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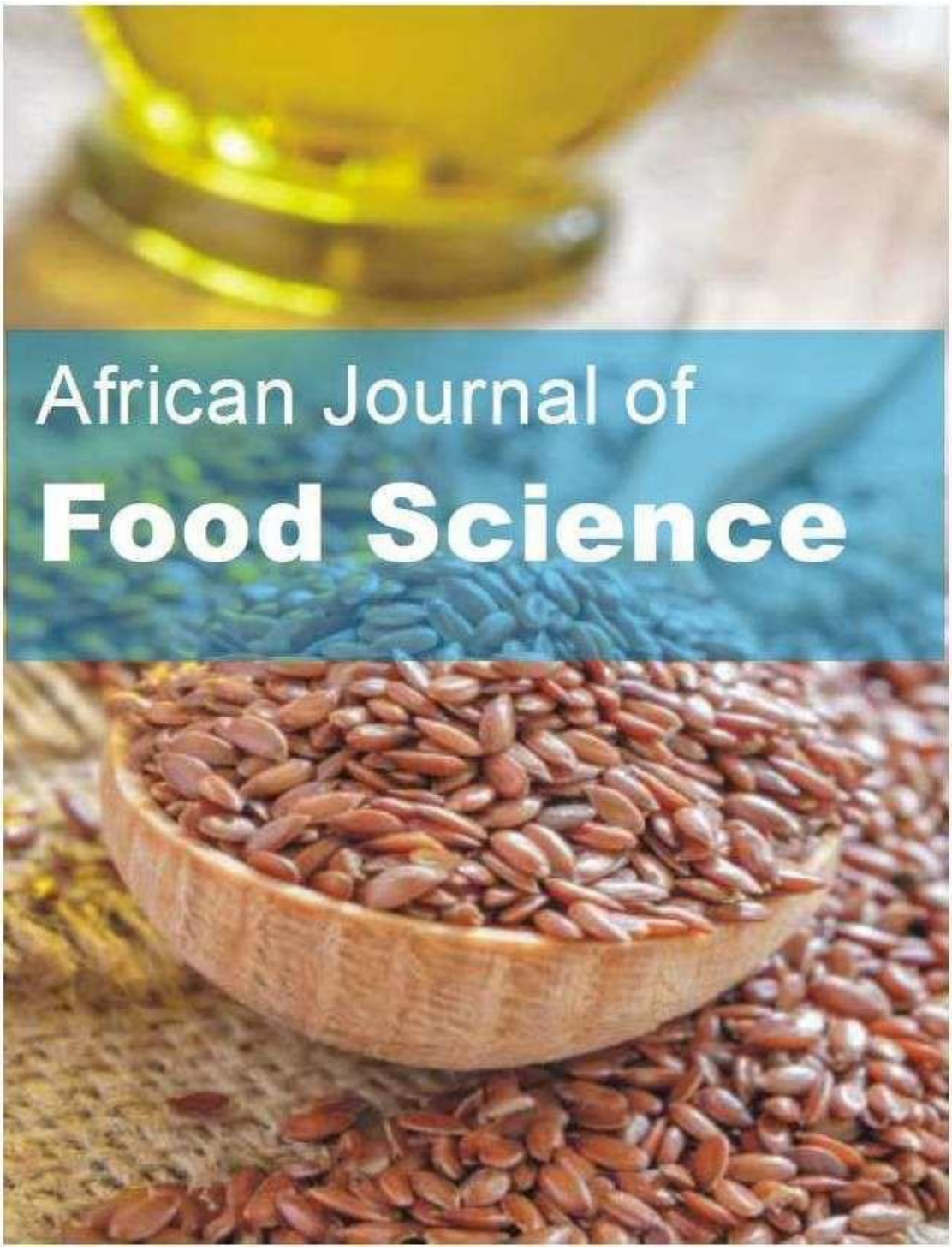

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African Journal of Food Science

Full Length Research Paper

Effects of sweetpotato (*Ipomoea batatas*) puree on bread shelf-life

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Bioactive compounds in sweet potatoes of different flesh colors possessed potential antioxidant and antimicrobial properties important for human health and food quality. The objective of this study was to determine the levels of bioactive compounds and their inhibitory effects on mold fungi in bread. Composite bread was made by replacing 10, 20, 30 and 40% wheat flour with puree from the sweet potato of different flesh colors. The loaves of bread were incubated over 8-days and analyzed for the fungi load. Physiochemical characteristics: Proximate composition, specific sugars, composites of carotenoids, flavonoids, phenols, and antioxidant activity, as well as ascorbic acid, were analyzed. Orange Fleshed Sweetpotato (OFSP) puree bread, at all levels of wheat flour substitution and Purple Fleshed Sweetpotato (PFSP) puree bread at 10 and 20% wheat flour substitution showed increased shelf-life up to six days, compared to 100% wheat bread that staled at day 3. Microbial inhibition properties exhibited by OFSP and PFSP are attributed to their respective phytochemicals and antioxidant activity of 303.38 and 32.29 mgTE/100 g. Yellow Fleshed Sweetpotato (YFSP) puree bread stayed up to day 4 with antioxidant activity of 64.456 mgTE/100 g whereas White Fleshed Sweetpotato (WFSP) and Cream Fleshed Sweetpotato (CFSP) puree breads had significantly lower ($p<0.05$) levels of phytochemical contents limiting their inhibition.

Key words: Phytochemicals, inhibition of fungi, antioxidant activity, sweet potato puree.

INTRODUCTION

Globally, sweet potato is positioned seventh among the major food crop in the world. As a root crop, it is

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important in contributing phytochemicals and energy to the human diet (Abong et al., 2020). There is evidence of increased production and consumption of sweet potatoes in Kenya because of its health benefits, this has led to sweet potato gradually shifting from a subsistence to a commercial crop (Makini et al., 2018). In Kenya, farmers and consumers have traditionally grown and consumed WFSP, CFSP, and YFSP variety that are starchy and have high dry matter content. There has been a steady increase in the production, trade, and consumption of OFSP and PFSP. PFSP is an important source of minerals, dietary fiber, and vitamins, with its major bioactive substances being anthocyanin and phenolic contents (Li et al., 2019).

OFSP is an excellent source of provitamin, a carotenoids, which is recommended for fighting Vitamin A Deficiency (VAD) that affected mostly children under five years of age, pregnant and lactating mothers in Sub-Saharan Africa (Wanjuu et al., 2018). According to Alam et al. (2016), 19.31-61.39 µg/g of beta-carotene content is found in OFSP roots as compared to 1.02-3.78 and 3.28-5.64 µg/g in white, cream, and yellow-fleshed sweet potato roots, respectively. This showed that OFSP provided very high levels of beta-carotene (carotenoids), that have a strong antioxidant capacity to scavenge free radicals because of their conjugated double bonds (Tang et al., 2015). Latest advances in baked and fried products have enabled the incorporation of OFSP purees as an ingredient to produce high-quality bread in terms of sensory acceptance and physiochemical quality (Owade et al., 2018). Wheat substitution through the incorporation of up to 30%-50% OFSP puree has led to reduced production costs and increased consumer demand (Wanjuu et al., 2018).

One of the challenges consumer and pastry industry face is major economic losses due to fungal spoilage of the bread (Melikoglu et al., 2013). Fungi can produce mycotoxins which in turn causes food spoilage and unpleasant flavors. Hence spoilt bread can pose a major health risk to consumer (Axel et al., 2017). Parameters in food such as water activity (a_w), and pH, determines the microorganisms likely to cause spoilage (Debonne et al., 2018). Baked products with a water activity (a_w) of 0.75-0.95 (Wanjuu et al., 2018) and pH greater than 5.3 (Saranraj and Sivasakthivelan, 2015) allows for *Penicillium* and *Aspergillus* species to become the most dominant food spoilage species (Science and Avenue, 2019). Physical methods like ultraviolet light, infrared radiation, microwave heating, or ultra-high-pressure treatments can destroy post-baking contaminants in bread.

In light of the developing consumer trend towards a healthier lifestyle, studies have increasingly set targets to replace traditionally used chemical preservatives with natural or controlled microbial and/or their antimicrobial compounds. Bio-preservation can also be applied to active plant ingredients or plant extracts (Axel et al., 2017). Various chemical groups of phytochemical compounds discovered till date include carotenoids, flavonoids, phytosterols, alkaloids, saponins, organic acids, proteases inhibitors, phenolic compounds, tocols, glucosinolates, chlorophyll, terpenoids as well as essential oils (Makhuvele et al., 2020). They may act directly or indirectly against pathogens due to their antimicrobial, anti-mutagenic, anti-genotoxic, anti-carcinogenic, anti-proliferative, antioxidant, and anti-inflammatory properties (Makhuvele et al., 2020).

The use of essential oils has gained popularity as an alternative to synthetic antimicrobials additive in the food industry. There are very few reports on the direct use of essential oils in dough systems of bread making, as part of natural antifungal preservatives, a typical example is the use of micro-encapsulated rosemary essential oil (made through spray-drying, using modified starch and maltodextrin as coating materials) to protect against the loss of antifungal activity at very high baking temperatures (Tyagi et al., 2012). The results showed that *Aspergillus* spp. were slightly more sensitive to the action of rosemary essential oil. Moreover, the gradual release of active constituents based on the encapsulation results in a longer shelf-life extension with reduced yeast and mold counts as of 15 days of storage (Debonne et al., 2018). Since there is an increasing demand for clean-label food products, the development of natural antimicrobial compounds will be of importance in contributing to such foods product. The objectives of this study were to deduce and quantify the major phytochemical components, responsible for the antimicrobial property of the respective purees, which influenced longevity of the respective bread' shelf life.

MATERIALS AND METHODS

Source of sweet potato root samples

Sweet potato storage roots with varying flesh colors white, cream, yellow, and orange (*White, Cream, Yellow, Kabode+Vitaa* species), respectively were harvested at full maturity. Approximately, 20 kg of each variety was randomly picked from a pool of five batches at Homabay County in Western Kenya and immediately transported to the International Potato Centre's Food and Nutritional Evaluation Laboratory (FANEL), in Nairobi. Purple-fleshed sweet potato (*Diedi*) was sourced from International Potato Centre (CIP) Ghana, since it is not widely grown in Kenya. The samples were cleaned and stored at 25°C awaiting analysis. The puree was prepared, vacuum packed, and stored at 5°C until it was needed for bread baking. The loaves of bread were made at the Food and Nutritional Evaluation Laboratory Kenya and International Potato Centre (CIP) Ghana. Wheat flour and ingredients used in bread making were purchased at a local supermarket. All chemicals were analytical-grade reagents.

Study design

This study employed an experimental design where orange, purple, yellow, white, and cream-fleshed sweet potatoes were prepared

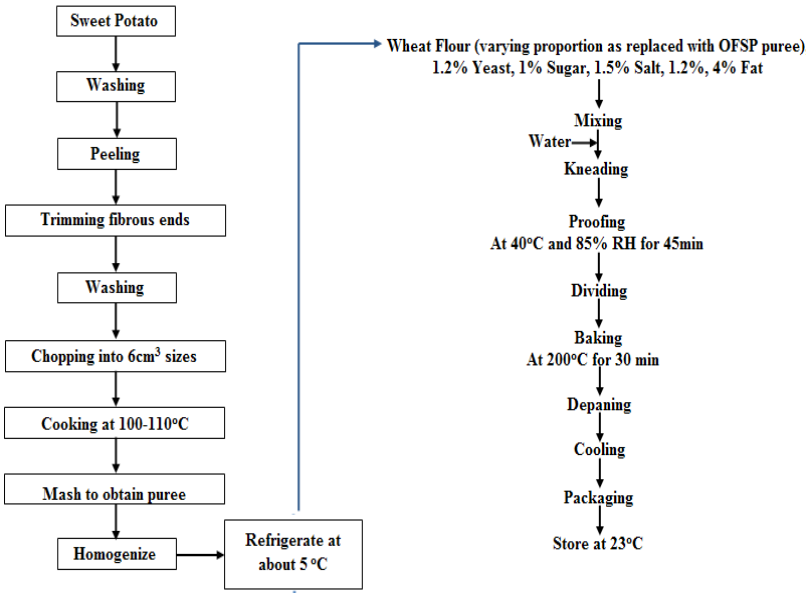

Figure 1. Flow diagram for puree formulation and bread making. Adopted from Muzhingi et al. (2018).

Table 1. Formulation of wheat and puree bread substitutions.

Ingredient	Percentage puree wheat substitution				
	Wheat	10%	20%	30%	40%
Flour	2500	2250	2000	1750	1500
Sugar	125	75	75	75	75
Salt	25	25	25	25	25
Fat	100	75	75	75	75
Yeast	37.5	50	50	50	50
Bread improver	0	7.5	7.5	7.5	7.5
Puree	0	250	500	750	1000

Source: Muzhingi et al. (2018).

into a puree. Bread making with sweet potato puree of wheat substitution at various levels was done followed by microbial analysis for all loaves of bread.

The design involved the use of independent variables (orange, yellow, white, cream, and purple-fleshed sweet potato) and the dependent variables such as mold count, proximate composition, and phytochemical composition (carotenoids, antioxidant activity, anthocyanin, sugars, ascorbic acid, flavonoids, and phenolics.

Sample preparation

Sweet potato puree preparation

Process operations for preparation of sweet potatoes puree as shown in Figure 1 involved reception of the raw material, weighing, and washing using running tap water to remove any soil. Sorting was also carried out where the unhealthy and bruised substrate was separated from the batch. Cleaned sweet potato roots, approximately 20 kg of each flesh color were peeled with sharpened kitchen knives. The peeled sweet potatoes were cubed into 6.0cm blocks before being steamed for 30-45 min at 100 - 110°C in a stainless-steel convection oven (MBM BRESCELLO ITALY CHEFMATE, model: CME6, 9500W, 400V), equipped with a stainless steel base. The steamed sweet potato was cooled, mashed, and homogenized, before being vacuum packaged (MINIPACK-TORRE S.p. A MVS 45X, ANNO-2015-Euro ingredients, Italy) into 300micron polythene bags and stored at 5°C in the refrigerator, readied for used in bread formulation.

The process of bread making

Composite bread with varying substitution levels of sweet potato puree was prepared according to Muzhingi et al. (2018) as observed in Table 1. The control bread was made solely of wheat flour. The ingredients were added as a proportion of the total quantity of wheat flour + sweet potato puree as seen in Figure 1. The ingredients were weighed and mixed in a mixer as the water was continuously added to achieve the desired dough consistency. The dough was further kneaded to ensure proper mixing, cut, and weighed in 450 g portions. The dough portions were moulded into rolls and placed into greased baking tins before being loaded in a proofing cabinet for 30 to 45 min to allow it to rise to a desirable

volume. The rolled dough was baked at 200°C for 30 min through heat transfer (Anishaparvin et al., 2010). The loaves were de-panned, cooled to room temperature then packaged in polythene bags followed by storing at 23 to 25°C before being assayed for microbial composition.

Microbial analysis

Mold count

The packaged breads were stored at 23 - 25°C in a clean, well-ventilated, secure store for over 8 days to establish the shelf-life. After every 48 h, a 25g sample of each bread was homogenized in buffered peptone water, and a 1ml aliquot was used to prepare a serial dilution up to a 10^{-6} dilution, according to AOAC (2012). From each dilution, 1ml was cultured onto Potato Dextrose Agar using the pour plate technique. The plates were incubated at 30°C for 72 h. After the incubation period, plates were observed for fungal growth as illustrated by Mpamah et al. (2019). A colony counter machine was used in counting the microbial colonies and the results presented as a mean base of 10 logarithms of colony forming units per g (log_{10} CFU/g).

Proximate analyses

Moisture content determination

A standard operating procedure for moisture (934.01) was used (International, 1990). Two grams of the sample were weighed in triplicate into a pre-weighed empty container (W_2) as well as weight of dried sample (W_3) was recorded. The container with the sample was placed in the air oven preheated to 135°C ±2 for 2 h ±5 min for a constant weight to be achieved. The container with the dried sample was placed into a desiccator to cool for 30 min and weighed (W_3). The dried sample's weight was provided by W_2-W_3.

$$\text{Total moisture } \% = \frac{(W2 - W3)}{W2} x100 \quad (1)$$

Where, W_2 = Weight of wet sample and W_3 = Weight of dried sample.

Protein content determination

The Kjeldahl method as described in International (1990) was used. 0.5 g of sample was weighed in a digestion tube. A Kjeldahl tablet and 10 ml concentrated sulfuric acid was added for digestion until the solution was clear The solution was taken to a distillation unit with 50% sodium hydroxide for the release of ammonia. The distilled solution was collected in the receiver with 50 ml of 4% boric acid with an indicator. The distillate was titrated with standardized 0.1 N hydrochloric acid until the first appearance of purple color. The volume of acid used was recorded to the nearest 0.05 ml.

N (g %) = (ml 0.1 N HCl sample - ml 0.1 N HCl blank) × N HCl ×1.4007 (2)

Protein (g per 100 g) = % total nitrogen × 6.25

Crude fat determination

A procedure of 920.39 from International (1990) was used. The sample was weighed in triplicate, 2 g each (W_1) and 1 g of Celite 545 added to it. Exactly 70 ml of 4 N HCl was added to the sample before connecting the tube to a hydrolysis unit at (170°C, 60 min). The digestion mixture was filtered and the residue was washed with warm water. The Whatman filter paper No. 4 used, was dried in an oven overnight and then transferred into an extraction thimble in the reservoir part of the Soxhlet apparatus. The extraction cups were dried in a 100°C oven for 1 h, cooled in a desiccator, and weighed (W_2). Solvent extraction was achieved by 60 ml petroleum ether which was added to the pre-weighed extraction cups. The extraction cups were then placed into the fat extraction system, thereafter dried in a 100°C oven for 30 min, cooled in a desiccator, and weighed (W_3) to obtain the crude fat.

$$\text{Total fat } (\frac{\text{g}}{100\text{ g}}) = \frac{(W3 - W2)}{W1} x100$$

Where, W_1 = Weight of sample, W_2 = Weight of dried extraction cup before fat extraction, and W_3 = Weight of dried extraction cup after fat extraction

Crude fiber determination

A procedure for fiber (978.10) was used (International, 1990). A sample of 2 g was weighed into a graduated 600 ml beaker ,boiling distilled water was added, as well as 25 ml 1.25 sulphuric acid solution and made up to 200 ml. The contents were filtered, then rinsed again with boiling distilled water plus 25 ml of 1.25 N sodium hydroxide solution. Filtration was done with the glass wool as well as washing off with ethanol followed before drying in an oven,then ignition at 550°C to constant weight. The item was cooled and weighed to obtain the fiber content.

Ash determination

A procedure for ash (942.05) was employed (International, 1990). Two grams of the sample were weighed in triplicate into a pre-weighed crucible dish. The crucibles with the samples were placed on a hot plate and charred gradually until smoking ceased. The charred samples were incinerated in a furnace at 550°C for 6 h. The samples were removed and cooled down in the desiccator and then weighed and the difference obtained gave the ash content.

Carbohydrate and energy determination

The difference from the other nutritional content gave the carbohydrate value.

Carbohydrate(mg/100 g) = 100 - (protein + fat + moisture + fibre + ash) mg/100 g (4)

Energy content was calculated as reported by Nakalembe et al. (2015).

