



Non-dairy Milk from Sprouted and Un-sprouted Soybeans Boiled in Plant Ash and Sodium Bicarbonate Solutions: A Comparative Nutrient, Physicochemical and Acceptability Responses

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Abstract:

Conventional uses of sodium bicarbonate in food processing result in gastrointestinal (GI) upset with such symptoms as nausea, stomach diarrhea, and vomiting to the venerable. Plant ash from empty palm bunch which is readily available, economical, underutilized, nutrient dense, and a tenderizer without side effects becomes the best alternative. Sorted and 12 h steeped soybean was sprouted (72 h) on a jute bag spread on the floor and covered with black polythene. The sprouts and un-sprouted soybeans were boiled (20 min) in water extract of plant ash of concentrations 30%, 50%, 70% and 0.5% sodium bicarbonate separately and hand de-hulled after cooling. The hulls were removed by water floatation and their cotyledons were milled with 93°C water (1 kg in 2.7 L) in a variable kitchen blender, sieved with a double layer calico cloth and the soymilk boiled for 20 min in an open pan with continuous steering. The soymilk samples were separately analyzed. The results showed that with decrease in plant ash concentration, moisture, fiber, ash, fat, energy, pH, viscosity and appearance of soymilk from sprouted soybean increased more than their un-sprouted counterparts. Also, soymilk from sprouted soybeans boiled in 0.5% sodium bicarbonate increased more than its un-sprouted counterpart in protein, vitamin C, calcium, phosphorous, iron, total soluble solids, pH, vitamins A and B1. There was no significance ($p>0.05$) in the acceptability of the entire soymilk samples, but those from sprouted soybeans were slightly better with more nutrient improvement at low ash concentration levels.

Keywords: Soymilk, sprouted soybean, un-sprouted soybean, plant ash, sodium bicarbonate.

1. Introduction

Soymilk is essentially an off-white water extract of whole soybeans containing all its components except some insoluble fibers (okara) removed during processing. Soymilk, a stable emulsion of oil, water and protein has long been reported as an economic protein source and a good economic breast milk substitute from the vegetable origin (Osuji & Ubaonu, 2004). When properly prepared, Okwunodulu et al. (2020a) reported protein content of 4.43%, fat 33.50%, fiber 0.05%, carbohydrate 4.75% and energy 6.39Kcal/g from sprouted TGX923-2E soybean variety boiled with 0.5% sodium bicarbonate. Soymilk composition varies depending on processing conditions and bean variety (Akintunde & Akintunde, 2002; Lakshmanan et al., 2006). Soymilk components decide the physicochemical characteristics as a result of complex interactions between their various colloidal molecules and particles.

Legume sprouting has long been adopted and utilized as a traditional technology to increase nutrient, energy and reduce soybean beany flavor, trypsin inhibitor, phytic acid, flatulence among others which have

undermining soymilk production and utilization (Yang, 2001, Agrahar & Jha, 2014). Sprouted soybeans provide readily consumed and pre-digested soymilk thereby making it a potential option for complementary food (Osuji & Ubbaonu, 2004; Okwunodulu & Akobundu, 2015). Consumption of soymilk produced from sprouted soybean is assumed to provide numerous health benefits such as reducing the risk of cardiovascular diseases and cancer, due to the presence of several health-promoting photochemical with high antioxidant properties (Osuji & Ubbaonu, 2004). Sprouting predigests while plant ash boiling of soybeans tenderizes them for maximum soymilk liquefaction (yields) with better quality due to increased soluble solids evenly dispersed within it. Changes in soluble solids dispersion affect the quality, stability and physical properties of soymilk. Particle size distribution is therefore a useful parameter in soymilk composition (Okwunodulu et al., 2019). Heat treatment decreases the fraction of large particles, increases the fraction of medium sized particles and removes off-flavor.

Boiling with plant ash extends soymilk shelf-life and can substitute the use of conventional sodium bicarbonate which has been found to be detrimental to human health. The conventional use of sodium bicarbonate in food processing causes gastrointestinal upset, nausea, stomach pain, diarrhea and vomiting (Burke & Pyne, 2017). Boiling as a heat treatment of soymilk during and after extraction is necessary to achieve higher soymilk quality, maximize yield as regards TSS and protein recovery (Camacho et al., 1992; Okwunodulu & Abasiokong, 2015). Also, boiling soymilk considerably destroys most of the anti-nutritional factors in soymilk, improves soy protein digestibility, but never reduces effectively phytic acid, which interferes with the availability of calcium (Onuorah et al., 2007). Boiling and sprouting also improve soymilk shelf-life by destroying spoilage microorganisms, improving sensory qualities by minimizing off-flavors and color (Kwok et al., 2002; Okwunodulu & Abasiokong, 2015). Therefore, adequate heat treatment is a balance between inactivation of anti-nutritional factors and satisfactory retention of nutrients. Excessive boiling can cause adverse effects on sensory attributes such as development of browning and cooked flavor, and destruction of essential amino acids and vitamins (Kwok et al., 2002).

Ash from plant materials has been incorporated in human diet over time during which people eat a lot more minerals in the form of ashes and charcoal either intentionally or by chance. Ever since this practice was abandoned, man has become weaker and sicker (Neil, 2012). Mexicans use plant ash in processing of corn, cooking grains (Passy, 2012) and as a tenderizer in cooking hard-to-cook foods like African bread fruit, ukwa meat, and corn. It is also used as a leavening agent, flavor enhancer and emulsifying agent (Baker, 2011; Passy, 2012). Other uses of plant ash have been recognized (Udeotok, 2012). Plant ash makes niacin more available, protein more digestible, and alters the amino acids balance of the protein to increase its quality and nutritional value of soymilk (Passy, 2012). This work aimed at comparing the effects of boiling sprouted and un-sprouted soybeans with plant ash and sodium bicarbonate on the processes and sensory properties of soymilk prepared from them.

