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The Effect of Fermentation and Extrusion on the Microbiological Composition of Millet and Defatted Soybean Blends

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Authors' contributions

This work was carried out in collaboration between all authors. Author JUO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors II and AOO managed the analyses of the study. Author AOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the effect of fermentation and extrusion on the microbiological properties of Millet and defatted soybean blends and to determine the storage stability of the plant product.
Methodology: Flour blends were prepared from millet and defatted soybean and at different proportions (100, 90:10, 80:20, and 70:30). A portion of the blends was extruded, and the other portion was preconditioned (using solid state fermentation method) and then extruded in a laboratory single screw extruder.
Results: The pH of the raw flour blends during fermentation, recorded a decrease from a range of 9.2-9.3 at day 1 (0 hour) to a range of 6.6 - 6.8 at day 4 (72 hours). *Bacillus cereus, Bacillus subtillis*, *Enterococcus* sp*., Micrococcus* sp*.* and *Lactobacillus* sp were isolated during fermentation. Also in the fermenting flour were some microorganisms which may not have played any part in the fermentation process such as *Pseudomonas* sp., *Proteus* sp., and coliforms (*Enterobacter* sp. and *Klebsiella* sp.). The microbiological load as measured by the total plate count per gram of the

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extrudates was generally low in all the extrudates stored at room temperature (35±2℃). The total plate count at room temperature steadily increased (0-20 days). The total plate count of the extrudates at day 0 showed an initial total plate count of 2.06×10^4-2.72×10^4CFU/g in the extruded (E) blends and 1.48×10^4-3.68×10^4CFU/g in the fermented-extruded (FE) blends. Highest count was observed in both extrudates in E3 and FE3 with 2.72×10^4CFU/g and 3.68×10^4CFU/g respectively.
Conclusion: The extrudates were stable under the storage condition investigated in this study.

Keywords: Millet; defatted soybean; microorganisms; extrudates.

1. INTRODUCTION

Foods including cereals and legumes are processed for different reasons. One very obvious reason for processing food is to improve their storage potential. Most raw foods intended for future use are subjected to some form of processing; and special conditions for their extended storage. They are provided to ensure their usefulness and wholesomeness overtime with or without further culinary treatment. Other reasons for processing food include to: (i) make the raw materials of food more suitable for use in the manufacture of composite foods; (ii) provide variations using same material; (iii) provide a basis for the production of more nutritious food items; and (iii) improve the safety and wholesomeness of food.

Extrusion cooking is a high-temperature, short time process in which moistened expensive, starchy and/or proteinacious food materials are plasticized and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions [1].

Extrusion has some unique positive features compared to other heat processes because the material is subjected to intense mechanical shear. It is able to break the covalent bonds in biopolymers and the intense structural disruption and mixing facilitate the modification of structural disruption and facilitate the modification of functional properties of food ingredients [2,3]. In many instances, the use of only one method of processing may not impact the desired level of removal of antinutritional compounds, improvement of nutritional quality and digestibility as well as bioavailability of minerals. New food processing technologies such as extrusion cooking combined with fermentation can provide alternatives for improving the nutritional quality of the food materials.

One of the oldest known ways of processing food is fermentation. Fermentation as defined by Ojokoh et al. [4] is a complex chemical transformation of organic substances brought about by the catalytic action of enzymes either naturally or elaborated by the microorganisms fermenting the raw materials. The processing of food by fermentation has been practiced by man for centuries and it involves the activities of microflora which may be bacteria or fungi. Achi [5] noted that fermented products remain of interest since they do not usually require refrigeration during distribution and storage.

Contrary to the other methods of food preservation, where the activities and growth of microorganism are usually discouraged, the activities of certain desirable microorganisms are encouraged during fermentation. Thus, instead of increasing or decreasing the temperature to destroy or retard the activities of microorganisms, the optimum conditions of temperature, hydrogen ion concentration (pH), oxygen supply, nutrient and water activity are maintained to promote desirable activities of selected microorganisms [6]. The research works reviews the effect of such processing techniques on the shelf life stability of food plant products.

2. MATERIALS AND METHODS

2.1 Materials

Millet (*Pennisetum glaucum*) was purchased from Uchi market, Auchi, Edo State; while defatted soy bean flour (Variety TGX 1448-2E) was purchased from IITA (International Institute of Tropical Agriculture), Ibadan, Oyo State.

2.1.1 Processing of millet flour

Dried millet grains were sorted and cleaned by removal of grasses, stones and other foreign materials. The sorted grains were washed and directly sun dried for 24 hours. The clean dried millet grains were then fed into an attrition mill. The milled flour was sieved with 120µm mesh into fine flour.

