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MICROBIOLOGICAL AND TECHNO-FUNCTIONAL ASSESSMENT OF UNFERMENTED AND FERMENTED GLUTEN-FREE FLOUR MIXES

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Abstract: **Globally, the zeal to develop vastly-enriching gluten-free, scrumptious therapeutic food forms from multiple botanicals, processed into different flour mixes increased after COVID-19 outbreak. The microbiological and techno-functional properties of unfermented and fermented sweet potato, maize and pigeon pea were accessed using customary test protocols for food-based products in order to appraise their nutritional status as flour mixes providing alternative to wheat flour. Samples purchased in their dry forms from food vendors, were validated, subjected to processing and a portion was fermented naturally. Results of the techno-functional properties revealed that the unfermented pigeon pea had the highest percentage of carbohydrate (80.0%) while the fermented pigeon pea had the highest percentage of protein content (11.44%). The fermented flours showed significant improvements in protein constituent, water and Oil absorption capacities when compared with the unfermented flours for all samples. All unfermented flours showed higher foaming capacity. The microbiological assessment showed that the unfermented maize flour had the highest microbial load followed by the fermented sweet potato flour and the bacteria isolates includes *Streptococcus feacalis, Staphylococcus aureus, Lactobacillus casei, Klebsiella pneumoniae, Staphylococcus saprophyticus, Bacillus cereus* and *Staphylococcus epidermidis* while the fungi isolates includes *Mucor mucedo, Penicillum italicum, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Bdellospora helicoides, Candida stellata, Schizosaccharomyces pombe* and *Saccharomyces cerevisiae. Staphylococcus aureus and Saccharomyces cerevisiae* had the highest prevalence percent. The techno-functional properties of all the flours play significant roles in manufacturing, transportation, storage, stability, texture, taste and flavor of food products. Therefore, we recommend that countries with comparatively-advantageous agro-bioresources experiencing massive post-harvest losses, could utilize these findings for the purpose of using their flour mixes to produce various gluten-free food meals.**

Keywords: **Microbiological assessment, Techno-functional assessment, Flour mixes.**

1. INTRODUCTION

The interest in the development of food products using flour mixes/blends has increased globally and is attracting much attention from food researchers, especially for the production of bakery and pasta food products (Hasmadi *et al.*, 2020). It is a Food and Agricultural Organization (FAO) conceptualized economic initiative for developing countries which entails percentage blending of different flours processed from various botanical sources to produce variety of baked and pasta

foods (Noorfarahzilah *et al*., 2014; Hasmadi *et al.,* 2020). Countries with adequate technology and lots of raw botanical sources can develop such blends leading to improved usage of various under-utilized indigenous food crops (Yuliana *et al*., 2018). Researchers have reported that flour blends could be used in the preparation of therapeutic food products (Adeola and Ohizua, 2018; Edun *et al*., 2019;Asouzu *et al*., 2020).

The positive effects in the application of flour mixes can be seen in the final product in relation to some improvements made on the nutritional values, health benefits, techno-functional and physicochemical properties as well as its overall acceptance on different types of food products (Hasmadi *et al.,* 2020). Generally, blending flours is a good new approach to utilizing uncommon botanicals into food products, as the application of these flour mixes produce products with different characteristics and quality depending on the types and percentage of flours used in formulation (Sulieman *et al.,* 2019). The increasing number of flour blends applied in many confectionary products has stimulated a number of studies on the effects of different types of materials used to produce these flours (Hasmadi *et al.,* 2014;Tien *et al.*,2019; Rowan and Galanakis, 2020).

Wheat the flour of choice is not easily grown in most tropical developing nations, it is poor in protein when compared with other grains and lacks some necessary amino acids like lysine and threonine. Long-term intake among wheat intolerant persons has been connected to the immune disorder of the abdominal called celiac disease (Gorgitano and Sodano, 2019). It has become necessary to develop and process enriched flour blends from comparatively-advantageous botanicals as alternative to wheat flour.

Sweet-potato (*Ipomoea batatas*), is a carbohydrate- rich tuberous root crop, consisting mainly of starch and sugar (occurring as sucrose, fructose and glucose) and low amounts of hemicellulose, cellulose and pectins (Onabanjo and Ighere, 2014). Some other chemical constituents include dietary fiber, β-carotene, protein, vitamins B, C, and E; minerals such as manganese, potassium, and iron (Ohizua, *et al.,* 2017). It has significant social importance because it has low economic value and used as a versatile snack food (Adeyeye and Akingbala, 2014). Medically, it is a beneficial food for the diabetics as its ability to assist in stabilizing blood sugar level and lowering insulin resistance in animals (de-Albuquerque *et al*., 2019).

Maize/corn (*Zea mays* L., Poaceae) flour contains high level carbohydrates, starch, protein, many important minerals and vitamins such as potassium, phosphorus, zinc, calcium, iron, thiamine, niacin, vitamin B_6, vitamin A (yellow maize) and folate (Păucean and Man, 2013). The maize flour (Corn starch) is a major ingredient in many industrialized food products and home cooking; it can be combined with wheat flour to produce healthy and nutritious bread (Adeyeye and Akingbala, 2014).

Pigeon pea (*Cajanus cajan L.*), an underutilized, highly drought-tolerant, fiber-rich legume, and a member of the family *Fabaceae*, is often grown and widely consumed in Africa. (Fasoyiro and Arowora, 2013). It provides high quality plant protein (18-35%), and contains 20-22% of all essential amino acids particularly lysine in diets which makes it especially beneficial in overcoming the incidence of protein-energy malnutrition in Africa and to the vegetarian populations (Ohizua *et al*., 2017). It is rich in dietary minerals such as calcium, phosphorus, sulfur, magnesium, iron, copper and potassium. It also has water-soluble vitamins such as niacin, riboflavin, thiamine and ascorbic acid (Kaushal *et al*., 2012). Medically, it has been widely used for many years for effectively managing diabetes, hemorrhoids, high blood pressure, sores, skin irritations, hepatitis, measles, jaundice, dysentery, expelling bladder stones, obesity, stabilizing menstrual period and many other illnesses (Chukwu *et al*., 2013; Oke, 2014; Jaja and Yarhere, 2015). Its slow-release of carbohydrates, makes it a good source of suitable raw material used for the formulation of food products low in glycemic index (Morales-Medina *et al*., 2012).

The main objective of this research study is to develop vastly-enriching gluten-free flour mixes from comparatively-advantageous indigenous agro-processed botanicals which could be used to prepare therapeutic foods.

