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Physicochemical and Microbiological Quality of Raw, Pasteurized and UHT Milks in Shops

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Abstract: This study was conducted to evaluate physicochemical and microbiological quality of raw, pasteurized and UHT milks in Shahrekord (Iran) in the spring 2005. All types of milk samples were collected from different part of the city were selected and in three consequent periods. The physicochemical parameters including fat, protein, temperature, Titrable Acidity (TA), Solid Not Fat (SNF) and Specific Gravity (SG) were determined. Additionally, the microbiological evaluations were based on the total bacterial count, total coliform count and *Escherichia coli* (*E. coli*). The results (mean values of 81 measurements) for raw milk samples showed fat content 2.6, protein 3.5, T 18°C, TA 0.17, SNF 7.71 and SG 1.030 and total bacterial count 13×10^6 cfu mL^{-1}, total coliform count 1300 cfu mL^{-1} and *E. coli* positive. Those values for the pasteurized milks were the fat content 2, protein 3.5, T 15°C, TA 0.16, SNF 7.5 and SG 1.033 and total bacterial count 71×10^4 cfu mL^{-1}, total coliform count 800 cfu mL^{-1} and *E. coli* positive. Furthermore, the values of UHT milks were the fat content 2.8, protein 3.1, T 19°C, TA 0.15, SNF 8.3, SG 1.029, total bacterial count 71 cfu mL^{-1}, total coliform count 9 cfu mL^{-1} and *E. coli* negative. Statistical analysis of data revealed that there is significant difference between results of different shops at the level of $p<0.01$ which implies the contamination of raw and pasteurized milk is above standards.

Key words: Specific gravity, *E. coli*, solid not fat, standard plate count, milk microbiology

INTRODUCTION

Milk is one of the most important food products with livestock origin which enjoys special significance in terms of its various nutritional properties such as protein, lactose, fat, minerals and vitamins. Many studies have been done on its constituents and physicochemical characteristics. For instance, the milk protein can affect the coagulability state of it alongside with the fat content in quality studies of milk (Walstra *et al*., 1999). From other important measures of quality in food industries and between customers, are microbial content and hygiene condition of the raw milk which can affect on the quality of raw, pasteurized and UHT milk and consequent products of them. Nowadays to conserve the customers' health, measures other than protein and fat content such as specific gravity, acidity, coliform and *E. coli* count, Solid Not Fat and total bacterial count are being determined for milk. There are two conflicting demands in terms of consumers for higher quality products and retails for products with extended shelf life so that manufacturers are willing to formulate products with these characteristics. Clearly, the initial step for producing higher quality milk and dairy products is at the farm where we can obtain minimum somatic cell and bacteria counts. As a consequence, only reaching the legal standard is not ideal to stay in global market of dairy products.

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According to milk legislation, milk and cream products for sale must not have sign of being watery, flaky, stringy, bloody, thick, or adulterated. Milk legislation differs in terms of treatment and delivery of milk and dairy products for example in the USA, there are two categories of drinking milk in the market including pasteurized ultrapasteurized and aseptic. It is generally considered that the major sources of contamination of raw milk are the milking machine, the bulk tank (Bramley and McKinnon, 1990; Douglas *et al*., 2002), lack of cooling facilities and unsatisfactory transportation means (Oghaiki *et al*., 2007). Processing methods can affect microbiological status and keeping ability. In pasteurized milk, a minimum temperature of 71.7°C for a short time (15 sec) should be applied and straightforward the temperature must reduce to 6°C or below. The shelf life of pasteurized milk is a function of a hygienic transfer from process to filling machines. Though, the thermoduric bacteria influence the holding time mainly by high temperature during distribution. It was reported that the aseptic transfer and packaging with the temperature of distribution is below 6°C could make longer shelf life of pasteurized milk. Pasteurized milk has a shelf life ranged from 2 to 20 days which depends on the local legislation and technological factors for instance raw milk quality, processing methods, hygiene in filling as well as the quality of the cold chain. In the USA, the standard pasteurized milk has the longest shelf life of 20 days due to a very good cold chain (Roberts and Graham, 2001).

