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Assessment of Imitation Milk from Sprouted Soybean (*Glycine max*) and Boiled African Breadfruit Seeds (*Treculia africana*) Milk Blends

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Abstract

This work examined the quality of imitation milk from blends of sprouted soybean (glycine max) and boiled African breadfruit fruit (*Treculia africana*). Three formulations of 95:5, 90:10 and 50:50 were prepared while soymilk (SSM) and breadfruit milks (BBM) were used as controls. Physicochemical properties of these formulations were done using standard methods. Sensory evaluation was conducted using consumer preference test. The results of increasing substitution with breadfruit milk showed increase in moisture content (MC) from 92.60 to 93.00%, 2.68 to 3.33% for protein, 0.55 to 2.18% for carbohydrate and decrease ash from 0.57 to 0.52%, 2.15 to 3.00% for fat . Calcium, phosphorous, zinc and iron decreased respectively from 0.65 to 0.59 mg/100 g, 8.20 to 6.82mg/100 g, 0.80 to 0.59mg/100 g and 0.6 to 0.59 mg/100 g. Vitamin A activity increased from 0.56 to 0.78 µg/100 g while vitamins B1 decreased from 0.11 to 0.07 mg/ˆ100 g and vitamin C from 2.91 to 3.82 mg/100 g. All the anti-nutrient contents decreased respectively from 0.12 to 0.07 mg/100 g, 0.11 to 0.06 mg/100 g, 0.37 to 0.28 mg/100 g, 0.20 to 0.15 mg/100 g and 0.25 to 0.12 mg/100 g respectively for flavonoid, saponin, tannin, phytase and oxalate. Titratable acidity and visible coagulation tome (VCT) increased respectively from 0.24 to 0.26 and 18 to 19 days while pH, total solids, viscosity and colour decreased respectively from 6.36 to 6.15, 7.20 to 7.00, 0.48 to 0.46 and 15.51 to 12.03. Similarly, general acceptability increased from 5.80 to 7.85. Sample 50:50 soymilk-breadfruit milk was the most acceptable.

Practical application

Multiple nutrient fortification of food is the best way to prevent nutrient deficiency within a locality. Soymilk is more popular than breadfruit milk; both are very much relished and can upgrade nutrients through complementation between the two. The blends can be likened to multiple fortification liable to checkmate nutrient deficiency within a particular community. The blends were compatible with improved nutrients and acceptability which are the primary goal of multiple fortification of food.

Keywords: Quality, Soybean, Bread fruit, Imitation, Milk, Sensory evaluation

1. Introduction

Imitation milk describes white coloured non-animal milk from plant sources. Some include soymilk, rice milk, almond milk, coconut milk and vegetable milk (Olivia, 2014).Similarity of their functional properties, nutritive value and sensory characteristics allow them to be used as substitutes for animal milks (Enwere, 1998). They could be used for babies who do not take human milk for either ethical or medical reasons like lactose intolerance and galactosemia (Obizoba & Ayinka, 1994). They are recommended for those suffering from

degenerative heart diseases that need unsaturated milk fat (Akinyele & Abudu, 1990).

Imitation milks are produced from vegetables or plant products including oil seeds and legumes such as soybeans, cowpea, bambara groundnut, melon and cotton seeds (Akinyele & Abudu, 1990). The most popular imitation milk is soymilk which has been prepared for hundreds of years in the orient by soaking the beans in water for several hours, draining and grinding with fresh water. Other methods of processing have been advanced (Iwe, 2003).Imitation milk production and widespread utilization in tropical countries are limited among others by beany flavour, phase separation flatulence, tedium in home preparation. Among others, remedies to these include steeping/germination and boiling sodium bicarbonate (Nsofor *et al.,* 1997; Osuji & Ubbaonu, 2004).

Soybean (*Glycine max*) belongs to the family Leguminosae, subfamily papilionoid and contains 40% protein, 20% fat, 20% carbohydrates, 5% crude fibre and 10% moisture). Soybean is an economic source of quality macronutrients and micronutrients (Iwe, 2003). Soymilk resembles breast milk in health benefits and cow's milk in appearance, flavour and nutritive value when properly processed. It is also regarded as a good candidate for infant food formulation. Among other sources of vegetable milk, soymilk has received very high research attention as reference vegetable milk due to absence of cholesterol and preponderance of polyunsaturated fatty acids that hint to the heart health implication of soymilk components (Osuji & Ubbonu, 2004).

African breadfruit is a leguminous crop which seeds are known as afon, ediang or ukwa in Nigeria. The seeds have recently gained attention as dietary component because they are rich sources of vegetable oil (10%), protein (17%), carbohydrates (40%) as well as several minerals and vitamins.

The need to source for alternative and cheaper source of milk and to reduce over dependency on animal protein necessitated this study. Therefore, this work aimed at producing and evaluating the proximate, nutrients, anti-nutrients, physicochemical and acceptability of soymilk-breadfruit milk blends from sprouted soybean and soaked African breadfruit seeds respectively.

2. Materials and Methods

2.1. Samples collection

Soybean and African breadfruit seeds were purchased from Urbani main market in Umuahia, Abia State, Nigeria, and brought to Food Processing Laboratory of Michael Okpara University of Agriculture Umudike, Abia State Nigeria where all the analyses were carried out using analytical grade reagents.

2.2. Sample preparation

2.2.1. Soybean milk

Sprouted soymilk was produced according to Okwunodulu *et al.* (2017) method (Figure 1). Cleaned and sorted soybeans were steeped (12 hours) in clean tap water, drained and sprouted at room temperature for 72 hours on jute sack spread on the floor. The beans were covered with black polyethylene and sprinkled with water regularly as soon as their surfaces were dried during sprouting. The sprouts were washed with tap water, boiled thereafter in 0.5% NaHCO3 solution for 20 min, drained and hand dehulled. The hulls and the shoot were removed by water flotation to obtain soybean cotyledons which

