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Microbiological and physicochemical properties of fermented and unfermented sweet potato flour

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ABSTRACT

This research accesses the suitability of using processed flours from fermented and unfermented yellow-fleshed sweet-potato as alternative flour based on their physiochemical and microbiological properties. Raw yellow sweet potato tubers were obtained from a local Nigerian market and processed into fermented and unfermented sweet potato flours. Their microbiological and physiochemical (proximate and functional) properties were analyzed using standard methods. The microbiological results identified six bacterial genera; (*Bacillus*, *Klebsiella*, *Micrococcus*, *Staphylococcus*, *Lactococcus*, and *Enterobacter*) and five fungal genera (*Aspergillus*, *Penicillium*, *Mucor*, *Candida*, and *Saccharomyces*). *Bacillus* and *Aspergillus* spp. were the most dominant bacterial and fungal genera respectively. The physiochemical result reveals that fermenting sweet potato flour resulted in improved protein, moisture content, and water and oil adsorption capacities compared to the unfermented flour. The pH also decreased from 8.8 to 5.0 after 72 h of fermentation. Most of the microorganisms isolated are associated with plants, water, environment, and fermentation, they are generally recognized as safe (GRAS) organisms and their potentials can be further exploited in the food industry. These findings highlights the potentials of incorporating fermentation techniques in enhancing the nutritional and techno-functional attributes of sweet potato flour as a healthy alternative gluten-free flour-based staple diet.

1. Introduction

Flour is the most important ingredient in bakery production and is a mainstay in the diets of many countries, including Nigeria (Asouzu et al., 2020; Igbabul et al., 2014; Sulieman, 2019; Tufan et al., 2019). The flour used for producing bakery products is usually obtained directly from wheat or as innovative composite flour blends (Adeola & Ohizua, 2018; Asouzu et al., 2020; Hasmadi et al., 2020; Noorfarahzilah et al., 2014; Shrivastava & Chakraborty, 2018). Recently, owing to the Russian-Ukraine armed combat and global climatic changes, there has been a supply challenge with wheat triggering double-digit inflations globally (Caldara et al., 2022; Macchiarelli, 2022). If this trend goes unchecked, some international food monitoring agencies of the United Nations Organization (UNO) like World Health Organization (WHO), Food and Agricultural Organization (FAO), and World Food Program (WFP) are already warning of a looming hunger catastrophe (FAO, 2022).

In 2020, Nigeria was ranked as the 4th largest wheat importer in the world with an import bill of $2.15 billion (Knoema.com, 2021). Reports from Nigeria's National Bureau of statistics (NBS) showed that to meet up with their food security needs, from 2017-2021, between $394.15 billion – $1.29 trillion was spent on importing durum wheat (a variety of spring wheat mostly consumed in different Nigerian diets) (Oyekanmi, 2022). With the current exchange rate of the United States dollars to the Nigerian naira resulting in the astronomical price increase of wheat-based products, the need to look inwards for a suitable substitute for wheat becomes imperative.

Countries are studying the properties of locally grown crop flours as a substitute for wheat flour, examining their physical and chemical properties and microbial enhancement (Anaemene & Fadupin, 2020; Awuchi et al., 2019; Kwofie et al., 2020; Nkhata et al., 2018; Nwosu, 2013; Ohizua et al., 2017; Padmaja et al., 2012; Vaughan et al., 2014). This can reduce the cost of wheat imports, improve national GDP, and enhance the

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nutrient value of diets, particularly for those with wheat intolerance or celiac disease. Studies in this area are becoming increasingly relevant post-COVID-19 (Adeleke & Odedeji, 2010; Asouzu et al., 2020; Butnariu & Sarac, 2019; Cámara et al., 2021; Igbabul et al., 2014; Rowan & Galanakis, 2020).

Sweet potato (SP) *Ipomoea batatas* is a dicotyledonous root crop of the family Convolvulaceae that is widely grown in temperate, tropical, and sub-tropical regions (Chandrasekara & Kumar, 2016; Ejechi et al., 2020; Rose & Vasanthakaalam, 2011; Sanoussi et al., 2016; Syme, 2022). It is nutritionally enhancing and promotes food security in Sub-Saharan Africa (Obomeghei & Ebabhamiegbebho, 2020; Low et al., 2017). When consumed, it provides a healthy diet for proper mental and physical well-being (Padmaja et al., 2012). SP flour has a dark coloration and low loaf volume when used alone in baking, but this can be improved through composite blending (Amal, 2015; Trejo-González et al., 2014; Yuliana et al., 2018).

