International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 3 (3): 137-143, 2008
ISSN 1811-9743

Comparison of Chemical Composition and Microbial Profile of Raw and Pasteurized Milk of the Western Cape, South Africa

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Abstract: During the present study raw and pastuerized milk samples were collected from the three major dairy factories during March- August 2001. The raw milk supplied by farmers to the selected factory was collected from bulk tank. Similarly pasteurized milk samples, processed and distributed by those factories, were randomly collected from three different food stores and retailers of the Western Cape of South Africa. The frequency of the isolation of the microorganisms, from both raw and pasteurized milk, revealed a higher prevalence of *S. aureus* in the raw milk (15.38%) followed by those of *E. coli* (14.3%) that was also isolated at a rate of 3.6% form pasteurized milk. Also, other mastitis-isolated pathogens found were *Streptococcus agalactiae* (8.79%), *Streptococcus dysglactiae* (12.09%), *Streptococcus uberis* (6.72%), *Enterococcus faecalis* (8.35%) which was also found in 2.2% of the pasteurized milk samples and *Staphylococcus epidermidis* (8.79%) that were also found in pasteurized milk (2.2%). Other identified isolates, were also represented. However, all samples revealed negative results for the growth of Salmonella and *Listeria monocytogenes*. Descriptive and frequency analysis showed higher means, standard error of means and standard deviation for the somatic cell counts, total bacterial counts, coliform counts and *E. coli* counts, although their minimum values revealed a negative or a very low levels. A lower level was also obtained for the chemical content (fat, protein, lactose, SNF and total solids) of the pasteurized milk compared to the raw milk samples for all studied companies. Also the percentage of the added water was very high in the processed milk compared to the samples from herd raw bulk milk. Moreover the significant variation between the measurements were estimated.

Key words: Raw milk composition, pasteurization, bacteriological quality, Western cape, South Africa

INTRODUCTION

Food production processes are increasingly influenced by quality and safety concerns, for dairy production, one of the food quality outcomes is low level of bacteria in unprocessed milk (Finger and Sischo, 2001). Milk and dairy products are the major sources of nutrition and energy; however, it is well known that they can become sources of zoonotic infections (Mohamed and El Zubeir, 2007a). They recommended that milking should be done under hygienic conditions and milk should be cooled immediately after milking and should be heat treated to control bacteriological quality. This to ensure that milk is produced, distributed, handled and marketed under the control of milk commission and the commission must have a sanitary inspector and veterinarian to enforce its methods and standards (Mohamed and El Zubeir, 2007b).

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The microbiological contents of raw milk affect quality, shelf life and safety of processed milk and other dairy products (Gunasekera *et al*., 2000). Mohamed *et al.* (1997) reported the effect of mastitis on the compositional quality of milk and concluded that mastitis has a great influence on milk composition. Infection and disease are the results of failures in proper application of milk production hygiene (El Zubeir *et al*., 2006). However, independently to the milk production situation in any place, milk should not be drunk or used to manufacture of any products without previous pasteurization or boiling (Giovannini, 1998). Moreover, pasteurization of milk provides protection for the consumers against pathogens that might be present in the raw milk and improves its keeping quality (IDF, 1994).

Gunasekera *et al.* (2000) reported that several methods available for detection and enumeration of microorganisms in raw and processed milk. However, as they reported culture techniques are the most common. Similarly, somatic cell counts (SCC) are widely used to predict the mammary health status of quarters and cows, the suitability of milk for human consumption (Heeschen, 1996). On the other hand, Berning and Shook (1992) cited that infections by major pathogens are often associated with SCC below the threshold for mastitis diagnosis (500,000 cell mL^{-1}). Moreover, Dekkers *et al.* (1995) indicated that efforts to reduce bulk milk Somatic Cell Count (SCC), resulted in substantial extra milk revenues. Further, a bulk SCC target of 250,000 cells mL^{-1} was advocated. Since they estimated a level 200,000 and 450,000 cells mL^{-1} in Ontario, Canada. However, Ma *et al.* (2000) stated that in general standard plate counts, coliform counts and psychrotrophic bacterial counts of both high and low SCC milk remained low (<100,000 cfu mL^{-1}) during 5°C storage.

The present study is meant to evaluate and discuss the present hygenic and compositional situation of milk produced and consumed in the Western Cape of South Africa.

