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Microbiological characterization of the Egyptian soft white cheese and identification of its dominant yeasts

Mohamed I. Hegazy* and S. A. Mahgoub

Department of Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt.

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We conducted this study to evaluate the differences in microflora and physicochemical properties of some traditionally manufactured soft white cheeses upon purchase from Zagazig city, Egypt, in 2010 and 2011. A total of 168 samples were analyzed for both spoilage (total viable count, lactic acid bacteria, yeasts and moulds, staphylococci, *Enterobacteriaceae*, and coliform group) and pathogenic (*Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and fecal Streptococci) microorganisms. Physicochemical analyses showed low levels of pH and high levels of salt. Two cheese samples were unsatisfactory due to levels of *Staphylococcus aureus* at 4.00 log cfu/g, and fecal streptococci at 4.3 log cfu/g. Despite the much lower spoilage microorganisms counts in the pasteurized cheeses, soft Feta of dairy M showed the highest contamination level of 4.11 and 3.72 log cfu/g of total viable count and staphylococci, respectively. Fifty-two isolates of the yeasts were identified using the physiological and biochemical tests, and were classified into seven species. Ten of the species were selected for identification by sequencing the 26S rRNA, where nine of them were identical to the phenotypic identification. These results emphasize the need for applying more strict hygienic practices especially in thermized cheese processing to minimize microbial contamination.

Key words: Egyptian soft white cheese, spoilage microorganisms, pathogenic bacteria, yeast identification.

INTRODUCTION

The provision of plenty good quality food is of primary importance in the modern world. Microbial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, feces and grass (Oliver et al., 2005; Yagoub et al., 2005; Coorevits et al., 2008). The presence of food-borne pathogens in milk can originate from direct contact with contaminated sources in the dairy farm environment and/or the excretion from the udder of an infected animal (El-Zubeir et al., 2006). There have been outbreaks of infections associated with the consumption of cheese, and the predominant organisms included *Salmonella, Listeria monocytogenes, Escherichia coli*, and *Staphylococcus aureus* (Zottola and Smith, 1991; De Buyser et al., 2001).

In Egypt, soft white cheese is manufactured from cow or buffalo milk or a mixture of them according to the Egyptian cheese-making technology. Production may be artisanal or industrial, depending on whether the cheeses

*Corresponding author. E-mail: mhegazy7@hotmail.com. Tel: +201111032288.

are manufactured with raw thermized (heated below pasteurization level) or pasteurized milk. The microbiological quality of cheese is influenced by equipments and environmental hygiene during manufacturing, packaging and handling (Robinson and Tamime, 2002). Some microorganisms, including lactic acid bacteria, yeasts, moulds and coliforms have been isolated from dairy products (Gadaga et al., 2000). Soft cheeses are available in Asia, Africa, the Middle East, and Northern and Eastern Europe. The microbiological characteristics of milk products have been studied in Indonesia (Hosono et al., 1989), Africa (Hamama, 1992; Beukes et al., 2001; Mathara et al., 2004) and Inner Mongolia, China (Shuangquan and Miyamoto, 2004). The implementation of ‘‘Good Manufacturing Practices’’ in the production of traditional cheese is fundamental for preventing contamination. The ‘‘Good Manufacturing Practices’’ are regulations, concepts and procedures that aim at promotion and certification of quality in services, processes, and products (Lima et al., 2008). So far, there is no comprehensive study on the microbial evaluation and comparison of the Egyptian soft white cheeses from different dairies, and identification of its dominant yeasts. Therefore, the aim of this study was (1) to evaluate the microbiological and physicochemical criteria of the retail Egyptian soft white cheeses, and (2) to identify and characterize the yeasts associated with it.

MATERIALS AND METHODS

Source of cheese samples

This study was conducted during the summer season of 2010 and confirmed at 2011. The cheese samples (~ 250 g) were purchased from different retail markets at different locations in Zagazig City, Egypt, pertaining to 18 of the common Egyptian Dairies as shown in Table 1. In dairies A through K, the soft and semi hard cheeses were produced from thermized milk and undefined ripening cultures, whereas in dairies L through R, the soft cheeses were produced from pasteurized milk, defined ripening cultures, and contained potassium sorbate as food preservative. About 5 to 15 samples were collected randomly from each company during the period of study. A total of 168 samples were collected and transported in ice boxes for analyses in the Laboratories of Microbiology Department, Faculty of Agriculture, Zagazig University. The microbiological quality of soft cheeses was assessed using the criteria of the European Commission recommendations (EC, 2004a, b) as shown in Table 2.