Energy value (kcal/100 g) = (2.62 × %protein) + (8.37 × %fat) + (4.2 × % carbohydrate) (5)

Determination of specific sugars

Extraction of sugars was done according to the method by Montesano et al. (2016). The precise weight of 2 g of the puree sample was weighed into a falcon tube. One gram of $CaCO_3$ was added to neutralize the sample. Then 10 ml 85% ethanol was added to the sample, capped, and placed in a water bath for 1 h at 85°C. The samples were centrifuged at 100.62 G force for 5 min and the supernatant was filtered through Whatman filter paper No. 4 into 50 ml falcon tubes. The extraction was repeated with 5 ml

boiling 85% ethanol and returned into the water bath for 30 min then centrifuged and filtered, and finally topped up to 10 ml of the 85% ethanol before being transferred into vials for analysis. ACN:H_2O: EtOH in ratio 82:17:1 was used as mobile phase with Luna -NH_2 sugars column for separation of eluates on Shimadzu LC, ELSD-LTΠ (low-temperature evaporative light scattering) detector. The program ran for 10 min at 30°C at 1.2 ml/min.

Ascorbic acid determination

Exactly, 2 g of the sample was weighed in a Falcon tube and 10 ml of 3% metaphosphoric acid was added. The sample was homogenized and sonicated in an ultrasonic bath for 5 min. Vortexing was done for 2 min and centrifuged for 10 min at 100.62 G force followed by filtration using Whatman No. 4 filter paper in separate clean falcon tubes. The extraction was repeated twice with 5 ml of 3% metaphosphoric acid and topped up to the mark with the acid. Then 1 ml was transferred into vials before loading in the HPLC Waters 2695 separation module and 2996 PDA Detector Reverse phase gradient HPLC method 50 mm × 4.6 mm, c30 5.0 µm particle size HPLC column (Waters Corporation). Injection volume was 30µl and the mobile phase used was 0.3 mM potassium dihydrogen phosphate in 0.35% (v/v) ortho- phosphoric acid at flowrate: 0.8 ml/min and 10 min runtime. Using the retention time for ascorbic acid and/or coeluted added reference ascorbic acid, at λ = 243 nm (Gazdik et al., 2008).

Extraction of phenolic compounds, flavonoids, and antioxidants

Exactly, 0.5 g of the sample was weighed into clean propylene tubes. A colorimetric assay with a microtiter plate reader was adopted. Approximately, 10 ml of 80% methanol was added followed by mixing on a vortex mixer (SI-0166, USA), and agitated for 24 h at 25°C in a mechanical shaker (Innova 43, USA). The samples were then centrifuged at 170.0478 G force for 10 min and the supernatant was aliquoted for determination of the total phenolic contents, total flavonoids, and DPPH scavenging activity.

Determination of antioxidant activity

Exactly, 50 µl of the blank, standards (5-50 µg/ml Trolox) and samples were pipetted into their respective wells in a microtiter plate followed by the addition of 50 µl of 0.002% 2, 2 diphenyl-1-picrylhydrazyl (DPPH) with gentle mixing by priming. Absorbance reading was done at 515 nm in a microtiter plate spectrophotometer reader (Synergy HT, USA) after 15 min. The calibration curve of Trolox used as a standard was used to calculate the concentration of total antioxidant activity in mg per 100 g of the fresh sample converted into dry weight basis and expressed as mg of Trolox Equivalent (mg TE). The total antioxidant activity of sweet potato roots was determined using the DPPH procedure and the results were expressed as Trolox equivalent. A concentration of 0.002% DPPH (Shalaby and Shanab, 2013) was adopted with modification.

Determination of total phenolic compounds

The total phenolic content was determined using a modified Folin-Ciocalteu procedure according to Baba and Malik (2015). Twenty microliters of the sample blank solution (80% methanol), gallic acid standards (10-80 µg/ml), and samples were pipetted into their respective wells in a microtiter plate followed by the addition of 100 µl of 10% Folin-Ciocalteu phenol reagent with gentle mixing. 80 µl of 7% of sodium carbonate was added and primed gently after 5 min before the plate was covered using an aluminum foil and the reaction was left to incubate for 90 min at room temperature.

Absorbance reading was done at 725 nm in a microtiter plate spectrophotometer reader (Synergy HT, USA). The concentration of total phenolic compounds was expressed in mg/100 g of the fresh sample converted to dry basis as Gallic Acid Equivalent (mg GAE).

Determination of flavonoids

The total flavonoid content was determined using the aluminum chloride colorimetric procedure (Medini et al., 2014). Twenty microlitre of catechin standards (10-100 µg/ml) and samples were pipetted into respective wells in a microtiter plate followed by the addition of 80 µl of deionized distilled water and 10 µl of 5% sodium nitrite and gently mixed by priming. After 5 min, 10 µl of 10% aluminum chloride was added and primed gently before the addition of 80 µl of 2 M sodium hydroxide. The plate was covered with aluminum foil and the reaction left to proceed at room temperature for 30 min. Absorbance readings were obtained at 510 nm in a microtiter plate spectrophotometer reader (Synergy HT, USA). External standard calibration technique was used to quantify the concentration of total flavonoids in mg/100 g of the fresh sample converted to dry basis as Catechin Equivalent (mg CE).

Anthocyanin content determination

Assay of anthocyanins was done according to Polivanova and Gins (2019) where two grams of fresh samples were weighed in a glass tube followed by the addition of 5 ml of extracting solution (80% ethanol) and sonicated for 10 min. Extraction was repeated using 5 ml of 80% ethanol and refrigerated overnight at 4°C protected from light. Centrifugation was done at 136.955 G force for 10 min and the supernatant was filtered through Whatman filter paper No. 2 in a 25 ml volumetric flask. Five milliliters of 80% ethanol were added to the sample, sonicated for 5 min, placed in a water bath for 5 min at 80°C then filtered. Extraction was repeated and the filtrate was brought to 25 ml using the extracting solution. One milliliter of each sample was aliquoted twice, 1 ml of potassium chloride (0.025 M) at pH 1, was diluted to the first portion and to the second portion sodium acetate (0.4 M) at pH 4.5. Vortexing was done and allowed to react for 5 min before transferring into microtiter plates and equilibrating for 15 min and the absorbance read at 530 and 700 nm from the spectrophotometer. The concentration of monomeric anthocyanin pigment was obtained:

Monomeric anthocyanin pigment (mg/l) = (A × MW × DF × 1000)/(ε × 1),

where A = (A530 nm − A700 nm) pH1.0 − (A530 nm − A700 nm) pH4.5 MW is the molecular weight, DF is the dilution factor, ε is the molar absorptivity(30200), and 1 is for a standard 1-cm pathlength.

Carotenoids determination

Carotenoid extraction was done according to Bhatnagar-Panwar et al. (2013) with modification. 0.5 g of the sample was weighed followed by the addition of 6 ml ethanol with 0.1% butylated hydroxytoluene (BHT) and homogenized for a minute. Two hundred and fifty microlitres of Echinenone (internal standard, Absorbance ≤0.2 at λ=460 nm) were added, vortexed, and incubated for 10 min at 85°C. 120 µl of potassium hydroxide 80% (w/v) was added and mixed by vortexing. Incubation for 5 min at 85°C then cooling in ice followed immediately and addition of 4 ml deionized water mixed by vortexing. Hexane of 5 ml was added then centrifugation of 100.62 G force for 5 min followed. The upper phase (hexane) to the

Table 2. Proximate composition (g/100 g on dry weight basis) of purees from colored sweet potatoes.

Variety	Puree colour	Protein	Fiber	Ash	Fat	CHO	Energy (Kcal)	Moisture
White spp.	White	1.67±0.09^{a}	2.81±0.10^{a}	5.03±1.94^{a}	1.96±0.88^{c}	54.45±1.51^{bc}	249.58±10.66^{b}	60.26±0.41^{a}
Cream spp.	Cream	1.88±0.09^{a}	2.15±0.47^{a}	3.43±0.18^{a}	1.53±0.10^{bc}	54.46±0.96^{bc}	246.60±3.76^{b}	61.16±0.72^{a}
Yellow spp.	Yellow	1.51±0.09^{a}	4.63±0.46^{b}	2.37±1.26^{a}	0.59±0.08^{ab}	58.09±1.46^{c}	252.88±6.82^{b}	59.82±1.04^{a}
Kabode+Vitaa(50:50)	Orange	3.03±0.36^{b}	2.89±0.15^{a}	4.35±1.20^{a}	0.52±0.06^{ab}	41.19±2.12^{a}	185.36±9.94^{a}	65.78±0.26^{b}
Diedi	Purple	4.44± 0.12^{c}	4.43±0.32^{b}	3.90±0.26^{a}	0.29 ±0.06^{a}	54.00±1.00^{b}	240.94±3.75^{b}	59.84±1.54^{a}

*Values with the same superscript along the column are not significantly different at P< 0.05.
Source: Authors

centrifuge tube was transferred using a Pasteur pipette. Extraction was repeated 3 more times with 4×3×3 ml hexane pooling the extract into the 25 ml tube. Addition of 5 ml deionized water into the extract and then vortexing followed before centrifugation for 5 min at 100.62 G force. The hexane layer was recovered in a clean test tube and evaporated under nitrogen in the N-Evap to complete dryness. Then, reconstitution in 10 ml of methanol and tetrahydrofuran (85:15 v/v) was done. Then, 1 ml was transferred into vials for analysis in the HPLC at 450 nm.

HPLC system: Waters 2695 separation module and 2996 PDA Detector was used with a YMC C30, carotenoid column (3 µm, 150×3.0 mm, YMC Wilmington, NC). The oven temperature was turned off and the flow rate was at 0.4 ml/min. Reverse phase gradient HPLC method was employed where Mobile phase A: methanol/tert-butyl methyl ether/water (85:12:3, v/v/v, with 1.5% ammonium acetate in the water) and Mobile phase B: methanol/tert-butyl methyl ether/water (8:90:2, v/v/v, with 1% ammonium acetate in the water) were taken into account and 30 µl became the injection volume.

Statistical analysis

All assays were carried out in triplicate. Data were analyzed by analysis of variance (ANOVA) using Minitab® 19.2020.1 software. Tukey's test was used to determine the significant difference in mean values. The significance level was expressed at 5% level. XLSTAT 2021 was used to determine the strengths of the linear associations between variables using Pearson's correlation. The correlation coefficient ranges from -1 (perfect negative correlation) to +1 (perfect positive correlation), with 0 representing no correlation.

RESULTS AND DISCUSSION

Proximate composition of sweet potato purees

Table 2 shows the proximate compositions of the different sweet potato purees on a dry weight basis (g/100 g). Significant differences (P< 0.05) observed across moisture, protein, ash, fiber, fat, carbohydrate, and energy contents are attributed to difference in veraity and steam processing of the roots at 100°C.

Moisture content

Moisture content was highest in OFSP puree at 65.78% with the lowest value from YFSP at 59.82% but did not significantly differ (p>0.05) from the other puree. The variations in the moisture content among the sweet potato varieties could be due to the differences in the genetic composition and cultivation practices.

Carbohydrate and energy content

OFSP had a lower carbohydrate level at 41.19%. The other purees had higher but relatively similar carbohydrate values ranging from 54.00 to 58.09%. YFSP registered the highest enegy level, 252.88 Kcal. This did not significantly differ (p>0.05) from the other purees except for orange-fleshed sweet potato (OFSP) at 185.36 Kcal. The significantly (p<0.05) lower carbohydrate and energy contents observed in OFSP could be associated with the difference in tissue morphology, structure, and chemical composition that influenced moisture migration (Kuyu et al., 2018). Consequently, the high moisture content observed, resulted in low dry matter content. OFSP is easily handled in the food industry due to its moist texture after cooking, this is a result of its viscous yet flowable attributes as compared to the yellow, purple, white, and cream sweet potato puree (Truong and Avula, 2010).

Crude ash content

YFSP ash content was lowest at 2.37%. It did not significantly differ (p>0.05) from the other purees that ranged (3.43 - 5.03%). Reported values (Alam et al., 2016) of ash content of OFSP varieties range from 1.17 to 1.31%. The observed and the reported values differed greatly. This could be attributed to different soils, climatic conditions as well as fertilizer application practice

Table 3. Individual sugar content in mg/100 g dry weight basis.

Variety	Puree color	Fructose	Glucose	Sucrose	Maltose	Total sugar
Diedi	Purple	5.37±0.09^{c}	1.52±0.02^{ab}	16.77±0.45^{b}	20.79±0.61^{a}	44.46±1.16^{a}
Kabode+Vitaa (50:50)	Orange	5.79±0.21^{c}	1.78±0.03^{bc}	15.54±1.43^{b}	48.16±4.19^{d}	71.28±5.87^{c}
Yellow spp.	Yellow	5.86±0.03^{c}	2.1±0.20^{c}	26.70±1.04^{c}	33.13±0.98^{b}	67.79±2.25^{c}
White spp.	White	3.34±0.10^{a}	1.97±0.03^{c}	12.90±0.62^{a}	43.20±1.04^{c}	61.42 ±1.80^{b}
Cream spp.	Cream	4.01 ±0.16^{ab}	1.25±0.06^{a}	11.09±0.75^{a}	42.25±1.12^{c}	58.62±2.10^{b}

*Values with the same superscript along the column are not significantly different at P< 0.05.
Source: Authors

during the planting phase of these sweet potatoes. Several authors have observed that increased application of fertilizers leads to increased mineral content in the sweet potato roots and leaves (Agbede, 2010).

Crude fiber

YFSP had the highest crude fiber content at 4.63% significantly different (p<0.05) from the other purees ranging from 1.51 to 4.43%. Malavi et al. (2022) reported that dietary fiber in sweet potato ranged of 5% which is slightly higher than the observed values on a DW basis. This could be attributed to genetic and cultivation differences. Also, the cooking procedure could have led to the polysaccharides being broken down into soluble components causing a reduction in the fiber. Recently dietary fiber has received much attention as it is believed to reduce cases of colon cancer and other gastrointestinal tract diseases (Kunzmann et al., 2015).

Fat content

The observed values ranged from 0.29 to 1.96% DW basis with significant (p<0.05) difference among the varieties. PFSP showed an unusually low-fat content at 0.29% while white-fleshed puree showed a considerably high-fat content at 1.96%. This could be due to difference in variety. According to Truong et al. (2018), raw sweet potato had a fat content of 0.05% FW basis which increased to 0.14% FW basis after boiling. Triasih and Utami (2020) also noted that not much fat decomposed when the roots are steamed hence retaining as much fat in the resultant puree compared to other processing methods such as roasting. Higher temperature and longer processing duration resulted in more intense damage to the fat content in the roots (Triasih and Utami, 2020).

Protein content

PFSP showed a significantly (p<0.05) higher protein level at 4.44% than the other purees ranging from 1.51 to 3.03% DW basis. Alam et al. (2016) reported that protein content of as high as 5% DW basis. The steaming procedure only made the protein available and did not contribute to its loss.

Sugar contents in sweet potato purees

According to Ridley et al. (2005), maltose is the most predominant sugar in sweet potato purees. In Table 3 OFSP showed that the high levels of the total sugar (71.28 mg/100 g), significantly different (p<0.05) from all other purees. YFSP had a significantly (p<0.05) high sucrose level attributed to the increased sweetness level of its bread. Increased maltose content in the purees could be due to varietal differences.

Phytochemicals in sweet potato purees

Ascorbic acid content

Ascorbic acid (mg/100g) was significantly high (p<0.05) in YFSP, WFSP and CFSP at 34.64, 30.27 and 25.56, respectively while the lowest at 1.75 for PFSP. Ascorbic acid is known to be a heat-sensitive bioactive component, this is contrary to what Dincer et al. (2011) observed. The fresh sweet potato samples exhibited low amounts of ascorbic acid compared to the heat-treated samples. This is related to the naturally occurring oxidizing enzyme, ascorbic acid oxidase, Which has been reported to be highly present in sweet potato and its active form on fresh samples (Dincer et al., 2011). PFSP exhibited the lowest ascorbic acid content due to the degradation of anthocyanins during heat treatment affecting ascorbic acid analyses (Dincer et al., 2011).

Carotenoid content

In Table 5, OFSP showed that the highest carotenoid level (mg/100g) 15.38, compared to PFSP and YFSP at 0.19 and 3.88 respectively. Lutein was found only in PFSP while *Trans* β-carotene was highest in OFSP at 11.75. This compared to Abong et al. (2020) where *trans* content of *Vitaa* and *Kabode* roots were 9.86 and 4.65, respectively. The lower levels registered in the purees could be due to the reduction in provitamin activity to the

Table 4. Comparison of flavonoids, phenolic, anthocyanin, ascorbic acid and antioxidant content (mg/100 g dry weight basis).

Variety	Puree color	Flavonoids (mgCE/100 g)	Phenolic (mgGAE/100 g)	Ascorbic acid (mg/100 g)	Carotenoids (mg/100 g)	Anthocyanin (mg/100 g)	Antioxidant (mgTE/100 g)
Diedi	Purple	575.57±5.24^{c}	452.10±17.80^{c}	1.75±0.09^{a}	0.19±0.03^{a}	88.26±5.14^{b}	32.29±10.09^{b}
Kabode+Vitaa(50:50)	Orange	46.55±4.04^{a}	6.63±6.63^{a}	15.06±2.26^{b}	15.38±2.39^{c}		303.38±2.27^{e}
Yellow spp	Yellow	58.16±1.12^{b}	198.07±1.80^{b}	34.64±2.59^{d}	3.88±0.18^{b}	1.93±0.35^{a}	64.45±1.99^{d}
White spp	White	-	-	30.27±1.43^{cd}	-	-	38.18±0.86^{c}
Cream spp	Cream	-	-	25.56±1.63^{c}	-	-	22.77±0.51^{a}

*Values with same superscript along the column are not significantly different p<0.05
Source: Authors

Table 5. Variations in carotenoid content (m g/100 g dry weight basis*)*.

Variety	Puree color	Lutein	Beta- C	13-Cis	Trans	9-Cis	Carotenoids
Diedi	Purple	0.07±0.02^{a}	0.04±0.008^{a}	-	0.07±0.0001^{a}	-	0.19±0.03^{a}
Kabode+Vitaa(50:50)	Orange	-	0.77±0.05^{b}	2.70±0.14^{b}	11.75±2.09^{c}	0.16±0.10^{a}	15.38±2.39^{c}
Yellow spp	Yellow	-	0.02±0.003^{a}	0.13±0.03^{a}	2.25±0.11^{b}	1.47±0.036^{b}	3.88±0.18^{b}

*Values with the same superscript along the column are not significantly different at P< 0.05.
Source: Authors

cis configuration of 9,13,15 position of carbon due to exposure to heat (Truong and Avula, 2010).

Table 5 shows that the content of different carotenoid content (mg/100 g) as captured in the purees.

