IMPACT OF FERMENTATION ON FOOD BORNE PATHOGENS

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Abstract: Microorganisms have been utilized to modified food through the process of fermentation. This study was carried out to assess the effect of fermentation on cereal-based food and lactic acid bacteria (LAB), isolates from the cereal-based food, on pathogenic bacteria species. Steeped grains were inoculated with *Escherichia coli* and *Salmonella typhimurium.* This was allowed to ferment at ambient temperate for 72 h; secondary fermentation was carried out at the souring stage for 24–28 h. LAB was isolated and screened for antibacterial potency against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium.* Results obtained showed that *Escherichia coli* and *Salmonella typhimurium* could not be detected after 48 and 72 h in fermenting maize and sorghum grains respectively due to a decrease in pH reduction in the total titratable acidity which increases with time. The results of the antibacterial activities of LAB against the test organisms revealed the inhibitory activities against all the test isolates except *L. lactis*with no inhibitory activity against *E. coli, S. typhimurium* and *B. subtilis. Leuconostoc mesenteriodes*, *Lactobacillus fermentum* and *Lactobacillus plantarum* exhibited highest zone of inhibition of 18.00 & 19.00 mm, 18.00 & 18.00 mm and 19.00 & 19.00 mm against *E. coli* and *Staphylococcus aureus*, respectively. This study revealed that presence of LAB in the food can prevent or reduce the contaminant in fermented foods. Adequate hygienic practices should be encouraged by food handlers to ensure health safety control among consumer.

Keywords: Fermentation, growth, lactic acid bacteria, maize, microorganisms, pathogens, sorghum

Introduction

Food fermentation has been from ancient time which is traditional norm in many towns in sub-Saharan Africa. Several reports have identified several fermentable foods in Nigeria which are fermented to edible food or food supplements. Some of these fermentable foods are not edible until they are fermented due to their high level of toxicity when in unfermented state (Oyewole and Isah, 2012). Fermentation enhances food longevity, pleasant texture, taste, aroma and improves the nutritional status of food (Egwim *et al*., 2013). In Nigeria, indigenous fermented foods are derived from cereals, legumes, cassava tubers, legumes and so on (Ijabadeniyi, 2007). Fermented foods also provide some beneficial effects during production which include carbohydrate digestibility, elimination of toxic compound and safety against disease causing organisms (Oyewole and Isah, 2012).

The supplement of these local available cereals with legumes which are good sources of protein will gives raise to weaning food that gives the infant enough energy and it's nutritional requirements (Umeta *et al.*, 2003). The widespread problem of infant malnutrition in developing countries has stirred effort in research development and extension by both local and international organization (Umeta *et al*., 2003). As a result, the formulation and development of nutritious weaning food from local and readily available raw-material, this have receive a lot of attention in many developing countries and it contributes to infant mortality, poor physical and intellectual development of infant as well as lowered resistance to disease and consequently stifles development (Umeta *et al.*, 2003). Protein energy malnutrition (PEM) generally, occurs during the crucial transitional phase when children are weaned from liquid, semi-solid or fully adult foods in addition to mother milk, because of the increasing nutritional demands of the growing body (Sajilate *et al.*, 2002). Apart from protein and energy lysine but it has sufficient sulphur contain amino acids, weaning diets of infant in developing countries requires more calcium, vitamins A and D, Iron and some important trace elements, which can be obtain by combining the local available material (Sajilate *et al.*, 2002). Combination of commonly used cereals with inexpensive plant protein source like legumes can be used, cereals are deficient in acid which are limited in legumes, where as legumes are highly rich in lysine. The effects of the fortification are highly beneficial since nutritive value of products is also improved (Wang and Daun, 2006). Legumes are nutritious food and a substitute for animal protein arises from the knowledge of the functional properties of the seed flour and other products.