were milled in Q-link (Japan) kitchen blender with hot water (93 ^{o}C) in a ratio of 2.7 L hot water to 1 kg cotyledons (v/w) to obtain sprouted soybean slurry. The slurry was screened through a double layered muslin cloth to obtain soymilk extract.
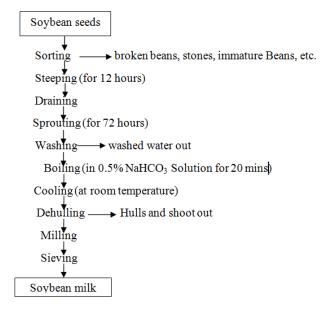
```
┌────────────────┐
│ Soybean seeds  │
└────────────────┘
        │
     Sorting ──────► broken beans, stones, immature Beans, etc.
        │
   Steeping (for 12 hours)
        │
     Draining
        │
   Sprouting (for 72 hours)
        │
     Washing ──────► washed water out
        │
     Boiling (in 0.5% NaHCO3 Solution for 20 mins)
        │
     Cooling (at room temperature)
        │
    Dehulling ──────► Hulls and shoot out
        │
      Milling
        │
      Sieving
        │
┌────────────────┐
│  Soybean milk  │
└────────────────┘
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Figure1: Flow chart for the production of soymilk from sprouted whole soybean. Source: Okwunodulu *et al.* (2017).

2.2.2. Preparation of boiled breadfruit milk

The method described by Onweluzo & Nwakalor (2009) with slight modification was used. African breadfruit seeds were washed in excess volume of water to remove extraneous materials and immature seeds, drained and parboiled in water at 95 ^{o}C for 15 min with constant stirring (Figure 2). Parboiled seeds were drained, allowed to cool and hand dehulled. The hulls were removed by winnowing to obtain the cotyledons which were preserved for milk production. One kilogram (1kg) of the cotyledons was washed and boiled with 0.2% NaHCO3 solution for 30 min and wet-milled in a variable speed blender (SB-736, Sonic, Japan),

with distilled water all in the ratio of 1kg of cotyledon to 3 L of water. The slurry was filtered through double layer linen cloth and the residue wet-milled twice or more for maximum extraction. The filtrate was boiled for 20 min with continuous stirring and filtered to obtain boiled breadfruit milk. Soymilk-boiled breadfruit milk was prepared using formulation ratio of 95:5, 90:10 and 50:50 respectively for soymilk and boiled breadfruit milk. Soymilk and boiled breadfruit milk were used as controls.
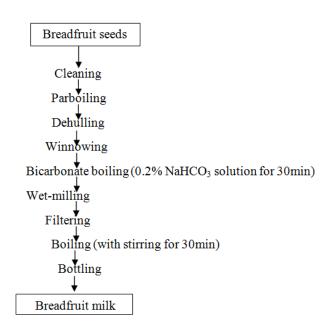
```
┌──────────────────┐
│ Breadfruit seeds │
└──────────────────┘
        │
     Cleaning
        │
    Parboiling
        │
    Dehulling
        │
    Winnowing
        │
  Bicarbonate boiling (0.2% NaHCO3 solution for 30min)
        │
    Wet-milling
        │
     Filtering
        │
    Boiling (with stirring for 30min)
        │
     Bottling
        │
┌──────────────────┐
│ Breadfruit milk  │
└──────────────────┘
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Figure 2: Flow chart for production of boiled breadfruit milk. Source: Onweluzo & Nwakalor (2009)

2.3. Analysis

2.3.1. Proximate composition

2.3.1.1. Moisture content

Moisture content was determined according to AOAC (2010).Ten grams (10 g) of the milk sample (W_2) in a previously weighed moisture can (W_1) were dried in the oven at 105°C for 3 h, cooled in desiccators and reweighed. This process was repeated at 30 min interval for

several times until a constant weight was obtained. The dried sample was cooled in desiccators, reweighed and final dry weight recorded (w_3) which was used to calculate the percentage moisture content of the sample using the formula presented below:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} X \frac{100}{1}$$

Where:

W_1 = initial weight of empty can
W_2 = weight of can + sample before drying
W_3 = weight of can + sample after drying

2.3.1.2. Crude protein

The method described by AOAC (2010) was used to determine total nitrogen (N_2) which was multiplied with factor 6.25 to obtain the protein content. One gram (1.0 g) of the milk sample was mixed with 10 ml of concentrated H_2SO_4 in a digestion flask with a tablet of selenium catalyst added and heated in a fume cupboard until a clear solution or digest was obtained. The digest was diluted to 100 ml in a volumetric flask and 10 ml aliquot was mixed with equal volume of 45% NaOH solution in a kjeldahl distillation apparatus. The mixture was diluted into 10 ml of 4% buric acid containing 3 drops of mixed indicator (bromoscressol green/methyl red). A total of 50 ml of distillates was collected and titrated against 0.02 N EDTA from green to deep red end point. The N_2 obtained was used to calculate protein thus:

$$\% \text{ Protein} = \% N_2 \text{ x } 6.25$$

$$\% N_2 = \frac{(100 \text{ X } Nx14 \text{ X } Vt)}{W \quad 1000 \quad Va} TBK$$

Where:

W = weight of sample

N = normality of titrant (0.02 H2SO4)
Vt = total digest volume (100m/s)
Va = volume of digest analysed (10ml)
T = titre value of sample
B = titre value of blank

2.3.1.3. Ash

The method described by Onwuka (2018) was used. Three grams (3 g) of the sample were added to a weighed dried porcelain crucible and ignited in the muffle furnace at 550 °C. The sample was allowed for 3 h to ash to a greyish white ash, brought out from the furnace using a forceps and left in a desiccator to cool. The ash was weighed and percent ash was calculated as presented below:

$$\% \text{ Ash} = \frac{w_3 - w_1}{w_2 - w_1} X \frac{100}{1}$$

Where:

W_1 = weight of empty crucible
W_2 = weight of crucible + food before drying or ashing
W_3 = weight of crucible + ash

2.3.1.4. Fat

The fat content was determined by continuous solvent extraction in a soxhlet reflux apparatus described by James (1995). Exactly 2 g of the sample was wrapped in a porous paper and carefully placed inside a soxhlet reflux flask. The reflux was mounted on a weighed extraction flask containing 200 ml of petroleum ether on the electro thermal heating mantle. The set was connected to a condenser that cools the evaporated petroleum ether while boiling which in turn fill up the reflux flask. The solvent will extract the oil during the reflux into the boiling flask. This process of boiling, vaporization, condensation and subsequent oil extraction was allowed to continue for 4 hours thereafter the solvent was recovered and the extracted oil was

obtained by drying the flask in the oven at 60^0C for 30 minutes. After cooling in the desiccators, the flask was reweighed. The fat content was calculated thus:

$$\% \text{ Fat content} = \frac{W_2 - W_1}{W_3} \times \frac{100}{1}$$

Where:

