

MICROBIOLOGICAL QUALITY ASSESSMENT OF SOME COMMERCIALY PRODUCED YOGHURT RETAILED IN ILORIN, NORTH CENTRAL NIGERIA.

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Abstract

Quality of processed food is one of delicate subject being considered all over the world. The present study is mainly concerned with the physicochemical and microbiological analysis of commercially produced yoghurt retailed in Ilorin, North Central part of Nigeria. Nine samples of these commercial yoghurt were collected from the metropolis and were analyzed. The pH, titratable acidity and microbiological analysis were carried out to ascertain the presences of microbiological contaminant including bacteria and fungi. The mean pH is 4.3 and the titratable acidity ranges from 1.30 – 2.30. The organisms detected includes *Aerobacter aerogenes*, *Pseudomonas fluorescens*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bacillus subtilis* and *Staphylococcus aureus*, while the fungi species includes *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Mucor racemosa*, *Rhizopus stolonifer* and *Penicillium italicum*. The study revealed the presence of pathogenic organisms in the collected samples which poses danger to the consumers. The attention of regulatory body is needed to bring sanity in the production, distribution and retailing process to eliminate contamination.

Keywords: Fermented milk, lactic acid bacteria, contaminantion, fungi, pH.

Introduction

Milk and milk products serves as important food substances for all age group due to the quantity of nutrients they contain (Torres-Vitela *et al.*, 2012). Consumption of fermented milk by man dates back to the beginning of civilization since the residues were found in pottery fragments from Neolithic Bronze and Iron Ages settlements in Britain (McKinley, 2005). It is generally accepted that the consumption of fermented or cultured milk products such as yoghurt, cheese and buttermilk are effective means of prolonging the shelf-life of milk (Tamine and Robinson, 2007).

Yoghurt has traditionally been considered a probiotic carrier food with health promoting ability which is derived from the action of bacteria on all or parts of the lactose to produce lactic acid, carbondioxide, acetic acid, diacetyl, acetaldehyde and several other components that gives the product its characteristic fresh taste (Tamine and Robinson, 2004). It is a ready to drink food commonly taken for energy production, a product made from heat treated milk that may be homogenized prior to the addition of lactic acid bacteria (LAB) cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Code of Federal Regulations, 2011), it is among the most common dairy products consumed around the world (Tamine, 2002; Saint-Eve *et al.*, 2006) and is consumed both as a food and as a thirst quenching beverage (Alfa-Lawal, 1984).

Yogurt has also been described as a notoriously balanced food containing almost the nutrients present in milk but in a more assimilable form and they can be produced from skimmed or whole milk and there is a large range of flavors available commercially (Oyeleke *et al.*, 2009). As the popularity of yogurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers (Allgeyer *et al.*, 2010).

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Science Focus: An International Journal of Biological and Physical Sciences; www.sciencefocus.com.ng

It is the fermentation of lactose (milk sugar) into lactic acid that gives yoghurt with its gel-like texture and characteristic tang (Fiszman *et al.*; 2001). Yoghurt has also been described as a balanced food containing almost all the nutrients present in milk but in a more assimilable form and they can be produced from skimmed or whole milk and there is a large range of flavours available commercially (Anthar, 1986).

Due to high nutritional constituents of yoghurt, they serve as a good breeding medium for microorganisms, hence, the utilization of such nutrients by the microorganisms which result in the deterioration of yoghurt and according to Salle (1993) deterioration involve change which render food unpalatable, unwholesome and unattractive. One of the important factors through food manufacturing is hygienic management, thus food manufacturers proof their hygienic activities by taking certification like a hazard Analysis and critical control point (HACCP). Yoghurt can be produced from the milk of cow, sheep, goat or buffalo. In Nigeria, cow's milk is usually used for the production of yoghurt (Igwe, 2002).

This project research is aimed at investigating the microbiological quality of some commercial yoghurt available in the markets in Ilorin metropolis, North central part of Nigeria due to large scale consumption milk and milk products, considering the level of microbial contamination.

MATERIALS AND METHODS

Study area and Sample Collection

This research was carried out in Ilorin, North central part of Nigeria. Nine samples of commercially packed sachet yoghurt were purchased from vendors from different parts of the metropolis. They were collected in triplicates and were transported in ice pack to the laboratory for analysis. All manufacturer details such as National agency for food and drugs administration control (NAFDAC) registration number, manufactured date, batch number, expiry date and manufacturer address were taken note of.