Contaminations of raw milk within milking process are originated for the udder, the exterior of the udder and the milking equipment used. Further contaminations increase for some reasons including the cooling and storage temperature plus holding time (Murphy and Boor, 2000). The bacterial count is a useful method to measure milk quality. High bacteria count in milk originates from milking wet, dirty udders plus inadequately cleaned and sanitized inflations, milking claws, hoses, pipelines and bulk tanks. The worldwide standard method to obtain bacterial numbers present in raw milk is the SPC method. According to this method, milk from grade A farms has the SPC less than 100,000 cfu mL^{-1} and grade B milk has the SPC with less than 300,000 cfu mL^{-1}. It was stated that a sensible target for SPC is less than 5,000 cfu mL^{-1} and a count of greater than 10,000 cfu mL^{-1} is generally a sign of milk quality problem in terms of difficulty in cooling milk and cleaning milking equipment. Milk media are often contaminated by *Escherichia coli* bacteria under lack of sanitary conditions which can affect public health. The coliform count is related to the unsanitary milking process and dirty cow's enviroument (Smiddy *et al*., 2007).

Milk is normally sold as fresh, pasteurized or UHT to consumers in Shahrekord. Approximately raw milk yield is around 194033 ton/year in Shaharekord which 70% of the milk produced, is collected by dairy industry in order to produce different type of milk and dairy products such as whole and skim milk, flavoured milk, yoghurt, ice cream, butter and Kashk (made form yoghurt) and Ghara (from whey proteins). The rest raw milk is traditionally boiled, then it is sold in plastic bags.

This study is useful for development of HACCP systems for healthy milk products. Also there is useful information on physicochemical parameters consisting of protein, fat, solid not fat, temperature, acidity. The results are valuable for considering risk of contamination relating to health problem of consumers. However, it is not reported many of food-borne illness since people are not fully understood about contaminated food and therefore bacterial infections (Mensah *et al*., 2002; Oliver *et al*., 2005). Milk quality in Shahrekord is still assessed by physical and chemical tests (milk density and fat content). Despite importance of hygienic and microbiological criteria at the farm level, there is not such information in retail milk products in Shahrekord. The objective of present study was to evaluate physicochemical properties as well as microbiological quality of three types of commercial milk in the retails of Shahrekord city (which some reports of their contamination were received) and compare the levels found with the Standards.

MATERIALS AND METHODS

Sampling Procedure

The study population in this research was the milk samples presented for sale at the shops of Shahrekord in the spring of 2005. The sea level was approximately 2066 m. The mean minimum temperature was 8°C and maximum 23°C, respectively. In this cross sectional study sampling was done by selecting the shops with random cluster sampling from retails representing raw, pasteurized and UHT milk to customers. Three shops in the northern, central and southern regions of the city were chosen randomly and in three consequent periods, three replications from 3 types of their milk were collected (total samples 81 = 3×3×3×3). Each sample was 200 mL and divided into two 100 mL parts in sterile tubes, one for microbial and other for physicochemical tests. Then the tubes were immediately transported to laboratory in an ice box and then kept at 6-8°C before being analyzed within 24 h of sampling. For the microbiological analysis of milk, 100 mL samples were aseptically stored in sterilized glass flasks.

Physicochemical Analysis

The protein, fat and SNF values were determined by Lactostar (Automatic Milk Analyzer, Funke, Dr. Gerber, Germany). Milk temperature was measured by a thermometer, specific gravity by thermo lacto-densitometer and Titrable Acidity (TA) by using 0.1 N NaOH according to the Association of Official Analytical Chemists (AOAC, 1997).

Microbiological Analysis

Total bacterial count was done by pour plate method. The coliform and *Escherichia coli* count with standard methods (AOAC, 1997). For the SPC method, an accurate volume of milk is poured to a given quantity of media and the plate incubated at 32°C for 48 h. The number of colonies is precisely counted and calculated as cfu mL^{-1} of milk.

Statistical Analysis

According to the importance of ANOVA test in statistical analysis of quantity variables, the data resulted from our study were analyzed by simple ANOVA test. The standard deviations were also calculated to control the precision of examination and provide the possibility of comparing the contamination of different types of Shahrekord shops' milk. The SPSS (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Descriptive statistics including mean, standard deviation, minimum and maximum were obtained.

RESULTS AND DISCUSSION

According to the results of study and ANOVA test, all the measures were significant at the level $p<0.01$. By calculating the standard deviation, it can be concluded that the study has been done precisely and with controlling the confounding variables (Coefficient of Variation, CV, <25%, values were not shown).

