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Seasonal Variation in Physico-chemical and Microbiological Properties of the Raw Milk from the Street Vendors of Jabalpur (India)

[1]Karuna S. Verma, [2]Apurva K. Pathak and 3* Anand Kumar Soni

[1]Deptt. of Biological Science, R. D. University, Jabalpur, India

[2]Deptt. of Microbiology, Modern Dental College and Research Centre, Gandhinagar, Indore, India

[3]*Central Pollution Control Board, Zonal Directorate (North), Ground Floor, PICUP Bhawan, Vibhuti Khand, Gomti Nagar, Lucknow-226010, India

Email : dr.anandkumarsoni@gmail.com

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Abstract : The household milk consumers of India are mostly depend upon the supply of unprocessed bovine milk from unorganized street milk vendors that bypasses the quality control regime. Many factors affect the quality of traditionally collected milk, in which changes in environmental parameters is of utmost important. The aim of this study was to investigate the effect of different Ritus (Season) on the physico-chemical properties and microbial load of raw milk. About 600 raw milk samples were collected from the street milk vendors of the city of Jabalpur, throughout the year in order to cover all the six Ritus (Season) i.e. Shishira (late winter), Vasanta (spring), Greeshma (summer), Varsha (rain), Sharad (autumn) and Hemanta (early winter) and their physico-chemical properties including the Temperature, MBRT, Acidity, Fat (%age), CLR, SNF as well as microbial load (Coliform and Total count) were examined. The results showed that milk marketed during the spring (*Vasanta*) season; have good physico-chemical quality compared to all other seasons, and the parameters that tested for microbial quality of milk were reported best during the autumn (Sharad), shows the variations in the quality of the milk sampled might be attributed to the seasons.

Keywords: Milk, Season, MBRT, CLR, SNF, Coliform and SPC.

Introduction

Agriculture is the base of Indian economy. Livestock production including dairy plays a multipurpose role in the agriculture system of India. Dairy plays a dynamic role in India's agro-based economy. Today, India ranks the first in the world in terms of milk production (Chye *et al.,* 2004). According to FSSAI (2011), milk can be defined as the normal mammary secretion derived from complete milking of healthy milch animal, without either addition thereto or extraction there from, unless otherwise provided and it shall be free from colostrums.

The seasonal variation in cows diet confirms that milk properties such as taste, color, fat content and even kinds of fats differ by season for example milk will be richer in valuable fatty acids like omega 3's and antioxidants in the summer (Rajeevie *et al.,* 2003). The effects of the various season of the year have been studied by different authors (Nateghi *et al.,* 2014) for the reason that climatic and geographic conditions that cannot be affected should be considered. The different seasons of the year are often related to different food regimes for cows. Food intake, kind and quality of fodder are connected to the food regime. This regime offers different possibilities to the breeder because using suitable diets that contain mineral and nutritional component according to the needs of the cows and the structure of the diets that enables good digestion, adequate intake and metabolism are enabled which on the other hand effects on the milk composition (Rajeevie *et al.,* 2003; Nateghi *et al.,* 2014).

The assessment of microbial load at various stages of manufacturing or processing may serve as a useful tool for quality assessment and improvement which will result in longer shelf life which is a desirable market requirement. Keeping fresh milk at an elevated temperature together with unhygienic practices in the milking process may result in microbiologically inferior quality. Apparently, these are common practices for small-scale farmers who produce fresh milk and sell it to local consumers or milk collection centers (Chye *et al.,* 2004).

Despite of presence of various regulatory mechanisms, dairy industry is one of the highly unregulated industries in India because of major player of this industry is dominated by private sectors. The major fraction of the unprocessed milk marketed to the Indian urban consumers comes from the producers of peri-urban area which include small, marginal farmers and landless laborers. Urban retailers or middlemen procured this milk to deliver urban consumers. Ineffective quality control systems of state, lack of knowledge about clean milk production, lack of post milking chilling facilities, mal-practices and greediness of these middlemen were resulted into poor quality of milk distribution in the community (Siva and Sannabhadti, 1994; Pathak *et al.,* 2012).

Keeping in the mind of above facts, the aim of this work was to ascertain the variation in physico-chemical and microbial properties of the traditionally collected un-certified raw milk from the street vendors of Jabalpur (India) based on Indian seasons.

Materials and Methods

Jabalpur (Latitude: 23.2; Longitude: 79.95; Altitude: 391.) also known as *Sanskaardhaani* is the third largest urban agglomeration in the state of Madhya Pradesh, India. Based on Indian tradition, the climate of Jabalpur is divided into six main season's also known as *Ritus* (Table:1), on the basis of the predominant effect of Sun and Moon each *Ritu* (season) consists of two months (Nathani *et al.,* 2012; Singh *et al.,* 2012).

