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# 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 x $10^6$cfu/ml), total heterotrophic fungal counts (3.0 x $10^6$cfu/ml), total coliform counts (11.6 x $10^6$cfu/ml), and total microbial isolates were all higher in the raw flour compared to the processed flour (4.2 x $10^6$cfu/ml, 1.0 x $10^6$cfu/ml, 4.2 x $10^6$cfu/ml, 1.0 x $10^6$cfu/ml and 3.0 x $10^6$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 ($10^4$ - < $10^6$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

## Introduction

Complementary foods are those foods given to a child in addition to breast milk during the vulnerable period of the child (Okoronkwo *et al*., 2017). The process by which the food is administered to the child is referred to as complementary feeding (WHO, 1998). 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 (Motuma *et al*., 2016). 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 (Okoronkwo *et al*., 2017). 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 (WHO, 2003). 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 (Monte and Giugliani, 2004).

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, vegetable as option (WHO, 2009).

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 (Sheth and Dwivedi, 2006).

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 (Lutter and Dewey, 2003). In the developing countries (Nigeria as example), 42% of children are stunted, 10% wasted and 25% underweight due to poor and unhygienic production/contamination of these complementary foods (NFCNS, 2004).

The high incidence of diarrhea in the second semester of life coincides with the increase in the intake of complementary foods (Oluwafemi and Ibeh, 2010). 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 (Onyelana and Cooker, 2012). 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 by Okoronkwo *et al.,* (2016) 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 aluminum foil and the content all allowed to ferment separately at room temperature ($29^0$C for 48hours). 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 were dried at $35^0$C for 12hours and the dried samples were passed through the local mill and sieved with 150µm. Before fermentation process, pigeon pea samples were boiled in water for 2 hours to remove the off taste.

### Germination of Maize-Pigeon Pea Seeds

The method of Mallechi and Desikachar, (1982) was adopted. Maize and pigeon pea seeds were sorted manually, washed in potable water and soaked separately in tap water at room temperature for 16hrs. 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 48hrs, water was spread at 2hrs interval to keep the germinated grains moist. The germinated grains were turned at 8hrs interval to

discourage the growth of molds. Later, pigeon pea spreads were dehulled manually, cooked for 2hrs, sundried alongside the maize sprouts for 2days. The rootlets in both cases were removed while the malt were kilned in the oven at 65$^0$C for 20minutes. The kilned malts (maize and pigeon pea) were ground in a local mill and sifted through a 150µm 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, 1978). 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. 1ml of each diluent was inoculated unto sterile standard petri dish in triplicate with a sterile pipette. 20ml 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$^0$C for 24hrs and one plate was incubated at 22$^0$C for 36hrs 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.

### Coliform Test

The presence of coliform bacteria was determined using the method described by Cruickshank *et al.,* (1980). 1ml of the test samples were inoculated into a lactose broth in fermentation tubes containing durham tubes. They were incubated at 37$^0$C for 24hrs. The formation of gas in the Durham tubes and a change in the colour of the medium (yellow to pink) indicates the presence of acid. Production of gas and acid is a positive indication of presence of coliform bacteria.

### Identification of Microbial Isolates/ Cultural/Morphological Characteristics

The isolated organisms were identified using standard microbiological methods (Chessbrough, 2006). Cultural and morphological, Gram stain reactions and biochemical examinations were carried out.

### Statistical Analysis

Data generated were analyzed using one-way analysis of variance (ANOVA). Turkey 95% simultaneous confidence interval was also applied to the results to differentiate each sample flour from the other.

## Results

**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 | $13.6x10^{6a}$ | $3.0 \times 10^{6a}$ |
| RPPF | $12.8 \times 10^{6a}$ | $3.0 \times 10^{6a}$ |
| FMF | $6.9 \times 10^{6b}$ | $2.5 \times 10^{6a}$ |
| FPPF | $5.2 \times 10^{6b}$ | $2.0 \times 10^{6a}$ |
| GMF | $4.6 \times 10^{6c}$ | $1.0 \times 10^{6b}$ |
| GPPF | $4.2 \times 10^{6c}$ | $1.5 \times 10^{6b}$ |
| MAPEA a | $4.0 \times 10^{6a}$ | $2.5 \times 10^{6a}$ |
| MAPEA b | $5.4 \times 10^{6b}$ | ND* |
| MAPEA c | $3.8 \times 10^{6a}$ | $3.5 \times 10^{6a}$ |
| MAPEA d | $3.2 \times 10^{6a}$ | $1.0 \times 10^{6b}$ |
| MAPEA e | $5.2 \times 10^{6b}$ | $2.0 \times 10^{6b}$ |
| MAPEA f | $5.2 \times 10^{6a}$ | ND |

