Production, isolation and identification of microbes in home-made complementary food flour based on maize-pigeon pea flour.

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Abstract

Maize (zea-mays) and pigeon pea (Cajanus cajan) were processed into flour by local methods (malting/ germination and fermentation) and used in formulating composite complementary foods at different proportions (75:25%), (25:75%) and (50:50%) respectively. The blends were formulated according to fermented maize to fermented pigeon pea, germinated maize to germinated pigeon pea respectively. The microbial contents of the raw maize flour, raw pigeon pea flour and the processed samples were evaluated using standard microbiological methods. The total heterotrophic bacterial count (13.6 × 106 cfu/ml), total heterotrophic fungal counts (3.0 × 106 cfu/ml), total coliform counts (11.6 × 106 cfu/ml), and total microbial isolates were all higher in the raw flour compared to the processed flour (4.2 × 106 cfu/ml, 1.0 × 106 cfu/ml, 4.2 × 106 cfu/ml, 1.0 × 106 cfu/ml and 3.0 × 106 cfu/ml) respectively. The bacteria isolated were predominantly in the raw flour mainly *Bacillus spp, Staphylococcus aureus, Lactobacillus sp, Pseudomonas sp, Escherichia coli, Klebsiella sp, Proteus sp* and *Streptococcus sp*. The fungal isolates were *Aspergillus niger, Aspergillus flavus, Penicillin sp, Geotrium sp, Trichophytum rubrium, candida sp and Rhizopus sp*. Indicator microorganisms were isolated only in the raw maize and pigeon pea but were not observed in the germinated, fermented and composite blends. Microbial load in the processed and composite blends fall within the level of acceptance (104-<106 cfu/ml) of the microbiological reference criteria for such foods. This work therefore concludes that raw flour samples are not suitable as complementary food in the feeding of children.

Keywords: Complementary food, Maize flour, Pigeon pea flour, Microbes, Germination, Fermentation.

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Introduction

Complementary foods are those foods given to a child in addition to breast milk during the vulnerable period of the child. The process by which the food is administered to the child is referred to as complementary feeding. Exclusive breast milk practiced today by the most of nursing mothers is ideal through a period of six months for optimal health, growth and development of the child. As the child grows, breast milk may no longer satisfy his hunger. Then addition food to the breast milk will be added which is called complementary foods. The children targeted are between the age of six months and 23 months which often continue alongside with breast milk or replaces breast milk automatically. Complementary food could be designed to boost the nutritional and health status of the child. In Nigeria, many factors is affecting complementary food formulation and feeding. Some of these include poor feeding practice. Poor timing of complementary foods, hygienic aspect of the food and child care practices, poor dietary quality of the foods and the microbial content of the complementary food is often not guaranteed [1].

Complementary foods could be formulated at household level by the mothers and caregivers using the traditional processing methods available (germination, fermentation). For small children between 6 to 11 months, complementary foods of thick porridge can be produced from maize, cassava, millet, rice, pea, with the addition of sugar, fish, and vegetable as option.

Contamination of complementary foods leads to the occurrence of diarrhoeal diseases in children. This could result from improper processing methods, food handling/handlers and belief system.

Food safety practice should be put in place in the production of complementary foods hence that it is of high priority for infant growth, preventing mortality and enhancing development. In the developing countries, 42% of children are stunted, 10% wasted and 25% underweight due to poor and unhygienic production/contamination of these complementary foods [2].

The high incidence of diarrhea in the second semester of life coincides with the increase in the intake of complementary foods. Proper maternal practices regarding the management, preparation, administration and storage of complementary foods may reduce the contamination. Fungi, yeast and bacteria have been isolated at critical points of Ogi production. We therefore studied production, isolation and identification of microorganisms in complementary foods based on maize and pigeon pea flour as the aim of this work.

Materials and Methods

Raw material selection

Yellow maize seeds were chosen because of their high carbohydrate content while brownish pigeon pea seeds were selected due to their easy availability and protein content.

Fermentation of maize and pigeon pea seeds

The method of fermentation adopted was described in the production of fermented Ogi flour. Ten kilograms each of cleaned yellow maize and pigeon pea grains were steeped in tap water of plastic buckets. The bucket was covered with aluminium foil and the content all allowed fermenting separately at room temperature (29°C for 48 hours). The steeping water was decanted and the fermented cereal ground to smooth slurry in local attrition mill. The slurries were allowed to settle for 3 hours. The sediment was dried at 35°C for 12 hours and the dried samples were passed through the local mill and sieved with 150 µm. Before fermentation process, pigeon pea samples were boiled in water for 2 hours to remove the off taste [3].

