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INFLUENCE OF PARTIAL SUBSTITUTION OF SUGAR WITH SERENDIPITY BERRY (*DIOSCOREOPHYLLUM CUMMINSII*) EXTRACT ON THE QUALITY ATTRIBUTES AND SHELF-LIFE OF WHEAT BREAD

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ABSTRACT

Serendipity berry (*Dioscoreophyllum cumminsii*) contains a protein sweetener termed monellin which could be substituted for sugar in foods for diabetics and dieters. Therefore, effects of partial substitution of sugar with serendipity berry extract on quality of white wheat bread were investigated. The pasting properties of wheat flour treated with mixtures of 5 % sugar solution and serendipity berry extract (100:0 control, 80:20, 70:30, 60:40, 50:50 and 40:60) were investigated. Wheat flour were blended with other ingredients and mixtures of 5 % sugar solution and serendipity berry extract to form dough which were allowed to rise prior to baking. Chemical, microbiological and sensory properties of the breads were evaluated. Pasting temperature, peak, final and setback viscosities of the control (sugar solution treated-flour) and serendipity extract treated flour were significantly ($p<0.05$) different. Moisture, ash, protein, fat, fibre and carbohydrate contents of the breads ranged from 11.93–15.22 %, 0.75–3.06 %, 11.67–14.13 %, 4.29–9.06 %, 1.24–2.50 % and 55.01–68.14 %, respectively. Although the bacterial (0.0–61 $\times 10^3$ cfu/g) and fungal (2.0–76.0 $\times 10^3$ cfu/g) counts of the breads increased throughout the 5 days storage period, the serendipity berry extract exerted antimicrobial activities in the treated breads. The proximate, except carbohydrate, of the breads increased while the bacterial and fungal counts decreased with increase in concentration of the serendipity extract. The 60 % serendipity extract treated-bread (mean sensory scores ≥ 8.0) compared favourably with the control (sugar solution treated-bread). This study revealed that 60 % serendipity berry extract could be substituted for sugar for production of high quality bread with extended shelf life.

Keywords: Bread, *Dioscoreophyllum cumminsii*, Preservation, Quality attributes, Substitution, Wheat flour, Sweetener

INTRODUCTION

The ever increasing consumers' demand for wholesome and safe foods with extended shelf-life and without the use of synthetic preservatives still remains a major challenge to most food industries. The fruit, serendipity berry (*Dioscoreophyllum cumminsii),* belonging to the family menispermaccae is an unpopular and under-utilized tropical West African indigenous plant (**Oselebe and Nwankiti, 2005; Abiodun and Akinoso, 2014**). It grows in humid and heavily shaded understorey vegetation of closed forest towards the end of the raining season usually between May and October (**Abiodun *et al.*, 2014**). It is regarded as a low acid food due to its high pH (6.6) and low titratable acid values (**Abiodun and Akinoso, 2014**). Like many other fruits, serendipity berry has high moisture content (80 %) (**Abiodun and Akinoso, 2014**) which makes it highly perishable. It contains considerable amounts of carotenoids and its vitamin C content has been reported to be higher than those of local orange, watermelon and banana (**Abiodun and Akinoso, 2014**). **Abiodun and Akinoso (2014)** have reported that the fruit contains low quantities of sugars (fructose, glucose and fructose) and alkaloids (solasodine, tomatidenol and soladulcidine) with fructose and soladulcidine being the major sugar and alkaloid, respectively. The intense sweetness of serendipity berry has been attributed to a protein called monellin (**Inglett, 1976**) which is mainly found in the mucilaginous mesocarp. This protein is the sweetest known naturally occurring substance, up to 3,000 times sweeter than sucrose, and approximately 100,000 times as potent as sugar on a molar basis (**Inglett and May, 1969, Faruya *et al.*, 1983, Penarrubia *et al.*, 1992**). Therefore, relatively lower concentrations of its extract may be required to achieve desired sweetness and these have facilitated its recommendation for use as a sugar substitute in foods for dieters and diabetics.

Bread is a widely consumed staple food which is mainly produced by baking fermented dough of flour (usually hard wheat), yeast, salt, sugar and water. Other ingredients that may be added include fat, milk and milk products, malt and malt products, oxidants and improvers (such as ascorbic acid and calcium propionate), surfactants and anti-microbial agents (**Kučerová, 2015**). Bread serves as one of the most important sources of nutrients such as carbohydrate, protein, minerals, fibre, and vitamins in the diets of many people worldwide (**Correia *et al.*, 2015**). Bread is an appealing, convenient and ready-to-eat food (**Correia *et al.*, 2015**). These attributes have led to a steady increase in its consumption in most parts of the world including Nigeria. However, the shorter shelf life and perishability nature of bread compared to most other bakery products poses a serious threat in terms of economic loss to its producers as well as its marketers. After production and during storage, bread swiftly loses its freshness and subsequently loses its organoleptic qualities (**Pateras, 1999; Ho *et al.*, 2013**). Generally, the changes in chemical or physical properties that subsequently resulted in the reduction of the crumb softness during storage time (staling), and spoilage, which result from microbial attack are the two major factors that accelerate the rate of freshness loss in bread (**Pateras, 1999**). Microbial spoilage of bakery products such as bread is mainly attributed to the growth of moulds (**Mentes *et al.*, 2007**) which could result to substantial economic loss in the bakery sector and also posing health challenges to consumers owing to the production of mycotoxins.

