BIOPRESERVATIVE EFFICIENCY OF *LACTOBACILLUS BULGARICUS* FMB1 ON NONO AND WARA COLLECTED FROM BOSSO METROPOLIS – NIGER STATE, NIGERIA

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Abstract
The biopreservative efficiency of Lactobacillus bulgaricus FMB1on Nono (fermented milk) and Wara (white cheese) was investigated. The L.bulgaricus FMB1 was inoculated asceptically from the standard inoculum of the culture using Mc farland standard into nono and wara and was stored at refrigeration temperatures of 2,4,6,8 10^0C and at room temperature of 24±1^0C.The inoculated milk products were observed for bio preservative efficiency. The total viable bacterial counts (TVBC) of nono and wara decrease drastically after 24 hours of the inoculation with 10^8 cells of L.bulgaricus FMB1 culture from 1.1 x10^6 Cfu/ml to between 7.2 x10^5 Cfu/ml and 8.6 x10^5 Cfu/ml and from 1.0 x10^6 Cfu/g to between 6.0 x10^5 Cfu/g and 7.8 x10^5 Cfu/g respectively.The shelf life of nono was extended between 2 to 6 days at the different storage temperatures employed while the shelf life extension of 2 to 4 days was recorded for wara at different temperature levels employed. The bio preservative efficiency of L.bulgaricus FMB1 was achieved more on nono than in wara.The lactic acid bacteria (LAB) play very important role in the biopreservation of food products due to the numerous type of metabolites (eg.bacteriocins) it secret when present in food products.

Keywords: biopreservative, *Lactobacillus bulgaricus,* inoculum, Nono, Wara, lactic acid bacteria, bacteriocins

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1. INTRODUCTION

Biopreservation refers to extended shelf life and enhanced safety of foods using microorganisms and/or their metabolites (Ross *et al*., 2002). Lactic acid bacteria (LAB) have a major potential for use in Biopreservation because they are safe to consume and during storage they naturally dominate the micro flora of many foods. In milk, brined vegetables, many cereal products and meats with added carbohydrate, the growth of LAB produce a new plant product (Hurst, 1981).

Lactic acid bacteria (LAB) are a group of gram-positive bacteria, non-spore forming, non-respiring, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrate. Historically, bacteria from the genera *Lactobacillus,Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus* are the main species involved. Several more have been identified but minor role in lactic fermentations (Aexlson, 1998). LAB provide protection against spoilage microorganisms by producing varieties of antimicrobial compounds, including bacteriocins and also due to pH decrease and competition for substrates. LAB produce various compounds such as organic acid and bacteriocin during lactic fermentation (Lindgren and Dobrogosz, 1990).

Fermentation of various foods by LAB is one of the oldest forms of Biopreservation practised by mankind.Bacterial antagonism has been recognized for over a century but in recent years this phenomenon has received more scientific attention,particularly in the use of various strains of lactic acid bacteria.One important attribute of LAB is their ability to produce antimicrobial compounds called bacteriocin.In recent years ,interest in the compound has grown substantially due to their potential usefulness as natural substitute for chemical food preservatives in the production

of foods with enhanced shelf life and/or safety. This balance is achieved by it inhibitory effect upon the harmful pathogenic microorganisms (Savadogo *et al.*, 2006). Fermented beef is the culinary name for fermented meat from bovines. For example cattle (cow) meat that has under gone fermentation due to exposure to microorganisms (World cancer research fund report, WCRFR, 2007). Meat and meat product serve as excellent growth media for variety of bacteria, although the outer surface of meat is generally covered by microorganisms, the inner parts of the meat contains few organisms. The contamination in meat come mostly from external sources during bleeding, handling and processing. The main sources of microorganisms in meat are exterior of the animal and the intestinal tract. Microorganisms that contaminate meat very widely but include molds and bacteria. Mold such as *Cladosorium, Sporotrichum, Geotrichum, Penicillium, Mucor* e.t.c grow on the meat surfaces. Bacteria such as specie of *Pseudomonas, Micrococcus, Streptococcus, Sarcina, Lactobacillus, Salmonella, Escherichia, Clostridium* and *Bacillus* are most common. (Wikipedia, 2010).Similarly,Asahan (2010) reported that LAB species such has *Lactobacillus sakei, Lactobacillus curvatus, Pediococcus acidilactici and Pediococcus pentosaceus* are associated with fermented meats.

