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Quality Attributes of *Pupuru* Analogue Produced from Orange Fleshed Sweet Potato (OFSP) as Influenced by Fermentation with *Rhizopus oligosporous*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this work, orange fleshed sweet potato (OFSP), rich in β -carotene and other micronutrients was used in producing *pupuru* analogue with the aim of improving the micronutrient and other quality attributes of the product. an analogue of *pupuru* normally a cassava-based food was developed from Orange Fleshed Sweet Potato (OFSP). The OFSP tubers were peeled, washed, grated and fermented with *Rhizopus oligosporous* for varying periods (0, 24, 48, 72, 96, 120 h). After fermentation period, each sample was subsequently smoke-dried and milled into *pupuru* analogue flour. The flour was analysed for proximate, physicochemical, minerals, vitamins and anti-nutritional factors. Sensory evaluation was carried out on the *pupuru* meals prepared from the flour samples by reconstituting in boiling water. The protein content ranged from 3.4 to 5.41% with increased in fermentation period. Similarly, there was increase in β -carotene content from 117-126 mg/100g after 120 h fermentation period. Vitamins B₆ and C ranged from 1.73 to 3.20 mg100g and 12 .3 to 21.1 mg/100g respectively. Oxalate, tannin and phytate decreased from 123.0 to 72.5 mg/100g, 1.10 to 1.05mg/100g and 1.62 to 1.16mg/100g respectively. Sensory scores showed that *pupuru* analogue fermented for 96 and 120 h were preferred most in terms of overall acceptability.

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Keywords: Orange fleshed sweet potato; Pupuru analogue; fermentation period; chemical composition.

1. INTRODUCTION

Roots and tubers are agricultural crops that play a significant role in facilitating food and nutrition security in many developing countries. Records indicated that 494.6 million tons of roots and tubers (including potato) were produced in the year 2017 worldwide [1]. They form significant part of diet for majority of the global population, with world average per capita consumption of 19.4 kg/year (2013-2015) and projecting to achieve 21.0 kg/year by 2025 [2]. They are also widely used in animal feeds formulation and for various industrial needs such as starch production [3]. Among the roots and tubers, sweet potato (Ipomoea batatas) is very significant on the basis of production and consumption. Sweet potato is a dicotyledon that belongs to Convolvulaceae family and ranks worlds' seventh most important. It is a potential energy contributor and considered as fifth essential crop (fresh weight basis) after rice, wheat, maize, and sorghum [4].

Orange Fleshed Sweet Potato (OFSP) has been adjudged to rank first among all vegetables due to its dietary significance and nutritional composition. OFSP tubers are considered as a significant dietary resource of pro-vitamin A carotenoids and non pro-vitamin A carotenoids [5]. OFSP is appreciated due to the vitamin A contribution and role in vitamin A Deficiency (VAD) eradication in developing countries [6.7.8]. Research on OFSP has been intensified in present times due to the many positive aspects related to agriculture, food and nutritional security to scale up its production and consumption in many parts of the world [9,10,11,12]. OFSP is also attractive with yellow to orange colour with sweet taste in comparison with other varieties of sweet potato [13]; hence, OFSP has been reported to have potential in alleviating the problems of calorific and VAD malnutrition particularly among the children in targeted communities. Orange-fleshed sweet potato is also a good source of nondigestible dietary fibre specific minerals, different vitamins, and antioxidants [14,15].

Cassava (*Manihot esculenta*) is a root that is usually processed through fermentation before consumption as a means of detoxification, preservation and modification. Various fermented cassava products are available including *garri*, *lafun* and *pupuru* [16]. *Pupuru* is a traditional fermented, smoked dried cassava molded product which is usually made into porridge in boiling water before consumption with any desired soup. It is unique in processing compared to other cassava-based fermented foods. It is a local food product in Nigeria, eaten mainly by the people of Ilaje and Ese-Odo (Ikale) area of Ondo State, Nigeria [17]. *Pupuru* has high carbohydrate content, the tuber containing 20 to 25% starch and very minor quantities of proteins, fats, and other biochemical constituents [18].

Application of solid-state fermentation process using non-pathogenic micro-fungi has been explored by many researchers and has been shown to result in the improvement of the nutritional and sensory value of a wide variety of products obtained from them [19,20,21,22,23,24]. The aim of this study was to produce pupuru analogue, a product that is similar to cassava pupuru from OFSP by fermentation with a micro fungus with a view to improve the nutritional status and examine the characteristics of the products obtained with the objective of assessing its physico-chemical, nutritional, functional and sensory properties.

