when it contained only margarine. Except for the bread containing 20%, all the breads containing SSO had greater specific volumes (2.40-2.90cm³/g) than the control. The maximum specific volume, 2.90cm³/g was found in the bread with 10% SSO. The dough's enhanced viscosity, which increases gas retention, was presumably caused by the SSO addition [21]. This can result in a bigger loaf and more bread per batch. On the other hand, found that the addition of African oil beans reduced the volume of the bread and the expansion of the dough. The wheat flour bread and just with margarine had a 32.4% proofing ability. Except for the bread containing 5% oil, the loaves' proofing capacities were lower than those of the control. Similarly, the bread containing 5% oil had the highest proofing ability of 38. 9cm³. It might be because the amount of added to reduced levels of margarine may have reduced the ability of the gluten component to capture carbon dioxide produced by yeast during fermentation. According to Ooms and Delcour [31], the gluten network has an impact on the dough's qualities, resulting in better water absorption, mixing tolerance, and tenacity.

Phytochemical Composition of Seed Oil and Bread

The phytochemical composition of SSO and bread is presented on figure 2. The SSO contained 41.36mg/100g carotenoids. This value is higher than that reported by Barkatullah et al. [32], where the ethanolic extracts of fruit seed was 21.68mg/100g. The bread containing 100% margarine had 0.22mg/100g carotenoids content while bread containing 10% SSO had 0.04mg/100g carotenoids. Maqbool et al. [33], reported that citrus contains many carotenoids, which are terpenes (tetraterpenoids) and carotenoids which are antioxidants that have positive effects on the immune system, promote eye health and lower the risk of cancer [34]. Carotenoids are the precursors of vitamin A and are lipophilic antioxidants with strong anti-cancer properties [35,36]. Carotenoids are powerful antioxidants which protect the cells by reacting with oxidizing gents and neutralizing their effects [33].

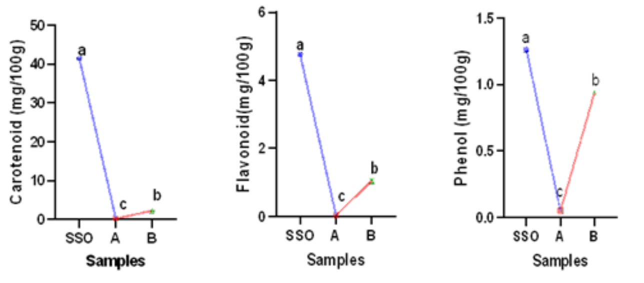

Figure 2: The phytochemical composition (mg/100g) of SSO and bread. Means within a row with the same superscript were not significantly different. Values with different letters are significantly different ($p<0.05$).

The flavonoids content of the SSO was 4.75mg/100g. Flavonoids were not detected in the breads due to the high temperature used in baking, which probably destroyed the flavonoids in the SSO and wheat flour. This corroborate with the findings of Sharma et al. [37], high proportion of the flavonoids was probably destroyed because of harsh heat generated during cooking, and further concluded that it is improper to expose flavonoid source at temperature higher than

120°C. Flavonoids are powerful antioxidants with anti-inflammatory, immune system benefits and are effective in preventing cancer and degenerative disease [34]. Flavonoids were reported to lower precursors of vitamin A and are lipophilic antioxidants with strong anti-cancer properties [38]. Some carotenoids could prevent photosensitization in certain skin diseases, increase immune response to certain infection types as well having anti-aging properties [39].