2. MATERIALS AND METHODS

2.1 Collection and confirmation of Samples

Dry tubers from white-fleshed variety of sweet potato (*Ipomea batatas*), white-grain variety maize (*Zea mays*), and pigeon peas *(Cajanus cajan)*, were bought from foodstuff sellers in Auchi, Etsako-West Local Government Area, Edo State,

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Nigeria; and were confirmed taxonomically by the head Botanist Department of Science Laboratory Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

2.2 Preparation and Fermentation of Samples

The botanical samples were fermented spontaneously to produce their fermented flour forms at temperature 28±2 ^{o}C for 72 hours using the modified method of (Ajayi *et al.*, 2018) for sweet potato (Fig. 1), while the modified method of (Odion-Owase *et al.*, 2018) was used for maize and pigeon pea (Fig. 2), while the unfermented flours of each sample did not undergo fermentation

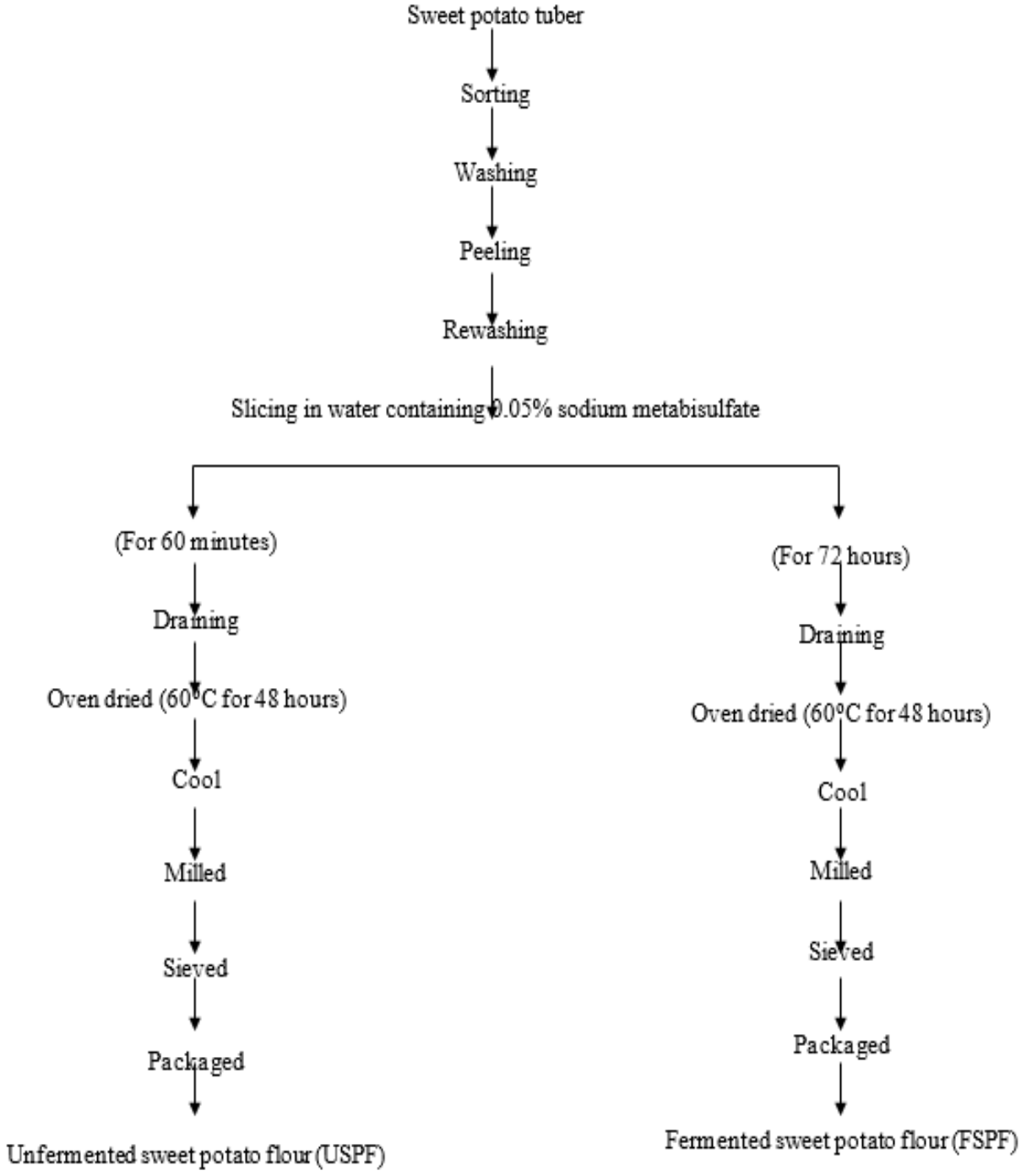

Fig 1: Preparation of unfermented and fermented Sweet Potato flour

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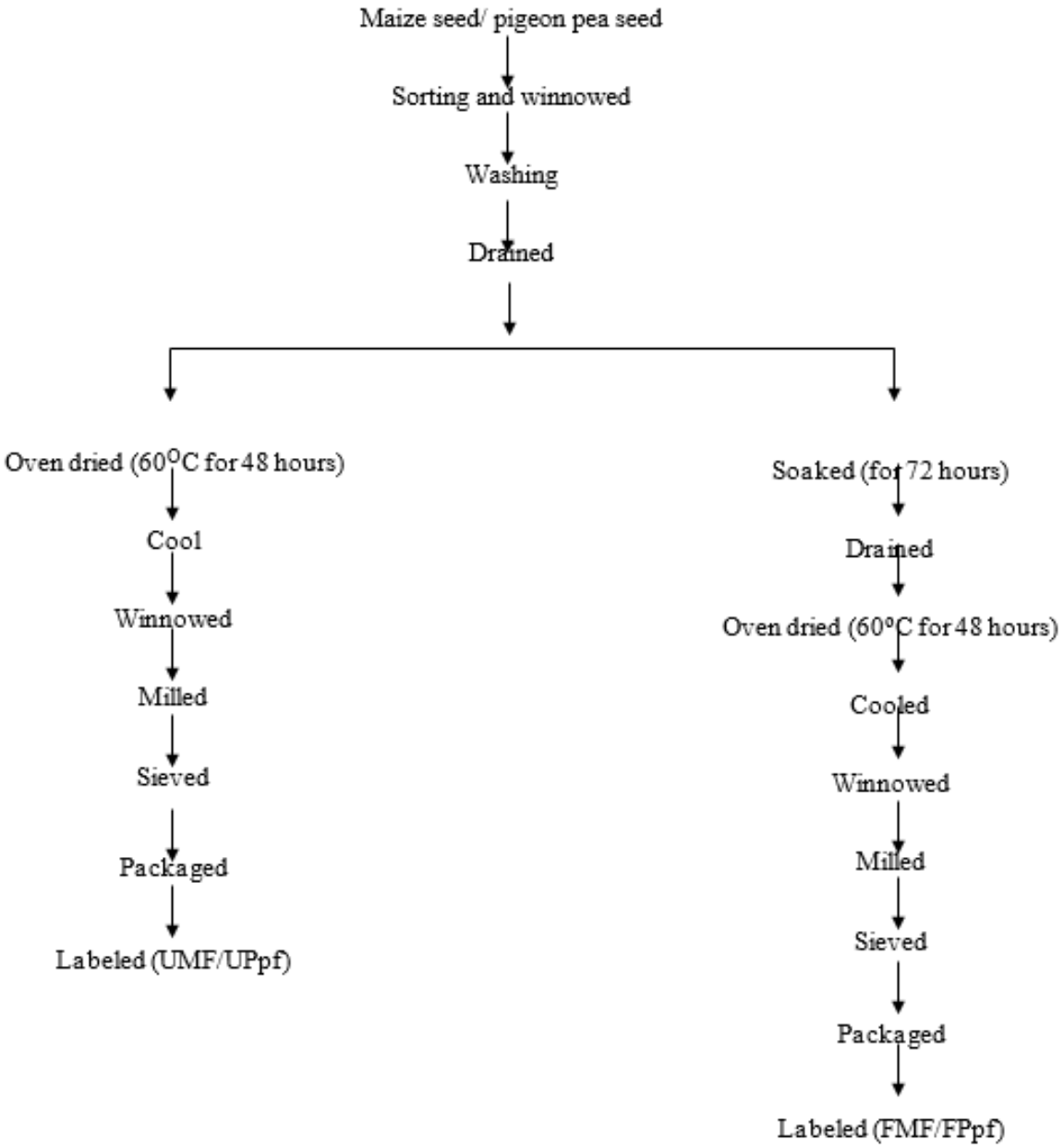

