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Evaluation of the Hygiene Level of Microbial Contamination of Traditionally Fermented Cottage Cheese in Ilorin Metropolis, Kwara State Nigeria

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Abstract: *Introduction: Traditionally fermented cottage cheeses are ready-to-eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption and as such, contamination of cottage cheese "wara" may occur from the equipment used in the production of "wara" and from the environment where it is being produced. The purpose of this research was to determine the hygiene level of microbial contamination of traditionally fermented white and red cottage cheese in Ilorin metropolis. Methodology: Forty (40) traditionally fermented white and red cottage cheese were randomly collected from ten (10) selected markets in Ilorin Metropolis. Four (4) cheeses were collected per market in the proportion of two (2) white and two (2) red cheese. The samples collected were cultured on four culture media (Mackonkey Agar, Mannitol Salt Agar, Potato Dextrose Agar and Nutrient Agar) and incubated at 37°C. Bacteria counts were obtain from direct culture to obtain the colony forming units. Characterization of isolates was done using their morphological appearance, colour of colonies, growth pattern and biochemical test. Also antibiotic sensitivity test was also done using the disc diffusion method. Result: The colony forming unit of white cottage cheese ranges from 5.0 x10 4 - 6.25 x10 5 CFU/ml while the colony forming unit of red cottage cheese ranges from 5.5 x10 4 - 5.1 x10 5 CFU/ml. Ten (10) bacterial species were isolated from both red and white cottage cheese "wara". 5% Klebsiella edwardsii, 15% Streptococcus spp, 5% Enterococcus feacalis, 5% Lactobacillus acidophilus, 10% Bacilus cereus, 5% Pseudomonas putida, 30% Shigella spp, 5% Pseudomonas fluorescens, 15% Escherichia coli and 10% Staphylococcus aureus. The organisms isolated were resistant to some commonly used antibiotics such as Tarvid, Gentamycin, Septrin, Amplicin, Ciproflox, Chloramphenicol and Ampliclox and also sensitive to Reflacine, Augumentin, Ceporex and Erythromycin. Discussion: Generally, this study reveals that red cottage cheese has the lowest pathogenic organisms present. The pathogenic microorganisms among them such as S. aureus, P. putida, and E. coli demonstrate a potential health risk which could result to foodborne illness. Improvement in the sanitary practices during the preparation of traditionally fermented cottage cheese is recommended. Keywords: Cottage cheese, Bacteria, Fungi, Contaminants, Sensitivity, Resistant, Staphylococcus aureus, Shigella spp, Streptococcus spp, Escherichia coli.*

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I. Introduction

The West African soft cottage cheese, "wara", is an important component of the diet among the nomadic Fulani of Nigeria and a much cherished delicacy among the urban and peri-urban dwellers of northern region and southwestern Nigeria. The common coagulant used in making soft cheese in these parts is the vegetable rennet obtained from the leaf or stem juice of Calotropis procera (Omotosho et al., 2011). "Wara" is a Nigeria soft white unripened cottage cheese that originated from Fulani cattle rearers in the northern part of the country. Cottage cheese "wara" is commonly produced from unpasteurized cow milk and sold along the major streets of Nigeria. "Wara" is a fresh cheese, that is, moist curd that has been cut and drained of the whey but never ripened and is unsalted and uncolored. The cheese is prepared by coagulating fresh cow milk with the leaf extract of the Sodom apple (Calotropis procera) or pawpaw (Carica papaya), which is known as 'ewe bomubomu' among Yorubas and 'tumfafia' in Hausa language. About one kilogram of cheese will be obtained from about five liters of milk (Adetunji and Chen, 2011; Sangoyomi et al., 2010). Cottage cheese is the fresh solid or semi-solid product obtained from coagulating milk. Most cheese types are made by the use of rennet to coagulate the case in micelles in the milk and addition of starter culture to produce lactic acid (Chikpah et al., 2014). Cottage cheese (or local cheese "wara" as it may be called) making is thought to have started in the region and as a result of the nomadic lifestyle of the fulani's, it spreads to other parts of Nigeria, such as Kwara, Oyo, Ondo States (Bamidele, 2006). Cottage cheese is an excellent source of protein, fats and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids, thus making it an important food in the diet