Physicochemical analyses

The pH values of the products were determined in accordance with AOAC (2005). Briefly, 25 g of the product sample were blended with 225 ml sterile buffered-peptone-water (BPW) for 60 s. A pH meter (ORION 720A, Boston, USA) was used for the measurement. Moisture content was determined according to AOAC (2005) using the oven drying method. The difference in weight before and after drying at 100˚C for 1 h was recorded. Salt content was determined according to AOAC (2005). 10 g of cheese sample was mixed and stirred with 15 ml of warm water. 25 ml of distilled water was added and mixed, then completed to 100 ml, and filtered to collect a filtrate of 50 ml. 25 ml of the filtrate was mixed with 1 ml of potassium chromate indicator, and then titrated with 0.1 N $AgNO_3$ to the first visible pale red-brown color lasting for 30 s.

Microbiological analyses

The microbial analyses followed the procedures outlined in Marshall (1993). The samples were examined for total viable counts (TVC), lactic acid bacteria (LAB), yeasts and moulds (Y&M), Staphylococci (Staph.), *Enterobacteriaceae* (ENT), total coliform group (TCF), *S. aureus*, and fecal streptococci (FSC), while *Salmonella* spp. and *E. coli* were detected by enrichment technique according to the procedure outlined in Anonymous (1998) and ISO (2001). The samples (25 g) were transferred aseptically to a stomacher bag (Sewared, London, UK), and homogenized with 225 ml of sterile peptone solution (0.1% w/v) for 60 s at room temperature. Serial dilutions of peptone water (0.1% w/v) were prepared and duplicate of 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread on selective or non-selective agar plates. The following microbial counts and growth conditions were used: total viable count enumerated on plate count Agar (Merck, 1.05463) at 30°C for 48 h; lactic acid bacteria grown on de Man, Rogosa, Sharpe (MRS; Biolife) overlain with 5 ml of the same medium at 25°C for 72 h; yeasts and moulds grown on Rose Bengal Chloramphenicol Agar (Lab M, 36, supplemented with chloramphenicol, X009) at 25°C for 5 days; staphylococci determined on Baired Parker agar (Biolife) supplemented with egg yolk, at 37°C for 48 h; *S. aureus* detected by examining the plates of Baired Parker for typical black, convex colonies with a light halo, which were tested for positive coagulase reaction (Bactident Coagulase, Biolife); coliform group enumerated on Violet-Red Bile agar at 35°C for 24 h; *Enterobacteriaceae* counted on Violet-Red Bile Glucose Agar, using a double layer of the medium at 35°C for 24 h (Christen et al., 1992); fecal streptococci grown on Kanamycin Aesculin Azide Agar (LAB M 106) at 37°C for 48 h; counting the black colonies as fecal streptococci.

E. coli was detected using the method described in ISO 16654 (2001). In brief, the samples (25 g) were added to 225 ml volumes of buffered peptone water (BPW) (Merck), and then incubated at 37°C for 24 h. Following incubation, 0.1 ml of the previous BPW was spread onto Tryptone Bile Glucuronide (TBX) Agar and streaked on TBX Agar, incubated at 37°C for 4 h, and directly incubated at 44°C for 37 h. The detection of *Salmonella* spp. was achieved by suspending 25 g of the products into 225 ml buffered peptone water (Merck), and then incubated at 37 °C for 24 h (ISO 6579, 1991). Following incubation, 0.1 ml of each BPW incubation was transferred into culture tubes containing 10 ml of Rappaport Vassiliadis (RV) enrichment broth and tubes were incubated again at 42 °C for 24 h. The culture was then streaked on Xylose Lysine Deoxycholate (XLD) Agar (Merck, 1.05287), and incubated at 37°C for 24 h.

Yeast isolation and phenotypic identification

The yeasts were isolated and characterized according to Rohm et al. (1992). The isolates were identified using the computer program of Barnett (1985). Additional biochemical tests and morphological criteria (budding/splitting of cells and formation of pseudomycelium,

Table 1. Types of cheese samples.