The detrimental health effects of sucrose-rich diets and consumers' demand for safe products with extended shelf life and without chemical preservatives (**Axel *et al.*, 2017**), have prompted intensive search for plants with sweetening and preservative properties. Recently, acceptable watermelon juice sweetened with serendipity berry extract was reported to be microbiologically stable within the twelve weeks of storage (**Dauda *et al.*, 2017**). The present study was therefore designed to determine the effects of using serendipity berry extract as sugar substitute on the chemical, microbiological, sensory properties and shelf life of wheat flour bread.

MATERIALS AND METHODS

Materials

Fully matured serendipity berry was procured at Esa-Odo farm in Osogbo, Osun state, Nigeria. White wheat flour (Dangote), refined sugar (sucrose), salt, instant dry yeast and fats were procured from a local market (Oja-Oba) in Ilorin, Kwara State, Nigeria. The materials were brought to the food processing laboratory of University of Ilorin, Nigeria for processing and analyses. Distilled water was used throughout the experiment and all reagents used were of analytical grade.

Extraction of juice from serendipity berry fruit

The serendipity berry juice was extracted as described by **Dauda *et al.* (2017)**. The serendipity berry fruits were washed with distilled water to remove dirt. The thoroughly washed fruit was peeled and the seeds were removed. The juice (sweetener) was extracted and strained through double layered muslin cloth to obtain the fresh sweetener. The serendipity berry extract were measured with a measuring cylinder into portions of 60 ml, 50 ml, 40 ml, 30 ml and 20 ml and kept in the refrigerator at 4 ºC for further use.

Preparation of sugar solution

Five percent (5 %) sugar solution was prepared by dissolving 50 g of refined sugar (sucrose) in 500 ml distilled water in a 1000 ml volumetric flask and then made up to mark with distilled water. The solution was kept in the refrigerator at 4 ºC for further use.

Pasting properties of serendipity berry extract-treated flours

In order to examine the effect of partial substitution of sugar with serendipity berry extract on the pasting properties of wheat flour, six 25 ml of mixtures of 5 % sugar solution and serendipity berry extract (100:0, 80:20, 70:30, 60:40, 50:50 and 40:60) were prepared separately and their respective treated flour were coded SSB_0F, SSB_2F, SSB_3F, SSB_4F, SSB_5F and SSB_3F. Pasting properties of the flour samples were determined using a Rapid Visco Analyzer (RVA Model 3c, Newport Scientific PTY Ltd, Sydney) as described by **Arise *et al.* (2017)** with little modification. Briefly, 2.5 g of each flour sample was accurately weighed into a previously dried empty canister and 25 ml of sugar solution or mixture of sugar solution and serendipity berry extract was added. Samples were transferred onto the water surface of the canister after which the paddle was placed into the canister. The mixture was thoroughly mixed by the paddle fitted at the centre of the canister and the canister was fitted into the Rapid Visco Analyzer. Peak viscosity (RVU), Peak time (min), Peak temperature (°C), Trough (RVU), pasting temperature (°C) and final viscosity (RVU) were read on the instrument while breakdown and setback viscosities (RVU) were calculated.

Formulations for serendipity berry extract sweetened-bread

Six different samples (SSB_0, SSB_2, SSB_3, SSB_4, SSB_5 and SSB_6) of bread were produced from various recipe formulations as shown in Table 1. Sample SSB_0 served as the control while other samples were treated with serendipity berry extract at different concentrations.

Table 1 Recipe for the production of Serendipity berry extract-sweetened breads

Ingredients	Treatment samples of the serendipity extract-sweetened breads					
	SSB_0	SSB_2	SSB_3	SSB_4	SSB_5	SSB_6
Wheat flour (g)	200	200	200	200	200	200
Edible salt (g)	2.5	2.5	2.5	2.5	2.5	2.5
Yeast (g)	2.5	2.5	2.5	2.5	2.5	2.5
Vegetable Fats (g)	5.0	5.0	5.0	5.0	5.0	5.0
5 % sugar solution (ml)	100	80	70	60	50	40
Serendipity berry extract (ml)	0	20	30	40	50	60
Distilled Water (ml)	30	30	30	30	30	30

Legend: SSB_0 = 100 ml sugar solution, SSB_2 = 80 ml sugar solution + 20 ml serendipity berry extract, SSB_3 = 70 ml sugar solution + 30 ml serendipity berry extract, SSB_4 = 60 ml sugar solution + 40 ml serendipity berry extract, SSB_5 = 50 ml sugar solution + 50 ml serendipity berry extract, SSB_6 = 40 ml sugar solution + 60 ml serendipity berry extract.