One of the main causes of high morbidity and mortality in developing countries such as Nigeria is food borne diseases. Food safety is a priority in the consumption of fermented foods. Foods are most times exposed to pathogenic organisms as a result of poor hygienic practices, consumption of foods from unsafe source and so on. Most Food borne diseases are caused by pathogens such as *Esherichia coli, Listeria monocytogenes*, *Salmonella typhimurium*, *Clostridium botulinum*, *Staphylococcus aureus* causing food borne diseases (Lawal *et al*., 2009). Fermented foods contain LAB which are capable of providing growth inhibitory environments to pathogens. LAB produces organic acids, therefore able to survive in acidic environment and even in halophilic environment. These organic acids they produce reduce the pH of the environment enhancing an acidic environment which most pathogenic bacteria species cannot survive, as a result, help to eliminate these pathogens in foods (Hutkins, 2006), hence improving the shelf life of foods and improving the microbiological safety of fermented foods (Adam *et al.*, 2009). Due to this potency, this study is therefore aimed to assess the antibacterial effect of fermentation and LAB isolates on the cereal-based food under study on the viability of enteric pathogenic bacteria.

Materials and Methods

Sample collection

Two varieties of cereals (white maize and sorghum) were purchased randomly from three different markets in Ago-Iwoye, Ogun State, Nigeria. They were collected in clean sterile polyethene bags, properly sealed and brought to the microbiology laboratory II of the Department of Microbiology, Olabisi Onabanjo University. The grains were

sorted and winnowed manually to remove stones, debris and defective seeds.

Fermentation of maize and sorghum for ogi production

Traditional preparation of ogi was employed in this study as previously described by Odunfa and Adeyele (1985). One hundred grams of the grains were weighed into six sterile containers and steeped in 150 ml of sterile water for 48–72 h to ferment at room temperature (30 ± 2^0C), after which the steeped water was drained. The grains were wet milled and sieved to remove hulls, germ and bran (Egwim *et al*., 2013). The pomace was retained on the sieve and discarded as animal feed while the filtrate was further fermented for 24–48 h (souring) to yield ogi which is sour white starchy sediment depending on substrate.

Microbial cultures

Stock cultures *of Escherichia coli* and *Salmonella typhimurium* were obtained from microbiology laboratory of the department of microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun Sate and Nigerian Institute of Medical Research, NIMR, Yaba, Lagos respectively. The cultures were subcultured on MacConkey Agar and Eosin Methylene Blue Agar prior to use.

Pathogen inoculation

Pure cultures of each *Escherichia coli* and *Salmonella typhimurium* were inoculated into 10 ml sterilized Nutrient broth and incubated at 37^0C for 18–24 h. The broth culture was centrifuged at 4000 revolutions for 10 min and serially diluted using 9 ml of sterile Peptone Solution (Obinna-Echem *et al*., 2014). The microbial inoculums 1 ml, 18 h old pure cultures of the test organisms respectively were aseptically inoculated into all the steeped grains (100 g) in all the four labeled sterilized transparent buckets with lids and left to ferment at room temperature. The un-inoculated fermenting substrate served as control. The pathogens were each inoculated into the grains sample at a concentration of 1 $\times10^6$cfu/ml (Obinna-Echem *et al*., 2014).

Analysis of samples

The samples were analysed at 0, 6 and 24 h interval for determination of aerobic plate count, Lactic acid bacteria (LAB), yeasts counts, pH, temperature, total titratable acidity and growth pattern of inoculated pathogens during fermentation of ogi prepared using white maize and sorghum.

Determination of changes in physiochemical properties

During fermentation, the temperature was recorded using mercury thermometer. pH was assessed at 0, 6 and 24 h interval throughout the fermentation period with the use of H70071 pH meter after calibration using standard buffer of pH 4 and 7. The total titratable acidity was assessed by mixing 10 ml sterile distilled water with 2 g of the sample and titrating 10 ml of the supernatant against 0.1N NaoH using phenol indicator until a light pink colour was obtained.