Nigeria has the largest annual SP production capacity in Africa, estimated at 4.03 million tons per year, presenting a significant opportunity to develop a sustainable food, fiber, and feed industry that empowers farmers and improves national food security and GDP. The use of SP as raw material for value addition in the agro-based industries can produce flours for the bakery and pasta food industries, either alone or as composite blends, contributing to a more robust agribusiness sector (Ejechi et al., 2020).

The focus of this research is to accesses the suitability of using indigenous processed flours from fermented and unfermented yellow-fleshed Sweet-potato as an alternative flour based on their physiochemical and microbiological properties.

2. Materials and methods

2.1. Collection/sources of samples

The dry samples of starchy yellow-fleshed cultivar of sweet potato (*Ipomea batatas*) were purchased from local vendors in Jattu Market in Etsako-West Local Government Area, Edo State, South-South of Nigeria, in Polypropylene bags, and transported to the laboratory for processing. These botanicals were authenticated at the herbarium of Plant Biology and Biotechnology unit, Department of Biological Sciences, Edo State University Uzairue, Edo State, South-South, Nigeria.

2.2. Processing and production of unfermented and fermented flour samples

The modified methods of Ajayi et al. (2019) and Ohizua et al. (2017) were used to produce the unfermented sweet potato flour (USPF) and the fermented sweet potato flour (FSPF) respectively (Figure 1). This involved selecting the inferior sweet potatoes from the batch, washing them under clean running tap water (to remove any adhering soil particles), peeling them, and then rewashing them.

To prevent browning of the samples, the peeled roots were cut into chips and soaked in potable water containing 0.1% (w/v) sodium metabisulfite for 1.5 h at room temperature, washed and drained. The fermented samples were left to further ferment for 72 h at room temperature. The chips were drained, oven-dried for 48 h at 60 °C, and then allowed to cool. Prior to examination, the corresponding dry samples were milled, sieved, placed in a plastic bucket with a transparent cover, and labelled as unfermented sweet potato flour (USPF) and fermented sweet potato flour (FSPF).

2.3. Microbiological analysis

The fermenting sweet potato sample was analyzed for microbiology every 12 h for three days using the guidelines of American Public Health Association's (2001). Samples were dissolved in peptone water and diluted, and microbial density was measured using the pour plating method on Nutrient agar (NA), MacConkey agar (MCA) for bacteria, and Potato dextrose agar (PDA) for fungi. Colonies were counted and recorded as colony forming units per gram (CFU/g), and pure cultures were obtained by sub-culturing and streaking onto sterile agar plates, and then maintained at suitable temperatures.

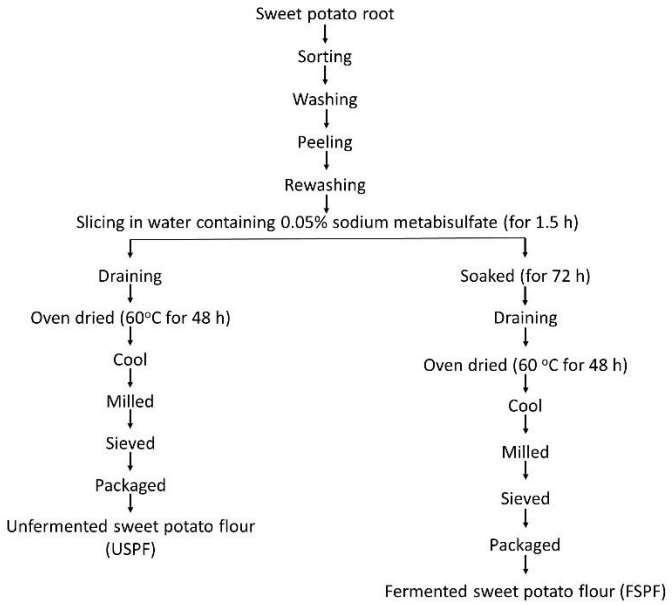

Figure 1. Preparation of unfermented and fermented sweet potato flour

2.4. Characterization of isolate and identification

Identification of the various microbial isolates was confirmed, using standard morphological and biochemical methods (Abdalla & Omer, 2017; Alsohaili & Bani-Hasan, 2018; Basavaraj et al., 2014; Salvamani & Nawawi, 2014).

2.5. Physicochemical analysis

2.5.1. Determination of proximate composition of flours

According to the Association of Analytical Chemists' (AOAC, 2005) standard methods, the proximate composition of the raw flours was assessed in triplicates. The following tests were carried out: crude fibre, protein, ash, moisture, lipid and starch.

2.5.2. Determination of pH

A pH metre (Jenway 3330, UK) was used to measure the pH, also in triplicate.