2. MATERIALS AND METHODS

2.1 Materials

Orange fleshed sweet potato (OFSP) was obtained from the Research and Teaching farm of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, while the inoculum of *Rhizopus oligosporus* was obtained from the Department of Food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

2.2 Methods

2.2.1 Preparation of subculture for sweet potato for *Pupuru* analogue

The subculture of *Rhizospus oligosporus* was prepared by the procedure described by Nout and Rombouts [25] as modified by Olanipekun et al., [26]. Fifty millimeters of distilled water was added to 50g of rice in a beaker. This was covered with sterile muslin cloth firmly tied with a twine. The beaker and its contents were then sterilized at 115 Psi for 15 min at a temperature of 120°C cooled and inoculated with the inocula of *Rhizospus oligosporus* before being incubated for 4 days at 30°C It was dried in an oven at 40°C for 48 h, pulverized in a clean sterile dry blender for about 2 min until uniform grey granules were obtained. This was then stored in a sterile sealed polythene bag in a sterile jar and stored at room temperature.

2.2.2 Preparation of fermented sweet potato *Pupuru* analogue

The sweet potato was processed into pupuru analogue by a modified method of Osundahunsi [27] for the traditional cassava pupuru. The orange fleshed sweet potato was peeled, washed and grated, transferred into a jute sack, tightly sealed and heavy weights placed on it for proper dewatering for about 24h. After dewatering it was weighed and divided into six equal portions and then transferred separately into sterilized plastic containers and then inoculated in portions with the culture of Rhizopus oligosporous. The portions were allowed to ferment for varying periods (0, 24, 48, 72, 96, 120 h). At each of the intervals a sample was moulded into balls and smoke dried on an oil drum smoker constructed of metal until the weight became lighter. The dark brownish outer crust of the dried balls was scrapped off and the inner crust was sieved and fried and then milled into flour. Similar operation was carried out on all the batches while control sample was also prepared from cassava using the same procedure.

2.3 Analyses

The proximate composition; protein, crude fat, ash, crude fibre, moisture and carbohydrate determined by difference according to the method of AOAC [28].

Determination of pH and Titratable acidity: Ten grams (10 g) of each sample was mixed with 100 ml of distilled water and pH was determined using the pin electrode of pH meter (JENWAY Instrument, model 3505). Titratable acidity of each *pupuru* analogue was determined by allowing the mixture to stand for 15 minutes, with shaking at 5 minutes intervals and filtered with Whatman No. 4 filter paper. Ten milliliter aliquots (triplicates) were pipette from the filtrate into conical flask and then titrated against 0.1M NaOH using 1% phenolphthalein as the indicator in order to determine the amount of acid (as lactic acid) in the sample. The titratable acidity was calculated by multiplying the titre value by 0.09 [29] and expressed as % lactic acid.

Swelling power and solubility index, bulk density, and water absorption capacity were determined according to the methods of Takashi and Sieb [30], Akpapunam and Markakis [31] and Ruales et al. [32] respectively.

Antinutrient contents (phytates, oxalates and tannins) were determined using the methods of Latta and Eskin [33], Maxon and Rooney [34], and Munro [35] respectively.

Beta-carotene was determined using the method described by Imungi and Wabule [36] using the High Performance Liquid Chromatography (HPLC model BLC -20G).

The determination of water soluble vitamin B6 and Vitamin C was done by reversed-phase HPLC using the <u>prontoSIL</u> C18 AQ column and smart line HPLC as described by Staroverov et al., [37].

Pasting properties of flours were determined according to the method of Ocheme et al., [38] using a Rapid Visco Analyzer (Model RVA-4; Newport Scientific Pty. Ltd, Warriewood, Australia).

The mineral contents (Fe, Ca, K, P and Na) of the pupuru analogue samples were determined using Atomic Absorption Spectrometer (AAS, BULK 210 VGA). The ash obtained after the determination of ash content was first dissolved in 5 ml concentrated hydrochloric acid (11.8M) and filtered into a 50 ml volumetric flask. The solution was made up to the 50 ml mark with more distilled water and transferred into a plastic sample bottle with a lid. The concentrations of minerals of iron, calcium, potassium, and phosphorus in the pupuru samples were measured atomic absorption by spectrophotometer following flame atomization using air acetylene flame and single element hollow cathode lamp.

