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A comparative study on the microbiological and chemical composition of cow milk from different locations in Madurai, Tamil Nadu

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Abstract: Sixty samples of raw cow milk were collected from four different locations in Madurai for their microbiological and chemical compositions analysis. The average levels of major chemical components were: fat (6.14%), crude protein (3.77%), lactose (4.25%), total solids (18.10%) and ash (0.80%). Microbiological enumeration revealed for the counts of total mesophilic aerobic bacteria, 5.84 log cfu/ml; bacterial endospores, 2.37 log cfu/ml; lactic acid bacteria, 4.46 log cfu/ml; coliforms, 2.76 log cfu/ml; *Escherichia coli,* 1.63 log cfu/ml and *Staphylococcus aureus,* 1.92 log cfu/ml. *Listeria* spp. were below detection level in all of the samples. The microbiological quality of raw cow milk was judged marginal and indicates the need for improved hygienic standards.

Keywords: cow milk, chemical composition, microbiological quality, safety.

Introduction

Raw milk (RM) often contains microorganisms which may cause food borne diseases (Adesiyun *et al*., 1995: Steele *et al*., 1997; Headrick *et al*., 1998). Milk producers, processors, regulators and consumers share a common objective: the production and sale of safe, high quality milk and dairy products. Bacterial enumeration of bulk raw milk samples forms the basis of many dairy regulatory programs. The application of an accurate and efficient method to quantify the microbiological load of raw milk is essential to this objective. Historically, bacterial analysis of raw milk used the standard plate count (SPC) (IDF standard 100B, 1991; Houghtby *et al.,* 1992), the plate loop count (PLC) (Thompson, 1960: Wright *et al*., 1970: IDF Standard 100B, 1991) and spiral plate count (SPL) (Gilchrist *et al*., 1973; Jarvis et al., 1977). The main goal of milk pasteurization or other heat treatment for cheese making is the elimination of pathogens, which may be present in milk. Apart from post- pasteurization contamination, the growth of milk pathogens depends strongly on the type of dairy technology (Grappin *et al*., 1987).

Pathogens that have been involved in food borne outbreaks associated with the consumption of milk include *Listeria monocytogenes, Salmonella spp., Escherichia coli* and *Staphylococcus aureus.* The presence of these pathogenic bacteria in milk emerged major public health concerns, especially for these individuals (Ryser, 1998).

China is the largest producer of milk; with both buffalo herds and buffalo milk production listed third world wide in 2004 after those of India and Pakistan (FAO, 2004). In Tamil Nadu as per the 126th livestock and poultry census 2000 the total cattle population is 93.63 lakhs, which accounts for 35.8% of the total livestock population in the country. The milk production in Tamil Nadu has increased tremendously over the past 20 years. From only 1.74 million tones in 1981, it has risen to 5 million tones in 2001. This has resulted in increase in per capita availability of milk to 219gm per day, which is very close to the Indian Medical Council Research (ICMR) recommendation of 220g per day (TNMPFL, 2000). Fresh milk drawn from a healthy cow normally contains a low microbial load (less than 1000ml^{-1}), but the loads may increase up to 100 fold or more once it is stored for sometimes at normal temperatures (Richter *et al*., 1992).

The objective of this study is therefore, to investigate the occurrence and load of microorganisms in cow milk with special reference to food borne pathogens. The results obtained serve as baseline data will be useful in future studies dealing with pathways of contamination and in the development of HACCP systems for hygienic processing of raw cow milk. The study also presents information on physiochemical parameters such as total solids, crude protein, lactose and fat content to enable an appreciation of the nutritional value of raw cow milk.

Materials & Methods:

Samples of cow milk were obtained from Madurai district. Milking was done manually twice a day at 7.00am and 5.00pm. A total 60 samples of raw cow milk were collected at four locations (North [Samayanallur], East [Thirumangalam], West [Oormechikulam] and South [Survayar Coloney]). At each location, samples of approximately 500ml were taken aseptically from the bulk milk container into sterile glass bottles. The milk was collected within 15min of milking at ambient temperatures, kept on ice during ≈ 2h transport and was analyzed immediately after arrival at the laboratory (Biotechnology Laboratory, The American College).

Chemical analysis

Fat content: Fat content and crude protein were determined by the Babcock and Kjeldahl methods, respectively.

Lactose: Lactose was determined by colorimetric method according to the Chinese standard method (GB/T 16285-1996).

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Total solids: Total solids were determined by conventional over drying (60°C for 2-3h then 100°C for 6h) of 5g milk samples.

pH: The pH was measured using a digital pH meter (pH 510 micro processor pH meter, Cyberscan, Italy): calibration was done with buffers of pH 4.00 and pH 6.80.

Ash: A 10g sample was evaporated in a water bath and then heated in a muffle furnace at 55°C for 3h, until the ash residue remained.

