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Microbial Assessment and Microbiological Quality of Some Commercially Prepared Yoghurt Retailed in Ibadan, Oyo State, Southwestern Nigeria

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Abstract: Yogurts are ready to drink foods commonly taken for energy production and for health in Nigeria but there is paucity of studies done to evaluate their food safety. Therefore this study was carried out to determine the microflora of some available yogurts sold in Ibadan. Twenty types of commercially prepared yogurt products were purchased, from Ibadan in Oyo State, Nigeria and its' environs, transported, processed and analyzed using standard laboratory methods. A total of 25 different organisms were isolated from 20 yogurt samples with *Lactobacillus bulgaricus*, *Streptococcus lactis* and *Saccharomyces cerevisiae* each being the most frequently isolated with frequency of 16.0%. They were also tested to show if their pH production was lactose-dependent. There were significant decline in pH in tryptone soy broth ($t = -13.88$, $p<0.05$), peptone with lactose ($t = -16.61$, $p<0.05$), and peptone containing milk and lactose ($t = -10.41$, $p<0.05$). This study has shown that most yogurts in Ibadan contain probiotics isolates including *L. bulgaricus*, *S. lactis* and *S. cerevisiae*, which are therefore, beneficial for human consumption.

Key words: Nigeria, pH production, *L. bulgaricus*, *S. lactis*, *S. cerevisiae*, yogurts

INTRODUCTION

Yogurt is a cultured dairy product produced by lactic acid fermentation of milk and is also a means of preserving the nutrients in milk (Hui, 1992; Oyeleke *et al*., 2009). It is generally known as cultured milk, as it is derived from the action of bacterial on all or part of the lactose to produce lactic acid, carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other components that gives the product its characteristic fresh taste (Tamine and Robinson, 2004). It is produced by the lactic fermentation of milk using a combination of bacteria such *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Hui, 1992) 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 (Anthar, 1986; Oyeleke *et al*., 2009).

Fermented milks, like the fresh milk from which they are produced, are liable to contamination. Moulds and yeast are the primary contaminants in yogurt produced commercially in Nigeria (Suriyarachchi and Fleet, 1981; Oyeleke *et al*., 2009). Moulds and yeasts growing in yogurt utilize some of the acid and produce a corresponding decrease in the acidity, which may favor the growth of putrefactive bacteria (Oyeleke *et al*., 2009). Moulds and yeast are the primary contaminants in yogurt produced commercially in Nigeria (Suriyarachchi and Fleet, 1981; Oyeleke *et al*., 2009). Moulds and yeasts growing in yogurt utilize some of the acid and produce a corresponding decrease in the acidity, which may favor the growth of putrefactive bacteria (Oyeleke *et al*., 2009). Yogurts are ready to drink foods commonly taken for energy production and for health in Nigeria but there is paucity of studies done to evaluate their food safety. The general objective of this study is to assess the microbiological quality of some commercially available yogurt retailed in Ibadan, Oyo State, Southwesten, Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out in Ibadan, Oyo State, Southwestern Nigeria from January 2009 to December 2009. Ibadan city lies 3º5′ E and 7º23′ N. The city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and

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improper management of wastes especially in the indigenous core areas characterized by high density and low income populations.

Sample collection: Commercially prepared yogurt products packaged in plastic containers were purchased from retailers in Ibadan, Oyo State, Nigeria and its' environs. Twenty pieces of different types of yogurt were purchased in duplicate. The information, on their claim forms were recorded e.g., National Agency for Food, Drug, Administration and Control, NAFDAC registration number, manufactured and expiry date, address of producers, batch number, starter culture and pasteurization status.

Isolation, characterization and identification of isolates: De Man Rogosa and Sharpe (MRS) medium, Blood Agar, Nutrient agar, and Brain heart infusion broth, were prepared according to the manufacturer's instruction. The samples of the yogurt products were shaken vigorously to suspend the microbial content. These samples were separately inoculated into different culture media, which include Blood agar, Chocolate agar, McConkey agar, and Brain heart infusion broth. Cultures on the brain heart infusion broth were subcultured into Chocolate agar after 24 h of aerobic incubation. They were also inoculated into De Man Rogosa and Sharpe (MRS) medium in order to aid the growth of *Lactobacillus* species. The plates were incubated aerobically at 37ºC for 72 h, except for Chocolate agar plates, which were incubated in a microaerophilic condition with 10% CO_2 for 72 h. Stock cultures of the isolates, were maintained in MRS medium at 4ºC. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and biochemical tests. The isolates were identified by comparing their characteristics with those of known taxa, as described by Cheesbrough (2003) and Oyeleke and Manga (2008).

