EFFECT OF TREATMENT INTERACTION WITH COLIFORM COUNTS OF YOGHURT VARIETES WITH NO PRESERVATIVES DURING STORAGE

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ABSTRACT

Coliforms are easily destroyed in pasteurization process. Still the presence of coliforms in food products has been recognized for many years during processing and storage especially in milk products. The functional foods are having specific physiological effects. The present study been carried out to analyze the storage period, yoghurt varieties and storage period x yoghurt varieties with coliform count interaction during storage period of different yoghurt varieties with no preservatives or stabilizers. Dairy products play a potential role in the functional food market which carries probiotics, prebiotics and synbiotics which aid the gut by increasing the number of helpful bacteria and inhibiting harmful bacteria and may strengthen the body's immune response. Most coliforms do not cause disease, but can cause illness in people, especially vulnerable groups such as young children, the elderly and those with weakened immune systems. Two factorial analysis indicates that the coliform count showed significant treatment interaction effect with parameters storage period, yoghurt varieties and storage period X yoghurt varieties and concluded that significant interaction with parameters and storage period (p<0.05) was observed.

KEY WORDS: Coliforms, probiotics, synbiotics, Yoghurt varieties

INTRODUCTION

In view of increasing demand for functional food products, the industries are concentrating not only on the quantity but also ensuring safety and healthy products (Salinas, 1986). Also, the industrial scenario has considerably improved in terms of storage, transportation and commercialization of these products and also laid emphasis on the microbiological parameters such as coliform-free products (Tamime and Robinson, 2007). Coliform quantification allows the verification of raw milk quality and the processing efficiency. Coliform bacteria and yeasts metabolize diacetyl content of butter-milk, sour cream and other fermented products leading to yoghurt-like flavor (Wang and Frank, 1981). Coliforms are a group of bacteria commonly found in the environment, including soil, surface water, vegetation and the intestinal tracts of warm-blooded animals. Coliform bacteria are not a traditional taxonomic group, like *Salmonella*, *Escherichia coli*, or *Listeria*. Instead, the coliform bacteria are a collection of strains in the *Enterobacteriaceae* family. *E. coli*, *Enterobacter* spp., *Klebsiella* spp. and *Citrobacter* spp. are the most common coliforms. They are rod-shaped Gram-negative non-spore forming organisms. They can ferment lactose with the production of acid and gas when incubated at 35-37°C. In dairy products, the process of pasteurization easily kills coliform bacteria. Most coliforms do not cause disease, but a small percentage can cause illness in people, especially young children, the elderly and those with weakened immune systems.

Yoghurt is a coagulated product obtained from pasteurized or boiled milk or concentrated milk, pasteurized skimmed milk and /or pasteurized cream or a mixture of two or more of these products by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Steptococcus thermophilus*. It may also contain *Bifidobacterium bifidus* and *Lactobacillus acidophilus* cultures and other cultures of suitable lactic acid producing harmless bacteria and if added a declaration to this effect shall be made on the label. Sensory attributes is a primary concern to determine shelf life in product development (Grunert *et al.*, 2000). Among the important factors in dairy food products for inclusion of probiotics, their type, form and optimal population to derive beneficial effects, as well as production process effects on viability, stability during storage and possible changes in the sensory properties are considered important (Champagne *et al.*, 2005).

Even after pasteurization, the presence of coliforms in dairy products has been recognized for many years during processing and storage. These microorganisms are associated with the spoilage of yoghurts and milk based food products and reduces the shelf life. As a result, maintenance of the quality of the product is today's one of the major task to make consumer healthy. Coliforms count has been reported in many research studies are also associated with different independent parameters and its relation. Hence, the present study been carried out to analyze the treatment interaction with coliforms during storage period has been studied.

