

Microbial Contamination of Yoghurt-An Overview

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Abstract: Yoghurt has been considered to be one of the most consumed effective probiotics throughout the world. Considerable number of live microbes given through food which enhances human health are said to be probiotics. These beneficial microbes often improvise lactose digestion and inhibits lactose intolerance. Yoghurt consumption is necessary since the beneficial microbes *Lactobacillus bulgaricus* and *Streptococcus thermophilus* helps in the process of digestion. They are considered to be the best probiotics for human consumption. But due to errors in manufacturing processes like production, packaging, handling, raw material sources, improper sanitary conditions, poor hygiene of employees and unsterilized equipment the quality of yoghurt is slightly reduced since microbial contamination occurs at any point. Microbial contamination of yoghurt can be reduced only through proper sanitary measures and excellent good manufacturing process. The product should meet all the requirements of HACCP as per regulatory guidelines.

Keywords: Acetobacter aceti, coliforms, contamination, *Lactobacillus bulgaricus*, *Mucor* sp, *Streptococcus thermophilus*, yeasts

1. Introduction

Yoghurt has been considered to be one of the most consumed effective probiotics throughout the world. Considerable number of live microbes given through food which enhances human health are said to be probiotics. These beneficial microbes often improvise lactose digestion and inhibits lactose intolerance. The study done by (Francisco et al., 2005) [2] infers that mostly the strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the most used starter cultures in yoghurt production. Also, the consumption of yoghurt with live bacteria has its beneficial effects rather than pasteurized products. Contamination of yoghurt can be because of bacteria and fungi other than the species *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Contamination of yoghurt occurs in various stages which includes contamination during the production process or change in weather from cold to warm temperatures or duration from manufacturing to selling and insufficient refrigeration. The contamination level in both locally and industrially made yoghurt were studied using various protocols. Most studies suggest that contamination occurs due to unhygienic practices during the production level. Intake of contaminated yoghurt not only causes food poisoning but also serves as the most suitable medium for almost a wide range of microbes like yeasts, moulds, gram negative psychrophiles, coliforms, lactic acid bacteria and so on [1]. Since the yoghurt is eaten as such without heating or boiling the chances of getting ill are at high rate.

Contamination depending on weather

Yoghurt samples that were locally made was collected from different regions of Cameroon throughout the year including both dry and rainy season. The collected samples were serially diluted and enumerated for microbial count. Total aerobic count of bacteria, yeast and moulds were made. Organisms that were seen was isolated and identified by standard biochemical tests. Statistical analysis was also done using ANOVA (analysis of variance) and SPSS [5]. The

results showed that the total aerobic count of bacteria during the dry season (November to January) was high compared to other samples.

Local samples showed a count of 9.28 ± 0.00 to 11.63 ± 0.10 from Dschang and 8.80 ± 0.06 to 11.78 ± 0.02 in Bamenda and 11.18 ± 0.04 to 11.36 ± 0.14 from Bafoussam (log 10 cfu/ml). Whereas the commercial brand from Bafoussam has shown highest bacterial count of 9.54 ± 0.05 to 11.48 ± 0.11 . Locally prepared yoghurt samples collected during the rainy season from Dschang showed a count of 8.70 ± 0.09 to 11.55 ± 0.06 also the samples from Bamenda and Bafoussam showed 9.17 ± 0.13 to 11.73 ± 0.01 and 11.02 ± 0.08 to 11.75 ± 0.03 respectively. Commercially obtained yoghurt samples during the rainy season showed 10.34 ± 0.12 to 11.34 ± 0.06 (log₁₀ cfu/ml). The coliform count of locally processed yoghurt increased during the dry season at about 47.05% and decreased during the rainy season at about 41.17 percent. The coliform count of commercially processed yoghurt had no coliform growth during both dry and rainy season [5].

(Moreira et al., 2001) [8] in their study also suggested that contamination was due to warm weather and improper refrigeration. Other possibilities of contamination may be because of unsterilized equipment and raw water source [15]. The yoghurt sample collected from both locally and commercially prepared methods during dry and rainy seasons after analysing showed the presence of following organisms that were responsible for contaminating the yoghurt, viz., *Proteus penneri*, *Ochrobactrum anthropic*, *Shigella* sp., *Providencia stuartii*, *Pantoea* sp., *Klebsiella pneumonia* ssp. *pneumonia*, *Klebsiella oxytoca*, *Providencia rettgeri*, *Providencia alcalifaciens*, *Enterobacter aerogens*, *Serratia plymuthica*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter freundii*, *Burkholderiacapacia*, *Pasteurella pneumotropica*, *Pseudomonas ornithinolytica* and *Manheimahaemolytica*.

