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Hygienic Quality of Some Fermented Milk Products

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ABSTRACT

This study was assumed to evaluate the hygienic quality of some fermented milk products. One hundred and thirty random samples (30 each of large scale plain yoghurt, Butter milk, Labneh and 40 samples of skimmed milk Raieb) were collected from dairy shops and supermarkets in Cairo, Giza and El Monofiagovernorates. The collected samples were examined to evaluate their microbial status. The results revealed that Coliforms, total *Staphylococci*, *S. aureus*, aerobic spore formers, yeast and mold could be present in high numbers, while *Yersinia enterocolitica* couldn't be detected in all of the examined samples.

Key words: Fermented milk, microbiological quality, pH, acidity

INTRODUCTION

Fermented milk defined as a dairy product obtained by the fermentation of milk through the action of appropriate microorganisms (Lactic Acid Bacteria) which resulted in lowering of pH with or without coagulation. These microorganisms must be viable, active and abundant in the product (Codex Alimentarius, 2003). The popular fermented milk products are Yoghurt, Labneh, Acidophilus milk, Raieb, Butter milk, Cultured butter milk, Kefir, Koumiss and beverages based on bulgaricus or bifidus strains (Kosikowski and Mistry, 1997).

Consumption of fermented milk products such as yoghurt dates back manycenturies, although there is no precise record of the date when they were first made. In these ancient times the milk would be fermented spontaneously by natural microflora. Since ancient times, in Europe, Asia and Africa, sour milk was known as being more stable and advantageous than fresh milk. It preserved the high quality nutrients present in milks in a relatively stable form (Oberman and Libudzisz, 1998). Although, yoghurt has been around for many years, it has become a popular fermented milk product in Europe, Asia and Africa (Kosikowski, 1966; Savadogo *et al.*, 2004).

Fermented dairy products considered an excellent source of calcium, phosphorus and magnesium, which are highly bioavailable, because of the lower pH of fermented milk compared with that of milk. These minerals in optimum ratio are present in milk and its products and are required for optimum growth and maintenance of bones (Aneja *et al.*, 2002; McKinley, 2005). It's also considered an excellent source of protein, riboflavin (vitamin B2), thiamin (vitamin B1), vitamin B12, folate, niacin and zinc (McKinley, 2005). During fermentation, both heat treatment and acid production resulting in finer coagulation of casein, which may also contribute to the greater protein digestibility of yoghurt than that of milk. Fermentation also causes increase in accumulation of free amino acids, furthermore the concentrations of most amino acids of yoghurt are about twice that of fresh milk (Sulieman *et al.*, 2009).

Lactic Acid Bacteria (LAB) refer to a large group of beneficial bacteria that have similar properties and all produce lactic acid as an end product of the food fermentation (Nakazawa and Hosono, 1992). LAB species are now frequently used to give the final product unique characteristics, species display symbiotic relations during their growth in milk medium. Ingestion of yoghurt in sufficient amount exerts beneficial effects on the normal microbial population of the gastrointestinal tract (Bourlioux *et al.*, 2003). Some LAB and fermented milk play a significant role in suppressing carcinogenesis indirectly through an activation of the immune system whereby whole cells as well as cell-wall fragments of LAB can activate the macrophages in the host (Adachi, 1992; Tavan *et al.*, 2002), finally consumption of fermented dairy products improve cholesterol profile lowering the LDL (bad) cholesterol, while raising the HDL (good) cholesterol (Leblanc and Pedrigon, 2004).

Fermented milk products are likely to contamination with mold and yeast as main contaminants as a result of low pH of these products (Suriyarachchi and Fleet, 1981). Molds and yeasts growing in yoghurt utilize some of the acid and produce a corresponding decrease in the acidity, which may favor the growth of putrefactive bacteria. Because of the high consumption of different types of fermented milk products in Egypt and to ensure the hygienic condition under which the products were produced, this study was planned to throw light on the sanitary status of some fermented dairy products.

MATERIALS AND METHODS

Sample collection: One hundred and thirty samples of fermented milk products (30, each of large scale plain yoghurt, Butter milk, Labneh and 40 samples of Skimmed milk Raieb) were randomly collected from dairy shops, super markets and street hawkers in Cairo, Giza and El Monofiagovernorates. Collected samples were transported under aseptic conditions in an ice packed container to the laboratory and evaluation commenced immediately.

