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Research Article

Prevalence of Thermophiles and Mesophiles in Raw and UHT Milk

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Abstract: The objective of the study was to evaluate the contamination level of cow's raw milk and different brands of UHT milk in Baghdad local market for thermophilic and mesophilic bacteria. The numbers of colony counts in milk samples were determined by the culture method according to bacteriological standards methods. Investigations were carried out for seven weeks in college of veterinary dairy farms from March 2013 to May 2013. Six (29%) positive samples out of 21 samples were tested for thermophiles in raw milk while 36 (100%) milk samples of Ultra High Temperature (UHT) were positive for thermophiles. Twenty one (100%) of mesophiles in raw milk samples were positive while in UHT milk samples only 18 (50%) out of 36 were positive. Overall the means of mesophiles have more significant counts differences ($p<0.05$) than thermophiles in raw milk samples. In UHT brands indicated that the results revealed high numbers of thermophiles in Nada and KDD which indicated that these brands were low quality. Moreover the values of these brands have significant differences ($p<0.05$) comparing with the UHT milk brands Al-Maraee and Al-Safi. In the same time the UHT brands were shown high numbers of mesophiles in Nada and Al-Safi which indicated that these brands have significant differences ($p<0.05$) comparing with Al-Maraee and KDD. This study was concentrated on milk contamination occurred after milk producing as well as processing, transport and store of UHT.

Keywords: Cow, high temperature, raw milk

INTRODUCTION

An increase in the counts of bacteria in cow's raw milk is problematic because they produce thermo-resistant extracellular proteolytic and lipolytic enzymes that pose a qualitative risk during milk processing and cause the spoilage of the final products during storage (Chen *et al*., 2003). In raw and UHT milk, some faults might be seen due to the usage of microbiologically or physio-chemically abnormal raw milk and improper processing. In the dairy industry, the thermophilic bacilli are usually enumerated using an Aerobic Plate Count (APC) incubated at 55°C (Scott *et al*., 2007). Spore-forming microorganisms have a special position among total microflora of milk with regard to their greatest ability to survive thermal treatment of milk and subsequently to propagate in the final products (Abo-Elnaga *et al*., 2002). The thermophilic bacilli are potential contaminants in a variety of industries such as dairy product manufacture (Janštová *et al*., 2006) where elevated temperatures (40-65°C) prevail during the manufacturing process or when product is stored (Cempirkova, 2007).

The facultative thermophiles belong to the Bacillus genus and tend to grow at both mesophilic and thermophilic temperatures, depending on the strain. Some examples of species include *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus pumilus*, *Bacillus sporothermodurans* and *Bacillus subtilis* (Scheldeman *et al*., 2005). Although these contaminants do not constitute a health risk to the consumer but they are used as an index of hygienic measurements. Accordingly, dairy plants adopt specification limits for thermophile counts in their products and practice strict hygiene standards to meet them, in order to guarantee the marketability of their products (Rueckert *et al*., 2004). Spore-forming microorganisms were either strictly anaerobic (SPAN)-genus *Clostridium*, which caused problems mainly in long lasting ripening of cheeses, or they were facultatively anaerobic; it means that they were grown under aerobic as well as anaerobic conditions-genus *Bacillus*, which was characteristic by broad complex of physiologic variants that were reflected in a variety of mesophilic, thermophilic and psychrophilic species with the high taxonomic diversity (Scheldeman *et al*., 2006). These spores can survive in products given heat treatment (De-Jonghe *et al*., 2010) and by undesired growth can cause defect such as sweet curdling, coagulation and diarrhea and emetic toxin production (Stenfors Arnesen *et al*., 2008).

The transit time of the milk during processing is usually less than 30 min and growth of thermophilic bacilli is not possible in all sections of the processing line due to restrictive temperatures and/or viscosities of

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the product stream. Thus, significant growth in thermophile numbers cannot occur in the milk being processed but is thought to originate from bio-films (Langeveld *et al*., 1995; Parkar *et al*., 2003, 2004) in the process line in area suitable for growth-hence the emphasis on clean in place (CIP). Enumeration of Mesophilic Aerobes (MA) is the main quality and hygiene parameter for raw and pasteurized milk. High levels of these microorganisms indicate poor conditions in production, storage and processing of milk and also the presence of pathogens (Freitas *et al*., 2009). This study documented the numbers of mesophiles and thermophiles of the raw and imported UHT milk samples consumed in Baghdad.