2.1.2 Processing of defatted soybean flour

Soybean seeds were cleaned by sorting out dirts, leaves and stones. The clean soybean seeds were coarsely milled to separate the coat from the cotyledon. The dehulled seeds were milled to fine soybean flour using an attrition mill. The fine soybean flour was then deffated using cold extraction with n-hexane from a 21% fat content to 15.17%. The deffated flour was then air-dried and the clumps broken into fine flour, and then sieved through 60 µm mesh screen.

2.2 Formulation of Millet and Defatted Soybean Blend

The flour samples from millet and defatted soybean were mixed at four (4) level combinations: 100, 90:10, 80:20, and 70:30.

The blends were separated into two batches from each combination. The two batches of the flour blends were stored in a clean sterile polythene bags, tightened at tips, and kept in appropriately labelled plastic bowls with cover lids. A batch of the flour blends were separately hydrated and preconditioned by adding the appropriate amount of water, and manually mixed in a sterile wide bowl, to ensure even moisture distribution. They were then extruded in a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany). The extrudates were oven dried at 30℃ for 24 hours. They were then allowed to cool, and stored at 38℃±2℃ in a polythene bag, and kept in appropriately labeled air tight plastic containers with cover lids.

The second batch of the flour blends were separately fermented, using solid state fermentation method (75 ml of water to 100 g of the flour blend) for 72 hours. The fermented blend was then extruded in a Brabender 20DN single screw laboratory extruder (Brabender OHG Duisburg, Germany). The extrudates were then oven dried at 30℃ for 24 hours. They were stored at a temperature of 38±2℃ in sterile polythene bags and kept in air-tight plastic containers with cover lids.

2.3 Extrusion Process

Extrusion was carried out in a Brabender 20DN single-screw laboratory extruder (Brabender OHG, Duisburg, Germany).

2.4 Chemical Analysis

2.4.1 Determination of pH

The pH of the raw flour blends during fermentation, and the extrudates during storage were determined for 21 days (5 days intervals). The pH was determined as described by AOAC [7] for flours. 10 g of each sample was suspended in 90 ml sterile distilled water and homogenized. The mixtures were allowed to stand for 30 minutes before being filtered. The pH values of the filtrates were determined by a combined glass electrode probe and a pH meter (Mettler-Toledo, Essex M3509 Type 340). Three readings were taken per sample.

2.4.2 Determination of total titratable acidity (TTA)

The total Titratable acidity of the fermenting flour and extrudates (during storage) was determined by the method described by AOAC [8]. 2 grams of the samples were each weighed into 20mls of distilled water in a beaker. 10 mls of the filtrate was put in a beaker, and a few drops of phenolphthalein indicator was added. This was then titrated with 0.1 M sodium hydroxide (NaOH) solution. Readings of the titre values were obtained in triplicates. Acidity was expressed as lactic acid based on the conversion of 1 ml of 0.1 M NaOH being equivalent to 9.008×10^{-3}g of lactic acid.

2.5 Microbiological Analysis

Microbiological analyses were carried out on the raw flour blends, during fermentation and on the extrudates as described in the laboratory manual of Food Microbiology for Ethiopian Health and Nutrition Research Institute [9]. At different stages of the fermentation process, samples were collected in triplicates. Microbiological population of the total bacteria and fungi were determined using nutrient agar and sabourand dextrose agar respectively. Total LAB count was determined using De Man Rogosa sharpe agar, incubated anaerobically at 35℃ for 48 hours in an anaerobic jar. The extrudates were stored at a temperature range of 38±2℃ and were microbiological tested for a period of 20 days at 5 days interval. Organisms were enumerated by using appropriate serial dilution and pour plate techniques. The bacteria cultures were incubated in an inverted position at 25±2℃ for 72 hours. The organisms were characterized based on morphological observations and tests.

2.6 Statistical Analysis

All data obtained from the analyses were subjected to one way analysis of variance (ANOVA) with a probability of level of (P>0.05) using GENSTAT computer software package and SPSS (version 17) software package. The difference between means was separated using Duncan's multiple range tests.

3. RESULTS AND DISCUSSION

The results of the microbial analysis on the raw flour blends of millet/defatted soybean flour showed that the bacterial load ranged between 5.24×10^5cfu/g to 6.24×10^5cfu/g. The highest aerobic bacteria count was observed in R2 (90% millet/10% defatted soybean flour). Results of the fungal count ranged between 1.24×10^5 cfu/g to 1.80×10^5cfu/g. The bacteria isolated from the raw flour blends were *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Micrococcus* sp. *Aspergillus niger* and *Rhizopus fusarium* were the moulds isolated from the raw flour blends.