2. Materials and Methods

Soybeans were procured from the Onuimo main market in Umungwa Obowo Local Government Area of Imo State. Plant ash was obtained from empty palm bunch procured from a small-scale oil mill in Ogbuebule in Ikwuano Local Government Area, Abia State, Nigeria.

Sample preparation

Soymilk from both sprouted and un-sprouted soybeans was prepared according to Okwunodulu et al. (2017) as shown in figure 1. Previously cleaned and sorted soymilk was steeped (12 h in tap water) and sprouted (72 h) on a clean jute sack spread on the floor covered with black polyethylene. The beans were sprinkled with water regularly as soon as their surfaces were dried during sprouting. The sprouts were boiled separately for 20 min in 30%, 50%, 70% plant ash and 0.5% sodium bicarbonate (NaHCO_3) solution and hand-dehulled after cooling. The hulls and shoots were removed by water floatation leaving cleaned soybean cotyledons that were milled into slurry in Master Chef (China) variable speed kitchen blender with hot water (93°C) in a ratio of 1 kg/2.7 l. The slurry obtained was screened through a double-layered muslin cloth to obtain soymilk extract. The extract was boiled in open pan for 20 min, packaged in coded sterilized retortable transparent bottles with lids, sterilized at 121°C for 5 min, allowed to cool at room temperature and stored in refrigerator for analysis. The same processes were repeated for soymilk from un-sprouted soybean but without sprouting.

Plant ash

Empty palm bunch procured was sun-dried to remove moisture, incinerated in the open air and the ash obtained was sorted to remove un-burnt materials (Okwunodulu et al., 2018). The ash was weighed according to design and dissolved in water to obtain the working concentrations (filtrate) for boiling both sprouted and un-sprouted soybeans.

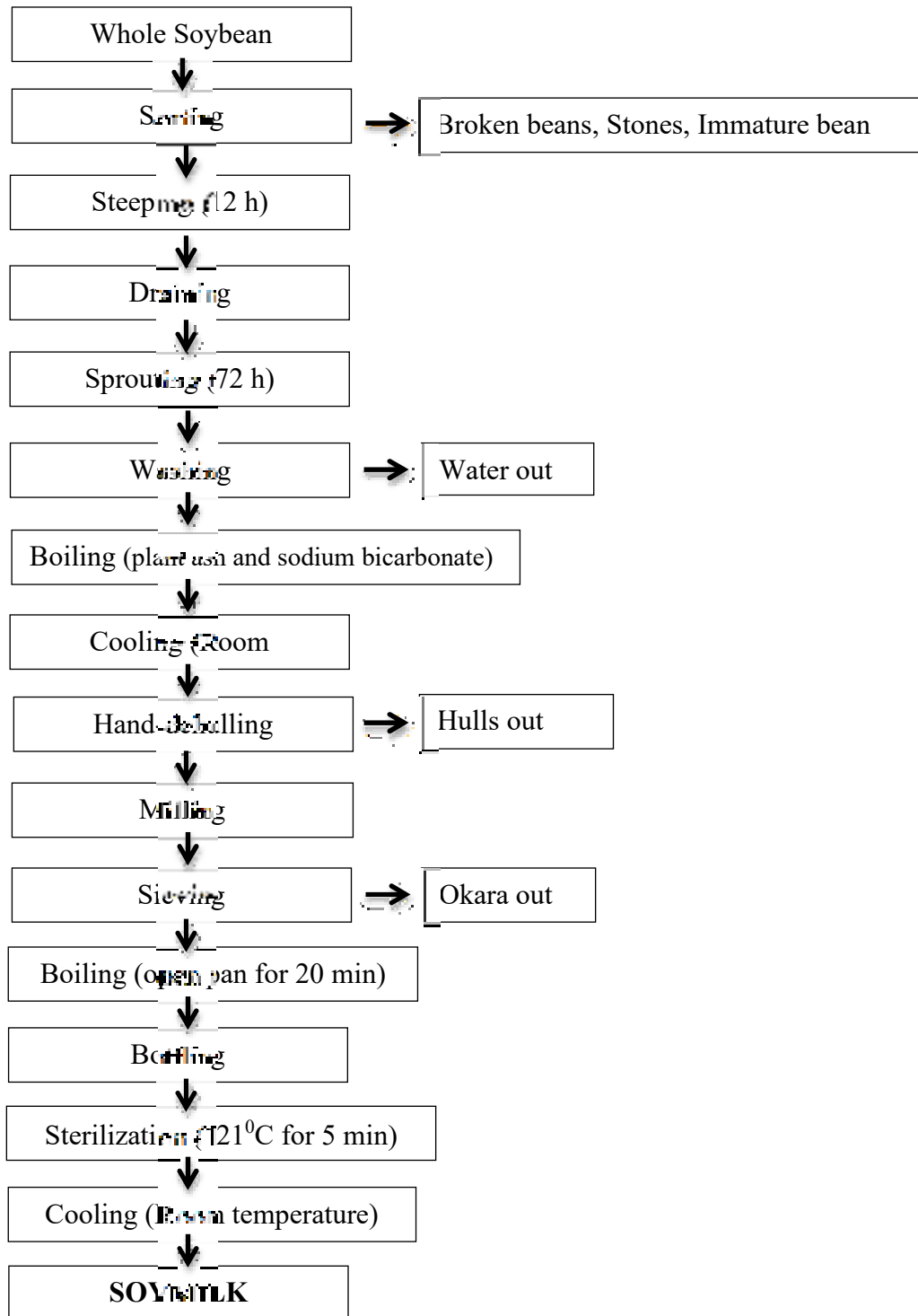


Figure 1. Flow chart for production of soymilk from sprouted whole soybean (Okwunodulu et al., 2017).

Moisture, crude fiber, fat and ash content

Moisture, crude fiber and ash content were analyzed using method described by Onwuka (2018).

Protein content

The Kjeldahl method which involved digestion, distillation and titration as described by James (1995) was used to determine the protein content of soymilk sample. Total nitrogen obtained from titration was multiplied with the factor 6.25 to obtain the crude protein content.