Microbiological analysis

Four culture media were used for microbiological analysis of the yoghurt samples. These includes de Mann Rogosa and Sharpe medium (MRS), MacConkey agar, Nutrient agar and Potato dextrose agar (PDA). All the media are of analytical standard and are prepared according to manufacturer instructions. The samples were transferred into peptone water and homogenized from where serial decimal dilution of the samples were carried out. The serially diluted samples were inoculated unto plates of different media using pour plate technique. MRS agar plate was incubated anaerobically for 48hrs, MacConkey and nutrients agar were incubated at 37°C for 24hrs while potato dextrose agar was incubated for 72hrs at 32°C.

pH analysis

The pH of the samples were determined at 20°C using digital pH meter after calibration with freshly prepared pH standard buffers of 5.0 and 9.0.

RESULTS

All the yoghurt samples were checked for their manufacturer information which includes National Food Drugs Administration and Control (NAFDAC) registration number, manufacturing and expiry date, batch number and manufacturer address. They were also checked for their wholesome status.

The pH and Titratable acidity (TTA) acidity of the yoghurt samples were shown in Table 1, in which there are significant differences in the sample tested for the pH and TTA.

Table 2 shows the microbial count of isolates from different brand of commercial yoghurt retailed in Ilorin metropolis. The microbial count ranges from 1.35×10^8 to 2.52×10^8 . Table 3, shows the frequency distribution of isolates from retailed yoghurt in Ilorin metropolis in which seven bacterial isolates and six fungi isolate were obtained from the ten samples. These are *Aerobacter aerogenes*, *Pseudomonas flourescens*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bacillus cereus* and *Staphylococcus aureus* *Aspergillus niger*, *Aspergillus flavus*, *Penicillium italicum*, *Saccharomyces cerevisiae*, *Mucor racemosa* and

Rhizopus stolonifer, *Lactobacillus acidophilus* 8(12.9%) were the most frequently isolated followed by *Aspergillus niger* 7(11.3%), *Lactobacillus bulgaricus* 7(11.3%), *Streptococcus thermophilus* 6(9.7%), *Penicillium italicum* 5(8.1%), *Mucor racemosa* 4(6.5%), *Saccharomyces cerevisiae* 4(6.5%) *Rhizopus stolonifer* 4(6.5%), *Aspergillus flavus* 4(6.5%), *Staphylococcus aureus* 4(6.5%), *Pseudomonas fluorescens* 4(6.5%), *Aerobacter aerogenes* 3(4.8%) and the least being *Bacillus cereus* 2(3.2%). The total microbial count of the yoghurt samples ranged from 1.35×10^8 cfu/ml for Winners yoghurt being the least to 2.52×10^8 cfu/ml for FanYogo yoghurt as the one having the highest bacterial count. The total fungi count of the yoghurt samples ranged from 2.35×10^7 cfu/ml for FanYogo being the least to 4.20×10^7 cfu/ml for MM as the highest fungal count. All these were shown in table 3. The assessment of the diversity of microflora available in commercially prepared yoghurt around Ilorin metropolis, North Central Nigeria revealed a lot of facts about the retailed products and their consequent implication on human health. The pH of the yoghurt samples as revealed in the research ranges from 3.50 – 4.99 with the average of 4.28 which agrees partly with the findings of Tamime (2002) and Ifeanyi *et al.*, 2013 who claimed that yoghurt's pH is less than or equal to 4.6. The variation may be as a result of inadequate addition of the starter culture. The titratable acidity of the samples ranges from 1.30 – 2.30, this differences could be due to variation in the growth of lactic acid bacteria during fermentation (James, 2003). The total bacteria count of the samples ranges from 1.35×10^8 to 2.52×10^8 cfu/ml while the fungi count ranges from 2.35×10^7 to 4.20×10^7 cfu/ml. This microbial count were higher than the findings of Khan *et al.*, 2008 whose total viable count ranged from 1.3×10^4 to 7.0×10^7 cfu/ml, Oyeleke, 2009, who recorded microbial count of 1.0×10^7 to 9.4×10^7 cfu/ml and Nwagu and Amadi, 2010 whose mean viable counts were from 1.4×10^6 to 2.2×10^7 cfu/ml.