Preparation of serendipity berry extract-sweetened breads

The breads were prepared according to the AACC (2000) straight dough method No 10-10B. The dougsh from each formulation was mixed for 5 min, raised for 30 min, punched for 4 min and raised for another 30 min. The dough was divided, punched again for 5 min, rounded and moulded. Then it was placed in baking pans and allowed to rise for 60 min at 30 °C. Loaves were baked for 10 min at 250 °C. The baked loaves were carefully removed from the pans and allowed to cool and packaged in polyethylene bags for analyses. The flow chart for the production of bread sweetened with serendipity berry extract is shown in Figure 1. Figure 2 shows the bread samples produced.

Proximate composition of the bread samples

The moisture content was determined by **AOAC (2000)** method, while the ash, crude protein, crude fat, crude fibre and carbohydrate contents of the breads were evaluated using **AOAC (1990)**. Briefly, the oven drying method at 105 °C for 5 hr was used for moisture determination. Protein content was determined using the micro-Kjeldahl method for crude protein. Total ash was obtained by igniting 2 g of sample at 600 °C for 2 hr using muffle furnace. Crude fat was determined by soxhlet extraction with petroleum spirit as the solvent. Crude fibre was determined using digestion method while carbohydrate was estimated by difference [100-(% water + % protein + % fat + % ash + % crude fibre)].

Estimation of energy values of the breads

The energy values of the breads were estimated using Atwater factors in which the percentage carbohydrate, crude fat and crude protein contents were multiplied by 4, 9 and 4, respectively. The energy values of the breads were expressed in kCal/g.

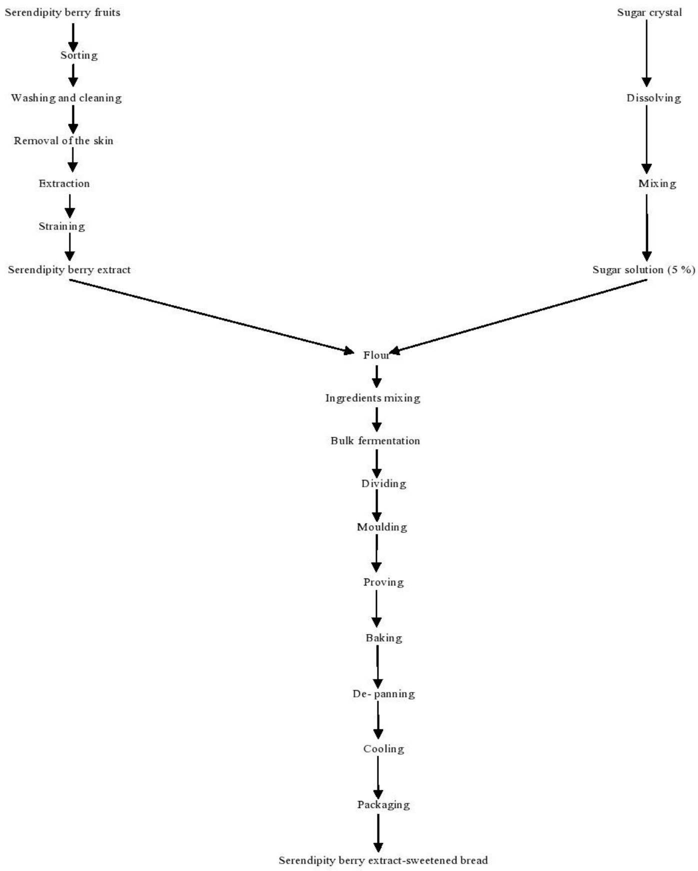

Figure 1 Flowchart for the production of serendipity berry extract-sweetened bread

SSB_6 SSB_5 SSB_4 SSB_3 SSB_2 SSB_0

Figure 2 Serendipity berry extract-sweetened breads.
Legend: SSB_0 − 100 ml sugar solution treated-bread, SSB_2 − 80 ml sugar solution + 20 ml serendipity berry extract treated-bread, SSB_3 − 70 ml sugar solution + 30 ml serendipity berry extract treated-bread, SSB_4 − 60 ml sugar

solution + 40ml serendipity berry extract treated-bread, SSB_5 − 50 ml sugar solution + 50ml serendipity berry extract treated-bread, SSB_6 − 40 ml sugar solution + 60 ml serendipity berry extract treated-bread.