Fig 2: Preparation of unfermented and fermented Maize/Pigeon pea flour

2.3 Determination of Techo-Functional Properties of the flour

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. The following tests were carried out: crude fibre, protein, ash, moisture, lipid and starch.

pH Determination

The pH was measured by weighing two (2) grams of each flour sample into 100ml of distilled water, mix to homogenize for 2- 5 minutes before pH determination. The pH metre used was the Jenway 3330, UK model.

Determination of Bulk Density

The methodology of Asoegwu *et al*., (2006) was followed in calculating bulk density. The samples were cautiously filled into a graduated cylinder (25 mL) by gentle tapping of the cylinder on a bench top; this is done until a height of 5 cm is attained. Thereafter the bulk density for each sample was calculated as g/mL

Water Absorption Capacity (WAC)

The modified method by Adebowale and Maliki (2011) was used. For each one (1 g) gram sample weighed, 10 mL of distilled water was added and placed into a centrifuged tube, then allowed to mix comprehensively for 30 minutes, before centrifuging (using Beckman GP, UK) for 15 minutes with 3000 rpm. After removing the top clean layer, each sample was reweighed. The sample's supernatant was measured with a 10 mL graduated cylinder. The WAC was calculated as the difference between the volumes of each supernatant against those of the water that was initially added to each sample.

Oil Absorption Capacity (OAC)

The OAC was analyzed using the modified centrifugal method by Asouzu *et al.,* (2020), by mixing (1 g) of each sample with 10 ml of pure canola oil for 60 seconds, then allowed to stand for 10 minutes at room temperature before centrifuging at 4000 rpm for 30 minutes. The oil supernatant was carefully allowed to drain for 10 minutes into tubes placed at an angle of 45°, before weighing. The percentage (%) rise in weight of the sample was taken as the OAC.

Swelling Index (SI)

The method by Yuliana *et al*., (2018) was used in evaluating swelling power and solubility. For each flour sample, (0.1 g) was placed into a centrifuge tube and 12.5 mL of distilled water was added. It was placed inside a water bath, and then heat is applied for 30 min at 60, 70, 80, and 90^{o}C, and then centrifuged at 3000 rpm for 15 min. The supernatants were dried at 105°C until the weight became constant. Solubility was calculated in percentage by dividing the weight of the dried supernatant by the weight of the dried flour (0.1 g). The precipitates were gathered immediately and weighed to determine the swelling power.

Least Gelation (LG)

The method of Adebowale *et al.*,(2005) was used in evaluating LG of the flour samples. A suspension of 2-20 % w/v of each flour samples was made using 5 mL distilled water using test tubes. Thereafter, heat was applied until boiling (100 °C) for 1 h, this was followed by quick cooling in ice for 24 h at 4°C. The least gelation concentration (LGC) was taken at the concentration of the sample in the test tube that did not fall or slip down from an inverted position.

Foam Capacity (FC)

The foam capacity (FC) was determined using the modified procedure by Asouzu *et al.,* (2020). At room temperature, each flour samples (2 g) was mixed with 50 ml distilled water inside a Braun blender. At 1600 rpm they are vibrated for 5 minutes, thereafter a 100 ml calibrated measuring cylinder was used to collect the foam and its content and its final volume recorded after 30 seconds. The content was allowed to stand for 30 minutes at room temperature and the volume of foam only was recorded. Foaming Capacity (FC) = (Vol. of foam After Whipping − Vol. of foam Before Whipping)/ Vol. of foam After Whipping x 100 after the 30 min standing is taken as foam stability.

Emulsion Capacity (EC)

The modified methodology of Shakpo and Osundahunsi, (2016) was used for Emulsion property determination for all samples of the flours. A cocktail preparation containing 20 g of flour and 20 mls each of distilled water and soybean oil was made on a calibrated centrifuge tube. This emulsion was centrifuged at 3,500 rpm for 5 min. The percentage of the emulsion property is calculated as the ratio of the emulsion layer's height to the mixture's total height.

Microbiological Analysis

Microbiological analysis was carried out on the flour samples to determine the total microbial counts as described by American Public Health Association, (2001) and Oleghe *et al*., (2022).

After processing the flour samples, one gram (1 gm) was weighed into 9 ml of peptone water, a sequential 10-fold serial dilution was carried out. An aliquot from each diluent was withdrawn and using the pour plate technique, introduced into the following agar mediums: Nutrient Agar (NA), MacConkey Agar (MCA) and De Mann, Rogosa and Sharpe Agar (MRS) for Bacteria; while Potato Dextrose Agar (PDA) for Fungi. They were later incubated at 37^{o}C for 24-48 hours and at room temperature (25± 2^{o}C) for Bacteria and Fungi respectively.

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2.4 Isolation and Enumeration of Bacteria and Fungi

All colonies with different morphologies was enumerated and expressed as colony forming units per gram (cfu/g) of samples, isolated as pure cultures and stored at 4°C and at room temperature as Agar slants for further characterization. Identification of the various bacterial and fungal species was confirmed, using standard morphological, biochemical and molecular methods (Abdalla and Omer, 2017; Alsohaili and Bani-Hasan, 2018).

All procedures were performed in triplicates

Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1 Results

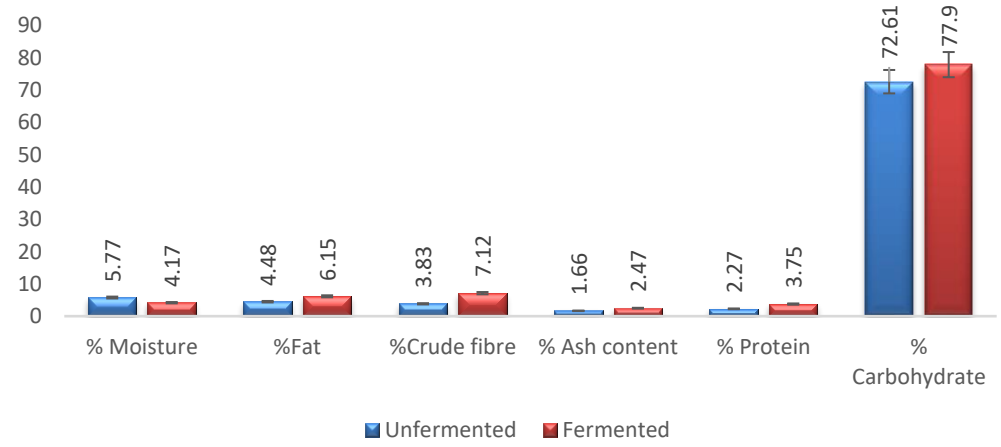

Figure 1a: Proximate content of Maize flours used for the formulation of flour mix.