The mean values of fat content (Table 1) were 2.6, 2 and 2.8%, for raw, pasteurized and UHT milk samples, respectively. The minimum value of fat content was 1.9 for pasteurized milk and the maximum value was 2.9 which were below the value on the package (3% fat content). Dairy farmers in Shahrekord normally boil raw milks and separate some fat from bulk milk before selling which resulted to obtain lower fat content of raw milk samples compare to standard level. This is due to prevent contamination of raw milk and high expenses of milk production for small- scale dairies in rural areas. They traditionally sell separated fat as cream to consumers in the local markets. The protein

Table 1: Physicochemical properties of raw, pasteurized and UHT milk (81 measurements)

Physicochemical properties		Raw	Pasteurized	UHT
Fat	Mean±SD	2.6±0.129	2.0±0.227	2.8±0.630
	Minimum	2.6	1.9	2.0
	Maximum	2.8	2.1	2.9
Protein	Mean±SD	3.5±0.296	3.5±0.259	3.1±0.296
	Minimum	3.2	2.9	2.8
	Maximum	3.8	3.8	3.4
Temperature (°C)	Mean±SD	18.0±1.9	15.0±2.9	19.0±2.01
	Minimum	16.0	8.0	16.0
	Maximum	20.0	22.0	22.0
TA	Mean±SD	0.17±0.24	0.16±0.03	0.15±0.28
	Minimum	0.15	0.14	0.14
	Maximum	0.19	0.18	0.16
SNF	Mean±SD	7.71±0.784	7.5±1.1	8.3±0.793
	Minimum	7.31	7.11	7.94
	Maximum	8.12	7.93	8.35

The standard deviation (±) was calculated for all measurements

contents of three type of milk were nearly similar (3.1 and 3.5%) however the range of protein content was about 1% differences (2.9-3.8% for pasteurized milk). The mean temperatures of milk samples were 18°C (raw milk), 15°C (pasteurized milk) and 19°C (UHT milk). The temperature of pasteurized milk was slightly lower than the others. Even minimum temperature of pasteurized milk (8°C) was above the standard value which affects the quality of milk. The acidity of raw milk, pasteurized and UHT milk samples were 0.17, 0.16 and 0.15, respectively. Only the acidity of raw milk samples was not within the standard range (0.14-0.16). The SNF values were 7.71% (raw milk), 7.5% (pasteurized milk) and 8.3% (UHT milk) which showed close values to normal range (7.9-10.0%) (Walstra *et al*., 1999).

When discharging from breast of healthy cow, the milk is sterile, after a while it can be contaminated by outer microorganisms, but this count is low don't go beyond tens and hundreds. In some cases infections can cause contamination and even make the milk not proper for use. The results of this study reveals that the contamination of different type of raw and pasteurized milk is above standards in all three study regions the milk of shops have severe contamination. The level of all variable studied, without any exception, had significant differences at the level of $p<0.01$ with these values obtained from developed countries. So these milks are unusable. According to US standards, each mL of raw milk (for pasteurization) must have less than $10^5/3\times10^5$ cfu mL^{-1} (Coast *et al*., 2004; Reinemann *et al*., 1999; Jayarao *et al*., 2001) Unfortunately, the resnlts presented in Table 1 showed that the SPC of raw milk (13×10^6) is almost far greater than the US standard. Due to the universal standards, the raw milk of Shahrekord shops does not have proper quality for use by consumers. High SPC of raw milk have been reported from some countries (such as India, Zimbabwe, Malaysia, Sudan and Bamako (Mali) as a result of improper handling and mismanagement practices and the unhygienic envirounent (Chatterjee *et al*., 2006; Gran *et al*., 2002; Chye *et al*., 2004; Elmagli *et al*., 2006; Bonfoh *et al*., 2003). If the bacterial count of milk increases to higher than standard level, its protein, fat and lactose content will be degraded. It causes poor taste and bad smell and affects the stability of milk and its products (Spreer, 1998).

In pasteurized, the SPC must be under 20,000 cfu mL^{-1} for the US standard (Pamela *et al*., 2002) and between 5000-50,000 cfu mL^{-1} for European Union (EU) standard (Hillerton and Berry, 2004). The mean SPC result of pasteurized milk was 71×10^4 cfu mL^{-1} greater than US and EU standards in Shahrekord retails. This confirms the severity of contamination of raw and pasteurized milk in Shahrekord. In the US and EU countries, producers and sellers may observe this strong relationship between the health of customers and their own economical benefit and they will be sure about the quality of milk. Thus, manufacturers give prizes to good producers. Overall, the production of high

quality milk is beneficial for both producers and sellers and they confirm the hygienic considerations (Anderson *et al*., 1995; Hittu and Punj, 1999). Moreover, the mean SPC of UHT milk samples was 71 cfu mL^{-1} and a range of 68-74 cfu mL^{-1} representing post-UHT contamination (Table 1).