of both old and young (Oladipo and Jadesimi, 2012). In view of its nutritional importance, milk or its products should form at least part of the daily diet for humans, particularly the young and growing. A major constraint to the regular consumption of fresh milk is its very short shelf life. Cheese provides an opportunity to extend the shelf life of milk and preserve its valuable nutrients in a concentrated form (Law and Tamime, 2010). Cheeses are ready-to-eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption. Contamination of "Wara" with food borne pathogens may occur at several stages. The global incidence of food borne illnesses is difficult to estimate but it has been reported that in 2007 alone 2.1 million people died from diarrhoeal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2000). Therefore, the aim of this study is to assess the level of microbial contamination in traditionally fermented cheese and their antibiotics sensitivity in Ilorin Metropolis.

II. Materials And Methods

Cottage cheese "wara" was randomly selected using purposive sampling method from ten different markets in Ilorin metropolis namely; Ojaiya, Mandate, Oja-tuntun, Ipata, Ago, Taiwo market, Oja-unity, Oja-gboro, Oja-oba and Yoruba road. A total of forty (40) traditionally fermented cheese were collected from ten (10) selected markets. Four (4) cheese were collected per market in the proportion of two (2) white and two (2) coloured cheese. The samples were kept in clean and sterile polythene bags to prevent contamination and the samples were transferred to the laboratory immediately for analysis. The cheese samples were collected in the afternoon between the hours of 12.00pm – 04.00 pm The isolation of bacteria was completed within 24 hours of samples collection. This was carried out by mixing 1ml of the cheese samples with 9mL of sterile distilled water and diluted serially up to 10 -10 . This was repeated for all the samples. 0.2mL (aliquot) of the suspension was plated out of Mackonkey Agar, Mannitol Salt Agar, Potato Dextrose Agar and Nutrient Agar. The plates were incubated at 35 0 C for 24 hours. Distinct colonies growing on each plate were counted, selected, sub cultured and stored on slants. Pure cultures of all the isolates were subjected to biochemical test such as gram staining, motility test, oxidase test, catalase production, indole production, nitrate reduction, starch hydrolysis, gelatin hydrolysis and urease activity.

Antibiotic Sensitivity Test

72g of Muller Hinton agar were measured into 1litre of distilled water, the conical flask containing the dissolved Muller Hinton agar was corked with cotton wool, wrapped with aluminum foil and it was then sterilized in the autoclave at 121 0 for 15minutes. The susceptibility of the bacteria Isolates was assayed using disc diffusion method as described by the British Society for Antimicrobial Chemotherapy (BSAC) (Andrews, 2008). A suspension of each Isolate in normal saline was compared with 0.5 McFarland standards to standardize the inoculums.

III. Results

3.1 TOTAL VIABLE COUNT IN WHITE AND RED LOCAL DAIRY CHEESE

From the figure 1, the least contamination was observed in OUW1 with 10 colonies and the highest contamination was recorded in OYW1 with 125 colonies representing 5.0 x 10 4 and 6.25 x 10 5 CFU/ml for OUW1 and OYWI respectively. From this result, it is abundantly clear that the white cheese from Ojaiya market is more contaminated than other cheese analyzed in this study.

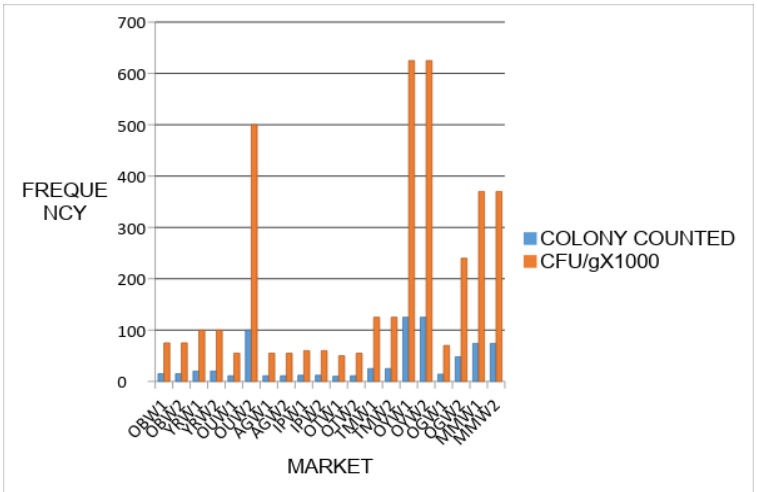

Figure 1: Comparison of level of contamination in White cheese from ten markets in Ilorin metropolis

From the figure 2, the least contamination was observed in OBR1, YRR1, OUR1 and OTR1 with 11 colonies and the highest contamination was recorded in OYR1 with 102 colonies representing 5.5 x 10 4 (OBR1, YRR1, OUR1 and OTR1) and 5.1 x 10 5 CFU/ml for OYR1. From this result, it is evidently clear that the red cheese from Ojaiya market is more contaminated than other cheese analyzed in this study. This followed the same contamination pattern with the result obtained from the white cheese.