Sensory analysis: Each sample of *'Pupuru'* flour analogue was made into meal in form of paste by reconstituting the flour in boiling water. Similar preparation was made from pupuru from cassava which served as control. The samples were then served to 20-semi-trained randomly selected judges who comprised of some members of staff and students of the Department. The judges were asked to score the sample for aroma, taste, appearance, and overall acceptability by using a nine- point Hedonic scale where 1-9 represent dislike extremely and like extremely respectively.

2.4 Data Analysis

Data obtained were subjected to statistical analysis using SPSS version 15.0 and means were separated using Duncan Multiple Range Test (p<0.05).

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The results of the proximate composition of *pupuru* analogue are as shown in Table 1. Protein content for the orange flesh sweet potato *pupuru* analogue increased from 3.4 to 5.41% within the period of fermentation. The highest protein content of 5.41% was recorded after 120 h of fermentation while the lowest protein content 3.4% was recorded from the unfermented sample. It is worth noting that the protein contents are higher than that of unsubstituted cassava *pupuru* as obtained by Lasekan et al. [39] in a similar work.

The high protein content could be attributed to the ability of the fungus to secrete some extracellular enzymes and also synthesized some protein during metabolic activities on the potato mash during fermentation of the potato by fungi. Similar increase in protein content of cassava fermented products was attributed to the possible secretion of some extracellular enzymes into cassava mash in an attempt to utilize cassava starch as a source of carbon [40]. Oyetayo [41] also suggested that increase in the microbial mass may also contribute to the increase in the protein content of the pupuru produce during fermentation. The fibre content of the fermented pupuru analogue from orange flesh sweet potato decreased from 0.91% in the unfermented 'pupuru' analogue flour at 0 h to 0.60% at 96 h of the fermented pupuru flour analogue. The decreasing trend was more significant (P<0.05) within the 0 to 96 h of fermentation. The fat content for the orange flesh sweet potato did not indicate any significant increase (P<0.05) between 0-96hours of fermentation and increases at 120hours of fermentation. Also, moisture contents of the unfermented and fermented pupuru analogue flour from orange flesh sweet potato further

reduced significantly (P<0.05) from 6.70 to 5.52% between 0 to 120 h of fermentation. The significantly low levels of moisture contents in all samples has the potential to cause reduction in microbial spoilage. The ash content of the unfermented and fermented *pupuru* analogue flour from OFSP increased significantly (P<0.05) from (2.64 to 4.14%) between 0 to 120 h of fermentation indicating increasing levels of minerals.

3.2 Physicochemical Properties of the Fermented *Pupuru* Analogue

The results of the physicochemical properties are presented in Table 2. The fermented 'pupuru' flour analogue from OFSP indicated that the pH decreased significantly with increasing period of fermentation from 4.01 to 3.25 indication of acidity in fermentation with increase R. oligosporus for 0 h of unfermented flour. Also no significant changes were observed as the fermentation period increased from day 0 to 48 h of fermentation. The titratable acidity increased significantly 0.0260 to 0.0431 (P<0.05) with increased fermentation period. A food with an inherently low pH would tend to be more stable microbiologically than a neutral food or alkaline food as reported by Frazier and Westhoff [42], likewise foods with low pH could more susceptible to spoilage by yeast and moulds [42]. The bulk density slightly varied from 1.07 to 1.43% between 0 and 120 hours of fermentation. The observation is useful in the selection of packages for the fermented pupuru flour analogue. Thus increased in fermentation time has the potential of adding space in terms of packaging for the same volume of product subjected to a lower period of fermentation. Firon et al. [43] also observed a correlation between decreased bulk density and fermentation in a weaning food formula. The reconstitution index of the fermented pupuru flour analogues were observed to increase as the fermentation period increased, the values ranging between 9.33 and 12.4 for 0 and 120 hours of fermentation respectively. It can therefore be inferred that increased fermentation time has the tendency of reducing the visco-elastic properties of the fermented pupuru flour analogue while less water will be required for reconstitution purpose.

Total sugar content reduced significantly (2.79 to 2.58%) at (P<0.05) within 0 to 120 h. This may be due to the utilization of the carbohydrates by *Rhizopus Oligosporus* as sources of energy for growth and metabolic activities.