Antioxidant Activity of Seed Oil and Bread

The antioxidant activities of SSO and the bread in which SSO substituted margarine analyzed using DPPH radical scavenging activity and FRAP assay are shown in figure 3. The antioxidant activities obtained by DPPH radical scavenging activity assay were 43.60%, 4.80% and 6.36% for the SSO, 100% margarine bread and 10% margarine respectively. The increase in DPPH value for the bread containing the seed oil may be due the higher radical scavenging activity of the seed oil (Table 1), which may be attributed to high contents of carotenoids and phenolic content [40]. Similarly, the SSO had higher FRAP value than the other samples, which was closely followed by the bread containing 10 % seed oil (Table 1). Olszowy and Dawidowicz [41], reported that seed oils with high FRAP values prevent several chronic diseases in presence of compounds that plays important roles in reduction of oxidative stress. Such oil can be used not only as edible additives to improve sensory and health promoting properties of foods but also as natural components for enhancing the antioxidant stability of foodstuffs, thereby increasing their shelf-life [42,43]. Goyal et al. [44], reported similar result for flaxseed oil, which ranged from 0.37-0.49mg/GAE/g. However, flaxseed oil contained higher phenolic compounds than other oilseeds. The variation in the antioxidant activity of flaxseed oil and sweet SSO might be due to the presence of different types of phenolic compounds present [45]. These antioxidant substances containing hydrogen-donating groups such as flavonoids and phenols reduced the DPPH solution due to the formation of non-radicals. Apart from antioxidant properties, flavonoids and other phenolics also exhibit several biological activities such as antimicrobial, antiviral, and anticancer [18,46]. These biological and pharmacological activities are usually associated with their ability to bind proteins and free radical scavenging properties [47]. Flavonoids show antioxidant properties through several mechanisms including chelation of metal ions, scavenging of free radicals and inhibition of enzymes that propagate the generation of free radicals [34]. The phenolic compounds probably acted as hydrogen donors and reducing agents in neutralizing the free radicals due to their hydroxyl groups [41]. Phenolics probably acted as antioxidant in scavenging radicals using the DPPH assay. This method is based on a decrease in alcoholic DPPH solution in the presence of H bonding. The DPPH radical scavenging assay is commonly used to evaluate the ability of antioxidant to scavenge free radicals [48]. The use of the DPPH free radical is advantageous in evaluating antioxidant effectiveness because it is more stable than the hydroxyl and super oxide radicals [18].

Instrumental Color of Bread

The instrumental color properties of bread are presented in figure 4. The bread containing 100% margarine had higher lightness value (72.91) than the bread containing 10% SSO (69.53). However, the 10% SSO is in range with the report of Silva et al. [49], who reported 54 to 64 as the acceptable range for bread lightness. The bread containing 10% SSO had higher redness, yellowness, chrome and hue angle values than the bread containing 100% margarine. The a*, b*, c*

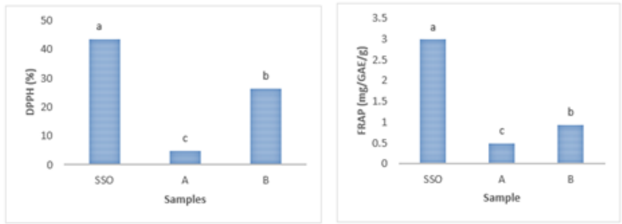

Figure 3: Antioxidant activity of Sweet SSO (SSO) and its effect on bread samples. Data are means ± standard deviation of two replications. Values with different letters are significantly different (p<0.05).

values of the bread containing 10% SSO were 4.49, 16.33 and 16.93 respectively, while the bread containing 100% margarine were 3.98, 14.21 and 14.75 respectively. This might be attributed to the color imparted by SSO. The color of bread containing 10% SSO showed significant (P<0.05) increase in a*, b*, c*, h* values. The color was golden brown for the bread containing 10% SSO.

Sensory Properties of Breads

The sensory properties of breads containing different levels of SSO are shown in table 1. The substitution of margarine with SSO had significant effect (p<0.05) on the bread color. The scores for the color of the breads ranged from 7.13-8.33. The scores for color of the breads incorporated with 10% and 40% SSO were not significantly (p<0.05) different from that of the control. The improved color of the breads with the addition of SSO may be attributed to the golden brown of the seed oil. The golden brown could also be ascribed to caramelization reaction and the reactions between amino acids and free sugar [50,51]. Color is very important parameter in judging properly baked bread that not only reflect the suitability of raw material but also provide information on the quality of the product.

The scores for the texture of the breads ranged from 7.00-7.73. The addition of SSO did not significantly (p<0.05) affect the bread texture. The bread with 100% margarine had the highest score of 7.73 and bread containing 50% SSO had the lowest score of 7.00.

Aroma and texture are important attributes for the acceptance of food product. The scores for taste of the breads ranged from 3.60-7.80. The substitution of margarine with SSO had significant effect (p<0.05) on taste of the breads. The scores for taste generally decreased with increase in the level of SSO in the bread. This may be due to the bitter taste and flavor of the SSO [9]. The bitter taste of the oil is due to the phytochemicals in the oil [52]. Similar result was reported by Heenan et al. [53], on the sensory quality of fresh bread. The scores for flavor significantly decreased from 7.73-5.87. The bread containing 100% margarine had the highest score of 7.73, which was followed by the bread with 10% SSO that had a score of 7.4. This could be due to presence of phytochemicals in the oil which affected the flavor of the breads containing the oil [52]. The scores for general acceptability of the breads ranged from 4.60-8.47. The scores significantly (p<0.05) decreased as the level of substitution of margarine with SSO increased. The decrease for the scores of the general acceptability was due to the low scores for taste and flavor of the breads. The score of the control was 8.33. The score increased to 8.60 for the bread containing 20 % seed oil and thereafter, decreased with the level of the seed oil. Similarly, the bread contain 10% seed oil was not significantly different (p<0.05) from the control for texture, taste, flavor and overall acceptability. This is similar to the findings of