Germination of maize-pigeon pea seeds

The soaked grains were separately spread on wet jute bags and the beds covered with moist Muslin cloths and left to germinate. They were allowed to germinate for 48 hrs, water was spread at 2 hrs interval to keep the germinated grains moist. The germinated grains were turned at 8 hrs interval to discourage the growth of molds. Later, pigeon pea spreads were dehulled manually, cooked for 2 hrs, sundried alongside the maize sprouts for 2 days. The rootlets in both cases were removed while the malt were kilned in the oven at 65°C for 20 minutes. The kilned malts were ground in a local mill and sifted through a 150 µm sieve to obtain malted maize and malted pigeon pea flours.

Microbiological analysis

Microbiological analysis was done using the method of International Commission on Microbiological Specification for Foods (ICMSF). Viable cell counts were carried out by direct plate count on plate count agar medium. Serial dilutions of each samples were done prior to inoculation. 1 ml of each diluent was inoculated unto sterile standard petri dish in triplicate with a sterile pipette. 20 ml of molten nutrient agar was poured aseptically over the inoculums. The plates were swirled for even mixing and allowed to cool and set. Two plates were inoculated at 37°C for 24 hrs and one plate was incubated at 22°C for 36 hrs respectively. The plates were incubated upside down to prevent the condensed water vapour from disrupting the surface of the medium. Plate containing 30-300 colonies were selected, counted and average recorded [4].

Identification of microbial isolates/cultural/ morphological characteristics

The isolated organisms were identified using standard microbiological methods. Cultural and morphological, Gram stain reactions and biochemical examinations were carried out.

Statistical analysis

The method of adopted. Maize and pigeon pea seeds were sorted manually, washed in potable water and soaked separately in tap water at room temperature for 16 hrs [6].

Results

Data generated were analyzed using one-way analysis of variance. Turkey 95% results to differentiate each sample flour from the other (Tables 1-5).

Table 1. *Total heterotrophic bacteria/fungal counts (Cfu/ml) of raw, processed and composite blends of complementary foods based on maize-pigeon pea flour.*

Samples	THBC (Cfu/ml)	THFC (Cfu/ml)
RMF	13.6 × 106a	3.0 × 106a
RPPF	12.8 × 106a	3.0 × 106a
FMF	6.9 × 106b	2.5 × 106a
FPPF	5.2 × 106b	2.0 × 106a
GMF	4.6 × 106c	1.0 × 106b
GPPF	4.2 × 106c	1.5 × 106b
MAPEA a	4.0 × 106a	2.5 × 106a
MAPEA b	5.4 × 106b	ND*
MAPEA c	3.8 × 106a	3.5 × 106a
MAPEA d	3.2 × 106a	1.0 × 106b
MAPEA e	5.2 × 106b	2.0 × 106b
MAPEA f	5.2 × 106a	ND

Abbreviation: N.D=Not detected
RMF=Raw maize flour
RPPF=Raw pigeon pea flour
FMF=Fermented maize flour
FPPF=Fermented pigeon pea flour
GMF=Germinated maize flour
GPPF=Germinated pigeon pea flour
MAPEAa=75%:25% (FMF+FPPF)
MAPEAb=50%:50% (FMF+FPPF)
MAPEAc=25%:75% (FMF+FPPF)

MAPEAd=75%:25% (GMF+GPPF)
MAPEAe=50%:50% (GMF+GPPF)
MAPEA f=25%:75% (GMF+GPPF)
THBC=Total heterotrophic bacteria count
THFC=Total heterotrophic fungal count
Tables bearing the same superscript letters are not significantly different.