Carbohydrate content

The carbohydrate content of the soymilk sample was calculated using by difference.

Mineral Content

Multiple nutrient extractions by wet-acid digestion method described by Ajai *et al.* (2012) was used to digest the samples used to determine the mineral compositions of the soymilk samples.

Trace elements

These were determined as described by James (1995) using a calibration graph prepared for each element from which their amount in each sample was read. The final computation of the metal concentration was carried out using eq. 1.

$$\text{Metal concentration} = \frac{C \times V}{\text{Weight of sample used}} \quad (\text{eq.1})$$

Note:

C = Concentration of the metal from the sample detected by the AAS; V = Volume of which the solution was diluted

Calcium

Calcium content of the sample digest was carried out by Versanate EDTA complexiometric titration described by James (1995) and calculated using eq. 2.

$$\% \text{ calcium} = \frac{100}{w} \times WE \times N \times \frac{V_f}{V_a} \times T - B \quad (\text{eq.2})$$

Note:

W = weight of sample analyzed; EW = Equivalent weight; N = Normality of EDTA; Va = Volume of extract titrated; Vf = Total volume of extract; T = Titer value of sample; B = Titer value of blank

Phosphorous

Phosphorous in the samples was determined by the molybodoovanadate colorimetric method (James, 1995). Phosphorus content was calculated using eq. 3.

$$\text{Phosphorus mg/100g} = \frac{100}{W} \times \frac{a_u}{a_s} \times C \times \frac{V_t}{V_a} \quad (\text{eq.3})$$

Note:

W = Weight of ash sample; Au = absorbance of test sample; As = Absorbance of standard phosphorus solution; C = Concentration of standard phosphorus solution; Vt = total extract volumes; Va = volume of extract analyzed

Vitamin A (Carotenoid)

The spectrophotometric method by Onwuka (2018) was employed and vitamin A was calculated using eq.4 below.

$$\text{Vitamin A (mg/100g)} = \frac{100}{w} \times \frac{a_u}{a_s} \times c \quad (\text{eq.4})$$

Note:

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

Thiamine (Vitamin B₁)

The method of Onwuka (2018) was adopted and thiamine was calculated using eq.5.

$$\text{Thiamine (mg/100g)} = 100/w \times A_u \times C/A_s \times V_f \times D/V_a \quad (\text{eq.5})$$

Note:

W = Weight of sample; A_u = absorbance of sample; A_s = Absorbance of standard thiamine solution, C = Concentration of standard thiamine solution; V_f = Total volume of filtrate; V_a = Volume of filtrate analyzed
D = Dilution factor where applicable

Vitamin C (*Ascorbic Acid*)

The titration method described by Okwu and Josiah (2006) was used. The concentration of vitamin C was using eq.6 below.

$$\text{Vitamin C (mg/100 g)} = 0.88 \times T \times V_f/V_a \times 100/W \quad (\text{eq.6})$$

Note:

V_f = Total volume of extract; T = Sample titre – blank titre; V_a = Volume of aliquot of extract analyzed; W = weight of sample used

Total soluble solids (TSS)

This was determined using the AOAC (2010) method. By weight difference, the weight of the soluble solids in the samples was determined as a percentage of the sample weight using eq.7.

$$\% \text{ total soluble solids} = (W_2 - W_1) / (\text{Volume of sample}) \times 100 \quad (\text{eq.7})$$

Note:

W₁ = weight of empty dish; W₂ = weight of dish + dried sample

Color

The spectrophotometric method was used which involved placing the sample at 25 to 30°C in a cuvette and absorbance readings were taken at four wave lengths of 460, 550, 620, and 670 nm. The photometric color index was calculated using eq.8 (Shimadzu, 2008).

$$\text{Color} = 1.29 (A_{460}) + 69.7 (A_{550}) + 41.2 (A_{620}) + 56.4 (A_{670}). \quad (\text{eq.8})$$

Sensory evaluation

Sensory evaluation of the soymilk was carried out as described by Iwe (2010) subjectively with 30 semi-trained panelists selected from students of the Michael Okpara University of Agriculture, Umudike. The samples were presented to them coded with similar tumblers in a well-illuminated room at the same time along with bottled water and questionnaires. They were instructed to rinse their mouths before and after testing each sample and rate their appearance, mouth feel and general acceptability on the questionnaires according to 9-point hedonic scale. Where 1 = like extremely, 5 = neither like nor dislike and 9 = dislike extremely.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and the least significant difference test was used to separate the means. Pearson correlation was used to evaluate the influence of pH and viscosity on acceptability (Okwunodulu et al., 2023).

3. Results and Discussion

Proximate composition

The proximate results in table 1 indicated that the dry matter of soymilk from plant ash boiled unsprouted soybean was significantly ($p < 0.05$) higher than their sprouted counterparts and the control (XU=unsprouted soybean boiled without ash). None sprouting which could have hydrolysed the soybeans and absence of ash to tenderize the beans could justify the variation. Therefore, sprouting decreases the dry matter. The dry

matter has a negative correlation with moisture, but positively with nutrients except minerals which implies that the higher the dry matter, the higher the nutrient the lower the moisture content. Therefore, high dry matter will result in lower refreshment of the soymilk from un-sprouted soybeans and may be the reason for slightly higher acceptability scores of soymilks from sprouted than un-sprouted soybeans in Table 1.

The moisture content of all soymilk samples from boiled ash plant sprouted soybeans were significantly ($p < 0.05$) higher than that from their un-sprouted counterparts and the control. Similar higher MC was obtained in soymilk from 0.5% sodium bicarbonate boiled sprouted soybean (Okwunodulu, & Nwabueze, 2019). These increments could stem from sprouting which hydrolyzed the soybean macromolecules thereby reducing the DM unlike in un-sprouted soybean with higher DM. More so, water imbibition during soybean steeping (Nonogaki et al., 2010) may have contributed as well. A similar increase was observed in sprouted chick pea and other legume seeds (Khalil et al., 2007, Khattak et al., 2008).