*Keys: 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 x $10^{6a}$ | MAPEAa | 3.2 x $10^{6a}$ |
| RPPF | 11.6 x $10^{6a}$ | MAPEAb | 3.0 x $10^{6a}$ |
| FMF | 4.2 x $10^{6b}$ | MAPEAc | 4.0 x $10^{6a}$ |
| FPPF | 4.4 x $10^{6b}$ | MAPEAd | 5.4 x $10^{6b}$ |
| GMF | 5.6 x $10^{6b}$ | MAPEAe | 4.4 x $10^{6a}$ |
| GPPF | 4.8 x $10^{6b}$ | MAPEAf | 3.4 x $10^{6a}$ |

*Keys: 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* specie |
| C | + | - | - | + | - | - | - | - | - | - | - | *Streptococcus* species |
| R | + | - | - | - | - | - | - | + | - | - | - | *Lactobacillus* species |
| R | - | - | + | + | + | + | - | + | + | - | - | *Proteus* spp. |

*Key: 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 | *Bacillusspp* | *Staphylococcusaureus* | *Lactobacillusspp* | *Pseudomonasspp* | *Escherichiacoli* | *Klebsiellaspp* | *Proteusspp* | *Streptococcusspp* |
|---|---|---|---|---|---|---|---|---|
| RMF | + | + | - | + | + | - | - | + |
| RPPF | + | + | - | + | + | - | + | + |
| FMF | + | - | + | - | - | + | - | - |
| FPPF | + | - | + | - | - | - | - | - |
| GMF | + | - | - | - | - | + | - | - |
| GPPF | + | - | + | - | - | - | - | - |
| MAPEAa | + | - | - | - | - | + | - | - |
| MAPEAb | + | - | + | - | - | - | - | - |
| MAPEAc | + | - | + | - | - | - | - | - |
| MAPEAd | + | - | + | - | - | - | - | - |
| MAPEAe | + | - | - | + | - | - | - | - |
| MAPEAf | - | - | + | - | - | - | - | - |

*Keys: + = 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)*

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

| Samples | *Aspergillusniger* | *Aspergillusflavus* | *Penicillinspp* | *Geotricumspp* | *TrichophytumRubrium* | *Candidaspp* | *Rhizopusspp* |
|---|---|---|---|---|---|---|---|
| RMF | + | + | + | - | - | + | + |
| RPPF | + | - | - | + | - | + | - |
| FMF | + | + | + | - | - | - | - |
| FPPF | + | - | - | + | - | - | - |
| GMF | - | + | + | - | - | - | - |
| GPPF | + | - | - | + | + | - | - |
| MAPEAa | + | - | - | - | - | - | - |
| MAPEAb | - | - | - | - | - | - | - |
| MAPEAc | + | - | + | - | + | - | - |
| MAPEAd | - | - | - | - | - | - | - |
| MAPEAe | - | - | - | - | - | - | - |
| MAPEAf | - | - | - | - | - | - | - |

*Keys: + = 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 x $10^6$cfu/ml) and raw pigeon pea flour (12.8 x $10^6$cfu/ml) respectively. Lower bacterial counts were observed in samples FMF (6.9 x $10^6$cfu/ml), GPPF (5.2 x $10^6$cfu/ml), GMF (4.6 x $10^6$cfu/ml) and GPPF (4.2 x $10^6$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. Omemu *et al.,* (2007) 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 x $10^6$cfu/ml to 5.4 x $10^6$cfu/ml. This result is in agreement with 2.5 x $10^5$ – 4.5 x $10^5$cfu/ml isolated in fermented complementary foods (Anigo *et al*., 2010).

Total heterotrophic fungal counts recorded a peak value in the raw maize flour (3.0 x $10^6$cfu/ml and raw pigeon pea flour (3.0 x $10^6$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 (Onyelana and Coker, 2012).

