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Colour Profile, Pasting and Sensory Properties of Processed Sweet Potato Flour

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Authors' contributions

This work was carried out in collaboration among all authors. Author OIA designed the study. Author SBK performed the statistical analysis. Authors OIA and SBK wrote the protocol and wrote the first draft of the manuscript. Authors BJA and TAO managed the analyses of the study. Author CPO managed the literature searches. Author AKL edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In this study, sweet potato was purchased and processed using different methods to obtain 4 different sweet potato flour samples. Sample A was unfermented sweet potato flour, sample B was fermented spontaneously, while sample C and D were produced by fermentation using indigenous starter cultures of lactic acid bacteria and yeast for 48 h and 72 h respectively. Colour profile, Pasting and Sensory evaluation was conducted. The whiteness (L* value) obtained in this study is within the range of value (87.29-89.52), Also, the redness value (a*) and yellowness value (b*) of

the sweet potato flour samples also showed a significant difference (p<0.05). Sample a had a higher value when compared with samples B,C and D. Pasting properties such as peak viscosity, trough viscosity, breakdown viscosity, setback, pasting temperature were determined. It was noticed that fermentation process and increase in fermentation time significantly (p<0.05) decrease the peak viscosity in this research work and all samples had a higher cooled paste viscosity than their corresponding hot paste viscosity. Nevertheless, sensory evaluation was carried out using thet 9-point hedonic scale, samples A,B,C,D are significantly different (P < 0.05).

Keywords: Starter culture; sweet potato flour; pasting properties; colour profile; sensory evaluation.

1. INTRODUCTION

Sweet potato (Ipomoea batatas (Lam)) is a dicotyledonous plant from the Convolvulaceae that grows in tropical and subtropical areas and even in some temperate zones of the developing world [1]. It is a starchy, sweet tasting, tuberous root vegetable with smooth skin and comes in different colours yellow, purple, orange and beige [2]. In developing countries, sweet potato ranks fifth economically after rice, wheat, maize, and cassava; sixth terms of dry matter production, seventh in terms of digestible energy production and ninth in terms of protein production [3].

World production is about 131 million tonnes per year, on approximately 9 million hectares with mean estimated yields of 13.7 tonnes per hectares [4]. Nigeria is the second largest producer of sweet potato in Africa with 4 million ton annually [5].

Sweet potato is also an excellent source of vitamin A (14187 IU/100 g) and it is highly nutritious in terms of vitamin C (2.4 mg/100 g), calcium (30 mg/100 g) and iron which is required for adequate energy (0.61 mg/100 g) and magnesium (25 mg/100 g) [6,7]. Despite its high carbohydrate content, sweet potato has a low glycemic index due to low digestibility of the starch making it suitable for diabetic or overweighed people [8,9,10,11,1]. The root has higher protein content than other roots and tubers, such as cassava and yams [12]. Sweet potato is readily available, inexpensive and delicious.

Lactic acid fermentation is a metabolic process by which glucose and other six-carbon sugars (also, disaccharides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolite lactate (lactic acid in solution) [13]. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells [14]. Fermentation of foods helps to eliminate anti nutritive factors [15] make the nutrients present more bioavailable [16] convert into shelf stable products with longer shelf life and improve the organoleptic properties [17].

Starter cultures are preparation or material containing large numbers of variable microorganisms, which may be added to accelerate a fermentation process [18,19]. They help to elicit specific changes in the chemical composition, nutritional value and sensorial properties of the substrate [7,20]. Moreover starter cultures help to reduce fermentation period [21], improve nutritional qualities of food [22] and provide consistency and reliability of performance [23]. Other functions of starter cultures may include the following: flavour, aroma, and alcohol production [7]. Starter cultures can be single (one selected strain of particular species of microorganism) or mixed cultures (two or more strains from a species and/or of different families and genera) with definite characteristics that are beneficial in the manufacture of a desired product [24]. The use of starter cultures adaptive to the substrate had been suggested to facilitate improved control of a fermentation process and predictability of its product [18].

Lactic acid bacteria are a group of bacteria related by certain morphological, metabolic and physiological characteristics [25]. They are typically Gram-positive, catalase-negative rods or cocci that grow under aerophilic to microaerophilic and strictly anaerobic conditions, producing lactic acid as a major metabolic products [26]. Lactic acid bacteria are generally considered as beneficial microorganisms and some called probiotics have been shown to impart certain health enhancing benefits [25].