Microbiological analysis

Serial dilution was carried out from the grain steeping to the resulting filtration from the sieved milled grain. 90 ml of sterile 0.1% peptone water was mixed with 10 ml of steeped water. This solution was used as stock. This procedure was repeated for ogi slurry. 90 ml of sterile peptone water was mixed homogeneously with 10 g of sample using a stomacher. Serial dilution in ten-fold was also carried out. Using pour plate technique, 1 ml of each dilution was dispensed directly into sterile petridishes for the isolation of mesophilic aerobes. Cooled molten nutrient agar (oxoid) was poured into the plates and mixed evenly with the dispensed sample. This was incubated at 37ºC for 24 h. De Man Rogosa Sharpe (MRS, oxoid) agar containing Tween 80 which served as a growth stimulant was used for the isolation of Lactic Acid Bacteria. Plates containing samples mixed with MRS agar was incubated an aerobically in an anaerobic jar for 48 h at 30ºC. Yeast extract agar supplemented with chloramphenicol (50 mg/l) to inhibit growth of bacteria was utilized for the isolation of yeasts. The presence of *Escherichia coli* and *Salmonella typhimurium* was evaluated using Eosin methylene blue agar and *Salmonella-Shigella* agar respectively. All analysis was carried out in duplicates. After incubation, colonies observed were counted and recorded in CFU/ml.

Preparation of cell free supernatant (CFS)

This was prepared based on the methods by Schillinger and Lucke (1989). The pure LAB isolates was inoculated into sterile MRS broth and incubated an aerobically for 35ºC for 48 h. CFS was obtained by centrifuging the broth culture at 5000 rpm for 15 min and was used immediately.

Antimicrobial screening of LAB

At the end of fermentation, the antimicrobial potency of LAB on the test organisms was done using Agar well diffusion method (Sam *et al.*, 2002). 0.1 ml of broth culture of the test organisms was dispensed on plates containing solidified nutrient agar. After 15 min, wells were created using sterile cork borer. CFS of the LAB isolates were dispensed into each well. These were incubated for 45 h at 30ºC. Clear zones of growth inhibition was checked for and recorded in millimeters

Characterization of isolates

According to Bergey's Manual of Determinative Bacteriology (1974), all isolates were identified and characterized based on colony morphology and biochemical characteristics which include production of catalase, oxidase, gas and acid production from carbohydrates and gram's reaction. Growth was also observed at 15 and 45ºC for 72–96 h, pH 4.4 and 9.6, 4 and 6.5% NaCl.

Results and Discussion

Survival of the food borne pathogens is depicted in Table 1. The presence of these pathogens in the substrates as a result of contamination prior to inoculation was tested. None of the pathogen tested was detected prior to inoculation and during fermentation of the un-inoculated grains. The counts of the pathogen found in the grains prior to inoculation and during fermentation are shown in the control Table 1.

The counts of *Escherichia coli* and *Salmonella typhimurium* inoculated into the steeped/fermenting maize increased from 9.6×10^5 to 1.1×10^6 and 9.8×10^5 to 1.2×10^6 within 6 h of fermentation respectively and decreased significantly to non-detectable levels at 48 hrs of fermentation (Tables 2 and 3). The counts of *Escherichia coli* and *Salmonella typhimurium* inoculated into the sorghum grains increased from 9.8×10^5 to 1.2×10^6 and 9.8×10^5 to 1.3×10^6 within 6 h of fermentation respectively and decreased to 3.2×10^2 at 48 h to non-detectable levels at 72 h of fermentation (Table 2). (The difference in the level of reduction of counts of the pathogens could be attributed to the differences in the pH and total titratable acidity of the samples particularly at the early stage) (Table 3).