2.6. Determination of techno-functional properties of the flour

2.6.1. Determination of bulk density

The approach by Asoegwu et al., (2006) was used to calculate bulk density. The volume of the sample was measured after the samples were carefully packed into a 25 mL graduated

cylinder by gently tapping it on the bench top ten times from a height of 5 cm. For each sample, the process was carried out three times, and the bulk density was calculated as g/mL of the sample.

2.6.2. Water absorption capacity (WAC)

The water absorption capacity was determined using a modified method by Adebowale & Maliki (2011), where each flour sample (1 g) was mixed with 10 mL distilled water, centrifuged at 3000 rpm for 15 min, and reweighed after removing the clean layer. The difference between the initial water added and the volume of the supernatant measured with a graduated cylinder was used to calculate the amount of water absorbed.

2.6.3. Oil absorption capacity (OAC)

To analyze the oil absorption capacity, the modified centrifugal method by Asouzu et al. (2020) was used. A mixture of 1 g sample and 10 mL canola oil was blended for 1 min and left for 10 min at room temperature before being centrifuged at 4000 rpm for 30 min. The separated oil was decanted, and the tubes were drained for 10 min at a 45° angle before being weighed. Oil absorption was calculated as a percentage increase in sample weight

2.6.4. Swelling index (SI)

Swelling power and solubility tests were conducted using the method described by Yuliana et al. (2018). Each flour sample (0.1 g) was mixed with 12.5 mL distilled water in a centrifuge tube, heated for 30 min at 60 - 90 °C, and then centrifuged for 15 min at 3000 rpm. The recovered supernatants were dried to a uniform weight at 105 °C. The solubility was calculated as a percentage by dividing the weight of the dried supernatant by the weight of the dried flour (0.1 g), while the swelling power was determined by weighing the precipitates.

2.6.5. Least gelation (LG)

To determine gelation properties, the method of Adebowale et al. (2005) was used. A suspension of the samples at 2-20% (w/v) was made in 5 mL distilled water. The suspension was heated in boiling water (100 °C) for 1 h and rapidly cooled in ice. The test tubes were then cooled for 24 h at 4 °C, and the least gelation concentration (LGC) was determined as the concentration at which the sample did not fall or slip from an inverted test tube.

2.6.6. Foam capacity (FC)

The foam capacity was determined using a modified procedure by Asouzu et al. (2020). A mixture of two grams of sweet potato flour sample and 50 mL distilled water was shaken for 5 min at 1600 rpm in a Braun blender, poured into a 100 mL graduated measuring cylinder, and the total volume was recorded after 30 sec. After standing at room temperature for 30 min, the volume of foam only was recorded.

$$FC = \frac{V_2 - V_1}{V_2} x100$$

where, V_1 and V_2 are the volume of foam before and after whipping, respectively.

2.6.7. Emulsion capacity (EC)

The emulsion property of the sweet potato flour samples was determined using the modified methods of Shakpo & Osundahunsi, (2016). The flour samples (20 g) as well as 20 mL distilled water and 20 mL soybean oil was prepared in a calibrated centrifuge tube. The emulsion was centrifuged at 3500 rpm for 5 min. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as the emulsion activity expressed in percentage.

2.7. Data presentation

Data obtained were subjected to descriptive and inferential statistics (ANOVA) using SPSS (version 20 incorporation, Chicago, Illinois, USA).

3. Results and Discussion

3.1. Processing and production of unfermented and fermented flour samples

Figure 2 displays raw processed fermented and unfermented sweet potato flours. The fermented flour has a more brighter (whitish) outlook than their unfermented counterpart

Figure 2. Showing processed fermented and unfermented sweet potato flour

3.2. Microbiological properties of the flours

Microbial analysis of the fermenting flour revealed the highest bacterial count at 72 h (7.5 to 10.6 $\log_{10}$ CFU/g) and the highest fungal count at 72 h (0 to 3.9 $\log_{10}$ CFU/g) (Figure 3). The total viable bacterial counts for raw unfermented and fermented samples were 7.33 and 7.5, respectively (Figure 4), possibly due to nutrient availability for microbial growth (Adebayo et al., 2013; Santos-Júnior et al., 2021).

The fermentation of sweet potato flour resulted in the isolation of a wide range of microorganisms. The Bacteria includes: *Bacillus cereus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Lactococcus latics*, *Bacillus subtilis*, *Enterobacter hormacchei*, *Bacillus polymyxa*, *Micrococcus luteus*, *Bacillus pumilus*, *Bacillus megaterium* and *Staphylococcus aureus* (Table 1) while fungi isolated includes*:* *Aspergillus nidulans*, *Candida stellata* and *Saccharomyces cerevisae*, *Penicillium* Spp., *Mucor* Spp., *Aspergillus niger*, *Aspergillus flavus* (Table 2).The Occurrence of the isolates at different fermentation time is shown in Table 3.