MATERIALS AND METHODS

Source of Milk Samples

The present study was conducted during March- August 2001 in the Western Cape of South Africa. It involves 3 different dairy companies, with their suppliers of the raw milk. Raw bulk tank milk samples were collected from some of the farmers (30 samples) that supply their milk to the selected dairy factories. The samples were collected in sterile Macarteny bottles directly from the bulk tank supplying raw milk to the factory. Similarly pasteurized milk samples, processed and distributed by three factories (30; 10 samples from each), were randomly collected from the three different food stores and retailers distributed in the different socioeconomical groups of the Western Cape of South Africa.

Analysis of Milk Samples

The milk samples were first brought to the Laboratory of the Medical Microbiology, University of Western Cape in an ice container. Part of the milk samples were spilt aseptically into 40 mL sterile bottles, coded and refrigerated over night for bacteriological analysis. All microbiological evaluations were done at Provincial Veterinary Laboratory, Stellenbosch. The somatic cell counts (SCC) were estimated using Coulter Counter (Beckman, Z1 series, England) according to the manufacturers recommended procedures. South African Bureau of Standards (SABS) methods were applied to the total bacterial count (ISO 6222, 1999), enumeration of coliforms (SABS ISO 4831,1991), detection of *Escherichia coli* (SABS ISO 7251, 1993), detection of Salmonella (ISO 6579, 1993) and detection of *Listeria monocytogenes* (SABS ISO 11290/ 1 and 2, 1996, 1998). The isolation and identification of *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp. and *Bacillus cereus* (their presence is coded as standard cultures; in order to facilitate their statistical analysis) were also done on blood agar according to Quinn *et al*. (1994) and Bergey's manual (Holt *et al*., 1994). Similarly another 50 mL of the same milk samples were coded and brought to the ARC-Animal Nutrition and Product Institutes,

Elsenburg for the determination of chemical composition of milk. Percentages of fat, protein, lactose and SNF were done using infrared spectrophotometer (Milko Scan 133B analyzer, A/S N. Foss Electric, Hillerford, Denmark), whereas total solids and ash contents were obtained by subtraction. The freezing point, to detect the percentage of the added water was also done by the Advanced Cryscope (Fiske, USA).

Statistical Analysis

The rate of isolation of each organism in both raw and pasteurized milk was calculated as a percentage of the total number of the isolates. The isolated bacteria were grouped into three categories (major pathogens: 1; minor pathogens: 2 and negative: 0), according to Berning and Shook (1992) to facilitate their statistical analysis. Due to the wide variation of the counts, a logarithmic function was estimated; using the Microsoft Excel computer program (2000) for SCC; TBC; *E. coli* count and coliform count. Descriptive statistical (mean, standard deviation, variance, maximum and minimum) and ANOVA test of the paired t-test analysis were also performed, using the Statistical Packages for Social Science (SPSS, 10). Correlation's and their significant level among the measurements, were estimated using Pearson correlation using the same program (SPSS, 10).

RESULTS

Comparison of Microbiological Contents of Raw and Pasteurized Milk

Table 1 shows the frequency of the isolation of the microorganisms, from both raw and pasteurized milk, which are produced and consumed in the Western Cape. The higher prevalence is that of *S. aureus* in the raw milk (15.38%), followed by those of *E. coli* (14.3%) that was also isolated at a rate of 3.6% form pasteurized milk. Similarly, other mastitis-isolated pathogens were *Streptococcus agalactiae* (8.79%), *Streptococcus dysglactiae* (12.09%), *Streptococcus uberis* (6.72%), *Enterococcus faecalis* (8.35%) that were also found in pasteurized milk (2.2%) and *Staphylococcus epidermidis* (8.79%) that were also found in pasteurized milk (2.2%). Other identified isolates, were represented in the same Table. However, all samples revealed negative results for the growth of *Salmonella* spp. and *Listeria monocytogenes*.

Descriptive and frequency analysis of the different measurements (Table 2), showed higher means, standard error of means and standard deviation for the somatic cell counts, total bacterial counts, coliform counts and *E. coli* counts, although their minimum values revealed a negative or a very low levels.