Table 2. *Total coliform counts (Cfu/ml) of raw, processed and composite blends of complementary foods based on maize pigeon pea flour.*

Samples	TCC (Cfu/ml)	Samples	TCC (Cfu/ml)
RMF	10 × 106a	MAPEAa	3.2 × 106a
RPPF	11.6 × 106a	MAPEAb	3.0 × 106a
FMF	4.2 × 106b	MAPEAc	4.0 × 106a
FPPF	4.4 × 106b	MAPEAd	5.4 × 106b
GMF	5.6 x 106b	MAPEAe	4.4 x 106a
GPPF	4.8 x 106b	MAPEAf	3.4 x 106a

Abbreviation: RMF=Raw maize flour
RPPF=Raw pigeon pea flour
FMF=Fermented maize flour
FPPF=Fermented pigeon pea flour
GMF=Germinated maize flour
GPPF=Germinated pigeon pea flour
MAPEAa=75%:25% (FMF+FPPF)
MAPEAb=50%:50% (FMF+FPPF)
MAPEAc=25%:75% (FMF+FPPF)
MAPEAd=75%:25% (GMF+GPPF)
MAPEAe=50%:50% (GMF+GPPF)
MAPEA f=25%:75% (GMF+GPPF)
Results bearing the same letter of superscript are not significantly different.

Table 3. *Characterization and identification of bacterial isolates from raw, processed and composite blends of complementary foods based on maize–pigeon pea flour.*

Cell morphology	Gram reaction	Spore	Mortility	Catalase	Coagulase	Citrate utilization	Indole	Urease	Oxidase	MR	VP	Probable organism
C	+	-	-	+	+	-	-	-	-	-	-	Staphylococcus aureus
R	+	+	+	+	-	+	-	-	+	-	+	Bacillus specie
R	-	-	+	+	-	+	-	-	+	+	+	Pseudomonas specie
R	-	-	+	-	-	-	+	-	-	+	-	Escherichia coli
R	-	-	-	-	-	+	-	+	-	+	-	Klebsiella sp ecie
C	+	-	-	+	-	-	-	-	-	-	-	Streptococcus species
R	+	-	-	-	-	-	-	+	-	-	-	Lactobacillus species
R	-	-	+	+	+	+	-	+	+	-	-	Proteus spp.

Abbreviation:
C=Cocci
R=Rod
+=Positive
-=Negative

Table 4. *Occurrence of bacterial isolates from raw, processed and composite blends of complementary foods based on maize pigeon pea flour.*

Samples	*Bacillus spp*	*Staphylococcus aureus*	*Lactobacillus spp*	*Pseudomonas spp*	*Escherichia coli*	*Klebsiella spp*	*Proteus spp*	*Streptococcus spp*
RMF	+	+	-	+	+	-	-	+
RPPF	+	+	-	+	+	-	+	+
FMF	+	-	+	-	-	+	-	-
FPPF	+	-	+	-	-	-	-	-
GMF	+	-	-	-	-	+	-	-
GPPF	+	-	+	-	-	-	-	-
MAPEAa	+	-	-	-	-	+	-	-
MAPEAb	+	-	+	-	-	-	-	-
MAPEAc	+	-	+	-	-	-	-	-
MAPEAd	+	-	+	-	-	-	-	-
MAPEAe	+	-	-	+	-	-	-	-
MAPEAf	-	-	+	-	-	-	-	-

Abbreviation:
+=positive
-=Negative
RMF=Raw maize flour
RPPF=Raw pigeon pea flour
FMF=Fermented maize flour
FPPF=Fermented pigeon pea flour
GMF=Germinated maize flour
GPPF=Germinated pigeon pea flour
MAPEAa=75%:25% (FMF+FPPF)
MAPEAb=50%:50% (FMF+FPPF)
MAPEAc=25%:75% (FMF+FPPF)
MAPEAd=75%:25% (GMF+GPPF)
MAPEAe=50%:50% (GMF+GPPF)

Table 5. *Occurrence of fungi isolated from raw, processed and composite blends of complementary foods based on maize – pigeon pea flour.*

Samples	***Aspergillus niger***	***Aspergillus flavus***	***Penicillin spp***	***Geotricum spp***	***Trichophytum Rubrium***	***Candida spp***	***Rhizopus spp***
RMF	+	+	+	-	-	+	+
RPPF	+	-	-	+	-	+	-
FMF	+	+	+	-	-	-	-
FPPF	+	-	-	+	-	-	-
GMF	-	+	+	-	-	-	-
GPPF	+	-	-	+	+	-	-
MAPEAa	+	-	-	-	-	-	-
MAPEAb	-	-	-	-	-	-	-
MAPEAc	+	-	+	-	+	-	-
MAPEAd	-	-	-	-	-	-	-
MAPEAe	-	-	-	-	-	-	-
MAPEAf	-	-	-	-	-	-	-