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 x $10^6$cfu/ml to 11.6 x $10^6$cfu/ml, raw pigeon pea flour recorded the peak value (11.6 x $10^6$cfu/ml) as well as raw maize flour 10.0 x $10^6$cfu/ml. Depreciation of the coliform counts were observed in the fermented, germinated and composite blends respectively. The composite blends had a lower values which ranged from 3.0 x $10^6$cfu/ml to 5.6 x $10^6$cfu/ml. The low counts observed in the processed flour is in tandem with 3.61x$10^6$cfu/g isolated in different flour samples (Ntuli *et al*., 2013) This can be further supported by the coliform and enterobacteriaceae numbers which by

definition are not spore formers (Tessi *et al.,* 2002). The level of coliform in processed and composite blends are low compared to $10^6$ to $10^7$/g referred to as been potential hazard to health (Bisen, 2014). The high level of coliform observed in raw maize (11.0 x $10^6$cfu/ml) and raw pigeon pea (11.6x$10^6$ cfu/ml) could be implicated to un-treatment/unprocessed state of the flour. Microflora of cereals and legumes includes molds, yeast, bacteria. Coliform and enterococci could occur as indicator of unsanitary and possible fecal contamination of the raw flour (Lloyd and Andreia , 2011).

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 with Fadh *et al*., (2019) who isolated coliform bacteria, *enterococcus* and *salmonella spp* in weaning food consumed in the Republic of Yamen.

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 *Proteussp*. Birhanu *et al., (2015)*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 (Setamou *et al*., *1997*). Reports showed that pathogenic (*Salmonella spp*, *Bacillus cereus* can contaminate unprocessed raw flour and it's products and the level of contamination are influenced by climatic conditions during cereal ripening and harvesting (Blackburn, 2006; Richer *et al*., 1993). 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 of Fandohan *et al.,* (2008) in food processing to reduce microorganisms.

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 was *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 (Blackburn, 2006). Mycotoxins producing fungi (*Aspergillus spp*, *Penicillin spp*, *fusarium spp*) can contaminate raw flour and cause food poisoning after consumption (Richar *et al.,* 1993).

High fungal isolates in the raw maize and raw pigeon pea flour is of public health concern, hence food cannot be consumed in it's 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 posses 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 (Omotola *et al*., 2018).

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.

## Conclusion

The isolation of *Staphylococcusaureus* 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 *Bacillussp* 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 *penicillinsp*, *Rhizopussp* and *Aspergillussp* 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 of *Salmonella* and *Shigellia* isolated in Anigo *et al*., (2007) and *Karok et al*., (2018).

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) were 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.

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## References

[1] Okoronkwo, C.U., Udensi, E.A., Okereke, H.C and Nwachukwu, N.O. (2017). Physicochemical characteristics and anti-nutritional factors of fermented complementary foods based on maize pigeon pea flour. *J. Advan. Food Sci Tech.* 4(11):38-43

[2] WHO (1998). Complementary feeding of young children indeveloping countries: A review of current scientific knowledge. WHO/NUT/98. I. Geneva World Health Organization.

[3] Motuma, A.A., Azeb, L., and Bekesh, G. (2016). Complementary feeding. Review of Recommendations, feeding practices and adequacy of home made complementary food preparation in Developing countries – lesson from Ethiopia. J. *Food*. *Nutri*. 3:4.

[4] WHO (2003). World Health Organization of the United Nation children's fund. Global strategy for infant and young child feeding. WHO press. Available from http:www. who.int/nutrition/publications/gs.infant feeding text org.pdf.

[5] Monte, C.M. and Giugliani, E.R. (2004). Recommendations for the complementary feeding of the breast child. J. *Pediatri*. 80(5):131-141.

[6] World Health Organization. Infant and Young Child feeding: Model chapter for Textbooks for medical students and allied health professionals. Geneva. WHO press. Available http://aps.who.int/iris/*bitstream*/10665/441171.

[7] Sheth, M. and Dwovedi, R. (2006). Complementary foods associated with diarrhea. *Indian* J. *Petriatr*. 73:61-64.

[8] Luther, C.K., and Deway, K.G. (2003). Supplement nutrient composition for fortified complementary foods. Proposed nutrient composition to fortified complementary foods. J. *Nutri*. 133:30115-30205.

[9] NFCNS, (2004). Nigeria food consumption and nutrition survey. 2001-2003. Summary UTA, pp 24-46.

[10] Oluwafemi, F., and Ibeh, I.N. (2010). Microbial Contamination of seven major Weaning foods in Nigeria. *J. Fd. Microbial (3)*:6-14.

[11] Onyelana, O. A. and Coker A. A. (2012). Microbial Contamination at different stages of production of ogi in Mowe. A rural community, South West Nigeria. *Bact.J,*2:1-11

[12] 12.Okoronkwo CU, Okereke HC, Nwachukwu NO. (2016). Production and isolation of microorganisms in ogi: A local cereal based complimentary food in Nigeria. *The International Journalof Science and Technologde.* 4 (5) : 156 - 159. *(PDF) Microbiology and Safety of Ogi Fermentation: A Review*. Available from: https://www.researchgate.net/publication / 34246 8146_ Microbiology_ and_ Safety_ of_Ogi_Fermentation_A_Review [accessed Aug 10 2020].