The genera *Bacillus*; a rod shaped gram positive aerobic ubiquitous bacteria had an overall prevalence of 33.3% (Figure 5). The growth of the different *Bacillus* species in the sweet potato flour could be ascribed to their presence in the environment particularly soil and water. *Bacillus* species are known to produce extracellular enzymes (protease and amylase) responsible for the breakdown of polypeptides and polysaccharides respectively. Their lipopeptides have antimicrobial action, which helps to enhance the safety and shelf-life of foods (Li et al., 2023).

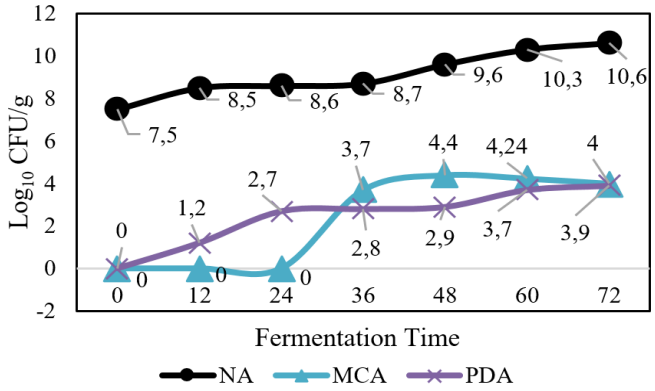

Figure 3. Microbial load of Fermenting Sweet Potato Samples (log_{10}cfu/g) (NA: Nutrient Agar. MCA: MacConkey Agar. PDA: Potato Dextrose Agar)

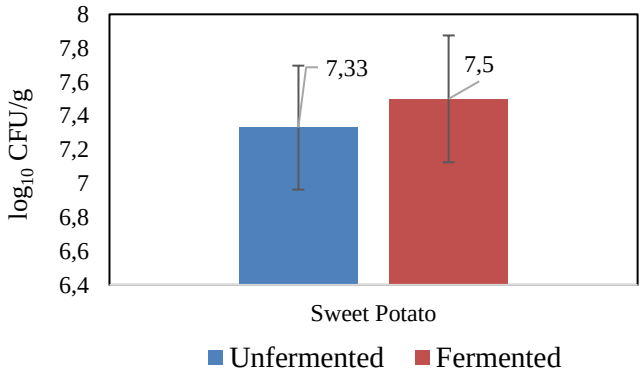

Figure 4. Viable bacterial load of unfermented and fermented raw botanical samples (log_{10} CFU/g)

Staphylococcus aureus is the second most prevalent bacteria in sweet potato flour 29.6%, (Figure 5). Staphylococci are found everywhere, and strains in the nose can contaminate hands, fingers, and face (Samanta & Bandyopadhyay, 2020; Wu et al., 2018). *Staphylococcus aureus* is linked to fermented plant-based foods, especially vegetables (Gupta et al., 2018; Skowron et al., 2022). Poor hygiene of food handlers, water, and utensils can lead to *Staphylococcus aureus* contamination in food (Okolcha & Ajide, 2006).

Aspergillus has the highest occurrence at 44.44% (Figure 6), and can proliferate beyond acceptable limits if food is mishandled (Oranusi & Braide, 2012). *Penicillium* and *Candida* are the second most prevalent at 16.67%. *Penicillium* is commonly found in soil, fresh fruits, vegetables, and indoor air (Skowron et al., 2022). Moulds play important roles in the fermentation of foods and they are most common in acidic foods (Yang et al., 2022). These findings are in line with those of (Ajayi et al., 2016; Hippolyte et al., 2022). *Candida* normally lives on skin and inside the body (de Melo Pereira et al., 2022); their presence in this study could be due to handling and environmental factors.