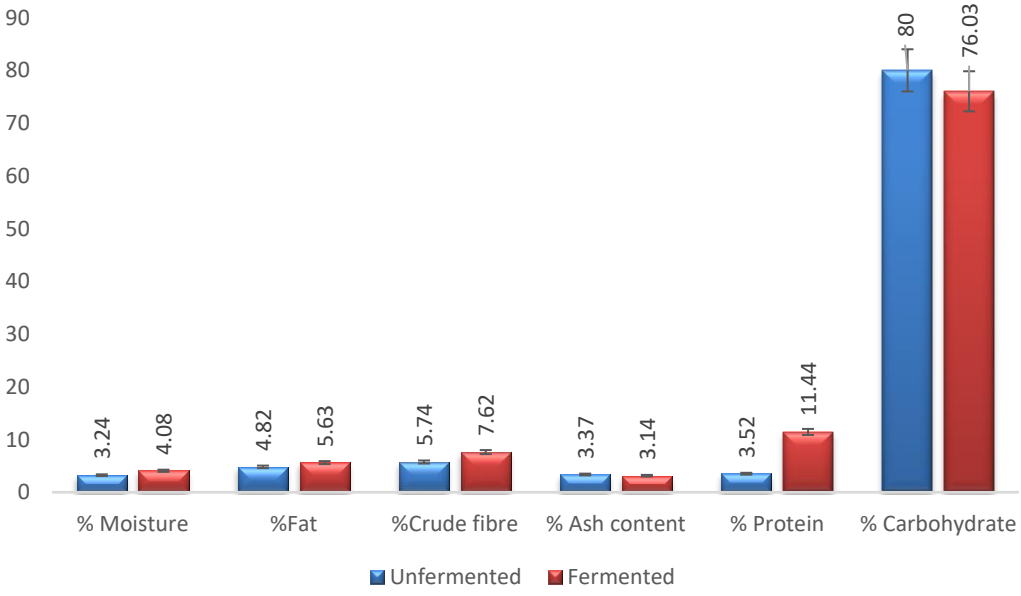

Figure 1b: Proximate content of Pigeon Pea flours used for the formulation of flour mix.

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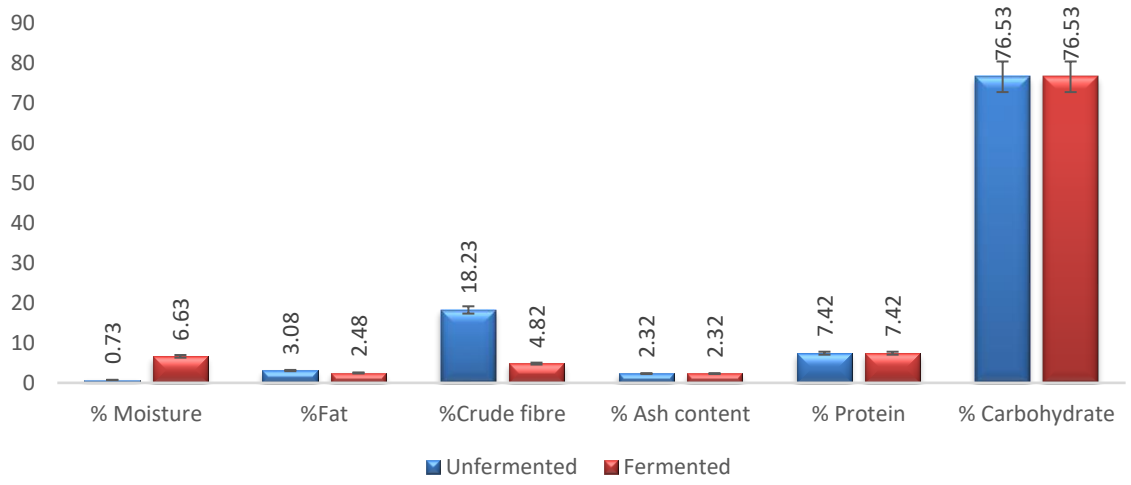

Figure 1c: Proximate content of Sweet Potato flour mix.