The percentage of bacterial isolates were low during the rainy season with 44.44% and high during the dry season with 55.55%. According to their study *Pantoea sp.* was found in almost every samples except commercial samples of dry season and Dschang of rainy season. The yeast culture however was found in 87.5% and 66.66% during the dry season of both locally and commercially prepared samples. Variation in yeast growth was observed during rainy and dry seasons. Yeast count decreased during the rainy season in sample collected from Bamenda and increased in sample collected from Bafoussam. The high growth count of yeast during the dry season was because of the warmer weather than compared to rainy season. No mould growth was seen in control samples in both seasons with 0.00 ± 0.00 to 4.26 ± 0.11 in Bamenda sample. Presence of these moulds and yeasts in examined yoghurt samples indicate that insufficient or inefficient preheating process had taken place.

The sample collected locally during the dry season showed the presence of yeast species viz, *Candida zeylanoides*, *Candida kruzei*, *Candida inconspicua*, *Candida dubliniensis*, *Candida lusitanae*, *Candida boidinii*, *Candida albicans*, *Stephanoascus ferrii*, *Trichosporon asahii*, *Rhodotorulamucilaginoso*, *Kodmaeaohmeri*, *Cryptococcus laurentii*, *Pichia angusta*, *Kloeckera sp.* and *Cryptococcus humicola*[5]. From this study it can be seen that yoghurt prepared locally constituted a high risk of health hazard when bought during rainy season and dry season. Also, commercialized yoghurt sample except few were not in the standard met, collected during dry season. This seasonal variation thus is another cause for contamination based on temperature, humidity and environmental changes.

Contamination due to handling

The manufacturing process of yoghurt is quite simple and easy which infers that any peon could do it. This paves way for higher contamination rates since yoghurt can be manufactured at any time, at any place, with any equipment by anyone. Thus, safe consumption of yoghurt is still an unanswered question. Research done by[4] clearly shows that high hygiene practices should be undertaken in the production process of both small- and large-scale industries. Random yoghurt samples obtained from both large and small industries in Qena district of Egypt showed maximum contamination rates. In their study [4] the microbiological quality was analysed from ready to sell yoghurt containers. 50 samples from small scale industries and 50 samples from large scale industries were put to test for the estimation of psychrotrophs, enterococcus, coliforms, yeasts and moulds.

Psychrotrophic bacteria that grow in refrigeration temperatures are known to cause food poisoning due to the products like lipase and protease after enzyme conversion by assimilating nutrients from dairy products [3]. The presence of enterococci may be as a result of poor sanitation of the industry since the organism is highly resistant to detergents, drying and freezing conditions [16]. Coliforms are often used as indicator organisms for faecal pollution. Their presence in dairy based products also indicates that other enteropathogenic microbes may be present [13]. The presence of *Staphylococcus aureus* in milk and milk-based products may be because of its initial contamination through

personal sharing and in handling and production procedures [4]. The samples examined from small scale industries had been contaminated with about 92% of psychrotrophs with count range of 1.7×10^3 to 3.0×10^5 with average count 3.9×10^4 /g. The samples examined from large scale industries had been contaminated with about 70% of psychrotrophs with count range of 4.0×10^2 to 6.0×10^4 with average count 6.8×10^3 /g. The high count was due to inadequate heat treatment, improper processing, careless handling or because of the presence of high psychrophiles in raw milk that was used for production.

The presence of enterococci in small scale industries ranged from 2.5×10^2 to 1.6×10^5 with an average count 1.7×10^4 /g with 58 percent in total. In large scale industries the average count was 2.0×10^3 /g ranging from 1.0×10^2 to 1.5×10^4 with 40 % in total. Contamination by enterococci in yoghurt often implies the negligence of hygienic control measures during handling and production.

Contamination by *Staphylococcus aureus* in small scale level was 72% with 8.5×10^3 /g as an average count. Whereas in large scale industries *Staphylococcus aureus* contamination was 36% with 9.4×10^2 /g as average count. Presence of *Staphylococcus aureus* in yoghurt is because of humans who act as mediatory channel for infection by way of coughing, sneezing, or handling equipment after touching lesions infected with *Staphylococcus aureus*. Yeast and mould count were also estimated which gave an average count of 1.4×10^4 /g ranging from 2.5×10^2 to 1.4×10^5 in small scale and 3.9×10^2 /g as average count in large scale with range of 1.00×10^2 to 1.40×10^3 . Poor sanitary practices are the major cause for deposition of yeasts and moulds in high acid product like yoghurt.

Coliforms an indicator of faecal pollution was also found in both small- and large-scale levels. High frequency distribution of 36.8% with range of 10^3 - $< 10^4$ cfu/g in small scale industries and high frequency distribution of 60% with range 10 - $< 10^2$ cfu/g in large scale industries were seen. Their presence also shows that improper sanitary measures had been taken by the industries. Totally their study has clearly shown that contamination has occurred due to handling, processing and improper sanitation in the production of yoghurt from any market.