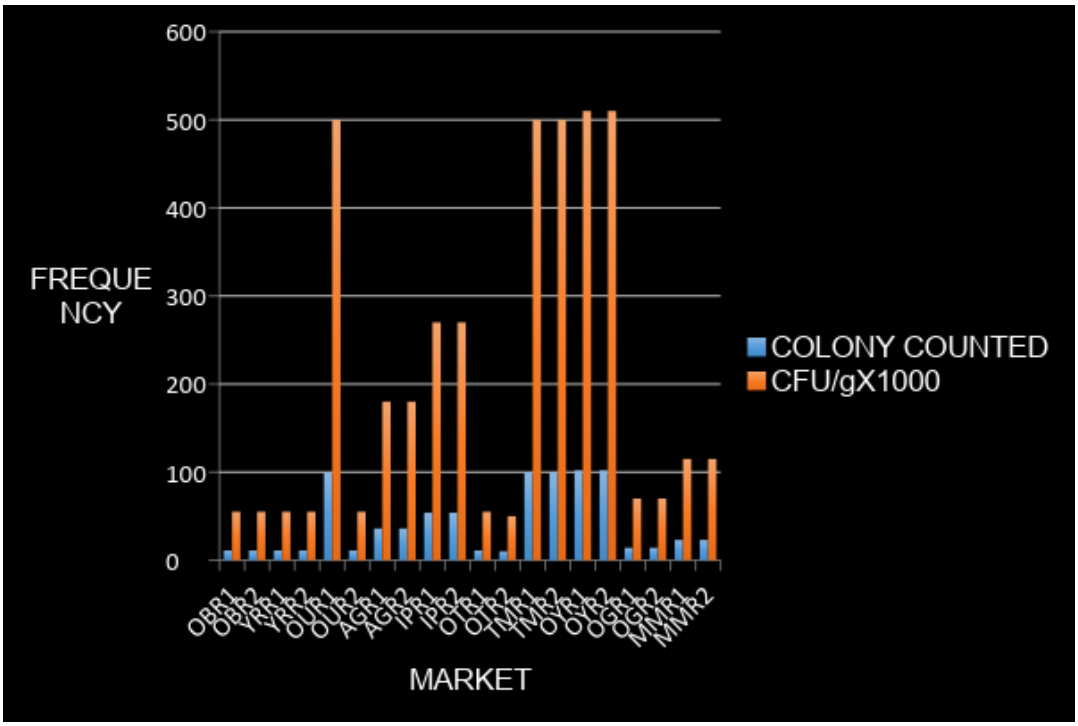

Figure 2: Comparison of level of contamination in Red cheese from ten markets in Ilorin metropolis

3.2. RELATIVE OCCURRENCE OF ISOLATES

The percentage occurrence of bacteria and fungi isolated from sampled cheese are shown in the graph below.