MATERIALS AND METHODS

Preparation of yoghurts

Yoghurts were developed with no preservatives or stabilizers and using starter culture as control, experimental varieties of yoghurts with *Lactobacillus casei*, *Bifidobacterium bifidum* along with fructo oligosaccharides and onion extracted prebiotics in different combinations which are named as C = yoghurt starter culture (control); V1 = yoghurt starter culture + *Lactobacillus casei17*; V2 = yoghurt starter culture + *Bifidobacterium bifidum 231*; V3 = yoghurt starter culture + fructo-oligosaccharides; V4 = yoghurt starter culture + *Lactobacillus casei17* + Fructo oligosaccharides; V5 = yoghurt starter culture + *Bifidobacterium bifidum 231* + fructo oligosaccharides; V6 = yoghurt starter culture + onion extracted prebiotics; V7 = yoghurt starter culture + *Lactobacillus casei17* + onion extracted prebiotics; V8 = yoghurt starter culture + *Bifidobacterium bifidum 231* + onion extracted prebiotics.

Shelf-life studies

These yoghurts were incubated at 37^0C, followed by cooling at -4^0C and stored for a period of 4 weeks in cold storage for the conducting shelf-life studies.

Evaluation of coliform count

Coliform count of all yoghurt samples was recorded after making a decimal serial dilution of each sample. MacConkey agar was used for coliform count. The plates were incubated at 37ºC for coliform bacteria for a period of 24h for counting total microbial colonies.

MacConkey agar was used for the enumeration and detection of coliform bacteria. The media was prepared according to the manufacturer's instructions and the pour plates incubated for 24±2 h at 37±1^0C. One ml of each yoghurt sample was diluted in 9 mL sterile saline solution and 1ml of the diluted sample (10^{-7}) was added in Petri dishes and incubated. Enumeration of coliform counts was done after 24h. Discrete colonies that developed on the plates (overnight culture) were recorded as bacterial counts. The determination of microbial contamination in yoghurt variations was made by using the plate count agar for the total viable bacteria counts, MacConkey agar for the coliform counts, selective enriched media of MRS agar for the lactic acid bacteria counts, as outlined in compendium of methods for the microbiological examination of foods (AMPH, 1992) with some modifications. The colonies were counted on a colony counter and expressed as colony forming units per mL (CFU mL^{-1}).

Statistical analysis

Two factorial completed randomized design was carried out for independent variables storage period, yoghurt varieties, storage period x yoghurt varieties with mean values of dependent variable coliform count. Interaction between dependent and independent variables done by INDOSTAT software.

RESULTS AND DISCUSSION

Coliform counts in yoghurt varieties were nil as desirable in the yoghurt on the day of preparation (Table 1) and continued same till 21 days. However, in all the varieties of yoghurt on 28th day of storage, V3, V5 and V8 showed counts. Among them, V3 showed highest (5.0) coliform count followed by V5 and V8 (2.0 each). This might be due to contamination or lack of sanitation of utensils and working area.

Comparison between storage periods also reflected significant variability in coliform counts from 28th day. These results showed that yoghurt types made in combination with Bb 231 with YSC and with OEP reflected higher susceptibility in terms of higher coliform counts. Slow lactic acid production by starter cultures in accumulated media may favor the growth and production of gas by coliform bacteria, because they have short generation times and found favorable in combination with Bb 231. In most of yoghurt samples coliform bacteria were absent due to pasteurization prior to its incubation and some yoghurt samples contained less count of coliform

Similar results have been reported by Lopez *et al*., (1997) and Younus *et al.*, (2002) who reported low number of coliforms in yoghurt samples. The presence of coliforms in V3, V5 and V8 on 28th day might be because the contamination of milk or utensils or working and storage areas. El Bakri and El Zubeir, (2009) evaluated the quality of yoghurt market samples and found the total bacterial count and yeast and molds count were not significantly different between different manufacturers, the coliform count of samples varied significantly ($p<0.001$) between manufacturers and with a significantly higher ($p<0.05$) coliform count in samples collected from traditional manufacturers than that collected from modern manufacturers.

Two factorial analysis was carried out with storage period, yoghurt varieties, storage period x yoghurt varieties and coliform count. Table 2 indicates the treatment interaction effect with coliforms at 0, 7, 14, 21 and 28 days of storage period. Coliform count showed significant interaction with parameters storage period, yoghurt varieties and storage period X yoghurt varieties. Hence not only contamination, lack of sanitation of utensils and working area but also the independent variables such as storage period due to its time, varieties of yoghurt due to its combination of different types of bacterial probiotic species and storage period verses yoghurt varieties do show the remarkable significant interaction with coliform count. Incubation time also show the effect on presence and increase of coliform count.