During the fermentation process of white maize inoculated with the pathogens, the mean aerobic mesophiles counts increased from 1.4×10^5 to 1.5×10^5 cfu/ml within 6 of fermentation (Table 3). At the end of the fermentation, the counts had decreased to 3.7×10^2 cfu/ml. In the sorghum grains inoculated with the pathogens, the mean aerobic mesophiles counts increased from 1.5×10^5 cfu/ml to 1.6×10^5 and thereafter dropped to 3.6×10^2 cfu/ml at the end of the fermentation.

Table 1: Inoculated test bacteria on maize and sorghum grains at different time intervals (hrs)

Substrates	Selective medium	Isolation method	Colonial Morphology	Fermentation time (hrs)				
				0	6	24	48	72
Maize with *E. coli*	EMB	Pour- plate	Green metallic sheen colonies on EMB	+	+	+	-	-
Sorghum with *E. coli*	EMB	Pour- plate	Green metallic sheen colonies on EMB	+	+	+	+	-
Maize with *S. yphimurium*	SSA	Pour- plate	Transparent Colonies with black centers	+	+	+	-	-
Sorghum with *S. typhimurium*	SSA	Pour- plate	Transparent Colonies with black centers	+	+	+	+	-
Maize(c)	EMB	Pour- plate	ND	-	-	-	-	-
	SSA		ND					
Sorghum(c)	EMB	Pour- plate	ND	-	-	-	-	-
	SSA		ND					

EMB: Eosin Methylene Blue agar; SSA: Salmonella-Shigella agar; Maize(c): Un-inoculated maize grains; Sorghum(c): un-inoculated sorghum grains; ND: Not detected; +: detected; –: not detected

Table 2: Microbial enumeration during inoculation of *Escherichia coli* maize and sorghum grains for ogi production

Fermentation (hrs)	Cereals	Control	TAM (cfu/ml)	LAB (cfu/ml)	Y.C (cfu/ml)	*E.C* (cfu/ml)
Prior to inoculation						
0	Maize	0.00	4.0×10^3	4.0×10^3	3.2×10^2	0.00
	Sorghum	0.00	3.6×10^3	3.0×10^3	3.5×10^2	0.00
After inoculation						
0	Maize	0.00	1.3×10^5	4.0×10^3	3.2×10^2	0.00
	Sorghum	0.00	1.4×10^5	3.0×10^3	3.5×10^2	0.00
6	Maize	0.00	1.5×10^5	6.8×10^4	4.2×10^2	1.1×10^6
	Sorghum	0.00	1.6×10^5	5.2×10^4	4.4×10^2	1.2×10^6
24	Maize	0.00	1.3×10^4	1.6×10^5	8.0×10^4	7.4×10^4
	Sorghum	0.00	1.2×10^4	1.3×10^5	8.6×10^4	8.6×10^4
48	Maize	0.00	9.6×10^4	1.9×10^6	1.2×10^5	ND
	Sorghum	0.00	8.8×10^4	1.8×10^6	1.0×10^5	$<3.4 \times 10^2$
72	Maize	0.00	8.0×10^4	2.1×10^7	1.5×10^5	ND
	Sorghum	0.00	7.6×10^4	2.3×10^7	1.2×10^5	ND
Souring period						
0	Maize	0.00	6.8×10^4	1.8×10^6	1.2×10^5	ND
	Sorghum	0.00	6.2×10^4	2.0×10^6	1.3×10^5	ND
24	Maize	0.00	4.2×10^2	2.7×10^7	1.6×10^5	ND
	Sorghum	0.00	3.6×10^2	2.9×10^7	1.4×10^5	ND

TAM: Total Aerobic mesophiles count; LAB: Lactic acid bacteria count; Y.C: Yeast count; E.C: Escherichia coli count; ND: Not detected

Table 3: Microbial enumeration during inoculation of *Salmonella typhimurium* maize and sorghum grains for ogi production