The variations in pH at solid state fermentation of the raw flours blends during fermentation showed that at day 1 (0 hour) the pH of the raw flour blends had a range of 9.2 to 9.3. The pH of the fermenting raw flour blends dropped at day 2 (24 hours) at a range of 9.0 to 9.1. At day 3(48 hours), the pH of the fermenting raw flour blends further dropped to a range of 7.8 to 8.8. However, the pH of the blends was lowest at day 4 (72 hours) with a range of 6.6 to 6.8.

In general, acidity increased within the first 48 hours and the lowest pH value (6.6) was reached in R1(100%millet flour),while the highest value of 6.8 was observed in R3(80%millet/ 20% defatted soybean flour blend). The decrease in pH and increase in titratable acidity (TTA) up to 48 hours, and a decrease at 72 hours in pH and TTA respectively, may be attributed to inhibition of the growth of the microbial population at a lower pH. This may be due to microbial activity which has been well documented in cereals (such as millets) and cereal-legume mixtures fermented spontaneously (Achi(5). The observed increase in TTA could be due to dominance of the environment by LAB, which degrades carbohydrates resulting in acidification. These observations are in agreement with earlier studies by Nout and Motarjemi [10]. According to Sharpe [11], the ability to produce acid from carbohydrates is used routinely to differentiate and identify species of Lactobacillus as well as other lactic acid bacteria.

A diverse group of microorganisms were isolated during the fermentation process (Table 1). The different microbial species encountered during the fermentation may be due to the spontaneous fermentation of the flour blends. The microorganisms isolated during the fermentation include; *Bacillus cereus, Bacillus subtillis*, *Enterococcus* sp*., Micrococcus* sp*.* and *Lactobacillus* sp. In the fermenting flour, were some microorganisms which may not have played any part in the fermentation process such as *Pseudomonas* sp., *Proteus* sp., and coliforms (*Enterobacter* sp. and *Klebsiella* sp.).

Table 1. Bacteria succession during fermentation of millet and defatted soybean flour blends

Sample	Time (hours)			
	0	**24**	**48**	**72**
A	*Bacillus subtilis* *Pseudomonas* sp.	*Lactobacillus plantarum* *Enterococcus* sp*.* *Pseudomonas* sp.	*Bacillus subtilis* *Lactobacillus* sp. *Streptococcus* sp.	*Lactobacillus* sp *Bacillus subtilis.* *Streptococcus* sp*.*
B	*Bacillus cereus* *Enterobacter* sp. *Proteus* sp	*Bacillus* sp. *Lactobacillus* sp *Klebsiella* sp *Enterobacter* sp	*Bacillus* sp*.* *Lactobacillus* sp*.* *Micrococcus* sp*.*	*Lactobacillus* sp. *Bacillus* sp.
C	*Bacillus* sp *Micrococcus* sp *Enterococcus* sp	*Micrococcus* sp *Bacillus* sp. *Staphylococcus* sp. *Lactobacillus* sp	*Bacillus* sp*.* *Lactobacillus* sp.	*Bacillus* sp. *Lactobacillus* sp.
D	*Bacillus* sp. *Pseudomonas* sp. *Klebsiella* sp.	*Bacillus* sp. *Klebsiella* sp. *Pseudomonas* sp.	*Enterococcus* sp. *Bacillus cereus* *Lactobacillus* sp.	*Enterococcus* sp. *Bacillus cereus* *Lactobacillus* sp.

Key: A -100% millet flour; B -90% millet/10% defatted soybean flour; C -80% millet/20% defatted soybean flour; D-70% millet/30% defatted soybean flour

Table 2. Fungal succession during fermentation of millet and defatted soybean flour blends

Sample	Time (Hours)			
	0	**24**	**48**	**72**
A	−	−	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
B	−	*Aspergillus niger*	*Aspergillus niger* *Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
C	*Aspergillus niger*	*Aspergillus niger*	*Saccharomyces cerevisiae* *Candida utilis Penicillium* sp.	*Candida* sp. *Saccharomyces cerevisiae*
D	*Aspergillus niger* *Rhizopus* sp.	*Aspergillus niger* *Rhizopus* sp.	*Penicillium* sp. *Rhizopus* sp.	*Rhizopus* sp..

Key: A- 100% millet flour; B- 90% millet/10% defatted soybean flour; C- 80% millet/20% defatted soybean flour; D- 70% millet/30% defatted soybean flour; − - No isolation

No Enterobacter was isolated from the fermenting flour blends after 24 hours. The inability to detect Enterobacter in the fermented blends, agreed with the observations of Usha and Chandra [12], Wakil et al. [13]. The disappearance of Klebsiella and Enterobacter may be attributed to changes in the environmental conditions of the fermenting flour blends. Moulds constitute very low proportions of the microbial population. The moulds isolated were *Aspergillus niger* and *Penicillium* sp. (Table 2).