Fermented milk (Nono) is an ideal medium for the growth of microorganisms and contamination by harmful bacteria such as *Salmonella* or *a* fungus is always a possibility. In addition, some bacteria which are not harmful nevertheless affect the quality of milk if allowed to grow in it (Taylor *et al;* 1997).

Cheese (wara) can be defined as a consolidated curd milk solid in which fat is entrapped by coagulated casein. The physical characteristics of cheese are far removed from those milk, this is because protein coagulation proceeds to a greater extent as a result of the use of proteolytic enzymes and much of the water content of the milk separates and its removed in the form of whey (Taylor *et al;* 1997). Some examples of cheese, soft ripened cheese which include camemberti and blue cheeses. Wara is highly perishable due to contamination from farm and processing, this often leads to spoilage and low keeping quality of the cheese (Olatunji *et al;* 2006). Wara has been found to harbour bacteria including LAB such as *Lactobacillus,Streptomyces* species as well as yeasts and moulds (Shiawoya *et al;* 2004, Olatunji *et al;* 2006).

This study is therefore focused at using *Lactobacillus bulgaricus* FMB1culture as Bio preservative for nono and wara as an alternative to chemical preservatives/additives used as shelf life extender in food products.

2. MATERIAL AND METHODS

Collection of Samples

Samples of beef were purchased and deposited in sterile stomacher bag from Bosso Market and were transfer to the Laboratory for the Isolation of (LAB).Samples of nono and wara were also collected in sterile bottles for the biopreservation studies.

Culture Media

The culture media used in this research were prepared following the standard laboratory methods as prescribed by Daba *et al.*(1991) and Cheesebrough (2003). The media used in this study include Nutrient agar (NA) (Oxoid), Urea agar base (Analar), Mannitol salt agar (MSA) (Oxoid), Simon's citrate agar (Oxoid), De Man Rogosa sharpe (MRS) broth (Oxoid) and Lactic acid medium (LAM) (Oxoid). Lactic acid medium (LAM) is a selective medium for the growth of lactic acid bacteria.

Isolation of Lactic Acid Bacteria (LAB)

Twenty five grams (25g) of fermented beef were aseptically transferred separately into sterile stomacher bag and 225ml of buffered peptone water,Bpw (Oxoid) was added to obtain 1:10 dilution. The samples were blended for 1 minute respectively. Serial dilution of the samples were done in 0.1% peptone water. Serially diluted samples were plated on lactic acid medium (LAM) and incubated at 37^{o}C for 24 hours. Colonies that appeared on the plates were counted using the colony counter (Stuart,

6339, Co. Ltd. Great Britain) and the result recorded as colony forming units per gramme (cfu/g). Pure culture was obtained by repeated sub-culturing of the isolate on fresh media. Pure culture was maintained on agar slant for further characterization and identification (Bromberg *et al;* 2004, Oyeleke and Manga, 2008).

Characterization and Identification of microbial isolates

The isolates were characterized based on colony morphology, cell morphology and biochemical tests (Hammes *et al*., 1999; Cheesbrough, 2003; Oyeleke and Manga, 2008). The biochemical tests include Gram's reaction, motility, ammonia from argenine, carbohydrate utilization profiles, production of catalase, oxidase, coagulase, citrate utilization, Indole test, mannitol activity, gelatin liquefaction (Fawole and Oso, 1998., Oyeleke and Manga, 2008). The LAB was identified as *Lactobacillus bulgaricus* FMB1using the scheme of Cheesebrough (2003).

Inoculum Preparation of LAB

The Bacteriocins producing LAB were inoculated into nutrient broth medium separately, and then incubated at 37°C overnight., serial dilutions was carried out in each case. The total count of microorganisms per milliliter (ml) of the stock suspension were determined by means of the surface viable count (SVC) technique. 0.5 Mcfarland standard is comparable to bacterial suspension of 10^8 cells/ml or cells/g (1.5ml of 0.5 Mcfarland standard is 10^8 cfu/ml of bacterial suspension).So 10^8 cells/ml or cells/g were inoculated into the 'Nono' and 'Wara' under study. (Mcfarland,1907., Sanaa *et al*., 2008) .