Phenolic content

Phenolic compounds are primary antioxidants found in grains, fruits, and vegetables (Musilova et al., 2020). Sweetpotato has also been reported to contain these compounds. Phenolic content (mgGAE/100 g) was significantly high (p<0.05) in PFSP at 452.1. YFSP followed at 198.07 and OFSP at 6.63. Phenolic content for white and cream sweet potato puree was not detected (Table 4). The variation could be attributed to the phenolic extraction method, growing conditions, and sweet potato genotype. Heat treatments like steaming, baking, microwaving, and boiling have been shown to cause a significant increase in the phenolic content. This is explained by the hydrolysis of glycoside bonds to release phenols during treatment and the induction of total polyphenol content oxidation in fresh samples through the catalytic activity of the enzyme polyphenol oxidase (Musilova et al., 2020).

Anthocyanin, flavonoids, and antioxidant activity

PFSP had significantly (p<0.05) high levels of flavonoids at 575.57 (mgCE/100 g) and anthocyanin content of 88.26 (mg/100 g). OFSP had 46.55 (mgCE/100 g) and 1.93 (mg/100 g) flavanoids and anthocyanin contents, respectively. Yellow fleshed sweet potato puree had 58.16 flavonoid content and the rest of the other purees had undetectable levels of flavinoid. This could be attributed to genetic variation in sweet potato, storage, and pre-treatment procedures on the tubers. Anthocyanins are highly reactive molecules and their stability is influenced by their structure, concentration, temperature, oxygen, presence of enzymes, and pH (Musilova et al., 2020). Cooking treatments have been found to increase bioactive components in sweet potatoes as compared to raw tubers (Musilova et al., 2020).

Flavanoids, phenolics, ascorbic acid, and

Table 6. Pearson Correlation between bioactive compounds and antioxidant activity.

Parameter	Flavanoids	Phenols	Carotenoids	Ascorbic acid	Anthocyanin
Antioxidant activity	-0.60	-0.87	0.99	-0.20	-1

Source: Authors

anthocyanin showed a negative correlation to antioxidant activity (Table 6). Sugars showed a positive correlation with antioxidant activity. Carotenoids exhibited a strong positive correlation with antioxidant activity.

As explained by Tang et al. (2015) where high carotenoid levels result in a strong antioxidant capacity to scavenge free radicals because of their conjugated double bonds. OFSP (*Kabode* + *Vitaa*) scored the highest antioxidant activity 303.38 (mgTE/100 g) followed by YFSP at 64.45 (mgTE/100 g), agreeing with the concept; that the more colored the root the higher the antioxidant activity. Contrary to what was observed with PFSP which recorded 32.29 (mgTE/100 g). According to Fidrianny et al. (2018), PFSP could exhibite lower antioxidant activity based on the structure of the anthocyanin (flavanoids) compounds. Where only flavanoid with a certain OH position resulted in high antioxidant activities. Based on this study, it could be the anthocyanin compounds in the PFSP did not have ortho di OH at C3'- C4' resulting in reduced antioxidant activity or more flavonoid glycoside present other than flavonoid aglycone. This could be explained by the negative correlation observed in flavonoids, anthocyanins, and phenolics with antioxidant activity.

Variation in microbial load of sweet potato puree wheat bread

Wheat bread acted as a control for all sweet potato puree wheat composite bread. On day 6 all the OFSP bread substitution levels and PFSP 10 and 20% levels had microbial counts below the acceptable limit accepted by Kenyan Standards for bread which allows for 1000 CFU/g. Table 7 shows that the average $\log_{10}$ microbial counts of all bread across the six days.

At 10% bread formulations, on day zero, no microbial colony was detected in all resultant bread substitutions. This implied the effectiveness of the baking process at 200°C for 30 min where the high temperatures managed to inactivate and destroy spoilage microorganisms. On day-2, wheat and PFSP bread recorded no microbial count. There was an increase of microbial count in all bread but very significant in CFSP10% where it recorded a mean $\log_{10}$ microbial count of 3. This is the limit of the recommended statutory count of 1000 CFU/g. On day 4, the microbial count was increasing. All WFSP, CFSP 10% puree bread substitution and wheat breads had a microbial count of mean $\log_{10}$ 3.1, 5.0, and 4.9 (CFU/g), respectively; making them unsuitable for consumption. YFSP 10% scored 2.5 making it suitable for consumption on day 4 and unfit for consumption on day-6 with a mean $\log_{10}$ microbial count of 4.7 CFU/g. Moreover, only PFSP and OFSP 10% level breads were fit for consumption for all study days. They recorded mean microbial count of 0.8 and 2.0 CFU/g on day 4, 1.9 and 2.9 CFU/g, respectively on day-6.

At 20% bread formulations, on day zero, there was no microbial colony observed. On day 2, PFSP 20%, and wheat breads recorded no microbial count. All breads were fit for consumption as they recorded mean log microbial counts of 1.1, 2.7, 2.0, and 0.7 CFU/g for WFSP, CFSP, YFSP, and OFSP 20% breads, respectively, on day 2. An increment of growth was observed for CFSP 20% and wheat breads on day 4, the mean $\log_{10}$ microbial count was 5.1 and 4.9 (CFU/g), respectively. Bread fit for consumption at this stage was YFSP, PFSP, and OFSP at 2.8, 1.7, and 1.7, respectively. On day 6, only PFSP and OFSP 20% breads were suitable for consumption at mean $\log_{10}$ microbial counts of 2.4 and 2.8 CFU/g, respectively. WFSP, CFSP, and YFSP breads on day 6 were observed at 4.6, 6.0, and 5.7 CFU/g, respectively while YFSP showed exponential growth of 2.8 to 5.7 in only two days.

At 30% bread formulations, the microbial load was not detected on day 0. On day-2, OFSP at 30% puree substitution and wheat breads had no microbial colony observed. All breads were fit for consumption except for CFSP 30%, which recorded a mean $\log_{10}$ microbial count of 4.5 CFU/g. This depicted an increase in CFSP puree in the bread and increased microbial count; resulting in a negative correlation between the puree and its antimicrobial property. On day 4, only WFSP, YFSP, PFSP, and OFSP were fit for consumption, recording means $\log_{10}$ microbial count 2.3, 2.7, 1.9, and 1.9 (CFU/g), respectively. On day 6, only OFSP 30% bread was suitable for consumption with a mean $\log_{10}$ microbial count of 2.5 CFU/g. This was an increase from 1.9 on day 4 but did not exceed 1000 (CFU/g) per the statutory level. Breads of WFSP, CFSP, YFSP, and PFSP had mean $\log_{10}$ microbial counts of 4.6, 4.5, 5.7, and 3.3 CFU/g, respectively.

At 40% bread formulations, on day zero, the microbial load was not detected. On day 2, OFSP 40% and wheat breads also showed no growth of the microbes. An increase in OFSP puree substitution in bread increased its microbial inhibition property. WFSP, YFSP, PFSP 40% breads showed mean $\log_{10}$ microbial count 2.0, 1.9, 1.0 CFU/g fit for consumption except CFSP 40% bread at 4.3 CFU/g. On day 4, only WFSP, PFSP, and OFSP 40%

Table 7. Microbial counts (log_{10} CFU/g) of resultant breads.

Formulation	Bread composition (%)	Storage duration (Day)			
		0	2	4	6
WFSP	10	0±0.0^{a}	1.0±0.0^{b}	3.1±0.01^{i}	4.71±0.00^{e}
	20	0±0.0^{a}	1.1±0.1^{b}	3.09±0.02^{hi}	4.57±0.01^{e}
	30	0±0.0^{a}	1.9±0.0^{c}	2.32±0.03^{defg}	4.58±0.0^{e}
	40	0±0.0^{a}	2.0±0.0^{c}	2.86±0.07^{ghi}	3.69±0.56^{d}
CFSP	10	0±0.0^{a}	3.06±0.02^{d}	5.00±0.00^{k}	6.01±0.02^{g}
	20	0±0.0^{a}	2.79±0.08^{d}	5.15±0.00^{k}	6.05±0.02^{g}
	30	0±0.0^{a}	4.50±0.02^{e}	4.91±0.01^{k}	4.51±0.07^{e}
	40	0±0.0^{a}	4.36±0.03^{e}	4.58±0.00^{jk}	4.50±0.17^{e}
YFSP	10	0±0.0^{a}	1.86±0.51^{c}	2.49±0.19^{efgh}	4.86±0.00^{ef}
	20	0±0.0^{a}	2.01±0.06^{c}	2.77±0.07^{ghi}	5.69±0.00^{g}
	30	0±0.0^{a}	2.21±0.11^{c}	2.66±0.05^{fghi}	5.68±0.00^{g}
	40	0±0.0^{a}	1.95±0.04^{c}	4.01±0.06^{j}	5.47±0.05^{fg}
PFSP	10	0±0.0^{a}	0.0±0.00^{a}	0.76±0.68^{a}	1.89±0.11^{a}
	20	0±0.0^{a}	0.0±0.00^{a}	1.65±0.56^{bc}	2.38±0.07^{ab}
	30	0±0.0^{a}	1.1±0.17^{b}	1.94±0.09^{cde}	3.28±0.04^{cd}
	40	0±0.0^{a}	1.0±0.00^{b}	1.81±0.13^{cd}	3.41±0.01^{cd}
OFSP	10	0±0.0^{a}	1.69±0.08^{c}	2.07±0.07^{cdef}	2.99±0.59^{bc}
	20	0±0.0^{a}	0.66±0.57^{b}	1.66±0.05^{bcd}	2.82±0.10^{bc}
	30	0±0.0^{a}	0.0±0.00^{a}	1.93±0.07^{cde}	2.46±0.0^{ab}
	40	0±0.0^{a}	0.0±0.00^{a}	1.10±0.17^{ab}	1.96±0.05^{a}
Wheat bread		0±0.0^{a}	0.0±0.0^{a}	4.90±0.58^{k}	5.0±0.00^{ef}

*Values with the same superscript along the column (similar days) are not significantly different at P< 0.05. Bread represented in the table at 10, 20, 30 and 40% sweet potato puree substitution where CFSP - Cream fleshed sweet potato puree, WFSP-White fleshed sweet potato puree bread, YFSP- Yellow fleshed sweet potato puree, Wheat- Wheat bread, PFSP- Purple fleshed sweet potato puree, OFSP-Orange fleshed sweet potato puree.
Source: Authors

breads were fit for consumption at the microbial count of 2.9, 1.8, and 1.1 CFU/g, respectively. On day-6 only OFSP 40% was suitable for consumption with a mean log_{10} microbial count of 1.9 CFU/g. WFSP, CFSP, YFSP and PFSP 40% recorded mean log_{10} microbial counts 3.7, 4.5, 5.5 and 3.4 CFU/g, respectively, making them unsuitable for consumption. PFSP bread showed a negative correlation between increments in puree substitution in the bread to its microbial inhibition property (Table 7). Post contamination of the breads was limited due to the hygienic-sanitary conditions of the production area (Science and Avenue, 2019). On day 4 for example, wheat bread was not suitable for consumption. Its average log_{10} microbial colonies were 4.90 ±0.58 CFU/g. This could be attributed to increased water activity during storage allowing for increased microbial activity as indicated by Wanjuu et al. (2018). The greater the water activity the greater the mold growth which causes spoilage (Abu-Ghoush et al., 2008).

Fungal growth observed in bread

Molds grow and throve in bread, since it is an excellent source of nutrition based on its composition. Due to its low moisture content, mold growth in bread is favored instead of bacteria and yeast (Mpamah et al., 2019). The fungus species referenced (Mpamah et al., 2019) were observed in most of the different puree bread levels.

Aspergillus spp. was mostly encountered. At its conidial stage, the mycelia were green in color (Table 8). The yellow and reddish yellow pigment in its ascosporic stage was also observed (Mpamah et al., 2019). *Aspergillus* spp. contamination on bread is as a result of inadequate preservation of cereals since raw materials are the main source of fungal spore dissemination (Science and Avenue, 2019). Basic principles in controlling fungal deterioration in baked products included restricting access of deteriorating fungi to products (Science and Avenue, 2019). This was achieved by ensuring all

Table 8. Morphological characteristics of fungal isolates observed in puree breads.

Fungus spp.	Color of mycelia	Appearance of mycelia
Aspergillus	Green	Web
Rhizopus	Black	Web like
Monilia	White	Cotton wool

Source: Authors

Table 9. Pearson correlation between day-4 average microbial count and sugar content .

Parameter	Average Log Microbial Count at day 4			
	10% Bread	20% Bread	30% Bread	40% Bread
Sugar content	0.04	0.17	0.06	-0.03

Source: Authors

ingredients and equipment were clean and safe for use. Reducing the exposure of the baked bread to raw materials by baking and packaging in separate sections of the production area used to help in this restriction (Science and Avenue, 2019). Slicing of the bread was avoided for the same reason to prevent post contamination and after a few hours of cooling the bread were packaged in polythene bags to imitate bread production in the Kenyan market. It is to be noted that the production area and length of exposure of the bread to the air around production environment after removal from the oven are crucial in influencing the fungal load (Science and Avenue, 2019). Intrinsic factors like pH, and water activity (a_w), as noted by Wanjuu et al. (2018), increased during the storage duration resulting in increased water activity for the wheat bread but not significantly in the OFSP bread. The higher the water activity the more chemical reactions and microbial growth causing deterioration of bread hence allowing growth of *Rhizopus* and *Monilia* spp. (Mpamah et al., 2019) .

Relationships observed in breads, purees, microbial counts, and bioactive compounds

Sugar contents present in relation to bread shelf-life

At 40% level substitution, increased sugar content in the bread resulted in a decrease in microbial count as observed in Table 9; this is based on the observed negative correlation between the sugar content in purees and average $\log_{10}$ microbial counts. Bystrom et al. (2009) indicated that high sugar concentrations have an antimicrobial effect with a lower water activity that deprived microorganisms of water thus inhibiting growth. This explained why all the OFSP bread (Table 3 for sugar content) combinations inhibited bread spoilage microorganisms up to day-6 of the recorded shelf-life. This does not imply that high sugar concentrations alone are responsible for microorganisms growth inhibition since 10, 20% PFSP bread combinations showed microbial inhibition hence attributing inhibition to an antioxidant activity as well. This is seen in Table 9 where there is observed a weak negative correlation between the sugar content in the breads of 40% puree substitution and average $\log_{10}$ microbial count at day-4 of recorded shelf-life. Mizzi et al. (2020) also observed that sugars' osmotic effect is not the only property inhibiting bacterial growth. Sucrose at optimum levels was found to be the best inhibitor to the other three common sugars against bacterial growth (Bystrom et al., 2009). It might be due to the high sucrose levels as observed in YFSP bread, which makes them suitable for consumption even for 4 days of storage.

Table 9 shows the (r) correlation between day 4 average microbial count and sugar content. Days 3 to 4 in bread served as a turning point as most bread degraded at this stage.

Phytochemicals present in relation to bread shelf-life

According to Wanjuu et al. (2018), carotenoids like lutein, zeaxanthin and β-cryptoxanthin were identified in wheat bread as a result of wheat flour being used as an ingredient. These xanthophylls could be attributed to the behavior of the wheat bread showing lower than the statutory limit mean log microbial count up to day-3 of its shelf-life. Alam et al. (2016) reported that 19.31 to 61.39 µg/g beta-carotene content in orange-fleshed sweet potato roots as compared to 1.02, 3.78, and 3.28 to 5.64 µg/g in white, cream, and yellow-fleshed sweet potato roots, respectively. This was not comparable to the observed non-detectable results in WFSP and CFSP. Abong et al. (2020) reported that processing methods of the sweet potato roots resulted in a 100% increase in the

Table 10. Pearson correlation between day-6 average microbial count and carotenoid content.

Parameter	Average Log Microbial Count at day 6			
	10% Bread	20% Bread	30% Bread	40% Bread
Carotenoid content	0.04	-0.17	-0.50	-0.65

Source: Authors

Table 11. Pearson correlation between day-4 average microbial count of WFSP, YFSP and ascorbic acid.

Parameter	Average Log Microbial Count at Day 4			
	10% Bread	20% Bread	30% Bread	40% Bread
Ascorbic acid	-0.96	-0.92	-0.79	-0.36
Antioxidant activity	-0.90	-0.85	-0.68	-0.20

Source: Authors

Table 12. Comparison with bread for its day-6 microbial count, percentage puree substitution and its respective sweet potato puree color.

	Flesh color	Percentage Puree	Day 6 Microbial count
Flesh color	1.00		
Percentage Puree	0.18	1.00	
Day 6 Microbial count	-0.64	-0.14	1.00

Source: Authors

carotenoid content due to the increased availability brought about by the release of bioactive compounds after cooking. Consequently, non-detectable levels of carotenoids in WFSP and CFSP also observed by Ooko et al. (2020) would not translate into the bread formulations; resulting in poor microbial inhibition as seen in Table 7.

YFSP showed significantly ($p<0.05$) high levels of carotenoid content at 3.88 µg/100 g; this could be explained why all bread levels could be consumed up to day-4 with an exception of 40% which had a mean $\log_{10}$ of 4.0. High levels of carotenoids as observed in Table 5 for YFSP and OFSP resulted in a strong antioxidant capacity to scavenge free radicals because of their conjugated double bonds (Tang et al., 2015).

Table 10 shows that a correlation between carotenoid content and day 6 mean $\log_{10}$ microbial count.

An increase in the puree level substitution in the bread and consequently carotenoid content resulted in a negative correlation with the day 6 average $\log_{10}$ microbial count. This is desirable because a low microbial count in the bread will resulte in reduced microbial activity hence spoilage. OFSP showed a decline in average $\log_{10}$ microbial level from 2.9 (10%) to 1.9 (40%) bread level shown in Table 7; due to significantly high carotenoid content in the puree 15.385 µg/100 g consequently increasing its availability in the bread. Li (2011) noted that flavonoids were better than ascorbic acid at inhibiting lipid peroxidation but weaker at reducing power and scavenging hydroxyl free radicals, superoxide anions, and DPPH radicals. Antioxidant properties of flavonoids depend on their structure and content in the sample. Significantly ($p<0.05$) lower ascorbic acid levels in PFSP as a result of anthocyanin degradation during treatment consequently affected negatively its antioxidant activity. In 30 and 40% WFSP, 10, 20 and 30% YFSP were suitable for consumption at day-4 of recorded shelf-life as observed. This is supported by the strong negative correlation between ascorbic acid and average $\log_{10}$ microbial count for day-4 in bread shown in Table 11. Where an increase in ascorbic acid and antioxidant activity resulted in a microbial count decrease in the bread.

The flesh colors of sweet potato purees were numbered according to their intensity: 1. White fleshed, 2. Cream fleshed, 3. Yellow fleshed, 4. Orange fleshed, 5. Purple fleshed.

As observed in Table 12, percentage puree had a weak positive correlation (0.18) to flesh color. It is expected if the flesh color intensified it could be due to an increment of the percentage puree. The correlation is weak because the intensity of the color is essentially influenced by the

Table 13. Comparison with flesh color of different sweet potato purees to their respective bioactive compounds.