Contamination by adulterants

A study conducted by (Rahman et al., 2020) [7] in Bangladesh cities revealed the presence of few adulterants in addition to high protein levels. The study showed local, unbranded yoghurt sample contained about 29% urea, 19% ammonium sulphate, 33% starch and 19% hydrogen peroxide. Likewise, branded, commercial yoghurt samples contained 29% starch, 14% urea, 36% ammonium sulphate and 21% hydrogen peroxide. Commercial urea is mostly added to increase the level of non-protein nitrogen content [11] and hydrogen peroxide is used for long term storage that acted as a preservative [12]. In this study adulterants like starch, hydrogen peroxide, ammonium sulphate and urea were found which is an indication of health hazard. Because high level of starch could be a reason for diarrhoea because of its effects like indigestion of starch in colon. Ammonium sulphate and hydrogen peroxide can cause intestine to get

inflamed [14]. From this study it can be seen that both branded and unbranded yoghurt were adulterated with chemical substances like urea, ammonium sulphate, starch and hydrogen peroxide. The microbiological analysis of the starter culture viz., *Lactobacillus bulgaricus* and *Streptococcus thermophilus* revealed that the amount of starter culture was extremely low in few samples and thus uniformity of the culture should be maintained to get sweetened yoghurt of high quality [7].

Signs of contaminated yoghurt

A detailed report on inward collapse and swelling of yoghurt packs was done by [6]. Before buying yoghurt, it is essential to see the manufacturing and expiry date and certain signs of contamination that could be observed with our naked eye. Typically, the research shows that samples analysed within expiry date had contaminants responsible for inward collapsing of the lid and swelling seal due to anaerobic condition by release of CO₂ by certain microflora. Three different brands studied showed comparative yield of bacteria, yeasts and moulds. From the samples collected, one brand had bacterial count that reached a level of 10⁷cfu/g, the second brand had no bacterial contaminants but the main contaminants were moulds with colony count range of (10² to 10⁵cfu/g) while the third brand had both yeasts and moulds ranging between 10⁶ and 10⁵cfu/g.

Contaminating strain of brand one was found to be *Acetobacter aceti* and samples from brand two had organisms like *Mucor hiemalis*Wehmer, *Mucor racemosus*Fres and *Penicilliumverrucosum*Dierckx var *cyclopium* were identified. In brand three samples, moulds like *Mucorhiemalis* and *Mucor racemosus* and yeasts like *Debaryomyceslanseni* were isolated. The inward collapse of the yoghurt pack was mostly because of *Acetobacter aceti* and swelling was because of yeasts and moulds. However, after swelling inward collapse was seen after five to seven days of incubation at refrigeration temperature [6]. From their study it is evident that certain alterations in the yoghurt packets can be taken as a sign of contamination, but then again if the manufacturing practices were good and in strict correct conditions of refrigeration there can always be a way to slow down such kind of alterations.

2. Discussion

Yoghurt consumption is necessary since the beneficial microbes *Lactobacillus bulgaricus* and *Streptococcus thermophilus* helps in the process of digestion. They are considered to be the best probiotics for human consumption. But due to errors in manufacturing processes like production, packaging, handling, raw material sources, improper sanitary conditions, poor hygiene of employees and unsterilized equipment the quality of yoghurt is slightly reduced since microbial contamination occurs at any point. In many or some cases local as well as branded yoghurt industries supply contaminated yoghurt that may not have been certified by HACCP. Even if some yoghurt is in good conditions during the ready to sell process, environmental factors also affect the quality due to improper refrigeration, transportation and climatic conditions. Consumers who are not aware of the contaminated product even if the product is not expired are prone to more diseases like diarrhoea, fever

and other conditions. Locally manufactured yoghurt should be given a thorough inspection by the health inspector before it comes to the market. Awareness among the people should be created about the health hazards of consuming low-quality yoghurts.

3. Conclusion

Microbial contamination of yoghurt can be reduced only through proper sanitary measures and excellent good manufacturing process. The product should meet all the requirements of HACCP as per regulatory guidelines. Workers should be clean and disease free while they handle any equipment in the production unit. Every containers and equipment required for manufacturing should be heat sterilized to avoid spore forming microbes or suitable sterilization technique must be carried out to eliminate bacteria, fungi, yeasts and moulds. The source or raw material like milk, artificial flavours, water etc should be pasteurized at required temperatures. Also, the sealed and ready to go yoghurt packets must be properly stored at freezing or refrigeration temperatures until they reach the consumer. Consumption of yoghurt bought from local markets should be avoided since no proper safety measures will be taken by the vendor during the production process. Yoghurt bought from branded sellers should be consumed within the date of expiry and should be eaten at once after the lid is opened. Even if the opened yoghurt is stored in refrigeration chances of contamination are at high rates. Thus, every consumer should be well aware of the product they ought to consume.

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