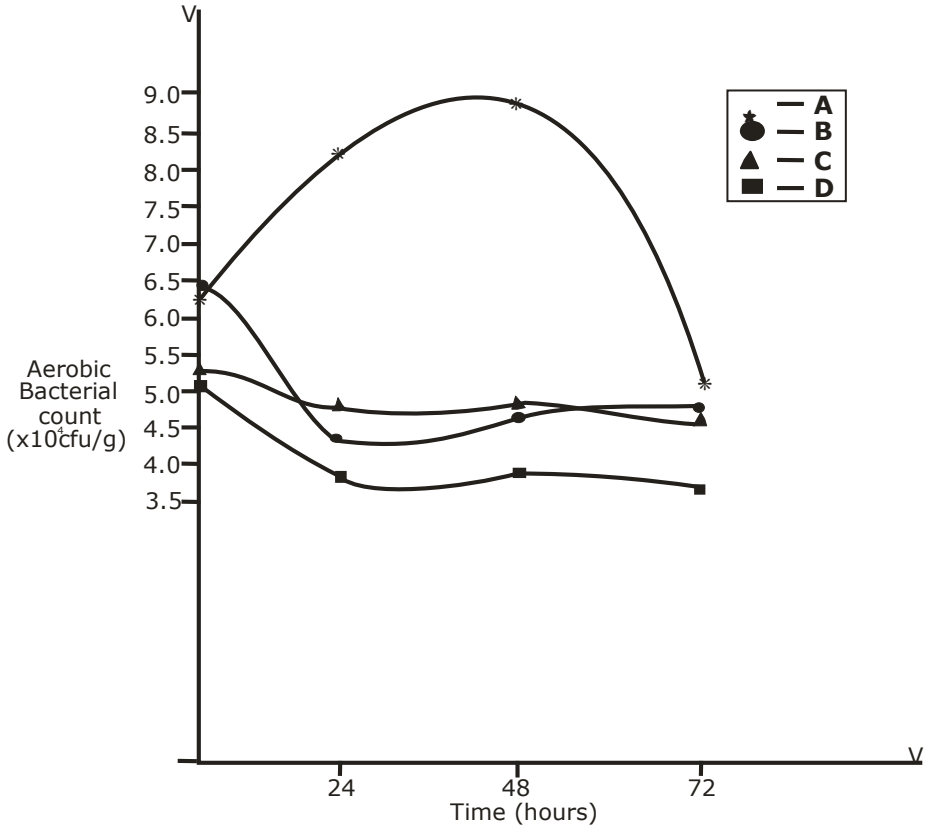

Fig. 1. Changes in the aerobic bacteria count of the flour blends during fermentation

Key: A-100% millet flour; B-90% millet/10% defatted soybean flour; C-80% millet/20% defatted soybean flour; D-70% millet/30% defatted soybean flour