Discussion

The disparity in mean value of total viable count of bacteria is a reflection of variation in manufacturing practices and preservation protocols which may be as a result of the distribution and handling of the products by the vendors who usually transport and preserved them above the recommended 4°C due to epileptic nature of power supply in the country couple with the attendant high cost of alternative power supply which makes the organisms to continually multiply. Inability to store the product at the recommended temperature is also a vehicle of addition of contaminants into the products which confirmed the claim of the findings of Moreira *et al.*, (2001) who reported that warmer weather and inadequate refrigeration are the principal causes of higher levels of contamination, increased species diversity and alteration in microbial flora. Cousin, 1982 noted that the presence of spoilage bacteria can have considerable negative effects on the quality of milk and dairy products while the presence of pathogens can have more severe repercussions on human health(Quigley *et al.*, 2011)

A total of thirteen (13) different organisms were isolated, out of which seven (7) were bacteria and six (6) fungi. These include *Aerobacter aerogenes*, *Pseudomonas fluorescens*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bacillus subtilis* and *Staphylococcus aureus* while the fungi species are *Aspergillus niger*, *Aspergillus flavus*, *Penicillium italicum*, *Saccharomyces cerevisiae*, *Mucor racemosa* and *Rhizopus stolonifer*. The presence of the microorganisms in the yoghurt could be due to the availability of nutrients required which include sugar and other nutrient source such as protein, amino acids, minerals and vitamin C (Ihekoronye *et al.*, 1985).

The samples shelf-life is also a factor of the products which maybe as a result of inappropriate keeping conditions the starter cultures that tends to increase their development provoking high acidity and consequently the killing of themselves (Tamime and Robinson, 2007).

The presence of *L. bulgaricus* and *S. thermophilus* in the samples agrees with the claim that they are commonly used in yoghurt production (Perdigan *et al.*, 1995). Also the presence of *saccharomyces* spp agrees with Trema and Musa, 1998 that says it helps in fermentation of milk to produce yoghurt while *Lactobacillus* spp. are responsible for flavor and aroma development. Other species of fungi present in the yoghurt sample also agrees with Oyeleke, 2008 and Foschino *et al.*, 1993 that moulds are the primary contaminant of yoghurt. The reason for this is not far fetched, low level of hygiene of the handlers and vendors coupled with constant exposure of the product at road side contribute greatly to this. The presence of *S. aureus* and *B. subtilis* may be as a result of bad production practices or post production contamination (Tamime and Robinson, 2007). Due to standardization and enlightenment by NAFDAC, the general consumers are getting more aware of quality and hygienic nature of processed product they consumed. Umol *et al.*, (1990) reported that dairy foods are frequently contaminated with *staphylococcus* and cases of *staphylococcal* contamination in milk and milk product have been recorded. *Staphylococcus aureus* is responsible for food poisoning as a result of food intoxication (Ahmed *et al.*, 2009). Enterotoxin production by *S. aureus* is promoted by the presence of starch and protein (Wistreich and Lechtman, 1980). Oyeleke, 2009 reported that contaminants of yoghurt includes *Bacillus* sp, *Staphylococcus* sp. and *Pseudomonas* sp. which were in support of this work.

Table 1: Physicochemical Characteristics of Different Brands of Yoghurt in Ilorin Metropolis

Name of Sample	pH Determination	Titrateable Acidity
Abide yoghurt	4.99	1.40
VanVanilla yoghurt	4.21	1.30
Savanna yoghurt	3.53	2.10
MM yoghurt	4.43	1.60
Winners yoghurt	4.45	1.30
SuperYogo yoghurt	4.36	2.20
Smoozy yoghurt	3.50	1.40
Abarim yoghurt	4.31	2.30
FanYogo yoghurt	4.72	1.50

Table 2: The Microbial Count of Different Brands of yoghurt obtained from Ilorin Metropolis

Sample	Bacterial Count (cfu/ml) x 10 ⁻⁸	Fungi Count (cfu/ml) x 10 ⁻⁷
Abide	1.45 ± 0.35	2.85 ± 0.36
VanVanilla	2.20 ± 0.39	2.85 ± 0.35
Savanna	1.40 ± 0.40	2.50 ± 0.40
MM	1.50 ± 0.42	4.20 ± 0.42
Winners	1.35 ± 0.30	3.43 ± 0.33
SuperYogo	2.34 ± 0.31	2.68 ± 0.31
Smoozy	1.38 ± 0.41	3.55 ± 0.44
Abarim	2.44 ± 0.34	2.43 ± 0.32
FanYogo	2.52 ± 0.45	2.35 ± 0.30

The table above is the mean of all the samples and the standard deviation.