Yeasts are unicellular fungi that are mostly identified on the basis of their morphological and cultural characteristics [27,28]. Yeast and lactic acid bacteria (LAB) occur as part of the natural

microbial population in spontaneously fermented food and as starter cultures in the food and beverage industry [29]. Yeast significant impact food quality parameter such as taste, texture, odour and nutritive value when yeasts are abundant alone or in stable mixed population with bacteria (usually LAB) [30]. The main objectives of this study is to evaluate the colour profile, pasting properties and sensory properties of starter culture fermented sweet potato flour so as to for better utilization determine its application for various food production.

2. METHODOLOGY

Materials: Sweet potato, microbiological media, potable water, fermentation vats, cotton wool, ethanol, Petri dishes, spatula, spoon, aluminium foil, micropipette, sieve and knife.

Sample Collection: Sweet potato tubers used for this work were purchased from Arena market, Bolade, Oshodi, Lagos State Nigeria.

Preparation of Samples: Sweet potato tubers were thoroughly sorted to remove bad ones from

the lot. The sorted tubers were washed to remove adhering soil particles, and then weighed accordingly into four different portions. 5 Kg of tubers after weighing were thereafter peeled and sliced into small pieces, transferred into sterile bowls, 1.5 L of clean water was added to the sweet potato samples to have four different the samples namely unfermented sweet potato flour (A), 72 h spontaneously fermented sweet potato flour (B). 48 h starter culture fermented sweet potato flour (C) and 72 h starter culture fermented sweet potato flour (D) as shown in Figs. 1 and 2.

2.1 Preparation of Inoculum

Starter cultures (Lactic acid bacteria and Yeast) used for this study were previously isolated from fermented sweet potato broth, after isolation the organisms were sub cultured by streaking on MRS agar (Oxoid) for Lactic acid bacteria and incubated anaerobically at 37°C for 24 h. Pure culture of yeast isolates was cultivated by streaking on potato dextrose agar PDA (Oxoid) and incubated at 25°C for 3 days.

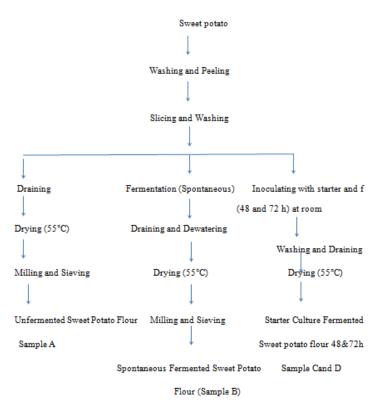


Fig. 1. Flow diagram for the production of unfermented, spontaneous fermented sweet potato flour samples [31] and starter culture fermented sweet potato flour

Colonies were picked from each pure culture plates of MRS and PDA plates and inoculated aseptically into MRS broth and YEPD respectively then incubated at 37°C and 25°C respectively for 24 h. After incubation, the organisms were harvested from the broth media by centrifuging at 5000 rpm for 15 minutes. The supernatants were decanted and the cell biomass dis logged using sterile distilled water. Appropriate volume of sterile distilled water was used to wash the organisms into the various fermentation bowls containing the sweet potato samples. It was left to ferment for 48 h and 72 h [32].

2.2 Analysis

Colour determination of the sweet potato flour samples: The surface colour (CIE I*, a^* and b^*) of the flour samples was measured using Konica Minolta Chroma Meter (Model CR-410). The chroma meter was calibrated before the measurements, using the white calibration plate (Y = 93.5, X = 0.3132, y = 0.3198). Five random readings were taken from each type of flour. The measurement were averaged for each surface and the result were expressed as I* (Whiteness), a^* (redness) and b^* (yellowness) as described by Collado et al. [33].

Determination of pasting properties of the fermented sweet potato flour: Pasting profile of the samples was determined using a Rapid Visco Analyzer (RVA-4; Newport Scientific Pty. Ltd., Australia) as described by Shittu et al. [34]. The RVA was interfaced with a personal computer equipped with the Thermocline for Windows software provided by the same manufacturer. 3 g of the sample (14% moisture basis) and 25 ml of distilled water was used.

