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Development and microbial quality evaluation of local weaning foods flour based on Maize, Pigeon Pea Seeds fortified with Crayfish Blends

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Abstract

Microbial quality of locally developed weaning food flour based on fermented and malted maize, pigeon pea, fortified with crayfish was investigated, the effects of fermentation and malting on microbial populations and the stability of these blends during a four-week storage period was also analyzed using standard microbiological techniques. Results of the fermented sample flour show Total Heterotrophic Bacteria Count (THBC) ($1.80x10^5$ – $4.50x10^5$Cfu/ml), Total Coliform Count (TCC) ($1.17x10^3$cfu/ml -$4.10x10^3$cfu/ml), Total Fungal Count (TFC) ($2.65x10^6$cfu/ml – $3.27x10^6$cfu/ml). Malted sample flour showed THBC (1.23 – $4.50x10^5$cfu/ml), TCC (2.23 -$4.03x10^4$cfu/ml), and TFC (2.67 -$4.77x10^2$cfu/ml). Fermented blends generally exhibited lower coliform counts ($1.17x10^3$ - $4.10x10^3$cfu/ml) and greater microbial stability during storage (3.00 - $1.26x10^6$cfu/ml) compared to malted blends (2.95 -$7.17x10^5$), highlighting the antimicrobial effects of the fermentation process. The microbial dynamics during storage in both processing methods fluctuated over the four-week period, with fermented samples showing a decline in microbial counts in later weeks (3.00 -1.57 $x10^6$cfu/ml), while malted blends displayed variable regrowth, particularly in Week 4 (2.73 -$7.17x10^5$cfu/ml). Microbial isolates revealed the presence of beneficial microorganisms such as Lactobacillus spp., Pediococcus spp., Bacillus spp, and Saccharomyces spp., alongside potential contaminants including E. coli, Enterobacter spp., Aspergillus spp., and Penicillium spp. The study concluded that fermentation is more effective than malting in controlling microbial contamination and enhancing the microbiological safety of the weaning food blends. However, proper handling, drying, and storage remain critical to minimizing microbial risks. The findings provide important insights for the development of safe, locally sourced, and microbiologically stable complementary foods for infants.

Keywords: *Weaning, food, maize, pigeon pea, crayfish, fermentation, malting, microbial*.

INTRODUCTION

Weaning is a critical stage in infant feeding, occurring between six and twenty-four months of age, when breast milk alone no longer satisfies the increasing nutritional and physiological needs of the child [1]. During this period, infants are highly susceptible to foodborne infections because their immune systems are still developing, making the microbial safety of complementary foods a paramount concern in public health. Complementary foods prepared from locally available ingredients such as maize, pigeon pea, and dried crayfish are widely used due to their affordability and accessibility. However, these raw materials are inherently susceptible to microbial contamination at multiple points along the food production chain, including harvesting, processing, drying, milling, storage, and handling [2][3}.

Microorganisms commonly associated with cereal-legume-animal-based flours include total aerobic bacteria, coliforms, pathogenic bacteria such as *Salmonella* and *Staphylococcus aureus*, as well as yeasts and molds. Some microorganisms are harmless or even beneficial, such as lactic acid bacteria produced during fermentation [4]; others can pose serious health risks if consumed, particularly by infants with immature immune systems [5][6]. Fungal contamination is of particular concern in cereal-based products, as molds such as *Aspergillus* and *Penicillium* can produce mycotoxins, which are highly toxic even at low concentrations and can cause acute and chronic health issues [7][8]. Bacterial contamination, on the other hand, can result from inadequate hygiene during processing or poor storage, leading to proliferation of pathogens capable of causing diarrhea, vomiting, and systemic infections [9][10].

Processing methods, including fermentation, roasting, drying, and milling, are critical in reducing microbial loads in complementary food flours. Fermentation lowers the pH, creating conditions unfavorable for pathogenic bacteria, while roasting and drying reduce moisture content, limiting fungal growth. Despite these measures, improper handling or storage can allow microbial proliferation, making continuous monitoring of microbial quality essential to ensure food safety [11].

Assessment of microbial quality involves determining the total viable bacterial load, the presence of yeasts and molds, and the detection of pathogenic microorganisms.

Such evaluations provide important information on the hygienic status of the flour, identify stages of processing that are vulnerable to contamination, and guide recommendations for safe storage and handling practices. In many African countries, traditional complementary foods are predominantly cereal-based, with maize (*Zea mays*) being a common staple; though maize provides a good source of carbohydrates and energy, it lacks sufficient quantities of essential amino acids such as lysine and tryptophan, which are necessary for proper protein synthesis and tissue growth in children [12]. Again, reliance on cereal-based gruels alone often results in protein deficiency and micronutrient inadequacies. This limitation highlights the need to enhance local weaning foods through fortification with nutrient-rich, affordable, and culturally acceptable ingredients.