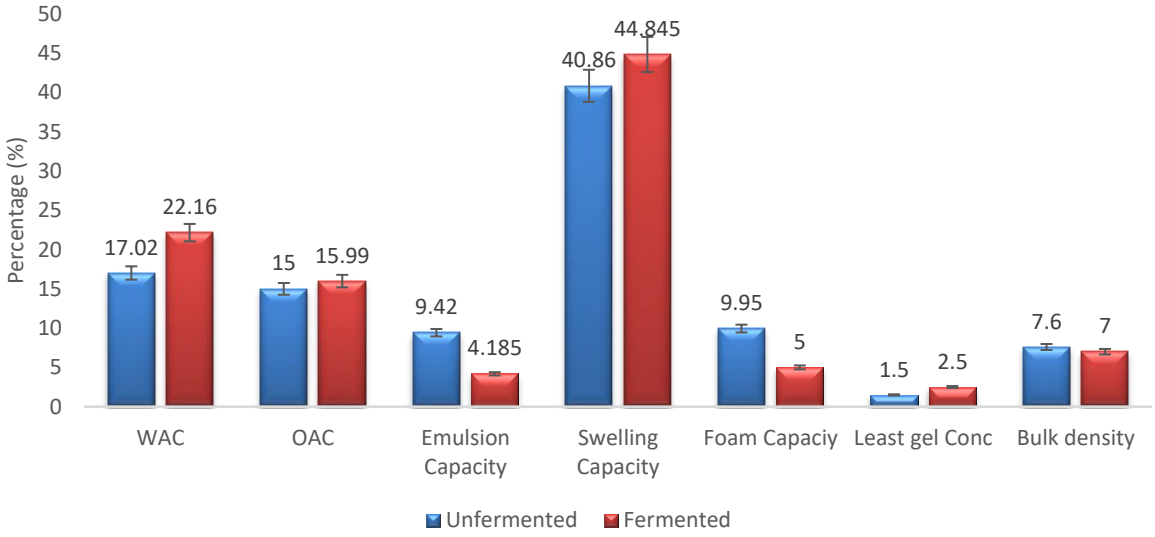

Fig. 2a: Functional properties result for fermented and unfermented maize flour

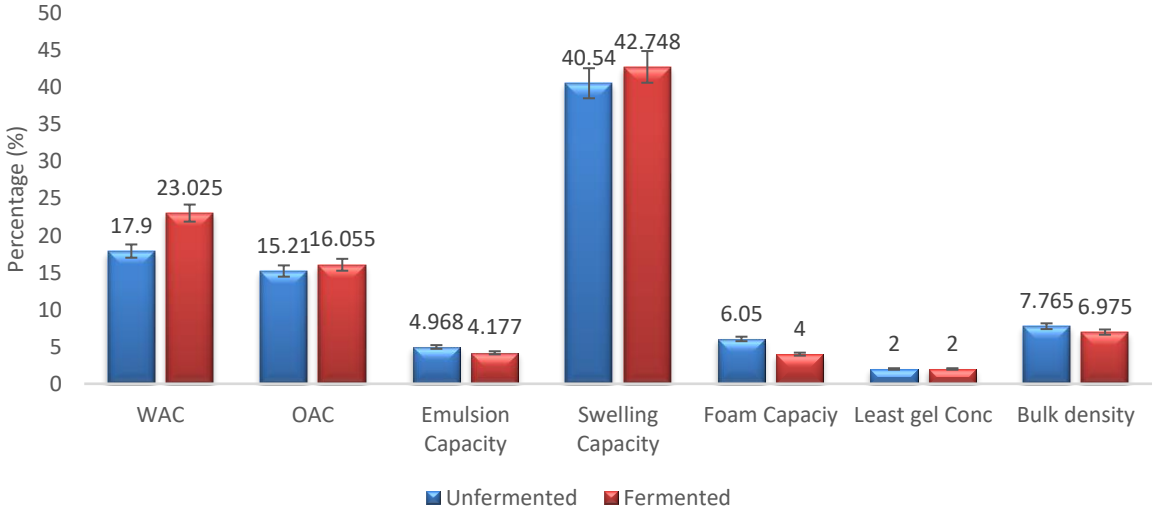

Fig 2b: Functional properties of Fermented and Unfermented Pigeon Pea Flour

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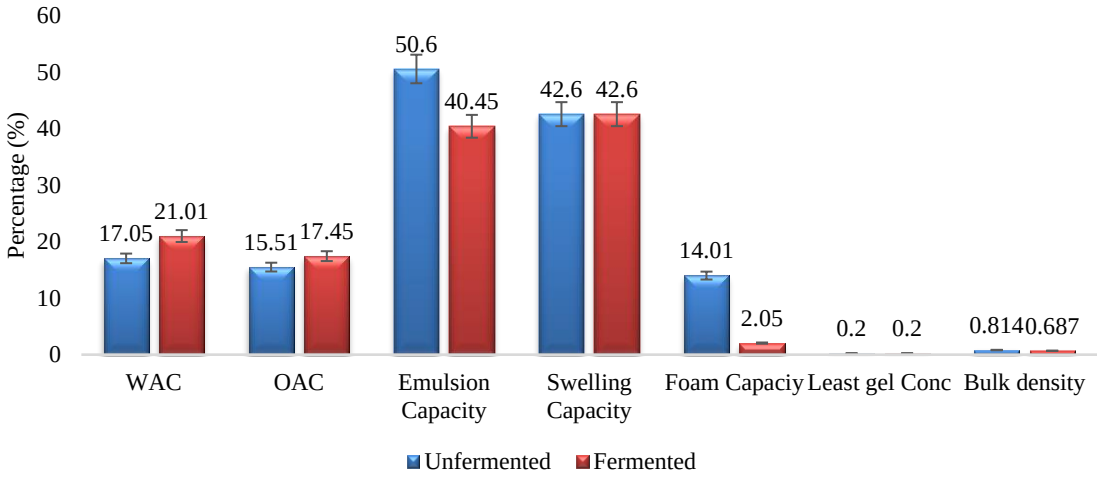

Fig 2c: Functional properties of Fermented and Unfermented Sweet Potato Flour

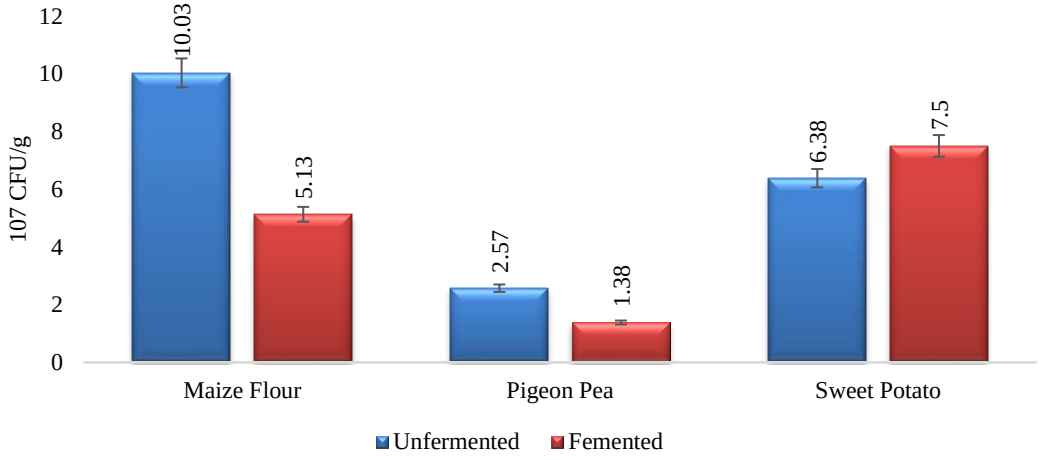

Figure 3: Mean heterophilic bacteria count from maize, Sweet Potato and Pigeon Pea flour used in the formulation flour mix (CFU/g)

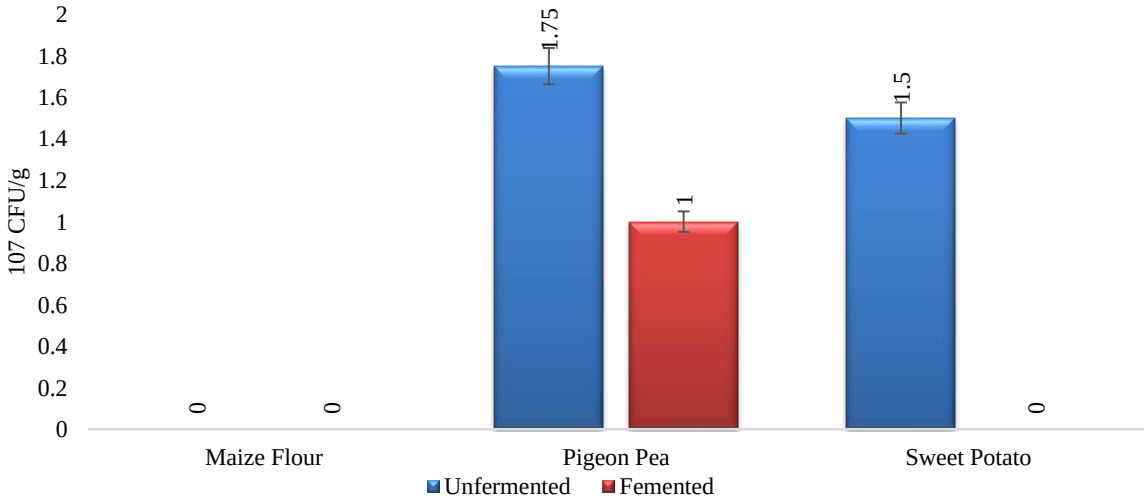

Figure 4: Mean coliform bacteria count from maize, Sweet Potato and Pigeon Pea flour used in the formulation of flour mix (CFU/g).