The equivalent sample mass (S) and water mass (W) corrected for 14% moisture basis was calculated using the formula:

$$S = 86 \times A/100-M$$
, $W = 25 + (A-S)$

Where: S = corrected sample mass, A = sample weight at 14% moisture basis, M = actual moisture content of the sample (% as is), W = corrected water mass.

A programed heating and cooling cycle was used at constant shear rate, where the slurry was held at 50°C for 1 min, heated to 95°C within 7.5 min, and then held at 95°C for 5 min. It was subsequently cooled to 50°C within 8.5 min and

held at 50°C for 2 min, while maintaining a rotation speed of 160 rpm. Total cycle time was 23 min. Duplicate tests were performed in each case. The viscosity is expressed as Rapid Visco Units (RVU).

The parameters measured automatically by the RVA were: peak viscosity (the highest viscosity of the paste during the heating phase), trough (lowest viscosity of the paste during the heating phase), breakdown viscosity (the difference between the peak viscosity and the trough), setback viscosity (the difference between the final viscosity and the trough), final viscosity (the viscosity at the end of the cycle), pasting temperature (°C) (the temperature at which there is a sharp increase in viscosity of flour suspension after the commencement of heating), and peak time (min) (time taken for the paste to reach the peak viscosity).

Determination of sensory properties of various sweet potato flour meals: Sensory analysis studies parameters like taste, texture, colour, mouth feel, flavour, mouldability was evaluated using a 9-point hedonic scale was designed to measure the degree of preference of the samples. The 9-point hedonic scale ranged from 1 to 9, with 1 as extremely unacceptable and 9 as extremely acceptable. The various samples of sweet potato flour were served to a 20 semi trained panellists made of staffs and IT students of Federal Institute of Industrial Research, Oshodi (FIIRO) from other departments to avoid bias.

Statistical analysis: The measurements were done in triplicate. All data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) was performed for post hoc multiple comparison using SPSS version 17. Statistically significant difference was established at P < 0.05. Correlation between the qualities was determined using a two-tailed Pearson's correlation test.

3. RESULTS

Table 1 presents the colour profile of the various sweet potato flour samples. The value ranged from 87.33 to 89.52 for L*, - 0.02 to 0.45 for a* and 7.60 to 10.28 for b* respectively.

The pasting profile of the various sweet potato flour samples (Table 2) revealed that peak viscosity, trough viscosity, breakdown viscosity,

final viscosity and setback viscosity ranged from 2302.00 to 7160.00 cP, 1796.50 to 4173.50 cP, 505.50 to 3460.50 cP,, 2249.50 to 5444.50 cP and 453.00 to 1565.00 cP respectively. Also, peak time ranged from 4.47 to 4.57 Mins while pasting temperature 80.68 to 81.90°C.

Table 3 revealed that the sensory analysis rating of reconstituted meal from sweet potato flour

samples ranged from 5.80 to 8.00 for colour, 6.00 to 6.80 for flavor, 4.40 to 7.15 for for mouldability, 5.60 to 6.60 for taste, 5.70 to 6.80 for mouthfeel and 5.45 to 7.30 for overall acceptability.

Table 4 presented the correlation matrix of colour profile, pasting and sensory properties of sweet potato samples.

Table 1. Colour profile of various sweet potato flour samples

Sample	L*	a*	b*
Α	87.29 ^c ± 0.18	$0.45^{a} \pm 0.04$	10.28 ^a ± 0.02
В	$89.52^{a} \pm 0.06$	$0.08^{b} \pm 0.03$	$8.90^{b} \pm 0.03$
С	$87.33^{c} \pm 0.06$	$-0.02^{\circ} \pm 0.03$	$7.60^{d} \pm 0.03$
D	$87.83^{b} \pm 0.03$	$0.04^{bc} \pm 0.04$	$8.07^{c} \pm 0.28$

Values are mean of triplicate determinations. Values in the same column with different superscript are significantly different at 5% probability level.

