Vol. 16(10) pp. 226-240, October 2022
DOI: 10.5897/AJFS2022.2204
Article Number: 4E39D3A69779
ISSN: 1996-0794
Copyright ©2022
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJFS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Food Science

Full Length Research Paper

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

Laura Mkabili Mwakamu[1], George Ooko Abong[1], Michael Wandayi Okoth[1], Mukani Moyo[2], Lucy Mwaura[2], Derick Malavi[3,4] and Tawanda Muzhingi[2,5*]

[1]Department of Food Science, Nutrition and Technology, University of Nairobi, Kangemi, Kenya.
[2]Food and Nutritional Evaluation Laboratory (FANEL), International Potato Center, Sub-Saharan Africa Regional Office, ILRI Campus, P. O. Box 25171 - 00603 Nairobi, Kenya.
[3]Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.
[4]Food Chemistry and Technology Research Centre, Department of Molecular Biotechnology, Environmental Technology and Food Technology, Ghent University Global Campus, Incheon Global Campus, 119, Songdomunhwa-Ro, Yeonsu-Gu, Incheon, 21985, South Korea.
[5]Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, 27695, USA.

Received 9 June; 2022; Accepted 20 September, 2022

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

*Corresponding author. E-mail: tmuzhin@ncsu.edu .

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

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

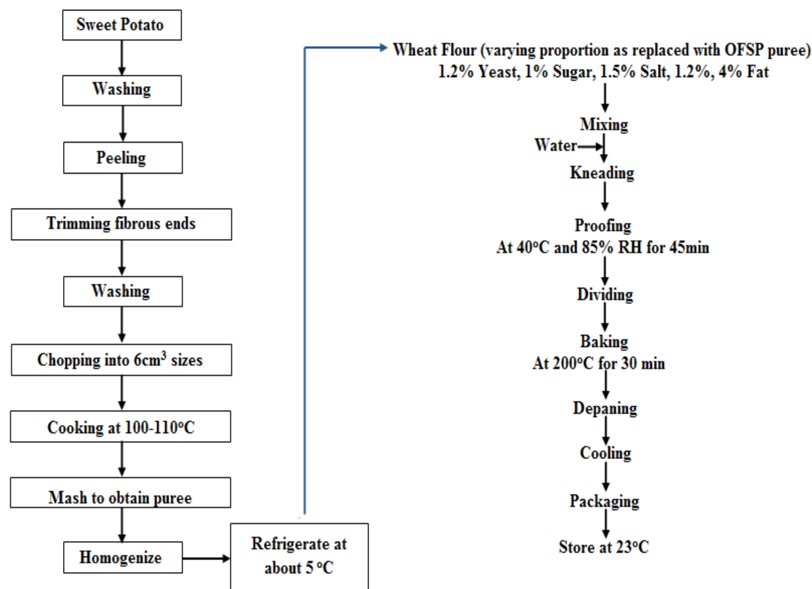

Figure 1. Flow diagram for puree formulation and bread making. Adopted from Muzhingi et al. (2018).

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.

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

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) = $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$,

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 practic

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 exhibit 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.

REFERENCES

Abong GO, Muzhingi T, Okoth MW, Ng'ang'a F, Ochieng E, Mbogo DM, Malavi D, Akhwale M, Ghimire S (2020). Processing methods affect phytochemical contents in products prepared from orange-fleshed sweetpotato leaves and roots. Food Science and Nutrition pp. 1-9.

Abu-Ghoush M, Herald TJ, Dowell F, Xie F, Aramouni FM, Madl R (2008). Effect of preservatives addition on the shelf-life extensions and quality of flat bread as determined by near-infrared spectroscopy and texture analysis. International Journal of Food Science and Technology 43(2):357-364.

Agbede TM (2010). Tillage and fertilizer effects on some soil properties, leaf nutrient concentrations, growth and sweet potato yield on an Alfisol in southwestern Nigeria. Soil and Tillage Research 110(1):25-32.

Alam M, Rana Z, Islam S (2016). Comparison of the proximate composition, total carotenoids and total polyphenol content of nine orange-fleshed sweet potato varieties grown in Bangladesh. Foods 5(3):64.

Anishaparvin A, Chhanwal N, Indrani D, Raghavarao KSMS, Anandharamakrishnan C (2010). An investigation of bread-baking process in a pilot-scale electrical heating oven using computational fluid dynamics. Journal of Food Science 75(9):E605- E611.

Axel C, Zannini E, Arendt EK (2017). Mold spoilage of bread and its biopreservation: A review of current strategies for bread shelf life extension. Critical Reviews in Food Science and Nutrition 57(16):3528-3542.