W_1= Weight of the empty flask
W_2= Weight of flask oil T extract
W_3= Weight of sample used

2.3.1.5. Carbohydrate

The carbohydrate content of the samples was calculated by difference (James, 1995) as shown below:

$$\% \text{ CHO} = \% \text{ NFE} = 100 - \% (a+b+c+d+e)$$

Where a= protein content, b= fat content, c= ash content, d= crude fibre content, e= moisture content

2.3.2. Determination of vitamins

2.3.2.1. Vitamin A (provitamin)

The spectrophotometric method by Onwuka (2018) was employed in determination of vitamin A with 5 g of milk sample which was dissolved in 30 ml of absolute alcohol (ethanol) and 3 ml of 5% potassium hydroxide added to the mixture. The mixture was boiled under reflux for 30 minutes, cooled rapidly with running water and filtered. 30 ml of distilled water was added, transferred into a separating funnel and 3 portions of 50 ml of ether were used to wash the mixture. The lower layer was discarded and the upper layer was washed with 50 ml of distilled water. The extract was evaporated to dryness, dissolved in 10 ml of isoprophyl alcohol and its absorbance was measured at 325 nm. The vitamin A content of the samples was calculated using the formula below.

$$\text{Vitamin A (mg/100g)} = \frac{100}{w} \times \frac{au}{as} \times c$$

Where:

au = absorbance of test sample
as = absorbance of standard solution
c = concentration of the test sample
w = weight of sample

2.3.2.2. Vitamin C

The method of Okwu & Josiah (2006) was used. Ten grams (10 g) of the milk sample was extracted with 50 ml EDTA/TCA extracting solution for 1 hour and filtered through a Whatman filter paper into a 50 ml volumetric flask and made up to the mark with the extracting solution. 20 ml of the extract was pipette into a 250 ml conical flask into which 10 ml of 30% KI and 50 ml of distilled water were added. This was followed by 2 ml of 1% starch indicator and titrated against 0.01 ml $CuSO_4$ solution to a dark end point. The vitamin C content of the samples was thereafter calculated using the formula:

$$\text{Vitamin C (mg/100g)} = 0.88 \times \frac{100}{10} \times \frac{Vf}{20} \times \frac{T}{1}$$

Where:

Vf = Volume of extract
T = Sample titre - blank titre

2.3.2.3 Thiamine

Determination of thiamine (Vitamin B1) The spectrophotometric method, described by Onwuka (2018) was used for determination of the B Vitamins. Exactly 5 g of each sample was homogenized with 50 ml of 1N ethanolic sodium hydroxide and the homogenate was filtered to obtain the filtrate to be used for the analysis. An aliquot (10 ml) of the filtrate was treated with

equal volume of 0.1N $K_2Cr_2O_7$ solution in a flask. Standard thiamine solution was prepared and diluted to a chosen concentration (0.5). An aliquot of the standard thiamine solution was also treated with 10 ml of the dichromate solution ($K_2Cr_2O_7$) in a separate flask while a reagent blank was set up by treating l0 ml of the ethanolic sodium hydroxide with the potassium dichromate solution. The absorbance of the sample and the standard solutions was measured in a spectrophotometer at a wavelength of 360 nm with the reagent blank to be used to calibrate the instrument at zero. The thiamine content was calculated using the formula:

Thiamine (mg/100g) = 100/w x Au x C/As x Vf x D/Va

Where:

W = Weight of sample
Au = Absorbance of sample
As = Absorbance of standard thiamine solution
C = Concentration of standard thiamine solution
Vf = Total volume of filtrate
Va = Volume of filtrate analyzed
D = Dilution factor where applicable

2.4. Determination of minerals

2.4.1. Calcium

Calcium was determined using the method described by Pearson (1976). Twenty-five (25) milliliter of the digested sample was pipetted into 250 ml conical flask and a pinch of Eriochrome Black-T-Indicator (EBT) was added. Thereafter, 2 ml of 0.1N NaOH solution was added and the mixture titrated with standard EDTA (0.01M EDTA) solution. Calcium was calculated using the formula shown below:

$$Ca\ (mg/I) = \frac{T \times M \times E \times 1000}{Volume\ of\ sample\ used}$$

Where:

T = Titre value
M = Morality of EDTA
E = Equivalent weight of calcium

2.4.2. Phosphorus

Molybdate method of Onwuka (2018) using hydroquinone as a reducing agent was used. Five millilitres (5 ml) of the test solution was pipette into 50 ml graduated flask. Then 10 ml of molybdate mixture was added and diluted to mark with distilled water. It was then allowed to stand for 30 minutes for colour development. The absorbance was measured at 600 nm against a blank. A curve relating absorbance to mg phosphorus present was plotted. Using the phosphorus standard solution, and following the same procedure for the sample, a standard curve was plotted to determine the concentration of phosphorus in the sample. Phosphorus was calculated using the formula presented below:

$$\%\ Phosphorus = \frac{graph\ reading \times solution\ volume}{100}$$

2.4.3. Iron

James (1995) spectrophotometric method was used. Absorbance readings of the iron standards prepared at different concentration of 2 ppm to 10 ppm by diluting with distilled water along with 3 ml of buffer solution, 2 ml of hydroquinone solution and 2 ml of bipyridyl solution were taken at 520 nm. The readings were used to plot a standard iron curve for extrapolation.

2.4.4. Zinc

The method of AOAC (2010) was used. One gram of the sample was first digested with 20 ml of acid mixture (650 ml concentrated HNO_3, 80

ml perchloric acid (PCA). Five (5) ml of the digest was collected and diluted to 100 ml with H_2O. This now served as sample solution for AAS reading. Also, a standard solution of respective elements concentration of 0.0, 0.2……….and 1.0 was taken. The readings were used to plot a standard iron curve for extrapolation.

$$Fe = \frac{Vf}{Vs} \times \frac{1}{10} \times \frac{100}{W} \times Df$$

Where:

W= Weight of sample analysed
Vf = Volume of extract
Vs = Volume of extract used
Df = Dilution factor

2.5. Determination of anti-nutrients

2.5.1. Phytate

Spectrophotometric method of Oberlease (2013) was used. One gram (1g) of the sample was extracted into two two tubes containing 20 ml of 0.1M nitric acid with constant agitation for 4 hours. The tubes were covered, placed in a boiling water bath for 20 min and allowed to cool. About 5.0 ml of amyl alcohol was added to each tube followed by 1.0 ml of ammonium thiocyanate (100 g/l). The tubes were shaken thoroughly, centrifuged at 2000 rpm and the absorbance of the supernatant taken at 465 nm against amyl alcohol exactly 15 min after the addition of the ammonium thiocyanate using spectrophotometer. Standard solution was also prepared with which percent phytic acid was calculated using the absorbance of the test sample.