Table:1 List of different Ritus (Seasons) in India.

Ritu (Season)	Hindi months	Date
Shishira (late winter)	Magha-falguna	15^{th} Jan-14^{th} March
Vasanta (spring)	Chaitra-vaishakh	15^{th} March-14^{th} May
Greeshma (summer)	Jyestha-ashadha	15^{th} May-14^{th} July
Varsha (rain)	Shravan-bhadrapada	15^{th} July-14^{th} Sep
Sharad (autumn)	Ashwina-kartika	15^{th} Sep-14^{th} Nov
Hemanta (early winter)	Margashisha-pausha	15^{th} Nov-14^{th} Jan

Source of Milk Samples: Raw milk samples were obtained from domestic and market area of five different community settlements at Jabalpur. The sampling carried out randomly, fortnightly and in triplicate, for a period of one year, in order to cover all the six seasons; that made a total of 600 samples in a year. Each batch was collected and after recording initial temperature in terms of °Celsius, kept in an ice-box and transported to the laboratory for physico-chemical and microbiological analysis.

Physico-chemical Analysis: Fat Percentage, Solid Not Fat (S.N.F.), Corrected Lactometer Reading (C.L.R.), Titratable Acidity (T.A) and Methylene Blue Reduction Test (M.B.R.T.) for microbial activity were determined according to the modified method of Association of Official Analytical Chemists. Washington, DC (AOAC, 2000).

Microbiological Examination: After receiving the sampled milk in the laboratory, the milk was kept at ambient temperature for 15 minutes to bring milk at ambient temperature level. The samples were further subjected to the serial dilution before inoculation by transferring one ml milk serially in sterile buffered peptone saline (0.5% w/v; peptone; 0.85% w/v; NaCl) to make dilution of 10^{-1} to 10^{-8}. 0.1 ml and 1ml of the milk from each diluted samples were transferred to surface of Plate Count Agar medium for Standard Plate Count (SPC) (Hi Media, Mumbai, India) and Violet Red Bile Agar (VRB) (Hi Media, Mumbai, India) plates for the detection and enumeration of coliform organisms in milk. The sample on the plate was spread evenly using sterile L-spreader. After incubation of each plate at 37±2°C for 24-48 hours, the colonies were counted in terms of colony forming units (cfu), from the plate having 20-200 colonies (Collee *et al.,* 1999; Dworkin *et al.,* 2006; Forbes *et.al.,* 2002; Jones and Sackin, 1980; Krieg and Holt, 1984). The numbers of bacteria per ml in milk samples calculated by taking average of three plates and the colonies were counted in terms of cfu per ml of milk by the methods as described previously by Khan *et al.* (2008).

Statistical Analysis: In order to analyze the relationship between various physico-chemical and microbiological parameters, Spearman correlation coefficients were done by using the SPSS Win 16.0 program.

Result

Physico-chemical and microbiological qualities of consumable raw milk supplied at the urban community area were monitored for a period of one year to cover a full cycle of the season. The physical properties of raw milk collected in Jabalpur city are shown in **Table 2**. The study revealed that a mean ± standard deviation (SD 3203.77±741.30 cfu/ml and 16.73±9.75 million cfu/ml.) the coliform count and total plate count in million respectively of bacterial count in the sampled raw milk collected from community area of Jabalpur; ranges from 2000.00-4440.00cfu/ml. of the coliform count and the total plate count was 4.00-40.00x10^6cfu/ml. of the sampled milk.

Table 2: Descriptive Statistics of Raw milk analyzed.

Parameters	Mean± Std. Deviation	Minimum	Maximum
Initial Temperature (ºC)	17.5±6.3	5.0	31.0
MBRT (min.)	63.8±31.6	30.0	120.0
Acidity	0.135±0.011	0.108	0.153
Fat (%)	4.9±1.6	2.0	8.0
CLR	25.3±1.6	21.5	29.0
SNF	8.0±0.6	6.7	9.4
Coliform/ml	3203.77±741.30	2000.00	4440.00
Total count in millions/ml	16.73±9.75	4.00	40.00

MBRT- Methylene Blue Reduction Test; CLR-Corrected Lactometer Reading, SNF-Solid Not Fat.

Table 3: The mean±SD values of seasonal variation of physicochemical and microbiological parameters of raw milk.