Legumes, particularly pigeon pea (*Cajanus cajan L.*), offer a promising solution as protein enhancers in complementary feeding. Pigeon pea is rich in lysine and complements the amino acid profile of cereals, thereby improving overall protein quality in cereal-legume mixtures [13].

Crayfish (*Astacus* spp.) is another valuable ingredient due to its high-quality animal protein and essential minerals, including calcium, phosphorus, iron, and zinc, which are important for bone formation and immune function. The inclusion of animal protein in complementary diets has been shown to enhance growth, improve hemoglobin levels, and increase the absorption of key micronutrients [14]. More so, crayfish contributes desirable sensory attributes, including characteristic flavor and aroma, which enhance the palatability and overall acceptability of fortified complementary foods among infants and young children [15]. Storage stability is particularly important, as microbial populations may increase over time if moisture control and packaging are inadequate, posing a risk to infant health.

The high susceptibility of infants to foodborne illness is the main focus of the development and microbial evaluation of maize–pigeon pea–crayfish weaning food blends, with a view to producing a safe, locally prepared complementary food and analyzing the changes in microbial counts during storage. This research aims to provide a locally produced complementary food that is both acceptable for infants and safe from harmful microorganisms.

Materials and Methods

Source of Raw Materials

Freshly harvested maize (*Zea mays*) and pigeon pea (*Cajanus cajan*) seeds were purchased from a local market in Uturu, Abia State, Nigeria. Dried crayfish (*Astacus spp.*) was also obtained from the same market to ensure availability and uniformity. All crops and crayfish samples were authenticated at the Department of Crop Science and Animal Science, Faculty of Agriculture, Abia State University, Uturu, Abia State, Nigeria, to confirm species identity and quality prior to processing

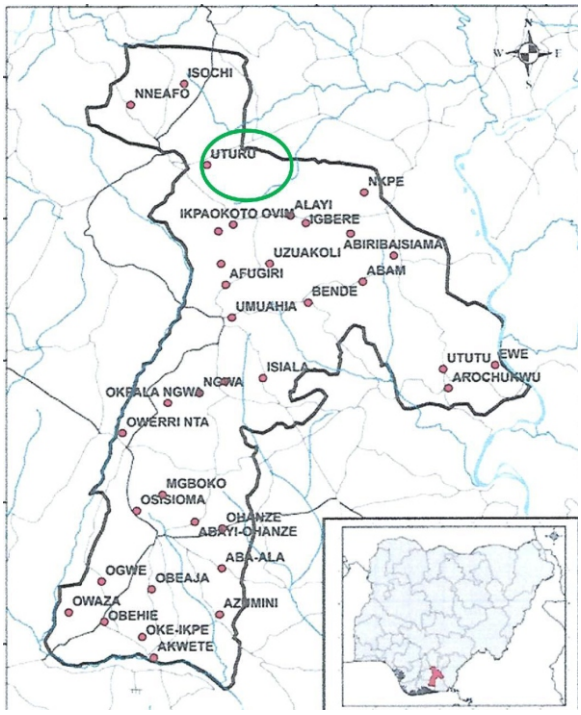


Fig 1: Map showing the study area where maize, pigeon pea and crayfish were collected (Created from Department of Geography and Planning, Faculty of Environmental Studies, Abia State University, Uturu)

Sample Preparation and Processing of Raw Materials

The maize grains were first thoroughly cleaned to remove dirt, stones, broken kernels, and other foreign materials. The cleaned grains were soaked in clean water for 12 hours to soften, after soaking, the grains were allowed to germinate for 48 hours at room temperature, the germinated maize grains were then oven-dried at 60 °C for 24 hours to lower the moisture content and prevent microbial growth, then, milled into fine flour using a laboratory hammer mill to ensure uniform particle size suitable for infant porridge.

Similarly, the pigeon pea seeds were cleaned to remove dirt, stones, and damaged seeds, and then soaked in clean water for 12 hours to soften them and reduce cooking time. The soaked seeds were boiled for 20 minutes to deactivate anti-nutrients such as trypsin inhibitors and tannins. After boiling, the seeds were oven-dried at 60 °C until the moisture content was below 10%, and subsequently milled into fine flour for uniform incorporation into the composite blend.

The crayfish (*Astacus* spp.) were carefully cleaned to remove sand, dirt, and impurities. If not already dried, the crayfish were oven-dried at 60 °C for 24 hours to prevent microbial contamination, after which they were milled into powdered form to ensure even distribution in the maize–pigeon pea flour blend.