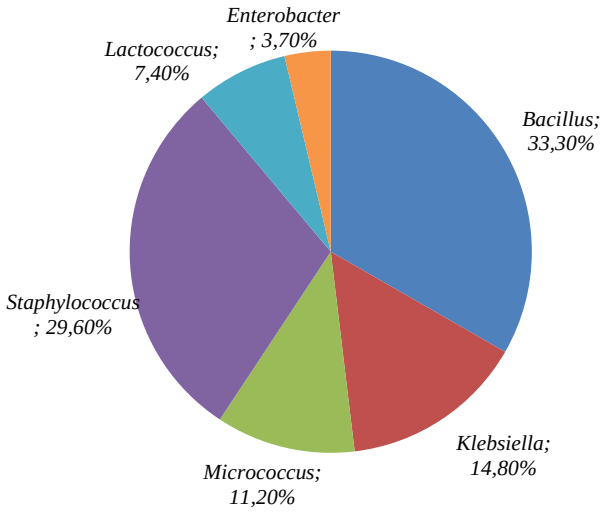

Figure 5. Occurrence of bacterial genera in sweet potato samples

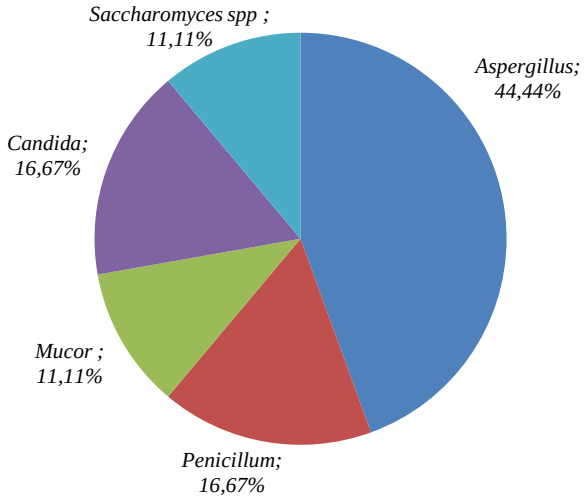

Figure 6. Occurrence of fungi genera in sweet potato samples

3.3. Physiochemical composition fermented and unfermented sweet potato flour

The comparison of the proximate composition of fermented and unfermented sweet potato flour showed that the fermented sweet potato flour has higher protein content (3.32%) than the unfermented one. Fermentation using microorganisms and their enzymes improves the nutritional value of food by converting it into easier-to-digest fermented products (Lasekan & Shittu, 2019; Rollán et al., 2019; Tamang et al., 2019; Voidarou et al., 2021). The fermentation process lead to an increase in moisture content and protein in the fermented sweet potato flour, but a decrease in other parameters (Figure 7). A higher moisture content supports a greater number of microorganisms and enhances the fermentation process. This result is in agreement with several studies on proximate analysis during fermentation (Agblemanyo & Abrokwah, 2019; Gomes et al., 2020; Lasekan & Shittu, 2019; Ogodo et al., 2017).

The pH of the fermenting sweet potato (Figure 8), indicates a gradual fall in pH values from the initial value of 8.8 at 0 h down to 5.0 at 72 h. The decrease in pH values is attributed to the production of organic acid (lactic acid) which is the characteristics of carbohydrates food fermentation as a result of the amylolytic activity of the fermenting microorganisms (Kwofie et al., 2020; Yuliana et al., 2014). Therefore, as fermentation time increases the pH value decreases.

Table 1. Cultural, morphological and biochemical characteristics of isolates

Cultural characteristics	Morphological gram reaction	Moti.	Cata.	Indo.	S.S.T.	Glu.	Suc.	Lac.	Fruc.	Mal.	Ara.	Identified organism
Concave, smooth and milk white	Gram positive rods with central spores	+	+	-	+	A	A	A	A	A	A	*Bacillus megaterium*
Pinkish red, mucoid colonies on MCA	Gram negative short rod	-	+	-	-	A	A	A	A	A	A	*Klebsiella pneumoniae*
Forms gray to deep golden yellow colonies on nutrient agar	Gram positive cocci	-	+	-	+	A	A	A	A	A	-	*Staphylococcus aureus*
Rough opaque, fuzzy or white, slightly yellow with jagged edges on NA	Gram positive rods	+	+	-	+	A	A	A	A	A	A	*Bacillus pumilus*
Concave and creamy yellow pigmented colonies on NA	Gram positive cocci arranged in tetrads	-	+	-	+	-	-	-	-	-	-	*Micrococcus luteus*
Gray white, round opaque flat drying colonies on NA	Gram positive rods with spores in colonies	+	+	-	+	A	A	V	A	A	A	*Bacillus subtilis*
Forms pink colonies on MCA	Gram positive short rods	+	+	+	+	A	-	A	A	A	-	*Bacillus polymyxa*
It forms gray white granular colonies on NA	Gram positive rod with spores	+	+	-	+	A	V	-	A	A	-	*Bacillus cereus*
It forms white raised cohesive colonies on NA	Gram positive cocci	-	+	-	+	A	A	A	A	A	-	*Staphylococcus epidermidis*
Appears bright orange on NA	Gram positive cocci	-	-	-	-	A	A	A	A	A	A	*Lactococcus latics*
Greyish to white coloured large circular and concave colonies on NA	Gram positive rod with spores	+	+	-	-	A	A	-	A	A	A	*Enterobacte rhormaechei*