	Flesh color	Sugars	Carotenoids	Ascorbic acid	Anthocyanin	Phenolic	Flavanoids	Antioxidant activity
Flesh color	1.00							
Sugars	-0.32	1.00						
Carotenoids	0.38	0.68	1.00					
Ascorbic acid	-0.81	0.63	-0.15	1.00				
Anthocyanin	0.72	-0.87	-0.29	-0.84	1.00			
Phenolic	0.73	-0.71	-0.30	-0.61	0.90	1.00		
Flavanoids	0.77	-0.82	-0.24	-0.83	1.00	0.93	1.00	
Antioxidant activity	0.36	0.63	0.99	-0.21	-0.26	-0.33	-0.22	1.00

Source: Authors

occurrence of the natural pigments for instance carotenoids and flavonoids found in the puree. A weak negative correlation between percentage puree to day 6 microbial count at (-0.14) is observed. Increment in percentage puree caused a decrease in the microbial count on day 6. The correlation was weak because 10 and 20% PFSP breads were safe for consumption at day 6 recorded shelf-life and not the 30 and 40% PFSP bread substitutions. Whilst day 6 microbial count to flesh color at (-0.64) showing a strong negative correlation. This is attributed to the intense color in PFSP and OFSP consequently showing the longevity of shelf-life even at day 6 of the microbial count. As also observed 10, 20, 30 and 40% OFSP breads were all fit for consumption at day six of recorded shelf-life.

Table 12 shows that a comparison of different bread for
their day 6 microbial count, percentage puree substitution, and their respective sweet potato puree color.

The intensity of the color influenced the quantification of the bioactive compounds significantly as observed in Table 13. According to Steed and Truong (2008), PFSP purees had polyphenolic content and antioxidant capacity within range reported for various purple-colored fruits and vegetables. Phenolics are antioxidant molecules with at least one aromatic ring and one or more hydroxyl groups. Anthocyanins are a group of water-soluble flavonoids that are acylated with various phenolic acids making them pH and heat-resistant, light-sensitive, possessing elevated antioxidant and anti-mutagenic activity differing largely from anthocyanin possessed in berries (Xu et al., 2015).

As observed in Table 13, anthocyanin strongly correlated with flavonoid at 1 due to its nature as a water-soluble flavonoid. Anthocyanin is also a naturally strong free-radical scavenger, providing many pharmaceutical values including anti-oxidation, anti-tumor capacities, prevention, and treatment of cardiovascular diseases (Li et al., 2019). Anthocyanin, phenolic, and flavonoid components showed a strong linear correlation to the flesh color of the purees whilst carotenoids and antioxidant activity had a weak positive correlation at 0.38 and 0.36, respectively as seen in Table 13. Carotenoids and sugars showed a strong linear correlation of 0.99 and 0.63, respectively with antioxidant activity alluding to the increased antioxidant activity of OFSP at 303.38 mgTE/100 g as observed in Table 4. Table 13 shows that the comparison of different sweet potato puree colors to their respective bioactive compounds.

Moreover, Huang et al. (2006) observed that total phenols, flavonoids, and anthocyanins of sweet potato flours were positively correlated with the reducing power and scavenging DPPH radical effects. The high levels of polyphenolic compounds found in PFSP and high levels of carotenoids in OFSP as observed in Table 4 have led to their increased utilization as a healthy food commodity and source of natural food colorants. There are growing interests in exploring these market opportunities for OFSP and PFSP sweet potato purees are preferred over flour forms for nutrition and economic reasons (Bocher et al., 2017).

Mode of action of phytochemicals on Fungi spp.

Bioactive compounds released by plants are either primary or secondary metabolites depending

on their functional role in the growth of the plant. Phytochemicals are non-nutritive secondary metabolites produced by plants as a defense mechanism against adverse environmental conditions, insects and pathogenic microorganisms (Makhuvele et al., 2020). Baba and Malik (2015) indicated that plant rich in secondary metabolites like carotenoids, phenols, and flavonoids exhibit redox activity due to their redox potential and structures. This explained why OFSP and PFSP bread exhibited high inhibition activity due to their significantly high content of the bioactive compounds shown in Table 4.

Flavonoids, and phenolics, of *yellow* spp*.,* are significantly lower compared to the values in PFSP and OFSP. This could be a result of differences in genetic makeup, agronomic practices, and production area (El Sheikha and Ray, 2017). Also, *white* spp. and *cream* spp. recorded 30.27 and 25.56 mg/100 g ascorbic acid and antioxidant activity of 38.18 and 22.77 mgTE/100 g, respectively while the rest of the other bioactive compounds were not detected as seen in Table 4. This described the resultant impact of significant reduction to no inhibition activity as observed in WFSP and CFSP breads. OFSP (*Kabode* + *Vitaa*) scored the highest antioxidant activity value at 303.38 mgTE/100 g agreeing with the concept of the more colored the root the higher the antioxidant activity. But this is contrary to what was observed with PFSP which recorded 32.29 mgTE/100 g shown in Table 4. According to Fidrianny et al. (2018), PFSP could be exhibited lower antioxidant activity based on the structure of the anthocyanin (flavanoids) compounds as earlier stated and also observed in Table 13; a negative correlation with antioxidant activity. It is suggested that only a flavanoid with a certain OH position, results in high antioxidant activities. Based on our study it could be the anthocyanin compounds in the PFSP did not have ortho di OH at C3'- C4' resulting in reduced antioxidant activity or more flavonoid glycoside present other than flavonoid aglycone (Fidrianny et al., 2018).

The phytochemicals present in PFSP and OFSP shown in Table 4 were responsible for the antagonistic effect against the growth of bread spoilage microorganisms. The mode of action as indicated by Loi et al. (2020), was an alteration of osmotic and redox balance, inhibition of cytoplasmic and mitochondrial enzymes as well as enzymes responsible for cell wall components causing cytotoxicity in fungi. This is made possible due to the hydrophobic nature of phenols and aldehydes that allows them to pass the phospholipid bilayer. Upon entering, they get to the nucleus and take the role of regulation of their biosynthesis. Consequently, fatty acid profiles are altered, the cell membrane is modified due to interaction with its ergosterol, and osmotic imbalance also occurs causing irreversible damage to conidiophores, hyphae membranes, and death of the fungi (Loi et al., 2020). These bioactive compounds present in PFSP and OFSP can be used in the prevention of fungal growth in food when used as an additive. This will act to reduce both the mutagenicity and carcinogenicity brought about by fungal species (Makhuvele et al., 2020).

Conclusion

Based on the results of this study, sugar, anthocyanin, carotenoids, ascorbic acid, phenols, and flavonoid content differed significantly in various sweet potato purees used in the bread formulation. Phytochemicals found in the sweet potato purees (OFSP and PFSP) are responsible for antimicrobial properties exhibited against the bread spoilage fungi. Further studies are recommended to determine the full characterization of the bioactive components observed, to exhibit inhibition of the fungi which causes bread spoilage. Moreover, determine the MIC of these purees.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Typology of Côte d'Ivoire dishes integrating Néré pulp (*Parkia biglobosa* L.) into the preparation process

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The Néré (*Parkia biglobosa* L.) is a tree of the Mimosaceae family, typical of the semi-arid and sub-humid zones of West Africa generally found in the northern of Côte d'Ivoire. The yellow pulp on the seeds of Néré is consumed raw or used for the preparation of drinks or enters into the composition of many cereal foods. The objective of the present study was to identify the dishes integrating Néré pulp in the formulation, the process and the food habits based on its consumption. Thus, a food consumption survey was carried out in 135 households per department (Korhogo, Séguéla and Bouna) in the northern of Côte d'Ivoire. Data revealed that five cereals dishes and a beverage based on Néré pulp were consumed in investigated areas. The process and the consumption form of foods depend on consumers' ethnic group and their origin region. Most people consume foods based on Néré pulp to have a good health. Among dishes, *Nammigue* was the most known and consumed food (36.79%) as a lunch with a consumption frequency of two times per month. Six foods based on Néré were identified and their processes were described.

Key words: Pulp, Néré, foods typology, consumption practices, household survey, Côte d'Ivoire.

INTRODUCTION

Néré (*Parkia biglobosa* L.) is a tree of the Mimosaceae family, typical of the semi-arid and sub-humid zones of West Africa. It is a wild edible fruit tree generally found in the North where it occupies a prominent place in people's lives (Kouamé et al., 2015; Arbonnier, 2009). The wild fruit tree *Parkia biglobosa* L (Néré) is widely distributed and consumed in the Poro (Korhogo), Worodougou (Séguéla) and Bounkani (Bouna) regions (Kouakou et al., 2020; Ambé, 2001). The fruits of *P. biglobosa* are long pods of about 45 cm, 2 cm wide, slightly arched, hanging in clusters on the club-shaped flower. During the ripening, the endocarp constantly fills the cavity between the seeds of the whole pod, which becomes powdery and light yellow at maturity. At maturity the flattened black seeds are embedded in a yellow pulp rich in sucrose (Mabetty, 2018). According to Touré (2020), the pulp of Néré is the sweet and yellow floury substance surrounding the seeds. It is rich in energy value and consumed raw or processed into a refreshing drink. The pulp of *P. biglobosa* constitutes a means of subsistence for local

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populations. The harvested mature fruits are used as food in the dearth season (Nyadanu et al., 2017). The pulp of the Néré has an interesting nutritional value, rich in sodium, potassium, calcium, zinc, b-carotene, ascorbic acid, riboflavin and also phenolic compounds. This pulp could therefore contribute to reducing malnutrition caused by micronutrient deficiencies and extreme hunger in developing countries (Bamigboye et al., 2012; Kouassi et al., 2018a). In addition, Néré pulp contains high dietary fibre content, making it essential for intestinal transit, prevention of obesity, intestinal diseases such as colon cancer and other gastrointestinal conditions and helps prevent diabetes (Nyadanu et al., 2017). *Parkia biglobosa* pulp can therefore contribute to human nutritional needs and be a good source of energy due to its high carbohydrate content. It can be used as a nutritional supplement (Aja et al., 2015). Thus, this pulp could fill micronutrient deficiencies in Côte d'Ivoire where malnutrition is a real public health problem. Indeed, some areas in Côte d'Ivoire can reach up to 40% for global acute malnutrition and over 5% for severe acute malnutrition. These deficiencies mainly affect women of childbearing age and children (N'goran, 2014; Schwartz and Grellety, 2010; WHO, 2009; FAO, 2008). An alternative to this situation could be the valorization of traditional wild fruit dishes such as Néré. The yellow pulp surrounding the seeds is eaten raw or used to make cold drinks (Andon et al., 2018) or used in many other cereal foods such as fritters and cakes (Lamien et al., 2011). There is very little data on dishes incorporating Néré pulp. Only recent studies (N'goran, 2014; Kouassi et al., 2018b) based on juices derived from Néré were available. There is a lack of literature on cereal foods made from Néré. Thus, in order to promote their access to consumers, the objective of this study was to inventory dishes incorporating Néré pulp in the formulation and determine their characteristics, that is, process, dietary habits and food consumption.

MATERIALS AND METHODS

The technical material consists of a household survey form with consumption practices sections such as food modes, food forms, consumption frequencies, and production processes of Néré-based dishes.

Survey areas

In each Néré widely distributed zone (Korhogo, Séguéla and Bouna), three villages were selected after a pre-survey based on the consumption of Néré pulp. Thus, the villages of Nahouokaha, Lataha and Kotchiéri (Korhogo), Sifié, Sélakoro and Bobi (Séguéla) and Niandégué, Bouko and Panzarani (Bouna) were surveyed.

Selection of surveyed households

Households were selected using the snowball technique. The size (n) of the households surveyed was calculated according to Dagnelie (1998) formula for an independent non-exhaustive sample based on Côte d'Ivoire population and housing census (RGPH, 2014):

$$n = t^2 . \frac{p.(1 - p)}{m^2}$$

Where, n = minimum sample size sought; t = 95% confidence level (standard value of 1.96); P = proportion of the target population in the study area; p estimated at 50% due to lack of knowledge of the number of households processing wild fruit; m = margin of error at 5%.

Thus, the minimum sample size (n) was:

$$n = \frac{1.96^2 \times 0.5(1 - 0.5)}{0.05^2} \approx 385 \text{ individuals}$$

To compensate errors in questionnaires that were incorrectly filled out, 20 additional households were added to calculate sample size (n). Thus, a total of 405 households were surveyed with 135 per department (Table 1).

Statistical processing

Data were entered using SPHINX Plus² (V5) software and then exported to SPSS 20.0 software for database. Statistical analyses were performed using XLSTAT 2016 software. The relationship between the dependent variables was assessed by Chi-square test and Z test at 5% threshold. Correspondence factor analyses (CFA) were performed for the comparison of more than 3 variables. Excel software was used to produce graphs of periods, consumption frequencies and level of knowledge of foods.

RESULTS AND DISCUSSION

Collection of Néré

In all the departments, most of consumers prefer to collect Néré in field (92.86-98.25%) than the payment into the market (2.63-8.33%) (Table 2). Néré is generally collected in pod (92.86 - 98.25%) than those of powder (1.75 - 7.14%) and pulp (0 - 1.19%) (Table 3). In Séguéla, consumers do not use Néré in pod form.

Reasons of Néré consumption

Data showed that Néré is mainly consumed in the three different localities for its health benefits and then for its taste. However, Néré is also consumed as a soldering food (Figure 1).

Dishes based on Néré

Six different Néré based dishes were identified. These dishes encountered in the different departments are corn or millet couscous with Néré, corn cake with Néré, Néré cerelac, wafer, Néré beverage and Néré cake. These dishes were called in different names depending on the

Table 1. Number of households surveyed per zone and per village.

Department	Village	Respondents	Total
Korhogo	Nahouokaha	48	135
	Lataha	51	
	Kohotiéri	36	
Séguéla	Sifié	41	135
	Sélakoro	44	
	Bobi	50	
Bouna	Niandégué	18	135
	Bouko	62	
	Panzarani	55	
Total		405	

Source: Authors

Table 2. Places of supply of Néré.

Place	Bouna	Séguéla	Korhogo
Market (%)	8.33[a]	6.25[a]	2.63[a]
Field (%)	92.86[b]	95[b]	98.25[b]
z	-20.217	-24.027	-48.859
P	<0.001	<0.001	<0.001

The values of column with the same letters are not significantly different at the 5% threshold.
Source: Authors

Table 3. Forms of procuration of the Néré.

Form	Bouna	Séguéla	Korhogo
Pulp (%)	1.19[a]	0.00[a]	0.88[a]
Powder (%)	7.14[a]	5.00[a]	1.75[a]
Pod (%)	92.86[b]	95[b]	98.25[b]
χ^2	197.73	205.8	233.82
dl	2	2	2
P	<0.001	<0.001	<0.001

The values of column with the same letters are not significantly different at the 5% threshold.
Source: Authors

locality or ethnicity (Table 4).

Production process of Néré based dishes

Production of Wasséra

Wasséra is a corn couscous with Néré. For its production, 1 kg of corn flour is mixed with 1 kg of Néré pulp powder. After homogenization, two processes can be applied: either the mixture of flours is directly overturned in a pot on soft fire containing 100 to 200 g of shea butter for a cooking of 5 to 10 min during which the whole is constantly stirred to obtain the floury *Wasséra* or the mixture of flours is sprinkled with water for the formulation of granules for 10 to 20 min steam cooking. After cooking, the granules are relayed in 100 g of melted butter or 100 mL of refined vegetable oil to obtain the granular *Wasséra* (Figure 2).

Production of Nammigue

Two processes are used to obtain *Nammigue*. On the first hand, a kilogram (83 g) of millet is roasted, ground and sifted to produce flour. This flour was mixed with 17 g of Néré powder then the whole was relayed in 300 mL of water. On another hand, 100 g of Néré powder was

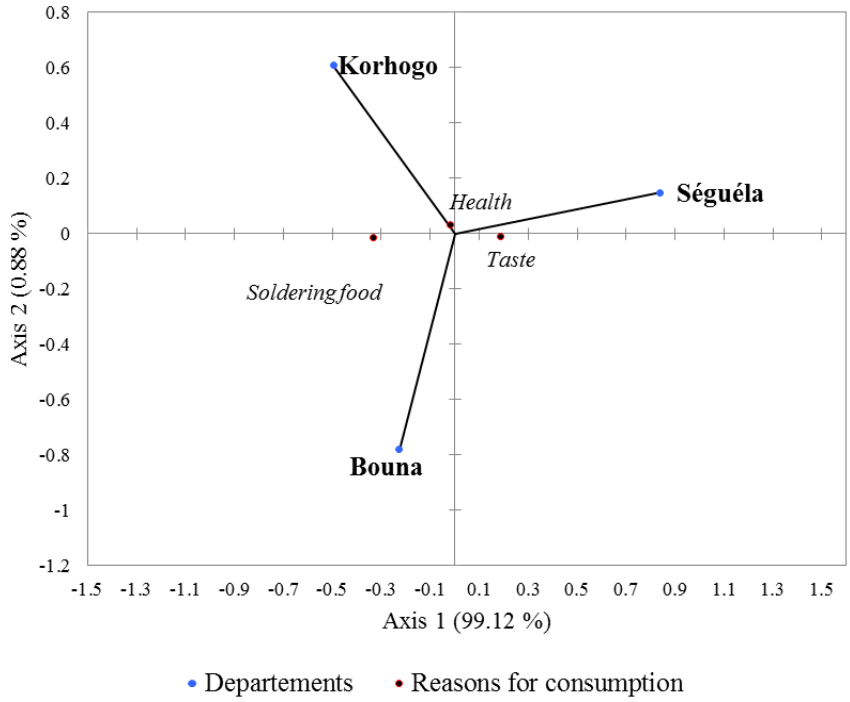

Figure 1. Reasons for consumption of Néré per locality.
Source: Authors

Table 4. Description and vernacular names of Néré-based foods.

Food	Locality	Consumers	Vernacular names
Corn couscous with Néré	Bouna Séguéla	Lobi Niaraforo	*Wasséra* ou *Pia-pia* *Tawigué nammiguin*
Corn cake with Néré	Bouna Korhogo Séguéla	Malinké Sénoufo Woroudougou	*Sakoula* *Kawaragu*e *Faléni-nèrèho*
Néré Cerelac	Bouna Korhogo Séguéla	Lobi Sénoufo Woroudougou	*Miyéh* *Nammigue* *Nèrèdji*
Néré beverage	Bouna Korhogo	Lobi Sénoufo	*Doun-gnon* *Nammigue* -lor
Baobab and néré donut	Bouna	Peulh	*Bafouratou*
Néré cake	Bouna	Wolof	Cake au Oul

Source: Authors

directly relayed in 300 mL of water. In both cases, the slurry was consumed sweetened or spiced with chili powder (Figure 3).

*Production of Kawaragu*e

One kilogram of Néré powder was mixed with 1 kg of corn flour and 30 g of salt. 1.5 L of water was gradually added for the homogenized mixture. The mixture was kneaded and the dough obtained was kneaded by hand for 10 to 15 min before frying to obtain doughnuts in the form of patties (Figure 4).