2.5.2. Saponin

Spectrophotometer method of AOAC (2010) at 620 nm was used. The quantity of saponin contained in each sample was estimated from the standard saponin curve obtained from plotting the concentration of the standard concentration against the absorbance. Saponin content was calculated as follow:

$$P_S = A_b \text{ x S x } D_F \text{ x } 100 \text{ (mg g}^{-1}\text{saponin)}$$

Where:

PS = Percent saponin
Ab= Absorbance
S = Slope
DF = Dilution factor

2.5.3. Tannin

The spectrometric method of Pearson (1976) was used. One gram (1 g) of each sample was weighed into a tube with 2 ml of distilled water and centrifuged at 1500 rpm for 10 min. The supernatant was dispersed and1 ml of $NaCO_3$ and Folin Denis reagent was added in the beaker and allowed to settle. Thereafter, the Absorbance readings obtained were used for calculating tannin using the formula:

$$\% \text{ Tannin} = \frac{A_n}{A_s} \text{ x C x } \frac{100}{W} \text{ x } \frac{V_f}{V_a}$$

Where:

An= absorbance of test sample
As = absorbance of standard sample
C = concentration of standard solution
Vf = total volume of extract
Va = volume of extract analyzed
W = Weight of sample

2.5.4. Oxalate

The oxalate contents of the samples were determined using the method of Iwuoha & Kalu (1994). This method involved three steps: digestion, oxalate precipitation and

permanganate titration. Oxalate content was calculated using the formula presented:

$$CA_S = \frac{T \times V_{mc} \times DF \times 10^5}{ME \times mF}$$

Where:

T= liter of $KMnO_4$ (mL)
Vmc = Vol.-Mass equivalent
DF= Dilution factor
A= Aliquot used (125ml)
ME=mola equivalent of $KMnO_4$ in oxalate
mF= Mass of the sample used

2.6. Physicochemical analysis

2.6.1. pH

The pH was determined as described by Akpakpunam & Dedeh (1995). Ten grams (10 g) of the sample was dissolved in 100 ml of distilled water. The mixture was allowed to equilibrate for 3 minutes at room temperature. The pH was then determined with a pH meter in the sample then taking the result displayed on the pH meter.

2.6.2. Titratable acidity

The titration method described by AOAC (2010) was used. The soymilk-breadfruit milk blends were titrated against standard sodium hydroxide solution until pink colour persisted for about 10-15 seconds for complete neutralization. Values of titratable acidity were calculated as shown in the formula:

Lactic acid (mg)/100ml of sample= Vg x N x 90 x100/Vm

Where:

Vg = volume of NaOH solution added
N= concentration of sodium hydroxide standardized solution expressed in Eq/L
90 = equivalent weight of lactic acid
Vm = volume of milk blend used for titration

2.6.3. Total solid

Total solid was determined as described by AOAC (2010). Three grams (3 g) of the sample was weighed into a dry Petri dish (W) of a known weight (W1) dried for 3 hours at 100oC in forced draft air oven (W2). Total solid of the sample was calculated as the weight of the dried sample residue thus:

$$\% \text{ Total Solid} = \frac{W_2 - W_1 \times 100}{W_1 - W}$$

Where:

W = Weight of the dish
W_1 = Weight of dish and sample test portion
W_2 = Weight of dish and dry sample

2.6.4. Colour

Sample colour was assessed with spectrophotometer at 620 nm after 1.50 dilutions with distilled water using Genesys 10vis thermo corporation spectrophotometer (Okwunodulu *et al.,* 2015).

2.6.5. Visible coagulation time (VCT)

The VCT was determined by visual observation at every 24 hours for time the samples will separate into visible coagulum during storage (Okwunodulu *et al.,* 2015).

2.6.6. Sensory evaluation

Sensory evaluation of milk samples was conducted using a randomly selected 30-member semi trained panelists. Selection was from student and workers of Food Science and Technology of the University aged between 18 to 35 years who are familiar with both milk. The sensory attributes of the milk samples tested were colour, taste, flavour, mouth feel and over all acceptability. The panelists were required to

observe and taste each coded sample and grade them on a 9-point Hedonic scale which ranged from like extremely for 9 to dislike extremely for 1, with 5 as neither like nor dislike (Iwe, 2010).

2.7. Statistical analysis

Data were statistically analyzed using Completely Randomized Design (CRD). One way ANOVA was conducted using SPSS version 22.0. Duncan Multiple Test was used to separate the means at 5% acceptable level.

3. Results and discussion

3.1 Proximate composition

Proximate results of soymilk-breadfruit milk blends are presented in Table 1.

3.1.1. Moisture content (MC)

The MC (93%) of 100% breadfruit milk (BBM) was significantly (0<0.05) higher than 92% from SSM probably due to soybean cotyledon: water ratio used during soymilk extraction and higher total solids of SSM (Table 5) resulting from sprouting of soybean. The MC has an inverse relationship with total solids. This may also explain the reason for increasing MC of the milk blends with increase in breadfruit milk. The increase were significantly (p<0.05) higher than 92.60% from SSM, but lower than 93.00% from BBM which is MC improvement to soymilk. The difference could be traced to cotyledon: water ratio used in the preparation. High MC will aid swallowing and acceptability which was enhanced by increasing bread fruit milk substitution.