Key:+ = positive, = negative, Moti = Motility, A = production of acid, Cata = Catalase, V = viable, S.S.T = Spore Staining Techniques, Glu = glucose, Mal = maltose, Suc = sucrose, Ara = Arabinose, Lac = lactose, Fru = fructose, NA= Nutrient Agar MCA= MacConkey Agar

Table 2. Microscopic physical appearance of identified fungi

	Physical appearance	Microscopic appearance	Identified organism
1.	Powdery light green buff to yellow colonies which spread on the surface of the medium	Hyphae and septate with the conidiophore born laterally on the hyphae	*Aspergillus nidulans*
2.	White and become blue-green, gray-green, olive-gray and yellow with time	Hyphae septate hyaline, conidiophores. Simple or branched	*Penicillum* spp
3.	White wooly colony covering the whole petri dish in few days	Hyphae is non septate and thick with elliptical conidial contained in large numbers in the sporangia	*Mucor* spp
4.	Grayish-white to brownish glossy soft and smooth	Yeast with budding cells	*Candida stellata*
5.	White-yellow green velvety to flashy surface due to intense sporulation	Septate hyphae with conidiophores inflates to form vesicles	*Aspergillus flavus*
6.	Powdery dark-brown black colonies with intense sporulation	Septate hyphae with conidiophore born on hyphae	*Aspergillus niger*
7.	Flat smooth moist cream color on PDA petri dish	Unicellular globose and ellipsoid to elongate in shape with budding cells.	*Saccharomyces cerevisiae*

Table 3. Occurrence of the isolates at different fermentation time

	Fermentation time (h)						
Identified organism	***0**	**12**	**24**	**36**	**48**	**60**	**72**
Bacillus megaterium	+	-	-	-	-	-	-
Klebsiella pneumonia	+	+	-	+	+	-	-
Staphylococcus aureus	+	-	+	+	-	+	+
Bacillus pumilus	+	-	-	-	+	-	-
Micrococcus luteus	+	+	-	-	-	-	-
Bacillus subtilis	-	+	+	-	-	-	-
Micrococcus luteus	-	-	+	-	-	-	-
Bacillus subtilis	-	-	-	+	-	-	-
Bacillus polymyxa	-	-	+	-	-	-	-
Bacillus cereus	-	-	-	+	-	+	-
Staphylococcus epidermidis	-	-	-	-	+	+	+
Lactococcus latics	-	-	-	-	-	+	+
Enterobacter hormaechei	-	-	-	-	-	-	+
Aspergillus nidulans	-	+	+	-	+	-	-
Penicillum spp	-	+	-	+	-	+	-
Mucor spp	-	+	-	+	-	-	-
Candida stellata	-	-	+	+	+	+	-
Aspergillus flavus	-	-	+	-	-	-	+
Aspergillus niger	-	-	-	+	+	+	-
Sacchoremyces cerevisiae	-	-	-	-	+	-	+