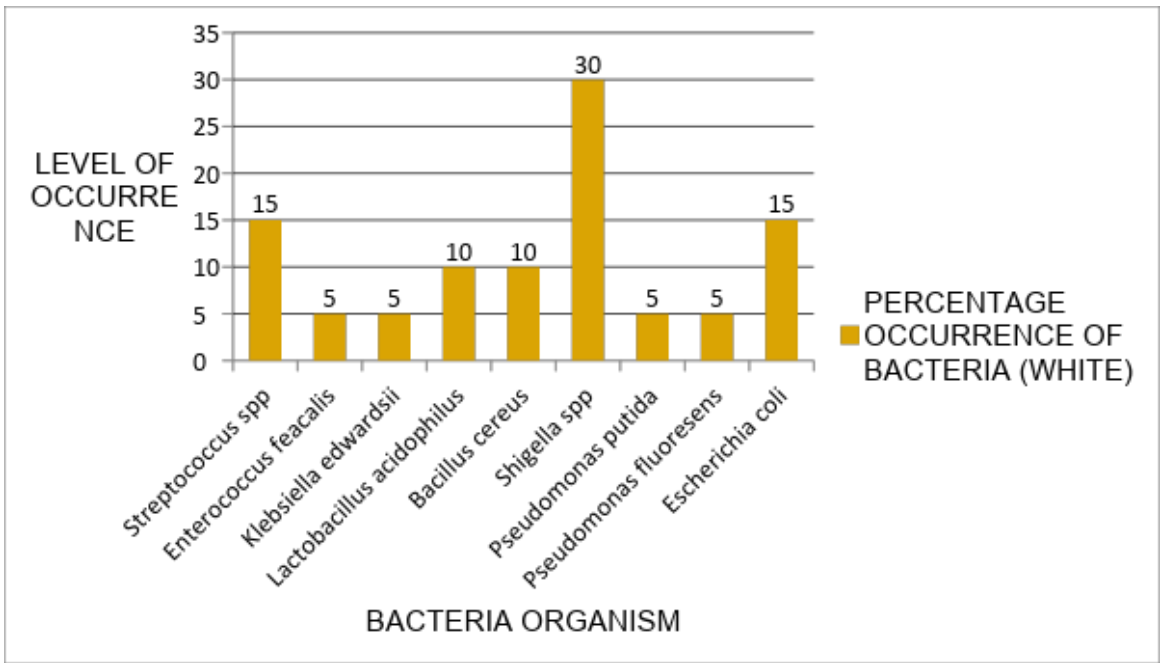

Figure 3: Percentage relative occurrence of organisms isolated from White cheese in Ilorin metropolis

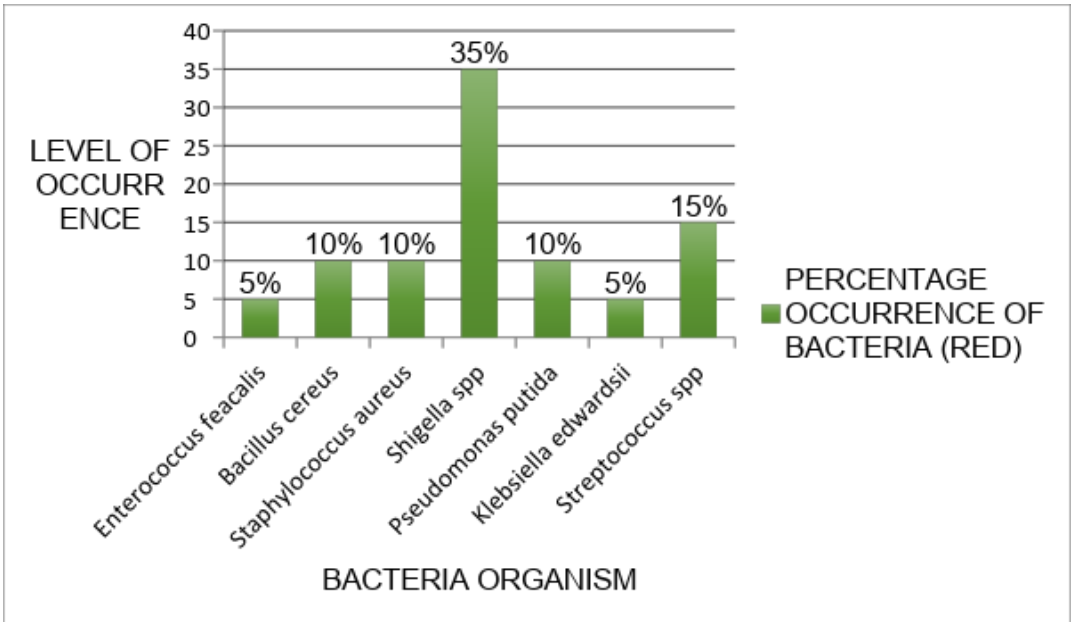

Figure 4: Percentage relative occurrence of organisms isolated from Red cheese in Ilorin metropolis