Flow chart of fermented maize/pigeon pea and crayfish

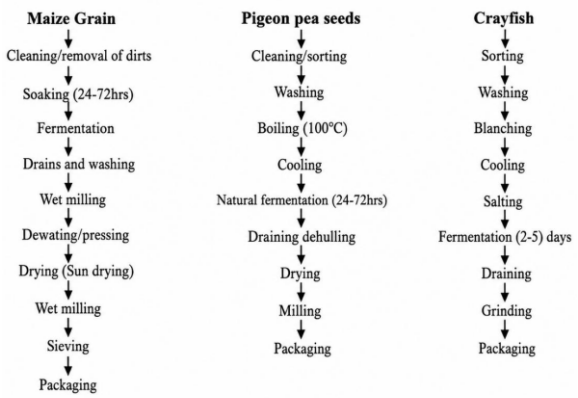


Table A: Blending of fermented maize/pigeon pea and crayfish flour into ratios

Option	Maize	Pigeon pea	Crayfish
A	60%	30%	10%
B	70%	20%	10%
C	80%	10%	10%
D	90%	5%	5%
E	100%	-	-

Flow chart of Malted maize/pigeon pea and crayfish

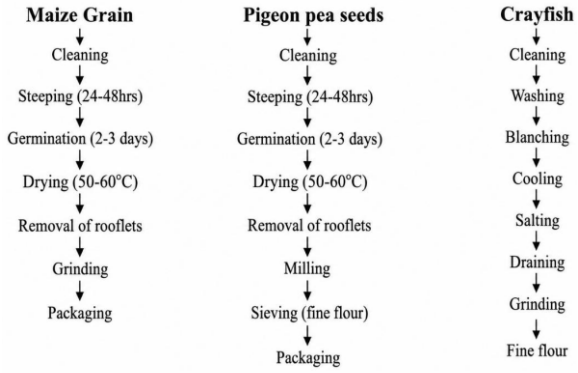


Table B: Blending of malted maize/pigeon pea and crayfish flour into ratios

Option	Maize	Pigeon pea	Crayfish
A	60%	30%	10%
B	70%	20%	10%
C	80%	10%	10%
D	90%	5%	5%
E	100%	-	-

FORMULATION OF COMPOSITE FLOUR

The maize, pigeon pea, and crayfish (*Astacus* spp.) flours were accurately weighed and mixed to create five composite blends for evaluation. The formulations were shown in the Table A and B respectively. Sample E served as the control consisting entirely of maize flour, while Options A–D contained varying ratios of pigeon pea and crayfish flour. All ingredients in each blend were thoroughly mixed to ensure uniformity, and the resulting flours were stored in airtight polyethylene bags at room temperature until further analysis.

Microbial Analysis

The microbial quality of the maize, pigeon pea fortified with crayfish flour were assessed using standard laboratory methods described by [16].

Total aerobic bacteria were enumerated on nutrient agar incubated at 37 °C for 24–48 hours, whereas yeasts and molds were cultured on potato dextrose agar at 25–28 °C for 3–5 days. Coliforms was determined using the Most Probable Number (MPN) method with MacConkey broth. All analysis were performed in triplicate, and results were expressed in colony forming units per ml (cfu/ml). The samples were prepared using the serial dilution method as described in [16]. A gram of the sample blend was weighed into a test tube, and 9mL of normal saline was added. Ten (10) test tubes standing on a rack were also filled with 9mL of normal saline. Using a sterile pipette, 1ml was taken from the first tube containing the food sample into the next tube, then from there also 1ml was taken to the next tube until the 9th tube. The sixth tube was used for the inoculation.

Inoculation:

This was done using the spread plate technique described in [16], and freshly prepared sterile plates were used for this study. 0.1ml was taken from the 6th tube and dropped onto the surface of the agar plates, then a well-flamed L-shaped glass rod was used to evenly distribute it on the surface of the agar. This was allowed to stand for 15 min to allow absorption, then they were incubated at their different temperatures. After incubation for the stipulated time, plates were observed for visible growth, and microbial load count was also taken and recorded.

Isolation of Pure cultures:

Isolation of pure bacterial colonies was done using the streaking method described in [16]. Using a flamed wire loop, single colonies were picked from the bacterial culture plates and streaked out onto freshly prepared nutrient agar plates and incubated at 37^{0}C for 24hours.

Identification of *Isolates*

Isolates were identified using their morphological characteristics, Gram staining, differential stains, and biochemical tests. Characterization was done by observing their morphology on agar plates, which included their shape, color, size, texture, and elevation.

Gram staining:

This was done to identify the bacterial isolates as either Gram-positive or Gram-negative, and the method described by [16] was used. A thin smear of the 24-hour culture was made on a clean grease-free slide and heat fixed. It was flooded with crystal violet and allowed to stand for 1minute. It was then rinsed with slow-running tap water, then flooded again with Gram's iodine for 30 seconds. It was rinsed with water again, then decolorized with Acetone for 5 seconds and again was rinsed under slow running tap water. It was then counter stained with safranin for 1minute then rinsed off again with tap water till no color comes off. It was then air dried and immersion oil was dropped, then it was viewed under the x100 objective lens of the microscope.

Biochemical Examination

Catalase test:

This was done according to the methods described by [16]. 2-3 drops of hydrogen peroxide were placed on a clean grease free slide, using a glass rod, colonies were taken from a 24hour old culture and dropped on the slide, bubbling within 5 seconds was recorded as positive.