Fermentation (hrs)	Cereals	Control	TAM (cfu/ml)	LAB (cfu/ml)	Y.C (cfu/ml)	*S.C* (cfu/ml)
Prior to inoculation						
0	Maize	0.00	4.2×10^3	4.0×10^3	3.2×10^2	0.00
	Sorghum	0.00	3.6×10^3	3.2×10^3	3.6×10^2	0.00
After inoculation						
0	Maize	0.00	1.4×10^5	4.0×10^3	3.2×10^2	9.2×10^5
	Sorghum	0.00	1.5×10^5	3.2×10^3	3.6×10^2	9.8×10^5
6	Maize	0.00	1.5×10^5	6.8×10^4	4.0×10^2	1.2×10^6
	Sorghum	0.00	1.6×10^5	5.2×10^4	4.4×10^2	1.3×10^6
24	Maize	0.00	1.4×10^4	1.6×10^5	8.0×10^4	7.8×10^4
	Sorghum	0.00	1.2×10^4	1.3×10^5	8.6×10^4	8.6×10^4
48	Maize	0.00	7.6×10^4	1.9×10^6	1.2×10^5	ND
	Sorghum	0.00	8.8×10^4	1.8×10^6	1.1×10^5	$<3.2 \times 10^2$
72	Maize	0.00	8.0×10^4	2.1×10^7	1.5×10^5	ND
	Sorghum	0.00	7.6×10^4	2.2×10^7	1.2×10^5	ND
Souring period						
0	Maize	0.00	6.8×10^4	1.8×10^6	1.2×10^5	ND
	Sorghum	0.00	6.2×10^4	1.9×10^6	1.3×10^5	ND
24	Maize	0.00	3.2×10^2	2.8×10^7	1.6×10^5	ND
	Sorghum	0.00	3.6×10^2	3.0×10^7	1.3×10^5	ND

TAM: Total Aerobic mesophiles count; LAB: Lactic acid bacteria count; Y.C: Yeast count; E.C: *Salmonella* count; ND: Not detected

The lactic acid bacteria increased in numbers at the beginning within 24 h of fermentation to the maximum numbers of 2.9×10^7 and 3.0×10^7 cfu/ml in the inoculated Sorghum grains with *E. coli* and *S. typhimurium* at the end of the fermentation (Table 4). In maize inoculated with the pathogens (Table 3 and 4), the maximum levels ranged between 2.7×10^7 cfu/ml in *E. coli* and 2.8×10^7 cfu/ml in *S. typhimurium*- inoculated maize. The yeasts increased in numbers in the fermenting maize and sorghum grains reaching maximum counts of 1.6×10^5 and 1.4×10^5 cfu/ml, respectively at the end of the fermentation.

Table 4: Changes in pH and titratable acidity during fermentation of maize and sorghum grains for ogi production at room temperature

Fermentation	Steeping/Primary fermentation					Souring/ Secondary	
Cereals	**0 h**	**6 h**	**24 h**	**48 h**	**72 h**	**0 h**	**24 h**
Maize grains							
pH	6.4	6.4	5.4	3.9	3.6	4.5	3.5
TTA (%)	0.03	0.03	0.18	0.77	0.82	0.60	0.83
Sorghum grains							
pH	6.4	6.4	5.9	4.2	3.5	4.5	3.5
TTA (%)	0.02	0.02	0.09	0.74	0.90	0.60	0.84

Table 5: Antibacterial activity of lactic acid bacterial isolates on test organisms (mm)

Isolate Code	Identified organism	*S. typhimurium*	*E. coli*	*S. aureus*	*B. subtilis*
W23E	*L. mesenteriodes*	10	18	19	12
S23E	*L. bulgaricus*	8	10	10	9
S42S	*L. cuslactis*	–	–	7	–
S72S	*L. acidophilus*	15	13	18	16
S72E	*L. plantarum*	12	13	10	10
S73E	*P. cusacidilactici*	9	10	10	10
W91E	*L. fermentum*	10	18	18	10
W92E	*L. plantarum*	10	19	19	10

ZOI: Zone of inhibition in millimetres; Letters starting with W, isolates from Maize; Letters starting with S, isolates from Sorghum

Table 6: Summary of microorganisms isolated at different stages of fermentation of white maize.