Determination of titratable acidity (%) and pH: Determination of titratable Acidity was performed using the method mentioned by AOAC (1990). While measuring pH value of the examined samples was applied according to Kirk and Sawyer (1991).

Microbiological examination: Preparation of food homogenate and decimal dilutions were applied according to (APHA, 2004). Total coliform count with completed test for *E. coli* was determined according to (BAM, 2002). Enumeration of Aerobic Spore Formers was applied according to (APHA, 2004) using tryptone dextrose agar medium (Oxoid Mannual, 2010), with identification of their isolates according to (Holt, 1986). Enumeration of total *Staphylococci* count with isolation of *S. aureus* according to (AOAC, 2000). Total yeast and mold count was applied according to (ISO, 1994). The technique recommended by APHA (2004) was used for isolation of *Yersinia enterocolitica*.

RESULTS AND DISCUSSION

Titratable acidty (%) and pH: The data recorded in Table 1 showed that the mean titratable acidity percentage of the examined samples (Large scale plain yoghurt, Raieb, Labneh and Butter milk) were 0.70±0.024, 0.68±0.014, 1.08±0.070 and 1.22±0.056, respectively. Nearly similar results

Table 1: Statistical analytical results of titratable acidity percentage and pH value in the examined samples of fermented milk

Total No. of samples	Minimum	Maximum	Mean±SEM
Titratable acidity			
Large scale plain yoghurt	0.34	0.93	0.70±0.024
Raieb	0.57	0.81	0.68±0.014
Labneh	0.59	1.80	1.08±0.070
Butter milk	0.64	1.60	1.22±0.056
pH			
Large scale plain yoghurt	3.90	4.42	4.19±0.020
Raieb	4.20	5.52	4.52±0.042
Labneh	4.11	4.72	4.45±0.035
Butter milk	3.50	4.60	3.92±0.053

of large scale plain yoghurt were reported by Mugampoza *et al.* (2011), higher results were obtained by Abdalla and Abdel Nabi Ahmed (2010) and Mohammad and El-Zubeir (2011), while lower findings were obtained by Ayana and El Deen (2011).

The mean pH value of the examined samples of Large scale plain yoghurt, Raieb, Labneh and Butter milk were 4.19±0.02, 4.52±0.042, 4.45±0.035 and 3.92±0.053, respectively. Nearly similar findings of large scale plain yoghurt were recorded by Eman (2007) and Mugampoza *et al.* (2011), higher results were reported by Younus *et al.* (2002) and Ayah (2010), while lower results were obtained by Lopez *et al.* (1993) and Abeer (1997). Lower findings of Raieb were recorded by All and Dardir (2009).

The acidification of milk is primarily linked to the conversion of lactose into organic acids, consisting mainly of lactic acid and acetic acids. This lowers the pH of milk from a value of 6.8 to less than 4.6, thus protecting the fermented milk against the risk of contamination by pathogens and rendering it hygienically safe (Garbutt, 1997; Al-Kadamany *et al.*, 2001; Wilton, 2004).

Microbiological examination

Coliform count: Table 2 revealed that coliforms were present in 18 (60%), 7 (17.5%), 9 (30%) and 21 (70%) in the examined samples of large scale plain yoghurt, Raieb, Labneh and Butter milk, respectively, with a mean value of $2.6\times10^4\pm8.1\times10^3$, $2.5\times10^5\pm4.5\times10^4$, $1.6\times10^4\pm1.6\times10^4$, $1.4\times10^2\pm98.4$ and $8.8\times10^2\pm7.1\times10^2$ MPN g^{-1}, respectively. Nearly similar results of Large scale plain yoghurt were recorded by Aboubaker (2004), higher results were reported by Farag (1991), while lower findings were assessed by Al-Hawary *et al.* (2005), Eman (2007) and Ayah (2010). Nearly similar results of Raieb were reported by All and Dardir (2009).

It is clear from the obtained results that most of examined yoghurt samples contain high numbers of coliforms and disagree with the Egyptian Standards (EOS, 2005), which recommend that coliforms count should be less than 10 cells g^{-1} in the product. The high number of coliforms is a sign of poor hygienic processing conditions and post-processing contamination.

