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Microbial Analysis of Yoghurt Samples in Trivandrum City, Kerala

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Abstract

Yoghurt is one of the oldest fermented milk products, popular all over the world and it is a rich source of protein, calcium and vitamins. However very careful processing is required for the production of safe and high quality yoghurt. In fact, even a little contamination may deteriorate the quality of yoghurt and may have negative effects on consumer health. Evaluation of the microbial quality of yogurt become very important due to the high risk associated with consuming yogurt containing pathogenic organisms. This becomes clinically significant if a micro organisms isolated from an assessed sample is resistant to conventional antibiotics. Thus, it can confer antibiotic resistance to the affected host. Early detection of food contamination will contribute greatly to safety of foods and thus to an improvement of social health. The microbiological quality of dairy product is influenced by the initial flora of raw milk, the processing conditions, post-heat treatment contamination. This study was carried out to determine the bacteria contaminants in yogurts sold in Trivandrum city which pose danger to public health. The microbial quality of yoghurt indicates the quality and acceptability of the yoghurt. For this purpose some branded (industrial) and unbranded (locally produced) samples of yoghurt from Trivandrum city were collected and were assessed for their quality. Various biochemical tests carried out to identify the isolated organisms. The microbiological analysis of one of the branded yoghurt samples showed the presence of E.coli, indicating some type of mishandling even at the industry. In contrast, a higher quantity of microbes was observed in unbranded yoghurt samples showing the intensity of high mishandling. The higher count could be attributed to the unsanitary conditions prevailing at the time of manufacturing process. In addition, this may also reflect the post-process contamination. The average microbes count varied between $1.25x10^8$ to 1.85×10^8cfu/ml in branded yoghurt samples. While in case of unbranded yoghurt samples it is 5.05×10^8cfu/ml.The bacteria isolates include: Bacillus sp, Staphylococcus sp, Streptococcus sp, E.coli, and Pseudomonas sp. High aerobic bacterial count in yoghurt samples were attributed to inadequate hygienic measures in production or inadequate processing recontamination.

Introduction

Yoghurt is one of the oldest fermented milk products, popular all over the world and it is a rich source of protein, calcium and vitamins. Yoghurt is mainly fermented by lactic acid producing bacteria, S. thermophilus and L. bulgaricus. The natural yoghurt is characterized by a smooth and viscous gel like texture. In fact, the fermentation of lactose by lactic acid bacteria results in the production of lactic acid, carbon dioxide, acetic acid and several other components giving a characteristic flavour to yoghurt.

Yoghurt is a valuable healthy food for both infants and adults. For children, it is a good and balance source of protein, fats, carbohydrates, and minerals, For senior citizens who usually have more sensitive colons or whose intestines have run out of lactase, yoghurt is also a valuable food. Yoghurt may help prevent osteoporosis and reduce the risk of high blood pressure. Dairy products mainly provide 23% of thiamine, 40% of riboflavin and 14% of nicotinic acid in an

average diet. Now a days, there has been increasing demand for a new range of dairy products, including yoghurts which are similar to traditional products but have a low fat content. However very careful processing is required for the production of safe and high quality yoghurt. In fact, even a little contamination may deteriorate the quality of yoghurt and may have negative effects on consumer health.

Yoghurts, like other dairy products are frequently contaminated by microorganisms and this often led to food poisoning. Evaluation of the microbial quality of yogurt become very important due to the high risk associated with consuming yogurt containing pathogenic organisms; health problems associated with consumption of inadequately pasteurized milk products causes serious infections that are hard to treat with antibiotics. This becomes clinically significant if a micro organisms isolated from an assessed sample is resistant to conventional antibiotics. Thus, it can confer antibiotic resistance to the affected host. Early detection of food contamination will contribute greatly to safety of foods and thus to an improvement of social health. The microbiological quality of dairy product is influenced by the initial flora of raw milk, the processing conditions, post-heat treatment contamination. Undesirable bacteria that can results in spoilage of dairy products include Gram-negative psychrotrophs, coliforms and lactic acid bacteria.

This study was carried out to determine the bacteria contaminants in yogurts sold in Trivandrum city which pose danger to public health.The microbial quality of yoghurt reflects towards the quality and acceptability of the yoghurt. Due to unhygienic conditions there is possibility of bacterial contamination, which may have serious effects on the health of consumers. In contrast there is an increasing demand for taste, quality, stability and shelf life of the yoghurt from consumer side. Hence the analysis in the field of quality assessment of yoghurt marketed is the basic need to create awareness among society. For this purpose some branded (industrial) and unbranded (locally produced) samples of yoghurt from Trivandrum city were collected and were assessed for their quality.