Sensory evaluation of the bread samples

A panel of 20 members who were regular consumers of white wheat bread was selected among students and members of staff of the Department of Home Economics and Food Science, University of Ilorin for the sensory analysis. The panellists were instructed to evaluate the coded bread samples for acceptability of colour, taste, after taste, aroma, crumb texture, crust colour and overall acceptability using a nine point hedonic scale where 1 represented dislike extremely and 9 represented like extremely. The panellists were also instructed to rinse their mouths with drinking water after evaluating each sample.

Determination of total bacterial and fungal counts of the breads

The total viable bacterial and fungal (yeast and moulds) counts of the bread were determined on days 0, 2 and 5, using the pour plate technique (**Adegoke, 2004**). One gram of each bread sample was dissolved in 1 ml of 2 % sterile sodium citrate solution in order to prepare a suspension. One millilitre of the suspension was then used for the serial dilution of between 10^{-1} and 10^{-3}. At about 44 − 50 °C, 0.1 ml of the dilution was transferred from each dilution bottle into the corresponding plates and about 15 ml of nutrient agar and potato dextrose agar which were already prepared according to manufacturers' instructions for total viable bacterial and fungal (yeast and moulds) counts, respectively were poured and mixed thoroughly with the inoculums by rocking the plates. The plates were incubated at 37 ºC for 24 hr and at room temperature (28±2 ºC) for 72 hr for nutrient agar and potato dextrose agar, respectively. Bacterial and fungal colonies were counted using a Stuart scientific colony counter and expressed as colony forming units per gram (cfu/g) of samples.

Shelf life of the bread samples

The bread samples were stored under ambient temperature (28±2 °C) and observed for 10 days. Visual observations for mould growth were carried out on the samples throughout the storage period as stated by **Ijah *et al*. (2014)**.

Statistical analysis

Data obtained for the pasting properties of the mixed dough, proximate composition and sensory evaluation of the breads were subjected to one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS v.16.0) and means were separated using Duncan Multiple Range Test ($p<0.05$).

RESULTS AND DISCUSSION

Pasting properties of serendipity berry extract-treated wheat flour

The appearance, texture, digestibility and application of starch-based food materials depend largely on their pasting properties (Onweluzo and Nnamuchi, 2009; Ajanaku et al., 2012). The pasting properties of the serendipity berry extract-treated and the control flours are presented in *Table 2*. The peak viscosity of the flours ranged from 110.7−195.4 RVU for SSB_2F and SSB_5F respectively. The peak viscosity of the serendipity berry extract treated flours increased with increase in the concentration of the extract up to 50 % substitution level. SSB_2F had a significantly ($p<0.05$) lower peak viscosity value compared to SSB_0F (control) while 30−60 % partial substitution of sugar with serendipity berry extract resulted into significant ($p<0.05$) increment in the peak viscosity of the wheat flour. Peak viscosity is the measure of the swelling capacity of starch-based foods soon after heating prior to physical breakdown (**Sanni *et al*., 2006**). Starch, starch components such as amylose and amylopectin, and degree of starch damage are some of the factors that could influence the pasting viscosity of flour. Trough viscosity is the minimum viscosity value in the constant temperature phase of RVA which indicates the ability of paste to withstand breakdown during cooling (**Ekunseitan *et al*., 2016**). Sample SSB_0F had the lowest trough viscosity (79.20 RVU) while SSB_5F had the highest (179.4 RVU). Partial substitution of sugar with serendipity berry extract generally increased the trough viscosity of the flour, though the increments were not proportionate with the concentrations of the serendipity berry extract. This result showed the ability of SSB_0F paste to withstand breakdown during cooling compared to the pastes of the serendipity berry extract-treated samples.

The breakdown viscosity of the flours ranged between 16.85 RVU for SSB_5F and 39.90 RVU for SSB_6F. No significant difference existed between SSB_4F and SSB_0F (control). It was observed that 20, 30 and 50 % partial substitution of sugar with serendipity berry extract significantly ($p<0.05$) decreased the breakdown viscosity of the flour while significant ($p<0.05$) increase in breakdown viscosity was recorded with 60 % serendipity berry extract substitution. Lower breakdown viscosity is an indication of higher heat and shear stress stability during cooking (**Adebowale *et al*., 2005**). Final viscosity indicates the ability of a starch-based food material to form paste after cooling (*26*). The final viscosity of the flours ranged from 153.85 RVU for SSB_0F (control) to 274.7 RVU for SSB_6F. The final viscosity of the treated flours increased with increase in the concentration of serendipity berry extract and they were significantly ($p<0.05$) higher than that of the control (SSB_0F). This implied that the treated flour pastes were less stable after cooling. The lowest (73.35 RVU) and the highest (158.45 RVU) setback viscosities were recorded for SSB_0F and SSB_6F respectively. Significant ($p<0.05$) differences existed among the setback viscosities of the flours with the serendipity berry extract-treated flours having higher values than the control (SSB_0F). Higher setback values are synonymous to reduced dough digestibility (**Shittu *et al*., 2001**), while lower setback during cooling of the paste indicates lower tendency for retrogradation (**Sanni *et al*., 2001**).