Test for pH change: The isolates on stock cultures were inoculated into Brain Heart Infusion Broth (BHIB). The turbidity of the broth culture was then adjusted to equal that of #1 MacFarland standard. This test was to determine if pH change was dependent on the presence of sugar. BHIB, peptone broth containing 5% lactose as the only sugar, and "milk and lactose" medium were prepared according to manufacturer's instruction. The pH of all medium was measured after preparation. Twenty-five bottles of each medium was prepared and inoculated with each isolates. The cultures were incubated aerobically at 37ºC for 24 h and the pH was measured using multisticks.

RESULTS

Table 1 showed the results of evaluation of yogurt companies with manufacturing ethics. The table showed that 3(15.0%) out of 20 different yogurts had no NAFDAC's registration number while all (100.0%) indicated manufacturing and expiry dates, and 3(15.0%) do not have batch number. The manufacturer's address was not indicated in 2(10.0%) of the 20 different yogurts.

Table 1: Yogurts company's information

Information	N	Yes (%)	No (%)
Nafdac number	20	17 (85.0)	3 (15.0)
Manufacturing date	20	20 (100.0)	0 (00.0)
Expiry date	20	20 (100.0)	0 (00.0)
Batch number	20	17 (85.0)	3 (15.0)
Manufacturing address	20	18 (90.0)	2 (10.0)

N: Number of yogurt brands

Table 2: Frequency distribution of isolates from yogurts in Ibadan, Nigeria

Isolates	Number of isolates (%)
Lactobacillus acidophilus	2 (8.0)
Lactobacillus bulgaricus	4 (16.0)
Lactobacillus cremoris	2 (8.0)
Lactobacillus fermentum	2 (8.0)
Lactobacillus lactis	1 (4.0)
Lactococcus mesenteroides	1 (4.0)
Micrococcus acidophilus	2 (8.0)
Rhodosporium spp	1 (4.0)
Saccharomyces bayanus	1 (4.0)
Saccharomyces cerevisiae	4 (16.0)
Streptococcus lactis	4 (16.0)
Streptococcus thermophilus	1 (4.0)
Total	25 (100.0)

Table 2 showed frequency distribution of isolates from yogurts in Ibadan and its environs. Twenty five (25) isolates comprising 12 different organisms were obtained from 20 yogurt samples, with *Lactobacillus bulgaricus* 4(16.0%), *Streptococcus lactis* 4(16.0%) and *Saccharomyces cerevisiae* 4(16.0%), being the most frequently isolated. This was followed by *Lactobacillus acicophilus* 2(8.0%), *Lactobacillus cremoris* 2(8.0%), *Lactobacillus fermentum* 2(8.0%), and *Micrococcus acidophilus* 2(8.0%). *Lactobacillus lactis* 1(4.0%), *Lactococcus mesenteroides* 1(4.0%), *Rhodosporium* spp 1(4.0%), *Saccharomyces bayanus* 1(4.0%), and *Streptococcus thermophilus* 1(4.0%) were the least frequently encountered organisms. Overall, the isolates includes organisms from the genus *Lactobacillus* (20.0%; n = 5 species), *Streptococcus* (8.0%; n = 2 species), *Saccharomyces* (8.0%; n = 2 species), *Micrococcus* species (4.0%; n = 1 specie), *Lactococcus* species (4.0%; n = 1 specie) *and Rhodosporium* species (4.0%; n = 1 specie).

Table 3 showed the probiotic content of yogurt in Ibadan, Nigeria. Most of the yogurt samples contained only one organism except Ivory and Milly products, which contained two organisms each and Star products, that contains three types of organisms.

Table 4 showed pH changes of the microbial isolates from yogurts in different media. There were significant decline in pH for all types of media inoculated as shown in the table. Also, significant decline in pH was observed with all the organisms used. Table 5 showed the analysis of the acid production of the isolates from yogurts in