MATERIALS AND METHODS

Media: Plate count agar (Oxoid) was weighed 23 g dissolved into 1litre of distilled water, then on hot plate with magnetic stirrer was dissolved completely until homogenized. The medium pH was adjusted to be 7.0±1 with either NaOH or HCl. Plate count agar was sterilized by autoclave at 121°C for 15 min. The agar was cooled down to 45°C in water bath and then poured into sterile Petri dishes with appropriate sample dilution or on Petri dishes agar plates were left to set out then label and kept in refrigerator until use.

Broth: Peptone water broth (Oxoid) was weighed 1g dissolved into 1 L distilled water, homogenized with hot plate and magnetic stirrer. Peptone water broth dispensed 9 mL into universal test tubes, then autoclaved at 121°C for 15 min. The broth in test tubes were left to cool down then kept in refrigerator and used as a diluent for milk samples.

Milk samples: Twenty one raw milk samples were collected randomly at weekly intervals (3 samples/week) for seven weeks from the animal field of the college of veterinary medicine. Thirty six imported UHT milk samples that distributed from different local markets in Baghdad were collected randomly at weekly intervals from four different brands for three weeks (3 samples/week/brand). All samples were collected in sterile 250 mL sterile plastic bags and transported to the laboratory in ice-cooled box. Microbiological analysis was performed to determine the average counts of thermophilic and mesophilic organisms with a special emphasis on determining the prevalence level of each of them in both raw and imported UHT milk.

All 21 raw milk samples were examined for the isolation and identification of *B. cereus* by heating to 80°C for 12 min and cooled down to room temperature and then diluted to (10^{-1}-10^{-2}) by peptone water (Oxoid) 0.1% (wt/v) using 10-fold serial dilutions.

Method: All Milk samples were serially diluted 10^{-1}-10^{-5} in peptone water (0.1% wt/v) and poured plated on plate count agar. All plates were incubated aerobically at 37°C for 24 h for mesophiles and at 55°C for 48 h for thermophiles. The colonies were counted after incubation using colony counter.

Statistical method: Simple student *t-test* was used for data analysis and the significant differences were determined at ($p<0.05$). The statistical analysis of the data was performed by F test analysis (ANOVA) and then the least differences (LSD) were conducted to find the significant differences among the different mean values.

RESULTS AND DISCUSSION

The prevalence level of both the thermophilic and mesophilic organisms in both raw milk and imported UHT is shown in Table 1. Out of 21 samples were examined only 6 (29%) samples were positive for thermophiles from raw milk while all the 36 imported UHT milk samples examined (100%) were positive for thermophiles (Table 1). out of 36 were positive imported UHT milk samples examined only 18 (50%) samples were positive for mesophiles while all the 21 raw milk samples examined (100%) were positive for mesophiles. Considering that this group of microorganisms is not eliminated by the thermal treatment of milk before processing to milk foodstuffs, it must be kept in mind, that also seemingly low mesophilic counts may have serious negative consequences for quality and storability of milk foodstuffs (Banykó and Vyletělová, 2009).

The laboratory studies of the cultural isolation during the period of the study revealed that there was a significant ($p<0.01$) differences in the percentages of thermophiles and mesophiles between the raw and UHT milk samples, when the highest prevalence level of thermophiles was found in UHT milk while the highest prevalence level of mesophiles was found in raw milk. The unhygienic practices and poor sanitation techniques in the production of milk were reflected on the highest significant ($p<0.01$) prevalence level of mesophiles (100%) in raw milk and of thermophiles (100%) in imported UHT milk. These results were agreed with the results discussed by Foltys and Kirchnerová (2011) whom showed that the thermophiles and the mesophiles were associated with the quality of the milk even the milk was treated with high temperature. Loose slatted-floor litter-less housing was connected with the insufficient environmental hygiene and with the subsequent higher fouling of dairy cows, which was reflected, together with a failure to observe the hygienic principles of milking process, in the highest milk contamination by all groups of the investigated microorganisms. Nevertheless, the influence of deficiencies in herd management and milking on the microbial quality of milk was confirmed (Holm *et al*., 2004). Microbial populations in log cfu/mL of total