Table 1. Proximate composition and energy values of the soymilk samples

Sample	Dry mater (%)	Moisture (%)	Crude protein (%)	Crude fiber (%)	Fat (%)	Ash (%)	CHO (%)
XU	13.64 ^c ±0.02	86.37 ^b ±0.02	5.34 ^g ±0.03	0.02 ^a ±0.01	4.50 ^c ±0.22	0.42 ^a ±0.03	3.35 ^c ±0.16
XU ₇₀	14.66 ^b ±0.01	85.34 ^c ±0.01	5.89 ^e ±0.01	0.01 ^a ±0.00	6.05 ^a ±0.04	0.18 ^b ±0.00	2.54 ^e ±0.04
XU ₅₀	13.98 ^c ±0.03	86.02 ^c ±0.03	5.72 ^f ±0.02	0.01 ^a ±0.01	5.22 ^b ±0.01	0.12 ^b ±0.15	3.48 ^c ±0.26
XU ₃₀	14.85 ^{ab} ±0.74	85.65 ^d ±0.03	5.35 ^g ±0.07	0.02 ^a ±0.01	4.00 ^d ±0.00	0.24 ^{ab} ±0.01	4.74 ^a ±0.09
XU _{0.5}	15.34 ^a ±0.01	84.66 ^f ±0.01	5.27 ^h ±0.02	0.03 ^a ±0.00	5.34 ^b ±0.01	0.27 ^{ab} ±0.02	4.45 ^b ±0.01
XS ₇₀	13.62 ^c ±0.02	86.39 ^b ±0.02	8.42 ^a ±0.02	0.02 ^a ±0.00	2.00 ^f ±0.00	0.25 ^{ab} ±0.01	2.93 ^d ±0.06
XS ₅₀	11.60 ^d ±0.02	88.40 ^a ±0.01	8.09 ^b ±0.01	0.03 ^a ±0.01	2.02 ^f ±0.01	0.26 ^{ab} ±0.00	1.21 ^f ±0.02
XS ₃₀	11.67 ^d ±0.05	88.38 ^a ±0.01	7.83 ^c ±0.01	0.02 ^a ±0.01	2.05 ^f ±0.01	0.29 ^{ab} ±0.02	1.43 ^f ±0.04
XS _{0.5}	13.62 ^c ±0.01	86.38 ^b ±0.01	6.38 ^d ±0.02	0.03 ^a ±0.01	2.34 ^e ±0.01	0.17 ^b ±0.19	4.58 ^b ±0.01

Values are means of triplicate determinations ±standard deviation. Means with different superscripts within the same column are significantly different ($p < 0.05$). XU= Soymilk from un-sprouted soybean boiled without ash, XU_{0.5}= Soymilk from un-sprouted soybean boiled with 0.5% sodium bicarbonate, XS_{0.5}= Soymilk produced from sprouted soybeans boiled with 0.5% sodium bicarbonate

There was significant ($p < 0.05$) protein enrichment of all soymilk samples from sprouted soybeans than their un-sprouted counterparts irrespective of boiling medium and the control. This implied that the enrichment was primarily from sprouting which was analogized with the report of (Okwunodulu et al., 2020b) on protein increase of *akamu* paste fortified with sprouted beans. Protein increase could be associated with sprouting which may have hydrolyzed soybean protein to amino acids. Also, synthesis of protein enzymes like proteases (Bau et al., 1997), hydrolysis of soybean anti-nutrients content and protein synthesis during soybean imbibition (Nonogaki et al., 2010) may have contributed too. Protein content decrease in both soymilk samples with decrease in plant ash concentration levels could be aligned to reduced tenderization and hydrolysis of protein. Therefore, boiling soybean with 70% ash may give a higher protein level liable to reduce protein-energy malnutrition when consumed provided soymilk acceptability is not compromised.

There was no significant ($p > 0.05$) effect of the boiling medium on the crude fibre content of all soymilk samples from sprouted and un-sprouted soybeans. Also, boiling with the convectional sodium bicarbonate had no significant ($p > 0.05$) variation. These may probably mean that hydrolysis during sprouting had no significant ($p > 0.5$) effect on crude fibre. This notwithstanding, crude fibre content of soymilk from sprouted and un-sprouted soybean increased slightly with decreased in plant ash concentrations unlike when boiled with sodium bicarbonate. This could be due to reduced tenderization and liquefaction of crude fibre at lower concentrations. Fibre hydrolysis due to plant ash boiling at higher concentration may have more effect than the increasing effect due to sprouting.

There was significant ($p < 0.05$) higher fat content of all soymilk samples from un-sprouted soybeans than their sprouted counterparts and control. Decrease in fat content level by sprouting has been acknowledged (Chung et al., 1998; Okwunodulu & Ukeje, 2018) as well as interactions of fat and calcium (Igyor et al., 2011). The decrease was substantiated by higher fat content of soymilk from un-sprouted soybeans than those from sprouted soybeans. The interaction may have been more in soymilk from sprouted soybean than un-sprouted

and may validate their fat content increase with decreasing ash concentration unlike in un-sprouted counterparts.

Ash is an indication of minerals or inorganic components of food samples which are very much desired for their health benefits (Iheanacho & Udebuani, 2009). There was significant ($p < 0.05$) ash improvement in all soymilk samples from sprouted soybeans than their un-sprouted counterparts. The improvement tallied with El-Adawy et al. (2002) who reported significant increase in ash content of sprouted mung-bean, pea and lentil seeds. Plant ash also contributed to the improvement. But boiling with conventional sodium bicarbonate contradicted ash improvement with sprouting. Significant ($p < 0.05$) and non-significant ($p > 0.05$) increase in ash content of soymilk from un-sprouted and sprouted soybean respectively with decrease in plant ash levels may mean that ash improvement is better at lower concentration levels. Higher ash content of the control than the rest soymilk samples justified oil interaction with calcium (Igyor et al., 2011).