Season	**Initial Temp(ºC)**	**MBRT (min.)**	**Acidity**	**Fat%**	**CLR**	**SNF**	**Coliform /ml**	**Total count (in millions)**
Autumn (Sharad ritu)	19.2±5.9	66.3±31.1	0.133±0.010	4.8±1.7	25.1±1.8	7.90±0.6	3123.2± 746.38	15.81±10.02
Early winter (Hemanta ritu)	16.6±6.1	69.9±34.4	0.135±0.012	4.8±1.6	25.2±1.5	7.96±0.6	3261.0± 747.13	14.98±9.33
Late winter (Shishira ritu)	14.4±4.7	66.0±33.3	0.137±0.010	4.8±1.6	25.3±1.6	7.98±0.5	3270.4± 741.87	15.94±9.58
Spring (Vasanta ritu)	15.6±5.3	59.7±31.5	0.138±0.011	4.9±1.6	25.4±1.4	8.00±0.5	3129.6± 717.10	18.14±10.12
Summer (Greeshma ritu)	20.1±6.9	61.2±28.9	0.135±0.010	4.9±1.6	25.3±1.7	7.99±0.6	3200.8± 707.75	17.67±9.66
Rain (Varsha ritu)	19.0±6.5	59.4±29.5	0.133±0.011	4.9±1.7	25.2±1.7	7.95±0.6	3237.6± 789.32	17.98±9.56

Table 4: Correlation between physicochemical and microbiological parameters of raw milk.

Parameters	Initial Temp (ºC)	MBRT (min.)	Acidity	Fat%	CLR	SNF	Coliform /ml	Total count (in millions)
Initial temp (ºC)	--	NS	S	NS	S	NS	NS	NS
MBRT (min.)	NS	--	NS	NS	NS	NS	NS	S
Acidity	S	NS	--	NS	NS	NS	NS	S
Fat%	NS	NS	NS	--	NS	S	NS	NS
CLR	NS	NS	NS	NS	--	S	NS	NS
SNF	NS	NS	NS	S	S	--	NS	NS
Coliform /ml	NS	NS	NS	NS	NS	NS	--	NS
Total count (in million)	NS	S	S	NS	NS	NS	NS	--

MBRT- Methylene Blue Reduction Test, CLR-Corrected Lactometer Reading, SNF-Solid Not Fat, NS-Non-significant and S-significant.

Mean of the physico-chemical and microbiological parameters of raw milk samples based on season were also analyzed. There were very little variation in these parameters recorded during the study period in the milk sampled from the market and venders, as shown in the **Table 3**. In raw milk samples; significant negative correlation at the 0.01 level (1-tailed, Pearson) were established between initial temp and acidity and total count with MBRT, and significant positive correlation between SNF with fat% and CLR (**Table 4**).

Discussion

The analysis of raw milk brings together information on the public health risks associated with the consumption of raw milk, and estimates the resulting burden of illness that may occur. This information provided an overall picture of the public health risks associated with consumption of raw milk. In order to estimate the likelihood of illness for consumers following consumption of raw milk, quantitative microbiological estimation was undertaken.

Analysis of raw milk obtained from domestic and market area of five different community settlements at Jabalpur, shows that most of the samples were below the standard. Variation in temperature depends upon climate (NEERI, 1988). In this study the mean ± std deviation, minimum and maximum temperature were recorded as 17.5±6.3, 5 and 31°C respectively and the mean temperature of raw milk was recorded high (20.1 °C) in summer (*Greeshma*) and low (14.4 °C) in late winter (*Shishira*) that is probably due to the lack of cooling system in dairy farms and inefficient use of refrigerator by milk vendors. According to Reinemann *et al.* (2005), inadequate cooling will increase bacterial counts by allowing a better environment for bacterial growth during storage, thus resulted into decreased self-life.

Methylene Blue (MB) dye has been employed to check for the overall microbial load and quality control of milk and other liquid foods (Impert *et al.,* 2002). The shorter the methylene blue reduction test, the lower the quality of milk (Benson, 2002). In present study the methylene blue reduction test performed for raw milk samples revealed that the mean reduction time of methylene blue was recorded 63.8 min. and the minimum and maximum methylene blue reduction time were 30 and 120 min., respectively which are not below the standard for raw milk and the mean reduction time is high (69.9 min.) in early winter (*Hemanta*), indicative of low bacterial load in milk during this season and low (59.4 min.) in Rain (*Varsha*).