Omokoro and Telema (2014) studied the microbiology of three yoghurt samples and found that the bacterial, fungal and thermo tolerant coliform counts were highest in the Mary gold samples which had an acidic pH, the bacterial and fungal counts were lowest in

Green field samples with a neutral pH and the highest total coliform count. While the total coliform and thermo tolerant coliform counts were lowest in the home victory yoghurt samples. Rodrigues *et al*., (2010) analyzed 36 samples in Brazil for coliforms, Lactic acid bacteria, numeration and pH and observed in the samples with less than 15 days for 7.4 log CFU/g LAB counts and 4.1 pH values and they were lower when compared to samples with more than 16 days of commercial viability, but without significant differences.

CONCLUSION

Industrially produced some dairy products are absent of coliform bacteria due to pasteurization of pre-mix, additives, preservatives prior to its incubation and some yoghurt samples contains less count of coliform. In this study, effect of treatment interaction with coliform counts of yoghurt varieties which were developed with no preservatives or stabilizers but natural with probiotics and prebiotic combinations were studied. Coliform counts in yoghurt varieties were nil as desirable in the yoghurt on the day of preparation and continued same till 21 days. But, in all the varieties of yoghurt on 28th day of storage, V3, V5 and V8 showed coliform counts. The interaction of coliform count in these yoghurts was significantly related with storage period, yoghurt varieties and storage period X yoghurt varieties. Comparison between storage periods also reflected significant variability in coliform counts from 28th day. These results showed that yoghurt types made in combination with Bb 231 with YSC and with OEP reflected higher susceptibility in terms of higher coliform counts. Hence not only contamination, lack of sanitation of utensils and working area but also the independent variables such as storage period due to its time, varieties of yoghurt due to its combination of different types of bacterial probiotic species, storage period verses yoghurt varieties and incubation do show the remarkable significant interaction with coliform count.

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Table 1. Microbiological quality of coliform counts (10^{-7}) in yoghurt

	Yoghurt type	**Coliform count (10^{-7}) CFU mL^{-1}**				
		Day-0	Day-7	Day-14	Day-21	Day-28
Control	C-YSC	0	0	0	0	0
Probiotic Batch	V1-YSC + Lc 17	0	0	0	0	0
	V2-YSC + *Bb* 231	0	0	0	0	0
FOS Batch	V3-YSC + FOS	0	0	0	0	5
	V4-YSC + *Lc* 17 + FOS	0	0	0	0	0
	V5-YSC + *Bb* 231 + FOS	0	0	0	0	2
OEP Batch	V6-YSC + OEP	0	0	0	0	0
	V7-YSC + *Lc* 17 + OEP	0	0	0	0	0
	V8-YSC + *Bb* 231 + OEP	0	0	0	0	2

Note: C = yoghurt starter culture (control); V1 = yoghurt starter culture + Lactobacillus casei17; V2 = yoghurt starter culture + Bifidobacterium bifidum 231; V3 = yoghurt starter culture + fructo- oligosaccharides; V4 = yoghurt starter culture + Lactobacillus casei17 + Fructo oligosaccharides; V5 = yoghurt starter culture + Bifidobacterium bifidum 231 + fructo oligosaccharides; V6 = yoghurt starter culture + onion extracted prebiotics; V7 = yoghurt starter culture + Lactobacillus casei17 + onion extracted prebiotics; V8 = yoghurt starter culture + Bifidobacterium bifidum 231 + onion extracted prebiotics.

Table 2. Effect of treatment interaction with coliform counts of yoghurt varieties during storage period

Details	**Coliform**
Parameters	
Storage Period	196.807***
Yoghurt varieties	166.007***
Storage period x Yoghurt varieties	166.007***

****** indicates significant interaction with parameters and storage period (p<0.05)***

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