Materials and methods

Sample Collection

Ten samples of branded (industrial) yoghurt and ten samples of unbranded yoghurt (handmade local yoghurt) were collected from Trivandrum city under sterilized conditions. Three to four random samples were taken from each branded and unbranded yoghurt sample. The branded samples were in their original packing and unbranded samples were collected in sterilized containers. The samples were analysed as soon as possible after the collection.Samples were fresh with proper expiry date, taken into the laboratory. Care was taken as much as possible to avoid contamination or spoilage of the yogurt samples from external sources. Information on the labels of the yogurt was recorded. The culture medium used in carrying out this analysis is: nutrient agar

Bacteriological Analysis

The laboratory work area and the containers of the yogurt were swabbed thoroughly with 70% ethanol before opening to avoid contaminating the sample. Each sample was serially diluted using sterile saline as diluents. 9ml of saline was measured into several test tubes and sterilized by autoclaving along with 250ml of nutrient agar. After sterilization, the samples were diluted by measuring 1ml of the sample from the container into the first test tube containing 9ml of sterile saline using a pipette pump. The tube was properly mixed and using a different sterile tip, 1ml from the first test tube (10^{-1}) was introduced in to the second test tube containing 9ml of sterile saline (10^{-2}), this is continued following the same procedure till the last dilution (10^{-6}) for all the

samples. Using the spread plate method 0.1ml each of the diluent was poured on the nutrient agar plates and spread using sterile L shaped glass rod. The plates were incubated at 37°c for the 48hr.

Standard plate count

After 48 hours of incubation the plates were examined and counted the total number of colonies which appeared on the surface of agar media in each dilution.

After incubation the representative colonies on the plates were sub cultured on fresh nutrients agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for biochemical identification. The various biochemical tests carried out to identify the isolated organisms.

Identification of SPC bacteria

Selected colonies of all morphological types were picked from agar plate. Isolates were purified by streaking on nutrient agar. Pure cultures were maintained on nutrient agar slants at 5°c. The cultures were identified by using **Bergy's manual of systematic bacteriology**, the main resource for identifying unknown bacteria. According to it, the first approach, identification of bacteria involves preliminary microscopic analysis of the gram-stained preparation for its categorization into gram negative and gram positive groups. After knowing this, the identification was done with the help of various key charts so as to confirm the bacterial identity.

1. **Catalase Test**
   A portion of the colony of the test organism grown in nutrient agar plate was pricked with a sterile glass rod and immersed in 10% H_2O_2 taken in a tube. Noted the effervescence produced.
2. **Oxidase test**
   The test disc impregnated with NNNN-tetra methyl-phenylenediaminedihydrochloride was wetted with one drop of sterile water. A portion of the colony of test organism from nutrient agar plate was pricked with a sterile glass rod and rubbed on the test disc. A purple colour developed within 10 seconds was considered as positive reacton.
3. **Indole test**
   The test organism was incubated in sterile tryptone media. Aftter 24-48 hours incubation, Kovac's reagent was added to the culture. The development of red colour in the reagent side was considered as positive for indole production.
4. **MR-VP test**
   The test organism was inoculated to 2 tubes containing glucose phosphate peptone water media. After 48 hours of incubation to 1 tube five drops of MR reagent and formation of red colour taken as MR positive reaction and yellow colour formation as negative. To the second tube 1ml Barritt's reagent A and 3ml of Barritt's reagent B were added and mixed thoroughly. A red colour development indicated VP test positive.
5. **Citrate utilization test**
   The test organism was inoculated to sterile slants of Simmon'scitrate agar and incubated for 48 hours. Prussian blue colour development was taken as citrate utilization.
6. **Sugar fermentation test**
   Peptone water media containing 0.5% test sugar and 0.0025% bromothymol blue was used to test the ability of the organism to ferment various sugars. The test organism was inoculated to the sterile media containing tubes with inverted Durham's tubes and incubated for overnight. The colony change from blue to yellow was taken as production and air bubble in Durham's tube as gas production due to the sugar fermentation.
7. **Urease test**

Sterile Christensen's urease media slants were inoculated with the test organisms and incubated for 24 – 48 hours. Development of pink colour indicated urease production.

8. **Nitrate reduction test**
   To sterile nitrate broth, the test organism was inoculated and incubated for 48 hours. To the culture, nitrate reagent was added and developed of red colour indicated the reduction of nitrate to nitrite.
9. **Mannitol motility test**
   The test organism inoculated by stabbing into stabbing into mannitol motility test media and incubated for 24 hours. Yellow colour developed indicated mannitol fermentation and diffused growth form the stab line indicated motility of the organism.
10. **Use of TSI media**
    The ability of utilization of sugars & H_2S production was analysed by using TSI media. The test organism was inoculated into TSI media first by stabbing into the butt and then streaking on slant portion. The inoculated media were incubated for 24 – 48 hours and noted the results.