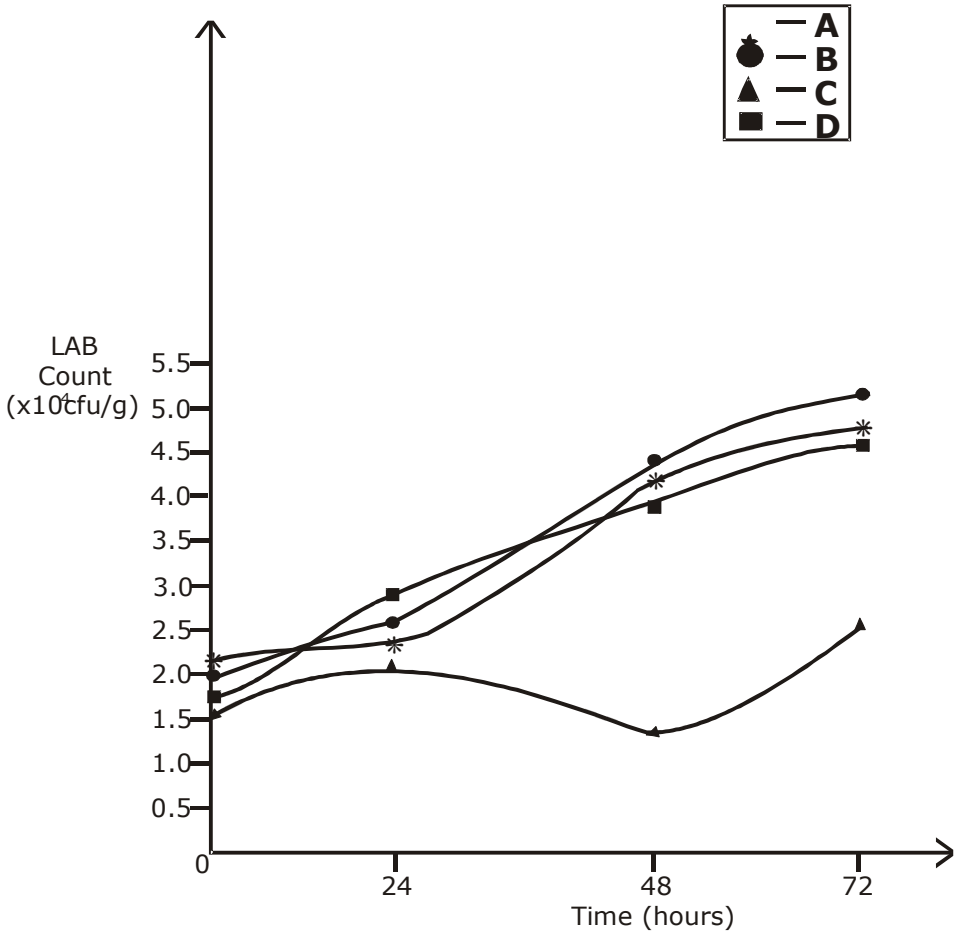

Fig. 2. Changes in the lactic acid bacteria count of the flour blends during fermentation

Key: A-100% millet flour; B-90% millet/10% defatted soybean flour; C-80% millet/20% defatted soybean flour ; D-70% millet/30% defatted soybean flour

A concomitant increase in lactic acid bacteria and yeast counts was observed during the spontaneous fermentation of the cereal-legume blends, an association that has been noted in several cereal foods [14] Figs. 2 and 3. The higher LAB and yeast count recorded is a result of reduction in pH, which has an inhibitory effect on the growth of some natural microflora other than LAB and yeast. Report has also shown that the development of lactic acid bacteria is stimulated by yeast, which provides soluble nitrogen compounds and other growth factors [14]. However, as the fermentation progressed, the LAB and yeast count increased and reached the peak at 72 hours (Fig. 2). The high count of LAB suggests their involvement in the solid state fermentation of the flour blends. The least count was however observed in R4 (70% millet/ 30% defatted soybean flour blends) at 3.81×10^4-4.73×10^4CFU/g. The microbial diversity in the flour blends is similar to the findings of Efiuvwevwere and Akoma [15] that soybean supplemented products had a greater microbial diversity and higher microbial populations.

The aerobic bacteria count of flour blend D changed from 5.24 x 10^4CFU/g (0 hour) to 3.81 x 10^4CFU/g (24 hours), and 3.93 x 10^4CFU/g (48 hours) Fig. 1. A slight decrease was observed at 72 hours to 3.73 x 10^4CFU/g. The highest aerobic bacteria count was observed in flour blend A, at 48 hour, while the least aerobic bacteria count was observed in flour blend D at 72 hours.