Microbiological analysis

Sample treatment: Representative 20g portions were aseptically weighed, mixed with 180ml peptone saline (0.1% neutral peptone, 0.9% NaCl), and homogenized by shaking. Subsequent decimal dilutions were prepared with the same diluents, and in all cases duplicate-counting plates were prepared of appropriate dilutions.

Total count of mesophilic aerobic bacteria (TC): TC was enumerated according to Chinese standard method GB/T 4782.2-2003, in pour plates of plate count agar (Hi Media, India), after incubation at 37°C for 2 days.

Bacterial endospores (BS): Raw cow milk samples were pasteurized (80°C for 10min) and BS was enumerated in pour-plates of PCA, after incubation at 37°C for 2d.

Lactic acid bacteria (LAB): LAB was enumerated according to Chinese Standard method GB/T4789.35-2003, in pour-plates of de Man, Rogosa and Sharpe medium (MRS, Merck, Germany), after incubation at 37°C for 3 days.

Coliform bacteria: Coliforms were enumerated using 3M petrifilm *E. coli* Coliform plates (3M, USA) after incubation at 37°C for 1d, and *E. coli* for 2d.

Staphyloccus aureus: S. aureus were enumerated using 3M petrifilm staph express count plate and disk (3M, USA). After incubation at 37° C for 1d, red-violet colonies on the plate were counted and presumed *S. aureus* colonies (having pink zones) were confirmed using the staph express disk after incubation at 37°C for 3h.

Statistical analysis

All microbial counts were converted to the base -10 logarithm of the number of colony forming units per ml of raw cow milk samples (log cfu/ml), and from these means and their standard deviations were calculated. Data were analyzed using analysis of variance (ANOVA) through the general linear models (GLM) procedure of the statistical analysis system software (SPSS version-11.5, 2003). Least significant differences were used to separate means at $p<0.05$

Results and discussion

Chemical analysis

Results obtained from the chemical analysis of raw cow milk are presented in Table1. Cow milk had lower crude protein, fat, lactose and total solid contents than those reported for milk (Fundora *et al*., 2001; Landmark *et al.,* 2003). The average fat (6.14±1.17% v/v) and lactose (4.25±0.40 w/w) contents were slightly higher than those reported by Najdenova and Dimitrov (2003), but lower than found by Supino *et al.,* (2004). The average crude protein (3.77±0.40 w/w), ash (0.80±0.05 w/w), and total solids (18.10±1.40 w/w) content and the average pH (6.44±0.25) of raw milk were similar to values reported elsewhere (Han & Ding, 1994; Fundora *et al*., 2001).

Chemical composition data were studied by analysis of variance. Total solids, fat, crude protein, and ash of southern cow milk sample were significantly higher ($p<0.05$) than those of northern and western regions. The lactose content of northern was significantly higher ($p<0.05$) than those of other milk. It is not difficult to see that the total solid, fat, crude protein and ash content of the southern milk samples were higher than the values of western but lower those of southern.

Our findings are in agreement with those of (Zhang *et al*., 2004) who reported that the total solids, protein, fat, lactose, ash and non-fat solids content of south were higher than those of pure western, but lower than the southern. This ranking was to be expected: considering that the mixed southern milk has higher nutrient levels than the western milk (Han & Ding, 1994; Amerjit & Tshihiko, 2003), the milk composition of the southern is expected in between that of milk from southern milk.

Similar as for raw cow milk (Landmark *et al.,* 2003), in addition to the type of breeds, other factors such as forage, feeding systems, milking frequency, milking method, seasonal changes and lactation period will exert an effect on the physio chemical parameters

Table1. Chemical analysis of cow milk in Madurai district

Region	Fat (%v/v)	Protein (%w/w)	Lactose (%w/w)	Total Solids (%w/w)	pH	Ash (%w/w)
North (n=15)	$5.57\pm1.21^{*,a}$	3.67 ± 0.43^{a}	4.22 ± 0.21^{a}	19.61 ± 1.11^{a}	6.60 ± 0.14^{ab}	0.83 ± 0.03^{a}
West (n=15)	5.53 ± 1.28^{a}	3.69 ± 0.20^{a}	4.47 ± 0.41^{b}	18.45 ± 1.24^{a}	6.45 ± 0.33^{b}	0.79 ± 0.06^{a}
East (n=15)	6.56 ± 0.90^{a}	4.01 ± 0.53^{b}	3.71 ± 0.65^{b}	17.25 ± 1.31^{ab}	6.36 ± 0.28^{b}	0.78 ± 0.04^{ab}
South (n=15)	6.90 ± 1.30^{ab}	3.71 ± 0.45^{bc}	4.60 ± 0.35^{b}	17.11 ± 1.96^{b}	6.36 ± 0.26^{ab}	0.83 ± 0.07^{b}
Average	6.14±1.17	3.77±0.40	4.25±0.40	18.10±1.40	6.44±0.25	0.80±0.05

**Means ± SD; [abc] Means bearing different superscripts in the same column differ significantly (p<0.005)*

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of raw cow milk (Suman *et al*.,1998).