There was significant ($p < 0.05$) higher carbohydrate level of soymilk from un-sprouted soybeans than their sprouted counterparts. Sprouting may have hydrolyzed some of the carbohydrate (Okwunodulu & Ukeje, 2018). This agreed with Harmuth-Hoene et al. (2007) who reported a decrease in dry matter and carbohydrates from sprouted wheat, mung bean and chickpea seeds. Increase in carbohydrate content of soymilk from un-sprouted soybean with decreasing levels of plant ash justified improvement at lower concentration. Decrease in carbohydrate levels of soymilk from sprouted soybeans with decrease in plant ash concentration signified double carbohydrate hydrolyses by sprouting and plant ash. There was no significant ($p > 0.05$) effect of bicarbonate boiling on soymilk carbohydrate, but from sprouted soybean was slightly higher

Vitamin and Mineral

There was more significantly ($p < 0.05$) vitamin B₁ improvement of soymilk from sprouted soybean (10.27-10.05 mg/100g) than un-sprouted (10.25, 10.21-10.15 mg/100g) (Table 2). The improvements were more at higher ash concentration than lower in sprouted soybeans above the control (9.95 mg/100g). Soymilk from bicarbonate boiled sprouted soybean was higher. These are clear indication of lower vitamin B interactions amidst more elaboration by sprouting. Decrease in vitamin B₁ content of soymilk from sprouted and un-sprouted soybeans with decrease in plant ash concentrations could be attributed to reduced soybean hydrolysis and interactions. Therefore, plant ash and bicarbonate boiling improved soymilk vitamin B₁ more in sprouted than un-sprouted soybean. General lower value of vitamin B₁ than A is because it is water soluble with more loss during boiling.

Table 2. Vitamin content of un-sprouted and sprouted soymilk samples

Sample	Vitamin A ($\mu\text{g}/100\text{g}$)	Vitamin B ₁ (mg/100g)	Vitamin C (mg/100g)
XU	41.03 ^a ±0.01	9.95 ^g ±0.01	26.18 ^f ±0.01
XU ₇₀	37.23 ^d ±0.02	10.25 ^{ab} ±0.01	26.48 ^e ±0.01
XU ₅₀	37.12 ^f ±0.02	10.21 ^c ±0.01	26.38 ^e ±0.13
XU ₃₀	37.01 ^h ±0.01	10.15 ^d ±0.01	26.11 ^g ±0.01
XU _{0.5}	39.41 ^c ±0.01	10.00 ^f ±0.01	26.06 ^g ±0.02
XS ₇₀	37.16 ^e ±0.03	10.27 ^a ±0.01	28.12 ^a ±0.03
XS ₅₀	37.07 ^g ±0.01	10.24 ^b ±0.07	27.86 ^b ±0.01
XS ₃₀	36.98 ^h ±0.03	10.05 ^e ±0.01	27.31 ^c ±0.01
XS _{0.5}	40.07 ^b ±0.01	10.03 ^{ef} ±0.01	27.02 ^d ±0.02

Values are means of triplicate determination \pm standard deviation. Means with different superscripts within the same column are significantly different ($p < 0.05$). XU= Soymilk from un-sprouted soybean boiled without ash, ash, XU0.5= Soymilk from un-sprouted soybean boiled with 0.5% sodium bicarbonate, XS0.5= Soymilk from sprouted soybeans boiled with 0.5% sodium bicarbonate.

Same higher vitamin C content of soymilk from sprouted soybeans, decrease with decrease in plant ash concentrations and higher value in bicarbonate boiled sprouted than un-sprouted soybean as in Vitamin B₁ was obtained here. This could be because it is also water soluble. Sprouting which increases vitamin C may have contributed (Fernandez *et al.*, 2005). Higher vitamin C content of all the soymilk samples than the control testified improvement due to sprouting, plant ash and bicarbonate boiling in both sprouted and un-sprouted

soybean. Like other vitamins, vitamin C increased with increase in plant ash concentration in both sprouted and un-sprouted soybeans.

Table 3 presents the mineral results. With increase in plant ash concentration, calcium values of soymilk from sprouted (14.74-8.84 mg/100g) increased significantly ($p < 0.05$) more than their un-sprouted counterparts (12.44-8.23 mg/100g) and control (6.86 mg/100g). Sprouting and plant ash must have been the major source of the increase. Calcium is the second most abundant mineral in empty palm bunch ash (Okwunodulu *et al.*, 2018). Similar calcium increase was observed in bicarbonate boiled sprouted soybean than un-sprouted which also projected sprouting and sodium bicarbonate as primary sources. Besides, significant ($p < 0.05$) calcium increases with increase in plant ash concentration in both sprouted and un-sprouted soybean than the control justified plant ash as calcium contributor. Therefore, boiling sprouted soybean with plant ash will produce soymilk with improved calcium content than un-sprouted soybean.

Like calcium, phosphorous values of soymilk from plant ash boiled sprouted soybean (80.16-71.42 mg/100g) were significantly ($p < 0.05$) higher than those from un-sprouted soybean counterparts (73.66-67.12 mg/100g) and control (49.05 mg/100g). Sprouting and plant ash must have contributed to that. Also, substantiating this is the significant ($p < 0.05$) higher phosphorous content of soymilk from bicarbonate boiled sprouted than un-sprouted soybeans. Increasing phosphorous content in soymilk from sprouted and un-sprouted soybean with increase in plant ash also attested to the discrepancy. Phosphorus helps maintain strong bones, balances the body's pH level and improves digestion (Kanbay *et al.*, 2012). Higher phosphorous content of the entire soymilk content than calcium is in consonant with the report that calcium is the second most abundant mineral in balm bunch ash (Okwunodulu *et al.*, 2018).