E. coli couldn't be isolated from all of the examined samples and this is because of the low pH value of these products.

Aerobic spore formers count: Data depicted in Table 2 revealed that all samples were contaminated with aerobic spore formers, the maximum counts were 85×10^3, 75×10^4, 96×10^4 and 25×10^5 with a mean value of $1.2\times10^4\pm38\times10^2$, $10.4\times10^4\pm34.1\times10^3$, $20\times10^4\pm38.8\times10^3$ and $68.3\times10^4\pm1.3\times10^4$ CFU g^{-1} in the examined samples of large scale plain yoghurt, Raieb, Labneh and Butter milk, respectively. Nearly similar findings of large scale plain yoghurt were reported by

Table 2: Statistical analytical results of the microbiological parameters in the examined samples of fermented milk

	Positive samples				
Total No. of samples	No.	%	Minimum	Maximum	Mean±SEM
Coliform count (MPN g^{-1})					
Large scale plain yoghurt	18.0	60.0	2.3×10^2	11×10^4	$2.6\times10^4\pm 8.1\times10^3$
Raieb	7.0	17.5	40.0	11×10^4	$1.6\times10^4 \pm1.6\times10^4$
Labneh	9.0	30.0	30.0	9.3×10^2	$1.4\times10^2\pm98.4$
Butter milk	21.0	70.0	40.0	15×10^3	$8.8\times10^2\pm7.1\times10^2$
Aerobic spore formers (CFU g^{-1})					
Large scale plain yoghurt	30.0	100.0	50.0	85×10^3	$1.2\times10^4\pm38\times10^2$
Raieb	30.0	100.0	10^4	75×10^4	$10.4\times10^4\pm34.1\times10^3$
Labneh	30.0	100.0	10^4	96×10^4	$20.0\times10^4\pm38.8\times10^3$
Butter milk	30.0	100.0	10^5	25×10^5	$68.3\times10^4\pm13\times10^4$
Total staphylococci count (CFU g^{-1})					
Large scale plain yoghurt	24.0	80.0	5×10^2	9.5×10^9	$1.4\times10^9\pm5.2\times10^8$
Raieb	28.0	93.3	5×10^2	86×10^3	$11.9\times10^3\pm39.5\times10^2$
Labneh	30.0	100.0	10^3	42.6×10^4	$89.0\times10^3\pm18.3\times10^3$
Butter milk	29.0	96.7	10^4	43×10^5	$1.2\times10^6\pm2.02\times10^5$
Total yeast count (CFU g^{-1})					
Large scale plain yoghurt	14.0	46.7	50.0	10^4	$23.0\times10^2\pm7.8\times10^2$
Raieb	12.0	40.0	5×10^2	32×10^3	$69.2\times10^2\pm26.4\times10^2$
Labneh	21.0	70.0	6×10^3	19×10^4	$42.1\times10^3\pm26.4\times10^2$
Butter milk	30.0	100.0	4×10^3	12×10^4	$42.1\times10^3\pm58.5\times10^2$
Total mold count (CFU g^{-1})					
Large scale plain yoghurt	12.0	40.0	50.0	4×10^3	$750.0\pm3.1\times10^2$
Raieb	18.0	60.0	5×10^2	11×10^3	$27.2\times10^2\pm7.1\times10^2$
Labneh	20.0	66.7	2×10^2	77×10^3	$21.2\times10^3\pm61.1\times10^2$
Butter milk	17.0	56.7	10^3	92×10^3	$21.7\times10^3\pm62.9\times10^2$

Essam (2008) and Ayah (2010), higher findings were obtained by Abeer (1997), while lower results were obtained by Halawa *et al.* (1993). Nearly similar results of Raieb were recorded by Sabreen (2001), while lower outcomes were reported by Abeer (1997). Nearly similar findings of Labneh were recorded by Ayana and El Deen (2011). Lower findings of Buttermilk were observed by Abeer (1997).

Biochemical identification of isolates of aerobic spore formers revealed that *Sporosarcina halophila* was the most frequent one 27 (36%), followed by *Sporolactobacillus inulinus* 24 (32%) in large scale plain yoghurt samples, while *Sporosarcina ureae* was the most frequent one in Raieb, Labneh and Butter milk samples (Fig. 1).

All fermented milk samples under examination are highly contaminated with aerobic spore formers which considered as a mirror of the neglected awareness of good sanitation during manufacture and storage.