Biopreservative Efficiency of Bacteriocin Producing LAB

From the inoculum preparations, 10^8 cells/ml or cells/g Mcfarland standard were inoculated into the food products under study i.e, fermented milk (nono) and cheese (wara) in each case respectively to determine the shelf life elongation of the food products under study. This products, after inoculation were kept at refrigeration temperature (2,4,6,8 and 10°C) and room temperature (24±1°C) with the experimental control (nono and wara without LAB) set aside (Mcfarland,1907., Technoserve, 1994., FSTGL, 2003; FSAI,2005., Sanaa *et al.,* 2008).

Monitoring of Parameters of Fermented milk Products

(i) Physical Appearance of Dairy Products

The physical appearances base on colour of nono (dairy product) was examined before and after inoculating (every 24 hours after first Inoculation of products) them with nisin and at expiration shelf life elongation of the products (Mohammed *et al*.,2013).

(ii) Flavour Determination

The flavour of dairy products under study were determined by perceiving the products before and every 24 hours after first inoculation of the product with nisin. The nono under study was also be perceived after the expiration of the shelf life. The results generated was recorded as either pleasant or unpleasant (Mohammed *et al*.,2013).

(iii) Microbial Counts

The pour plate method were used. Serially diluted sample of the fermented milk (nono) and cheese (wara) was inoculated asceptically into nutrient agar and incubated at 37°C for 24 hours for the presence of aerobic viable bacteria for each sample respectively. Colonies which appeared on the plates were counted using colony counting chamber and were recorded as colony forming unit per milliter (cfu/ml) or per gram (cfu/g) of samples (Cheesbrough,2003.,Oyeleke and Manga, 2008). Microbial counts were taken before inoculation of product, every 24 hours after first inoculation of nono and wara with culture of *L.bulgaricus* FMB1and at the expiration of shelf life of nono under study.

3. RESULTS

Isolation,Characterization, Identification of LAB and it Selection for Biopreservation Studies

The Fermented Beef (FMB) analyzed contained lactic acid bacteria (LAB) in varying numbers. *L.bulgaricus* FMB1was isolated

based on colony morphology on lactic acid medium (LAM), characterized based on cell morphology and biochemical tests (Table 1). The *L.bulgaricus* FMB1 was selected after vigorous screening based on it ability to grow in De Man Rogosa Sharpe broth to produce bacteriocin, also through spectrophotometric analysis at 580nm wave length, pH,bacteriocin activity (AU/mL) and with potential for use as food biopreservative. It was observed that *L. bulgaricus* FMB1 had growth ability of 0.89,at pH of 4.20 and bacteriocin activity of 7000 AU/mL was better bacteriocin producer (Table 2).

Biopreservative Efficiency of LAB on Fermented milk and Cheese

TVB of fermented milk (nono) and cheese (wara) decreased drastically after 24 hours of inoculation with 10^8 cells of *L. bulgaricus* FMB1 from 1.1 x 10^6 cfu/ml to between 7.2 x 10^5cfu/ml and 8.6 x 10^5cfu/ml, 1.0 x 10^6 cfu/g to between 6.0 x 10^5cfu/g and 7.8 x 10^5cfu/g for fermented milk (nono) and cheese (wara) respectively.Shelf life extension days of milk products were observed at storage temperatures of 2^0C (5 days),4^0C (6 days),6^0C (4 days),8^0C (4 days),10^0C (5 days) and 24±1^{0}C (2 days) for fermented milk (nono) while at 2^0C (4 days),4^0C (3 days),6^0C (2 days),8^0C (2 days),10^0C (3 days) and 24±1^{0}C (2 days) was also observed for cheese (wara) (Table 3).

4. DISCUSSION

The Fermented beef analyzed contained lactic acid bacteria (LAB) in varying numbers,which include. *L.bulgaricus* FMB1. The presence of LAB in locally fermented foods has been reported by other researchers (Odunfa, 1985; Kuboye, 1985; Olukoya, 1993). This is similar to the findings of Oyeleke *et al.*(2006) on occurrence of lactic acid bacteria in some locally fermented foods who reported frequent isolation of *L.bulgaricus* and *L.acidophilus* with 29% each of occurrence,followed by *S.thermophilus* (25%),*S.cremoris* (10.6%) and *L.lactis* (6.4%) products.