Comparison of the Chemical Composition of Raw and Pasteurized Milk

The comparison of different levels of the bacteriological quality on milk constituents (Table 3), revealed significant differences in the fat % for the somatic cell counts ($p<0.05$), coliform counts

Table 1: Frequency of the isolation of microbial organisms from raw and pasteurized milk in Western Cape, South Africa

Organisms	Raw milk	Pasteurized milk	Total
Staphylococcus aureus	14 (15.38%)	0	14 (15.38%)
Streptococcus agalactiae	8 (8.79%)	0	8 (8.79%)
Streptococcus dysglactiae	11 (12.09%)	0	11 (12.09%)
Streptococcus uberis	6 (6.723%)	0	6 (6.723%)
Staphylococcus epidermidis	8 (8.79%)	2 (2.2%)	10 (10.99%)
Staphylococcus haemolyticus	6 (6.723%)	0	6 (6.723%)
Enterococcus faecalis	8 (8.35 %)	2 (2.2%)	10 (10.99%)
Streptococcus bovis	8 (8.79%)	0	8 (8.79%)
Staphylococcus intermedius	4 (4.4%)	1	5 (5.49%)
Staphylococcus chromogenes	3 (3.6%)	0	3 (3.6%)
Escherichia coli	13 (14.29%)	3 (3.6%)	16 (17.58%)
Total	83 (91.2%)	8 (8.79%)	91

Table 2: Descriptive statistical analysis of some quality control tests on raw and pasteurized milk produced and consumed in the Western Cape

Measurements	Minimum	Maximum	Mean	SD
Log somatic cell counts	4.38	6.25	5.3420	0.5783
Log total bacterial counts	1.78	7.00	4.0366	1.3772
Log coliform counts	0.00	6.00	2.3457	1.5316
Log *E. coli* counts	0.00	4.59	0.7295	1.3426
Salmonella	0.00	0.00	0.0000	0.0000
Listeria	0.00	0.00	0.0000	0.0000
Fat (%)	1.03	5.47	3.1928	1.1352
Protein (%)	1.39	3.54	2.6808	0.6335
Lactose (%)	2.12	5.11	3.8966	0.9078
Solid not fat (%)	4.23	9.17	7.22975	1.5342
Total solids	5.35	15.28	10.5607	2.5913
Ash	0.71	0.73	0.7198	4.73E-03
Added water	ND	53.30	20.9556	14.6415

SD = Standard Deviation, ND = Not Detectable

Table 3: Comparison of the significant differences of bacteriological quality on raw and pasteurized milk constituents, using t-test analysis

Measurements	Somatic cell counts	Total bacterial counts	Coliform counts	*E. coli* counts	Standard cultures
Fat (%)	0.049*	0.843^{NS}	0.003**	0.331^{NS}	0.001***
Protein (%)	0.183^{NS}	0.33^{NS}	0.129^{NS}	0.588^{NS}	0.001***
Lactose (%)	0.232^{NS}	0.325^{NS}	0.150^{NS}	0.490^{NS}	0.001***
Solid not fat (%)	0.200^{NS}	0.329^{NS}	0.138^{NS}	0.530^{NS}	0.001***
Total solids	0.318^{NS}	0.107^{NS}	0.073^{NS}	0.146^{NS}	0.001***
Ash	0.456^{NS}	0.958^{NS}	0.563^{NS}	0.389^{NS}	0.315^{NS}
Log somatic cell counts	0.181^{NS}	0.031*	0.014**	0.022**	0.001***

NS = Non Significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

($p<0.01$) and bacteriological status (standard cultures) of the milk ($p<0.001$). However protein %, lactose %, total solids % and solids not fat % revealed significant differences only for the bacteriological status (standard cultures) at $p<0.001$. The only significant difference obtained for the ash % was due to the variation of the SCC ($p<0.05$). However the added water showed significant differences due to *E. coli* counts ($p<0.05$).

Correlation Between Milk Constituents and Microbiological Contents of Raw and Pasteurized Milk

The log SCC revealed a positive correlation with log total bacterial counts; log coliform counts; standard cultures; fat %; protein %; lactose %; total solids % and solids not fat % ($p<0.001$) and log *E. coli* counts ($p<0.01$). Positive correlation's were found for the log *E. coli* counts and each of the total solids % ($p<0.001$), log SCC; solids not fat % and protein % ($p<0.01$) and fat %; lactose %; ash % and standard cultures at $p<0.05$. Similarly, the standard culture showed a positive correlation ($p<0.01$) towards log SCC.