Abbreviation:
+=Positive
-=Negative
RMF=Raw maize flour
RPPF=Raw pigeon pea flour
FMF=Fermented maize flour
FPPF=Fermented pigeon pea flour
GMF=Germinated maize flour
GPPF=Germinated pigeon pea flour
MAPEAa=75%:25% (FMF+FPPF)
MAPEAb=50%:50% (FMF+FPPF)
MAPEAc=25%:75% (FMF+FPPF)
MAPEAd=75% : 25% (GMF+GPPF)
MAPEAe=50% : 50% (GMF+GPPF)
MAPEA f=25% : 75% (GMF+GPPF)

Discussion

Table 1 presents the results of the total heterotrophic bacterial/ fungal counts (cfu/ml) of raw, processed and composite blends of complementary foods based on maize-pigeon pea flour. Highest bacterial counts were isolated from raw maize flour sample (13.6 × 106 cfu/ml) and raw pigeon pea flour (12.8 × 106 cfu/ml) respectively [7]. Lower bacterial counts were observed in samples FMF (6.9 × 106 cfu/ml), GPPF (5.2 × 106 cfu/ml), GMF (4.6 × 106 cfu/ml) and GPPF (4.2 × 106 cfu/ml). The decrease in the total heterotrophic bacterial counts could be attributed to the processing steps such as replacement of steeped liquor prior to milling, removal of chaffs and sieving respectively. reported similar microbial reduction during the spontaneous fermentation of Akamu. However, lower bacterial counts were observed in the composite blends MAPEAa–MAPEAf which ranged from 2.2 × 106 cfu/ml to 5.4 × 106 cfu/ml. This result is in agreement with 2.5 × 105–4.5 × 105 cfu/ml isolated in fermented complementary foods [8].

Total heterotrophic fungal counts recorded a peak value in the raw maize flour (3.0 × 106 cfu/ml and raw pigeon pea flour (3.0 × 106 cfu/ml). Reduction of the fungal counts were also recorded in the fermented, germinated flour samples as well as composite blends. The presence of the fungi (although very minimal) could be implicated to air around the laboratory and culture media.

Table 2 presented the results of total coliform counts of raw, processed and composite blends of complementary foods based on maize-pigeon pea flour flour. The results ranged from 3.0 × 106 cfu/ml to 11.6 × 106 cfu/ml, raw pigeon pea flour recorded the peak value (11.6 × 106 cfu/ml) as well as raw maize flour 10.0 × 106 cfu/ml. Depreciation of the coliform counts were observed in the fermented, germinated and composite blends respectively [9]. The composite blends had a lower values which ranged from 3.0 × 106 cfu/ml to 5.6 × 106 cfu/ml. The low counts observed in the processed flour is in tandem with 3.61 × 106 cfu/g isolated in different flour samples. This can be further supported by the coliform and enterobacteriaceae numbers which by definition are not spore formers. The level of coliform in processed and composite blends are low compared to 106 to 107 g referred to as been potential hazard to health. The high level of coliform observed in raw maize (11.0 × 106 cfu/ml) and raw pigeon pea (11.6 × 106 cfu/ml) could be implicated to un-treatment/unprocessed state of the flour. Microflora of cereals and legumes includes molds, yeast, bacteria [10]. Coliform and enterococci could occur as indicator of unsanitary and possible fecal contamination of the raw flour.

Table 3 shows the characterization and identification of bacterial isolates from raw, processed and composite blends of complementary foods based on maize–pigeon pea. *Staphylococcus aureus*, *Bacillus sp*, *Streptococcus sp* and *Lactobacillus spp* were all positive to gram stain reaction.

Spore formation was only observed in *Bacillus sp* while *Pseudomonas sp, Bacillus sp, Escherichia coli* and *Proteus sp* were all motile in the test. All the bacterial isolates in Table 3 were positive to catalase test except *E. coli, Klebisella* and *Lactobacllus sp*. Our result is in agreement who isolated coliform bacteria, enterococcus and salmonella spp in weaning food consumed in the Republic.