Production of *Bafouratou*

One kilogram of Néré powder was mixed with 1 kg of baobab powder and 1 kg of millet flour to constitute a homogeneous medium. To the mixture obtained, 1.5 L of

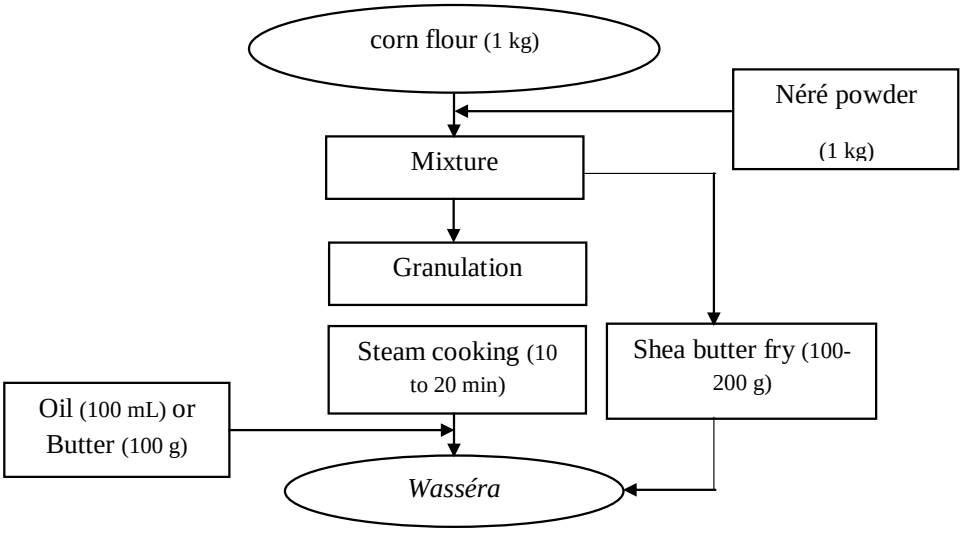

Figure 2. Production diagram of *Wassera*.
Source: Authors

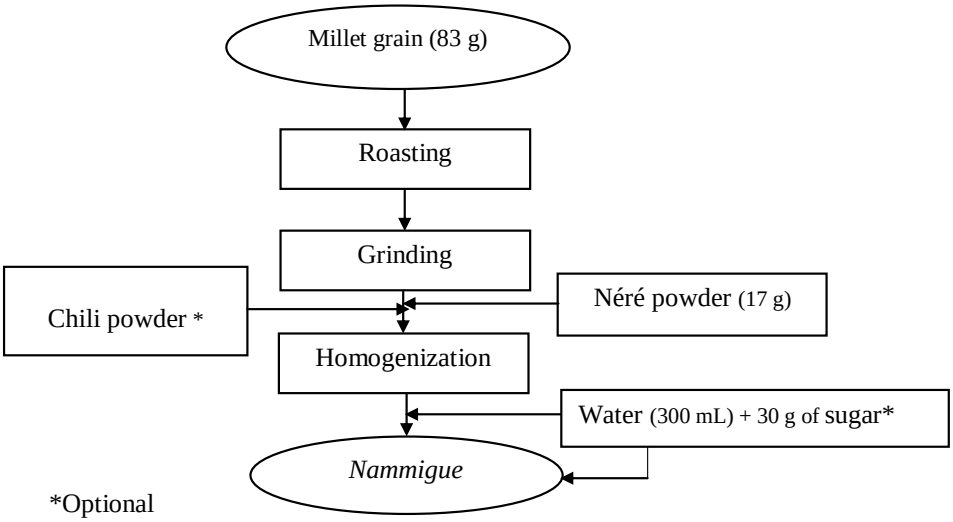

Figure 3. Production diagram of *Nammigue*.
Source: Authors

water was added and the whole was mixed and kneaded by hand for 10 to 15 min. The resulting dough was fried to obtain fritters in the form of patties (Figure 5).

Production of Cake au Oul

A mass of 700 g of butter was melted and mixed with 250 g of sugar and 5 to 10 g of baker's yeast. To this mixture, 6 to 7 fresh eggs and 0.5 L of water were added and the whole was stirred until homogeneous medium was obtained. To this medium, 1 kg of Néré powder and 500 g of durum wheat flour were gradually added. The final dough obtained was kneaded by hand for 10 to 15 min and then left to rest (incubation of 5 to 10 min) before cooking in the oven for 20 to 30 min (Figure 6).

Production of Nèrèdji

The production process of *Nèrèdji* is described in Figure 7. Ten grams of yellow Néré powder were diluted in 100 ml of water or milk and the mixture was homogenized. The resulting solution was consumed by adding a

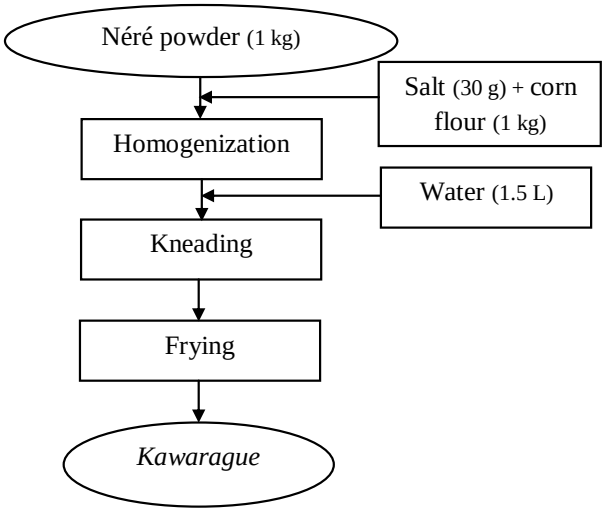

Figure 4. Production diagram of *Kawaragué*.
Source: Authors

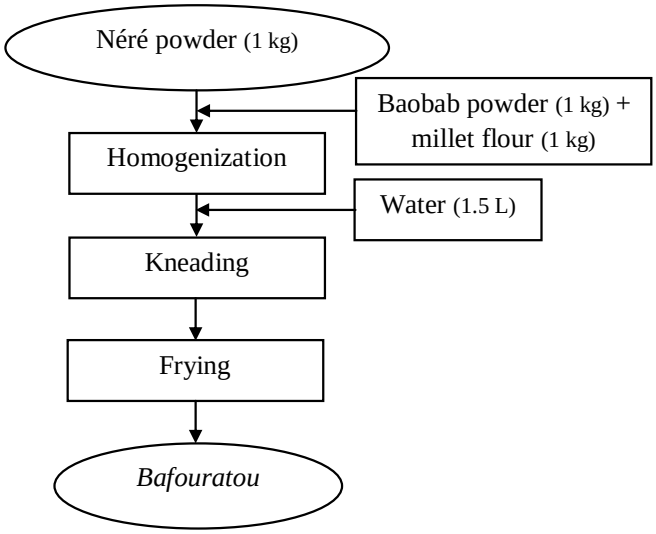

Figure 5. Production diagram of *Bafouratou*.
Source: Authors

tablespoon of honey or 5 g of sugar.

Classification of Néré-based foods

Among foods based on Néré, PCA showed that *Nammigue* and *Kawaragué* are specific to Korhogo, Séguéla and Bouna consumers. *Wasséra*, *Bafouratou* and *Cake au Oul* are specific to Bouna, while *Nérédji* is specific to Korhogo than Séguéla and Bouna (Figure 8).

Level of knowledge of Néré-based dishes

With a proportion of 36.79% *Nammigue* is a food that is moderately known in all the investigated localities where *Wasséra* (8.89%) tops the list of little-known foods (Table 5).

Periods and frequencies consumption

Data in Figure 9 reveal that *Nammigue* is the most consumed food with a frequency of 2 times/month followed by *Wasséra* with a frequency of consumption of 1 time/month. All dishes are consumed at any time except *Cake au Oul* which is consumed at breakfast. *Nammigue* (32.59%) is more consumed at lunch (Figure 10).

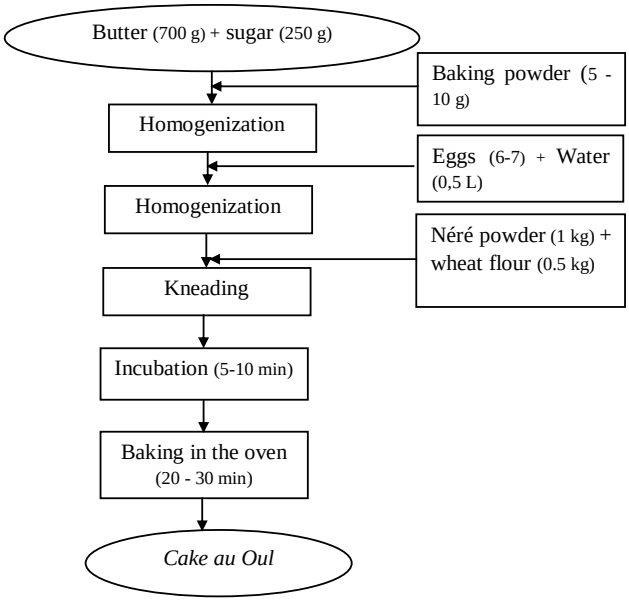

Figure 6. Production diagram of *Cake au Oul*.
Source: Authors

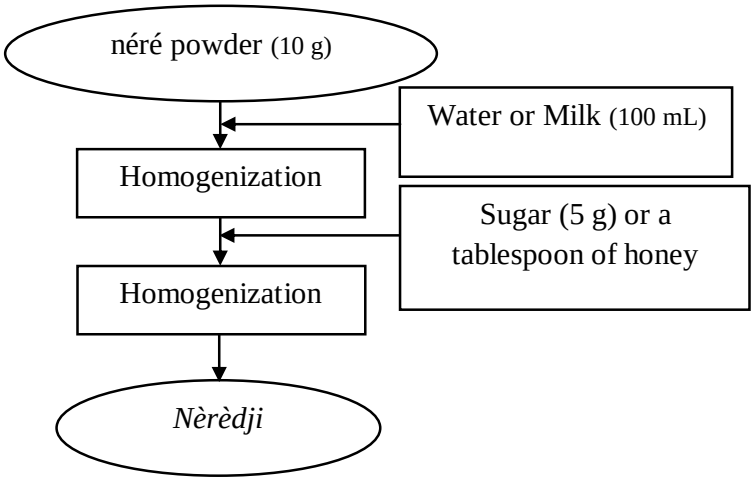

Figure 7. Production diagram of Nèrèdji.
Source: Authors

DISCUSSION

The study was conducted to identify the consumption practices of Néré pulp (*Parkia biglobosa* L.) in the departments of Korhogo, Séguéla and Bouna in northern Côte d'Ivoire. The data showed that six Néré-based dishes were identified (maize or millet couscous in Néré, maize fritters in Néré, cerelac in Néré, fritters in Néré, drinks in Néré and cakes in Néré) in the localities studied. However, the names of these foods differ according to locality/ethnic group. This diversity of foods produced from Néré pulp could reflect the importance of this fruit in the diet of rural populations. These foods would therefore contribute to the food security of these populations. These results corroborate the studies of Ouattara et al. (2016) which revealed the importance of wild fruits in human nutrition. Nyadanu et al. (2017) also confirmed the importance of *P. biglobosa* pulp in the diet of rural populations. According to these authors, Néré pulp is used as a subsistence food in times of food scarcity and is also used as infant food. While according to studies of Sackou et al. (2020) on food insecurity in the city of Abidjan, only 3.8% of households were food secure. Thus, the popularisation of these foods could contribute to the reduction of food insecurity in developing countries, particularly in Côte d'Ivoire. The identified Néré pulp-

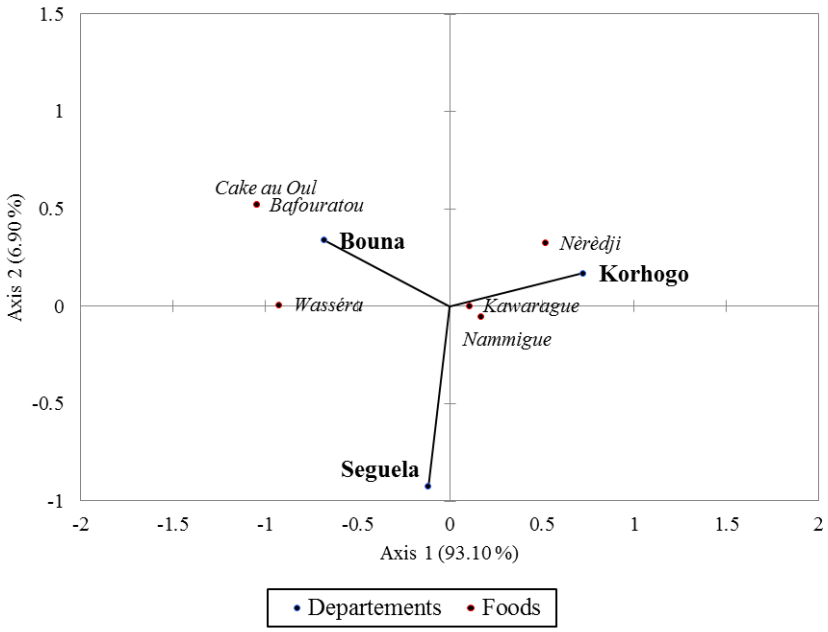

Figure 8. Factor analysis of Néré-based dishes.
Source: Authors

Table 5. Level of knowledge of Néré-based dishes.

Cowpea-based dish	Korhogo	Séguéla	Bouna	Number	Proportion (%)	Level of knowledge
Nammigue	78	21	50	149	36.79	Moderately known
Wasséra	0	6	30	36	8.89	
Nèrèdji	14	0	5	19	4.69	
Kawarague	8	2	6	16	3.95	Less known
Bafouratou	0	0	2	2	0.49	
Cake au Oul	0	0	1	1	0.25	

Source: Authors

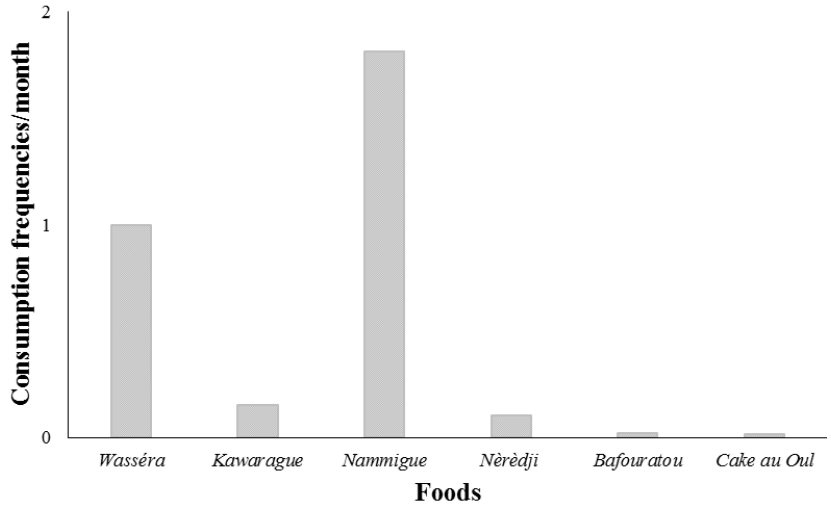

Figure 9. Consumption frequencies of Néré-based dishes.
Source: Authors

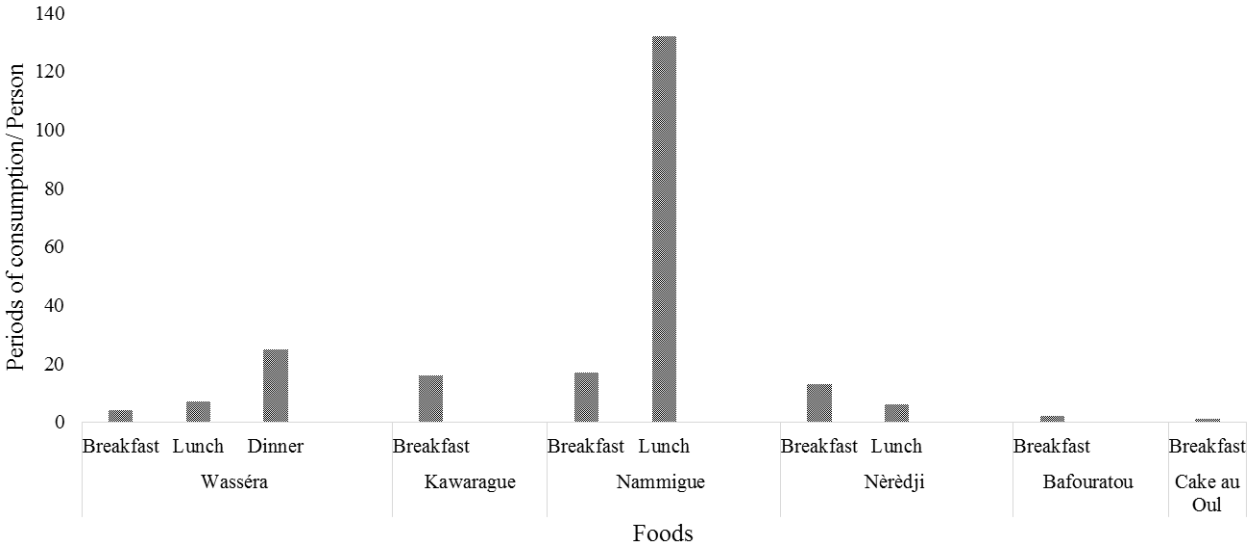

Figure 10. Periods of consumption of Néré-based foods.
Source: Authors

based foods are consumed more as meals than as drinks, with a meal/drink ratio of 5:1. Néré pulp is consumed as fritters, cakes, cereals and drinks. These different forms of food use in the form of drinks or meals are in line with those described by several authors such as Toure et al. (2020) and Vivien and Faure (2011). These authors confirm that the mealy pulp of the Néré fruit can be consumed raw, in jam or diluted in a liquid to prepare a sweet and refreshing drink. Similarly, Touré (2018) and Lamien et al. (2011) found that the flour of Néré fruit pulp mixed with cereal flour can be consumed in couscous, porridge, fritters and cakes. Studies of Arinola et al. (2019) also revealed that Néré pulp has appreciable dietary and functional properties, both in oil and water, that may qualify it for use in food. Five of the six foods identified are specific to each locality studied. It is believed that the specificity of these foods in each locality is related to differences in culture, customs and also eating habits of the populations. Al-Fatimi (2021) state that there is a strong relationship between local people and their environment, and that traditional knowledge is therefore passed on from generation to generation. Kruger and Gericke (2002) and Thurber et al. (2016) with a meal/drink ratio of 5:1. Cowpea pulp is consumed as fritters, cakes, cereals and drinks. These different forms of food use in the form of drinks or meals are in line with those described by several authors such as Touré (2020) and Vivien and Faure (2011). These authors confirm that the mealy pulp of the cowpea fruit can be consumed raw, in jam or diluted in a liquid to prepare a sweet and refreshing drink. Similarly, Touré (2018) and Lamien continue this same view by stating that it is evident that there is a divergence in the foods consumed, as culture is one of the important factors that influence consumer attitudes towards a given food. For the preparation of all these dishes, wild fruits are mainly collected in the field (92.86 to 98.25%). This result is explained by the fact that the surveys were conducted in rural areas and the abundance of this fruit in these localities. Indeed, Avana-Tientcheu et al. (2019) and Koura et al. (2013) confirm that in Côte d'Ivoire, savannah populations preserve the dwarf tree for its numerous virtues. Koné et al. (2020) make a similar observation on the biodiversity of plant species in the north of Côte d'Ivoire, showing that wild fruit trees such as the dwarf tree are protected by local populations because of their high ethnobotanical value. The use of the dwarf tree in the preparation of dishes is explained by the satisfaction of health problems and the search for particular tastes. In addition, the dwarf tree is considered an emergency food. This diversity in the reasons for consumption of this fruit in different localities could be related to the low level of information about the importance of this fruit. According to Soma Massieke et al. (2017), the lack of knowledge of all the benefits of the fruit may influence food uses. Indeed, as a health product, Néré pulp is used as a mild diuretic and purgative (Arbonnier, 2009; Vivien and Faure, 2011; Aubréville, 1950). It also has a beneficial effect on health due to its high content of flavonoids, which have antioxidant activity on hydroxyl radicals and free radicals. It can also prevent congestive heart failure due to its composition of cardiac glycosides (Arinola et al., 2019). These foods developed by these populations are most often consumed at any time of the day. However, with a consumption rate of 36.79%, Nammigue appears to be the best known and most consumed food in Néré, with a consumption frequency of about 2 times per month. This low consumption rate of the main staple food of Néré could be explained by the fact that Néré is not a food of

choice for consumers. This hypothesis is supported by the results of Lamien et al. (2011) which reveal that yellow Néré pulp is much more used only to cover the food deficit of some families during the lean season. This result could also be explained by the modernisation of the rural areas surveyed. It is in this vein that Muhammad et al. (2015) argue that modernisation disrupts and creates conflicts with the cultural traits of rural people by changing their lifestyles. According to Oryema et al. (2015), changing lifestyles promote neglect of wild food resources, thereby reducing their use. It is therefore important to encourage rural populations to consume these foods in order to reduce the rate of food insecurity in Côte d'Ivoire.