Total *Staphylococci* count: *Staphylococci* was present in 80, 93.3, 100 and 96.7% of the examined samples of large scale plain yoghurt, Raieb, Labneh and Butter milk, respectively, with a mean count of $1.4\times10^9\pm5.2\times10^8$, $11.9\times10^3\pm39.5\times10^2$, $89\times10^3\pm18.3\times10^3$ and $1.2\times10^6\pm2.02\times10^5$ CFU g^{-1}, respectively, (Table 2). Nearly similar results of Large scale plain yoghurt were reported by Ayah (2010), lower findings were recorded by Al-Tahiri (2005) and Eman (2007), while higher findings were obtained by Nwamaka and Chike (2010). Higher results

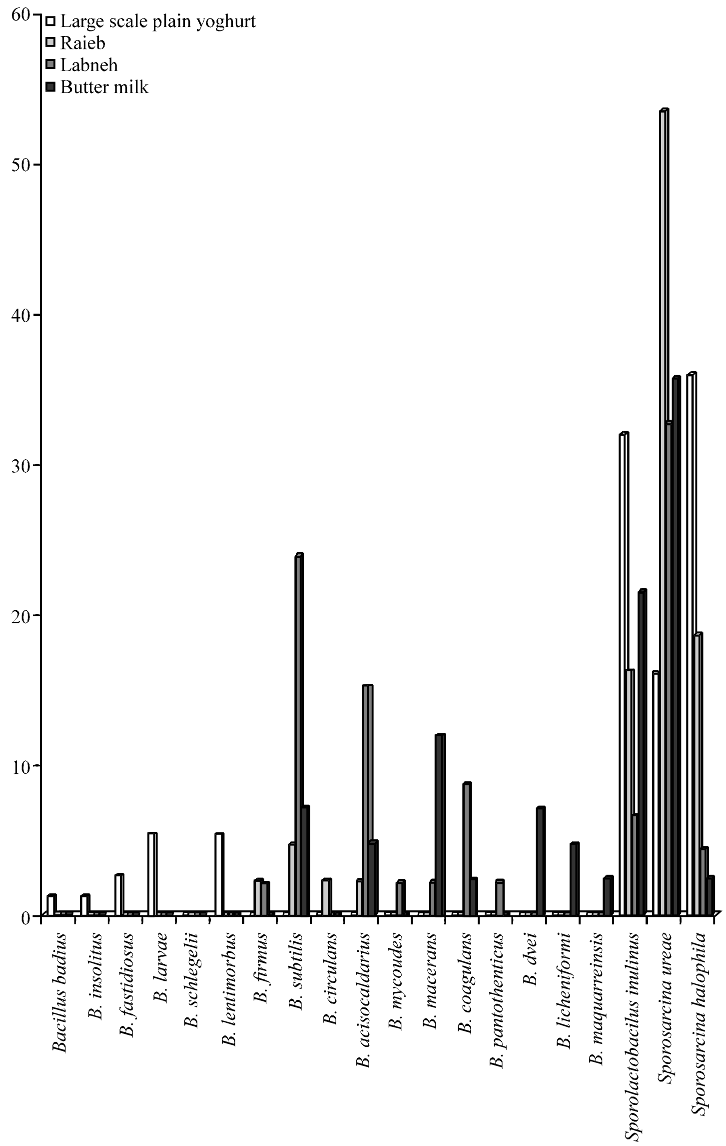

Fig. 1: Incidence of isolated aerobic spore formers from examined samples of fermented milk products

of Raieb samples were obtained by Abeer (1997) and All and Dardir (2009). Lower results of Labneh were reported by Al-Tahiri (2005). Lower results of Butter milk were reported by Saudi *et al*. (1986) and Abeer (1997).

From the previously mentioned results nearly all samples were containing high numbers of *Staphylococci*, which reflect poor sanitary conditions during manufacture and storage. The presence of *Staphylococcus* spp., in nearly all samples is probably as a result of the dominance of the genus on parts of the human body such as hands, nose, skin and clothing (Nwamaka and Chike, 2010).