Where: Sample A: Unfermented sweet potato flour; Sample B: 72 h spontaneously fermented sweet potato flour; Sample C: 48 h starter culture fermented sweet potato flour; Sample D: 72 h starter culture fermented sweet potato flour

Table 2. Pasting properties of various sweet potato flour samples

Sample	Peak viscousity (cP)	Trough viscousity (cP)	Breakdown viscousity (cP)	Final viscousity (cP)	Setback viscousity (cP)	Peak time (Min)	Pasting temperature (°C)	
A	7160.00 ^a	3699.50 ^b	3460.50 ^a	4936.00°	1236.50 ^b	4.47 ^b	81.58 ^{ab}	
, ,	±101.82	± 65.76	± 36.06	± 11.31	± 54.45	± 0.00	± 0.04	
В	6625.50 ^b	4173.50 ^a	2452.00 ^c	5444.50 ^a	1271.00 ^b	4.47 ^b	81.50 ^{ab}	
	± 4.96	± 3.54	± 8.49	± 12.02	± 8.49	± 0.00	± 0.00	
С	6297.50 ^c	3731.00 ^b	2566.50 ^b	5296.00 ^b	1565.00 ^a	4.63 ^a	81.90 ^a	
	± 28.99	± 29.70	± 58.69	± 91.92	± 121.62	± 0.05	± 0.71	
D	2302.00 ^d	1796.50 ^c	505.50 ^d	2249.50 ^d	453.00 ^c	4.57 ^a	80.68 ^b	
	± 39.60	± 17.68	± 21.92	± 9.19	± 8.49	± 0.05	± 0.04	

Values are mean of triplicate determinations. Values in the same column with different superscript are significantly different at 5% probability level.

Where: Sample A: Unfermented sweet potato flour; Sample B: 72 h spontaneously fermented sweet potato flour; Sample C: 48 h starter culture fermented sweet potato flour; Sample D: 72 h starter culture fermented sweet potato flour

Table 3. Sensory analysis rating of reconstituted meal from sweet potato flour samples

Parameters	Sample A	Sample B	Sample C	Sample D
Colour	5.80 ^b	8.00 ^a	6.05 ^b	7.30 ^a
Flavour	6.00 ^a	6.85 ^a	6.20 ^a	6.80 ^a
Mouldability	4.40 ^b	7.15 ^a	6.75 ^a	6.60 ^a
Taste	5.60 ^a	6.65 ^a	6.60 ^a	6.30 ^a
Mouth feel	5.70 ^a	6.80 ^a	6.55 ^a	6.50 ^a
Overall acceptability	5.45 ^c	7.30 ^a	6.35 ^b	6.65 ^{ab}

Values in the same column not followed by the Same Superscript are significantly different (P < 0.05). Sample A: Unfermented sweet potato flour; Sample B: 72 h spontaneously fermented sweet potato flour; Sample C: 48 h starter culture fermented sweet potato flour; Sample D: 72 h starter culture fermented sweet potato flour

Table 4. Correlation matrix of colour profile, pasting and sensory properties of sweet potato samples

	L*	a*	b* Pea		Trough	Break	Final	Setback	Peak	Pasting	Colour	Flavou	r Mouldability T	aste Mo	uth	Overall
				Vis. (cP)			Vis.		time (Min)	Temp (°C)			•	fee	feel	acceptability
L*	1					,	•	` '	` ,	, ,						
a*	281	1														
b*	.007	.940**	1													
Peak Viscousity	.077	.458	.506	1												
Trough Viscousity	.301	.238	.341	.960**	1											
Breakdown Viscousity	119	.617	.613	.971**	.865**	1										
Final Viscousity	.206	.192	.267	.959**	.990**	.872**	1									
Setback Viscousity	019	.075	.083	.890**	.899**	.827 [*]	.951**	1								
Peak Time	462	649	843 ^{**}	370	340	372	220	.061	1							
Pasting Temp	084		.094	.785 [*]	.765 [*]	.752 [*]	.830 [*]	.916**	.152	1						
Colour	899**	469	- 225	360	128	534	218	403	267	425	1					
Flavour			382	568	344	723 [*]	418	550	091	547	.970 ^{**}	1				
Mouldability			800 [*]	335	066	543	062	050	.391	107	.705	.751 [*]	1			
Taste		918 ^{**}		162	.103	377	.121	.152	.427	.073	.592	.614	.979** 1			
Mouthfeel			742 [*]	285	009	502	016	030	.311	093	.750 [*]	.775 [*]		75 ^{**} 1		
Overall		739 [*]	524	276	.001	494	047	150	.034	207	.914**	.900**		67 ^{**} .95	0**	1
acceptability																

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

*. Correlation is significant at the 0.05 level (2-tailed)

4. DISCUSSION

According to Wrolstad and Smith [35] colour is an important quality attribute of flour which affects the appearance and consumer acceptability of its final products. Based on the tristimulus colour parameter where I* indicate whiteness, (a*) represents redness while (b*) represents yellowness value of flour sample [33], there are significant difference (p<0.05) in the mean colour profile values obtained for the sweet potato flour samples.