Isolated/identified species	Fermentation period (hrs)				
	0	**6**	**24**	**48**	**72**
Lactobacillus fermentum	+	+	+	+	+
Lactobacillus plantarum	–	–	+	+	+
Lactobacillus bulgaricus	–	–	+	+	+
Leuconostocmesenteriodes	+	+	+	+	+
Staphylococcus aureus	+	+	+	–	–
Bacillus subtilis	+	+	+	+	+
Candida tropicalis	–	–	+	+	–
Candida krusei	+	+	+	+	+
Saccharomyces cerevisiae	–	–	+	+	+

Table 7: Summary of microorganisms isolated at different stages of fermentation of sorghum

Isolated/identified species	Fermentation period (hrs)				
	0	**6**	**24**	**48**	**72**
Lactobacillus plantarum	+	+	+	+	+
Lactobacillus bulgaricus	+	+	+	+	+
Lactobacillus acidophilus	–	–	+	+	+
Pediococ cusacidilactici	–	–	–	–	+
Lactococ cuslactis	–	–	–	+	+
Leuconostoc mesenteriodes	+	+	+	+	+
Staphylococcus aureus	+	+	+	–	–
Bacillus subtilis	+	+	+	–	–
Candida tropicalis	+	+	+	+	–
Candida krusei	–	–	+	+	+
Saccharomyces cerevisiae	–	–	+	+	+

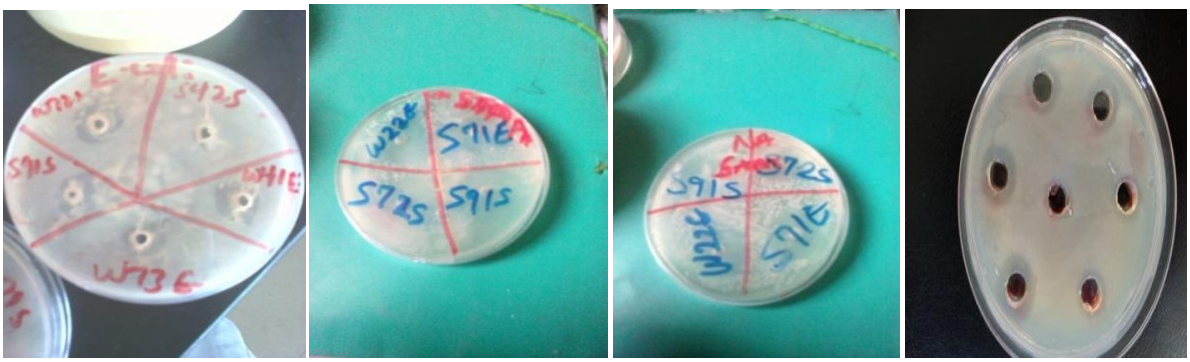

Plate A **Plate B** **Plate C** **Plate D**

Plate A: Antibacterial activity of some Lab isolates against *Escherichia coli*
Plate B: Antibacterial activity of some Lab isolates against *Staphylococcus aureus*
Plate C: Antibacterial activity of some Lab isolates against *Salmonella typhimurium*
Plate D: Antibacterial activity of some Lab isolates against *Bacillus subtilis*

During food fermentation, microbial species capable of spoilage and disease causation are encountered, but most times fermented foods provide high level of health safety against food borne diseases and according to Gadaga *et al*. (2004) from ancient times these foods are believed to be safe for consumption. For example, diarrhea cases among consumers of fermented gruel are very low as reported by Kingamkono *et al*. (1994).