Table 3. Frequency distribution of isolates from commercial yoghurts in Ilorin, Nigeria.

Isolates	Number of isolates(%)
<i>Aerobacter aerogenes</i>	3(4.8)
<i>Pseudomonas fluorescens</i>	4(6.5)
<i>Lactobacillus bulgaricus</i>	7(11.3)
<i>Streptococcus thermophilus</i>	6(9.7)
<i>Lactobacillus acidophilus</i>	8(12.9)
<i>Bacillus subtilis</i>	2(3.2)
<i>Staphylococcus aureus</i>	4(6.5)
<i>Aspergillus niger</i>	7(11.3)
<i>Aspergillus flavus</i>	4(6.5)
<i>Saccharomyces cerevisiae</i>	4(6.5)
<i>Mucor racemosa</i>	4(6.5)
<i>Rhizopus stolonifer</i>	4(6.5)
<i>Penicillium italicum</i>	5(8.1)
Total	62(100)

Conclusion

This research revealed that the commercial yoghurt sampled contained arrays of microorganism in which some causes food spoilage while the others are pathogenic which have adverse effects on consumers causing health hazard. The presence of these microorganisms are indicative of bad production practices and post production contamination by the handlers and transportation. Contamination of this products need to be checked by the regulatory authority to safeguard the health of the consumers. Also the handlers and vendors of the finished products needs to be educated on measures to be taking to prevent the contamination of the products.

Acknowledgement

The authors wish to thank the technical staff of Department of Microbiology University of Ilorin for their support during the course of this research.