Conclusion

At the end of this study, six foods based on Néré were identified. The production processes and consumption patterns of these foods are largely cultural, with a clear preference for *Nammigue*, which is consumed more during the lean season. However, beyond their overall use during the lean season, the lack of knowledge of the biochemical composition of these foods is an obstacle to better dissemination.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Optimization of fermentation and malting process of sorghum beverage and effects on nutritional quality

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Sorghum is a nutritious and under-utilized cereal whose potential in development of nutritious ready-to-drink beverages remains unexplored. The objective of the study was to optimize malting and fermentation conditions to obtain a nutritionally superior new sorghum product. Four beverage formulations containing sweetened sorghum malt extracts were developed through fermentation using *kombucha* culture at different temperatures (between 20 and 35°C). The formulations were also malted for three, four and five days and analyzed for nutritional characteristics. The iron, total phenolics and vitamin C contents in the formulations ranged from 0.125 ± 0.292 to 2.956 ± 13.83 mg/100g, 0.1328 ± 0.00594 to 1.6601 ± 0.0640 mg GAE/100 ml and 4.3505 ± 0.2797 to 6.1304^{c} ± 0.2797 mg/100 ml respectively. Iron was used in the process optimization to select the best beverage which had high nutritional and sensory quality. The mean contents of iron in the beverages ranged from 1.25 ± 0.292 mg/kg (formulation 5D1) to 29.56 ± 13.83 mg/kg (formulation 4D). The best formulation was obtained from an optimum of four days malting and fermentation temperature of 25°C (4D). The findings indicate that nutritious beverages can be developed from sorghum by employing different malting days and fermentation conditions to come up with products having varying levels of iron, vitamin c and phenolics depending on your process or consumer needs.

Key words: Sorghum, kombucha, malting, fermentation.

INTRODUCTION

Sorghum is a major source of minerals, calories and proteins for many people in Africa and Asia (Hadebe et al., 2017). It has drought tolerance and is adapted to both tropical and subtropical ecosystems hence it is a very important subsistence crop (Amelework et al., 2016). In terms of cereal production in Kenya, it is ranked third after maize and wheat and it's a staple crop for many low income households (Kilambya and Witwer, 2013). It is the only indigenous cereal to Kenya, being cultivated even in areas considered poor in terms of potential for agriculture. This cereal has great potential to stimulate regional development and improve food security.

Sorghum in addition to being a protein and minerals source, has potential in functional constituents that promote health, such as fibers, B group of vitamins, waxes that lower cholesterol and antioxidant phenolics (Hassani et al., 2014). The composition and contents of starch in the grain are influenced by the grain's growth

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conditions (Hill et al., 2012). It usually ranges between 32.1 and 72.5 g/100 g with amylopectin being (81.0 to 96.5%) and amylose (3.5 to 19.0%) (Shegro et al., 2012). Due to starch granules, proteins and tannins being bound together strongly, it has the lowest starch digestibility among cereals (Mkandawire et al., 2013). Prolamins and non-prolamins are the main proteins present in the sorghum grain. Of the total protein composition, prolamins range between 77 to 82% (7-15 g/100 g) with glutelins, globulins and albumins occupying the other minor proportion (Mokrane et al., 2010).

The cereal's mineral content varies depending on area of cultivation with the minerals being phosphorus, potassium, iron and zinc. Their bioavailability is still unknown and availability of iron and zinc in the grain ranges from 6.6 to 15.7% and 9.7 to 17.1%, respectively (de Morais Cardoso et al., 2014). The phenolic acid content in some sorghum varieties varies from 135.5 to 479.40 mg/g with ferulic and protocatechuic acids as the major contributors with 120.5 to 173.5 mg/g and 150.3 to 178.2 mg/g, respectively (Afify et al., 2012). Tannins are a group of phenolic compounds and are found in many plants. It plays part in defense against pathogens and predators.

They reduce the availability of minerals, starch and proteins. Despite their anti-nutritional effect, they are better radical scavengers compared to other simple phenolics (Kaufman et al., 2013). Sorghum has a lot of health benefits some of which include alleviation of the negative effects brought about by cardiovascular disease, many chronic diseases and obesity (Salazar-López et al., 2018).

The lag in commercialization of sorghum compared to other cereals in Kenya is mainly due to grain prices that cannot compete with the other cereals levels of production which are low and which also vary, high costs of assembly and high costs of processing (Njagi et al., 2019). Production of sorghum is also conducted majorly by subsistence farmers who produce just enough for their domestic use and seldom excess for sale purposes. Thus, production limitations vary from conventional to commercial scales (Omoro, 2013).

Malting involves germination, under controlled conditions. Its main objective is modification of the chemical composition of the grain through mobilization of the endogenous enzymes, as a result, physical and rapid solubilization are enabled during brewing resulting in a nutritionally rich medium for yeast fermentation which produces carbon dioxide and ethanol (Taylor and Kruger, 2019). In hydrolysis of malt, the most important enzymes are alpha and beta amylases that cause production of fermentable maltose from starch (Taylor and Kruger, 2019).

Fermentation of cereals is usually aimed at preservation, which comes from acids production. The acids include lactic, acetic and propionic or alcohol production which is often combined with a reduction in water activity, safety enhancement of the final products by inhibition of pathogenic microorganisms, enhancement of sensory properties (color, aroma, texture and taste), nutritional value improvement by removal of anti-nutrients such as tannins, phytic acid, enzyme inhibitors and poly-phenols, bio-availability enhancement of some components of carbohydrates, indigestible poly and oligosaccharides reduction (Liptáková et al., 2017).

Epidemiological studies have shown that consumption of fermented foods leads to an improvement in health and a decline in the risk of disease contraction. Probiotics consumption in adequate doses can confer health benefits to the consumer (Rezac et al., 2018). The probiotics use carbohydrates that are available to produce short chain fatty acids, out-compete pathogens for resources, produce antimicrobial agents, they balance the immune system and also produce vitamins (Derrien et al., 2015).

In terms of sorghum beverages, in Kenya there's locally produced sorghum beer targeted at consumers who want to upgrade to bottled beers from illicit drinks (Orr et al., 2014), coffee substitutes from sorghum for those sensitive to caffeinated beverages (Omer and Abou-zaid, 2022). There is also the malt extract from sorghum which is a sweet wort which is rich in sugar and is also a beverage by itself, but can be made into a flavored syrup through concentration or powder by evaporation of the extract into a product which is dark-colored (Elgorashi et al., 2016). This beverage development will contribute to the sorghum value chain by adding onto the list of existing sorghum products.

MATERIALS AND METHODS

Study design

A completely randomized block design was used with malting and fermentation chosen as the blocks in the experimental design.

Sample collection and preparation

Sorghum *(Sorghum bicolor L. Moench)* purchased from Busia, finger millet (*Eleusine coracana*) from the local market in Kangemi, *kombucha* culture bought from Kombucha Kenya Company (a mushroom-like consortium of yeasts and acetic acid bacteria which are in a symbiotic relationship suspended in previously fermented broth), previously fermented *kombucha* broth and white sugar purchased from a local supermarket.

Methodology

Product development

Sorghum grains were malted for 3, 4 and 5 days after which malt extraction was conducted, resulting into malt extracts which were further subjected to 7 days fermentation at temperatures between 20 and 35°C by the SCOBY (Table 1). Resulting beverages were analyzed for their nutritional components and the nutrient which had a significant number of reported deficiencies nutritionally and also

Table 1. Experimental design used for this study.

Formulation	Malting temperatures and days	Fermentation temperatures and days	Culture (%)
1	20°C for 3 days	20°C, 7 days	2.4
2	20°C for 4 days	25°C, 7 days	2.4
3	20°C for 5 days	30°C, 7 days	2.4
4	20°C for 5 days	35°C, 7days	2.4

Equal sorghum and finger millet ratios were used in all formulations and also equal culture inoculation rates.
Source: Author

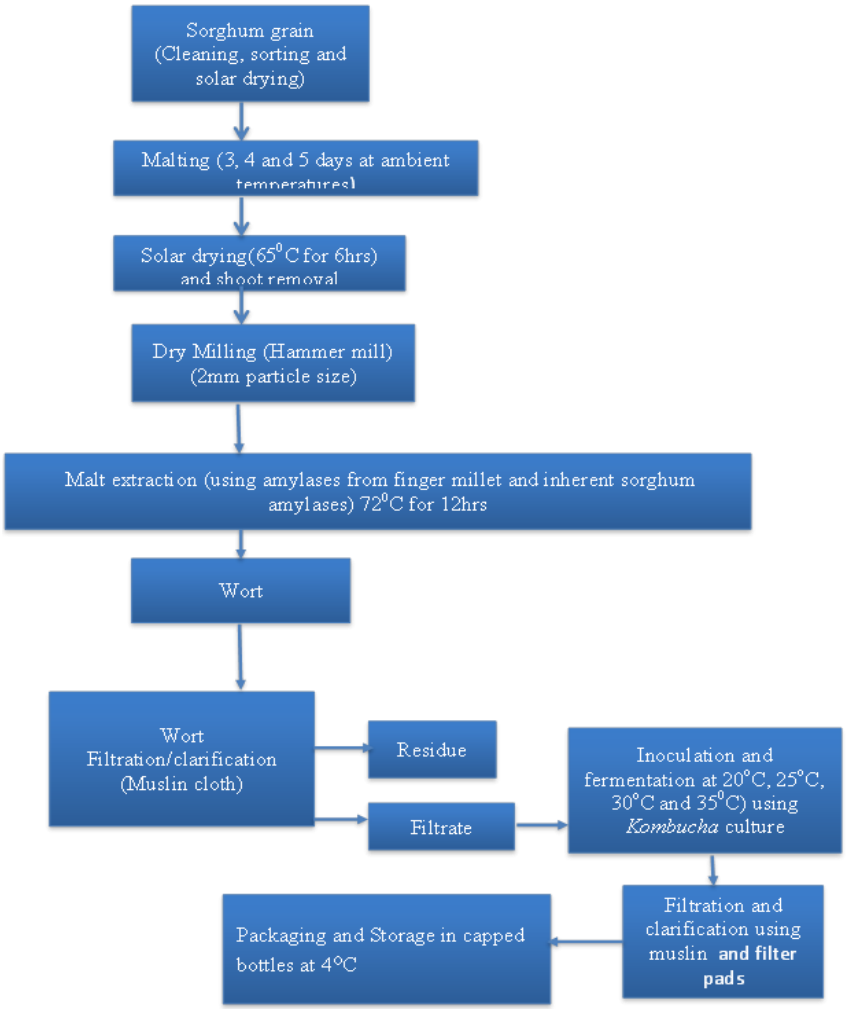

Figure 1. Process flow diagram for production of a ready to drink sorghum beverage.
Source: Author

had significant differences between the formulations was chosen as the determinant factor during the process optimization (Figure 1).

Malting

The method of Aluge et al. (2016) was used, with variations being applied in the germination days per formulation. Foreign matter was sorted out and grain soaking done in buckets with potable water for 12 h in the ratio 1:2. The grains were rid of water by draining then sprouted by spreading on wet blankets on malting trays on wooden benches and finally covered for the sprouting process. Daily sprinkling was done onto the grains and absolute ethanol sprayed onto the grains to prevent growth of mold. Oven drying (memmert oven supplied by GmbH and Co. Stavendamm22 model Schutzart DIN 40050-IP20) for 6 h at 65°C was done to the germinated grains. After drying, meshed trays were used to remove the shoots by rubbing the sprouts on them (Hassani et al., 2014).

Malt extraction and brewing

Slight modifications were applied to the hot water extraction method by Sarkodie et al. (2014). 2 L of portable water at ambient temperature was mixed with ½ a kilo of milled sorghum malt (supplemented with 40% malt from finger millet). The mash was left to sediment for 1 h. 40% (1 L) of the supernatant extract composed of enzymes was decanted and kept aside using calibrated jars. 30 min boiling in stainless steel cooking vessels of the remaining mash which was thick was conducted and the enzyme extract added after cooling.

The cooked mash was then incubated at 72 ± 1°C (memmert oven supplied by GmbH and Co. Stavendamm22 Schutzart DIN 40050-IP20) overnight for saccharification then cooled to room temperature and an adjustment made to the weight to reach 3 kg with distilled water. Filtration was done to the resulting wort using a muslin cloth then finally through filter pads.

Inoculation and fermentation

The methods according to Jayabalan et al. (2014) and Kumar and Joshi (2016) with slight modifications were used. 1 L of the cool sorghum malt sweetened with 100 g sucrose at ambient temperature was poured Into a wide mouthed clean vessel which had been sterilized with boiling water, 100 ml of previously fermented *kombucha was* added to prevent the growth of undesirable microbes by lowering the pH and a mat of the culture about 24 g placed on the surface of the infusion and the jar was hygienically covered using clean muslin cloths and properly fastened using rubber bands. The concentrates were placed at different incubation temperatures (between 20 to 30°C) for 7 days.

Analytical methods

Nutritional analysis

Protein: AOAC 2012 method 991.20 was used for determination of crude protein. Into a Kjedahl flask, 0.5 g of the accurately weighed samples was placed while folded in a nitrogen free filter paper. Sulphuric acid and a catalyst tablet were added to digest the sample in a fume chamber. Phenolphthalein indicator was used to indicate the end point before connection of the flask to a distillation unit. For back titration, 40% NaOH solution was used against 0.1N NaOH solution. The standard conversion factor used was 6.25.

Vitamin C: AOAC 967.21 (2006) was used whereby 5 ml of the test solution was titrated against prepared standard solution of ascorbic acid until the end point which was a faint pink color.

Iron and zinc: The method AOAC 999.11 according to 2006 AOAC was used. The beverage samples were subjected to ashing in a muffle furnace overnight and residue collected, acidified with nitric acid to remove acid soluble minerals and heated on a hot plate. The resulting clear solutions were diluted up to 100 ml in volumetric flasks with distilled water then subjected to Buck Scientific Atomic Absorption Spectrophotometer (Model 210VGP) to obtain the mineral content readings directly using the different cathode tubes made from the elements of interest (Fe and Zn).

Total sugars: Done according to Islam et al. (2013) with slight adjustments. 4 ml of anthrone reagent was added into an aliquot of pipetted 1 ml beverage extract in test tubes. This was cooled after boiling for ten minutes. Preparation of a reagent blank was done and it was treated the same way. The resulting solutions' absorbances were measured at 630 nm in a Perkin Elmer UV-VIS spectrophotometer model 166351. A glucose standard curve was also prepared and used in calculating the concentrations from absorbances obtained. Total sugar content per 100ml sample was calculated using the formula:

$$\% \text{ Total sugar} = \frac{\text{Quantity of sugar obtained}}{\text{Sample weight}} \times 100$$

Alcohol content determination

The procedure according to Park et al. (2004) was used. Distillation was performed to a final volume of 50 ml after filtration of 100 ml samples through a strainer. Distilled water was used to readjust the distillate to 100 ml. An alcohol hydrometer was used to determine the strength at room temperature.

Calorific value

This was done according to Mohammed et al. (2011). Calculations of calorific value were done using the Atwater factors: 4 Kcal/g for carbohydrates, 4 Kcal/g for protein, 9 Kcal/g for fat and 7 kcal/g for alcohol.

Total phenolics

Done according to Singleton et al. (1999). The Folin-Ciocalteu method was used with some modifications. 50 ul of the diluted sample was mixed with Folin-Ciocalteu reagent (100 µl) and deionized water was used as the diluent and control. Final dilution was done to a total volume of 1,150 ul with deionized water and mixed thoroughly. 10 min incubation at room temperature was done then 500 µl of 20% Na_2CO_3 solution added with mixing immediately and this was further incubated for 2 h at room temperature. Absorbance was recorded at 765 nm with all samples being measured in duplicate. Gallic acid (1 mg/ml) was used as the standard and the quantification of total phenolic compounds was done in milligrams per 100 ml gallic acid equivalents (mg GAE/100 ml).

Tannins determination

The method according to Adeyeye et al. (2019) was used. 1 ml sample was weighed and soaked with a solvent mixture 100 ml with acetic acid and acetone in the ratio 1:4 respectively for 5 h so as to extract the tannins. The samples were filtered and absorbance of the filtrate determined using a Perkin Elmer UV-VIS spectrophotometer model 166351 according to AOAC. A calibration curve for the standard (tannic acid) was prepared.

Statistical analysis

Data obtained was analyzed using one-way ANOVA on Genstat statistical software version 15.1. Means obtained were compared using least significant difference at 5% under Tukey test.

RESULTS

Nutritional quality of developed sorghum beverage

Most nutritional components in the raw materials were

Table 2. Average means obtained for the different nutrients on analysis of the raw sorghum (60%) and finger millet (40%) composite flour before development of the beverage.

Protein (%)	Total sugar (%)	Calories [kCal/100 g)]	Iron (mg/100 g)	Zinc (mg/100 g)	Tannin (mg/100g)	Total phenolics (GAE/100g)
8.44±0.34	74.55±0.44	331.95±0.80	60.66±0.75	20.88±0.94	105.66±0.58	0.83±0.134

Source: Author

Table 3. Means obtained for the different nutrients present in the beverages on blending the raw finger millet (40%) and raw sorghum (60%).

Beverage formulation	Protein (g/100 ml)	Total sugars (%)	Alcohol (%)	Calories (Kcal/ml)	Vitamin C (mg/100 ml)	Iron (g/l)	Zinc (g/l)
1	3.28^{a}±0.06	2.47^{c}±0.001	ND	23.00^{b}±0.21	5.04^{b}±0.14	13.31^{b}±1.37	1.51^{a}±0.08
2	3.63^{a}±0.56	1.99^{b}±0.001	ND	22.48^{b}±2.17	4.35^{a}±0.28	29.56^{c}±13.83	2.11^{a}±0.84
3	3.63^{a}±0.56	2.02^{b}±0.001	ND	22.59^{b}±2.20	6.13^{c}±0.28	1.25^{a}±0.29	1.21^{a}±0.59
4	2.01^{a}±0.62	1.42^{a}±0.001	ND	13.19^{a}±1.65	4.65^{a}±0.14	1.32^{a}±0.26	0.70^{a}±0.36

Mean values with common superscript letters in a column indicate no significant difference among samples (P>0.05) from Tukey's mean test. ND - not detected
Source: Author

significantly increased while the non-nutritional components and sugars were reduced after processing. There was no significant difference between the protein contents of the developed beverages (Tables 2 and 3).