Peak time indicates the time required for cooking to be achieved (**Adebowale *et al*., 2005**). The peak time recorded for the flours varied between 5.62 min (SSB_2F) and 6.82 min (SSB_0F). Partial substitution (20−60 %) of sugar with serendipity berry extract resulted into decrease in the peak time of the flour, although the peak time obtained for SSB_3F and SSB_5F were comparable with that of the control (SSB_0F). Hence, partial substitution (20-60 %) of sugar with serendipity berry extract reduced the time required to cook wheat flour paste. Pasting temperature is the minimum cooking temperature at which there is first observable increase in viscosity due to the swelling of starch granules (**Ikegwu *et al*., 2009; Ekunseitan *et al*., 2016**). The pasting temperature of the flour samples ranged from 89.72 ºC for SSB_0F to 95.82 ºC for SSB_6F. Partial substitution (20-60 %) with serendipity berry extract significantly increased the pasting temperature of the flour. The highest pasting temperatures were recorded for the flours at 10 and 60 % serendipity berry extract substitution levels. Higher pasting temperatures obtained for the treated flour samples are indication of higher energy cost, low components stability, higher gelatinization tendency and low swelling properties of the starch granules.

Table 2 Effect of serendipity berry extract on the pasting properties of wheat flour

Sample	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Setback viscosity (RVU)	Peak time (min)	Pasting temperature (ºC)
SSB_0F	$114.5^{d}\pm 0.56$	$79.20^{e}\pm0.71$	$36.90^{b}\pm 0.71$	$153.85^{e}\pm0.92$	$73.35^{f}\pm0.21$	$6.82^{a}\pm0.35$	$89.72^{d}\pm0.59$
SSB_2F	$110.7^{e}\pm0.35$	$82.00^{e}\pm2.12$	$31.90^{c}\pm 0.71$	$195.4^{d}\pm 1.06$	$113.2^{c}\pm0.57$	$5.62^{d}\pm5.62$	$95.70^{ab}\pm0.63$
SSB_3F	$156.4^{b}\pm0.14$	$134.0^{b}\pm2.12$	$23.50^{d}\pm 0.71$	$237.4^{c}\pm0.71$	$105.4^{d}\pm1.34$	$6.48^{ab}\pm0.22$	$94.92^{abc}\pm0.46$
SSB_4F	$166.3^{b}\pm0.29$	$129.3^{c}\pm1.27$	$36.80^{b}\pm0.28$	$252.8^{b}\pm0.78$	$124.2^{b}\pm0.42$	$6.18^{bc}\pm0.71$	$94.18^{bc}\pm0.74$
SSB_5F	$195.4^{a}\pm0.14$	$179.4^{a}\pm0.35$	$16.85^{e}\pm0.92$	$273.3^{a}\pm0.92$	$93.05^{e}\pm1.06$	$6.50^{ab}\pm0.49$	$94.06^{c}\pm0.57$
SSB_6F	$155.5^{c}\pm0.28$	$116.5^{d}\pm0.01$	$39.9^{a}\pm0.71$	$274.7^{a}\pm1.41$	$158.45^{a}\pm1.63$	$5.98^{c}\pm0.78$	$95.82^{a}\pm0.74$

Values are means of triplicate determinations ± standard deviation. Means with different superscript in each column are significantly different ($P< 0.05$). SSB_0F = 100 ml sugar solution treated-flour, SSB_2F = 80 ml sugar solution + 20 ml serendipity berry extract treated-flour, SSB_3F = 70 ml sugar solution + 30 ml serendipity berry extract treated-flour, SSB_4F = 60 ml sugar solution + 40ml serendipity berry extract treated-flour, SSB_5F = 50 ml sugar solution + 50ml serendipity berry extract treated-flour, SSB_6F = 40 ml sugar solution + 60 ml serendipity berry extract treated-flour.