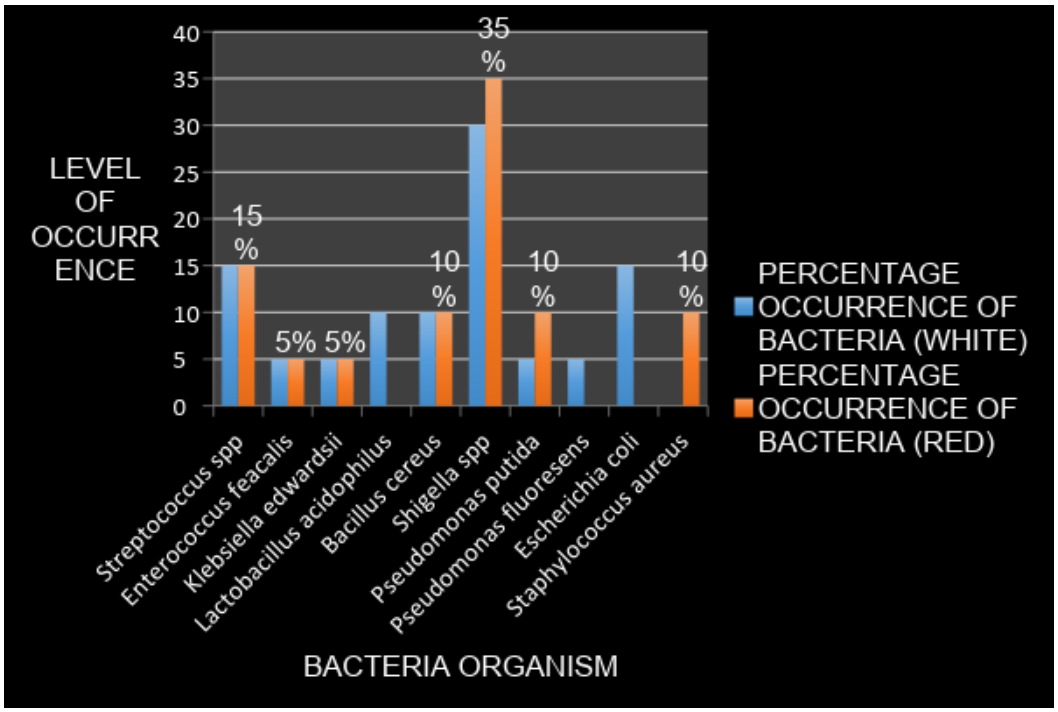

Figure 5: Comparison Percentage relative occurrence of bacteria isolated from the two types of cheese in Ilorin metropolis