Table 3. Mineral composition and calcium to phosphorous ratios of the soymilk samples (mg/100g)

Samples	Calcium	Phosphorus	Fe	Ca: P
XU	6.86 ⁱ ±0.01	49.05 ^g ±0.02	3.370.01	1:7 ^c ±0.02
XU ₇₀	12.44 ^b ±0.05	73.66 ^c ±0.01	5.04 ^b ±0.01	1:6 ^{ab} ±1.01
XU ₅₀	10.09 ^d ±0.01	70.05 ^e ±0.01	4.86 ^d ±0.01	1:7 ^{ab} ±0.01
XU ₃₀	8.23 ^f ±0.02	67.12 ^f ±0.02	4.39 ^f ±0.01	1:8 ^a ±0.02
XU _{0.5}	6.92 ^h ±0.01	46.05 ^h ±0.03	4.05 ^h ±0.02	1:7 ^{ab} ±0.02
XS ₇₀	14.74 ^a ±0.02	80.16 ^a ±0.02	5.41 ^a ±0.01	1:5 ^b ±0.10
XS ₅₀	11.17 ^c ±0.01	75.28 ^b ±1.40	4.95 ^c ±0.01	1:7 ^{ab} ±0.01
XS ₃₀	8.84 ^e ±0.01	71.42 ^d ±0.02	4.47 ^e ±0.01	1:8 ^a ±0.02
XS _{0.5}	7.03 ^g ±0.01	49.26 ^g ±0.03	4.12 ^g ±0.02	1:7 ^{ab} ±0.02

Values are means of triplicate determinations ± standard deviation. Means with different superscript within the same column are significantly different ($p < 0.05$). XU= Soymilk from un-sprouted soybean boiled without ash, XU₇₀= Soymilk from un sprouted soybean boiled with 70% plant ash, XU₅₀= Soymilk from un sprouted soybean boiled with 50% plant ash, XU₃₀= Soymilk from un-sprouted soybean boiled with 30% plant ash and XU_{0.5}= Soymilk from un-sprouted soybean boiled with 0.5% sodium bicarbonate, XS₇₀= Soymilk produced from sprouted soybean boiled with 70% plant ash, XS₅₀= Soymilk produced from sprouted soybean boiled with 50% plant ash, XS₃₀= Soymilk from sprouted soybean boiled with 30% plant ash, XS_{0.5}= Soymilk from sprouted soybean boiled with 0.5% sodium bicarbonate. Ca: P =calcium to phosphorous ratio.

Same trend of results as obtained in calcium and phosphorous was obtained here. Iron values of soymilk from plant ash boiled sprouted soybeans (5.41-4.47 mg/100g) significantly ($p < 0.05$) improved better than their un-sprouted counterparts (5.04-4.39 mg/100g) and control (3.37 mg/100g). Iron increase with increase in plant ash concentration and sodium bicarbonate in both sprouted and un-sprouted soybean attested to their contributions in iron increase. Besides, sprouting may have contributed as well. Least iron values obtained from control also confirmed the significant ($p < 0.05$) iron contribution by plant ash and sprouting. High iron values obtained in this study agreed with the report of Okwunodulu *et al.* (2018) that empty palm bunch ash is an excellent iron source. Therefore, soymilk from this study can be used as strategic food against the prevalence anemia in developing countries where resource constrained poor cannot afford to include animal products in their daily diets. It can be inferred that iron content of soymilk can be significantly ($p < 0.05$) improved by sprouting and boiling soybean with higher concentrations of plant ash provided it does not affect acceptability.

The Ca: P ratios of the soymilk from sprouted (1: 50-1: 8) and un-sprouted (1: 6-1: 8) soybeans increased significantly ($p < 0.05$) with decrease in plant ash concentration in the boiling water extract. This may connote peak ratio at 30% ash concentration as it was higher than the control (1:7) as well. It is worthy to note that the ratios' increasing trends observed, were at variant with the corresponding calcium decrease which also point at 30% ash increase as the peak ash level. However, higher ratios may be possible at less than 30% ash concentrations. Lower Ca: P at higher ash concentrations could be due to more calcium interactions with soybean components (protein and fat). Plant ash is a good source of mineral most especially calcium and phosphorous (Okwunodulu *et al.*, 2018). Boiling sprouted and un-sprouted soybeans with 0.5% sodium bicarbonate did not differ their Ca: Ps (1: 7). The Ca: P obtained in both soymilk samples were within 1.5: 1, 1:1, 1:2 and 2:1 recommended by the nutritionists for bone health and development (Koletzko *et al.*, 2005; Ebeledike *et al.*, 2010). Adequate Ca: P is associated with infant bone growth and development (Sax, 2001) and a determining factor for calcium absorption and retention (Bass & Chan, 2006). Abnormal and low Ca: P leads to increased risk of bone fracture and rickets in children, and osteoporosis in adults, (Abrams & Atkinson, 2003). Low bone density is another consequence (Sax, 2001). Therefore, the Ca: Ps improvement is a welcome development in this study and may be improved more by decreasing the plant ash concentration. Conclusively therefore, consumption of the milk samples over time may be a prophylactic measure against the risk of bone fracture, rickets and osteoporosis.

Total soluble solids (TSS)

The results in table 4 showed that soymilk samples from plant ash boiled sprouted soybeans had significant ($p < 0.05$) higher TSS (15.45-15.28%) than their un-sprouted counterparts (15.28-14.88%) which may be attributed majorly to sprouting (Okwunodulu *et al.*, 2015). Significant ($p < 0.05$) higher TSS of soymilk from bicarbonate boiled sprouted soybeans than un-sprouted and the least TSS value of the control sample confirmed the positive correlation of sprouting and plant ash boiling to TSS increase.