3.1.2. Ash content

Ash content (0.60%) of sample SSM was significantly (p<0.05) higher than 0.45% from

Table 1: Proximate composition of soymilk and boiled breadfruit milk blends (%)

Samples	Moisture Content (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
SSM	92.60[b]±0.14	0.60[a]±0.00	3.20[a]±0.14	3.05[b]±0.00	0.55±0.28
BBM	93.00[a]±0.28	0.45[c]±0.00	1.08[d]±0.35	3.30[a]±0.14	2.18[a]±0.39
SBM1	92.80[ab]±0.14	0.57[a]±0.00	3.00[ab]±0.14	2.68[a]±0.74	0.53[b]±0.00
SBM2	92.90[ab]±0.14	0.54[b]±0.02	2.75[b]±0.07	3.10[b]±0.00	1.01[b]±0.18
SBM3	93.00[a]±0.14	0.52[b]±0.00	2.15[c]±0.07	3.33[a]±0.04	1.14[ab]±0.83

Values are means of triplicate determinations ± standard deviation Values bearing different superscripts in the column are significantly different (p<0.05). SSM = 100% soymilk, BBM = 100% breadfruit milk, SBM1 = 95: 5 soybean-breadfruit milk, SBM2 = 90: 10 soybean-breadfruit milk, and SBM3 = 50:50 soybean-breadfruit milk

BBM which may have caused the ash content decrease with increasing breadfruit milk substitution. The decrease was significantly (p<0.05) lower than 0.60% from SSM. This is not an improvement compared to SSM. Ash content of a food material is a measure of mineral content or inorganic residue remaining after water and organic matter have been removed by open air incineration (Dabels *et al.*, 2016). The range of ash content obtained in this study is lower than 0.46% to 0.64% reported by

Lawal *et al.* (2016) from kunu-zaki formulated with sprouted breadfruit milk.

3.1.3. Fat

Fat content of soybean-breadfruit milk samples decreased with increasing breadfruit milk substitution below SSM which could stem from significant ($p<0.05$) higher fat content (3.20%) of SSM than 1.08% from BBM. Fat is an energy substrate which also boosts food flavour. Therefore, partial substitution with breadfruit milk may likely reduce the energy potential and flavour of the milk blends.

3.1.4. Protein content

Significant ($p<0.05$) higher protein content (3.30%) of BBM compare to 2.68% from SSM may have caused the increasing protein content of soybean-breadfruit milk with increasing substitution of breadfruit milk. The increase was protein improvement compared to SSM and BBM. Protein is importance for human health and growth. Frequent consumption of protein rich milk will help to alleviate protein malnutrition especially in developing countries such as Nigeria. Therefore, breadfruit milk substitution which enhanced the protein content will predispose the milk blend to a good complementary food. The protein content of the milk blends is comparable to complementary protein requirement of 9 .1 to 10.9 g/day (Adeshu *et al.,* 2016).

3.1.5. Carbohydrate content

Carbohydrate content of soymilk-breadfruit milk samples ranged from 0.55% (SBM1-95:5% soybean-breadfruit milk) to 1.14% (SBM3-50:50% soybean-breadfruit milk).Carbohydrate content of the soymilk-breadfruit milk blends increased more than SSM with increasing BBM

substitution which could be traced to higher carbohydrate content (2.18%) of BBM compare to 0.55% from SSM. The substitution was an improvement of carbohydrate content SSM which is an energy substrate. With this increase, the milk blends may help to address protein-energy malnutrition.

3.2. Vitamin composition

The results are presented in Table 3.

Table 3: Vitamin content of soybean-breadfruit milk samples

Samples	Vitamin A (µg/100g)	Vitamin B$_1$(mg/100g)	Vitamin C(mg/100g)
SSM	0.49^e±0.06	0.13^a±0.01	4.28^a±0.25
BBM	1.19^a±0.09	0.01^d±0.00	0.34^e±0.08
SBM1	0.56^d±0.00	0.11^{bc}±0.01	3.82^{bc}±0.04
SBM2	0.69^c±0.04	0.09^c±0.01	3.57^c±0.11
SBM3	0.78^b±0.03	0.07^c±0.01	2.91^d±0.16

Values are means of triplicate determinations.± standard deviation Values bearing different superscripts in same column are significantly different ($p>0.05$). SSM = 100% soymilk, BBM = 100% breadfruit milk, SBM1, 95: 5% soymilk-breadfruit milk, SBM2 = 90:10% soymilk- breadfruit milk and SBM3 = 50:50% soymilk- breadfruit milk.

3.3.1. Vitamin A (provitamin)

Vitamin A content of BBM (1.19 µg/100 g) was significantly higher than 0.49 µg/100 g from SSM which is in line with the report of Floyd & Brandon (1995) that breadfruit seeds are mainly induced by xanthophylls pigment (carotenoid) which is vitamin A precursor (Omueti & Ajomale, 2005). Higher vitamin A content of BBM may be the major reason for significant (p<0.05) increase in vitamin A content of soybean-breadfruit milk blends with increasing BBM substitution. The increase was lower than 1.19 µg/100 g from BBM but higher than 0.49 µg/100 g from SSM 100% soymilk which implied enhancement compared to SSM vitamin A. Vitamin A has many varied functions which include formation and maintenance of teeth, bones, soft tissues, white blood cells, immune system, boost vision, lowers cholesterol and powerful antioxidants for food preservation (Onyeka, 2008).

3.3.2. Vitamin B1

Vitamin B1 content of soy-breadfruit milk blends ranged from 0.07 mg/100 g in sample SBM3 (50:50% soymilk-breadfruit milk) to 0.11 mg/100 g in SBM1 (95:5 soymilk breadfruit milk). Low vitamin B1values in this study could stem from its water soluble nature and susceptibility to process losses (Badejo, 1999).Significant (p<0.05) decrease in vitamin B1 content of soymilk-breadfruit milk blends with increasing BBM substitution could be justified by higher (0.13 mg/100 g) vitamin B1 content of sample SSM than 0.01 mg/100 g from BBM. Vitamin B1is a part of the eight vitamins that make up the B-complex family which plays an important role in brain, nerve, muscle and heart function helps to boost the immune system and protects stress (Everyday Health, 2018).

3.3.3. Vitamin C

Vitamin C content of the soymilk-bread milk blends decreased significantly (<0.05) with increase in BBM substitution as a result of significant (p<0.05) higher vitamin C content (4.28 mg/100 g) of SSM than 0.34 mg/100 g from BBM. Vitamin C content range obtained in this study is within 3.38 to 4.21 mg/100 g reported by Onweluzo & Nwakalor (2009) from African breadfruit milk. The difference could be due to variety and processing methods as Vitamin C is water soluble. Vitamin C is a powerful antioxidant that helps the body to form and maintain connective tissues, including bones, blood vessels, and skin (Onyeka, 2008).