DISCUSSION

The present research is done as a trial to estimate the bacteriological and the chemical composition of the market milk, following its distribution and comparing that with the raw original milk supply. As represented in Table 1, a high frequency of pathogens were isolated from the raw milk and this confirmatory results for the presence of mastitis pathogens as stated by Berning and Shook (1992) and Gunasekera *et al.* (2000).

The higher level of the SCC in most of the bulk tank milk (Table 2), which exceeded the threshold, were also indicative of the presence of the infection within the herd as it was confirmed by the

presence of mastitis pathogens (Table 1). This was in accord to Berning and Shook (1992) who demonstrated that log SCC was discriminate infected from non-infected quarters of cows. Hence, the present study suggested that the quality control authority of each factory should inform and train their farmers to apply the proper hygienic practices. However, as represented in Table 2 that some of the farms yield very satisfactory milk as represented by the low SCC, TBC and coliform counts.

There is no evidence that any particular cell count per se has any significant effect on human health. However, the higher the cell count the greater the risk of raw milk contamination with pathogens and antibiotic residues (Savelle *et al*., 2000); toxins (Tood, 1992) and the resistant bacteria that could also be transfer to human (Manie *et al*., 1999). The risk of milk contamination was confirmed by the present findings as shown in Table 3, that significant differences and a positive correlation were obtained for the different bacteriological measurements.

Pasteurization as a practice has a positive effect on the bacteriological contents of milk since it improve the TBC, coliform and other pathogenic organisms (Table 1). Moreover, as stated by Hayes *et al*. (2001) that increase in TBC can reduce the price that farmer receives for milk. The presence of *E. coli* and other pathogens in pasteurized milk (Table 1) was either due to insufficient pastuerization or indication of post pastuerization contamination of milk, since as stated by Manie *et al*. (1999) that the Enterobacteiaceae do not survive pasteurization but contamination can be due to poor post pasteurization control. Moreover, the bacteriological content was found to influence the milk constituents (Table 3), which was in agreement to Mohamed *et al*. (1997). Lower levels of all the chemical compositional estimated in the pasteurized milk, compared to the original raw milk from all tested milk samples of the different companies. This might be due to either adulteration of milk by the addition of water and /or the steam that used as an indirect method of pasteurization. Since some of the factories, used the direct steam, as stated earlier by Lombard (1976) that systems in operation in South Africa use steam injection for heating and sterilizing temperature was the reason of this due to some technical faults. Regardless of the reason of the high percentage of the added water, the lower level of lactose (Table 2) also reflected the adulteration. Since the lactose level is the most non-variable of the milk constituents as stated by Mitchel *et al*. (1978). However, it is well known that it is influenced by bacterial status as demonstrated by Mohamed *et al*. (1997).

The present study suggested that monitoring program for evaluation and grading of milk is urgently needed for the consumed dairy products, since in those factories they pay for milk according to its quality including the somatic cell count. However, the lack or inefficiency of the quality assurance programs at factories may lead to production of law quality products. Similarly more attention should be directed towards the producers to ensure safety supply of milk. This could be a chivied by application of quality control at farm as well. The absence of *Salmonella* spp. and *Listeria monocytogenes* in both raw and pasteurized milk, during the present study was indication that the herds and the labors; deal with; are free from those pathogens. This was cope well with the results of the questionnaire that addressed the farmers' knowledge and practices (data not shown) that no entry for the person with such illness as stated by the farmers involved in the present study.

The present study concluded that milk is a vulnerable product, which in some cases may become dangerous to human health if it was subjective to adulteration and/or health hazards. Hence a quality assurance programs are essential for monitoring its quality at all steps from production to consumers. Training and education is also needed for all persons who deal with milk production and processing. Further research and extension is urgently needed to characterize critical quality points and hazards in order to ensure that good quality dairy products are produced and consumed.

ACKNOWLEDGMENTS

The present study was conducted through the UNESCO Pilot African Academic Exchange Program (UPAAE). The fund from the Royal Society/NRF of South Africa was also appreciated. The

authors would also like to thank all factories' mangers, quality control supervisors, dairy advisers and their farmers who allowed and facilitate collection of data and samples for the present study. The effort of the International office of the University of the Western Cape and the other departments was also acknowledged. The technical help of the staff of ARC-Animal Nutrition and Product Institutes, Elsenburg and Provincial Veterinary Laboratory, Stellenbosch were also appreciated with thanks.

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