Sample	Period of	Protein	Moisture	Fat	Crude Fibre	Ash	Carbohydrate
	fermentation	content	content	content	content	content	content
	(h)	(%)	(%)	(%)	(%)	(%)	(%)
Α	0	3.40 ^b	6.70 [†]	0.27 ^a	0.91 ^c	3.78 ^a	86.1 ^d
В	24	3.22 ^a	7.52 ^e	0.20 ^a	0.63 ^a	3.87 ^b	85.6 [°]
С	48	3.17 ^a	7.01 ^d	0.26 ^a	0.62 ^a	4.04 ^c	85.5 [°]
D	72	4.14 ^c	6.39 [°]	0.20 ^a	0.62 ^a	4.01 ^c	84.6 ^b
E	96	5.12 ^d	5.73 ^b	0.20 ^a	0.81 ^b	4.10 ^d	84.2 ^b
F	120	5.41 ^e	5.52 ^a	0.45 ^b	0.60 ^a	4.14 ^d	83.9 ^a

Table 1. Proximate Composition of Fermented 'Pupuru' Flour Analogue from (OFSP)

Values are means of three determinations

Values with the same superscript in the same column are not significantly different (P<0.05)

Table 2. Physico-Chemical Properties of Fermented "Pupuru" Flour Analogue from OFSP

Sample	Period of Fermentation	Bulk Density (h) g/ml	Reconstitution Index	Total Sug Content (Titratable Acidity
Α	0	1.07 ^a	9.33 ^a	2.79 ^c	4.01 ^d	0.0260 ^a
В	24	1.10 ^a	10.7 ^b	2.78 ^c	3.58 ^b	0.0321 ^b
С	48	1.40 ^b	11.1 ^b	2.73 ^c	3.44 ^b	0.0335 ^c
D	72	1.41 ^b	12.0 ^c	2.52 ^b	3.34 ^a	0.0361 ^d
E	96	1.42 ^b	12.1 [°]	2.42 ^a	3.64 ^c	0.0430 ^e
F	120	1.43 ^b	12.4 ^c	2.58 ^b	3.25 ^a	0.0431 ^e

Values are means of three determinations

Values with the same superscript in the same column are not significantly different (P<0.05).

3.3 Vitamins Content

The result of the Vitamin A content of pupuru flour analogue from OFSP during fermentation with *R. oligosporus* is as presented in Table 3. The values increased with increasing period of fermentation from 117 mg/100g of unfermented sample to 126 mg/100g after 120 h of fermentation. This might imply that consumption of fermented OFSP in terms of pupuru analogue can help in alleviating vitamin A deficiency and thereby combating night blindness and other diseases associated with VAD [44]. The result obtained for Vit B₆ and Vit C shows similar trends of increase as fermentation time increased. The vitamin levels of both Vit B₆ and Vit C were found to increase significantly at (P<0.05) from 1.73 to 3.20 mg/100g (Vit B₆ OFSP), 12 .3 to 21.1

mg100g (Vit C OFSP) respectively within the period of 0 to 120 h of fermentation.

3.4 Mineral Content

The result of the mineral content as influenced by fermentation with *R. oligosporus* as presented in Tables 4. It was observed that increase in fermentation time increased the mineral contents. Minerals such as Sodium, Iron, Magnesium, phosphorus and zinc were observed to follow increasing trends which was significant (P<0.05). As the fermentation increased from 0 to 120 h, the value of the minerals ranged between 39.8 to 49.4, 0.62 to 1.05, 24.4 to 280.0, 40.3 to 48.0 and 0.11 to 0.14 mg/100g for Sodium, Iron, Magnesium, phosphorus and zinc respectively.