The production of acid in milk is normally termed "souring" and the sour taste of such milk is due to lactic acid production. The percentage of acid present in milk is a rough indicator of its age and the manner in which it has been handled. Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid (O'Connor, 1995). In this study minimum value of acidity was recorded 0.108 and maximum was 0.153 and the mean value of acidity was 0.135 of raw milk. Acidity of milk is high (0.138) in spring (*Vasanta*) may be due to high microbial load and low (0.133) in autumn (*Sharad*) and rain (*Varsha*) respectively. The acidity of milk samples was not within the standard range (0.117-0.135), since its value has to be kept under fixed law limits. Hossain *et al.* (2010) reported higher acidity (0.216% lactic acid) for milk collected from vendors. Hand washing using detergents, washing of udder and teats using sanitizers was not practiced. On the other hand, the poor transportation system used in the study area might contribute to the contamination of milk by microorganisms. The growth of microorganisms in the milk might increase the acidity of the milk.

During the present study the mean ± standard deviation, minimum and maximum fat percentage was recorded as 4.9±1.6, 2.0 and 8.0 respectively and the amount of fat percentage contained in spring (*Vasanta*), summer (*Greeshma*) and rain (*Varsha*) milk was higher (4.9%) than autumn (*Sharad*), early winter (*Hemanta*), late winter (*Shishira*) milk as its amounts were reported during this study were 4.8% respectively. According to Yasmin *et al.,* (2012) the highest fat content (5.4%) was observed in February and lowest (4.6% and 4.3%) was recorded in June and July, the decrease in fat content is more pronounced from late June to early August. The average fat content of milk obtained from dairy farms is little higher with earlier findings of Janstova *et al.,* (2011) who reported a fat content of 3.79 ± 0.18% for milk produced in dairy farms. However, lower value of fat content (4.3%) was reported from milk of cows from dairy farms were reported by Mansson *et al.,* (2003) as compared to the present study. Some of the dairy farmers in Jabalpur city normally separate cream from the milk before marketing which resulted to lower fat content of raw milk samples compare to standard level. This malpractice is probably due to the high expenses of milk production for small scale dairies in this city. They traditionally sell separated fat as cream to consumers in the local markets to earn more profit.

The sellers adulterate water because its cheep rather than starch which may be expensive or difficult to be homogenized and obviously can be detected and discovered by consumers (Adam, 2009). In the present study the mean CLR value was recorded as 25.3 and minimum and maximum values were recorded as 21.5 and 29.0 respectively. The maximum value of CLR (25.4) was recorded in spring (*Vasanta*) and minimum value was recorded in autumn (*Sharad*) for corrected lactometer reading was 21.5. The CLR of milk samples was not found within the standard range (28.5-32). Observing by Murali *et al.* (2005) that there was a gradual but significant ($P<0.05$) decrease in the CLR of cow milk from 27.9 ± 0.35 (unadulterated) to 13.5 ± 0.07 (50% adulterated). The CLR of buffalo milk decreased significantly ($P<0.05$) from 32.9 ± 0.07 (unadulterated) to 15.5 ± 0.07 (50 % adulterated).

In this study the mean ± standard deviation, minimum and maximum SNF content were recorded as 8.0±0.6, 6.7 and 9.4 respectively and SNF content was recorded high (8.0) in spring (*Vasanta*) and quite low in other seasons was less than the findings (8.7) of Bille *et al,*. (2009) and Janstova *et al.,* (2011) who reported higher (8.96%) value of SNF content of raw cow milk. The difference observed in SNF content of milk could be due to difference in the feeding practices, season, milking method and lactation period exerted (Suman *et al.,* 1998). According to European Union quality standards for unprocessed whole milk, solids-not-fat content should not be less than 8.5% (Tamime, 2009)

The standard plate count is suitable for estimating bacterial population in most types of dairy products and it is the reference method specified in the Grade 'A' Pasteurized Milk Ordinance to be used to examine raw and pasteurized milk and milk products (Marshall, 1992). The European Union (EU) had set standards for raw milk offered for sale. The basic milk hygiene requirement in the EU for total aerobic bacterial count should be less than 10^5 cfu/ml and in many ways the directives are comparable to the US "Grade A" Pasteurized Milk Ordinance (Hillerton and Berry, 2004).