Table 3: Microflora spectrum of yogurts samples in Ibadan, Nigeria

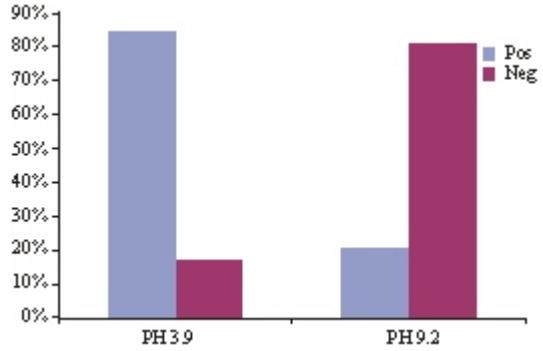
Yogurt	1st isolate	2nd isolate	3rd isolate
Fan	*L. lactis*	-	-
Meva	*S. thermophilus*	-	-
Star	*M. acidophilus*	*L. ceremonies*	*S. lactis*
Lino	*S. lactis*	-	-
Rubengo	*L. acidophilus*	-	-
Dammy	*S. bayanus*	-	-
Dandy	*L. bulgaricus*	-	-
Princess	*L. cremoris*	-	-
Miva	*S. cerevisiae*	-	-
Ivory	*L. bulgaricus*	*S. cerevisiae*	-
Milly	*L. fermentum*	*S. cerevisiae*	-
Cedar	*L. fermentum*	-	-
Lambak	*L. bulgaricus*	-	-
Honey	*L. mesenteroides*	-	-
Samal	L. bulgaricus	-	-
Queen	*S. lactis*	-	-
Peace & mercy	*M. acidophilus*	-	-
Temitope	L. acidophilus	-	-
Dereal	*S. lactis*	-	-
Rosefon	*S. cerevisiae*	*Rhodosporium* sp.	-

Table 4: pH changes of the microbial isolates from yogurts in different media

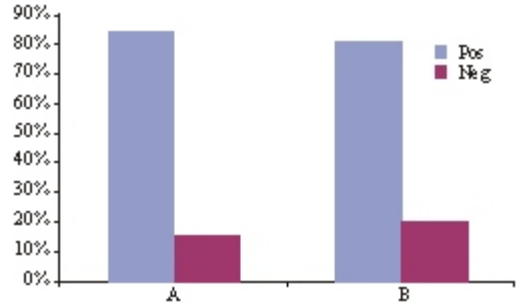
Isolates	TSB pH7.0	PWL pH7.0	MLM pH6.8
L. bulgaricus	6.5	<5.0	5.0
S. cerevisiae	6.5	5.0	5.5
L. fermentum	6.5	<5.0	5.0
S. cerevisiae	6.5	5.5	6.5
L. lactis	6.0	5.0	6.0
Rhodosporium spp	6.5	6.5	6.5
S. thermophilus	6.0	5.0	6.0
M. acidophilus	6.5	5.0	6.0
L. cremoris	6.5	6.0	6.0
S. lactis	6.0	<5.0	5.0
S. lactis	6.0	5.0	5.0
L. cremoris	6.5	6.0	6.0
L. bulgaricus	6.0	5.0	5.5
L. acidophilus	6.0	5.0	6.0
S. bayanus	6.5	5.0	6.0
L. fermentum	6.5	<5.0	5.0
L. bulgaricus	6.5	5.0	5.0
L. mesenteroides	6.5	6.0	6.0
L. bulgaricus	6.5	6.0	6.0
S. lactis	6.0	5.0	5.0
M. acidophilus	6.5	5.0	6.0
L. acidophilus	6.0	5.0	6.0
S. lactis	6.0	<5.0	5.0
S. cerevisiae	6.5	5.0	5.5
S. cerevisiae	6.5	5.5	6.5

TSB = Tryptone Soy Broth, PWL = Peptone with Lactose, MLM = Milk and Lactose Medium, MD = Mean deviation

relation to sugar content of the medium. It also showed that the final mean pH values in all the culture media were significantly lower than the initial pH values of the media before inoculating with the probiotic isolates; for tryptone soy broth ($t = -13.88$, $p<0.05$); for peptone with lactose only ($t = -16.61$, $p<0.05$) and for peptone with milk and lactose ($t = -10.41$, $p<0.05$).



Pos: Growth; Neg: No growth
Fig. 1: Bar chart of percentage growth of isolates at selected pH



A: Growth at 15ºC; B: Growth at 45ºC; Pos: Growth; Neg: No growth
Fig. 2: Bar chart of percentage growth of isolates at selected temperature

Figure 1 showed bar charts of percentages of isolates that grew at different pH. 84% of the isolates grew at pH 3.9 while 20% grew at pH 9.2.

Figure 2 showed bar charts of percentages of isolates that grew at different temperatures, respectively. Likewise, 84% of the isolates also grew at temperature of 15ºC, while 20% grew at 45ºC.