Inoculation of 10^8 cells/ml or cells/gram of bacteriocin producing LAB into nono and wara revealed that pH, storage temperature and microbial load played significant roles in shelf life determination. FSAI (2005) reported that the shelf life of many food products is dependent on storage temperature and microbial load. At refrigeration storage temperatures of 4^0C and 10^0C, fermented milk products in this study were better preserved than other storage temperatures (2,6,8 and 24±1^{0}C) employed in this study.

Table 1 .Morphology,Cultural and Physiological Characteristics of potential bacteriocin producing lactic acid bacteria in Food products

Isolate code	Colony Morphology	Cell morphology	Gram staining	Oxidation test	Mannitol activity	Catalase	NH_3 activity from arginine	Gelatine liquif action	Sugar Fermentation				
									Glucose	Sucrose	Fructose	Lactose	Probable organism
FM B 1	Circular, convex	Rods	G+	-	-	-	-	-	AG	-	AG	AG	*Lactobacillus bulgaricus*

Key : + = positive result; - = Negative result; A = Acid production; G = Gas production; Ag = Acid and Gas production; G+ = Gram positive; FMB: Fermented beef.

Table 2. Bacteriocin Producing Ability of Lactic Acid Bacteria (LAB)

Code isolate	Concentration (580nm)	pH of medium	Bacteriocin activity (AU/mL)
Lactococcus lactis FALB18	0.89	4.20	7000*

FMB:,Fermented Beef,AU/mL: Activity unit permillilitre, nm: nanometer*: potential bacteriocin producer

Table 3.Physicochemical and Microbial Qualities of Fermented Milk Products Enriched with Bacteriocin Producing LAB (Biopreservation)

Fermented milk products	Physical appearance of fermented milk products before inoculation with LAB	Flavour of fermented milk products before inoculation with LAB	Initial pH of fermented milk products before inoculation with LAB	Storage temperature (^{0}C)	Physical appearance of milk products after first inoculation with LAB	Flavour at the end of the shelf life of milk products	Microbial counts (cfu/ml or cfu/g)before inoculation with LAB	Microbial counts (cfu/ml or cfu/g) after 24 hours of inoculation of milk products with LAB	Microbial counts (cfu/ml or cfu/g) after shelf life of milk products	*Days of original shelf life of milk products	Improved shelf life (elongation) of milk products	Potential LAB used for improvement of fermented milk products
Fermented Milk (Nono)	White	Pleasant	6.2	2	Grey White	Unpleasant	1.1 x 10^6	7.2x10^5	1.3x10^6	14	19	*L.bulgaricus* FMB1
	White	Pleasant	6.2	4	Grey White	Unpleasant	1.1 x 10^6	7.4x10^5	1.1x10^6	14	20	*L.bulgaricus* FMB1
	White	Pleasant	6.2	6	Grey White	Unpleasant	1.1x 10^6	7.7x10^5	1.0x10^6	10	14	*L.bulgaricus* FMB1
	White	Pleasant	6.2	8	Grey White	Unpleasant	1.1 x 10^6	8.0x10^5	1.3x10^6	7	11	*L.bulgaricus* FMB1
	White	Pleasant	6.2	10	Grey White	Unpleasant	1.1 x 10^6	8.1x10^5	1.0x10^6	7	12	*L.bulgaricus* FMB1
	White	Pleasant	6.2	24 ±1	Grey White	Unpleasant	1.1 x 10^6	8.6x10^5	1.4x10^6	1	3	*L.bulgaricus* FMB1
White Cheese (Wara)	White	Pleasant	6.1	2	White	Unpleasant	1.0 x 10^6	6.0x10^5	1.0x10^6	7	11	*L.bulgaricus* FMB1
	White	Pleasant	6.1	4	White	Unpleasant	1.0 x 10^6	6.2x10^5	1.0x10^6	7	10	*L.bulgaricus* FMB1
	White	Pleasant	6.1	6	White	Unpleasant	1.0 x 10^6	6.5x10^5	1.0x10^6	7	9	*L.bulgaricus* FMB1
	White	Pleasant	6.1	8	White	Unpleasant	1.0 x 10^6	7.0x10^5	1.1x10^6	5	7	*L.bulgaricus* FMB1
	White	Pleasant	6.1	10	White	Unpleasant	1.0 x 10^6	7.2x10^5	1.2x10^6	5	8	*L.bulgaricus* FMB1
	White	Pleasant	6.1	24 ±1	White	Unpleasant	1.0 x 10^6	7.8x10^5	1.6x10^6	1	3	*L.bulgaricus* FMB1