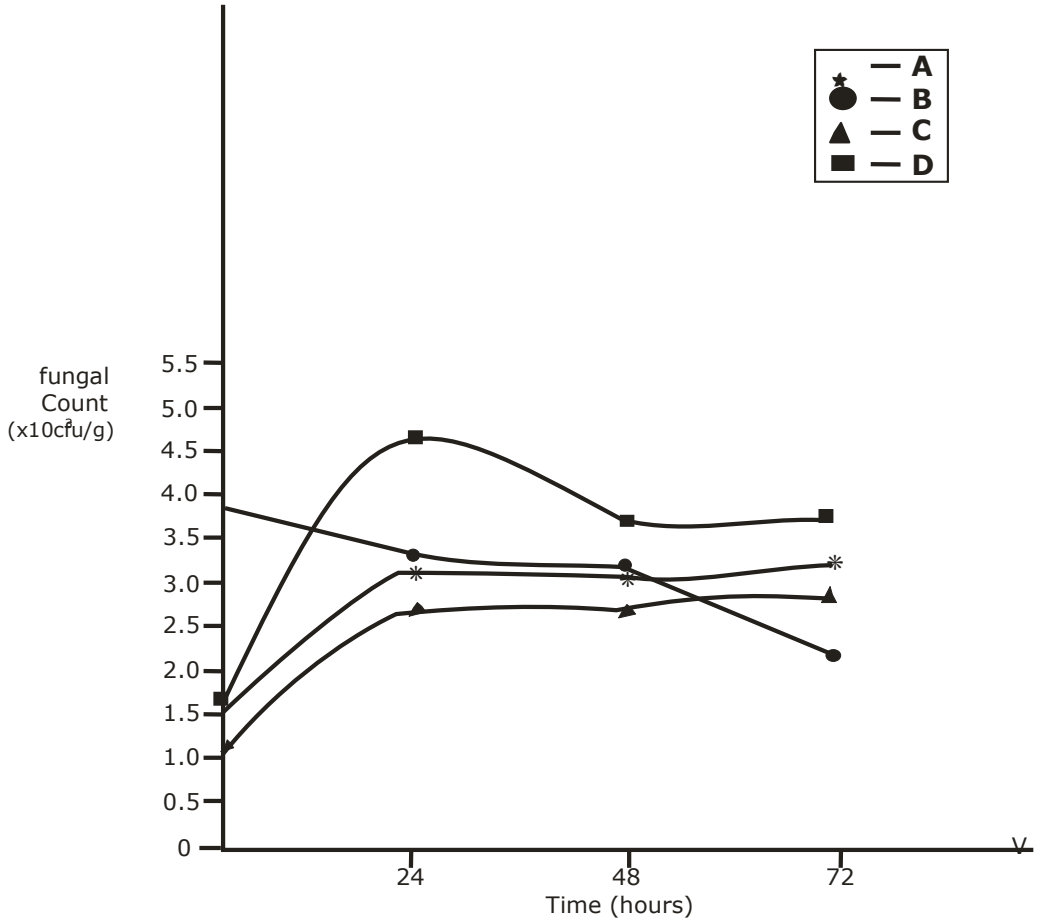

Fig. 3. Changes in the fungal count of the flour blends during fermentation

key: A-100% millet flour; B-90% millet/10% defatted soybean flour; C-80% millet/20% defatted soybean flour; D-70% millet/30% defatted soybean flour

Table 3. Occurrence of microbial isolates of the extrudates

Presumptive microbial isolate	E1	E2	E3	E4	FE1	FE2	FE3	FE4
Bacillus cereus	−	−	−	−	+	+	+	+
Bacillus subtilis	−	+	+	−	+	+	+	+
Enterobacter sp.	−	+	+	−	+	+	−	+
Pseudomonas sp.	−	−	−	−	+	−	−	+
Micrococcus sp.	−	+	+	−	−	−	−	−
Enterococcus sp.	+	+	−	+	+	−	−	−
Citrobacter sp.	+	+	+	+	+	−	−	−
Lactobacillus brevis	+	+	+	−	+	+	+	+
Lactobacillus plantarum	−	+	+	+	−	+	+	+
Candida utilis	+	−	−	−	−	−	+	−
Saccharomyces cerevisiae	−	−	−	−	+	+	+	+
Aspergillus niger	−	−	−	−	−	−	−	−
Rhizopus fusarium	+	−	−	−	−	−	−	+
Penicillium sp.	+	−	−	−	−	−	−	−

Key: E1− Extruded 100% millet flour; E2− Extruded 90% millet/10% defatted soybean flour; E3− Extruded 80% millet/20% defatted soybean flour; E4− Extruded 70% millet/30% defatted soybean flour; FE1−Fermented-extruded 100% millet flour;FE2−Fermented-extruded 90% millet/10% defatted soybean flour;FE3−Fermented-extruded 80% millet/20% defatted soybean flour;FE4−Fermented-extruded 70% millet/30% defatted soybean flour

Table 4. Changes in titratable acidity (tta) of fermented-extruded (fe) and extruded (e) blends of millet and defatted soybeanflour

Parameters	Storage day	E1	E2	E3	E4	FE1	FE2	FE3	FE4	SEM
pH	0	8.00±0.10^{a}	7.87±0.06^{abc}	7.93±0.06a	8.10±0.00^{ab}	7.77±0.06^{a}	7.80±0.00^{a}	7.83±0.06^{ab}	7.97±0.06^{b}	0.024
	5	8.87±0.06^{bc}	8.90±0.00^{c}	8.87±0.06^{bc}	9.00±0.00^{d}	8.70±0.00^{b}	8.80±0.00^{b}	8.87±0.06^{bc}	8.87±0.06^{bc}	0.018
	10	9.00±0.00^{d}	9.10±0.00^{d}	9.10±0.10^{c}	9.13±0.06^{c}	8.83±0.06^{b}	8.87±0.06^{a}	9.07±0.06^{d}	9.00±0.00^{c}	0.024
	15	8.40±0.00^{c}	8.50±0.00^{c}	8.50±0.00^{c}	8.53±0.06^{b}	7.90±0.00^{a}	8.07±0.06^{c}	8.10±0.00^{c}	7.97±0.06^{b}	0.052
	20	8.07±0.06^{a}	8.13±0.06^{bc}	8.17±0.06^{b}	8.23±0.06^{b}	7.37±0.06^{a}	7.47±0.06^{a}	7.43±0.06^{a}	7.57±0.06^{a}	0.074
Titratable	0	2.80±0.00^{bc}	2.52±0.00^{b}	3.09 ± 0.00^{b}	2.52±0.00^{b}	2.49±0.00^{a}	2.52±0.00^{ab}	2.49±0.00^{a}	2.54±0.00^{a}	0.053
acidity	5	2.80±0.00^{b}	3.52±0.00^{d}	3.09±0.00^{b}	2.49±0.00^{a}	4.27±0.00^{bc}	4.03±0.00^{b}	4.17±0.00^{c}	3.91±0.00^{b}	0.162
	10	2.09±0.00^{a}	2.15±0.00^{a}	2.22±0.00^{a}	2.13±0.00^{a}	4.27±0.00^{bc}	4.45±0.00^{cd}	4.13±0.00^{bc}	3.91±0.00^{b}	0.268
	15	3.69±0.00^{cd}	3.71±0.00^{c}	3.81±0.00^{cd}	3.70±0.00^{b}	4.07±0.00^{b}	4.33±0.00^{b}	4.18±0.00^{c}	4.13±0.00^{c}	0.061
	20	4.03±0.00^{d}	4.13±0.00^{d}	3.91±0.00^{cd}	3.72±0.00^{c}	4.77±0.00^{c}	5.33±0.00^{d}	6.17±0.00^{d}	6.03±0.00^{d}	0.236