References

- Ahmed, K., Hussain, A., Qazalbash, M. A. and Hussain, W. 2009. Microbiological quality of ice cream sold in Gilgit Town. *Pakistan Journal of Nutrition* 9 : 1397-1400.
- Alfa-Lawal, A.B. 1994. Dairy and Food Engineering. Division Lund-Sweden. P. 177.
- Allgeyer, L., Miller, M. and Lee, S. 2010. Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. *Journal of Dairy Science* 93: 4471-4479.
- Anthar, I. H. 1986. Preparation of Cheese and Yoghurt at Household Level. Pakistan Agricultural Research Council, Islamabad, p390 – 391.
- Chaudhary, A.K., Gupta, L. K., Gupta J.K. and Bunnerjee, U. C. 1997. *Journal of Industrial Microbiology and Biotechnology* 61: 348 – 352.
- Code of Federal Regulations. 2011. Low fat yogurt. Title 21, Chapter I, Subchapter B, Part 131, subpart b, 131.203.
- Çon, A.H. , Çakmakçı, A., Çalar A. and Gökalp, H.Y. 1996. Effects of different fruits and storage periods on microbiological qualities of fruit-flavored yogurt produced in Turkey *Journal of Food Protection* 6: 402-406.
- Cousin, M.A. 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. *Journal of Food Protection* 172-207.
- Fiszman, S.M., Lluch, M.A. and Salvador, A. 2001. Effect of gelatin on microstructure of acidic milk gels and yoghurt and on their rheological properties. *International Journal of Dairy Science* 11:985 – 903.
- Foschino, R., Garzaroli, C. and Ottogalli, G. 1993. Microbial contaminants cause swelling and inward collapse of Yoghurt packs. *Lait* 73:395-400.
- Igwe, E. C. 2002. The adoption of appropriate technology for dairy processing by Fulani nomads in Nigeria. *Journal of Nomadic studies*. 5: 61 – 66.
- Ihekoronye, A. I. and Nyoggy, P.O. 1985. Integrated Food Science and Technology for the Tropics. Macmillan Publisher Ltd., London and Basin Stoke p. 293 – 311.
- James, M.J. 2003. *Modern Food Microbiology*. 4th edn. Newage International Publishers Ltd, New Delhi, India. Pp. 224 – 248.
- Khan, M.T., Hussain, M., Wajid, A. and Rasool, S. A. 2008. Microbial population and enzyme production of indigenously isolated yeast. *Pakistan Journal of Botany* 40: 2225-2230.
- Moreira, S. R., Schwan, R. F., Carvalho, E. P. and Ferreira, C. 1999. Análise microbiológica e química de iogurtes comercializados em Lavras, MG. *Ciênc. Tecnol. Alim.* 19: 147-152.
- Moreira, S. R., Schwan, R. F., de Carvalho, E.P. and, Wheals, A. E. 2001. Isolation and identification of yeasts and filamentous fungi from yoghurts in Brazil. *Brazilian Journal of Microbiology* 32: 117-122.
- McKinley M.C. 2005. The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology* 58: 01-12.
- Nogueira, C., Albano, H., Gibbs, P. and Teixeira, P. 1998. Microbiological quality of Portuguese yogurts. *Journal of Industrial Microbiology Biotechnology* 21:19-21.
- Nwagu, T. N. and Amadi, E. C. 2010. Bacteria population of some commercially prepared yoghurt sold in Enugu State, Eastern Nigeria. *African Journal of Microbiology Research*, 4: 984-988.
- Oyeleke, S.B. 2009. Microbial assessment of some commercially prepared yoghurt retailed in Minna, Niger State. *African Journal of Microbiology Research* 3: 245-248.
- Oyeleke, S. B. and Manga, S. B. 2008. *Essentials of Laboratory Practical in Microbiology*. Tobest Publisher, Minna, Nigeria. Pp. 36-75.
- Perdigon, G., Alvarez, S., Rachid, M., Agüero, G. and Gobbato, N. 1999. Immune System Stimulation by Probiotics. *Journal of Dairy Science* 78:1597-1606.
- Quigley, L., O'Sullivan, O., Beresford T. P., Ross, R. P., Fitzgerald, G. F. and Cotter, P. D 2011. Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. *International Journal of Food Microbiology* 150: 81
- Rodrigues, L.A., Ortolani M. B. T and Nero L. A. 2010. Microbiological quality of yogurt commercialized in Viçosa, Minas Gerais, Brazil. *African Journal of Microbiology Research*. 4: 210-213.
- Salle, A.J. 1983. Bacteriology of water. In: *Fundamental Principle of Bacteriology*. 7th edition. McGraw Hill Book and Co. 687 – 710.
- Saint-Eve, A. Levy, C. Martin, N. and Souchon, I. 2006. Influence of proteins on the perception of flavored stirred yogurts. *Journal of Dairy Science* 89: 922-933.
- Tamine, A.Y. and Robinson, K. 2004. *Yoghurt Science and Technology*. Published by Institute of Applied Science. pp 32 – 56.
- Tamine, A. Y. and Robinson, R. K. 2007. *Yoghurt: Science and Technology*. 3rd ed. Cambridge, Woodhead Publishing Limited; 2007:808.
- Tamine, A. Y. 2002. *Fermented milks: a historical food with modern applications - a review* *European Journal of Clinical Nutrition* 56: 01-15.
- Tamine, A.Y. and Robinson, K. 2004. *Yoghurt Science and Technology*. Published by Institute of Applied Science. p32 – 56.

- Trema, A and Musa, A. 1998. Soybeans yogurt production using starter culture from nono. *Nigerian Journal of Biotechnology* 9: 17-22.
- Tamine.A.Y, and Robinson, R .K. 2007. *Yoghurt: Science and Technology*. 3rd ed. Cambridge, Woodhead Publishing Limited p. 808
- Torres-Vitela, M. R., Mendoza-Bernardo, M., Castro-Rosas, J., Gomez-Aldapa, C. A., Garay-Martinez, L.E., Navarro-Hidalgo, V. and Villarruel-Lopez, A. 2012. Incidence of *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:h7 and *Staphylococcal enterotoxin* in two types of Mexican fresh cheese. *Journal of Food protection* 75: 79-84.
- Umoh, V 1989. Contamination of 'fure da nono' by *staphylococcus aureus* in fura. *A cereal food*. *Zaria Veterinary* 4: 53-58.
- Wistreich, C. A. and Lechtman, M. D 1980. *Microbiology*. 3rd Ed. Glencoe Publishing Company 280pp.