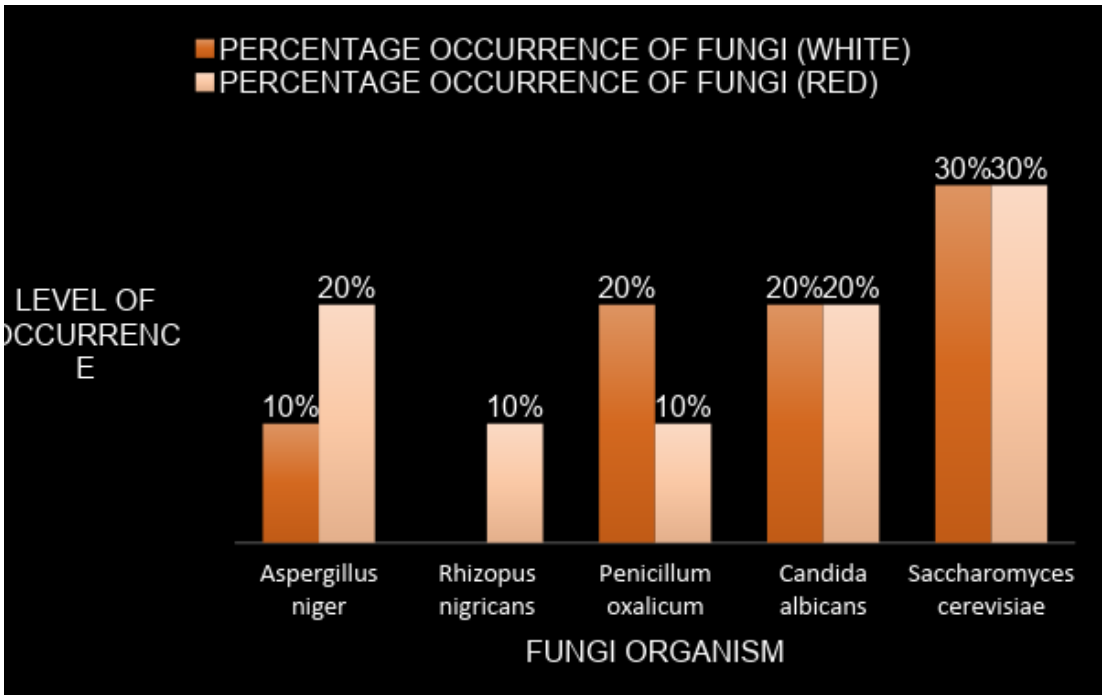

Figure 6: Comparison percentage relative occurrence of fungi isolated from the two types of cheese

Table 1: Microorganisms isolated from the sampled cheese

S/n	Cheese sampled	Microorganism isolated
1	White cheese	*Klebsiella edwardsii, Streptococcus spp, Enterococcus feacalis, Lactobacillus acidophilus, Bacilus cereus, Pseudomonas putida, Shigella spp , Pseudomonas fluorescens, Escherichia coli.*
2	Red cheese	*B. cereus, Streptococcus spp., Staphylococcus aureus, E. coli, E. feacalis, Shigella spp, K. edwardsii , P. putida.*

Table 1 shows that virtually all the bacteria isolated in white cheese are also present in the red cheese. Except for S. aureus that is present in red but absent in white cheese.

IV. Discussion

The percentage occurrence of bacteria isolates for white cheese as shown by figure 3 are as follows; 5% of E. feacalis, 10% of B. cereus, 15% of Streptococcus spp.5% of K. edwardsii, 10% of L. acidophilus, 30% of Shigella spp. 5% of P. putida, 5% of P. fluoresens, 15% of E. coli, while that of fungi in figure 6, 16% of Aspergillus niger, 21% of Penicillum oxalicum, 21% of Candida albicans, 32% of Saccharomyces cerevisiae. Figure 4, the percentage occurrence of bacteria isolated from the red cheese were recorded to be 5% of E. feacalis, 10% of B. cereus, 15% of Streptococcus spp. 35% of Shigella spp. 10% of S. aureus, 10% of P. putida, while that of fungi was recorded to be 20% of A. niger, 10% of Rhizopus nigricans, 10% of P. oxalicum, 20% of C. albicans, 30% of S. cerevisiae as presented in Figure 6. The percentage occurrences of all the bacteria isolated from white and red cheese was compared in Figure 5. The result of comparison reflected 15% of Streptococcus spp. in white and red cheese, 5% of E. feacalis in white and red cheese, 5% of K. edwardsii in white and red cheese, 10% of L. acidophilus present in white cheese and absent in red cheese, 10% of B. cereus present in white and red cheese, 30% of Shigella spp. present in white cheese and 35% present in red cheese respectively, 5% of P. putida found in white cheese and 10% found in red cheese , 5% of P. fluoresens present in white cheese and absent in red cheese, 15% of E. coli present in white cheese but absent in red cheese, 10% of S. aureus was present in red cheese but absent in white cheese. The occurrence of bacteria isolated from cheese was in consonance with a study done by Ogbolu et al., (2011) and the fungi isolated were also in line with the study. The reason could be attributed to poor environmental condition, handling and poor personal hygiene of sellers as observed during the surveillance study and also during the period of sample collection. All the bacterial species encountered in the cheese samples can affect the keeping quality or the health of the consumers (Willey et al., 2011). S. aureus are associated specifically with the hands and nasal cavity. The deposition of these microorganisms in cheeses can occur if good sanitary practices are not followed by the food handlers. Growth of microorganisms on food can lead to its decomposition and spoilage. Pathogenic microbes may result