Means within the same row with different superscripts are significantly different (p<0.05). Values are means ± standard deviations of triplicate means

Table 5. Microbial load of fermented-extruded (fe) and extruded (e) blends of millet and defatted soybean flour during storage at room temperature (38±2℃)

Microorganism	Storage day	E1	E2	E3	E4	FE1	FE2	FE3	FE4	SEM
Total aerobic	0	2.64±0.06^{cd}	2.06±0.06^{b}	2.72±0.06^{ab}	2.38±0.06^{a}	2.93±0.06^{ab}	1.48±0.06^{a}	3.68±0.06^{bc}	3.04±0.06^{b}	0.129
count(×10^{4}cfu/g)	5	2.96±0.01^{c}	2.37±0.01^{b}	3.13±0.01^{c}	3.43±0.03^{c}	2.90±0.06^{ab}	1.56±0.06^{a}	3.71±0.01^{bc}	3.14±0.05^{b}	0.130
	10	3.23±0.04^{d}	2.76±0.06^{b}	3.85±0.04^{d}	2.90±0.04^{b}	3.01±0.01^{c}	1.83±0.03^{ab}	3.99±0.02^{c}	3.44±0.01^{c}	0.133
	15	2.21±0.01^{b}	2.55±0.04^{c}	3.00±0.03^{c}	2.67±0.04^{b}	3.42±0.04^{d}	2.38±0.02^{c}	3.93±0.04^{c}	3.42±0.00^{c}	0.117
	20	1.41±0.01^{a}	1.06±0.05^{a}	2.61±0.01^{a}	1.28±0.06^{a}	2.80±0.04^{a}	1.61±0.02^{ab}	2.52±0.02^{a}	2.30±0.01^{ab}	0.117
Total LAB count	0	0.24±0.04^{a}	0.39±0.03^{b}	0.74±0.03^{a}	1.91±0.02^{b}	1.80±0.04^{b}	2.34±0.05^{c}	2.03±0.04^{c}	2.23±0.02^{c}	0.168
(×10^{4}cfu/g)	5	0.44±0.03^{b}	0.35±0.02^{a}	0.85±0.05^{a}	0.93±0.01^{a}	1.77±0.05^{b}	2.24±0.04^{c}	0.95±0.04^{ab}	1.94±0.02^{b}	0.138
	10	0.57±0.01^{b}	0.65±0.03^{c}	0.80±0.02^{a}	1.01±0.01^{b}	0.46±0.03^{a}	1.91±0.02^{bc}	1.03±0.01^{ab}	1.50±0.01^{b}	0.097
	15	2.73±0.10^{d}	2.84±0.08^{d}	2.24±0.03^{c}	1.97±0.06^{b}	0.51±0.01^{a}	1.80±0.01^{b}	076±0.02^{a}	0.94±0.02^{a}	0.175
	20	1.30±0.02^{c}	1.16±0.01^{cd}	1.03±0.01^{b}	0.95±0.06^{a}	0.67±0.01^{a}	1.64±0.02^{a}	0.81±0.02^{a}	0.84±0.00^{a}	0.061
Total fungal count	0	0.81±0.01^{ab}	1.02±0.02^{a}	1.16±0.01^{a}	0.73±0.01^{ab}	0.52±0.02^{a}	0.55±0.41^{ab}	1.07±0.00^{a}	0.74±0.00^{ab}	0.053
(×10^{3}cfu/g)	5	1.04±0.02^{ab}	1.25±0.05^{ab}	1.32±0.05^{b}	0.69±0.03^{a}	0.62±0.02^{a}	0.55±0.05^{a}	0.98±0.02^{a}	0.96±0.06^{b}	0.059
	10	1.53±0.00^{b}	1.50±0.01^{b}	1.83±0.02^{c}	1.07±0.02^{c}	1.16±0.03^{b}	1.10±0.01^{c}	0.89±0.02^{a}	1.03±0.03^{b}	0.062
	15	2.54±0.03^{bc}	2.35±0.02^{c}	2.89±0.04^{d}	2.12±0.02^{d}	1.81±0.01^{c}	1.61±0.01^{c}	1.15±0.04^{ab}	1.22±0.00^{c}	0.122
	20	2.78±0.14^{bc}	2.62±0.03^{cd}	3.16±0.03^{e}	3.05±0.05^{d}	3.94±0.04^{d}	2.72±0.04^{d}	2.20±0.01^{c}	2.31±0.01^{d}	0.107