Coagulase test:
The method described by [16] was also adopted. 2-3drops of serum will be dropped onto a clean, grease-free slide and then a loopful of the organism was added, visible clumping within 5 – 10 seconds was recorded as positive.

Motility test:
This was done using a hang drop method. A drop of the test bacteria in water suspension was placed on a cover slip. The cover slip were inverted and placed on a slide, and the preparation viewed under a microscope. A sharp darting movement in different directions and across the field of view of the microscope was indicative of a positive.

Oxidase test
This was done according to the method described by [16]. A few drops of 1% aqueous solution of tetramethyl-p-phenylene-diamine hydrochloride reagent was added to a piece of filter paper in a petri dish, a smear of the bacteria were made onto the impregnated filter paper in a petri dish using a glass rod. A purple coloration appearing within 5-10 seconds was a positive result.

Citrate test:
This was done as described by [16]. Simmons citrate agar was prepared according to the manufacturer's instruction in test tubes, then were then allowed to gel. The tubes were inoculated by streaking the isolates using a sterile wireloop. It was then incubated at 37°C for 24-48 hours. A color change from green to blue indicates a positive test.

Indole test:
The method described by [16] was used. A loopful of the bacteria isolates was inoculated into sterile peptone water and incubated for 24hours at 37°C. Then to this culture, 0.5ml of kovacs reagent (p-dimethyl amino benzaldehyde) will be added and thoroughly mixed, this was then allowed to stand. Presence of a deep red color is a positive result.

Urease test:
This was prepared according to the methods described by, [16]. Christensen's urea agar was prepared according to the manufacturer's instructions. It was then dispensed in tubes, slanted and allowed to gel. The slope were then inoculated with the bacteria isolates using the streaking method, this was then incubated at 37°C for 24hours. A positive test was indicated by a color change from yellow to pink.

Methyl red test:
This was done according to the method described by [16]. 5mls of peptone water was dispensed into a tube and a loop full of the bacteria isolate as inoculated into the medium. It was then incubated at 37°C for 3-5 days. 5 drops of the indicator, methyl red, was then added. A color change was indicative of a positive test, from yellow to red.

Vogues proskeur test:
This was done according to the method described by [16]. 5mls of peptone water was dispensed into a tube and a loop full of the bacteria isolate as inoculated into the medium. It was then incubated at 37°C for 3-5 days. Alpha naphthol was added first then KOH was added. A red coloration shows appositive reaction.

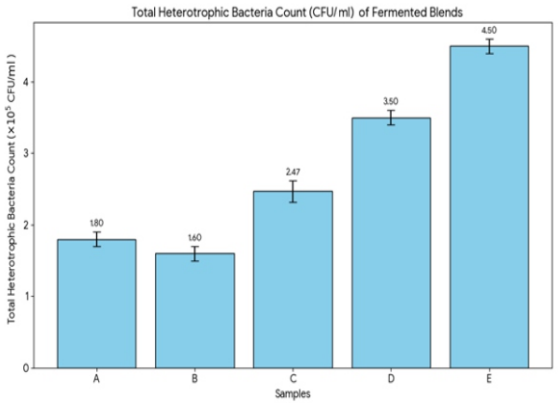
STATISTICAL ANALYSIS
All microbial counts were expressed as mean ± standard deviation (SD) of three replicates. Data were analyzed using one-way analysis of variance (ANOVA) to evaluate differences in microbial populations across the four-week storage period. Where significant differences were observed, means were separated using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p < 0.05$. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Table 1: Total Heterotrophic Bacteria Count (CFU/ml) of Fermented complementary foods based on maize, pigeon pea fortified with crayfish blends

Samples	THBC (CFU/ml)
A	$1.80\pm0.10\times10^{5a}$
B	$1.60\pm0.10\times10^{5a}$
C	$2.47\pm0.15\times10^{5b}$
D	$3.50\pm0.10\times10^{5c}$
E	$4.50\pm0.10\times10^{5d}$

Values are expressed as Mean ± Standard Deviation (n=3). Means followed by different superscript letters are significantly different (P<0.05).

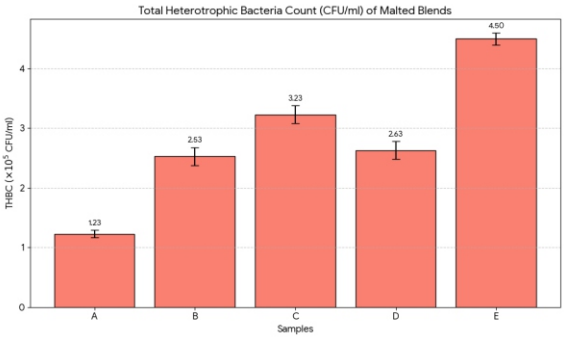


The bar chart above illustrates the Total Heterotrophic Bacteria Count (THBC) for the different fermented blends (Samples A through E). The values were presented in 10^5 CFU/ml, with the error bars representing the standard deviation provided in the table.