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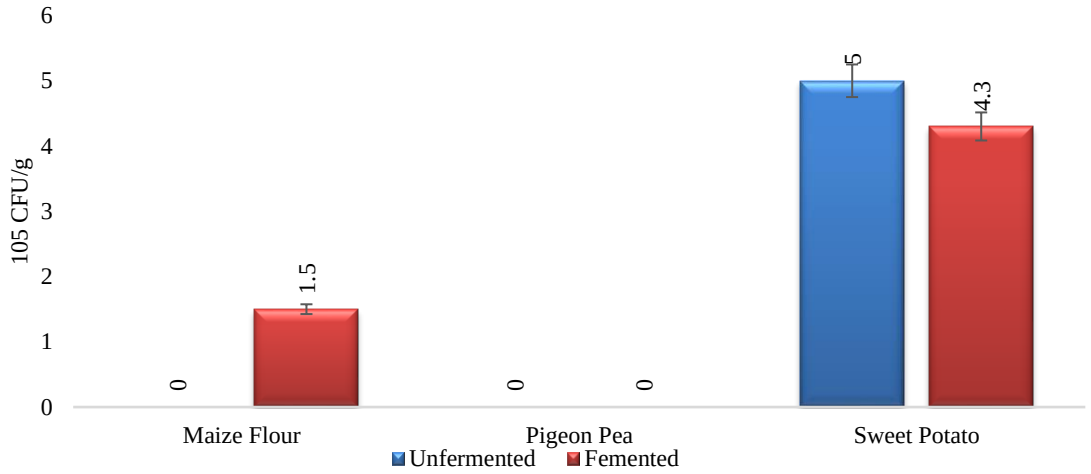

Figure 5: Mean Lactic acid bacteria count from unfermented maize, Sweet Potato and Pigeon Pea flour used in the formulation of composite flour (CFU/g).

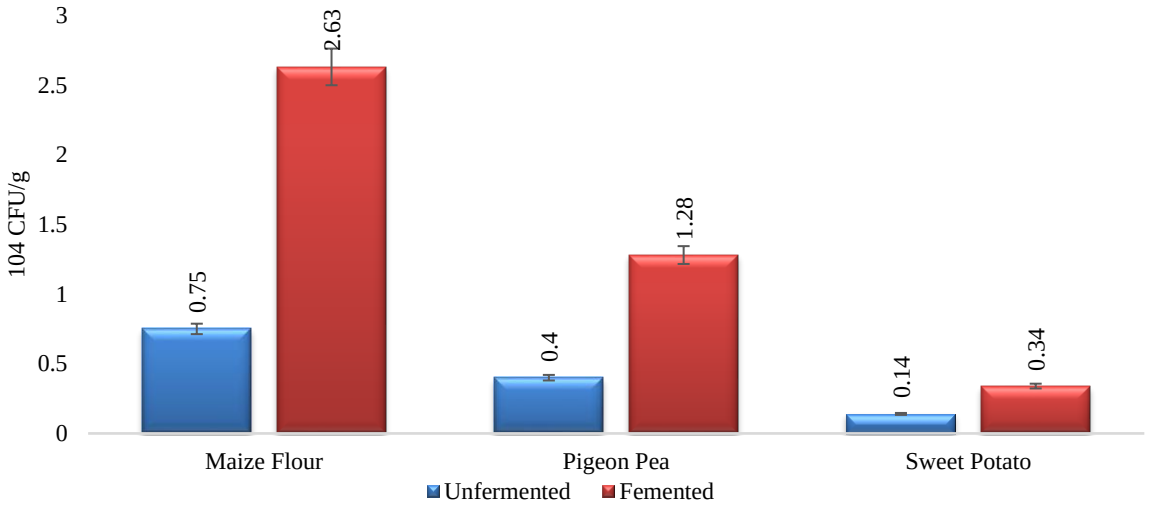

Figure 6: Mean fungi count from maize, Sweet Potato and Pigeon Pea flour used in the formulation of flour mix(CFU/g).

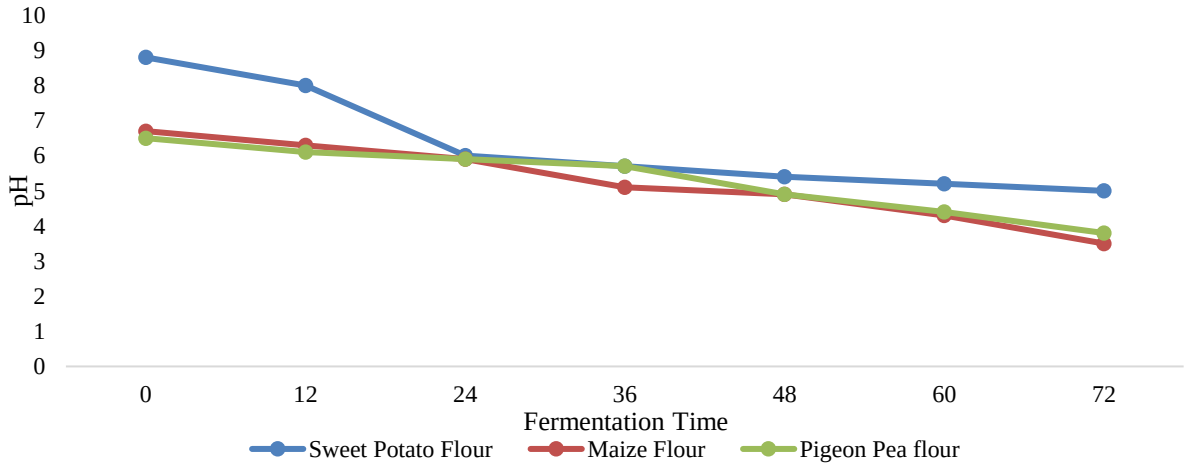

Fig 7: pH of fermenting flour samples

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Table1. Cultural, Morphological and Biochemical Characteristics of Bacteria Isolates From Flour Mix.

Parameter	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8
Cultural characteristics	Small creamy colonies on nutrient agar	Large Creamy, circular, elevated on nutrient agar	Tiny creamy colonies on Nutrient agar, round and pinkish on MRSA and MCA respectively	Small creamy colonies on nutrient agar	Pale white, round, raised smooth colonies on nutrient agar	Cream smooth colonies on Nutrient agar	Brown pigmented colony which diffuses into the Nutrient agar	Pale white, round, raised smooth colonies on nutrient agar
Morphological characteristics	Rod	Cocci	Rod	Cocci	Rod	Shorts rod	Rods	Rod
Cell arrangement	Pairs	Clusters	Pairs	Single	Chains	Pairs	Single	Chains
Gram reaction	-	+	+	+	-	-	+	-
Catalase	-	+	-	+	-	+	+	-
Coagulase	-	+	ND	-	ND	ND	ND	ND
Oxidase	ND	ND	ND	ND	ND	ND	ND	ND
Nitrate	ND	ND	ND	-	ND	ND	+	ND
Urease	ND	ND	ND	ND	ND	ND	-	ND
Motility	+	-	-	ND	+	+	+	+
Methyl Red	+	ND	ND	-	+	-	ND	+
Vogues Proskaur	-	ND	ND	ND	+	+	ND	+
Glucose	A	AG	A	A	A	A	A	A
Lactose	A	AG	A	Aw	A	-	A	A
Sucrose	-	AG	A	-	A	-	A	A
Maltose	-	AG	A	Aw	A	A	-	A
Mannitol	-	AG	A	A	A	-	-	A
Probable bacteria	*Streptococcus feacalis*	*Staphylococcus aureus*	*Lactobacillus casei*	*Micrococcus luteus*	*Klebsiella pneumoniae*	*Staphylococcus saprophyticus*	*Bacillus cereus*	*Staphylococcus epidermidis*