Means within the same row with different superscripts are significantly different (p<0.05)
Values are means ± standard deviations of triplicate means

Key:SEM- Standard error of means.;E1− Extruded 100% millet flour;E2− Extruded 90% millet/10% defatted soybean flour;E3− Extruded 80% millet/20% defatted soybean flour;E4− Extruded 70% millet/30% defatted soybean flour;FE1− Fermented-extruded 100% millet flour;FE2−Fermented-extruded 90% millet/10% defatted soybean flour;FE3−Fermented-extruded 80% millet/20% defatted soybean flour;FE4−Fermented-extruded 70% millet/30% defatted soybean flour.

3.1 Microbiology of the Extrudates

Table 3 represents the presumptive microbial isolates of the fermented-extruded (FE) blends and extruded (E) blends. *Lactobacillus species* were predominant in almost all the extrudates, and were isolated mostly in the FE blends. *Bacillus subtilis* was also predominant in the extruded (E) blends. *Aspergillus niger* was absent in all the extrudates. The fungus most predominant in the extrudates was *Saccharomyces cerevisiae.*

3.2 Microbiological Changes in the Stored Extrudates

Similar to the reports of Ojokoh and Udeh [16], as fermentation progressed, some extrudates exhibited undulating pH values. This could be attributed to the dispersion of different molecules during extrusion cooking after fermentation. Ezeama and Ihezie [17]; Omofuvbe et al. [18], had earlier reported that cereal fermentation is lactic type where pH of fermenting mass decrease with increase in total titratable acidity (TTA) and vice versa. However in this research, undulating patterns observed in pH and TTA (though not yet reported) may have been influenced by a number of factors such as fermentation temperature, aeration and composition of the blends. Environmental factors such as analytical error may also have influenced the concomitant undulating pH and TTA values in the reports (Table 4).

A report by the Australian Wine Research Institute revealed that pH is not correlated with the concentration of acids present in foods, but is influenced by their ability to dissociate it. AWRI [19], recorded increasing TTA and a concomitant pH increase during fermentation.

TTA and pH are both measures of acidity. However, for most purposes, pH is a better process control tool. It is also pH, rather than TTA which is the best indicator of preservation and safety effects of acidity. pH influences microbiological stability and the solubility of proteins [20].

The microbial load of all extrudates stored at room temperature (38±2℃) (as measured by the total plate count per gram) was generally low (Table 5), based on the maximum permissible level of microbial loads in ready-to-eat foods (less than 10^6CFU/g). The total plate count of the extrudates at day 0 had an initial total plate count range of 2.06×10^4-2.72×10^4CFU/g (extruded (E) blends) and 1.48×10^4-3.68×10^4CFU/g (fermented-extruded (FE) blends). Highest count was observed in both extrudates in E3 (2.72×10^4CFU/g) and FE3 (3.68×10^4CFU/g). A trend of steady increase in plate count from day 0 - day 10, and a subsequent decrease from day 15 - day 20 was observed in both extrudates. This may be attributed to the changes in the nutritional composition of the extrudates during storage, which may have inhibited the growth of certain bacteria, and encouraged the growth of others.

4. CONCLUSION

Extrusion being a high temperature short-time process can be employed in the production of better quality food materials. The results of the microbiological changes during fermentation of the flour blends were mainly characterized by initial increase in total bacterial count, lactic acid bacterial (LAB) population and yeast count. Apart from the flora present in the grains, microbial flora may also have developed during milling, blending, and defatting processes. This explained the initial higher count in the flour blends, which eventually decreased after extrusion.

The maximum permissible level of total aerobic colony of ready-to-eat foods given by Fylde Borough Council extracted from a manual of PHLSG [21] was 10^4 to less than 10^6CFU/g. Consequently, the extrudates were considered stable under the storage conditions investigated in this study. In conclusion, the extrudates has good potentials as cheap and safe source of ready-to-eat diets which could be utilized to improve food security against malnutrition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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