DISCUSSION

The study of the microflora of various available yogurts in Ibadan and it's environment and their implications on human health, showed a total of 25 different isolates of 12 species, with *Lactobacillus*

Table 5: Analysis of the acid production of the isolates from yogurts in relation to sugar content of the medium

MEDIUM	Total isolate number	Initial pH of medium	Mean final pH	M D	t-value	P Value
TSB	25	7.0	6.32±0.25	-0.68	-13.88	<0.05
PWL	25	7.0	5.16±0.55	-1.84	-16.61	<0.05
MLM	25	6.8	5.68±0.54	-1.12	-10.41	<0.05

TSB = Tryptone Soy Broth, PWL = Peptone with Lactose, MLM = Milk and Lactose Medium, MD = Mean Deviation

bulgaricus, *Streptococcus lactis* and *Saccharomyces cerevisae* having the highest number, 16.0% each, while *Lactobacillus acidophilus*, *Lactobacilus fermentum*, *Lactobacillus cereviase* and *Micrococcus acidophilus*, have 8.0% each, which agrees with the claims that they are the most commonly used organisms in the fermentation of milk into yogurt (Hilton *et al*., 1992; Perdigon *et al*., 1995; Bengmark, 1996; Mattila-Sandholm, 1999). Other organisms, which do not take prominent importance, which were isolated include *Lactobacillus lactis*, *Lactobacillus mesenteroides*, *Streptococcus thermophilus*, *Saccharomyces bayanus* which has also been quoted as probiotics (Bengmark, 1996), and *Rhodosporium* sp. all have 4% each. Most of the sampled yogurts had one isolate except for Ivory and Milly products which had two isolates as well as Star product that had three isolates. Also some of the yogurts producers did give adequate information about their products even though the probiotics present in the products were not specified. This could be associated with the good work of the regulatory agent involved although some were still found not to have the regulated number. The presence of *Lactobacillus* spp. is desirable as the first is responsible for flavour and aroma, while the latter is involved in the fermentation of milk for yoghurt production, as reported by Trema and Musa (1998). All the isolates have a favorable dominance against each potential pathogenic organisms of which they were challenged. This may be one of the reasons to support the claims of Barefoot and Klaenhammer (1983), Mel'nikova *et al.* (1993), Smirnov *et al.* (1993), which says they favorably alter intestinal microflora balance and inhibits the growth of harmful bacteria. It also agrees with the claims of Brashears and Durre (1999); that *Lactobacillus lactis* completely inhibits all pathogens at 37ºC in 24 h but not at refrigeration temperatures.

All the isolates show a considerable production of acid even without the presence of sugar (lactose), produce more with milk lactose medium and the highest production was observed with medium containing lactose only. The final mean pH values in all the culture media were significantly lower than the initial pH values of the media before inoculating with the probiotic isolates for tryptone soy broth ($t = -13.88$, $p<0.05$); for peptone with lactose only ($t = -16.61$, $p<0.05$) and for peptone with milk and lactose ($t = -10.41$, $p<0.05$); which agrees with Brashears and Durre (1999); that *L. lactis* produced significant reduction in pH in neutralized broth, (i.e., acid production), and that it was primarily responsible for inhibition properties. The pH of the acid they produced, were in the average pH of 5.5, which inhibit most potential pathogenic organisms (Chung and Geopfert, 1970; Juven, 1976). Also their pH values showed also correlates with the pH of acetic acid and lactic acid (Kawase, 1982; Rasic, 1983).

Eighty-four percent (84.0%) of the total isolates also grew at pH 3.9 which may indicate the reason why most of the yogurt microflora may survive the pH of the stomach. It should be noted that only *S. lactis* did not grow (i.e., negative) at pH 3.9 while *S. lactis* and *S. bayanus* grew (i.e., positive) at the alkaline pH of 9.2. This could be part of the reason for the pharmacokinetics of probiotics as described by Blum *et al.* (1999) and other workers (Dugas *et al.*, 1999; Gomes *et al.*, 1999). A wider range of growth was observed at temperature of 15 and 45ºC, which are 84 and 80%, respectively which may be the reason why the organisms survive storage in the yogurt for a longer period.

CONCLUSION

This study has shown that most yogurts in Ibadan contain probiotics isolates including *L. bulgaricus*, *S. lactis* and *S. cerevisiae*, which are therefore, beneficial for human consumption. The result revealed that yoghurt commercially produce in Ibadan are of high quality, in line with Oyeleke (2009) all effort should made to sustain the standard. In conclusion, the study on microflora of yogurts sold in Ibadan has shown that the yogurts contained isolates which has been documented as probiotics from literature. The acid production is independent of the presence of lactose. All the major isolates in this study were the starter culture used in the fermentation of the yogurts. Based on the results obtained in this study, yogurts should be served as part of daily diets. There is need to carry out *in vivo* study on the probiotic activities of yogurts. Further study to determine the probiotic shelf-life of yogurts needs to be carried out. Determination of extrinsic factors (e.g., temperature), that may affect probiotic activities of yogurts is also advocated.

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