Milk type	Packaging	Type of cheese	Number of samples	Dairy name	Ripening culture
Thermized	Cut to order	Soft Feta cheese	9	A	Undefined
	Cut to order	Soft Feta cheese	7	B	Undefined
	Cut to order	Semi-hard Old cheese	10	C	Undefined
	Cut to order	Semi-hard Old cheese	12	D	Undefined
	Cut to order	Semi-hard Old cheese	7	E	Undefined
	Cut to order	Semi-hard Old cheese	8	F	Undefined
	Cut to order	Soft Storage cheese	11	G	Undefined
	Cut to order	Soft Karish cheese	9	H	Undefined
	Cut to order	Soft Tallaga cheese	7	I	Undefined
	Cut to order	Soft Tallaga cheese	5	J	Undefined
	Cut to order	Soft Tallaga cheese	10	K	Undefined
Pasteurized	Packaged	Soft Feta with palm oil (Microbial rennet)	12	L	Defined
	Packaged	Soft Feta with palm oil (Cow rennet)	6	M	Defined
	Packaged	Soft Feta with palm oil (Cow rennet+ microbial culture)	15	N	Defined
	Packaged	Soft Feta with palm oil (Cow rennet + microbial culture)	7	O	Defined
	Packaged	Soft Barameli cheese (Microbial rennet)	9	P	Defined
	Packaged	Soft Tallaga cheese (Microbial rennet)	11	Q	Defined
	Packaged	Soft Istanbuly with palm oil cheese (Microbial rennet)	13	R	Defined
Total			168		

true hyphae or arthroconidia) were taken into account when strains gave poor identification scores. Traditional physiological characterization was performed at the Laboratories of Micro-biology Department, Faculty of Agriculture, Zagazig University.

Identification of selected strains by sequencing of the 26S rRNA

Ten species, identified using phenotypic approach, were selected for molecular-based identification by sequencing the 26S rRNA. Isolation and purification of DNA were performed using the hexadecyl trimethyl (CTAB) method (Messner et al., 1994). The purpose of such molecular-based identification was to confirm the fidelity of the phenotypic identification. Two primers ITS3p (5`GCATCGATGAAGAACGC AGC3`) and NL4 (5`GGTCCGTGTTTCAAG ACGG3`) were used to amplify a 1 kb fragment of the D1/D2 domain of 26S rRNA according to Lopandic et al. (2004). The PCR program was set as follows: 30 cycles of 98°C/15 s, 59°C/ 60 s, and 72°C/90s, with a final extension of 72°C/ 10 min. A fragment of 600 bp was sequenced using the NL1 (5`GCATATCAATAAGCGG AGGAAAAG3`) and NL4 primers (White et al., 1990). The sequence was used in a BLAST search in the GenBank, and the identification was based on a minimum identity score of 99%.

Statistical analysis

The data from microbiological analyses were entered into Excel and transformed into log cfu/g for all experiments. The numerical results are presented in the tables as means and standard deviations (SD) using the SPSS program for Windows, version 11.0 (SPSS Inc., Chicago, IL, USA) with “n” being the number of cheese samples.

RESULTS AND DISCUSSION

Physicochemical quality of the soft white cheese

Analyses of some physicochemical properties of cheese, namely, pH, salt content, and moisture content are shown in Table 3. There were fairly wide ranges of compositional differences, which seem to indicate

Table 2. Classification of retail cheese as recommended by EC microbiological criteria in 2004/24/EC and 852/2004 /EC.

Microorganism	Microbiological quality (cfu/g)		
	Satisfactory	Borderline	Unsatisfactory
E. coli			
Thermized milk	$< 10^4$	10^4 –$<10^5$	$\geq 10^5$
Pasteurized milk	$<10^2$	10^2–$<10^3$	$\geq 10^3$
S. aureus			
Thermized milk	$<10^3$	10^3–$<10^4$	$\geq 10^4$
Pasteurized milk	$<10^2$	10^2–$<10^3$	$\geq 10^3$
Salmonella spp.	Not detected	-	Detected

Table 3. Mean values ± standard deviations of physicochemical analyses data of the white cheese from dairies A to R.