In the milk hygiene condition the degree of cleanliness of the milking system probably influences the bacterial count as much. Milk residue left on equipment contact surfaces supports the growth of a variety of microorganisms. Organisms considered being natural inhabitants of the teat canal and teat skin. In this study mean standard plate count for raw milk were reported in Jabalpur retails was 16.73×10^6 cfu/ml. The present study showed that the total aerobic bacterial count of all milk samples exceeds the standards given for milk by European Union and US regulations. Microbial load of spring (*Vasanta*) milk was significantly higher (18.14×10^6 cfu/ml) than that of autumn (*Sharad*), early winter (*Hemanta*), late winter (*Shishira*), summer (*Greeshma*) and rain (*Varsha*) milks were 15.81×10^6 cfu/ml, 14.98×10^6 cfu/ml, 15.94×10^6 cfu/ml, 17.67×10^6 cfu/ml and 17.98×10^6 cfu/ml respectively suggesting that spring (*Vasanta*) milk was produced under more favorable hygienic conditions. Ahmed *et al.,* (2008) who found low total aerobic bacterial count of (9.089 ± 0.281 cfu/ml) in milk samples collected from dairy farms of Khartoum State. Furthermore, lower total aerobic bacterial count (10^6cfu/ml) was reported by Millogo *et al.* (2010) for bulk tank milk of dairy farms in Burkinafaso.

According to the European Union standards for total aerobic bacterial and coliform counts of raw milk should be less than 10^5 and 10^2cfu/ml respectively (Fernandes, 2009). Furthermore, based on the standards of US regulations for the quality of raw milk, coliform bacteria count per ml of milk must not be above 100 cfu/ml and *E. coli* must be negative (Hillerton and Berry, 2004). In this study total coliform density was recorded minimum (2,000 cfu/ml.) and maximum (4,440 cfu/ml.) in raw milk and the mean value was 3203.77 cfu/ml. is above the range. Coliform counts are associated with fecal and environmental contamination of the milk. Milching animals with coliform mastitis cause the milk coliform count to rise. According to Boor *et al.,* (1998) the acceptable limits of coliform counts in milk should be less than 100 cell/ml. In this study coliform count reported significantly higher 3270.4 cfu/ml. in late winter (*Shishira*). This higher count of vendor milk was reported by El Zubeir and Ahmed (2007) and Singh *et al.,* (1975) who reported a count of more than 5600 cell/ml in vendor milk. These findings were also in agreement with Beniwal *et al.,* (1998) for they reported the coliform count of 1000 to 4700 cell/ml.

This confirms the severity of contamination of raw milk in Jabalpur. The higher total aerobic bacterial count observed in the present study might be attributed to the initial contamination of the milk samples either from the udder of the cow, the milker`s hand, the milking area and the milking containers. The very high bacteria count observed in milk samples collected from vendors' could probably be due to further contamination of the milk during transportation, the use of poorly cleaned milk containers, lack of and improper cooling systems at milk vending areas and poor personal hygiene.

In order to analyzing the correlation between the factors (Initial temperature, methylene blue reduction test, acidity, fat percentage, corrected lactometer reading and solid-not-fat) and the quantity of bacteria isolated from raw milk. Karl`s Pearson correlation analysis is performed (Table 4). In raw milk there is low degree of positive correlation was established in between initial temperature and total count (r = + 0.034), M.B.R.T. and coliform (r = + 0.0173), acidity and total count (r = + 0.392), fat and coliform (r = + 0.109), C.L.R. and coliform (r = + 0.131), C.L.R. and total count (r = + 0.046), SNF and coliform (r = + 0.165), SNF and total count (r = + 0.059), low degree of negative correlation is established in between initial temperature and coliform (r = - 0.056), acidity and coliform (r = - 0.155) and coliform and total count (r = - 0.298) and strong negative correlation was established in between M.B.R.T. and total count (r = - 0.952).

In general the result of the present study indicates that seasons affect milk quality and spring (*Vasanta*) milk showed better quality regarding physico-chemical parameters. Regarding microbial load it was predicted that summer milk would show higher microbial load because of higher temperatures, but the results were the reverse of what had been expected as the microbial load of spring (*Vasanta*) milk was significantly higher than summer (*Greeshma*) milk due to ambient temperature for microbes. Changes in season are a natural process which could not be altered by farm management practices. Thus feeding of animals with special diet and transportation of milk in cool containers could result in better quality of milk throughout the year. Coliforms may grow in raw milk and reaches higher number in tropical countries or in the absence of cooling system. The isolation of coliform from milk poses a serious threat to food safety. Milk temperature is simple and easy criteria that could be used by milk vendor's in Jabalpur to determine milk quality. This could reduce the risk of change in quality of milk.

Conclusion: The unorganized dairy industry of Jabalpur is mainly in the hands of small and marginal farmers. These small and marginal farmers generally managed their livestock through grazing system. During the present study a wide variation was observed in physico-chemical and microbial quality of milk sampled from local milk venders. Since the pasture cattle feeding and managements are highly depend on environmental factors and seasonal availability of fodders and forage, and in absence of standard milk management and transportation practices, the variations in the quality of the milk sampled might be attributed to the seasons.

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