Table 4. Physicochemical properties of the soymilk samples

Samples	TSS (%)	pH	Viscosity (MPa)	Appearance
XU	14.11 ^f ±0.01	6.13 ^a ±0.01	186.15 ^a ±0.07	0.29 ^a ±0.00
XU ₇₀	15.28 ^b ±0.01	4.38 ⁱ ±0.01	147.30 ⁱ ±0.00	0.15 ^e ±0.01
XU ₅₀	15.03 ^c ±0.01	4.68 ^h ±0.01	153.75 ^h ±0.07	0.21 ^d ±0.01
XU ₃₀	14.88 ^d ±0.02	4.91 ^g ±0.01	162.35 ^g ±0.07	0.26 ^b ±0.01
XU _{0.5}	14.06 ^g ±0.03	5.86 ^c ±0.01	185.00 ^b ±0.00	0.28 ^a ±0.00
XS ₇₀	15.45 ^a ±0.02	5.13 ^f ±0.01	171.15 ^f ±0.07	0.11 ^f ±0.01
XS ₅₀	15.28 ^b ±0.01	5.34 ^e ±0.00	179.45 ^e ±0.07	0.17 ^e ±0.01
XS ₃₀	15.42 ^a ±0.02	5.61 ^d ±0.01	180.05 ^d ±0.07	0.20 ^d ±0.01
XS _{0.5}	14.23 ^e ±0.02	6.03 ^b ±0.01	184.15 ^c ±0.07	0.23 ^c ±0.00

Values are mean of triplicate determinations ±standard deviation. Means with different superscripts within the same column are significantly different ($p < 0.05$). XU= Soymilk produced from un-sprouted soybean boiled without ash, plant ash, XU_{0.5} = Soymilk produced from un-sprouted soybean boiled with 0.5% sodium bicarbonate, 30% plant ash, XS_{0.5} = Soymilk produced from sprouted soybeans boiled with 0.5% sodium bicarbonate.

pH

The pH of soymilk samples from plant ash boiled sprouted soybean (5.13-5.61) was significantly ($p < 0.05$) higher than their un-sprouted counterparts (4.38-4.91). Sprouting may have increased the pH. Similar higher pH was observed in soymilk from boiled bicarbonate sprouted than un-sprouted soybean. Higher pH value of control than the rest soymilk samples may imply that plant ash boiling reduced pH significantly ($0 < 0.05$). This may confirm the reason for pH decrease with increase in plant ash concentration in soymilk from sprouted and un-sprouted soybeans. Furthermore, it could be inferred that pH is maximized at lower ash concentration (>30%). Conversely, bicarbonate boiling significantly ($p < 0.05$) increased the pH as evident in higher pH value of soymilk from bicarbonate boiled sprouted soybean than un-sprouted counterpart.

Viscosity

Significant ($p < 0.05$) higher viscosity values of plant boiled ash sprouted soybean (171.15-180.05 Mpa) than their un-sprouted counterparts (147.30-162.35 Mpa) could be traced to sprouting which increases TSS. The TSS is a function of viscosity (Okwunodulu & Abasiokong, 2015). Besides, the difference confirmed maximum viscosity at lower plant ash levels ($>30\%$). Significant ($p < 0.05$) higher viscosity of boiled bicarbonate un-sprouted than sprouted soybeans could stem from larger particle sizes of the soymilk solids due to absence of sprouting. Plant cold ash be a better tenderizer as shown in the higher TSS of the sprouted than un-sprouted soybeans. This could also support the significant ($p < 0.05$) higher viscosity of the control soymilk than the rest. Higher viscosity correlates with good mouth feel consistency, and ambient stability (Okwunodulu & Abasiokong, 2015)

Appearance

The appearance of soymilk from plant ash boiled un-sprouted soybeans (0.15-0.26) was significantly ($p < 0.05$) higher than their sprouted counterparts (0.11-0.20). Similar results were obtained from soymilk prepared from bicarbonate boiled sprouted and un-sprouted soybeans. Sprouting could be the major cause of discrepancy as un-sprouted soybean results in relatively larger solid particles liable to absorb more light than smaller solid particles from sprouted soybean. This could also explain the higher appearance value of the control than the rest samples. Appearance is a function of TSS which itself increases with sprouting and plant ash concentrations. This therefore, substantiated the significant ($p < 0.05$) increase in appearance of soymilk from sprouted and un-sprouted soybean with increase in plant ash concentrations.

Correlation of pH and viscosity with acceptability for both soymilk samples

Table 5 presents the correlation results for soymilk from un-sprouted soybeans. Here, the pH of soymilk has a positive correlation with viscosity at the 0.01 level (2-tailed). This implies that with pH increase, the viscosity increases as well. This is analogized with the assertion that low pH precipitate and sediment the soymilk components thereby lowering the viscosity (Okwunodulu & Okwunodulu, 2016). Conversely, the pH is negatively correlated with acceptability which connotes precipitation of soymilk components at low pH often associated with low acceptability or rejection.

Table 5. Correlation of pH, viscosity with acceptability for soymilk from un-sprouted soybeans

		pH	Viscosity	Acceptability
pH	Pearson Correlation	1		
	Sig. (2-tailed)			
	N	5		
Viscosity	Pearson Correlation	.992**	1	
	Sig. (2-tailed)	.001		
	N	5	5	
Acceptability	Pearson Correlation	-.165	-.103	1
	Sig. (2-tailed)	.790	.869	
	N	5	5	5

** . Correlation is significant at the 0.01 level (2-tailed).

Table 6 presents the correlation results for soymilk from sprouted soybeans. Just like in soymilk from un-sprouted soybean, the correlation of pH and viscosity with acceptability is positive but not significant at the 0.01 level (2-tailed). This is an indication that the pH did not correlate as significantly as in soymilk from un-sprouted with viscosity and therefore will not precipitate and sediment soymilk components, but rather the soymilk will have an even distribution of total solids. This will increase acceptability and will increase the visible coagulation time thereby increasing the ambient storage stability unlike in soymilk from un-sprouted

soybean (Okwunodulu & Okwunodulu, 2016). Therefore, soymilk from sprouted soybean has an edge over that from un-sprouted soybean as regards acceptability and ambient storage stability.