Total sugars varied significantly between the different formulations being highest in beverage formulation 1. This could probably be due to the limited number of malting days such that sugars had not been used up a lot by the germinating seedling compared to the other formulations which have lesser sugars as the days of malting increase, the least being beverage formulation 4 (Tables 2 and 3).

For iron and zinc, there were significant differences between the formulations and the trend was similar for both nutrients. The mineral contents increased with days of malting and fermentation temperatures up to the fourth day then decreased sharply. For both minerals, formulation 2 had the highest concentrations, with 3 and 4 having the least concentrations that were not of significant difference between the two. From the above statistics, it is evident that formulation 2 (Four days malting and one week fermentation at 25°C) is superior in terms of the content of iron, which increases with days of malting and temperature of fermentation up to the four day of malting and 25°C fermentation then subsequently decreases with increase in both factors. Zinc had no significant difference between the different formulations (Table 2).

In terms of vitamin C content, formulation 3 (five days malting and one week fermentation at 35°C) was the most superior while 2 and 4 had the least quantities (Table 2).

Non nutritional composition of developed sorghum beverage

There was a significant difference in the quantities of tannins between the different sorghum beverage formulations with formulation 1 having the highest tannin contents (40 g/100 g) and Formulation 1 had the highest total phenolic content (1.66 mg/100 g). They were highest in formulation 3D and lowest in 4D and 5D2. The malting days for the cereals to have less tannin were 4 days as from the values obtained (Table 4).

For phenolics, despite significant differences between the formulations, the trend was not well defined since 4D and 5D1 had the least phenolics content and 5D2 the highest. Formulation 4 (5 days malting and 1 week fermentation at 35°C) is characterized by the highest total phenolics content (Table 4).

Malting effects on nutritional quality of developed sorghum beverages

Malting or fermentation as treatments yields nutritionally superior products. Fermentation specifically, has been used to improve the yield of different bioprocesses (Das et al., 2014). The contributions of malting to the different nutrients can be seen especially on tannins and iron content on which there are significant differences between the samples. The fermentation temperatures used also cause significant differences in the quantity of sugar, calories, vitamin c, iron, phenolics and tannins (Table 5). From the data processing on statistical

Table 4. Mean total phenolics and tannin contents of the different formulations.

Beverage formulation	Tannins (mg/100 g)	Total phenolics (GAE/100 ml)
1	40.08^{c}±0.26	0.35^{b}±0.01
2	1.29^{a}±0.04	0.13^{a}±0.05
3	2.17^{b}±0.07	0.17^{a}±0.02
4	1.31^{a}±0.07	1.66^{c}±0.06

Mean values with common superscript letters in the same column indicate no significant differences among the formulations (P>0.05) from Tukey's mean test.
Source: Author

Table 5. Effect of fermentation temperatures on the nutritional quality.

Temperature	Vitamin C	Phenolics	Tannins	Calories	Iron	Protein	Zinc	Sugar
30	6.13^{b}	0.17^{a}	2.17^{b}	22.59^{b}	1.25^{a}	3.63^{a}	1.12^{a}	2.02^{b}
35	4.65^{a}	1.66^{c}	1.31^{a}	13.19^{a}	1.32^{a}	2.01^{a}	0.70^{a}	1.42^{a}
20	5.04^{a}	0.35^{b}	40.08^{c}	23.001^{b}	13.31^{ab}	3.28^{a}	1.51^{a}	2.47^{c}
25	4.35^{a}	0.13^{a}	1.29^{a}	22.49^{b}	29.56^{b}	3.63^{a}	2.11^{a}	1.99^{b}
P	0.001	<0.0001	<0.0001	0.013	0.041	0.085	0.204	0
Significant	Yes	Yes	Yes	Yes	Yes	No	No	Yes

Source: Author

Table 6. Effect of malting days on the nutritional quality.

Malting days	Vitamin C	Phenolics	Tannins	Calories	Iron	Protein	Zinc	Sugar
5	5.39^{a}	0.92^{a}	1.74^{a}	17.89^{a}	1.28^{a}	2.82^{a}	0.91^{a}	1.72^{a}
4	4.35^{a}	0.13^{a}	1.29^{a}	22.49^{a}	29.56^{b}	3.63^{a}	2.11^{a}	1.99^{a}
3	5.04^{a}	0.35^{a}	40.08^{b}	23.01^{a}	13.31^{a}	3.28^{a}	1.51^{a}	2.47^{a}
P	0.309	0.407	<0.0001	0.381	0.009	0.566	0.105	0.061
Significant	No	No	Yes	No	Yes	No	No	No

Source: Author

software, there was no interaction between fermentation temperatures and the days of malting owing to the experimental design used.

Effects of fermentation on nutritional quality of developed sorghum beverages

Fermentation temperatures used were found to have a significant effect ($p<0.05$) on the levels of vitamin c, phenolics, tannins, calories, iron and sugars and no effect ($p>0.05$) on protein and zinc contents.

The number of malting days used were also found to have a significant effect ($p<0.05$) on the levels of tannins and iron content. There was no significant effect ($p>0.05$) on the levels of vitamin C, phenolics, calories, protein, zinc and sugars (Table 6).

DISCUSSION

Compared to most of the beverages currently in the market, the protein content of the developed beverages is higher and can contribute towards meeting the daily protein requirements. Protein content forms an important basis for the quality of a beverage. The interactions among proteins, amino acids and phenols greatly influence the stability and organoleptic characteristics of the beverage. The amino acids also form a great component of the beverage's aromatic compounds. There was increase in protein content with increase in fermentation period which can be attributed to an increase in microbial mass (Correia et al., 2010). This could be supported by the favorable pH for the growth of lactic acid bacteria with the progress of the fermentation which in turn could cause extensive

hydrolysis of the protein molecules to amino acid and other simple peptides (Das et al., 2014). It is also worth noting that increased fermentation time yielded lower protein content. This is because the fermenting microorganisms also uses amino acid which could lower the protein content and quality of some fermented food (Pranoto et al., 2013).

Sugars form an important part for calories provision in an individual. Beverage F1 had the highest sugar content. The initial days of malting facilitated the enzymatic breakdown of carbohydrates into simple sugars through activation of endogenous enzymes such as α-amylase thereby improving digestibility as a result of degradation of starch to provide energy for the seed development (Nkhata et al., 2018). The rest of the beverage formulations had decreased sugars, an occurrence that can be linked to the malting periods. In earlier stage of germination, large portions of soluble sugars are expected to be used up during respiration and not enough α-amylase has been synthesized or activated to hydrolyze starch, leading to less increase in sugars (Okolo et al., 2020). However, after 36 to 48 h of germination, the dormancy is lost as the amylolytic enzymes synthesized in the aleurone layer migrate into the endosperm and initiate the hydrolysis of starch granules. Glucose and fructose levels are generally low in the raw cereals at this moment, however, on germination, the two soluble sugars increase significantly such that their levels supersede that of sucrose activation of invertase which hydrolyzes sucrose into glucose and fructose during germination (Oseguera-Toledo et al., 2020). This explains the gradual increase then decrease to a further increase in sugar content in the formulations depending on malting periods. Calories were not significantly different between formulations 1, 2 and 3 while 4 had the least calories due to also having the least sugars.

Cereals have most of the nutritional elements bound. Malting ensures the bound mineral components are released. This explains the increase in mineral content (iron and zinc) up to day 4. The increase could be due to leaching of the anti-nutritional factors that bind the minerals. It has been hypothesized that the remarkable increase in phytase activity during germination helps reduce phytic acids, which bind minerals subsequently leading to increased mineral availability (Nkhata et al., 2018). After day four, the bound elements have been released hence accounting for the sharp decrease.

The initial increase in tannin content could be attributed to hydrolysis of condensed tannins such as proanthocyanidin. While the eventual decrease may be due to their binding with cotyledon endosperm that are usually undetected by routine method due to their insolubility in solvent or may be due to microbial phenyl oxidase action as explained by (Osman, 2011).

Phenolic content increased with increasing days of fermentation, a factor which may be attributed to an increase in the level of free soluble phenolics, due to hydrolysis of the glycosidic bonds of bound phenolics by hydrolytic enzymes secreted by microorganisms in the culture (Elkhalifa and Bernhardt, 2018). Phenolic compounds provide the antioxidant compounds in a beverage. These phenolic compounds have several functional properties in the beverage and influence its colloidal stability, flavor and color (Adebo and Medina-Meza, 2020). Phenolic compounds are also important antioxidants, and owing to this antioxidant capacity and low alcoholic content, consumption of beer helps to improve the plasma antioxidant activity and reduce the risk of cardiovascular diseases (Das et al., 2014). For phenolics, they affect the taste of products especially when they are very high in concentration. They were highest in this formulation due to increased number of malting days as suggested by Carciochi et al. (2016) and also fermentation temperature (Aguilar et al., 2019).

For vitamin C, consumption of 300 ml of either of the four beverages is enough to meet the daily requirements of the nutrient. Osman (2011) confirmed that malting and fermentation increase the quantity of vitamin C. Vitamin C can be synthesized during malting by the hydrolysis of starch using amylases and diastases that avail glucose for this process. This enhanced content of glucose is the one that acts as a precursor to formation of vitamin c. This study confirmed that C-6 of glucose could be oxidized to form the carboxyl carbon of the ascorbic acid concluded that the same could happen in plants during fermentation or malting.

For process optimization, iron content was chosen as the standard due to the fact that previous studies have shown that phenolics can contribute to bitter taste especially when in exceeding amounts, for example in olive oil as suggested by Shahidi and Ambigaipalan (2015) despite them having health benefits against cancer and cardiovascular diseases. Based on the recommended dietary allowances issued by WHO (8 mg/day), this beverage will easily meet the requirements of all individuals. Formulation 4D is well balanced in terms of phenolics which are lower in amounts compared to the rest hence the issue of bitterness may not be present and also in terms of the content of vitamin C it contains an amount which is almost similar to the other formulations when the means are compared.

Conclusion

It is quite interesting to note that varying the number of malting days and use of different fermentation temperatures can lead to considerable differences nutritionally in the final cereal products despite similarities in certain nutrient compositions as in the above scenario. The best formulation for the beverage development for good nutritional output is four days malting at 25°C fermentation temperature for one week. Sensory analysis

output can also be used for optimization purposes then the products during commercialization can be improved nutritionally via fortification as is done by most food industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Microbial quality and visual appearance of traditional baobab fruit nectar during storage

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In Senegal, traditional baobab (*Adansonia digitata* L.) fruit nectar (T-BFN) is the most popular drink from baobab fruit pulp and its consumption helps to fight poverty, malnutrition and generate income along the Senegalese value chain of non-timbered forest products. However, for a better competitiveness in the local market, traditional baobab fruit nectar must be microbiologically stable with an attractive visual aspect. The aim of this study is to evaluate the microbial quality during storage, and the impact of some treatments like homogenization on the visual appearance of traditional baobab fruit nectar processed in Senegal. The microbial shelf-life of pasteurized traditional baobab fruit nectar and the effect of homogenization at 0, 5, 8, 12, 13 and 14 MPa on the stability of visual appearance during storage have been studied. Pasteurized traditional baobab fruit nectar could be stored up to 190 days at 4°C without microbial spoilage. Homogenization at 14MPa stabilized the visual appearance of traditional baobab fruit nectar without sedimentation of the pulp or clarification for days at 4°C. In combination with pasteurization, homogenization may be addressed as an effective tool to prevent pulp sedimentation in traditional bottled baobab fruit nectar.

Key words: Baobab fruit nectar, microbial quality, visual appearance, storage, homogenization pressure, pulp sedimentation.

INTRODUCTION

In Senegal, traditional baobab (*Adansonia digitata* L.) fruit nectar (T-BFN) is the most popular drink from baobab fruit pulp. It is obtained by adding water (hot or fresh) to whole seeds, sugar, citric acid, milk or flavor generally according to consumer preference (Cissé et al., 2009; Diop Ndiaye et al., 2013; Maptouom et al., 2020; Cissé et al., 2021). It is often home made for self-consumption or processed at artisanal and sometimes at semi-industrial level. It is very popular and is found in all places of mass consumption, be it family and religious ceremonies, in hotels and restaurants, markets, supermarkets or in the street (Cissé et al., 2009).

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With an average registered production of 3 000 tons/year, baobab fruit is one of the most important non-timber forest fruits exploited in Senegal (DEFCCS, 2019). Baobab fruit pulp is rich in sugar (20-32%), calcium (254-655 mg/100g) and pectin (Haddad, 2000; Cissé et al., 2009; De Caluwé et al., 2010; Kaboré et al., 2011; Vincent et al., 2020; Adedokun et al., 2022). From the study of Cissé (2007), baobab fruit pulp has also an interesting antioxidant capacity around 88 µmol trolox/g due to its richness in Vitamin C and phenolic compounds. It is also a good source of fibers with prebiotics-like activity with respectively 22.5 and 22.04% dry weight for soluble and insoluble fibers (Manfredini, 2002; Kaboré et al., 2011). Several studies have been reported on biological properties of baobab fruit pulp like anti-pyretic, analgesic, antibacterial, anti-inflammatory, antioxidant, antiviral, etc. (Baky et al., 2021).

Therefore, the consumption of baobab fruit pulp and derived products will be helpful to fight poverty, malnutrition and generate income along the Senegalese value chain of non-timbered forest products.

Due to their high-water content and biochemical composition, fruit beverages are attractive medium for microbial growth, which could lead to various food borne diseases or changes in sensory quality (Khan et al., 2015; Kregiel, 2015; Fowoyo and Amadi, 2021). Microbial spoilage reduces the shelf-life of fruit beverages especially if the processing conditions are not controlled (Dudez et al., 2000; Kregiel, 2015). In Senegal, traditional fruit beverages, particularly T-BFN sold in the local market are often made at artisanal level by informal processors or at micro, small and medium scales. In most cases, Good Manufactured Practice (GMP) and Good Hygienic Practice (GHP) are not applied. In addition, few data are available on microbial shelf-life and storage conditions of T-BFN for informal and small and medium scale beverage processors who do not have enough money to control the microbial quality of products. Cissé et al. (2009) did accelerated aging test for 8 days at 37°C of T-BFN pasteurized at 80 ºC/10 min and 90ºC/5 min. They carried out stability test over several weeks to assess the stability of pasteurized T-BFN. James et al. (2022) studied the microbial contamination in processed baobab products in Kenya but their works focused on baobab candies and pulp. In this study of the microbial shelf-life of local fruit drinks in general and T-BFN in particular, there was an urgent need to improve their quality and ensure consumers' safety.

On the other hand, if microbial spoilage made fruit drinks unfit for human consumption, the visual aspect of the beverages is also a relevant parameter that contributed to the consumer's purchase decision (Da Silva et al., 2019). In Senegal, local fruit- beverages like T-BFN packed in bottles (plastic or glass) exhibited a fast decantation during storage.

In fact, fruit juices and beverages exhibited a rapid sedimentation as a result of particles movements that aggregated under gravity to form sediment (Sallaram et al., 2014; Da Silva et al., 2019). This technological problem due to the natural composition of fruits beverages contributed to the low competitiveness of local fruit drinks in Senegalese market and has to be addressed in order to propose solutions especially for informal and small and medium-scale beverage processors. Since local fruit drinks are not attractive in bottle, local consumers prefer imported drinks that are better in appearance even if they are not always better in terms of nutritional quality. To improve the physical stability of fruits drinks, reducing the size of suspended particles and/or increasing viscosity are common methods (Da Silva et al., 2019).

Studies have been found in the literature concerning the prevention of decantation in different fruit beverages like cupuaçu juice (Da Silva et al., 2019) or apple juice (Gössinger et al., 2018); but no data are available on traditional baobab fruit nectar.

To be more competitive in the local market, traditional baobab fruit nectar must be microbiologically stable with an attractive visual aspect. Scientific data on microbial stability and visual aspect over time must be produced to improve our knowledge but also to allow informal and micro, small, medium scale beverage processors to have technological information to improve their products. Improving the visual appearance of traditional fruit drinks can increase the market access and reduce the level of imported drinks. Therefore, the aim of this study is to evaluate the microbial quality and the impact of homogenization on the visual appearance of traditional baobab fruit nectar processed in Senegal.

MATERIALS AND METHODS

Baobab fruits seeds were purchased at the wholesale fruit market (Sandica, Pikine), in Dakar, Senegal.

Preparation of traditional baobab fruit nectar for microbial analyses

Products were prepared according to preparation methods standardized by the Institute of Food Technology in Dakar (Diop Ndiaye et al., 2013) and in compliance with the Senegalese standard on Guava, Baobab, Ditax and Mango nectars, which is equivalent to the General Standard for Fruit Juices and Nectars (CODEX STAN 247-2005). Baobab fruit pulp was extracted by adding hot water to the baobab seeds (7 l/kg). After kneading, sieving and refining, the baobab pulp extract (3°Brix; pH 2.8) was mixed with sugar (130 g/l) and citric acid (2 g/l). After preparation, T-BFN was pasteurized at 95°C for 2 min with a three-stage electric pasteurizer operating at a maximum rate flow of 150L/h (Gilson-Pierre-et-fills, France). Pasteurized T-BFN was then hot filled in 250 ml aluminized bags that were kept five minutes before cooling with cold water to ensure the auto pasteurization of packaging. Pasteurized samples were divided into two batches; one batch stored at room temperature (25°C) and another in the refrigerator (4°C) for a maximum total period of 190 days. At the time of microbial control, three T-BFN packs of 250 ml each were taken at random from each storage batch.

Unpasteurized T-BFN was collected aseptically, just after preparation, in sterile bottles. It was transferred to the laboratory and analysed just once to see the initial contamination before pasteurization.

Microbial quality of T-BFN during storage

Microbiological analyses were carried out according to classical AFNOR (2002) standards methods. Since the products to be analysed are liquid, each test sample represented a stock suspension. Samples were transferred into sterile closed containers and gently homogenized with hand before performing a 10-fold serial dilution (from 10^{-1} to 10^{-6}) under aseptic conditions in tryptone broth sterile salt (BIOKAR). For each serial dilution, inoculation was done in two petri dishes and each beverage sample was analysed three times. The following germs were enumerated: Total aerobic bacteria at 30ºC (TAB); yeast and moulds (YM); Thermo tolerant coliforms (TtC); Coagulase-positive pathogenic *Staphylococci* (CppS); Sulfite-reducing *Clostridium* (SrC); *Bacillus cereus* (Bc); *Salmonell*a spp. (S) and Mesophilic *Lactobacillus* (ML).

Standard enumeration methods by counting bacterial colonies on agar culture media were used. For this purpose, specific culture media were used to highlight the species of bacteria sought.