Proximate composition and energy values of freshly baked serendipity berry extract-treated bread

Table 3 shows the proximate composition and energy values of the freshly baked serendipity berry extract-treated breads. Moisture content of the breads ranged from 11.93−15.22 % for SSB_2 and SSB_6 respectively. The values fell within the acceptable moisture limit (15 %) for dry products (**Ijah *et al*., 2014**). Although the moisture content of the treated bread samples generally increased with increase in the concentration of the serendipity berry extract substitution, 20−40 % partial substitution of sugar with serendipity berry extract resulted into production of breads with lower moisture contents compared to the control (SSB_0). The significantly highest moisture contents recorded for SSB_5 and SSB_6 could be attributed to the liquid nature of the serendipity berry extract used as sugar substitute. **Olaoye and onilude (2008)** have also reported increased in moisture content with increase in the level of breadfruit substitution in wheat-breadfruit breads. Moisture content is an indicator keeping quality and high moisture could result into shorter shelf life of the breads. Ash is the inorganic material remaining after oxidation of organic matter and it is an indication of the total minerals (**Wilson, 1987**). The lowest (0.75 %) and the highest (3.06 %) ash contents were found in SSB_2 and SSB_6 respectively. Ash content of the treated bread samples generally increased with increase in the concentration of serendipity berry extract. This increment could be an indication of the presence of higher minerals in the serendipity berry extract. This is plausible because similar trend has also been reported for wheat-breadfruit flour breads (**Olaoye and Onilude, 2008**) and date palm fruit pulp treated-wheat cookies (**Peter *et al.*, 2017**). The ash contents of breads with 30−60 % serendipity berry extract substitution were observed to be higher than 1.43 % recorded for the control. This suggests that the inclusion of serendipity berry extract could boost the mineral content of bread.

The protein content of the breads ranged between 11.67 % for SSB_0 (control) and 14.13 % for SSB_6. The values of 12.26−14.13 % obtained for the protein contents of serendipity berry extract-treated breads were higher than 11.67 % recorded for the control (SSB_0). This may be due to the high protein content of the serendipity berry extract which must have contributed to the protein content of the wheat flour, thus increasing the protein level of the serendipity berry extract treated-bread samples. This is plausible since the sweetness of serendipity berry has been attributed to a protein known as monellin (**Wlodawer and Hodgson, 1975; Inglett, 1976**). The crude fat of the bread samples ranged from 4.29−9.06 % with the serendipity berry extract-treated breads recording significantly ($p<0.05$) higher values than the control (SSB_0). It was observed that the crude fat of the treated-breads with the exception of SSB_4, increased as the concentration of the serendipity berry extract substitution increased. This is an indication of higher fat content in the serendipity berry extract compared to wheat flour. This is similar to the report of **Peter *et al.* (2017)** on date palm fruit pulp treated-wheat cookies. High fat content could shorten the shelf life of baked foods such as bread (**Ihekoronye and Ngoddy, 1985**).

The fibre content of the breads fell within 1.24−2.50 %, recorded for the control (SSB_0) and SSB_6 respectively. The fibre content of the bread samples except SSB_5 increased with increase in concentration of the serendipity berry extract substitution. Partial substitution (20-60 %) of sugar with serendipity berry extract generally increased the fibre contents of the breads. Similar trend has been reported by **Olaoye and Onilude (2008)** for wheat-breadfruit flour breads and **Peter *et al.* (2017)** for date palm fruit pulp treated-whole wheat cookies Higher fibre content is advantageous in the diets as it increases faecal output, reduces faecal pH, diabetes, incidence of colon cancer, obesity, heart diseases and certain degenerative diseases (**Cummings *et al.*, 1996**). The results showed that the carbohydrate content of the breads ranged between 55.01 % and 68.14 %. Partial substitution (20−60 %) of sugar with serendipity berry extract resulted into the production of breads with significantly ($p<0.05$) lower carbohydrate contents. This could be due to the lower sugar content of serendipity berry (**Inglett, 1976; Oselebe and Nwankiti, 2005; Abiodun *et al.*, 2014**). It was observed that the carbohydrate content of the treated bread samples generally decreased as the concentration of the substituted serendipity berry extract increased. Similar trends have been reported for wheat-breadfruit flour breads (**Olaoye and Onilude, 2008)** and date palm pulp meal sweetened bread (**Obiegbuna *et al.*, 2013**).

Energy values of the bread samples

The energy values of the bread samples ranged from 352.21−376.83 kCal/g for SSB_4 and SSB_3 respectively (Table 3). Energy values of the breads significantly ($P<0.05$) increased at 20 % and 30 % serendipity berry extract substitution levels but decreased significantly ($P<0.05$) at 40 % and 50 % substitution levels compared to the control (SSB_0). **Bhise and Kaur (2014)** have also reported reduction in the calorific values of wheat bread with the incorporation of polyols as a sugar substitute. No significant difference existed between the energy values of SSB_6 and the control (SSB_0).