Baba SA, Malik SA (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. Journal of Taibah University for Science 9(4):449-454.

Bhatnagar-Panwar M, Bhatnagar-Mathur P, Bhaaskarla VVA, Dumbala SR, Sharma KK (2013). Rapid, accurate and routine HPLC method for large-scale screening of pro-vitamin A carotenoids in oilseeds. Journal of Plant Biochemistry and Biotechnology *24*(1):84-92.

Bocher T, Low JW, Muoki P, Magnaghi A, Muzhingi T (2017). From lab to life: Making storable orange-fleshed sweetpotato purée a commercial reality. Open Agriculture *2*(1):148-154.

Bystrom LM, Lewis BA, Brown DL, Rodriguez E, Obendorf RL (2009). Phenolics, sugars, antimicrobial and free-radical-scavenging activities of Melicoccus bijugatus Jacq. Fruits from the Dominican Republic and Florida. Plant Foods for Human Nutrition 64(2):160-166.

Debonne E, Bockstaele FV, Samapundo S, Eeckhout M (2018). The use of essential oils as natural antifungal preservatives in bread products. Journal of Essential Oil Research 2905:1-10.

Dincer C, Karaoglan M, Erden F, Tetik N, Topuz A, Ozdemir F (2011). Effects of baking and boiling on the nutritional and antioxidant properties of sweet potato [*Ipomoea batatas* (L.) Lam.] cultivars. Plant Foods for Human Nutrition 66(4):341-347.

El Sheikha AF, Ray RC (2017). Potential impacts of bioprocessing of sweet potato: Review. Critical Reviews in Food Science and Nutrition 57(3):455-471.

Fidrianny I, Suhendy H, Insanu M (2018). Correlation of phytochemical content with antioxidant potential of various sweet potato (Ipomoea batatas) in West Java, Indonesia. Asian Pacific Journal of Tropical Biomedicine *7*(1):25-30.

Gazdik Z, Zitka O, Petrlova J, Adam V, Zehnalek J, Horna A, Reznicek V, Beklova M, Kizek R (2008). Determination of vitamin C (ascorbic acid) using high performance liquid chromatography coupled with electrochemical detection. Sensors 8(11):7097-7112. https://doi.org/10.3390/s8117097

Huang YC, Chang YH, Shao YY (2006). Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. Food Chemistry 98(3):529-538.

International A (1990). Official Methods of Analysis: 15th Edition. TrAC Trends in Analytical Chemistry 9(4):VI

Kunzmann AT, Coleman HG, Huang WY, Kitahara CM, Cantwell MM, Berndt SI (2015). Dietary fiber intake and risk of colorectal cancer and incident and recurrent adenoma in the prostate, lung, colorectal, and ovarian cancer screening trial. American Journal of Clinical Nutrition 102(4):881-890.

Kuyu CG, Tola YB, Mohammed A, Ramaswamy HS (2018). Determination of citric acid pretreatment effect on nutrient content, bioactive components, and total antioxidant capacity of dried sweet potato flour. Food Science and Nutrition 6(6):1724-1733.

Li A, Xiao R, He S, An X, He Y, Wang C, Yin S, Wang B, Shi X, He J (2019). Research advances of purple sweet potato anthocyanins: Extraction, identification, stability, bioactivity, application, and biotransformation. Molecules 24(21):3816.

Li YC (2011). Antioxidant activity of flavonoids from sweet potato vines *in vitro*. Advanced Materials Research pp. 23*6*-238.

Loi M, Paciolla C, Logrieco AF, Mulè G (2020). Plant bioactive compounds in pre- and postharvest management for aflatoxins reduction. Frontiers in Microbiology 11:243.

Makhuvele R, Naidu K, Gbashi S, Thipe VC, Adebo OA, Njobeh PB (2020). The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxins. Heliyon 6(10):e05291.

Makini FW, Mose LO, Kamau GK, Salasya B, Mulinge WW, Ongala J, Makelo MN, Fatunbi AO (2018). Innovation opportunities in Sweet potato Production in Kenya. In Forum for Agricultural Research in Africa (FARA), Accra Ghana.

Malavi D, Mbogo D, Moyo M, Mwaura L, Low J, Muzhingi T (2022). Effect of orange-fleshed sweet potato purée and wheat flour blends on β-carotene, selected physicochemical and microbiological properties of bread. Foods 11(7):1051.

Medini F, Fellah H, Ksouri R, Abdelly C (2014). Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum . Journal of Taibah University for Science 8(3):216-224.

Melikoglu M, Lin CSK, Webb C (2013). Analysing global food waste problem: Pinpointing the facts and estimating the energy content. Central European Journal of Engineering *3*(2):157-164.