in the transmission of diseases. Jawadi et al., (2017) isolated B. cereus from local Yemeni cheese. B. cereus could come from soil dust since large number of Bacillus spp. occur in the soil. B. cereus can cause spoilage of dairy product due to its phospholipinase activity (Pelczar et al., 2005). The fungal species encountered in the cheese (A. niger, S. cerevisiae, and Candida sp.) was in conformance with a study done by Sule et al., (2015) in Ilorin Metropolis which could also come from the environment, production process, and the food handlers. S. cerevisiae occurred in the cheese samples than the other fungi and this may be due to the fact that they are one of the most dominant organisms found in curdled milk. C. albicans could come from the female handler's genital tract if their hands are not properly washed after urination. The Isolation of S. aureus, B. cereus, E. coli, S. cerevisae, C. albicans corroborate the findings of Tekinsen and Ozdemir (2006) in which these organisms were found in local vended cheese isolated from Abeokuta. According to Raheem (2006), the microbial quality of raw milk is crucial for the production of any high quality dairy food. It could also be as result of unsanitary conditions by the producers and vendors during processing and handling of the cheese. Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with foods and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer (Omemu and Aderoju, 2008). Contamination of foods could have resulted from inappropriate processing, incomplete heating, or secondary contamination via contact with contaminated equipment and utensils. Although in this study, Salmonella spp was not isolated, nevertheless the presence of E. coli and other Enterobacteria such as Shigella spp. is an indication of possible faecal contamination of food, water and poor hygienic processing practices (Little et al., 2007).; (Tambekar et al., 2007; EFSA,2007) In addition, the presence of S. aureus, an enterotoxin producer which can cause serious gastroenteritis (Balaban and Rasooly, 2000) and Pseudomonas putida, an opportunistic pathogen, is known to cause food spoilage and can lead to economic loss (Liao and Tai, 2006) must be of outmost concern.

Table 2: CHEESE ISOLATION AND CHARACTERIZATION OF BACTERIA, COLONY COUNT AND ANTIBIOTIC SENSITIVITY TEST

ANTIBIOTICS	***Shigella spp***	***Staphylococcus aureus***	***Streptococcus spp***	***Enterococcus feacalis***	***Escherichia coli***	***Pseudomonas putida***	***Klebsiella edwardsii***	***Bacillus cereus***	**Proteus vulgaris Zone size (mm)**	**% R**	**% S**
Tarvid (OFX)	R	R	S	S	R	R	S	S			
Reflacine (PEF)	S	S	S	S	S	R	S	S			
Augumetin (AU)	S	S	S	R	S	R	S	R			
Gentamycin (CN)	R	S	S	S	R	S	S	S			
Streptomycin (S)	R	R	S	R	S	R	R	R			
Ceporex (CEP)	S	R	S	S	S	R	S	S			
Nalidixic acid (NA)	R	S	S	S	R	R	S	S			
Septrin (SXT)	R	S	S	R	R	R	S	R			
Amplicin (PN)	R	R	S	R	R	R	S	R			
Ciproflox (CPX)	R	S	S	R	R	R	S	R			
Norfloxacin (NB)	R	R	R	R	R	R	R	R			
Amoxil (AML)	R	S	S	R	R	S	S	R			
Rifampicin (RD)	R	R	S	R	R	S	S	R			
Erythromycin (E)	S	S	S	S	S	S	S	S			
Chloramphenicol (CH)	R	S	S	S	R	R	S	S			
Ampliclox (APX)	R S	R S	S S	R S	R S	R R	S S	R S			
Levofloxacin (LEV)											

V. Conclusion

Based on the findings on this study, it is revealed that most of the cheese samples obtained from the different markets are not fit for human consumption since they have been contaminated by disease causing bacteria and fungi. This study revealed that the red cheese has lesser microorganisms present than the white cheese which is as a result of the "sorghum bicolar" used in coloring the cheese because it has antimicrobial properties which kills some certain organism. It is important to keep cheese fit for consumption by taking adequate measures to prevent contamination during and after production of cheese. Therefore, cheese vendors should be educated on the health effects of lack of personal hygiene, environmental hygiene and good handling practices especially hand-washing practices. 4.

VI. Recommendations

The following recommendations would help to maintain the keeping quality of cheese. There is need to improve on the hygienic conditions surrounding cheese production. Pre-production and post- production operations should be adequately checked. Care should be taken not to use contaminated water in the course of production; and contact with soil and dust should be avoided as much as possible. Clean bowls with covers should be used to keep the cheese and spoons should be used for the selection of cheese for the consumers. Healthy cows should be used for milking. Food handlers with open wound should not be involved either in the production or sales of cheese. Utensils used for preparation of cheese should be washed well after each use to prevent contamination.