Milk type	Dairy	pH	Salt (%)	Moisture (%)
	A	4.93±0.06	10.92±0.09	54.74±0.21
	B	4.25±0.07	10.33±0.08	56.14±0.22
	C	4.75±0.10	12.53±0.11	51.56±0.23
	D	4.52±0.10	11.89±0.03	53.44±0.32
	E	4.61±0.09	8.81±0.08	59.64±0.31
Thermized	F	4.30±0.11	12.44±0.07	68.04±0.24
	G	4.80±.012	8.11±0.08	50.21±0.25
	H	4.55±0.07	8.00±0.11	65.45±0.21
	I	4.53±0.08	8.78±0.09	63.14±0.22
	J	4.77±0.08	12.07±0.07	50.8±0.33
	K	4.87±0.07	14.08±0.21	55.64±0.12
	L	4.68±0.08	12.83±0.31	56.26±0.26
	M	4.97±0.07	11.96±0.11	61.32±0.29
	N	4.93±0.11	12.61±0.23	60.76±0.44
Pasteurized	O	4.91±0.13	10.86±0.32	62.05±0.21
	P	4.68±0.08	11.98±0.11	55.07±0.14
	Q	4.63±0.07	10.45±0.21	46.76±0.16
	R	4.09±0.06	13.06±0.11	57.31±0.15

different soft cheese manufacturing practices in making soft cheeses (Abou-Donia, 1991). In general, the pH values for all types of cheeses ranged from 4.09 to 4.97, being in harmony with previous data reported for Egyptian soft cheese (Ayad, 2009). Salt content was very high in all cheese samples as expected, with obvious variability within the same type of cheese, for example, Tallaga cheeses produced by dairies I, J and K, had 8.78, 12.07, and 14.08%, respectively. It seems that such a high salt concentration completely inhibited *Salmonella* spp. (Table 5), confirmed by the results obtained by Papadopoulou et al. (1993) as they studied the effect of different manufacturing processes of Feta cheese on *Salmonella enteritidis*. Abou-Donia (1991) also found that increasing salt level to 100 g/L was enough to destroy *Salmonella typhi* in 16 days. Even though most of the cheese-associated outbreaks is reported from unpasteurized or improperly pasteurized milks, white cheeses are not commonly involved in such outbreaks (Anonymous, 1998) most likely because of the high salt

Table 4. Mean values ± standard deviations for spoilage microbial counts (log cfu/g) of white cheeses from dairies A to R.

Milk Type	Dairy	Mean log (cfu/g)		Mean log (cfu/g)		Mean log (cfu/g)		Mean log (cfu/g)		Mean log (cfu/g)		Mean log (cfu/g)	
		TVC	SD	LAB	SD	Y&M	SD	Staph.	SD	ENT	SD	TCF	SD
	A	7.56	1.12	6.15	0.64	4.51	0.86	3.06	0.51	3.46	0.06	1.39	0.12
	B	4.88	0.33	2.65	0.07	2.80	1.13	3.48	2.09	ND	ND	ND	ND
	C	5.72	0.95	3.31	0.32	3.11	0.30	2.24	0.34	ND	ND	ND	ND
	D	6.60	2.05	4.36	1.12	3.73	1.76	2.15	0.21	2.74	0.80	1.74	1.04
	E	5.96	1.17	5.13	0.80	5.05	0.54	2.39	0.12	ND	ND	ND	ND
Thermized	F	4.86	0.03	3.54	0.09	4.11	0.76	1.24	0.34	ND	ND	ND	ND
	G	6.54	0.33	6.31	0.32	2.65	0.07	3.21	0.22	ND	ND	ND	ND
	H	7.42	0.38	7.44	0.13	5.67	0.44	4.21	0.67	4.40	0.14	2.58	0.06
	I	7.20	0.16	6.91	0.14	4.88	1.18	3.88	0.10	ND	ND	ND	ND
	J	7.03	0.12	6.15	1.20	5.13	0.07	2.58	0.67	3.67	0.79	1.54	0.76
	K	7.83	0.03	6.71	0.08	3.89	0.58	3.67	0.09	3.36	0.40	1.19	1.26
	L	3.99	0.78	3.52	0.74	1.15	0.21	3.42	0.60	ND	ND	ND	ND
	M	4.11	0.39	2.45	0.21	1.85	0.21	3.72	0.17	ND	ND	ND	ND
	N	2.98	0.31	2.39	0.12	2.22	0.11	2.54	0.09	ND	ND	ND	ND
Pasteurized	O	3.62	0.49	1.52	0.74	1.15	0.21	2.75	0.21	ND	ND	ND	NDD
	P	2.10	0.07	1.80	0.28	1.80	0.71	2.54	0.09	2.68	0.67	2.01	0.15
	Q	3.13	0.07	2.59	0.16	1.00	0.00	2.56	0.73	ND	ND	ND	ND
	R	3.93	0.06	3.44	0.11	1.98	1.38	1.15	0.21	ND	ND	ND	ND