In this study, there was a reduction in the number of the enteric pathogens. This report was similar to that of Adetunji (2011) and Omemu and Faniran, (2011). In their study, they observe total absence at the end of the fermentation. There was also an increase in number of LAB in the presence of the enteric pathogens and according to Abegaz (2007), this increase is essential in decreasing the pH of the food and to produce growth inhibitory compounds which will impede the growth of the pathogens. These growth inhibitory compounds include acetic acids, carbohydrate, ethanol, formic acid, bacteriocin, acetoin and so on (Dracheva *et al*., 2001). Gram negative bacteria are highly susceptible to the organic acids

and hydrogen peroxides produced by LAB impeding their growth (Boziaris and Adams, 1999). Caridi (2002) reported growth inhibition of both gram positive and gram negative bacteria by bacteriocin

The decrease in the aerobic counts as observed in this study was similar to Olsen *et al*. (1995) who reported decrease in the microbial load of steeped grain after 24 h of fermentation. This decrease was attributed to the interaction between the microbial flora of the fermented grains and the total microbial count of non-lactic acid bacteria species. In addition to the decrease in microbial load, a decrease in pH with time was also observed as the grains ferment. As a result, total titratable acidity increases as the fermentation time increases. According to Abegaz, (2007) this may be attributed to the production of lactic acid by lactic acid producing bacteria species present in the food substrates. The production of acid decreases the food pH (Adebayo *et al*., 2013). Wakil and Daodu (2011) report that specific constitute of the food, lactic acid bacteria act upon in releasing this organic acid is the carbohydrate constituent (sugar) converting sugar to lactic acid.

The anaerobic medium and sufficient nutrient available enhance the increase growth of LAB (Adebayo *et al*., 2013). Abegaz *et al*. (2002) reported that during fermentation, this increase growth of LAB can be facilitated by the nutritious metabolites produced by yeasts.

In this study, *Lactobacillus* species are the predominate species isolated. They include *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus and L.bulgaricus.* This observation was also reported by Nwokoro and Chukwu (2012) who stated that*Lactobacillus* species take the highest number of lactic acid bacteria among all others in all fermenting food due to their high acidophilic potency. Other lactic acid bacteria isolated which include *Leuconostoc mesenteriodes, Lactococ cuslactis* and *Pediococ cusacidilactici* were also reported by Adebayo *et al*. (2013). Yeasts were also isolated in this study. They include *Saccharomyces cerevisiae, Candida krusei, and Candida tropicalis.* These yeasts were also reported by Jespersen (2003). The yeast count was observed to increase after 24 h of fermentation. This increase could be as a result of a decrease in the pH, because yeasts are also acidophilic in nature. An acidic environment is an ideal environment for their growth.

According to Jespersen (2003), the beneficial roles played by yeast in fermenting food include improving flavor and aroma of the food. The isolation of *Bacillus subtilis* in this study could be associated with the role they play in the fermentation processes. Nwokoro and Chukwu (2012) also isolated some *Bacillus* species reporting their roles in carbohydrates metabolism during fermentation. *Staphylococcus aureus* isolated at early stage of fermentation may be as a result of contamination of the grains by the market women (Nwogwugwu *et al*., 2012).

Conclusion

This study shows that food fermentation enhances a decrease in food pH that eradicates non-acidophilic microbial species present in the food. The results reveal that the traditional fermented products can also result in the growth increase of LAB. It was also recorded that LAB species exhibited good antibacterial activities against *E. coli, S. aureus, S. typhi* and *B. subtilis* in this study. The presence of LAB in the food can prevent or reduce the contaminant in fermented foods. It should be noted that adequate hygienic practices must be encouraged by food handlers to ensure health safety among consumer. Therefore, the suggested fermented food is presumed to be safe for human consumption. Further work need to be carried out to ascertain the antibacterial agents present in the LAB species.

Conflict of Interest

Authors declare that there is no conflict of interest reported on this work.

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