References

[1]. Adetunji VO, Chen J (2011). Effect of temperature and modified vacuum packaging on microbial quality of wara a West African soft cheese. Research Journal of Microbioogy. 6(4):402- 409.
[2]. Balaban N, Rasooly A (2000). Staphylococcal enterotoxins. International Journal of Food Microbiology, 61(1):1-10.
[3]. Bamidele R (2006). Developments and microbiological applications in African foods-emphasis on Nigerian wara cheese. Nigerian food journal, 24(1):13-17.
[4]. Chikpah SK, Teye GA, Teye M, Mawuli FF (2014). Effects of different concentrations of fresh and dried Calotropis procera (Sodom apple) extract on cow milk coagulating time, cheese yield and organoleptic properties of West African soft cheese (WAGASHIE). European Scientific Journal, 10 (27):317-326.
[5]. European Food Safety Authority (EFSA) (2007). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. The EFSA Journal, 130, 10-310.
[6]. Jawadi AH, Addar AM, Alazzam AS, Alrabieah, FO, Al Alsheikh AS, Amer RR, Aldrees AAS, Al Turki MA, Osman AK, Badri M (2017).Prevalence of dietary supplements use among gymnasium users. Journal of Nutrition and Metabolism, 2017: Article ID 9219361, 8 pages, https://doi.org/10.1155/2017/921936
[7]. Journal of Antimicrobial Chemotherapy, Volume 62, Issue 2, 1 August 2008, Pages 256–278, https://doi.org/10.1093/jac/dkn194
[8]. Law BA, Tamime AY (2010). Technology of Cheese making, Second Edition, Print ISBN: 9781405182980; doi:10.1002/9781444323740. Blackwell Publishing Ltd.
[9]. Liao W-C, Tai, W-T (2006). Organizational justice, motivation to learn, and training outcomes. Social Behavior and Personality-an international journal, 34:545-556.
[10]. Little AC, Jones, BC, Burriss RB (2007). Preferences for masculinity in male bodies change across the menstrual cycle. Hormones and Behavior, 51:633-639
[11]. Oladipo IC, Jadesimi PD (2012): Microbiological analysis and nutritional evaluation of West African soft cheese (wara) produced with different preservatives. American Journal of Food and Nutrition, 3(1):13-21.
[12]. Ogbolu DO, Daini OA, Ogunledun A, Alli AO, Webber MA (2011). High levels of multidrug resistance in clinical isolates of Gram-negative pathogens from Nigeria. International journal of antimicrobial agents, 37(1):62-66.
[13]. Omemu AM, Aderoju ST (2008). Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. Food Control, 19:396-402.
[14]. Omotosho OE, Oboh G, and Iweala EEJ (2011). Comparative effect of local coagulants on the nutritive values, in vitro multi enzyme protein digestibility and sensory properties of wara cheese. International Journal of Dairy Science, 6(1):58-65.
[15]. Pelczar, M.J, Chan, E.C.S, and Noel, R.K.C, (2005). Microbiology (5th ed.) Tata Mc Graw Hill. New Delhi, 571
[16]. Raheem . B (2006). Developments and microbiological applications in African foods: emphasis on Nigerian Wara cheese. Academic dissertation, University of Helsinki, Finland. pp 45.
[17]. Sangoyomi TE, Owoseni AA, Okerokun O (2010). Prevalence of enteropathogenic and lactic acid bacteria species in wara: A local cheese from Nigeria. African Journal of Microbiology Research, vol. 4(15):1624-1630.
[18]. Sule HA, Akor JA, Toluhi OJ, Suleiman RO, Akpihi L, Ali OU (2015). Impact of sex education in Kogi State, Nigeria. Journal of Education and Practice, 6(3):34-41.
[19]. Tambekar DH, Shirsat SD, Suradkar SB, Rajankar PN, and Banginwar YS. (2007). Prevention of transmission of infectious disease: studies on hand hygiene in health-care among students. Continental Journal of Biomedical Sciences, 1:6-10.
[20]. Tekinsen K, Ozdemir Z (2006). Prevalence of microbiological and compositional status of Turkish foodborne pathogens in Turkish Van otlu (Herb) white cheese. Food Control, 17:707-711.
[21]. WHO (2000)."Cholera",Factsheetno.107.http://www.who.int/mediacentrefactsheets/fs107/en/Ac cessed on 9/10/2012
[22]. Willey, J. M., L. M. Sherwood, Woolverton, C. J. (2011). Prescott Microbiology. 8th ed. McGraw-Hill Companies, New York, p. 1070.