Table 2. Cultural and Morphological Characteristics of Fungal Isolates from Flour Mix

Sn	Cultural characteristics	Microscopy	Isolate
1	Cover agar surface. They are white and fluffy that later turned grey. Reverse side is white.	Sparsely septate, broad hyphae, sporangiophores, sporangia and spores were visualized	*Mucor mucedo*
2	Creamy powdery growth that later turned black	Aseptate hyphae, unbranched sporangiospores are from the foot of rhizoids that enlarged in a cup-shaped form with the mycellial region	*Penicillum italicum*
3	Cottony appearance; white to yellow and then turning black.	Septate hyphae with the conidial heads smoothly defined are radiate with conidiogenous cells biseriate	*Aspergillus flavus*
4	Gray-green velvety to flaky surface due to intense sporulation	Septate hyphae with conidiophores inflates to form vesicles giving rise to conidia	*Aspergillus fumigatus*
5	Dark green with orange to yellow in areas of cleistothecial production. Reverse is purplish to olive. Exudate is usually present and may be brown to purplish. Growth rate is slow to moderate	Hyphae are septate and hyaline. Conidial heads are columnar. Conidiophores are brown, short and smooth-walled.	*Aspergillus nidulans*
6	Powdery dark brown-black colonies with intense sporulation	Septate hyphae with conidiophores on the hyphae	*Aspergillus niger*
7	Cover agar surface. They are white and fluffy that later turned grey. Reverse side is white.	Sparsely aseptate, broad hyphae, sporangiophores, sporangia and spores were visualized	*Bdellosporahelicoides*

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8	White to cream-colored, soft, dull, smooth or slightly wrinkled	Multilateral budding; ellipsoidal to cylindrical in chains. Pseudohypae seen arranged like short tree-like branches	*Candida stellata*
9	Circular-cream colored to yellowish in colour	Small rod-shaped cells	*Schizosaccharomyces pombe*
10	Flat, smooth, moist, glistening or dull, and cream in color.	Blastoconidia (cell buds) are observed, globose, and ellipsoid to elongate in shape	*Saccharomyces cerevisiae*

Table 3. Distribution of the Isolates in the sample flour

Isolates	Sweet Potato Flour	Pigeon Pea	Maize flour
Streptococcus feacalis	$+^{a}$	$+^{a, b}$	-
Staphylococcus aureus	$+^{a, b}$	$+^{a, b}$	$+^{a, b}$
Lactobacillus casei	$+^{a, b}$	-	$+^{b}$
Micrococcus luteus	$+^{a, b}$	-	$+^{a, b}$
Klebsiella pneumoniae	$+^{a}$	$+^{a, b}$	-
Staphylococcus saprophyticus	$+^{a}$	$+^{a}$	$+^{a}$
Bacillus cereus	$+^{a,b}$	$+^{a}$	$+^{a, b}$
Staphylococcus epidermidis	$+^{a}$	-	$+^{a, b}$
Mucor mucedo	$+^{b}$	-	$+^{a, b}$
Penicillum italicum	-	-	$+^{a}$
Aspergillus flavus	-	+	$+^{b}$
Aspergillus fumigatus	-	-	$+^{b}$
Aspergillus nidulans	-	$+^{b}$	$+^{a, b}$
Aspergillus niger	$+^{b}$	$+^{a, b}$	$+^{a, b}$
Bdellospora helicoides	-	$+^{a, b}$	-
Candida stellata	-	$+^{b}$	$+^{b}$
Schizosaccharomyces pombe	$+^{a, b}$	-	$+^{a, b}$
Saccharomyces cerevisiae	$+^{a, b}$	$+^{a, b}$	$+^{a, b}$

Key: a: Unfermented , b: Fermented

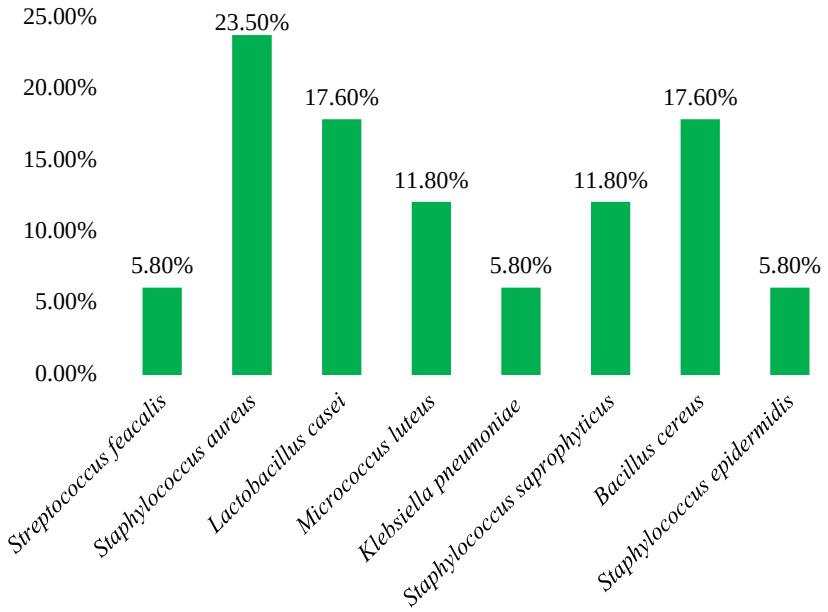

Figure 8a: Percentage occurrence of the Bacteria in the samples.

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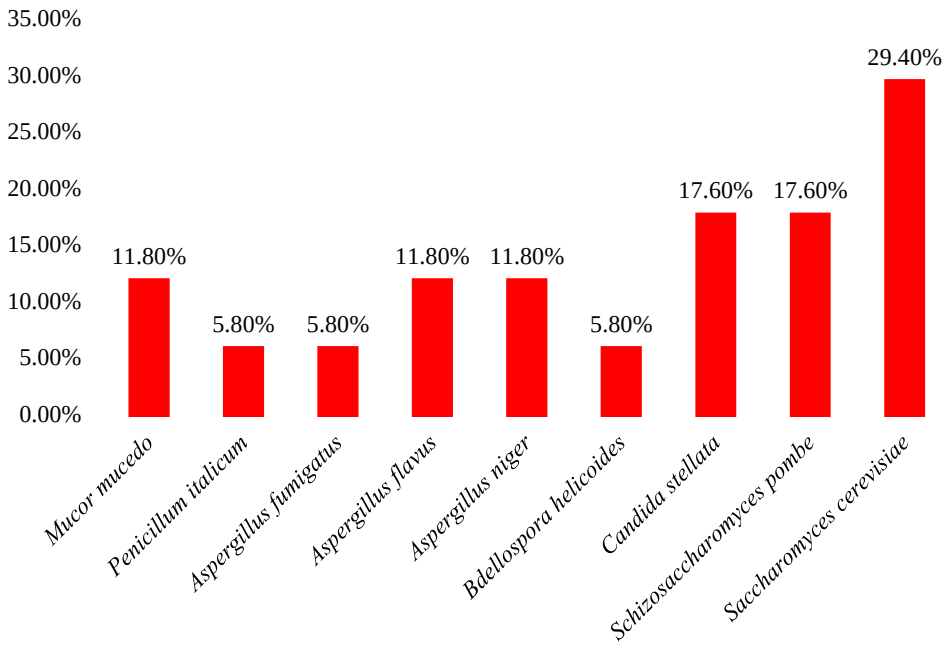

Figure 8b: Percentage occurrence of Fungi in the samples

4. DISCUSSION

The techno-functional and microbiological properties of unfermented and fermented sweet potato, maize and pigeon pea were accessed using customary test protocols for food-based products in order to appraise their nutritional status as flour mixes providing alternative to wheat flour.