3.3. Mineral composition

The results are presented in Table 2.

3.2.1. Calcium

Despite poor calcium sources of all the milk samples, that of SSM (0.66 mg/100ml) was significantly (p<0.05) higher than BBM (0.57 mg/100ml) which is still higher than 0.38 to 0.47 mg/100 reported by Onweluzo & Nwakalor (2009) for bread fruit milk. Breadfruit milk substitution did not improve calcium content of SSM rather BBM. Calcium is important for optimal bone health, teeth and proper nail growth which diets are the best source (Onyeka, 2008). Calcium content of the milk blends (0.59 to 0.63 mg/100g) is higher than 0.08 to 0.25 mg/100g reported for kunnu-soymilk blends (Sowonola *et al.,* 2005).

3.2.2. Phosphorous

Increasing substitution levels of BBM decreased the phosphorous content compared to SSM but an improvement to BBM. This could be attributed to significant (p<0.05) higher

Table 2: Mineral composition of soybean-breadfruit milk blends (mg/100g)

Samples	Calcium	Phosphorous	Zinc	Iron
SSM	0.66a±0.03	8.33a±0.18	0.79a±0.04	0.59a±0.04
BBM	0.57b±0.00	5.11d±0.29	0.35c±0.00	0.58a±0.00
SBM1	0.63b±0.00	8.20a±0.00	0.80a±0.00	0.60a±0.14
SBM 2	0.60b±0.14	8.03b±0.16	0.76a±0.00	0.59a±0.00
SBM3	0.59b±0.00	6.82b±0.13	0.59b±0.00	0.59a±0.01

Values are mean of triplicate determinations ± standard deviation. Values bearing different superscripts in same column are significantly different (p>0.05). SSM = 100% soybean milk, BBM = 100%=breadfruit milk, SBM1=95:5% soybean-breadfruit milk, SBM2=90:10% soybean-breadfruit milk and SBM3 = 50:50 soybean-breadfruit milk.

phosphorous content (8.33 mg/100ml) of SSM than BBM (5.21 mg/100 g). Statistical similarity between samples SSM and SBM1 (95:5% soymilk-breadfruit milk) may mean that 5% BBM substitution had no significant (p>0.05) effect on phosphorous content unless above. Phosphorous is the second most abundant mineral in the body needed for such functions as waste filtering and repair of tissues and cells. However, certain health conditions (diabetes and alcoholism) or medications (antacids) can lower phosphorous levels in your body (Onyeka, 2008).

3.2.3. Zinc

With significant (p<0.05) higher zinc content (0.79 mg/100ml) of SSM than 0.35 mg/100ml from breadfruit milk, zinc content of soybean-breadfruit milk blends decreased with increasing BBM substitution. Despite the decrease, zinc content of all the soybean-breadfruit milk blends was statistically similar which implied slight improvement compared to BBM. Zinc among others regulates body immune function, increases mental ability, replaces damaged tissues, and decreases the rate of age related chronic diseases as well as fertility (Onyeka, 2008).

3.2.4. Iron

Statistical similarity iron content between samples SSM and BBM also reflected in iron similarity between all the soybean-breadfruit milk blends despite the decrease with BBM substitution. The substitution only had slight iron improvement on the soymilk-breadfruit blends. Iron levels in this study were higher than 0.031 to 0.039 mg/100 g reported by Donald & Eucharia (2018) from African breadfruit-corn milk. Iron is an integral part of haemoglobin, red blood cell, that carries oxygen from the lungs to all parts of the body which shortage (anaemia) leads to fatigue and weakness (Onyeka, 2008).

3.4. Anti-nutrient composition

Results are presented in Table 4.

3.4.1. Flavonoid

Flavonoid content of soymilk-breadfruit milk blends varied without significant (p>0.05) variations but decreased with increasing

breadfruit substitution. The decrease could be traced to significant (p<0.05) higher flavonoid content of SSM (0.17 mg/100 g) than BBM (0.02 mg/100 g).Despite this, flavonoid content of all the samples were within the safe limit probably because of the processing techniques employed which may have destroyed some polyphenolic compounds like flavones. Flavonoids are anti-oxidants that fight against some cancers and cardiac diseases (Group, 2016).

Table 4: Anti-nutritional composition of soymilk-breadfruit milk blends (mg/100g)

Samples	Flavonoid	Saponin	Tannin	Phytate	Oxalate
SSM	0.17[a]±0.03	0.13[e]±0.01	0.40[a]±0.03	0.24[a]±0.01	0.28[a]±0.00
BBM	0.02[c]±0.01	0.02[e]±0.00	0.17[c]±0.03	0.06[c]±0.01	0.12[cd]±0.02
SBM1	0.12[b]±0.01	0.11[bc]±0.01	0.37[a]±0.01	0.20[a]±0.00	0.25[ab]±0.01
SBM2	0.11[b]±0.01	0.09[cd]±0.01	0.28[b]±0.01	0.18[b]±0.00	0.21[b]±0.02
SBM3	0.07[b]±0.00	0.06[d]±0.01	0.26[b]±0.01	0.15[b]±0.01	0.17[c]±0.00

Values are means of triplicate determinations ± standard deviation. Values bearing different superscripts in the column are significantly different (p>0.05). SSM = 100% soymilk, BBM = 100% breadfruit milk, SBM1- 95: 5% soymilk-breadfruit milk, SBM2 = 90: 10% soymilk breadfruit milk and SBM3 = 50:50 soymilk- breadfruit milk.

3.4.2. Saponin

Saponin content of SSM (0.13 mg/100 g) was significantly (p<0.05) higher than BBM (0.02 mg/100 g) which may justify the decrease saponin content of soymilk-breadfruit milk blends with the increase BBM substitution. Low saponin content observed in this study could be as a result of leaching and/or processing losses as saponin is heat labile, but not completely destroyed during cooking (Ikemefuna *et al.,* 1991). Due to its amphipathic nature, saponins are surfactant that enhances penetration of macro molecules such as proteins through cell membranes (Howes, 1998). However, saponin has health beneficial effect because of its hypocholesterolemic activity (Onimawo & Akubor, 2005).