TVC, total viable count; LAB, lactic acid bacteria; Y&M, yeasts and moulds; Staph., Staphylococci; ENT, *Enterobacteriaceae*; TCF, total coliform; ND, not detectable.

content and low pH (Bintsis and Papademas, 2002). As for the moisture content, it was relatively higher in Feta cheese as compared to the other types, and generally ranged for all cheeses from 46.76 to 68.04%. Similar results were found by Ayad (2009).

Microbiological quality of soft cheese

The results of microbial counts (total viable count, lactic acid bacteria, yeasts and moulds, staphylococci, *Enterobacteriaceae*, and total coliform group) of the cheese samples are presented in Table 4. In general, total microbial counts in the pasteurized soft cheeses (3.41 log cfu/g) were lower than those of the thermized soft cheeses. The mean log counts of TVC ranged from 4.86 to 7.83 log cfu/g, and 2.10 to 4.11 log cfu/g for thermized and pasteurized cheeses, respectively. The numbers of LAB in the cheese samples were higher in thermized cheeses (2.65 to 7.44 log cfu/g) than those in pasteurized cheeses (1.52 to 3.52 log cfu/g). The results suggest that in thermized and unpackaged cheeses, the higher total bacterial counts may be related to having little hygiene practices, such as handling and cut-to-order at room temperature. Thermized cheeses had higher numbers of yeasts and moulds (2.65 to 5.67 log cfu/g) than those in pasteurized cheeses (1.0 to 2.22 log cfu/g). In this study, it is obvious that in addition to the low heat treatment, the low pH values (4.09 to 4.97), moisture content, storage temperature and high salt level (Table 3) during the ripening of these cheeses contribute to the growth of yeasts in thermized cheese. These results are in agreement with those of Beresford et al. (2001). Coliform group and *Enterobacteriaceae* were not detected in all the cheese samples, except those from dairies A, D, H, J, K and P. The numbers of staphylococci, *Enterobacteriaceae*, and coliform group were lower in pasteurized cheese samples than those in thermized cheese samples. The low coliform numbers were probably due to the high salt content (8 to 14.08%), while, staphylococci and yeasts numbers were obviously high, which could be due to their inherent salt tolerance and survival at low pH values (Olerta et al., 1999; Turkoglu et al., 2003). Additionally, soft Feta (A, B, L to

Table 5. Prevalence of pathogenic bacteria of retail soft cheese from dairies A to R.

Milk Type	Dairy	*Salmonella* spp.		*E.coli*		*S. aureus*		Fecal streptococci	
		Number of positive samples	Log number cfu/g	Number of positive samples	Log number cfu/g	Number of positive samples	Log Number cfu/g	Number of positive samples	Log number cfu/g
Thermized	A	0	ND	1	1.7	1	1	0	ND
	B	0	ND	0	ND	0	ND	0	ND
	C	0	ND	1	1	1	2	0	ND
	D	0	ND	1	1.3	0	ND	0	ND
	E	0	ND	0	ND	0	ND	1	2.48
	F	0	ND	0	ND	0	ND	0	ND
	G	0	ND	0	ND	0	ND	0	ND
	H	0	ND	1	1.48	1	2	1	4.3*
	I	0	ND	0	ND	0	ND	0	ND
	G	0	ND	1	1.3	0	ND	2	3.3
	K	0	ND	0	ND	1	4*	2	2.48
Pasteurized	L	0	ND	0	ND	1	3	0	ND
	M	0	ND	0	ND	0	ND	0	ND
	N	0	ND	0	ND	0	ND	0	ND
	O	0	ND	0	ND	0	ND	0	ND
	P	0	ND	0	ND	0	ND	0	ND
	Q	0	ND	0	ND	0	ND	0	ND
	R	0	ND	0	ND	0	ND	0	ND

*Unsatisfactory retail cheese according to recommendations of 2004/24/EC and 852/2004 /EC; ND, not detectable.