Table 6. Correlation of pH, viscosity with acceptability of soymilk from sprouted soybeans

		pH	Viscosity	Acceptability
pH	Pearson Correlation	1		
	Sig. (2-tailed)			
	N	4		
Viscosity	Pearson Correlation	.899	1	
	Sig. (2-tailed)	.101		
	N	4	4	
Acceptability	Pearson Correlation	.779	.613	1
	Sig. (2-tailed)	.221	.387	
	N	4	4	4

Sensory evaluation

From table 7, the appearance of soymilk samples from plant ash boiled un-sprouted soybean (6.00-6.95) was higher than their sprouted counterparts (5.00-6.40). This could be attributed to larger macromolecules associated with soymilk from un-sprouted compared to sprouted soybeans. This is in consonant with the results obtained in Table 4. Similar results were obtained from bicarbonate boiled sprouted and un-sprouted soybean. Therefore, sprouting and lower plant ash levels (30%) maximize soymilk appearance which in turn will enhance acceptability better. Both may also explain the increasing appearance trend with decreasing in plant ash concentrations in soymilk from sprouted and un-sprouted soybeans. Colour of the ash at higher concentrations may have contributed in lowering soymilk appearance ratings.

The flavors of the entire soymilk samples were rated higher with decrease in plant ash concentrations, but more in sprouted (5.00-6.30) than un-sprouted (4.85-5.65) soybeans and the control (4.85). Slight variation was observed in bicarbonate boiled soymilk samples. Sprouting and plant ash level also positively influenced soymilk in both soymilk samples. Hence, the least flavor value of the control.

Same trend of results as obtained in flavor was observed here. Therefore, sprouting and lower ash concentration levels positively influence the soymilk mouth feel. Though there was no significant ($p > 0.05$) variation between the entire soymilk samples irrespective of boiling medium and control, slightly higher ratings of general acceptability were observed in sprouted than un-sprouted. Same decreasing trend with decrease in ash concentration was also observed. In all, soymilk from 30% plant ash boiled soybean was liked slightly (6.45), while 70% plant ash boiled un-sprouted soymilk was neither like nor dislike (4.90).

Table 7. Sensory evaluation of the soymilk samples

Samples	Appearance	Flavor	Mouthfeel	General acceptability
XU	6.00 ^{ab} ±1.52	4.85 ^b ±2.28	4.70 ^c ±1.87	5.00 ^a ±2.43
XU ₇₀	6.00 ^{ab} ±1.59	4.85 ^b ±1.69	5.05 ^{bc} ±1.64	4.90 ^a ±1.97
XU ₅₀	6.35 ^a ±1.39	5.65 ^{ab} ±1.50	5.60 ^{abc} ±1.64	5.95 ^a ±1.54
XU ₃₀	6.95 ^{ab} ±1.76	5.25 ^{ab} ±1.71	5.65 ^{abc} ±1.76	5.85 ^a ±1.57
XU _{0.5}	6.25 ^{ab} ±1.81	5.35 ^{ab} ±1.95	5.15 ^{bc} ±1.60	5.60 ^a ±2.04
XS ₇₀	5.00 ^b ±2.08	5.00 ^{ab} ±1.94	5.50 ^{abc} ±1.91	5.50 ^a ±2.04
XS ₅₀	5.60 ^{ab} ±2.14	5.00 ^{ab} ±2.22	5.40 ^{abc} ±1.88	5.40 ^a ±2.06
XS ₃₀	6.40 ^a ±1.64	6.30 ^a ±1.72	6.45 ^a ±1.64	6.30 ^a ±1.69
XS _{0.5}	6.10 ^{ab} ±1.71	5.50 ^{ab} ±2.09	6.20 ^{ab} ±1.82	6.15 ^a ±1.72

Values are mean values of triplicate determinations ± standard deviation. Means with different superscript within the same column are significantly different ($p < 0.05$). XU= Soymilk from un-sprouted soybean boiled without ash, XU₇₀= Soymilk

from un-sprouted soybean boiled with 70% plant ash, XU₅₀= Soymilk from un-sprouted soybean boiled with 50% plant ash, XU₃₀= Soymilk from un-sprouted soybean boiled with 30% plant ash and XU_{0.5}= Soymilk from un-sprouted soybean boiled with 0.5% sodium bicarbonate. XS₇₀= Soymilk from sprouted soybean boiled with 70% plant ash, XS₅₀= Soymilk from sprouted soybean boiled with 50% plant ash, XS₃₀= Soymilk from sprouted soybean boiled with 30% plant ash and XS_{0.5}= Soymilk from sprouted soybean boiled with 0.5% sodium bicarbonate.

4. Conclusion

Soymilk samples from sprouted soybean boiled in 30% plant ash water extract had better nutrient improvement than those from un-sprouted counterparts, but with same calcium to phosphorous ratio levels. Boiling with 30% plant ash and 0.5% sodium bicarbonate gave best accepted soymilk without significant variation. Conversely, sodium bicarbonate had been reported to cause gastrointestinal (GI) upset to some allergic people with such symptoms as nausea, stomach diarrhea, and vomiting. In this regard, soymilk from 30% plant ash boiled sprouted soybeans had better sensory properties with enhanced color, flavor and mouth feel. Though the acceptability level was rated liked slightly, higher rating may be achieved at lower plant ash level (>30%). Conclusively therefore, plant ash could be used to replace the conventional sodium bicarbonate as it is readily available, inexpensive, underutilized, nutrient dense and could be used as food fortification in food processing.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

Okwunodulu, Nwazulu Innocent conceived the idea, designed, monitored and finally proof read the work. Nwagwu, Okwudiri Francis prepared the samples, collected and analysed the results, and drafted the initial manuscript. Okwunodulu, Uchechukwu Felicia assisted in data collation, laboratory and statistical analysis while Iwe, Ofor Maduebibisi reviewed and edited the manuscript. All authors read and approved the final manuscript.

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