Antibiotic sensitivity testing by disc diffusion method

- **Preparation of the culture broth and bacterial inoculation**: Nutrient broth was prepared and inoculated with the test organism. A loop full of microorganisms was taken and inoculated in the nutrient broth and was incubated at 37°c for 24hrs to obtain viscous growth.
- **Preparation of agar plate**: The freshly prepared autoclaved nutrient agar media was poured in the petri plate, after cooling it to 45°c, and was kept to solidify.
- **Inoculation of agar plate**: The inoculum is spread over the entire surface of the petriplate by swabbing in three directions. Inoculated plates were allowed to dry before applying antibiotic discs. Disc should applied to the surface of the agar within 15 minutes of inoculation (BSAC method for antimicrobial susceptibility testing, 2008).
- **Application of discs**: Storage and handling of the discs should be very careful, so that there will be no loss of potency as a result. 4 antibiotic discs were firmly applied to the dry surface of the inoculated petriplate. The contact with the agar should be even.
- **Incubation**: If plates are left for extended times at room temperature after discs are applied, the antibiotics will diffuse out and the microorganisms starts to grow, results in larger zones of inhibition compared with zones produced when plates are incubated immediately (Andrews, 2004). Plates should therefore be incubated within 15 minutes of disc application. Plates were incubated at 35-37°c for 18-24hrs.
- **Measuring zones**: The diameters of zones of inhibition are measured to the nearest millimeter with a ruler. The zone edge is taken as the point of inhibition as judged by naked eye.

Results

The microbial quality assessment of yoghurt is mainly concerned with two aspects1) Protection of the customers against exposure to health hazard and 2) Ensuring that the yoghurt is not suffering microbiological deterioration during its shelf-life. The microbiological analysis of one of the branded yoghurt samples showed the presence of E.coli, indicating some type of mishandling even at the industry. In contrast, a higher quantity of microbes was observed in unbranded yoghurt samples showing the intensity of high mishandling. The higher count could be attributed to the unsanitary conditions prevailing at the time of manufacturing process. In addition, this may also reflect the post-process contamination. The average microbes count varied

between 1.25x10^8 to 1.85 x 10^8cfu/ml in branded yoghurt samples. While in case of unbranded yoghurt samples it is between1.96x10^8 to5.05 x 10^8cfu/ml.

Table 1and 2 shows the total viable count of the samples, which was obtained after incubation at 37°C for 48hours. Sample Brand1 has less viable growth after 48 hours, sample brand2 had the highest. Unbranded sample 7 has highest colony counts of 505.

Table 1: Total viable count of the organisms in branded and unbranded samples

Samples	Dilution factor (10^{-5})			
	Branded sample		Unbranded sample	
	Colonies per plate	Bacterial count (CFU per ml)	Colonies per plate	Bacterial count (CFU per ml)
1	125	1.25x10^8	408	4.08x 10^8
2	185	1.85x10^8	416	4.16x 10^8
3	122	1.22x10^8	492	4.92x 10^8
4	138	1.38x10^8	310	3.10x 10^8
5	142	1.42x10^8	196	1.96x 10^8
6	170	1.70x10^8	224	2.24x 10^8
7	148	1.48x10^8	505	5.05x 10^8
8	134	1.34x10^8	359	3.59x 10^8
9	160	1.60x10^8	228	2.28x 10^8
10	181	1.81x10^8	245	2.45x 10^8

Table 2: Colonial characteristics of the bacterial isolates from the yoghurt samples

Organisms	Shape	Elevation	Margin	Pigment
Bacillus sp	Round	Flat	lobate	Milky
Staphylococcus	circular	Convex	entire	Yellow
Streptococcus sp	circular	Raised	entire	Milky
Pseudomonas sp	circular	Raised	undulate	blue-green
E.coli	circular	Convex	smooth	No

Discussion

The bacterial viable count recorded a highest count of 5.05 x 10^8 cfu/ml in unbranded sample and lowest of 1.25 x 10^8cfu/ml is in sample Brand1. The bacteria isolates include: Bacillus sp, Staphylococcus sp, Streptococcus sp, E.coli, and Pseudomonas sp. High aerobic bacterial count in yoghurt samples were attributed to inadequate hygienic measures in production or inadequate processing recontamination. The sample recorded counts that ranged 5.05x10^8 cfu/ml is an indication of contamination of the dairy product either during packaging or at the preparatory stage or during handling.

The occurrence of Streptococci is in line with the works of Bramley et al., who showed that microorganisms that contaminate the surface teat and udders of the cow include Staphlococci, Streptococci, spore formers, coliforms and gram negative bacteria which can survive

pasteurization temperature and Streptococci which can grow under refrigeration at low temperature.