This might be due to the inability of some spoilage pathogenic organisms to grow at that particular temperatures and/or the presence of bacteriocin producing LAB. This finding is similar to the report of Technoserve (1994) that most commercial products are refrigerated at 10^0C which also encourage the growth of many psychrophiles like *Pseudomonas*, *Alkaligenes, Flavobacterium* and *Micrococcus* species.

At room storage temperature (24±1^{0}C) the fermented milk products (nono and wara) were also preserved probably due to the presence of LAB which grows optimally at 30-37^{0}C and produce metabolites - like bacteriocin which probably inhibited the growth of spoilage and pathogenic microorganisms in the milk products under study.This is similar to the findings of Ogunbanwo *et al.*(2003) and FSAI (2005) who reported that LAB grows optimally at 30-37^{0}C and produce metabolites like bacteriocin which probably inhibits the growth of spoilage and pathogenic microorganisms such as *Enterococcus faecalis, E. coli, Salmonella* and *S. aureus* in the milk products.Adams and Moss (1995) also reported that lactic acid bacteria (LAB) grow optimally at pH 5.8 to 6.5 and produce metabolites like lactic acid and bacteriocin (Biopreservative) which are used against food borne pathogens. Similar observations were made in the present

study where LAB inoculated into fermented milk products grew maximmaly and elaborated bacteriocin at pH 4.20.

The LAB (*L.bulgaricus* FMB1) inoculated into the fermented milk products (nono and wara) proved effective and extended the shelf life of nono and wara by 2 - 6 and 2 - 4 days for each respectively.The shelf extension of the milk products was more favourable in nono than wara and this could be as a result of growth of the LAB involved and extent of bacteriocin production in the milk products under biopreservation with LAB or this could be as a result of the wide area (liquid) of exposure to the LAB than the small area (solid) of exposure of wara under biopreservation studies.This is similar to the report of Maisnier-Patin *et al*.(1992) that as an alternative to using bacteriocin it self for biopreservation of foods,direct introduction of live bacteriocin-producing culture of LAB as a protection starter has been investigated extensively and has achieved favourable results in some food systems.For example,the nisin-producing starter has been shown to have the potential to inhibit *L.monocytogenes* in Camembert cheese manufacture.Futhermore,it was reported that *Lactobacillus* or *Pediococcus* strains producing an antilisterial class IIa bacteriocin could inhibit *L.monocytogenes* growth in meats and meat products (O'Sullivan *et al*.,2002).

Similarly, Dike and Sanni (2010) conducted research on shelf-life of agidi using the culture of lactic acid bacteria and revealed that the shelf - life of traditional fermented agidi (T-Ag), agidi produced using NBP-Ag and the samples BP-Ag, were compared.

It was observed that agidi prepared from maize fermented with the mixed culture of BP strains had the longest shelf life of 8 days. Agidi produced from maize fermented with single starter culture of BP *L. plantarum* had a shelf life of 6 days, while agidi prepared from maize fermented in the traditional way had a shelf life of 2 days.

On the other hand, samples prepared from maize fermented with NBP mixed culture starters had a shelf life of 4 days before spoilage occurred.

Dike and Sanni (2010) further revealed that agidi fermented with NBP mixed culture had a total bacterial load of 3.2×10^5 log10 cfu/g and fungi load of 1.6×10^4 $\log_{10}$ cfu/g when spoilage occurred after day 4. Agidi produced with single starter culture of BP *L. plantarum* had none on the same day. However, agidi produced with mixed culture of *Lactobacillus* strain had a bacteria load of 1.9×10^5 $\log_{10}$ cfu/g and fungi load of 3.3×10^6 $\log_{10}$ cfu/g when spoilage occurred after day 8, while the bacterial load and fungi load of agidi prepared from mixed culture of NBP *Lactobacillus* strain increased to 2.0×10^8 and 3.8×10^8 $\log_{10}$ cfu/g, respectively. In addition, samples prepared from traditionally fermented agidi increased to 1.0×10^8 and 3.0×10^8 $\log_{10}$ cfu/g, while those prepared using BP *L. plantarum* increased to 1.0×10^6 and 2.8×10^7 $\log_{10}$ cfu/g after day 8, respectively.