Table 1: The prevalence level of thermophilic and mesophilic bacteria in both raw and imported UHT milk

Bacterial types	Thermophiles		Mesophiles	
Type of milk sample	Raw milk	UHT Milk	Raw milk	UHT milk
No. of samples analysed	21	36	21	36
No. of positive samples	6	36	21	18
Incidence	29%	100%	100%	50%

Table 2: Microbial populations (log cfu/mL) of total thermophiles and mesophiles in cow's milk

Week	No. of samples examined	Counts (log cfu/mL) Mean±SE	
		Thermophiles	Mesophiles
1	3	2.392± 0.051	5.534±0.393
2	3	2.159±0.159	5.560±0.179
3	3	2.360±0.059	5.548±0.302
4	3	2.373±0.107	5.610±0.068
5	3	2.259±0.139	5.519±0.139
6	3	2.259±0.042	5.382±0.247
7	3	2.418±0.059	5.434±0.296
Total	21	2.317± 0.035	5.512±0.030

Differences between the columns of thermophiles and mesophiles counts in milk were significantly differences ($p<0.01$)

Thermophiles and Mesophiles in cow's raw milk were recorded. The mean total reading of thermophiles in 7 weeks was 2.317±0.035 cfu/mL while the mean of total reading in 7 weeks for mesophiles was 5.512±0.030 cfu/mL. There were significant differences ($p<0.01$) between mesophilic and thermophilic bacteria. Besides, there were no significant differences among the rows within the column of thermophiles or mesophiles (Table 2).

Moreover, the population of bacteria have been shown that the numbers of thermophilic were lower than the numbers of mesophilic bacteria (Table 2). In primaryproduction of raw milk the spore-forming microorganisms were originated from contaminated air, water, milking equipment, feed-especially silage, grass, soil and faeces. Their occurrence may be linked to differences in housing strategy (Coorevits *et al*., 2008).

Four brands (Al-Maraee, KDD, Al-Safi and Nada) of UHT milk samples were tested for thermophiles and mesophiles. The total of 36 UHT milk samples was examined for thermophiles in three weeks intervals (12 samples/week). The mean of thermophiles colonies counts of Al-Maraee UHT milk was 1.956±0.197 cfu/mL, while the mean of thermophiles colonies counts of KDD UHT milk sample was 2.477±0.042 cfu/mL. Al-Safi UHT milk thermophiles colonies counts mean were 2.187±0.046 cfu/mL. In case of Nada UHT milk sample the mean of thermophiles colonies counts were 2.526±0.051 cfu/mL. There was significant differences ($p<0.05$) among the results of Al-Maraee, KDD, Al-Safi and Nada but there were no significant differences among the results within the same product in the first, the second and the third weeks (Table 3). Also, these results indicated that the UHT brands have high numbers of thermophiles Colonies Forming Unit (cfu) such as Nada and KDD revealed low quality comparing with the UHT milk brands Al-Maraee and Al-Safi. So, even very law initial number of these bacteria in pasteurized milk during cooled storage will reach the critical level of 10^6-10^7cfu/mL within 10 days and contribute to milk spoilage (Eneroth *et al*., 1998). This level of bacterial contamination causes spoilage of pasteurized milk, which were associated with occur of bitter components, unclean flavour and shortening of shelf life.

Furthermore, it was evaluated the total number of UHT milk samples for mesophilic bacteria. The total of sample numbers were 36 for 3 weeks (12 samples interval/week). The mean of mesophiles in Al-Maraee UHT milk brand was 1.256±0.097 cfu/mL. The mean of mesophiles in KDD UHT milk brand was 1.556±0.111 cfu/mL. In Al- Safi UHT milk brand the mean was 3.117±0.017 cfu/mL. The means of mesophiles of Nada UHT milk brand was 5.265±0.390 cfu/mL (Table 4). There are significant differences ($p<0.05$) among the four brands of UHT milk mesophiles results but there are no significant differences ($p>0.05$) within the results of the same brands in the first, the second and the third weeks.