The whiteness (I* value) obtained in this study is within the range of value (87.79) reported by [36] for white fleshed sweet potato flour. It was observed that fermentation process improves the whiteness of the sweet potato flour samples when the results of the fermented samples are compared with the unfermented sample. This same observation was reported by Lu et al. [37] for rice flour, [38] for fufu cassava and [39] for sweet potato flour.

This could be as a result of purification of the flour by fermentation process. According to [39], during fermentation process bacteria would produce proteinase which degraded protein in sweet potatoes; convert free sugar to lactic acid thus the content of protein and free sugar in fermented flour was lower than those in unfermented sweet potato flour. A higher protein and free sugar content in unfermented sweet potato flour may cause non-enzymatic browning during flour drying which result in darker colour, thus reducing whiteness of flour.

The redness value (a*) and yellowness value (b*) of the sweet potato flour samples also showed a significant difference (p<0.05) with unfermented sweet potato sample showing a higher value when compared with all the other fermented samples.

Amylograph pasting profile remains the most utilized method of determining the pasting characteristics of any starch-based food products as it provides information on the functionality of starchy food ingredients in various food processes [40,41,42].

Peak Viscosity refers to the maximum viscosity attainable during the heating cycle; and reflects the ability of starch granules to swell freely before their physical breakdown [43] It was noticed that fermentation process and increase in fermentation time significantly (p<0.05) decrease

the peak viscosity in this research work. This decrease in peak viscosity value might be as a result of the degradation of some of the sweet potato components during fermentation including starch which is the main macromolecule. Bearing in mind that submerged fermentation used in this research work usually causes swelling of the sweet potato tuber making it porous and enhances its amylase accessibility [44] and the subsequent degradation and breakdown of some starch granules [12]. This relative decrease in peak viscosity with increase in fermentation time gives an indication of high enzymatic activities in the flour [45] as well as the fact that the flours can be used for the production of products that require low gel strength and elasticity like weaning foods [46,47].

However, this observation is contrary to the reported work of [39] who reported an increase in the peak viscousity in their work.

Trough viscosity is the minimum viscosity value that measures the ability of a paste to withstand breakdown during cooling. There are significant difference (P<0.05) in the mean value obtained with 72 hrs fermented sweet potato flour samples having the least value while spontaneously fermented sweet potato flour sample has the highest value. This indicated that spontaneously fermented sweet potato flour sample had a greater ability to withstand shear at high temperatures than the other samples [48].

Breakdown viscosity measures the decrease of starch viscosity when heated at 95°C. It is an indication of breakdown or stability of the starch gel during cooking [49]. Various authors [50,47] had reported that the higher the breakdown in viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking. The significant (P<0.05) decrease in the breakdown value of fermented sweet potato flour with increase in fermentation time (except 72 h sample) when compared with unfermented sweet potato flour has implication on increasing the ability of starch granules to be resistant to degradation during heating and shearing. This could be explained by the fact that fermentation had caused degradation of some starch granules; and the starch of the flours produced become less resistant to rupture/deformation and have lower paste stability during heating [51,52].

Final viscosity is used to define the particular quality of starch and indicate the stability of the cooked paste when in actual use; it also indicates the ability to form a various paste or gel after cooling [53] from the result obtained, all samples had a higher cooled paste viscosity than their corresponding hot paste viscosity. This is might be as a result of the high degree of association between the starch-water systems and their high ability to re-crystallize, resulting in progressively higher viscosities during cooling [54]. The final viscosity value of 72 hrs fermented sweet potato flour samples was significantly (P<0.05) lower when compared with the other samples suggesting that it would have least tendency to form more solid paste on cooling and will be least resistant to shear stress during stirring.