The presence of staphylococcus bacteria should be discouraged because it increases if the product is poorly stored. Also, Park C et al. reported the frequent contamination of milk products by the bacteria *Staphylococcus aureus*. The possible source of this bacterium may be from the nasal passage, skin and other mammals. Coughing and talking during production, transportation, storage and retailing produces droplets which settle on the yogurt. *Staphylococcus aureus* is resistant to temperature, drying and radiation. The presence of the bacteria *Staphylococcus aureus* in yogurt may cause food poisoning. It is a major type of food intoxification caused by ingestion of improperly stored or cooked food in which *Staphylococcus aureus* has grown.

The presence of coliform in yoghurt sample indicated contamination and the poor hygiene after processing. Coliform bacteria are not supposed to be present in yogurt sample because of pasteurization and better hygienic procedures, contamination might be from contaminated impure water source or equipments used or from storage and display/sale outlet. Coliforms are considered as normal flora of the intestinal tract of human and animals and their presence indicates direct faecal contamination in the sample. They have been used as an indicator organism for bacteriological quality of dairy products. In most foods, the total bacterial count is an indicator for the sanitary quality, safety and utility of foods. It may reflect the conditions in which the product is manufactured such as contamination of raw materials and ingredients, the effectiveness of processing and the sanitary conditions of equipment and utensils at the processing plants.

Conclusion

Based on the results of the present analysis, it can be concluded that the overall picture of yoghurt quality assessment needs emphasis on quality control during handling, processing and storage. Also standardization of milk for yoghurt production should be observed to meet the standards and adjustment of yoghurt mix should approach the standard of the yoghurt package labels. This study shows that there is large variation in the quality of unbranded yoghurt samples as compared to the branded samples. In unbranded samples, an uncontrolled growth of microorganisms has been observed. In addition, one of the branded samples was positive for Coliform count, which indicates insufficient sanitation and also raises concerns of consumer food safety and health.

From the available result, it can be concluded that some of the yogurt on sale do not meet adequate bacteriological quality. This suggests the need for :

•Strict hygienic measures applied during production, processing and distribution of yogurts and milk products to avoid contamination with unwanted materials and microorganisms.

• Periodical inspection of factory must be done by regulators in the industry to check the problem of poor hygiene.

• The manufacturers should make it as a duty upon themselves to aware their staff on clean and hygienic practices considering the high level of coliform contamination.

References

1. Agu K.C, Archibong E. J, Anekwe D.C, Ago C.A, Okafor A.C, Awah N.S, (2014) , Scholars Journal of Applied Medical Sciences (SJAMS) ISSN 2320-6691 (Online) Sch. J. App. Med. Sci, Assessment of Bacteria Present in Yoghurt Sold on Awka Metropolis, 2(6D):3071-3075.
2. Ahmad. I, M. Gulzar, F. Shahzad, M. Yaqub, T. Zhoor; (2013), The Journal of Animal and Plant Sciences, 23(1 Suppl.), quality assessment of yoghurt produced at large (industrial) and small scale, Page: 58-61 ISSN: 1018-7081
3. Alli, J.A et al, (2010.), British Journal of Dairy Sciences, Microbial Assessment and Microbiological Quality of Some Commercially Prepared Yoghurt Retailed in Ibadan, Oyo State, Southwestern Nigeria, 1(2): 34-38,
4. Bramley AJ, Mckinnon CW, (2004), Dairy Microbiology, The Microbiology of Raw milk, Volume I, Esevier Science Publisher, London, 163-208
5. Dobrea et al, (2009), Bulletin UASVM Agriculture, The Correlation between the Dynamic of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus CFU/mL and pH Value during the Shelf-Life of Yoghurt, 67(1)/2010.
6. Igbabul, Bibiana, Shember, Joseph and Amove,Julius, (2014), African Journal of Food Science and Technology ((ISSN: 2141-5455) ,Physicochemical, microbiological and sensory evaluation of yoghurt sold in Makurdi metropolis, Vol. 5(6) pp. 129-135,.
7. Maria A. El-Ansary, (2014), Alexandria Journal of Veterinary Sciences, Assessment of Microbiological Quality of Yoghurt Sold in El-Behera Governorate, 43: 52-57 ISSN 1110-2047, www.alexjvs.com DOI: 10.5455/ajvs.168635.
8. Mohammed et al; (2007) Research Journal of Animal and Veterinary Sciences, Assessment of Chemical and Microbiological Quality of Stirred Yoghurt in Khartoum State, Sudan;, 2: 56-60,
9. Oyeleke, S.B., (2009), Niger State. Afr. J. Microbiol, Microbial assessment of some commercially prepared yoghurt retailed in Minna,. Res., 3(5): 245-248.
10. Park C, Albano H, Gibbs P, Teixeira P; (2012), Journal of Industrial Microbiology and Biotechnology, Microbiological quality of Portuguese yogurts, 21: 19-21.