***S. aureus* count:** *Staphylococci* can be divided into two groups according to the production of Coagulase enzyme, which is capable of coagulating blood plasma. The synthesis of this enzyme is

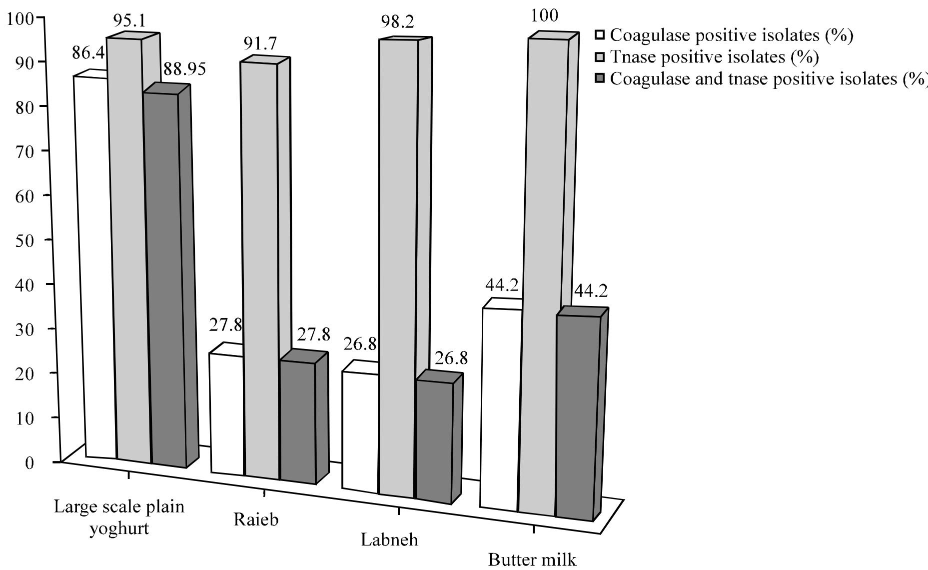

Fig. 2: Incidence of *S. aureus* depending on the results of coagulase and TNase tests

restricted to some species in the genus, among which *S. aureus*. The other *Staphylococci* that do not synthesize coagulase are referred to as Coagulase Negative Staphylococci (CNS) (Kloos and Bannerman, 1995; Koneman, 1997).

Regarding to the data presented in Fig. 2, it was obvious that the incidence of *S. aureus* in the examined samples of large scale plain yoghurt, Raieb, Labneh and Butter milk depending on the results of coagulase test were 70 (86.4%),10 (27.8%), 15 (26.8%) and 19 (44.2%), respectively, while depending on the results of TNase test were 77 (95.1%), 33 (91.7%), 55 (98.2%) and 43 (100%), respectively. It was found that the highest incidence depending on both tests was 68 (83.95%) in Large scale plain yoghurt samples, while the lowest incidence was 15 (26.8%) in Labneh samples.

Coagulase positive *S. aureus* is considered the most important species of *Staphylococci* due to its pathogenisity and enterotoxin production which cause food intoxication (Abeer, 1997; ICMSF, 1982).

Total yeast and mold count: Yeast and mold are widely distributed in the environment, as they grow at wide range of temperature and acidity, therefore, fermented milks are considered as an excellent medium for their growth and multiplication causing spoilage due to their lipolytic and proteolytic activity that might cause musty and other undesirable off flavor. Moreover, some species of yeasts and molds constitute a public health hazards (Comi *et al*., 1982; Hassan *et al*., 1994).

Total yeast count: Data depicted in Table 2 revealed that the highest contamination level was found in the samples of Butter milk (100%), followed by Labneh (70%) and Large scale plain yoghurt (46.7%), while the lowest level was recorded in Raieb samples (40%), with a mean values of $23\times10^2\pm7.8\times10^2$, $69.2\times10^2\pm26.4\times10^2$, $42.1\times10^3\pm26.4\times10^2$ and $42.1\times10^3\pm58.5\times10^2$ CFU g^{-1} in examined Large scale plain yoghurt, Raieb, Labneh and Butter milk samples, respectively. Nearly similar results of Large scale plain yoghurt were reported by Bahout and Moustafa (2003) and Ayah (2010), higher findings were obtained by Abeer (1997), Khan *et al*. (2008) and

Okonkwo (2011), while lower findings were recorded by Al-Tahiri (2005) and Eman (2007). Nearly similar outcomes of Raieb samples were reported by All and Dardir (2009), while higher findings were recorded by Sabreen (2001). Lower findings of Labneh samples were reported by Al-Tahiri (2005) and Al-Khatib and Al-Mitwalli (2009).