3.4.3. Tannin

Tannin content of SSM (0.40 mg/100 g) was significantly (p<0.05) higher than that of BBM (0.17 mg/100 g) which may have contributed to the decrease in tannin content with increasing BBM substitution. Tannin range obtained in this study was higher than 0.01 to 0.06 mg/100 g reported for African breadfruit seeds and is responsible for the astringency, colour, and some of the flavour in tea (Olapade & Umeonuorah, 2013). Low tannin content observed in this study could be attributed to leaching and processing loss as it is heat labile and water soluble (Ugwu & Oranye, 2006). Tannin quickens wound and burns healing in human body (Farquar, 1996).

3.4.4. Phytate

Phytate (salt of phytic acid) content of SSM (0.24 mg/100 g) was significantly (p<0.05) higher than that of BBM (0.06 mg/100 g), hence the decreasing effect of increasing BBM substitution. Lower phytate content implies more

nutrient availability with BBM having an edge over SSM. The range of phytate obtained in this study is lower than 0.76 to 0.78 mg/100 g reported by Osabor *et al.* (2009) from African breadfruit seeds. Phytic acid is the storage form of phosphorous in plant seeds such as beans/ legumes, nuts and grains which impairs absorption of iron, zinc and calcium and may promote mineral deficiencies (Osabor *et al.,* 2009).

3.4.5. Oxalate

Oxalate content of soymilk-breadfruit milk like other anti-nutrients was significantly ($p<0.05$) decreasing with increasing BBM substitution. This was justified by significantly higher oxalate content of SSM (0.28 mg/100 g)) than BBM (0.12 mg/100 g). However, oxalate content of all the various samples is within safe limit for human consumption. Lower value of the oxalate content in BBM could be attributed to leaching into soaking water prior to boiling. Oxalate content of 0.13 to 0.21 mg/100 g from African breadfruit seeds (Olapade & Umeonuorah, 2013) was lower than 0.28 mg/100 g from SSM and higher than 0.12 mg/100 g from BBM. Oxalate above safe limit becomes an anti-nutrient by chaleting with the protein and prevent their bioavailability and digestion (Ijieh *et al.,* 2010).

3.5. Physicochemical composition

Physicochemical results are presented in Table 5.

3.5.1. pH

The pH of soymilk-breadfruit milk blends decreased with increasing BBM substitution which could be attributed to significantly ($p<0.05$) higher pH (6.38) of SSM than BBM (5.75). The decrease in pH was significantly ($p<0.05$) below SSM but above BBM which may slightly affect acceptability and ambient storage

Table 5: Physicochemical properties of soy- breadfruit milk samples

Samples	pH	TTA	Total Solid (%)	Viscosity (MPa)	Colour	VCT (days)
SSM	6.38[a]±0.03	0.20[b]±0.01	7.40[a]±0.14	0.48[a]±0.01	18.47[a]±0.02	17.00[a]±0.14
BBM	5.75[bc]±0.01	0.26[a]±0.01	7.00[a]±0.28	0.46[a]±0.01	17.54[b]±0.06	18.00[a]±0.28
SBM1	6.36[a]±0.01	0.22[b]±0.01	7.20[a]±0.14	0.48[a]±0.00	15.51[c]±0.02	18.00[a]±0.14
SBM2	6.31[b]±0.02	0.23[b]±0.01	7.10[a]±0.14	0.48[a]±0.01	15.03[d]±0.03	19.00[a]±0.14
SBM3	6.15[d]±0.00	0.24[b]±0.01	7.00[a]±0.14	0.46[a]±0.00	12.03[e]±0.01	19.00[a]±0.14

Values are mean of triplicate determinations ± standard deviation. Values bearing different superscripts in the column are significantly different ($p>0.05$). SSM = 100% soymilk, BBM = 100% breadfruit milk, SBM1= 95: 5% soymilk breadfruit milk, SBM2 = 90: 10% soymilk- breadfruit milk and SBM3 = 50:50% soymilk- breadfruit milk.

stability compared to BBM as both are better at pH 7. Therefore, substituting above 50% with BBM may likely result in the decrease in ambient storage stability and acceptability. Lower pH has been reported to precipitate protein resulting to phase separation and decreased acceptability (Okwunodulu & Okwunodulu, 2016). The pH of SSM was lower than 6.82 obtained from 100% soymilk (Okwunodulu *et al.,* 2017).Soymilk-breadfruit

milk blends in this study will be more stable and may be acceptable to ulcer patents since it is less acidic.

3.5.2. Total titratable acidity (TTA)

The TTA of SSM (0.2) was significantly ($p<0.05$) lower than BBM (0.26) which may have been the major cause of significant ($p<0.05$) TTA increase in soymilk-breadfruit blend with BBM substitution increase. The TTA has an inverse correlation with pH and therefore justify the pH results which were near point of neutrality and therefore potent stability. The TTA (0.24) of SBM_3 (50:50 soymilk-breadfruit milk) was within 0.24 to 0.36% reported by Onweluzo & Nwakalor (2009) for breadfruit seed milk while SBM_1 (95:5 soymilk-breadfruit milk) was lower (0.22).

3.5.3 Total solid

Total solids (TS), an inverse of moisture content, decreased insignificantly ($p>0.05$) with increasing substitution of SSM with BBM due to slight higher TS value (7.40) of SSM than BBM (7.0). The decrease was lower than 7.40 from SSM, but higher than 7.00 from BBM and therefore an improvement to BBM. Increasing BBM substitution beyond 50% may therefore decrease mouth feel, viscosity and acceptability as they depend on TS.

3.5.4. Viscosity

Viscosity, just like TS decreased slightly with increasing substitution of SSM with BBM. Slight ($p>0.05$) difference between the viscosity values of SSM (0.48 mPa) and BBM (0.46 mPa) may have been the cause. Similarly, there was no significant ($p<0.05$) viscosity variation in all the soymilk-breadfruit milk blends, but their values were lower than 196 pas obtained by Okwunodulu & Abasiekong (2015) from soymilk from sprouted soybean. Lower viscosity values could be aligned to low TS which is a function of viscosity. Substitution with BBM above 50% may likely affect viscosity which in turn affects mouth feel, taste and acceptability of the milk blends as they are function of viscosity (Onweluzo & Nwakalor, 2009).