[13] Achi, O.K (1999). Quality attributes of fermented yam flour supplemented with processed soy flour. *Plant Foods Human Nutrition54*:151-15.

[14] Mallechi, W.G., and Desikachar, H.S.R (1982). Formulation of a complementary food with low hot-paste viscosity based on malted ragi and green grain. *J. Food Sci. Tech* pp. 19-193.

[15] ICMSF, (1978). International Commission on Microbiological Standards for Foods. Microorganisms in food sampling for microbiological Analysis. Principles and specific Application, University of Toronto Press, Toronto.

[16] Cruickshank, R., Ollguid J.P., Mamion, B.P., and Swain, R.H.A. (1980). The practice of Medical Microbiology., 12th ed. Churchill Livingston, N.Y. Vol. *2*, pp. 273-277.

[17] Okereke,H.C. and Okereke, J.I (2013) Biotechnological properties of probiotic lactic acid bacteria. *PsbJ.Sci*, 1(1):26-40

[18] Cheesbrough, M.(2006). District Laboratory Practice in Tropical Countries, part II. Cambridge University Press.

[19] Omemu, A.M., Oyewole, O.B and Bankole, M.O. (2007). Significance of yeast in the fermentation of maize for Ogi production. Food Microbiology. *24*: 571-576

[20] Anigo, K.M., Ameh, D.A., Ibrahim, S., Danbauchi, S.S. (2009). Nutrient composition of commonly used complementary foods in North western Nigeria. *African J. Biotechnol*. *8*(12):4211-4216.

[21] Ntuli, V., Mekibib, S. B., Molebatsi, N., Makotoko, M., Chatanga, P., and Asita, O.A., (2013). Microbial and Physicochemical Characterization of Maize and Wheat Flour from a Milling Company. *Internet Journal of Food Safety,* 15:11-19.

[22] Tessi, M.A., Aringoli, E.E., Pirovani, M.E., Vincenzini, A.Z., Sabbag, N.G., Costa, S.C., Garcia, C.C., Zannier, M.S., Silva,, E. R., and Moguilevsky, M.A. (2002). Microbiological quality and safety of ready-to-eat cooked foods from a centralized school kitchen in Argentina. *J. Food Prot.* 20-30.

[23] Bisen, P.S. (2014). Microbes in practice, New Delhi, IK *International*. Pp. 1089-1157.

[24] Lloyd, B., Bullerman and Adreia, B. (2011). Microbiology of cereal and cereal products. John Willey & Sons Publishers. 1-8.

[25] Fahd A. A., Amani M. A., and Najeeb, K. A (2019) Bacteriological evaluation of weaning dried foods consumed in Taiz City, Republic of Yemen. *Journal of the Saudi Society of Agricultural Sciences,* 18(3): 302-308

[26] Birhanu, D., Gosa, G., Delegn, W., and Ketema, B. (2015). Microbiological quality of safety of weaning foods of in-patient infants in Jimma University specialized Hospital, Jimma Town, Southern Ethiopia. *J. Biol. Chem. Res.* 32(2):673-682. Available http://www.sasjournal.com

[27] Setamou, M., Cardwell, K.F., Schuthess, F., Hell, K. (1997). *Aspergillusflavus* infection and aflatoxin contamination of pre-harvest maize in Benin. *Aspnet*. 18: 1323-1327.

[28] Blackman, C.D.W (2006). Food spoilage microorganisms: Woodhead Publishing Ltd. England.

[29] Richter, K.S., Doneanu, E., Eskidge, K.M., Ran, C.S. (1993). Microbiological quality of flours. Cereal Foods World 38:367-369.

[30] Fandohan, P., Hell, K., Marasa, W.F. (2008). Food processing to reduce mycotoxins in africa. In Lestie*etal* (edsmycotoxins detection methods, management, public health Wallingford, U.K. pp 302-309.

[31] Omotola, O., Nokuthula, M., Ponja, V., Benjamin, G., Noel, D., Isabella, M., Didier, M., and Oluwatosin, I. (2018). Fungal contamination of food commodities in Durham, South Africa. J *FoodSafety*. 38(6): 125-135.

[32] Karoki, W.H., Karanja, D.N., Bebora, L.C., and Njagi, L.W., (2018). Isolation, characterization and quantification of bacteria from African sausages sold in Nairobi County, Kenya. *Inter. J FoodScience*, pp9 http//doi.org/10.1155/2018/361265.

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