Citrate utilization were positive in *Bacillus spp, Pseudomonas spp, Klebisella spp and Proteus spp*. Probable microorganisms recorded were *Staphylococcus aureus, Bacillus sp, Pseudomonas sp, Streptococcus sp, Lactobacillus sp* and *Proteus sp*. isolated Enterococcus and other faecal coliform in weaning food of in–patient infant food in Jimma University.

Table 4 presented the occurrence of bacterial isolates from raw, processed and composite blends of complementary foods based on maize–pigeon pea flour. The bacterial isolates were too many in the raw maize and raw pigeon pea flour. Among the isolates recorded in the raw maize and pigeon pea were Bacillus sp, *Staphylococcus aureus, Escherichia coli, Lactobacillus sp and Streptococcus sp. Bacillus sp, Lactobacillus sp and Klebsiella sp* were isolated in the germinated and fermented pigeon pea flour. Isolation of different classes of bacteria in the raw sample (maize- pigeon pea flour) is in agreement with contamination of seeds during crop growth, pre-harvesting, post-harvesting, transportation and storage. Reports showed that pathogenic can contaminate unprocessed raw flour and its products and the level of contamination are influenced by climatic conditions during cereal ripening and harvesting. In the other hand, removal of the outer skin of the grain (dehulling), milling, decantaton, drying, malting, washing, sieving reduced the microbial isolates of the fermented, germinated and composite blends. This reduction in the microbial isolates agreed with the report in food processing to reduce microorganisms [11].

Table 5 Occurrence of fungi isolated from raw, processed and composite blends of complementary foods based on maize–pigeon pea flour. The probable fungal isolates were *Rhizopus spp, Penicillin spp, Aspergillus niger, Fusarium spp, Aspergillus flavus, Geotricum spp* and *Trichophytum rubrium*. The identification process adopted were colour of the aerial hyphae, colour of colony, nature of hyphae, shape and kind of sexual spores, presence of special structure, appearance of sporongiophore and characteristics of spore head. The prominent fungal isolates were *Aspergillus niger* which occurred repeatedly (Table 5). Other fungal isolates ranged from *Aspergillus flavus, Penicillin spp, Geotricum spp, Trichophytum rubrium, Candida spp and Rhizopus spp*. Raw maize and pigeon pea recorded high level of these fungal isolates whereas fermented and germinated flour recorded low level of fungi (Table 5).

Mould growth is the most common cause of microbial spoilage and deterioration of quality of cereal grains and flour during storage. Mycotoxins producing fungi (*Aspergillus spp, Penicillin spp, fusarium spp*) can contaminate raw flour and cause food poisoning after consumption [12].

High fungal isolates in the raw maize and raw pigeon pea flour is of public health concern, hence food cannot be consumed in its raw state. The high level of fungal isolates in all the raw samples could be attributed to the un-treatment of the samples. Fungal contamination of food products possess challenges to global security and are been destroyed by processing operations. The fungal load may be carried over into the processed food, though they may cause little or no harm to consumers.

The proper processing methods adopted reduced the level of fungi from raw state of the samples to the processed flour, hence the reduced numbers in Fermented Maize Flour (FMF), Germinated Maize Flour (GMF), Fermented Pigeon Pea Flour (FPPF), Germinated Pigeon Pea Flour (GPPF) and composite blends respectively [13].

Conclusion

The isolation of *Staphylococcus aureus* in the raw maize and pigeon pea flour do not indicate a sign of danger as raw food flour cannot be recommended for child's consumption. The repeated isolation of *Bacillus sp* in some samples may be due to the ubiquitous nature of microorganisms. The microbial load of the germinated, fermented and composite flour is below the level capable of causing health hazard.

Isolation of microbes could be implicated to the poor handling of the food during the processing techniques as well as the environment. The isolation of *Penicillin sp, Rhizopus sp* and *Aspergillus sp* could be implicated as ordinary environmental contaminants. The level of microbial contamination observed in the processed samples and composite blends were low compared to unacceptable level.

The results reviewed that complementary foods cannot be consumed in a raw state. The reduction of the microbial load in all the processed flour and composite blends reviewed that processing techniques adopted (germination, fermentation) was able to reduce the number of microbes to acceptable limit. Therefore complementary foods should be prepared using germination and fermentation to reduce the number of microbial load in the raw state.

Data Availability Statement

The data used in the research of this work are available to the corresponding author who is always willing to issue them upon request.

Conflict of Interest

Conflict of interest does not exist.

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