Mizzi L, Maniscalco D, Gaspari S, Chatzitzika C, Gatt R, Valdramidis VP (2020). Assessing the individual microbial inhibitory capacity of different sugars against pathogens commonly found in food systems. Letters in Applied Microbiology *71*(3):251-258.

Montesano D, Cossignani L, Giua L, Urbani E, Simonetti MS, Blasi F (2016). A simple HPLC-ELSD method for sugar analysis in goji berry. Journal of Chemistry. https://doi.org/10.1155/2016/6271808

Mpamah CI, Kanu C, Kanu KC, Uchendu UI, Ochor NO (2019). Bread Fungi : Phytochemical Constituents and Antimicrobial Activity of Isolates. Journal of Applied and Environmental Microbiology 7(1): 20-24.

Musilova J, Lidikova J, Vollmannova A, Frankova H, Urminska D, Bojnanska T, Toth T (2020). Influence of heat treatments on the content of bioactive substances and antioxidant properties of sweet potato (*Ipomoea batatas* L.) tubers. Journal of Food Quality. https://doi.org/10.1155/2020/8856260

Muzhingi T, Owade JO, Low J, Malavi D, Okoth MW, Mbogo D, Abong GO, Heck S (2018). Sensory Attributes of Composite Breads from Shelf Storable Orange-Fleshed Sweetpotato Puree. Open Agriculture *3*(1):459-465.

Nakalembe I, Kabasa JD, Olila D (2015). Comparative nutrient composition of selected wild edible mushrooms from two agro-ecological zones, Uganda. SpringerPlus 4(1).

Ooko AG, Muzhingi T, Okoth MW, Ng'ang'a F, Ochieng' PE, Mbogo DM, Malavi D, Akhwale M, Ghimire S (2020). Phytochemicals in leaves and roots of selected kenyan orange fleshed sweet potato (OFSP) varieties. International Journal of Food Science. https://doi.org/10.1155/2020/3567972

Owade JO, Abong GO, Okoth MW (2018). Production, utilization and nutritional benefits of orange fleshed sweetpotato (OFSP) puree bread: A review. Current Research in Nutrition and Food Science Journal 6(3):644-655

Polivanova OB, Gins EM (2019). Antioxidant activity of potatoes (Solanum tuberosum L.) and anthocyanin content, its biosynthesis and physiological role. Vegetable Crops of Russia 7132(6):84-90.

Ridley SC, Lim M, Heenan S, Bremer P (2005). Evaluation of sweet potato cultivars and heating methods for control of maltose production, viscosity and sensory quality. Journal of Food Quality 28(2):191-204.

Saranraj P, Sivasakthivelan P (2015). Microorganisms involved in spoilage of bread and its control measures. https://www.researchgate.net/publication/283122213_Microorganisms_Involved_in_Spoilage_of_Bread_and_Its_Control_Measures

Science F, Avenue R (2019). Alternative methods for mould spoilage control in bread and bakery products. International Food Research Journal 26(3):737-749.

Shalaby EA, Shanab SMM (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis. Indian Journal of Marine Sciences 42(5):556-564.

Steed LE, Truong VD (2008). Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweetpotato purees. Journal of Food Science 73(5):215-221.

Tang Y, Cai W, Xu B (2015). Profiles of phenolics, carotenoids and antioxidative capacities of thermal processed white, yellow, orange and purple sweet potatoes grown in Guilin, China. Food Science and Human Wellness 4(3):123-132.

Triasih D, Utami FD (2020). The effect of different processing techniques in sweet potato (*Ipomoea batatas*) of content nutrition. E3S Web of Conferences 142:3-6.

Truong VD Avula RY Pecota KV Yencho G (2018). Sweetpotato production, processing, and nutritional quality. Handbook of Vegetables and Vegetable Processing: Second Edition, *2-2*(February):811-838.

Truong VD, Avula RY (2010). Sweet potato purees and dehydrated powders for functional food ingredients. In Sweet Potato: Post Harvest Aspects in Food, Feed and Industry.

Tyagi AK, Malik A, Gottardi, D, Guerzon ME (2012). Essential oil vapour and negative air ions: A novel tool for food preservation. *Trends in* Food Science and Technology 26(2):99-113.

Wanjuu C, Abong G, Mbogo D, Heck S, Low J, Muzhingi T (2018). The physiochemical properties and shelf-life of orange-fleshed sweet potato puree composite bread. Food Science and Nutrition 6(6):1555-1563.

Xu J, Su X, Lim S, Griffin J, Carey E, Katz B, Tomich J, Smith JS, Wang W (2015). Characterisation and stability of anthocyanins in purple-fleshed sweet potato P40. Food Chemistry 186:90-96.