The implication of these findings is that re-inoculating these LAB into fresh product can extend the shelf life of these products particularly at refrigeration temperatures of 4^0C and 10^0C.

5. CONCLUSIONS

The growth of pathogenic and spoilage microorganisms in nono and wara under study were inhibited by *L. bulgaricus* FMB1. LAB are very important, in that their presence in foods enhance the improvement of the shelf life of the food products. The use of bacteriocin-producing strains of LAB are of great interest as they are generally recognized as safe (GRAS) organisms and their antimicrobial products as biopreservatives.Therefore,the presence of bacteriocin producing LAB in foods and/or fermented foods can enhance safety and shelf life extension of the food products and will also serve as alternative to chemical preservatives/additives in food preservation.

6. RECOMMENDATION

Based on the findings of this study the following recommendation are made:

1. The presence of lactic acid bacteria in foods are recommended, in that they produce bacteriocins that could inhibit the growth of spoilage and pathogenic organisms, and therefore increase the shelf life of such food products.
2. Food processing companies should embark on the use of bacteriocin producing LAB for bio-preservation of foods which may serve as alternative to using chemicals as food additives or preservatives.
3. More research should be carried out on bacteriocin producing lactic acid bacteria, particularly their involvement in food preservation and enrichment.

7. REFERENCES

[1] Adams M.R. and Moss, M.O. (1995).*Food Microbiology*. Royal Society of Chemistry, Gulford, pp. 252-284.
[2] Asmahan A. A.(2010).Beneficial role of lactic acid bacteria in food preservation and Human health:*A Review.Reseach Journal of Microbiology.*5(12): 1213-1221.
[3] Axelson, L. (1998). Lactic Acid Bacteria: Classification and Physiology of Lactic Acid Bacteria, *Microbiology and Functional Aspects*. Salminen, S. and Wright, A. (eds), Marcel Decker Inc., New York, USA.
[4] Bromberg, R. *et al* (2004): Isolation of Bacteriocin-producing Lactic acid bacteria from meat products and it spectrum of inhibitory activity. *Brazilian Journal of Microbiology.* Vol. 35. No.1-2.
[5] Cheesebrough, M. (2003).District Laboratory Practices in Tropical *Countries*. Cambridge University Press, Edinburgh, Uk. pp. 382-407.
[6] Daba, H., Pandian, S., Goisselinh, JF., Simard, RE., Huang, J. and Lacroix, C. (1991).Detection and activity of bacteriocin produced by *Leuconostoc mesenteroides.Applied* and *Environmental Microbiology,* 57: 3450-3455.
[7] Dairy Storage Guidelines ,DSGL (2004*). Safe Food Storage Time and Temperature:* Arizona Department of Health Services http://www.azdhs.govtphs/oeh/Fses/Sf-sttat5.htm
[8] Dike,K.S.and Sanni,A.I.(2010).Influence of starter culture of lactic acid bacteria on shelf life of agidi ,an indigenous fermented cereal product.*African Journal of Biotechnology*.9(46).7922-7927.
[9] Fawole, MO. and Oso, BA. (1998) laboratory manual of Microbiology. Spectrum book limited, Ibadan, Nigeria, pp 16 – 35.
[10] Food Safety Authority of Ireland, FSAI, (2005). *Determination of Product* Shelf life: Guidance Note No. 18 FSAI, Dublin, pp. 19 – 46.
[11] Food Storage and Time Guidelines, FSTGL (2003). *Food Service of America* http://www.FsaFood.com/foodsafety/049.html.
[12] Hammes, W., Weiss, N. and Holzepfel, W. (1999).*The Prokaryotes* Springer - Velag, New York, pp. 1 - 103.
[13] http://en.wikipedia.org/wikiIbacteriocin 2010http//www.microbiology.procedure.com/foodmicrobio1ogy//preservation- of-meat.htm.
[14] Hurst,A.(1981).Nisin.*Advances in Applied Microbiology*.27:85-123.
[15] Kuboye, AO. (1985). Traditional fermented foods and beverages of Nigeria. In: Prage L, ed. Proceedings of the IFA/UNU Workshop on the Development of Indigenous Fermented Foods Technology in Africa.*International Foundation for Science*, Stockhotm, pp. 22-36.
[16] Lindgren, S. W. and Dobrogeosz, W.L. (1990).Antagonistic activities of lactic acid bacteria in food and food fermentation.*Microbiology Review,* 87: 49 - 160.
[17] Maisnier-Patin, S., Deschamps, N., Tatini, S.R. and Richard, J. (1992) Inhibition of *Listeria monocytogenes* in Camembert cheese made with a nisin-producing starter. Lait 72, 249–263.
[18] McFarland,J.L.(1970).*Journal of America Medical association*,JAMA.49:1176.
[19] Mohammed,S.S.D.**,**Damisa,D.,Oyeleke,S.B.,Jigam,A.A.(2013).Biopreservation Studies of White Cheese (*Wara*) using Acidophilin produced by *Lactobacillus acidophilus* FCF2 isolated from Fermented Corn Flour. IRJSET.*Journal of Engineering and Applied Sciences*: 2(1). pp1-9.
[20] Odunfa, SA. (1985). African fermented foods. In: *B.J.B. Wood, (ed.)Microbiology of Foods.: Elsevier, London,* pp. 155-191.
[21] Ogunbanwo, ST., Sanni, A I. and Onilude, AA. (2003).Influence of cultural conditions on the production of bacteriocin by *Lactobacllus brevis* OG 1. *African Journal of Biotechnology,* 7: 179 – 184.
[22] Olatunji, E.A., Shiawoya, E.L. and Ijah, U.J.J (2006). Bacterial assessment and keeping quality of local cheese (wara) in whey and salt solution. *Journal of Sustainable Tropical Agricultural Research* 17:125-130
[23] Olukoya, DK. (1993). *Lactobacillus* in human health with reference to locally fermented foods. *Nigerian Medical Journal,* 24(1): 28-30.
[24] O'Sullivan L, Ross, R.P., Hill C. (2002). Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. 84, P. 593-604.
[25] Oyeleke, SB. Faruk, AK. Oyewole, OA. and Nabara, HY. (2006). Occurence of Lactic acid bacteria in some locally fermented food products sold in Minna markets. *Nigerian Journal of Microbiology,* 20 (2): 927-930.
[26] Oyeleke, SB. and Manga, BS. (2008).Essentials of Laboratory practicals in Microbiology (first edition).Tobest publishers, Minna, Nigeria, pp, 28- 62.