3.5.5. Colour

As shown in plates 1 and 2, colour of SSM (18.47) was significantly ($p<0.05$) higher than that of BBM (17.54 mpa) probably due to higher TS and pH of SSM. Soybean tenderization due to sprouting and boiling in 0.5% sodium bicarbonate unlike breadfruit seed that were only boiled in 0.2% without sprouting may have been the major source of TS variation (Okwunodulu *et al.,* 2015). Colour of SSM (18.47) was lower than 28.15 reported by Okwunodulu *et al.* (2017) from soymilk which may be due to variety, percent sproutability and milling. Colour of soymilk-breadfruit milk blends decreased significantly ($p<0.05$) as BBM substitution increased (Plates 3 to 5). Colour attracts and decides acceptability which will decrease with BBM substitution increase most especially beyond 50%.

3.5.6. Visible Coagulation Time (VCT)

The VCT which is time taken for visible coagulation to occur in the milk samples increased without significant ($p>0.5$) variation with increase BBM substitution. Samples SBM_1 (95:5% soymilk-breadfruit milk) showed visible separation on the 18th day while samples SBM_2 soymilk-breadfruit milk) and SBM_3 (50:50 soymilk-breadfruit milk) separated at 19th day. Increased VCT is an index of ambient storage stability which implies that increasing BBM

substitution will increase soymilk-breadfruit milk blends ambient storage stability. The VCT range obtained in this study is within 10- 20 days reported by Okwunodulu *et al.* (2017) for fortified soymilk and15.00 to 27.00 as reported by Osuji & Okafor (2012) from soymilk.

3.6. Sensory evaluation

Sensory scores results are presented in Table 6.

Table 6: Sensory evaluation of breadfruit-soy milk samples

Samples	Colour	Taste	Mouth feel	GA
SSM	7.85^a±1.14	8.15^a±1.57	8.10^a±1.12	8.01^a±0.79
BBM	6.32^c±1.05	6.72^b±0.03	6.81^b±0.52	6.77^b±0.51
SBM1	5.85^c±0.99	5.35^c±1.31	5.60^c±1.09	5.80^c±0.81
SBM2	6.89^b±1.14	6.72^b±0.03	6.95^b±0.83	6.91^b±0.52
SBM3	8.00^a±0.92	8.10^a±0.91	6.90^b±0.79	7.85^a±0.81

Values are mean of triplicate determinations ± standard deviation. Values bearing different superscripts in the column are significantly different (p>0.05). SSM = 100% soymilk, BBM = 100% breadfruit milk, SBM1- 95: 5% soymilk-breadfruit milk, SBM2 = 90: 10% soymilk-breadfruit milk and SBM3 = 50:50 soymilk- breadfruit milk.

3.6.1. Colour

The SSM colour rating (7.85) was significantly (p<0.05) higher than that of BBM (6.32) which

analogized with Plates 1 and 2. Colour scores increase with increasing BBM substitution in all the soymilk-breadfruit milk blends which were in line with Plates 3 to 5 which was an improvement in colour and acceptability of all the milk samples. Colour rating (8.00) of SBM3 (50:50% soymilk-breadfruit milk) was significantly (p<0.05) higher than all the milk samples. Colour is an important physical property of foods which decides acceptability as consumers eat with their eyes and use what they observed to assess quality (Oluwole, 2009).

Sample A

Plate 1: Milk produced with 100% Soy Bean

Sample C

Plate 2: Milk produced with 100% boiled breadfruit

Sample E

Plate 3: Milk blend of 95:5% soymilk- boiled breadfruit milk

Sample G

Plate 4: Milk blend of 90: 10% soymilk- boiled breadfruit milk

Sample I

Plate 5: Milk blend of 50:50% soymilk- boiled breadfruit milk

3.6.2. Taste

Taste score of SSM (8.18) was significantly (p<0.05) higher than that of BBM (6.72) which

may be that panelists were not familiar with breadfruit milk which is unpopular. The 8.15 score translate to "like very much" on the Hedonic scale. This notwithstanding, increasing BBM substitution increased the taste of soymilk-breadfruit samples most especially at above 10% BBM substitution level. Maximum taste score of 8.10 was obtained at 50% BBM substitution level which was most preferred by the panelists. The least preferred (5.35) was sample SBM1 (95:5 soymilk-breadfruit milk) that translated to "neither like nor dislike" on the Hedonic scale.

3.6.3. Mouth feel

Mouth feel of SSM (8.10) was significantly (p<0.05) higher than that of BBM (6.81) which could be traced to sprouting which increased TS and viscosity (0.48m Pa), and in turn the mouth feel (Onweluzo & Nwakalor, 2009). However, mouth feel of soymilk-breadfruit samples increased with increasing BBM substitution. The increase which was lower than SSM (8.10) may be due to higher oil, TS and viscosity content which enhance mouth feel.

3.6.4. General acceptability (GA)

General acceptability is an overall assessment of the sensory characteristics of samples and any product with highest acceptable levels in most of the attributes than others is expected to have highest overall acceptability (Oluwole, 2009). Therefore, with increasing trends in all the attributes with increase BBM levels of substitution implicated 50: 50 soymilk-breadfruit milk blends as the most acceptable (7.85). Sample with 95:5 soymilk-breadfruit milk blend had the least score (5.80) Therefore, breadfruit milk substitution improved soymilk-breadfruit milk blend acceptability. Substitution above 50% may be feasible provided acceptability and nutrient are not compromised.

4. Conclusion

Quality and acceptable vegetable milk is feasible from partial substitution of soymilk with breadfruit milk up to 50%. Increased breadfruit milk substitution increased the moisture, protein, carbohydrate and reduced the ash and fat content of the blends. Also, calcium, phosphorous, zinc and iron were decreased. Vitamins B1 and C were increased while vitamin A was decreased. Although the anti-nutrients were within safe limit, they were further reduced. Ambient storage stability and titratable acidity were increased despite the decrease in pH, total solids, viscosity and colour. Acceptability also improved with 50:50% blend being the most acceptable (7.85) which ranked between like moderately to like very much while 95:5% blend had the least rating (5.80) which ranked neither liked nor dislike. Therefore, blending ratio will depend on consumers' choice provided acceptability and targeted nutrients are not compromised.

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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