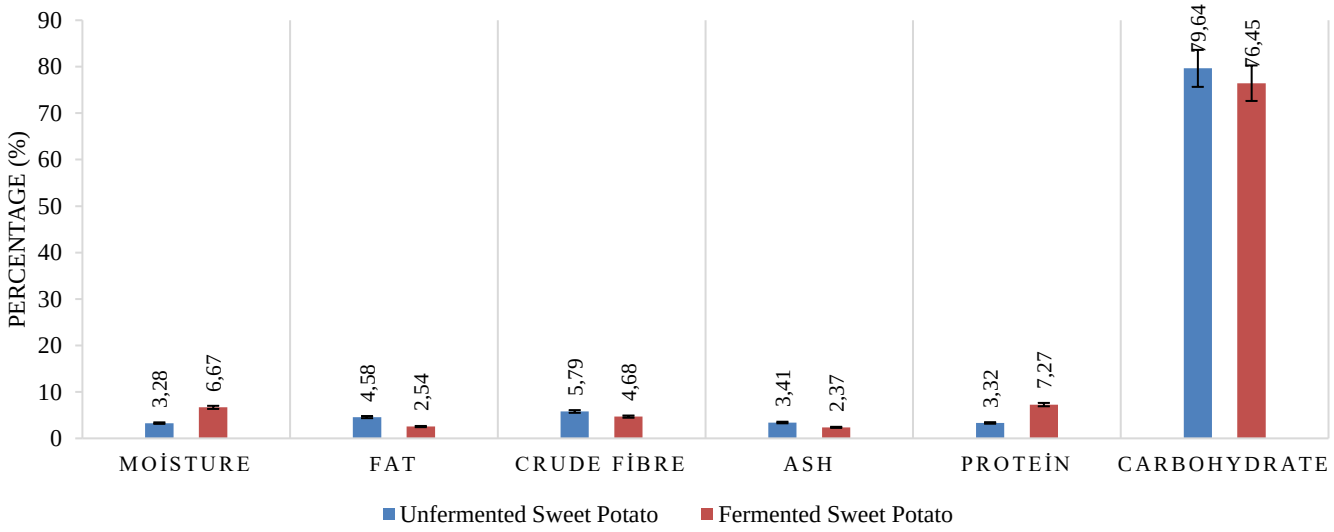

Figure 7. Proximate analysis results for fermented and unfermented sweet potato

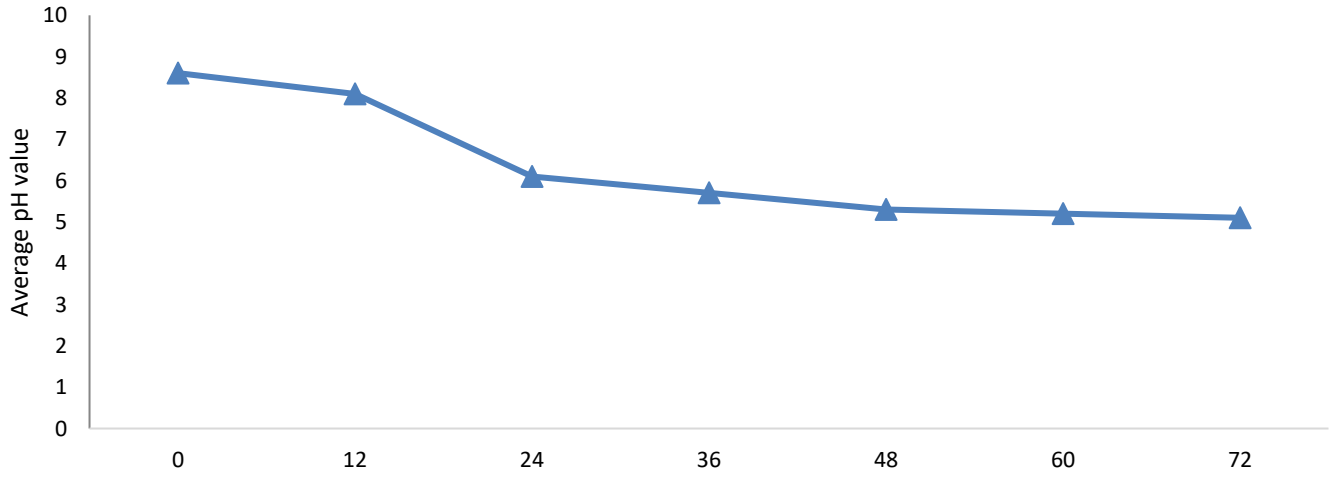

Figure 8. pH during fermentation of sweet potato

3.4. Techno-functional properties of the fermented and unfermented sweet potato flour

The techno-functional properties of fermented and unfermented sweet potato flour (Figure 9), revealed that fermented sweet potato flour showed higher water adsorption capacity (WAC) and oil adsorption capacity (OAC) than unfermented flour (21.01% vs 17.05%, and 17.45 vs 15.51 respectively). WAC measures the ability of a product to retain water under limited conditions (Awuchi et al., 2019; Singh, et al., 2011), and the higher WAC of the fermented flour may be due to its higher carbohydrate and fiber content (Figure 9). The fermentation process likely contributed to the higher WAC in the fermented sample, which agrees with the findings of Fasoyiro et al., (2006). OAC, which affects the binding of protein to fat in food preparations, is important for palatability and flavor retention in bakery products (Hasmadi et al., 2020).

The swelling capacity for both fermented and unfermented sweet potato flour were observed to be the same revealing that fermentation does not increase the swelling capacity of sweet potato flour. In this study, fermentation of sweet potato showed decrease in ash, crude fibre, fat and carbohydrate contents (Figure 9). These constituents are among factors affecting the whiteness of flours. Similar findings has been reported for rice flour (Lu et al., 2005), cassava fufu (Sobowale et al., 2007) and spontaneous lactic acid fermentation on sweet potato flour (Yuliana et al., 2014).