The results of the proximate techno-functional analysis for the fermented and fermented maize, pigeon pea and sweet potato were shown on figure 1a, 1b and 1c respectively. The unfermented pigeon pea had the highest percentage of carbohydrate (80.0%) followed by fermented maize flour (77.9%) while the least was recorded in unfermented maize with 72.61%. This agree with the work of Ufere *et al*., (2021) who carried out the carbohydrate content analysis of air-dried white and yellow maize and showed that the air-dried white maize had the highest carbohydrate content (80.48%) and was significantly different ($P < 0.05$) from the air-dried yellow maize which had the least carbohydrate content (71.35%). The carbohydrate content of all the samples used in this study were high. Carbohydrate supplies the energy needed by cells such as the brain, muscles and blood. It also contributes to fat metabolism and thus acts generally to the bulk of the diet (Ufere *et al.*, 2012). However, the fermented pigeon pea had the highest percentage of protein content (11.44%). As the fermented maize, sweet potato and pigeon pea showed significant increase in the percentage of protein constituent compared to their unfermented flours. This agrees with the work of Fasasi, (2009) who revealed that fermentation improves the protein content of fermented foods. Proteins are essential for the building up of new cells and worn out tissues as well as for the creation of hormones, DNA, digestive enzymes, vitamins, antibodies, and structural tissues.

The techno-functional properties of the flour mix as showed on figure 2a, 2b and 2c revealed that all the flour mixes had significant yields. These properties play significant roles in manufacturing, transportation, storage, stability, texture, taste and flavor of food products (Haq *et al*., 2015). Comparatively, the result of these findings also revealed that fermented flours have a higher water and Oil absorption capacity (WAC and OAC), while the unfermented flours showed higher foaming capacity for all the flours. The Consistency and stability of viscous foods and baked products depends entirely on the WAC of starch and protein present in the foods (Haq *et al*., 2015).

Figure 3 shows the mean growth of heterophilic bacteria as the unfermented maize flour had the highest bacterial load followed by the fermented sweet potato flour. This high load of heterophilic bacteria in maize may be due to the high moisture content (5.77%) of the unfermented maize used in the formulation as the fermented sweet potato flour with high moisture content (6.63%) also recorded increased in the load of heterophilic bacteria although to a lesser degree. The reason

for the lower load of these bacteria in the fermented potato flour could be as a result of the end product of fermentation such as lactic acid produced b lactic acid bacteria (LAB), and the increased in pH which is inhibitory/ antagonist to some bacteria (Mehra *et al.*, 2022). This could also be observed in the figure 4 as there were no growth of coliform bacteria recorded except on the unfermented sweet potato and both pigeon pea flours. This is an indication that the samples were not faecally contaminated. However, the case of both pigeon peas and the unfermented sweet potato flour were different as there were indications that the samples were contaminated with coliform bacteria either from the environment or the handling process. This agreed with the work of Kin *et al.*, (2018), which showed that coliform bacteria like *E. coli* can contaminated a sample when handled with and unwashed hand after contact with faecal material. Some material such as currency note or pen that is passed from one person to another could also be a vehicle (fomite) for the transfer of infectious agent from one place to another (Agholor *et al.*, 2020).

The bacteria isolates as shown in Table 1, includes *Streptococcus feacalis*, *Staphylococcus aureus*, *Lactobacillus casei*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Bacillus cereus* and *Staphylococcus epidermidis*. *Streptococcus feacalis* can serve as probiotics as they live as commensal in the gastrointestinal tract of man. It also produce bacteriocin which can be used as bio-preservative.

There is high prevalence of *S. aureus* (figure 7a.) observed from the microbiological analysis of the flour mix. This could be due to the ubiquitous nature of the organism (Agholor *et al*., 2020). This bacterium is commonly found in the soil, water and air and is also found as a commensal organism in the nose, skin and the gastrointestinal tract of humans. They can be propelled from the respiratory tract into the air when coughing or sneezing and eventually settle on the palm and transferred to food (Abubakar *et al*., 2018). This non-pathogenic microflora can cause opportunistic infection. It is also known to cause Staphylococcus food poison. A gastrointestinal illness caused by eating foods contaminated with toxins produced by *S. aureus*. The bacterium can contaminate food if handled with unwashed hands and once food is contaminated, they multiply in the food and produce toxins that causes illness even though the bacteria is killed by cooking.

Lactobacillus casei are found in many fermented foods and in the gut of man. They balance the gut microbiota, improve gastrointestinal dysfunction, prevent infection and cancer by improving the immune responses (Miyazaki and Matsuzaki, 2008).

Bacillus cereus is a common soil bacterium also found in foods such as meat, cereal dishes, vegetables and milk products. It is one of the important causes of food poisoning. These organisms may have find their way into to flour from the environment or through soil during harvest, or preparation process due to their ubiquitous nature and their ability to survive harsh environmental condition through the formation of spores (Agholor *et al*., 2020).

The fungi isolates from the flour are shown in Table 2. These includes *Mucor mucedo*, *Penicillum italicum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, *Bdellospora helicoides*, *Candida stellata*, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*.

There is high fungi count among the fermented sweet potato, maize and pigeon peas flour compared to the unfermented flours. The fermented maize flour had the highest colony forming unit (2.63×10^4cfu/g) as shown in figure 6. This is because some fungi are cellulose digesting and are responsible for the breakdown of fibrous material in maize and pigeon peas. Fungi are also involved in lactic acid fermentation (Obafemi *et al.*, 2022). As showed in figure 8, there is decreased in the pH with increased in the fermentation time and according to Sadiq *et al.*, (2017), the optimum pH for fungi growth is ranged from 5.5 - 6.8 as the enzyme activities is affected by hydrogen ion (H^+) concentration.

The *Saccharomyces cerevisiae* had the highest prevalence percent as revealed in figure 7b. The unicellular fungi also referred to as baker's yeast is used for making bread and other wheat-based products as well as alcoholic beverages through fermentation processes (Klosowski *et al*., 2017).

5. CONCLUSION

The techno-functional properties of all the flour mix had significant yields. These properties play significant role in manufacturing, transportation, storage, stability, texture, taste and flavor of food products. Therefore, developing countries experiencing food insecurity, malnutrition, burgeoning population and experiencing massive post-harvest losses because of insufficient technological know-how across their entire food value chain, but endowed with comparatively-advantageous agro-bioresources could utilize these findings for the purpose of using their flour mixes to produce various gluten-free food meals.

Declaration of conflicting 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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