Sample	Period of Fermentation (h)	Vit A mg / 100g	Vit B ₆ mg/ 100g	Vit C mg/100g
Α	0	117.0 ^a	1.73 ^a	12.3.0 ^b
В	24	118.0 ^b	1.90 ^b	11.10 ^a
С	48	120.0 ^c	2.20 ^c	11.50 ^a
D	72	124.0 ^d	2.40 ^d	12.40 ^b
E	96	125.0 ^e	2.80 ^e	15.20 [°]
F	120	126.0 ^f	3.20 ^f	21.10 ^d

Values are means of three determinations

Values with the same superscript in the same column are not significantly different (P<0.05)

Sample	Period of Fermentation (h)	Sodium mg/100g	lron mg/100g	Magnesium mg/100g	Phosphorus mg/100g	Zinc mg/100g
Α	0	39. 80 ^a	0.62 ^a	24.40 ^a	40.30 ^a	0.11 ^a
В	24	40.20 ^b	0.67 ^a	24.70 ^a	41.30 ^{ab}	0.11 ^a
С	48	41.80 ^{bc}	0.68 ^a	25.20 ^b	42.70 ^b	0.13 ^a
D	72	44.30 ^c	0.70 ^b	25.70 ^b	43.70 ^{bc}	0.13 ^a
Е	96	46.70 ^d	0.71 ^b	26.60 ^c	44.30 ^c	0.14 ^a
F	120	49.40 ^e	1.05 [°]	28.00 ^d	48.00 ^d	0.14 ^a

Table 4. Mineral Content of Pupuru Analogue from Fermented OFSP

Values are means of three determinations

Values with the same superscript in the same column are not significantly different (P<0.05)

3.5 Anti-nutritional Content

The results obtained in Table 5 shows that the three anti nutritional factors tested for were present in detectable amounts however in the samples, they reduced significantly from 123.0 to 72.5 mg/100g for oxalate 1.10 to 1.05 mg/100g for tannin and 1.62 to 1.16 mg/100g for phytate. This implies that fermentation reduced these anti nutritional factors in fermented OFSP as may be the case in other food materials. This is supported by earlier reports by IFS (1992). Therefore, due to the observed significant reduction on the anti-nutritional effects of *pupuru* flour analogue through fermentation, the process suggests that more nutrients may also be bio-available as fermentation time increased.

3.6 Pasting Characteristic of *Pupuru* Analogue from OFSP

The result of the pasting characteristic of *pupuru* analogue is shown in Fig. 1. The values of the pasting characteristics of pupuru analogue produced from OFSP ranged from 355.2 to 470.0, 115.5 to 194.8, 187.3 to 220.4, 255.4 to 320.1 and 50.55 to 85.55 RVU for peak viscosity, trough viscosity, breakdown viscosity, final viscosity and setback viscosity respectively. From the result, peak viscosity, final viscosity and setback viscosity decreased significantly (P<0.05) with increased fermentation period. While trough viscosity and breakdown viscosity showed unstable decreasing trend with increased period of fermentation. The pasting temperatures increased significantly from 78.55 to 82.92°C with increased period of fermentation while the peak time decreased from 7.99 to 5.99 min as fermentation period increased. The use of Rapid Visco Analyzer (RVA) was to give information on the paste viscosity characteristics of the starch in flours in relation to fermentation time. This could probably be due to high protein contents with increased fermentation time which

invariably influence the visco-elastic properties of the flour. The pasting characteristics data also suggest that increased fermentation period has tendency to reduce the heating time of the fermented flours. This is due to the fact that peak viscosity was observed to reduce as fermentation period increased [45]. This reduction is probably due to activities of amylase which break starch down into simpler sugars, releasing bound water and thus reducing viscosity. However Benjamin [46] observed low viscosity trend due to prolonged fermentation which also reduced peak viscosity for soy bean flour. The break down viscosity of flours from fermented 'pupuru' flour analogue from OFSP appeared to present a decreasing trend with the length of fermentation which might lead to cohesiveness during heating and stirring as a result of breaking down of starch molecules. This also suggests lower peak time causes early gelatinization of the starch [47] which is tantamount to, cohesiveness of products from the flour to breakdown reduces as fermentation time increases. Hence relatively more stable products are expected to be formed as it is subjected to long period fermentation.