Table 2: Total Heterotrophic Bacteria Count (CFU/ml) of Malted complementary foods based on maize, pigeon pea fortified with crayfish blends

Samples	THBC (CFU/ml)
A	$1.23\pm0.06\times10^{5a}$
B	$2.53\pm0.15\times10^{5b}$
C	$3.23\pm0.15\times10^{5c}$
D	$2.63\pm0.15\times10^{5b}$
E	$4.50\pm0.10\times10^{5d}$

Values are expressed as Mean ± Standard Deviation (n=3). Means followed by different superscript letters are significantly different (P<0.05).

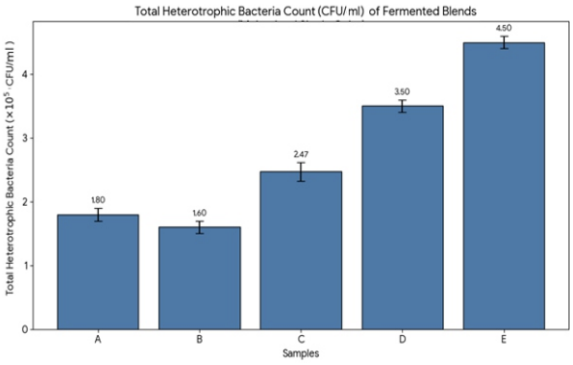


The bar chart above illustrates the Total Heterotrophic Bacteria Count (THBC) for the different malted blends (Samples A through E). The values were presented in units of 10^5 CFU/ml, with the error bars representing the standard deviation provided in the table.

Table 3: Total Coliform Count (CFU/ml) of Fermented complementary foods bases on maize, pigeon pea fortified with crayfish blends

Samples	TCC (CFU/g)
A	$1.17\pm0.15\times10^{3a}$
B	$1.60\pm0.10\times10^{3b}$
C	$2.43\pm0.15\times10^{3c}$
D	$3.70\pm0.10\times10^{3d}$
E	$4.10\pm0.10\times10^{3e}$

Values are expressed as Mean ± Standard Deviation (n=3). Values followed by different superscript letters are significantly different (P<0.05).

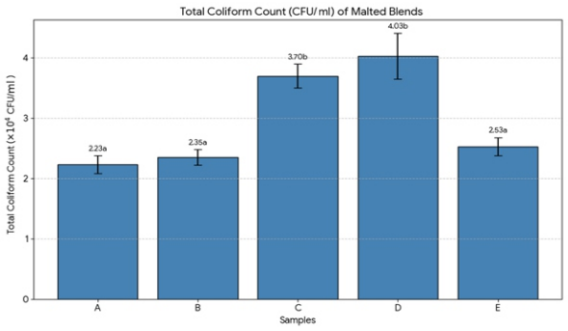


The bar chart above represents the Total Coliform Count (TCC) of the various fermented blends (Samples A through E). The values are presented in units of 10^3 CFU/ml and error bars depict the standard deviations.

Table 4: Total Coliform Count (CFU/ml) of Malted complementary foods based on maize, pigeon pea fortified with crayfish blends

Samples	TCC (CFU/ml)
A	$2.23\pm0.15\times10^{4a}$
B	$2.35\pm0.13\times10^{4a}$
C	$3.70\pm0.20\times10^{4b}$
D	$4.03\pm0.38\times10^{4b}$
E	$2.53\pm0.15\times10^{4a}$

Values are expressed as Mean ± Standard Deviation (n=3). Means followed by different superscript letters are significantly different (P<0.05).

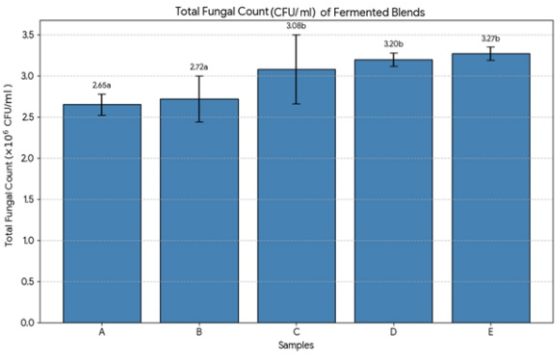


The bar chart above represents the Total Coliform Count (TCC) of the various malted blends (Samples A through E). The values were presented in units of 10^4 CFU/ml,

Table 5: Total Fungal Count (CFU/ml) of Fermented complementary foods based on maize, pigeon pea fortified with crayfish blends

Samples	TFC (CFU/ml)
A	$2.65\pm0.13\times10^{6a}$
B	$2.72\pm0.28\times10^{6a}$
C	$3.08\pm0.42\times10^{6b}$
D	$3.20\pm0.08\times10^{6b}$
E	$3.27\pm0.08\times10^{6b}$

Values are expressed as Mean ± Standard Deviation (n=3). Means followed by different superscript letters are significantly different (P<0.05).