Microbial quality of resultant nectar

1) For TAB enumeration, 1 ml of the decimal dilutions was inoculated in plate count agar medium (PCA, HIMEDIA) under aerobic conditions and incubated at 30°C for 72 h ± 3 h according to ISO 4833-1 (2013).
2) YM were enumerated after inoculation of 1 ml of samples in yeast extract, glucose and chloramphenicol agar (YGC, LD) under aerobic conditions and incubated at 25°C for 3 - 5 days (NF V08-059, 2002).
3) For ML enumeration, inoculation (1 ml sample) was done in depth on Man Rogosa and Sharpe agar medium (MRS, CONDA), poured into two petri dishes. After the mixture solidified, a second layer of the same medium was used to cover the medium. Plates were incubated at 30°C for 24 to 48 h (NF ISO 15214, 1998).

Hygienic status during nectar production

1) For the enumeration of TtC, 1 ml sample was inoculated in depth on bile, crystal violet, neutral red lactose (VRBL, BIO-RAD) agar medium into two petri dishes (double layer technique) and incubated at 44°C for 24 h (NF V 08-060, 2009).
2) For the detection of CppS, 1 mL sample was used for enrichment on hyper salted lactose broth (Chapman broth). After that, isolation was carried out on Baird Parker's (LD) selective glucose medium. Petri dishes were incubated under aerobic conditions at 37°C for 24 to 48 h. To perform the coagulase test, a portion of each selected colony was removed and inoculated in sterile tube containing heart-brain broth. After incubation at 37°C for 24 h ± 2 h, 0.1 ml of each culture was aseptically added to 0.3 ml of rabbit plasma in sterile hemolysis tubes and incubated at 37°C for 4-6 h. The reaction is considered positive when the coagulum occupies at least half the volume initially occupied by the liquid (ISO 6888-1, 1999).
3) SrC was detected by inoculating 1 mL sample in two tubes in which 20 ml of tryptose sulfite cycloserine agar (TSC, SCHARLEAU) was added. Incubation was performed at 37°C under anaerobic conditions for 20 h ± 2 h.
4) For the enumeration of Bc*,* 0.1 ml of sample was inoculated on solid selective *Bacillus cereus* medium (SCHARLEAU) and incubated at 30°C under aerobic conditions for 18 to 24 h and then 48h (NF EN ISO 7932, 2005).
5) The detection of *Salmonella* requires four successive phases. A pre-enrichment stock suspension (25 ml of T-BFN in 225 ml of buffered peptone water) was homogenized using a Stomacher and incubated at 37°C for 16 to 20 h (De Smedt et al., 1986). After that, 0.1 and 2 ml of the pre-enrichment culture were transferred into two different tubes containing respectively 10 ml of Rappaport-Vassiliadis broth and 20 ml of selenite-cystine medium. The two media were then incubated respectively at 42 and 37°C for 18-24h. Isolation was done on the selective solid medium by the streak method. Incubation was done at 37°C for 24 to 48h. Colonies presumed to be *Salmonell*a were isolated and purified on nutrient agar (ISO 6579-1, 2017).

The results were expressed in Log_{10} CFU/ml as the number of colony-forming units per milliliter of traditional baobab fruit nectar.

Effect of homogenization pressures on the visual aspect of T-BFN during storage

T-BFN was prepared and pasteurized as previously. After hot water extraction (7 l/kg), kneading, sieving, refining and formulation (130g/l sugar cane and 2 g/l citric acid), T-BFN was pasteurized at 95°C for 2 min with a three-stage electric pasteurizer operating at a maximum rate flow of 150 l/h (Gilson-Pierre-et-fils, France). The pasteurized traditional baobab fruit nectar was homogenized in a continuous process by connecting the outlet of the pasteurizer to the inlet of the homogenizer to avoid contamination after thermal treatment. The following homogenization pressures were applied to the pasteurized T-BFN: 0, 5, 8, 12, 13 and 14 MPa using an ALM2 Pierre Guérin homogenizer (France). After homogenization, T-BFN was packed into 250 ml cleaned plastic bottles and stored at 4°C. The impact of the homogenization pressures was evaluated directly by visual observation of the presence of the serum phase and pulp sedimentation. Photographs were taken using a Lumix camera.

Statistical analysis

All statistical analyses were performed using SPSS 20.0 (IBM stats software). The Student-Newman-Keuls (SNK) test was used to determine the difference at α=0.05.

RESULTS AND DISCUSSION

Microbial quality of traditional baobab fruit nectar during storage

Table 1 presents the microbial composition expressed in Log_{10} CFU/ml of pasteurized traditional baobab fruit nectar during storage at 4°C and 25°C between 0 (zero time) and 190 days. In the absence of microbiological criteria in the Senegalese standards applicable to local fruit juices, the results were compared to the microbiological criteria of foreign references such as the guidelines for interpretation (F0-54, 2018) and the Federation of Commerce and Distribution Criteria (FCD, 2019) as well as with data from the literature on similar products.

Indeed, in the Senegalese standard for fruit juices and nectars, it is only mentioned that baobab nectar must be free of microorganisms capable of growing under normal storage conditions and must not contain any substance originating from microorganisms in quantities that could

Table 1. Microbial composition of traditional baobab fruit nectar during storage at 4 and 25°C.

Microbiological parameters (Log_{10}CFU/ml)	Traditional baobab fruit nectar						
	Unpasteuri zed	4°C				25°C	
		Zero time	34D	62D	190D	0D	34D
Aerobic mesophilic counts at 30ºC	1.41	1.24^{aX} (0.017)	2.24^{bY} (0.04)	2.03^{bc} (0.15)	1.81^{c} (0.36)	1.24^{aX} (0.017)	5.43^{bZ} (0.03)
Yeast and molds	ND	ND	ND	ND	ND	ND	5.3^{a} (0.012)
Thermo tolerant coliforms	ND	ND	ND	ND	ND	ND	ND
Coagulase-positive Pathogenic *Staphylococci*	ND	ND	ND	ND	ND	ND	ND
Sulfite-reducing *Clostridium*	ND	ND	ND	ND	ND	ND	ND
Bacillus cereus (LOG_{10}CFU/0.1mL)	ND	ND	ND	ND	ND	ND	ND
Mesophilic Lactobacillus	ND	ND	ND	ND	ND	ND	ND
Salmonella (absence in 25 g)	ND	NA	NA	NA	NA	NA	NA

Means values ± standard deviation of three samples. Different letters in miniscule, denote significant differences between the storage times for the same storage temperature. Different letters in majuscule denote significant differences between storage temperatures for the same storage time.
Source: Authors

present a health risk without specifying microbiological criteria (NS 03-92, 2009; NS 03-96, 2009). However, the standard recommended that baobab nectar should be prepared in accordance with the Recommended International Code of Hygienic Practice for Canned Fruits and Vegetables and the general principles of food hygiene recommended by the Codex Alimentarius Commission (CAC/GL 21, 1997; CODEX STAN 247, 2005).

Depending on the type of ingredient, the level of bacterial population in fruits and vegetables ranged from 3 to 7 log_{10} CFU/g (Pla et al., 2005; Abadias et al., 2008; Korir et al., 2016; Krahulcová et al., 2021). Raw foods containing a number of spoilage microorganisms aerobic plate count at 21.1°C less than 4 log_{10} CFU/ml are rated as good and are safe for consumption (Buyukunal et al., 2015; Khadka et al., 2017). For non-bottled drinks, the number of foodborne pathogens such as *E. coli*, *Staphylococcus aureus* and *Clostridium perfringens* must be less than 2 log_{10} CFU/ml while *Salmonella* spp. must be not be detected in 25ml (CFS, 2014). In ready-to-eat food in general, *Clostridium perfringens*, *Staphylococcus aureus* and other coagulase-positive staphylococci and *Bacillus cereus* must be less than 1, 1.30 and 4 log_{10} CFU/ml respectively (CFS, 2014).

Only total aerobic bacteria (TAB) were detected while yeast and moulds (YM), thermo tolerant coliforms (TtC), Coagulase-positive Pathogenic *Staphylococci* (CppS), Sulfite-reducing *Clostridium* (SrC), *Bacillus cereus* (Bc), *Salmonella* (S) and Mesophilic *Lactobacillus* (ML) were not found both in unpasteurized and pasteurized samples.

These results showed the good hygiene level of unpasteurized and pasteurized traditional baobab nectar as TAB was 1.41 log_{10} CFU/ml in unpasteurized T-BFN and 1.24 log_{10} CFU/ml in pasteurized T-BFN. However, the results are different from those obtained by Cissé (2009) in unpasteurized baobab nectar (ratio fruit water 1/3) in which total aerobic bacteria (2.29 - 5.75 log_{10} CFU/ml), mesophilic *lactobacillus* (2.20 - 4.11 log_{10} CFU/ml) and moulds (1.30 - 3.53 log_{10} CFU/ml) were detected. Yeasts were detected at a level < 1 log_{10} CFU/ml, while in pasteurized baobab nectar (70°C/10 min), TAB and moulds were less than 2 log_{10} CFU/ml (Cisse et al., 2009). The results were also lower than those reported in papaya juice (6.3 log_{10} CFU/ml) by Khan et al. (2015). Krahulcová et al. (2021) reported also total aerobic bacteria from 2.9 to 7.3 log_{10} CFU/ml in different smoothie samples collected in six food service establishments in Slovakia. James et al. (2022) reported an amount of TAB at 3.08 and 4.3 log_{10} CFU/ml, respectively in baobab fruit pulp from formal and informal baobab processors sector in Kenya.

According to FCD (2019) criteria guidelines, YM and lactic flora in unpasteurized fresh fruit juices are respectively 4 and 4.69 log_{10} CFU/ml at the factory outlet in their initial industrial packaging. In pasteurized juices, the number of TAB varied from 4 to 6 log_{10} CFU/ml according to the requirements of F-054 Rev05 (2018). As TAB, mesophilic ML and coliforms are considered as process hygiene criteria, the present results pointed out the respective GMP and GHP during the process. As

the acceptable limit of bacteria load in drinks for human consumption should not exceed 7 $\log_{10}$ CFU/ml (ICMCF, 2005), traditional baobab fruit nectar processed in our study conditions is safe for consumption. However, the microbial safety of T-BFN has to be evaluated in order to assess the microbial shelf-life.

The microbiological problems encountered in beverages and nectars as well as in fruit juices are due to the presence and growth of microorganisms of alteration that lead to the degradation of the hygienic and marketable qualities of the product, and microorganisms responsible for food-borne illnesses (Philip, 2005). During storage at 25°C, only TAB and YM were detected among the microorganisms sought. The TAB increased significantly 4.19 $\log_{10}$ times after 34 days of storage compared to initial load (Table 1). However, this result was under acceptable limit of bacteria load in drinks for human consumption (7 $\log_{10}$ CFU/ml). The presence of YM implied unfitness for consumption. Therefore, in our conditions of study, T-BFN has a limited microbial shelf-life that was less than 34 days when it is stored at ambient temperature. Pasteurisation only killed vegetative microbes and pasteurized products should be stored below 20°C.

During storage at 4°C, only TAB was detected among the microorganism sought with significant variation after 34, 62 and 190 days compared to initial load. Nevertheless, after 190 days of storage at 4°C, the TAB level was still under than permissible limits in fruits juices. Therefore, in our conditions T-BFN can be stored up to 190 days at 4°C without microbial spoilage. These results are satisfactory and indicated good refrigeration (at 4°C) that kept the food in a state close to its initial state. The lower the temperature is, the slower the multiplication of microorganisms.

Effect of homogenization pressures on the visual aspect of T-BFN during storage at 4°C

Figure 1 presents the impact of homogenization pressures at 0, 5, 8, 12, 13 and 14 MPa on the visual aspect of pasteurized traditional baobab fruit nectar after 1D (day) and 54 D (days) of storage at 4°C. Homogenization is a unit operation commonly used in the processing of fruit and vegetable juices in order to improve their physical stability by reducing the size of suspended particles (Zamora and Guamis, 2015; Patrignani and Lanciotti, 2016; Da Silva et al., 2019).

High-pressure homogenization (HPH) application ranged from 3 to 500 MPa continuously in fluid products (Castagnini et al., 2014; Betoret et al., 2015). In our study, pressures below 20 MPa were applied since our homogenizer operated at 5, 8, 12, 13 and 14MPa. The impact of homogenisation pressures was assessed directly by visual observation of the presence of the serum phase after 1 and 54 days of storage at 4°C. From Figure 1, the non-homogenized traditional baobab fruit nectar showed a serum phase and a sediment phase that are more or less of the same height after 1 and 54 days of storage at 4°C. In homogenized T-BFN, the visual height of the serum phase decreased with the progressive increase of pressure (5, 8, 12 and 13 MPa). At 14 MPa no difference was found after 1 and 54 days of storage at 4°C but in comparison with the control sample, no serum phase occurred after 1 and 54 days of storage. The present results indicated a positive effect of homogenization on the visual appearance of traditional baobab fruit nectar which could be stored up to 54 days at 4°C without appearance of pulp sedimentation. Thus, homogenisation at 14 MPa is sufficient to stabilise the visual appearance of T-BFN. According to Siebert (1999), turbidity and sediment formation in fruit juice is due to the presence of starch, pectin, polyphenols and proteins especially in the absence of microbial growth.

Yu et al. (2018) obtained similar result in taro pulp where HPH treatment (0-60 MPa) improved the stability of the taro pulp suspension. On the other hand, different results were observed for pineapple pulp where homogenized samples showed sedimentation of the pulp after ten days of storage at 25°C (Silva et al., 2010). This can be explained by the accumulation of particle size aggregated, which are different in size for each fruit. Indeed, according to Lopez-Sanchez et al. (2011), each plant cell wall behaved differently when treated with HPH. Homogenisation reduced the particle size and improved the stability of the suspension in fruit drinks. During this operation the pulp is passed through a narrow orifice and then broken down into small particles, resulting in high stability, no sedimentation and a smooth texture. The results offered interesting prospects for small-scale beverage manufacturers as homogenizers working at a pressure below 20MPa are easier to have and more suitable for their production level than high-pressure homogenizers.

However, high-pressure homogenisation and ultra-homogenisation offered the dual advantage of microbiologically stabilization beverages while also allowing for physical stability of the beverage. The HPH could be used as a valuable tool to reduce particle sedimentation and serum separation (Kubo et al., 2013; Salehi, 2020). The reduction in particle size during homogenization can be related to the greater stability of the homogenized products. Therefore, homogenization may be addressed as an effective instrument for preventing pulp sedimentation in traditional baobab fruit nectar.

Conclusion

Pasteurized traditional baobab fruit nectar could be stored up to 190 days (6 months) at 4°C without microbial spoilage. When the storage was carried out at room

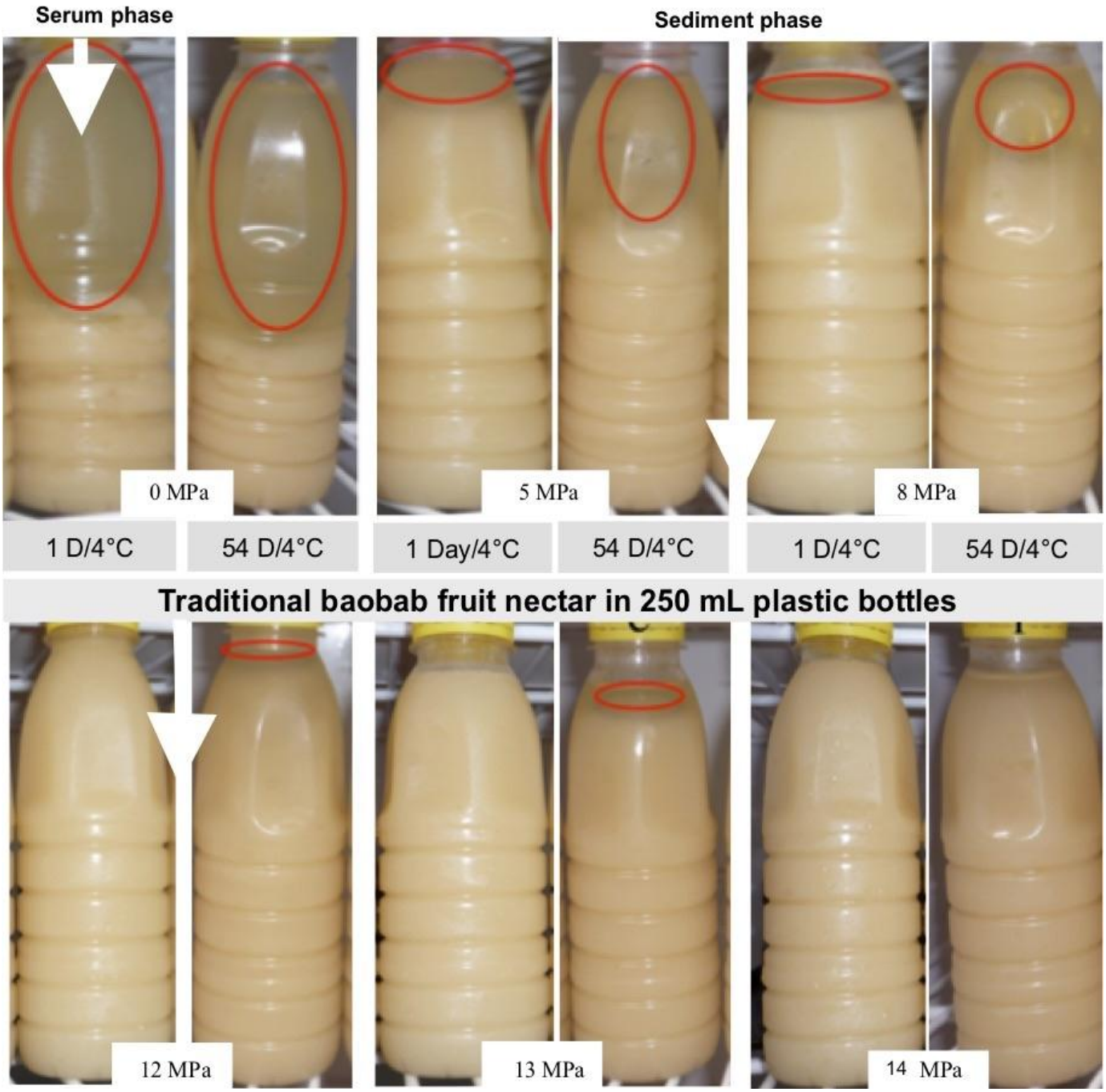

Figure 1. Effect of homogenization at 0, 5, 8, 12, 13 and 14 MPa on the visual appearance of traditional baobab fruit nectar after 1 (1D) and 54 days of storage at 4°C.
Source: Authors

temperature (25°C) its microbial shelf life was reduced less than 34 days. The present results have also demonstrated that the application of homogenization, particularly at 14MPa could be efficient to maintain during 54 days at 4°C the visual appearance of traditional baobab fruit nectar without appearance of pulp sedimentation and serum phase. These results would be useful for informal and micro, small, medium scale beverage processors, who will have technical information to produce safe and attractive traditional baobab fruit nectar. However, in order to generate more knowledge on traditional baobab fruit nectar, further studies should be carried out on the evolution of nutritional and sensory quality during storage at 4°C of pasteurized and

homogenized (14Mpa) traditional baobab fruit nectar.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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