Additionally, spore-forming bacteria in raw milk were examined for 7 weeks. The total number of raw milk samples 21 (3 milk samples/week) were examined. The total of maximum mean of *B. cereus* was 2.882950 cfu/mL and the total mean of *B. cereus* was 1.933±0.180 cfu/mL (Table 5). The results in the present study were disagreed with the findings recorded in the investigations (TeGiffel *et al*., 1996) due to the different breed of cows, different environment and used different methods for the isolation of *B. cereus*.

Similar outcomes were found in the research performed by Griffiths and Phillips (1990) whom demonstrate that *B. cereus* spores in raw milk were the major source of *B. cereus*. *Bacillus species* have been isolated from 58% of raw milk samples tested and 94% of the positive milk samples was stored at 6°C for 14 days. Abd and Ali (year) (in process) recorded that fifty seven out of ninety bovine milk samples were collected from different areas inside and surround Baghdad were positive for *Bacillus cereus.*

In general these results indicated that the UHT milk brands have high numbers of mesophiles Colonies Forming Unit (cfu) such as Nada and Al-Safi which were indicated that these UHT products have had low quality values comparing with Al-Maraee and KDD UHT milk brands which were contained low numbers of mesophiles cfu and have considered as better quality values. The sterilization method was determined by the

Table 3: Microbial populations (Mean±SE log cfu/mL) of total thermophiles in imported UHT milk from different brands (3 samples/brand/week)

		Counts (log cfu/mL) Mean ± SE			
Week	No. of samples tested	Al-Maraee	KDD	Al-Safi	Nada
S	12	2.201±0.100	2.519±0.042	2.100±0.100	2.460±0.087
2	12	2.100±0.100	2.519±0.042	2.201±0.100	2.625±0.074
3	12	1.566±0.296	2.392±0.296	2.259±0.042	2.492±0.115
Total	36	1.956±0.197	2.477±0.042	2.187±0.046	2.526±0.051

Differences in thermophiles counts of UHT milk in the columns significantly differences ($p<0.05$)

Table 4: Microbial populations (Mean±SE log cfu/mL) of total mesophiles in imported UHT milk from different brands (3 samples/brand/week)

			Counts (log cfu/mL) Mean±SE			
Week	No. of tested	No. of (+) ve	Al-Maraee	KDD	Al-Safi	Nada
1	12	6	1.233±0.233	1.333±0.333	3.100±0.201	5.943±0.472
2	12	6	1.434±0.133	1.667±0.333	3.150±0.201	5.259±0.139
3	12	6	1.100±0.100	1.667±0.202	3.100±0.100	4.593±0.210
Total	36	18	1.256±0.097	1.556±0.111	3.117±0.017	5.265±0.390

Differences in thermophiles counts of UHT milk in the columns significantly differences ($p<0.05$)

Table 5: Microbial populations of total *B. cereus* in cow's milk

		Counts of *B. cereus* in log	
Week	No. of samples examined	Maximum cfu/mL	Mean cfu/mL
1	3	2.954243	1.680±0.437
2	3	2.643453	2.101±0.268
3	3	2.863323	2.350±0.070
4	3	2.954243	1.503±0.472
5	3	3.184691	2.300±0.292
6	3	2.735599	2.405±0.316
7	3	2.845098	1.189±0.429
Total	21	2.882950	1.933±0.180

estimation of the total numbers of aerobic mesophilic microorganisms in the UHT milk consumed in Baghdad. In UHT sterilization methods, performing the effective heating process in closed arrangements provided very high bactericide effect and using aseptic package system decreased the contamination risk down to the zero. Tekinsen *et al*. (2007) reported that sensorial, chemical and, particularly microbiological qualities of the UHT milk under process affect directly the quality of the final products. Because of that, milk under process for the UHT milk should have very high quality characteristics. The researchers were indicated that the total number of the bacteria in the UHT milk used in the production of drinking milk to be 2.477 cfu/mL at most.

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