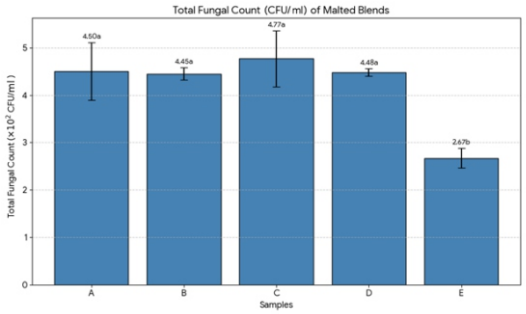


The bar chart below represents the Total Fungal Count (TFC) of the fermented blends (Samples A through E). The values were presented in units of 10^6 CFU/ml

Table 6: Total Fungal Count (CFU/ml) of Malted complementary foods based on maize, pigeon pea fortified with crayfish blends

Samples	TFC (CFU/g)
A	$4.50\pm0.61\times10^{2a}$
B	$4.45\pm0.13\times10^{2a}$
C	$4.77\pm0.59\times10^{2a}$
D	$4.48\pm0.08\times10^{2a}$
E	$2.67\pm0.21\times10^{2b}$

Values are expressed as Mean ± Standard Deviation (n=3). Means followed by different superscript letters are significantly different (P<0.05).

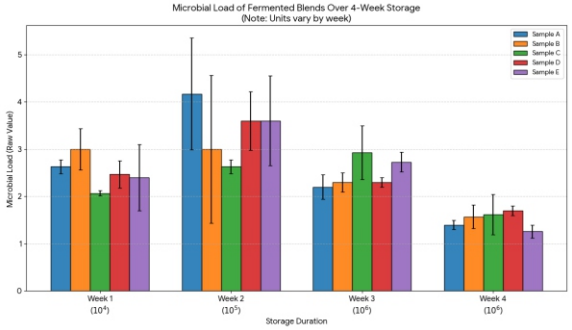


The bar chart above illustrates the Total Fungal Count (TFC) for the malted blends (Samples A through E). The values were presented in units of 10^2 CFU/ml

Table 7: Microbial Load/ shelf- life (CFU/ml) of Fermented Blends during 4-Week Storage

Samples	Week 1 (10^4)	Week 2 (10^5)	Week 3 (10^6)	Week 4 (10^6)
A	2.63±0.15	4.17±1.18	2.20±0.26	1.40±0.10
B	3.00±0.44	3.00±1.56	2.30±0.20	1.57±0.25
C	2.07±0.06	2.63±0.15	2.93±0.57	1.62±0.43
D	2.47±0.29	3.60±0.62	2.30±0.10	1.70±0.10
E	2.40±0.70	3.60±0.95	2.73±0.21	1.26±0.14

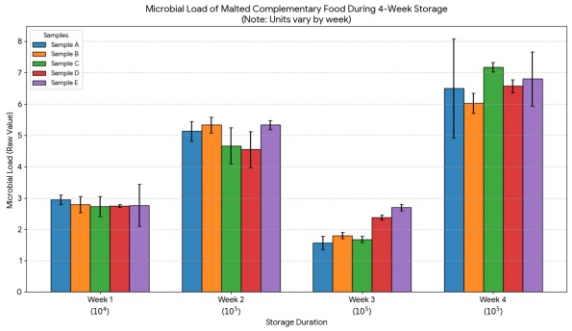
Values are expressed as Mean ± Standard Deviation (n=3). Means followed by different superscript letters are significantly different (P<0.05).



The chart above displays the Microbial Load/ shelf life of the fermented blends (Samples A through E) across a four-week storage period.

Table 8: Microbial Load/ shelf-life (CFU/ml) of Malted complementary food based on maize, pigeon pea fortified with crayfish blends

Samples	Week 1 (10^4)	Week 2 (10^5)	Week 3 (10^5)	Week 4 (10^5)
A	2.95±0.15	5.13±0.31	1.57±0.21	6.50±1.57
B	2.80±0.26	5.33±0.25	1.80±0.10	6.03±0.32
C	2.73±0.32	4.67±0.57	1.68±0.10	7.17±0.15
D	2.75±0.05	4.55±0.57	2.38±0.08	6.57±0.21
E	2.77±0.67	5.33±0.15	2.70±0.10	6.80±0.87



The chart above displays the Microbial Load/Shelf-life of malted complementary food blends (Samples A through E) over a four-week storage period. The values were plotted using the raw means and standard deviations from the table, with each sample distinguished by its own color.