Setback viscosity is the phase of the pasting profile that measures the re-crystallization of gelatinized starch during cooling [55]. Aryee et al. [56] reported that flour with a low setback viscosity will be unsuitable for products that require starch stability at low temperatures. Like final viscosity value, the setback viscosity value of 72 hrs fermented sweet potato flour samples have a significantly (P<0.05) lower than the other samples. This suggests that the flour sample its might be relatively more stable when cooked [57] and with less retrogradation tendency than the other samples [58] This view was also reported by [31] who reported that fermented flour upon gelatinization will not readily retrograde particularly if it is intended for a dough-like meal.

Pasting temperature is the temperature at which the first detectable increase in viscosity is noted which is an index associated with the initial change due to the swelling of the starch [59]. The temperature is one of the pasting properties which provide an indication of the minimum temperature required for sample cooking, energy costs involved and other components stability. There are significantly (P<0.05) different in the result obtained for the pasting temperature with 72 hrs fermented sweet potato flour sample having the least pasting temperature value when compared to the other samples. This implies that it has fewer associative forces and cross-links within its starch granule, hence has a faster cooking quality.

Peak time measures the cooking time [47]. It is the time it takes a paste to be cooked and corresponds to the time at which the maximum viscosity develops during or soon after cooking [60]. Significantly (P<0.05) difference was also observed in the result obtained for the peak time with 72 h fermented sweet potato flour sample

having the least value when compared to the other samples.

Generally, for technical and economic reasons, starches/flours with lower pasting time and temperature may be more preferred when all other properties are equal [61,62].

From the sensory analysis rating, meal from sample B was significantly (p<0.05) rated highest in all the measured sensory attributes. However, all the sensory attributes do not differ significantly from meal from sample D.

With reference to colour, Sample B was the most acceptable and significantly (p<0.05) differs from Sample A and Sample C. For flavour, Sample B had the highest value but do not differ significantly (p<0.05) from the other samples. The Mouldability rating of sample B was also highest and differs significantly (p<0.05) only from sample A. Sample B had the highest sensory rating for both taste and mouthfeel but do not differ significantly (p>0.05) from the other samples.

Based on overall acceptability, Sample B was rated highest also and differ significantly (p<0.05) from samples A and B.

Significant correlations between some of the colour profile, pasting and sensory properties of sweet potatoes samples were observed. The lightness (I*) of the flour samples was found to have a highly significant (p<0.01) and positive correlation with sensory properties of colour and overall acceptability; and a significant (p<0.05) and positive correlation with flavour only.

Redness value (a*) have a highly significant (p<0.01) and positive correlation with yellowness value (b*) and a highly significant (p<0.01) and negative correlation with mouldability, taste and mouthfeel and a significant (p<0.05) negative correlation overall acceptability. Yellowness value (b*) have a highly significant (p<0.01) and negative correlation with peak time and a significant (p<0.05) negative correlation mouldability, taste and mouthfeel.

Both peak and trough viscosities have a highly significant (p<0.01) and positive correlation breakdown viscosity, final viscosity and setback viscosity with a significant (p<0.05) positive correlation pasting temperature. Breakdown viscosity have a highly significant (p<0.01) and positive correlation final breakdown and a

significant (p<0.05) positive correlation with setback viscosity and pasting temperature; and significant (p<0.05) negative correlation with flavor. Final viscosity have a highly significant (p<0.01) and positive correlation setback and a significant (p<0.05) positive correlation with pasting temperature. Setback viscosity have a highly significant (p<0.01) and positive correlation pasting temperature.

Sensory property of colour have a highly significant (p<0.01) and positive correlation with flavor and overall acceptability as well as a significant (p<0.05) positive correlation with mouthfeel. Flavour have a significant (p<0.05) positive correlation with sensory properties of mouldability, mlouthfeel and overall acceptability. Mouldability have a highly significant (p<0.01) and positive correlation with taste, mouthfeel and overall acceptability. Taste and mouthfeel have a highly significant (p<0.01) and positive correlation with overall acceptability.

5. CONCLUSION

The 72 h starter culture fermented sweet potato flour sample has the least pasting temperature value when compared to the other samples. This implies that it has fewer associative forces and cross-links within its starch granule, hence has a faster cooking quality. Generally, for technical and economic reasons, starches/flours with lower pasting time and temperature may be more preferred when all other properties are equal hence the 72 h starter culture fermented sweet potato flour maybe preferable.

The meal from spontaneous fermented sweet flour has the highest sensory rating based on colour and overall acceptability

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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