[27] Ross, RP., Morgan, S., and Hill, C. (2002). *International Journal of Food Microbiology* **79,** 3-16 .
[28] Saana, OX., Fadous, A,B. and Ibtisarn, E, E. (2008). Effects of temperature and storage period on the constituents of milk inoculated with *Pseudomonas aeruginosa. Research Journal of Microbiology* 3 (1). Pp 30 - 34.
[29] Savadogo, A., Ouattara, C. A.T., Bassole, I. H. N., Traore, S. A. (2006). Bacteriocins and lactic acid bacteria - a minireview. *African Journal of Biotechnology*. 5 (9). pp. 678-683.
[30] Shiawoya, E.L., Ijah, U.J.J, Olatuji, E.A. and Laiidi, A.O. (2004). The occurrence of moulds and yeasts in Wara (Nigerian white cheese) preserved in whey and whey permeate with or without salt. in: Proceedings of the 29th *Annual Conference of the Nigerian Society for Animal Production,* pp.120-123. Eds.
[31] Taylor, D.J., Green, N.P.O., Stout, G.W. (1997). *Biological Science*. 3rd Ed. Cambridge University Press Cambridge, Pp401 – 402.
[32] Technoserve, (1994). Male milk manval, A *guide for establishing and operating small scale interprises for production of culture milk* .Technoserve, P.O. Box, 1482, Nairobi or P.O. Box 2117 Arusha, Tanzania.
[33] World Cancer Research Fund Report, WCRFR (2007): Strong evidence that red meat and processed meat are cause of bowed cancer and serving beef at Ayodhya, article from the times ofIndia.http://en.wikipedia.org/wiki/beef.