Table 3 Proximate composition and energy values of freshly baked serendipity berry extract-treated breads

Sample	Moisture (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Carbohydrate	Energy value (kCal/g)
SSB_0	$13.50^b±0.38$	$1.43^{de}±0.42$	$11.67^c±0.18$	$4.29^d±0.37$	$1.24^d±0.14$	$68.14^a±1.06$	$357.85^c±3.03$
SSB_2	$11.93^c±0.09$	$0.75^e±0.35$	$12.26^{bc}±0.19$	$5.73^c±1.03$	$1.58^{cd}±0.24$	$65.96^b±0.77$	$364.45^b±1.52$
SSB_3	$12.12^c±0.51$	$1.75^{cd}±0.35$	$13.10^b±0.10$	$7.87^{ab}±0.17$	$1.98^{bc}±0.21$	$63.40^c±0.47$	$376.83^a±1.02$
SSB_4	$13.25^b±0.31$	$2.26^{bc}±0.37$	$12.44^{bc}±0.16$	$7.37^b±0.53$	$2.12^{ab}±0.17$	$59.03^d±0.74$	$352.21^d±1.36$
SSB_5	$15.12^a±1.21$	$2.75^{ab}±0.35$	$12.52^{bc}±0.91$	$8.33^{ab}±0.24$	$2.11^{ab}±0.14$	$57.16^d±0.98$	$353.69^d±1.68$
SSB_6	$15.22^a±0.22$	$3.06^a±0.08$	$14.13^a±0.21$	$9.06^a±0.17$	$2.50^b±0.22$	$55.01^e±0.91$	$358.10^c±2.12$

Values are means of triplicate determinations ± standard deviation. Means with different superscript in each column are significantly different (*P< 0.05*). SSB_0 = 100 ml sugar solution treated-bread, SSB_2 = 80 ml sugar solution + 20 ml serendipity berry extract treated-bread, SSB_3 = 70 ml sugar solution + 30 ml serendipity berry extract treated-bread, SSB_4 = 60 ml sugar solution + 40ml serendipity berry extract treated-bread, SSB_5 = 50 ml sugar solution + 50ml serendipity berry extract treated-bread, SSB_6 = 40 ml sugar solution + 60 ml serendipity berry extract treated-bread.

Effect of serendipity berry extract on the consumer acceptability of wheat breads

Table 4 shows the mean sensory scores for the colour, taste, after taste, aroma, crumb texture, crust and acceptability of the bread samples. No significant differences existed between the mean scores obtained for the colour, aroma, crumb texture, crust colour and acceptability of SSB_6 and the control (SSB_0) but they were significantly ($p<0.05$) higher than the mean values recorded for other bread samples. Also, comparably high mean scores of 8.23 and 8.73 were recorded for the taste of SSB_6 and the control (SSB_0) respectively. The control bread (SSB_0) had the lowest mean score of 6.73 while SSB_6 recorded the highest mean score of 8.53 for after taste. This indicated that the partial substitution (20-60 %) of sugar with serendipity berry extract positively influenced the after taste of wheat bread samples. The bread (SSB_6) produced by partially substituting sugar with 60 % serendipity berry extract possessed similar or better sensory attributes (colour, taste, after taste, aroma, crumb texture, crust colour and acceptability) compared to the control bread sample (SSB_0).

Table 4 Mean acceptability scores of freshly baked serendipity berry extract treated-breads

Sample	Colour	Taste	After taste	Aroma	Crumb texture	Crust colour	Overall acceptability
SSB_0	$8.67^a±0.56$	$8.73^a±1.25$	$6.73^{bc}±1.11$	$8.67^a± 1.06$	$8.70^a±1.34$	$8.80^a± 1.13$	$8.70^a±1.15$
SSB_2	$7.03^{cd}±1.29$	$7.17^c±1.19$	$7.80^b±1.21$	$6.73^d±1.49$	$6.70^d±1.39$	$6.73^d±1.26$	$6.83^d±1.16$
SSB_3	$6.77^d± 1.31$	$6.97^c±1.30$	$7.33^b±1.16$	$6.43^d±1.20$	$6.77^{cd}±1.19$	$6.67^d± 1.27$	$6.73^d±1.17$
SSB_4	$7.27^{bcd}± 0.79$	$7.23^{bc}±0.89$	$7.87^b±0.94$	$6.93^{cd}±0.98$	$6.93^{cd}±0.98$	$7.03^{cd}±0.77$	$7.10^{cd}±0.76$
SSB_5	$7.43^{bcd}±0.68$	$7.30^{bc}±0.59$	$8.00^a±0.91$	$7.37^{bc}±0.93$	$7.33^{bc}±0.84$	$7.40^{bc}±0.81$	$7.43^{bc}±0.77$
SSB_6	$8.20^a±0.93$	$8.23^{ab}±0.63$	$8.53^a±1.03$	$8.13^a±1.07$	$8.00^a±0.79$	$8.03^a±0.81$	$8.37^a±0.67$

Values are means of twenty determinations ± standard deviation. Means with different superscript in each column are significantly different (*P<0.05*). SSB_0 = 100 ml sugar solution treated-bread, SSB_2 = 80 ml sugar solution + 20 ml serendipity berry extract treated-bread, SSB_3 = 70 ml sugar solution + 30 ml serendipity berry extract treated-bread, SSB_4 = 60 ml sugar solution + 40 ml serendipity berry extract treated-bread, SSB_5 = 50 ml sugar solution + 50 ml serendipity berry extract treated-bread, SSB_6 = 40 ml sugar solution + 60 ml serendipity berry extract treated-bread.