Table 9: Characterization of Bacterial Isolates from Fermented and Malted Weaning Food Blends

Morphology	Gram Stain	Catalase	Oxidase	Coagulase	Motility	Methyl Red	Voges-Proskauer	Maltose	Glucose	Xylose	Mannitol	Lactose	Sucrose	Most Probable Organism
Small, circular, smooth, cocci in chains	-	-	-	-	-	+	+	+	+	-	-	+	+	*Pediococcus spp.*
Small, circular, smooth, cocci in chains forming irregular chains	-	-	-	-	-	+	+	+	+	-	+	+	+	*Leuconostoc spp.*
Small, white, cocci in chains	-	-	-	-	-	-	+	+	+	-	+	+	-	*Streptococcus spp.*
Large, irregular, rod-shaped colonies	+	+	+	-	+	+	-	+	+	+	+	-	+	*Bacillus spp.*
Smooth, golden yellow, cocci in clusters	+	+	-	+	-	+	-	+	+	-	+	+	+	*Staphylococcus spp.*
Small, circular, pale yellow colonies, cocci in tetrads	+	+	-	-	-	-	-	+	+	-	+	+	-	*Micrococcus spp.*
Medium-sized, creamy, rod-shaped colonies	-	+	+	-	+	+	+	+	+	+	-	+	+	*Enterobacter spp.*
Medium-sized, creamy, rod-shaped colonies	-	+	-	-	+	+	-	+	+	+	-	+	+	*Escherichia coli*

Keys:

- + = Positive
- - = Negative

Table 10: Fungal Isolates from Fermented and Malted Weaning Food Blends

S/No	Appearance	Isolates
1	Conidiophore non-septate, unbranched with swollen apex (vesicles); vesicles produce chains of conidia from phialides	*Aspergillus spp.*
2	Conidial area seen as grey-green during growth; straight tubes bearing conidia	*Penicillium spp.*
3	Sporangiophores arising from stolons; dark and striate; sporangia spherical and large, found at rooting nodes of stolons	*Rhizopus spp.*
4	White, fluffy mycelium; sporangia round, non-septate; rapid cottony growth	*Mucor spp.*
5	Creamy, smooth, shiny colonies; budding cells forming pseudohyphae; yeast-like growth	*Saccharomyces spp.*
6	Creamy to white, pasty, smooth colonies; budding cells with pseudohyphae; yeast-like growth	*Candida spp.*

DISCUSSION

The study revealed that the Total Heterotrophic Bacteria Count (THBc) varied significantly ($p < 0.05$) across the fermented and malted maize–pigeon pea–crayfish blends. In the fermented samples, bacterial counts ranged from 1.60×10^5 to 4.50×10^5 CFU/ml, whereas the malted samples ranged from 1.23×10^5 to 4.50×10^5 CFU/ml. The lower counts observed in Samples A and B suggest that processing methods, such as fermentation and malting, can effectively reduce microbial load when properly applied. These findings aligned with [17], who reported that fermentation promotes beneficial microbial growth while suppressing pathogenic bacteria in cereal-based foods. Similarly, [18] reported that traditional processing methods, including fermentation and heat treatment, play important roles in reducing microbial contamination and improving the microbiological safety of cereal-based complementary foods.

An increase in bacterial counts from Samples A to E in both fermented and malted blends indicates that formulation differences, particularly higher inclusion of protein-rich ingredients like crayfish, may contribute to enhanced microbial proliferation. This is consistent with the observations of [19], who noted that protein-enriched complementary foods can support microbial growth if not processed or stored under hygienic conditions. In the malted blends, Sample A had the lowest microbial load ($1.23x10^5$Cfu/ml), suggesting that malting can also reduce microbial contamination, likely due to enzymatic activity and controlled drying, as noted by [20]. However, higher counts in Sample E (4.50cfu/ml) indicate that post-processing handling, moisture levels, and storage conditions may influence microbial growth. The bacterial counts, which were predominantly within the range of 10^5 CFU/g, are generally considered acceptable for dry complementary foods when produced under hygienic conditions and subjected to proper processing and storage practices [7].

The Total Coliform Count (TCC) showed significant differences ($p < 0.05$) across the samples, reflecting variation in hygienic conditions and microbial quality. In the fermented blends, TCC values ranged from 1.17×10^3 to 4.10×10^3 CFU/ml, with Samples A and B recording the lowest counts. The reduction in coliforms can be attributed to the activity of lactic acid bacteria, which produce organic acids, hydrogen peroxide, and bacteriocins that inhibit coliform growth. These results are in agreement with [21], who reported that fermentation lowers coliform counts in cereal and legume foods by creating acidic, competitive environments; also, [25], who noted that metabolites from fermentation suppress enteric bacteria.

The gradual increase in coliforms from Samples A to E may reflect higher levels of protein-rich ingredients and possible contamination during post-processing, packaging, or storage, supporting the observations of [23].

Again, malted blends exhibited higher TCC values, ranging from 2.23×10^4 to 4.03×10^4 CFU/ml, which suggests that malting alone does not provide the same antimicrobial effect as fermentation. [24] also observed that fermentation is more effective than malting in reducing coliforms due to acidification. Elevated counts in some malted samples may indicate lapses in hygiene or exposure to contaminated environments during processing, as highlighted by [25].