Fermented sweet potato flour has been observed to show good functional properties, which enhance its nutritional qualities. Yuliana et al., (2018), discovered that the fermentation of sweet potatoes flours influence their physicochemical and pasting properties. Also, they observed that fermented sweet potato flours in both composite and non-composite forms, generally decrease in pH, solubility, fat, temperature of maximum viscosity, but increased peak viscosity, amylose content, swelling power, water absorption capacity, break down, and set back value as well as whiteness. Similar results were obtained in this study for the parameters determined.

3. Conclusions

This study explores the suitability of using processed flours from fermented and unfermented yellow-fleshed sweet-potato as alternative flour by analyzing their physiochemical and microbiological properties. The findings highlighted the potentials inherent in incorporating fermentation techniques to enhance the nutritional and techno-functional attributes of sweet potato flour, which could serve as a healthy alternative gluten-free flour-based staple diet especially for gluten-intolerant persons. The results show that spontaneous sweet potato fermentation is dominated by *Bacillus* and *Aspergillus*, these microbes help in improving both the shelf-life and the nutritive composition of yellow fleshed sweet potatoes. Fermentation also improves the physicochemical properties of the flour, making it a suitable substitute for wheat flour in pastry and bakery products. Further research is recommended to explore other nutritional and health benefits of sweet potato as well as how to biotechnologically incorporate important features such as the dough-rising attribute for which wheat is known in order to improve the flour's capacity in producing instant food and bakery products.

Declaration of Competing Interest

There are no conflicts of interest from any of the authors concerning the conceptualization, research design and publication of this work

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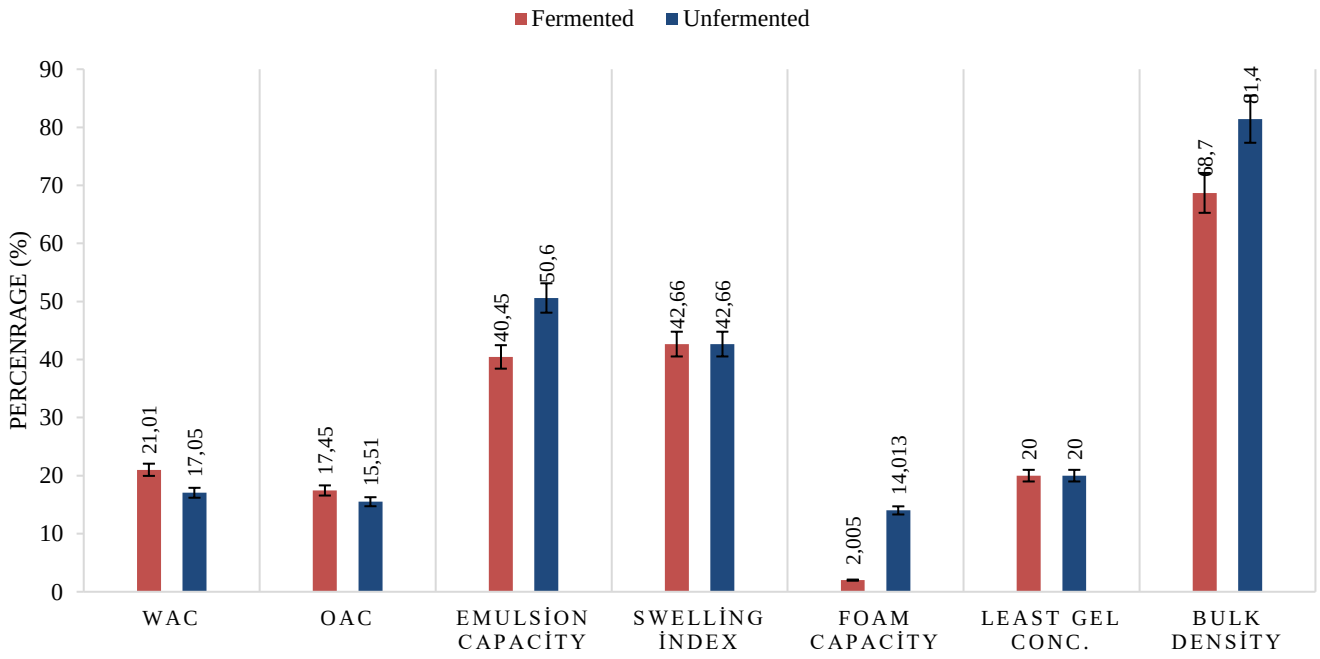

Figure 9. Techno-functional properties results for fermented and unfermented sweet potato flour (WAC: water absorption capacity, OAC: oil adsorption capacity

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