Microbial counts of serendipity berry extract-treated breads during storage

Microbial enumerations could be used as indication of food safety and spoilage. The results showing the influence of serendipity berry extract on the bacterial and fungal counts of white wheat breads during 5 days of storage are displayed in Table 5. The bacterial ($0.0–61×10^3$ CFU/g) and fungal ($2.0–76×10^3$ CFU/g) counts generally increased throughout the 5 days storage period. However, the values were within the safe limits stated by **Ihekoronye and Ngoddy (1985)**. The highest bacterial and fungal counts were recorded for the control bread sample before and during the storage period. In comparison with the control (SSB_0), the serendipity berry extract treated-breads recorded lower bacterial and fungal counts which were observed to decrease with increase in the concentration of serendipity berry extract substitution Similar trends have been reported for tigernut milk treated with different spices (**Kayode *et al.*, 2017**). The generally lower bacterial and fungal counts of the serendipity berry extract treated-breads before and during the storage period could be attributed to the preservative effect of the serendipity berry extract. This is similar to the report of **Dauda *et al.* (2017)** on watermelon juice where serendipity berry extract was observed to function as a sweetener and preservative. Bacteria and fungi are the major organisms responsible for the spoilage of bread and their contamination with bread could be from the raw materials used and during processing, handling and storage (**Ijah *et al.*, 2014**). The antimicrobial activities of serendipity berry extract observed in this study could probably be attributed to the synergetic action of secondary metabolites in the fruit extract.

Shelf life of serendipity berry extract-treated breads

The breads produced lasted for 6–8 days prior to observable visible spoilage (Table 5). Breads with 50 % and 60 % serendipity berry extract had the longest storage period of 8 days before visible spoilage was noticed. This might be due to the ability of the serendipity berry extract to inhibit microbial growth. The spoilage observed was indicated by black, yellow, and green coloration on the bread and this was suspected to be mould growth. Incorporation of polyols (glycerol, sorbitol and mannitol) has been reported to extend the shelf life of bread from 4 days to 10 days mainly through moisture retention and relatively water activity stability potentials of the polyols, although packaging materials and storage conditions were shown to be contributory factors to the observed shelf life elongation (**Bhise and Kaur, 2014**). However, caution must be taken in the use of visual observation in evaluating the shelf life of bread because mycotoxins and off-flavours might have been produced even before fungal outgrowth would be visible (**Magan *et al*., 2003**).

Table 5 Effect of serendipity berry extract on the microbial counts and shelf-life of white wheat breads during storage

Sample	Bacterial count			Fungal count			Storage days prior to visible spoilage
	0 day (× 10^3 cfu/g)	2 days (× 10^3 cfu/g)	5 days (× 10^3 cfu/g)	0 day (× 10^3 cfu/g)	2 days (×10^3 cfu/g)	5 days (×10^3 cfu/g)	
SSB_0	7	22	61	9	28	76	6
SSB_2	4	19	55	6	24	62	6
SSB_3	2	13	50	4	17	57	6
SSB_4	2	10	43	3	15	51	7
SSB_5	1	7	36	3	13	45	8
SSB_6	NG	8	24	2	9	31	8

Values are means of duplicate determinations. NG = No growth, SSB_0 = 100 ml Sugar solution treated-bread, SSB_2 = 80 ml Sugar solution + 20 ml Serendipity berry extract treated-bread, SSB_3 = 70 ml Sugar solution + 30 ml Serendipity berry extract treated-bread, SSB_4 = 60 ml Sugar solution + 40 ml Serendipity berry extract treated-bread, SSB_5 = 50 ml Sugar solution + 50 ml Serendipity berry extract treated-bread, SSB_6 = 40 ml Sugar solution + 60 ml Serendipity berry extract treated-bread.

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CONCLUSION

The use of serendipity berry extract as sugar substitute could be applied not only to improve nutritional and sensory qualities but also to inhibit microbial growth and thus extend the shelf life of wheat flour bread. This study showed that serendipity berry extract could be used as natural sweetener and as a preservative in bread. The sensory study also indicated that highly acceptable bread could be produced by substituting sugar (5 % sugar solution) with 60 % serendipity berry extract. This study therefore recommends serendipity berry extract as a natural sweetener and as a substitute for chemical preservatives in bread production and similar food applications.

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