The Total Fungal Count (TFC) also varied significantly ($p < 0.05$) in the fermented blends, counts ranged from 2.65×10^6 to 3.27×10^6 CFU/ml, with Samples A and B showing lower fungal loads compared to Samples C, D, and E. This suggests that these formulations were more microbiologically stable. Higher fungal counts in some fermented blends could be linked to moisture conditions during fermentation and post-processing handling, a pattern observed by [26] and. [27], who reported that inadequate drying and poor storage conditions facilitate fungal proliferation. Malted blends had considerably lower fungal counts, ranging from 2.67×10^2 to 4.77×10^2 CFU/ml. The low fungal load indicates that malting combined with proper drying effectively limits fungal growth, consistent with the findings of [28]. These results emphasize that moisture control and hygienic handling are critical in preventing fungal contamination, as discussed by [29]. The presence of molds like *Aspergillus* and *Penicillium* in some fermented samples highlights the potential health risks from mycotoxin production, in line with [30] recommendations.

The microbial populations also varied during the four-week storage period (Table 7 and 8). Fermented blends showed an initial increase in microbial counts from Week 1 to Week 2, followed by a gradual decline from Week 3 to Week 4. This pattern reflects initial nutrient availability and favorable moisture, followed by inhibitory effects from organic acids and antimicrobial metabolites, as reported by [31] and [32]. Malted blends, however, displayed more fluctuations, with increases in Week 4, likely due to limited antimicrobial activity, moisture absorption, and inadequate packaging, supporting the findings of [23] and [34].

Bacterial characterization revealed a mix of beneficial and potentially pathogenic organisms; *Pediococcus spp., Leuconostoc spp.,* and *Streptococcus spp*. were Gram-negative, non-motile lactic acid bacteria that contributed to fermentation and inhibited pathogenic growth, in agreement with [35] and [36]. *Bacillus spp*., *Staphylococcus spp*., and *Micrococcus* spp. were Gram-positive cocci or rods, with *Bacillus* showing spore-forming ability and resilience during processing [20], while *Staphylococcus* and *Micrococcus* may reflect handling contamination [37]. *Enterobacter spp*. and *E. coli* were also detected, indicating possible fecal contamination, consistent with [25].

Fungal analysis identified *Aspergillus spp., Penicillium spp., Rhizopus spp., Mucor spp., Saccharomyces spp.*, and *Candida spp.* Filamentous molds such as *Aspergillus, Penicillium, Rhizopus*, and *Mucor* thrived in high moisture conditions, whereas yeasts like Saccharomyces enhanced fermentation and can suppress spoilage organisms, which is in tandem with [26], [38], and [29]. The data show that fermentation effectively improves bacterial safety, while malting and proper drying are more effective in reducing fungal load.

The study demonstrates that both fermentation and malting improve microbial quality of maize–pigeon pea–crayfish weaning blends, though their effectiveness differs by microbial group. Fermentation favors bacterial safety through lactic acid bacteria activity, whereas malting enhances fungal control through moisture reduction. Proper formulation, hygienic processing, drying, and storage are essential to ensure the microbiological stability and safety of these complementary foods.

CONCLUSION

This study investigated the microbial quality of locally developed weaning food flour based on maize and pigeon pea, fortified with crayfish blends. The results demonstrated that both fermentation and malting significantly influence microbial populations, with notable differences between processing methods and storage periods. Fermented blends generally exhibited lower coliform counts and greater microbial stability during storage, indicating that fermentation effectively inhibits pathogenic bacteria through the production of organic acids and antimicrobial metabolites. Malted blends showed fluctuating microbial loads, suggesting that malting alone provides limited microbial control and may allow for regrowth under suboptimal storage conditions.

The identification of microbial isolates revealed the presence of beneficial organisms such as *Lactobacillus spp.*, *Pediococcus spp.*, and *Saccharomyces spp.*, which contribute to food safety and stability. However, potentially harmful bacteria (*E. coli*, *Enterobacter spp.*, *Staphylococcus spp.*) and fungi (*Aspergillus spp.*, *Penicillium spp.*) were also detected, highlighting the importance of strict hygiene, adequate drying, and proper storage in the production of weaning foods. The findings indicated that fermentation is more effective than malting in reducing microbial contamination and improving the microbiological safety of weaning food blends. Both processes require careful handling and storage to ensure safety, particularly for infant consumption. The study emphasizes that the development of safe and nutritious local weaning foods must integrate appropriate processing methods, good manufacturing practices, and effective storage strategies to minimize microbial risks.

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Authors' Contributions

OCU designed the study, developed the research topic, and wrote the introduction and literature review. He also contributed to the editing and discussion of the work. NNO wrote the materials and methods section, made corrections, and contributed to the discussion of some results. EHA proofread the manuscript, conducted most of the laboratory analyses, and provided the data generated from the bench.

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Data availability statement

The raw dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability: Not applicable

Declarations

Consent for publication: Not applicable
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