Yoghurt at the point of sale should not contain above 100 viable yeast cells mL^{-1}, while above 1000 yeast cells mL^{-1} would be judged as unsatisfactory (Davis *et al.*, 1971). In addition, according to Egyptian standards (EOS, 2005) the count of total viable yeast and mold in yoghurt samples should not exceed 100 cells g^{-1}. According to the obtained results, 46.7% of Large scale plain yoghurt samples were disagree with this standards.

Total mold count: Although the presence of mold in yoghurt constitute a serious economic losses because it associated with a visible spoilage, off flavor, discoloration and rejection of the product but also isolation of some species have raised the possibility that contaminated fermented milk samples could be source of mycotoxins which were implicated in outbreaks of human food poisoning and many several diseases such leukemia, cancer and kidney toxicity (Bullerman, 1980; Robinson, 1990).

Regarding to the data presented in Table 2, it was evident that the highest contamination level was observed in the examined Labneh samples 20 (66.7%), then the samples of Raieb and Butter milk which nearly at the same level of contamination 18 (60%) and 17 (56.7%), respectively, while the samples of large scale plain yoghurt was contaminated at a level of 12 (40%), with a mean values of $750\pm3.1\times10^2$, $27.2\times10^2\pm7.1\times10^2$, $21.2\times10^3\pm61.1\times10^2$ and $21.7\times10^3\pm62.9\times10^2$ CFU g^{-1}. in the examined samples of Large scale plain yoghurt, Raieb, Labneh and Butter milk, respectively. Nearly similar findings of Large scale plain yoghurt were obtained by Ayah (2010), higher findings were obtained by (Deeb *et al.*, 2004; Ali *et al.*, 2004; Okonkwo, 2011), whereas lower finding were recorded by Tarakci and Kucukoner (2003), Al-Tahiri (2005) and Eman (2007). Nearly similar results of Raieb samples were reported by All and Dardir (2009), while higher findings were obtained by Sabreen (2001). Lower findings of Labneh samples were reported by Sahan *et al.* (2004), Al-Tahiri (2005) and Al-Khatib and Al-Mitwalli (2009).

The highest contamination level within the examined samples of yoghurt was due to contamination from air day old culture used for yoghurt manufacture (Con *et al.*, 1996) and this agreed with Yaygm and Kilic (1980) who showed that yoghurt made from pure culture has no growth of yeast and mold up to 4 days of storage. Warmer weather and inadequate refrigeration are the principal causes of higher levels of contamination (Moreira *et al.*, 2001).

The fungi in commercial fermented milk samples generally correspond to poor cleaning practices and the use of unhygienic techniques or inadequate storage conditions. Montagna *et al.* (1998) and El Bakri and El Zubeir (2009), while Abou-Donia *et al.* (1980) attributed the contamination with yeast and mold in Egypt to post production contamination.

Incidence of *Yersinia enterocolitica*: *Yersinia* failed to be detected in all of the examined fermented milk samples, while (Hamama and Bayi, 1991; Hamama *et al.*, 1992; Abeer, 1997; All and Dardir, 2009) could isolate the organism from different types of fermented dairy products. The lower incidence of *Yersinia* in fermented dairy products may be explained by the low pH and the proper cooling which can inhibit the growth of such organisms (Moustafa *et al.*, 1983; Northolt, 1983; Robert, 1990).

CONCLUSION

From the obtained results we can conclude that a large portion of Egyptian fermented milk products exposed for sale in Cairo, Giza and El Monofiagovernorates are of poor hygienic quality due to the neglected sanitary measures adopted during their production, handling and distribution. Therefore, to safeguard the consumers from being infected and to save a lot of products from being spoiled on the market, the following suggestions may be considered:

- Public awareness targeting dairy factories and households that produce dairy products should encourage and help them to follow strict hygienic control measures during manufacturing, handling, transportation and storage
- Measures should be taken to interrupt the transmission of pathogens to fermented foods at both the household and commercial levels. At the commercial level, improvement of product quality and safety could be achieved by applying Good Manufacturing Practices (GMPs) and the Hazard Analysis and Critical Control Point (HACCP) system
- Application of Food Safety Management System eg. ISO 22000: 2005 in dairy plants

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