Based on standards of raw and pasteurized milk, the coliform bacteria count in each mL of it must not be above 100 and 10 (based on the US regulations) and 5 (based on the EU regulations), respectively and *E. coli* must be negative (Hillerton and Berry, 2004; Potter and Hotchkiss, 1995; Lues *et al*., 2003). Presence of coliform in pasteurized milk is from poor hygiene of pasteurization and packing process. According to Iran's standard, a coliform count less than 100-500 cfu mL^{-1} is appropriate for raw milk transferring to further processing (Iranian National Standard, Microbiological properties of milk No. 2406). The results of raw, pasteurized and UHT milk samples were obtained 1300, 800 and 9 cfu mL^{-1}, respectively representing a downward trend. A coliform count less than 500 is traditionally used for drinking (Table 1). The pasteurized milk should have a coliform count less than 1 to be safe for consumers. Other studies AL-Tahiri (2005) stated that a coliform count between 100 and 1000 generally expresses poor milking hygiene and a coliform count greater than 1000 depict growth of bacteria as a result of milk handling equipment (Pamela *et al*., 2002; Lues *et al*., 2003).

E. coli causes severe diarrhea in newborns and adolescents and originates from mastitis (Kornalijnslijper *et al*., 2004). So the raw and pasteurized milk (Table 2) not ouly are not top grade but also some kinds of them are unusable Heat-stable proteases breakdown casein and increase low-molecular weight nitrogen compounds which provide nutrients for post-pasteurization contaminants, which in turn cause spoilage (Esther *et al*., 2004). The results of *E. coli* are over the standard level (i.e., less than 1) and the contamination of pasteurized milk (*E. coli* does not exist in 1 mL) is worrisome. It is apparent that these values of the raw milk and pasteurized milk do not meet the standards (*E. coli* positive: 59 raw milk samples and 32 pasteurized milk samples). Anderson *et al*. (1995) pointed out that sometimes due to unhygienic procedure of processing, delivering, handling and selling milk, *E. coli* is found in milk especially in raw milk. In Pakistan, 57% of milk samples were at risk of *E. coli* as well as some other reports (Adesiyun, 1994). Chye *et al*. (2004) studied bacteriological qnality and safety of raw milk in Malaysia. They confirmed that fresh raw milks were highly contaminated by bacteria (a mean total plate count of 12×10^6 cfu mL^{-1}, regarding to improper milking systems and keeping quality of milk (such as temperature). They also stated 312 of 930 (33.5%) milk samples were examined to be positive for *E. coli* 0157:H7. Lastly, the packaging system needs to improve to reduce airborne contamination. These will enhance the quality of milk, reduce wastage and benefit the consumer monetarily (Table 3).

Table 2: Microbial measurements of raw, pasteurized and UHT milk (81 measurements)

Microbial measurements		Raw	Pasteurized	UHT
SPC	Mean±SD	$13\times10^6\pm2.86\times10^5$	$71\times10^4\pm2.06\times10^3$	71±2.03
	Minimum	12×10^5	67×10^4	68
	Maximum	16×10^6	75×10^4	74
Coliform count	Mean±SD	1300±23.493.2	800±16.21	9±1.326
	Minimum	1233	745	7
	Maximum	1276	855	11

The standard deviation (±) was calculated for all measurements

Table 3: The *E. coli* results of three type of milk including raw, pasteurized and UHT milk in Shahrekord retails

	E. coli			
	Positive		Negative	
Milk type	No. of samples	(%)	No. of samples	(%)
Raw	59	72.83	22	27.17
Pasteurized	32	39.50	49	60.50
UHT	-	-	81	100.00

CONCLUSION

The raw and pasteurized milk samples were shown to be unsafe and of poor quality within Shahrekord in three parts of the city. Despite low range of temperature (between 8 and 30°C) of Shahrekord in comparison with many cities in Iran (higher temperature, >30°C, in spring), The SPC, coliform and *E. coli* counts were as much as International standards. The results clearly manifested that milk hygienic quality is not satisfying for consumers because of milking methods, milk temperature, packaging, handling, cleaning, shops conditions (unproper refrigerators). The Food health organization must take more responsibility to control and reduce the risk of milk contamination in Shahrekord without any excuses in this regard.

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