	Level of Substitution of Margarine with Orange Seed Oil						
Sensory Attributes	0	5	10	20	30	40	50
Color	$8.33^{a}\pm0.98$	$8.00^{ab}\pm1.07$	$8.45^{a}\pm0.83$	$8.60^{ab}\pm0.86$	$7.93^{ab}\pm1.33$	$8.27^{a}\pm0.92$	$7.13^{b}\pm2.00$
Texture	$7.73^{a}\pm1.22$	$7.73^{a}\pm1.28$	$7.73^{a}\pm1.33$	$7.14^{a}\pm1.70$	$7.63^{a}\pm1.28$	$7.60^{a}\pm1.59$	$7.00^{a}\pm2.23$
Taste	$7.80^{a}\pm1.08$	$5.20^{b}\pm1.70$	$7.07^{a}\pm1.91$	$5.07^{b}\pm2.16$	$3.53^{c}\pm1.81$	$4.80^{bc}\pm2.31$	$3.60^{c}\pm1.80$
Flavor	$7.73^{a}\pm0.88$	$7.33^{ab}\pm1.88$	$7.40^{ab}\pm1.64$	$5.79^{b}\pm2.36$	$6.53^{ab}\pm2.10$	$6.60^{ab}\pm2.20$	$5.87^{b}\pm2.56$
General Acceptability	$8.47^{a}\pm1.06$	$6.20^{b}\pm2.68$	$8.13^{a}\pm1.06$	$4.79^{b}\pm2.72$	$4.87^{b}\pm2.59$	$5.87^{b}\pm2.80$	$4.60^{b}\pm2.72$

Table 1: Sensory properties of breads in which SOSO substituted margarine.

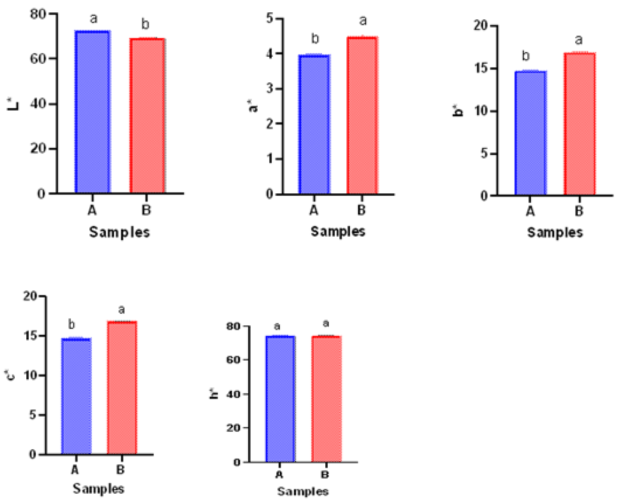

Figure 4: Data are means ± standard deviation of two replications. Means within a row with the same superscript were not significantly different. L*=lightness; a*=redness; b*=yellowness; c*=chrome; h*=hue angle; Values with different letters are significantly different ($p<0.05$).

Osuna et al. 2018 who reported a formulation that gives a good scores in the evaluated sensory attributes of a bread produce using canola oil [54,55].

Conclusion

Sweet orange seed also contains many beneficial nutrients, including unsaturated fatty acids, bioactive components such as phenolic acids, flavonoids, and carotenoids. Tonnes of Sweet orange seeds are obtained from the beverage industry as a by-product of juice. Researchers increase awareness is turning its focus toward food application. Its oil contains bioactive components that are of great interest, particularly for treating cardiovascular diseases. Apart from its useful potential health benefit, SSO possess many useful properties in the food industry. They can potentially be used as functional food. In conclusion, based on these findings, substituting 10% of margarine with SSO resulted in a significant increase in the bread's phytochemical composition and antioxidant activity. The addition did not also show any significant differences from the bread containing 100% margarine for all the sensory attributes evaluated.

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Author's Contribution

Bello Mohammed Yunusa: Writing - Original Draft, Writing - Review & Editing, Visualization, Graphics conceptualization.

Peter Isa Akubor: Conceptualization, supervision, Writing - Review & Editing

Sale Fojibaje: Conducted experiments, Writing - Original Draft,

Zakka Riyyang: Writing - Review & Editing

Bumbyerga Garboa Gargea: Writing - Review & Editing

Diamond Joseph Rimamshong: Writing - Review & Editing

Shahida Anusha Siddiqui: Writing - Review & Editing, financial support

Conflicts of Interest

The authors declare no conflicts of interest.

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