Table 2. Microbiological loads of cow milk (log cfu/ml) in Madurai district

Region	TC	BS	LAB	Coliforms	*E. coli*	*S. aureus*
North (n=15)	5.17±0.46*[a]	3.08±0.11[a]	4.54±0.64[a]	2.41±0.21[a]	1.63±0.13[a]	2.15±0.53a
West (n=15)	6.40±0.31[b]	2.44±0.21[b]	5.03±0.31[b]	3.36±0.08[a]	1.65±0.41[b]	2.34±0.47[a]
East (n=15)	5.45±0.34[b]	1.61±0.26[b]	3.69±0.20[ab]	2.21±0.29[a]	1.46±0.17[b]	1.40±0.72[b]
South (n=15)	6.37±0.14[b]	2.35±0.41[a]	4.61±0.61[ab]	3.09±0.11[a]	1.89±0.09[b]	1.81±0.17[c]
Average	5.84 ±0.31	2.37±0.27	4.46±0.44	2.76±0.18	1.63±0.20	1.92±0.47

**Means ± SD; [abc] Means bearing different superscripts in the same column differ significantly (p<0.05); TC-total count of mesophilic aerobic bacteria: BS-bacterial endospores: LAB- lactic acid bacteria.*

Table 3. Microflora of 60 samples of cow milk in Madurai district

Type of microorganism	% of samples with log N cfu /ml						
	<1	1-2	2-3	3-4	4-5	5-6	6-7
TC					10	74	16
LAB		12	35	27	18	6	
BS			3	67	25	5	
Coliforms		7	71	14	8		
E. coli	18	34	44	4			
S. aureus	21	29	40	10			
Listeria spp	100						

TC- Total count of aerobic bacteria; LAB- Lactic acid bacteria; BS- bacterial endospores.

Microbiological analysis:

The microflora of raw cow milk is presented in Tables 2 & 3. Differences among milks from different regions of cow raw milk were studied by analysis of variance (Table 2) of all regions. The highest ($p<0.05$) average loads of TC, LAB and coliforms were observed in northern. No significant differences were observed with respect to the average counts of *E. coli*.

Raw milk contained an average TC of 5.84 log cfu/ml. It is a high count of TC and should be due to inadequate sanitary conditions during milking, collection and transport. Raw cow milk in Italy (Supino *et al*., 2004) had total bacterial counts of 5.23 log cfu/ml, which is of the same as our data. LAB constituted a major part of the microflora with an average 4.46 log cfu/ml. Boycheva *et al*., (2002) observed that LAB and psychrotrophs predominated in Bugarian buffalo milk. As the result indicates relatively high numbers of LAB, it may cause undesirable fermentative acidification of raw milk. So effective measures should be taken to avoid this kind of spoilage.

The average count of bacterial endospores was 2.37 log cfu/ml; these usually originate from the soil, manure, and forages (Zhou, 1998). Although only 9% of the samples exceeded the tolerated level of 10^4 cfu/ml (Table 3), spores however pose a potential threat for the quality of buffalo milk products, since they might survive pasteurization and other heat treatments (Te geffel, 2003).

The average load of *S. aureus* was 1.92 log cfu/ml. Fook *et al*.,(2004) reported considerably higher levels of *S. aureus* in other cow milk, with 35% of the samples having 4.2 log cfu/ml. since *S. aureus* is potentially hazardous at > 10^4 cfu/ml (Han *et al*.,2005), all cow milk samples were within an acceptable level. However, since 79% samples contained *S. aureus,* it must be prevented to avoid potential risk. The presence of *Listeria* spp. was not detected in any samples. The average levels of coliform bacteria and *E. coli* were 2.76 and 1.63 log cfu/ml respectively (Table 2).

These counts were higher than those reported by Desmasures *et al*.,(1997), who reported that 84% of samples of French cow milk had coliform counts <100 cfu/ml and 80% had *E. coli* counts ≤10 cfu/ml. *E. coli* may be considered an indicator microorganism of faecal contamination and other entric pathogens. Its occurrence in milk may originate from machines, manual milking, handling, and inferior quality of water (Fook *et al*., 2004).

Conclusion

The chemical composition of raw cow milk indicates that it is a rich source of nutrients and thus offers excellent opportunities for the development of local dairy industry and to meet the public need for nutrition. The microbiological quality was only marginally acceptable with respect to the total bacteria count. Nevertheless, the presence of pathogenic and indicator bacteria, such as *E. coli*, coliforms and *S. aureus* indicate that to the growth of these organisms may lead to a hazard against public health. Therefore practice and regulations, such as on-site pasteurization and implementation of HACCP following established standards, should be introduced to facilitate the production of cow milk of high quality and safety.

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