O) and Istanbully white cheese (R) had relatively high salt and low moisture contents (Table 3). The counts of *Enterobacteriaceae* and coliform group in soft thermized cheese samples (11 dairies) ranged from 2.74 to 4.40 and 1.39 to 2.58 log cfu/g, respectively. However, in pasteurized cheese samples, *Enterobacteriaceae* and coliform group were detected only in dairy “P” with low number (2.68 and 2.01 log cfu/g, respectively). Such low numbers are most likely due to the pasteurization effect, the higher salt content (Table 3) and potassium sorbate in pasteurized cheese samples. The presence of staphylococci, *Enterobacteriaceae* and coliform group in the thermized cheeses may be due to the insufficient heat treatment of the raw milk during cheese manufacturing and/or contamination of the product during storage, cut-to-order at sale point. Moreover, staphylococci and *Enterobacteriaceae* numbers did not exceed 4 log cfu/g for all samples except for the cheeses produced by dairy H, where they reached up to 4.21 and 4.40 log cfu/g, respectively. In Caciocavallo Silano, southern Italy, a ripened and semi-hard cheese made from bovine raw milk, showed *Enterobacteriaceae* numbers ranging from 5.0 to 6.1 log cfu/g (Corsetti et al., 2001). Those numbers were higher than in the current study. Corsetti et al. (2001) mentioned that the staphylococci and enterococci in Caciocavallo Silano could be considered as microflora derived from the raw milk, the environment, or the manufacturing technology. Giraffa (2002) also stated that the presence of enterococci in dairy products may be due to insufficient sanitary conditions during the production and milk processing. These studies indicate that the reason for the high microbial counts in the thermized cheese samples should be due to the use of raw milk, insufficient hygienic conditions during the manufacturing, storage, and cut-to-order at sale point. *Salmonella* spp. was not detected in any sample examined (Table 5). Based on the EC microbiological criteria (EC, 2004a, b) shown in Table 2, 166 of the cheese samples were of microbiological satisfactory level, while 2 of them were of unsatisfactory level due to the high counts of *S. aureus* and fecal streptococci (4 and 4.3 log cfu/g, respectively). The presumptive *E. coli* ranged from 1.3 to 1.7 log cfu/g (Table 5).

Yeast identification

Fifty-two out of the 95 yeast isolates obtained from the different types of soft cheeses were identified using the phenotypic approach according to Rohm et al. (1992). These isolates showed either identical or single difference from the data found in the description of the species. The phenotypic identification resulted in three isolates identified as *Candida zeylanoides*, nine as *Debaryomyces hansenii*, six as *Kluyveromyces marxianus*, nine as *Pichia anomala*, 13 as *Saccharomyces cerevisiae*, six as *Candida inconspicua*, and six as *Issatchenkia orientalis*. To confirm the results of the phenotypic identification, ten species were selected for molecular-based identification by sequencing the 26S rRNA. The species used for sequencing were two species of each of *S. cerevisiae* (proved to be strain HA 817 and HA 820), *P. anomala* (HA 814)*, D. hansenii* (HA 764), and one strain of each of *K. marxianus* (HA 810), *I. orientalis* (HA 803), *C. inconspicua* (HA758), and *Candida zeylanoides* (HA 755). All the molecular strain identifications coincided with those obtained by the phenotypic approach except for one of the *D. hansenii* which turned out to be *S. cerevisiae* (HA 823). Such discrepancy between the phenotypic approach and molecular-based identification is not unexpected since the phenotypic characteristics are generally not as accurate as the genotypic method (Clarridge, 2004).

These results indicate the significance of applying strict hygienic practices during the manufacturing of the soft white cheese, and probably necessitates the pasteurization of the used milk rather than just heating below pasteurization level. It also shows the need for more Governmental surveillance on the cheese manufacturers and the cheese products in the markets.

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