3.7 Sensory Properties of Pupuru Meal

The results of sensory evaluation for pupuru meal analogue samples are presented in Table 6. This indicated that the most acceptable product in terms of sensory attribute in term of aroma came from fermentation of OFSP for 72 to 120 h, while in terms of taste came from fermentation for 120 h while the overall acceptability highest scores was from fermentation between 96 and 120 h of the fermented pupuru flour analogue. According to Belewu and Babalola, [20], fungal fermented sample especially the Rhizopus oligosporous fermented had greater acceptability in terms of colour, taste and flavour in comparison with samples that were naturally fermented. This indicates that depending on the microorganism

Sample	Period of fermentation (h)	Oxalate mg/100g	Tannin mg/100g	Phytate mg/100g
Α	0	123 [†]	1.10 ^{ab}	1.62 ^t
В	24	85.8 ^{cd}	1.74 ^c	1.44 ^e
С	48	83.2 ^c	1.90 ^d	1.40 ^d
D	72	65.3 ^b	1.36 ^b	1.35 [°]
E	96	37.5 ^a	1.07 ^a	1.30 ^b
F	120	72.5 ^e	1.05 ^ª	1.16 ^a

Table 5. Anti-nutritional factor of Fermented Pupuru Analogue from OFSP

Values are means of three determinations

Values with the same superscript in the same column are not significantly different (P<0.05)

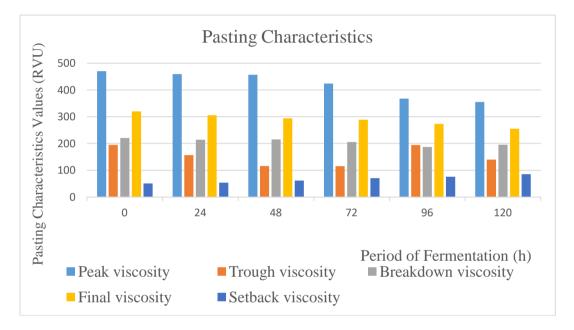


Fig. 1. Effect of Fermentation on Pasting Characteristics of *pupuru* Flour Analogue from Orange Flesh Sweet potato

A– Unfermented pupuru sample for 0 h; B– Fermented pupuru sample with Rhizopus Oligosporus for 24 h; C– Fermented pupuru sample with Rhizopus Oligosporus for 48 h; D– Fermented pupuru sample with Rhizopus Oligosporus for 72 h; E– Fermented pupuru sample with Rhizopus Oligosporus for 96 h; F– Fermented pupuru sample with Rhizopus Oligosporus for 120 h

Sample	Aroma	Taste	After taste	Appearance	Overall acceptability
Α	5.3 ^a	5.3 ^a	4.6 ^a	4.9 ^a	5.0 ^a
A1	5.1 ^ª	5.0 ^a	4.4 ^a	4.6 ^a	4.9 ^a
A2	5.1 ^ª	5.0 ^a	4.4 ^a	4.3 ^a	4.6 ^a
A3	4.3 ^c	4.8 ^b	4.3 ^a	4.1 ^a	4.4 ^a
A4	4.9 ^b	4.5 ^{bc}	4.5 ^a	3.6 ^b	3.4 ^b
A5	4.5 ^b	3.9 ^c	3.9 ^b	3.1 ^b	4.0 ^a
С	4.7 ^{bc}	3.6 ^{cd}	3.3 ^b	3.2 ^b	3.4 ^b

Table 6. Senso	ry attributes of	f fermented	pupuru analo	ogue from	OFSP and cassava
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Values are means of 20-member panel scores

Values with the same superscript in the same column are not significantly different (P<0.05) A – Unfermented pupuru sample for 0 h; A1– Fermented pupuru sample with Rhizopus oligosporus for 24 h A2– Fermented pupuru sample with Rhizopus oligosporus for 48 h; A3– Fermented pupuru sample with Rhizopus oligosporus for 72 h; A4– Fermented pupuru sample with Rhizopus oligosporus for 96 h; A5– Fermented pupuru sample with Rhizopus oligosporus for 120 h; C -Fermented pupuru sample from cassava used and the fermentation period there is an action of the microorganism on the flour which made the fermented samples more preferable. Similarly the sensory scores also showed that samples of pupuru meal analogues from OFSP were preferred above the *pupuru* prepared from cassava in terms of taste, aroma, appearance and overall acceptability.

4. CONCLUSION

Pupuru analogue with good consumer acceptability was successfully produced from orange fleshed sweet potato fermented with Rhizopus oligosporous. The most acceptable sample in terms of taste and aroma came from fermentation of OFSP for 72 to 120 h. Similarly, the increasing fermentation period also increased the protein, β-carotene and vitamin contents significantly of *pupuru* analogue from OFSP. This indicated that instead of concentrating on cassava alone for pupuru production, the emergence of OFSP can also be harnessed to produce quality *pupuru* with essential nutrients and good sensory attributes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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