

EFFECTS OF MILK AGE AND STORAGE TEMPERATURE ON SOMATIC CELL COUNT OF BOVINE MILK: A CASE STUDY FROM TURKEY

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ABSTRACT

The aim of this study was to determine the effects of milk age (MA) and storage temperature (ST) on somatic cell count (SCC) of cow milk. Raw milk samples were taken from a milk cooperative in the Black Sea region of Turkey, with monthly visits throughout a year. All samples were evaluated by direct microscopy according to five different MA (1d, 2d, 5d, 8d and 15d) at refrigeration (RT) and freezing (FT) temperatures (+4 °C and -20 °C). When SCC of the samples belonging to different MA were compared by SCC of fresh milk, no statistical difference was found at RT, but SCC decreased significantly ($P < 0.001$) at 8d and 15d post collection at FT. Highest SCC values were recorded in both RT and FT samples tested in the autumn season, and lowest SCC values were determined in the winter season, except for samples stored 2d at RT. The correlations among SCC values by MA were statistically significant ($P < 0.01$) at both RT and FT. As the result, SCC of cow milk obtained from 15d post collection at RT and 5d post collection at FT can safely be used as the ones determined from fresh milk for assessing raw milk quality and controlling herd management programs.

Key words: cow milk, somatic cell count, storage, temperature.

INTRODUCTION

Today, obtaining and saving raw milk quality are assumed as the main issues for dairy sector. In many countries, quality milk premiums are gradually focused and thus, determination of quality by convenient techniques and storage raw milk by proper methods has continuously been conducted. Of milk quality indicators, somatic cell count (SCC) is assumed as a reliable parameter to determine health status of cow or herd (Köster *et al.*, 2006), and thus, its elevated levels reflect lower milk quality (Ogola *et al.*, 2007). In EU countries, acceptable level of SCC per mL for human consumption has been confirmed to be 400 000 cells/mL (Sharma *et al.*, 2011). Besides, some study results (Svensson *et al.*, 2006; Rhone *et al.*, 2008) revealed that various factors are effective on SCC. Sanchez-Macias *et al.* (2010) emphasized that milk preservative, analytical or storage temperature and milk age have been identified as main factors affecting SCC of cows. Also, Sánchez *et al.* (2005) reported that information on the different methods of preservation, storage temperature, and interactions with storage time could help to optimize analyses, and the effects of these factors must be taken into account for quality control observations. While SCC records have been regarded for selecting cows for treatment or culling in dairy operations (Barkema *et al.*, 1997), determination of SCC in previously frozen samples would be advantageous to subclinical mastitis control programs. In spite of some studies had been conducted on storage temperature (Martinez *et al.*, 2003; Sánchez *et al.*, 2005;

Zeng *et al.*, 2007), these were especially on sheep or goat. However, studies on cattle (Barkema *et al.*, 1997; Hachana *et al.*, 2008; Malinowski *et al.*, 2008) were focused on the efficiency of electronic cell counters or on the udder quarter milk samples.

The purpose of this study was to reveal the effects of milk age and storage temperature on SCC of milk samples from tanks of dairy farms.

MATERIALS AND METHODS

Sampling and SCC Analysis: A total of 401 raw milk samples were collected from tanks of 34 dairy farms enrolled to a milk cooperative in Bafra district of the Middle Black Sea region of Turkey. For this aim, the farms were visited with 28 d intervals between April 2007 and March 2008 and about 100 ml raw milk samples were taken from each milk tank. No preservative added milk samples were immediately transported to laboratory for SCC analyses.

All collected samples were analyzed within test day and thus, fresh SCC values were recorded. In addition, the same samples were stored in a refrigerator for 1d, 2d, 5d, 8d and 15d at refrigeration (RT) and freezing (FT) temperatures (+4 °C and -20 °C). The temperatures of RT and FT units were controlled continuously and no deviations were observed during the study period. For SCC analysis, direct microscopic cell counting (Packard *et al.*, 1992) was performed. The dye solution prepared for microscopy was composed from 0.6 g of certified methylene blue chloride to 52 mL of 95%

ethyl alcohol, 44 mL of tetrachlorethane and 4 mL glacial acetic acid. Total number of fields counted per slide was 40 and the working factor (WF) was 13255.

Statistical Analysis: Due to wide ranges in the SCC data, SCC values were transformed to log10 for normality and homogeneity of variances. The data were tested by one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test based on the 0.05 level of probability. The model used in the study was:

$$Y_{ijkl} = \mu + A_i + T_j + S_k + e_{ijkl}$$

where Y_{ijkl} = depended variables for logSCC; μ = mean; A_i = milk age (MA) effect (six levels: fresh, 1d, 2d, 5d, 8d and 15d postcollection); T_j = storage temperature effect (two levels: RT and FT); S_k = storage season effect (four levels); and e_{ijkl} = random residual.

To compute correlations of logSCC values among different storage periods, Pearson's correlation coefficient analysis was applied. All statistical analyses were performed using SPSS 10.0 for Windows (SPSS, 1999)

RESULTS AND DISCUSSION

In this study, when the changes of seasonal logSCC values by different MA at RT were evaluated, it was observed that logSCC means were highest in the autumn and logSCC means were lowest in the winter, except for 2d after refrigeration (Table 1). This result was agreement with the result of Przysucha and Grodzki (2004), who obtained the best quality raw milk samples in winter months. However, no statistical difference was determined among logSCC means of fresh milk samples. This finding was similar with the results obtained for FT. When the season groups were evaluated by separately, significantly statistical difference ($P < 0.01$) was observed in 1d after refrigeration, and logSCC mean of winter season was different from the means obtained in the summer and autumn seasons. For 2d after refrigeration, logSCC mean of spring was found as different from logSCC mean of autumn, statistically ($P < 0.01$). Besides, for 5d after refrigeration, logSCC mean of autumn, which was the highest value in similar to the other MA groups, was recorded as significantly ($P < 0.05$) different from the mean logSCC determined in the winter and summer seasons. Interestingly, logSCC means among 8d MA values at both RT and FT were not statistically significant in the present study. Thusly, this finding was harmonic with logSCC means of fresh milk samples. However, significant ($P < 0.001$) difference was found among logSCC means for 15d MA, and the mean logSCC of winter season was also statistically ($P < 0.001$) different from those determined in the other seasons. These results indicated that when milk samples were stored at +4 °C,

8d MA was advised for SCC determination truly if raw milk samples evaluated by regarding the seasons.

Seasonal distribution of logSCC for different MA at FT is given in Table 2. According to this, no statistical difference was determined among logSCC means of fresh milk samples. However, logSCC means of autumn seasons were higher ($P < 0.01$) than those identified in the other seasons. For 1d after freezing, logSCC mean of autumn was different from those tested in the winter and spring seasons. Besides, for 2d after freezing, logSCC mean belonging to winter was found to be different ($P < 0.01$) from those determined in summer and autumn, statistically. While 5d after freezing logSCC means between winter and autumn was different ($P < 0.01$), no statistical difference was found among means for 8d after freezing by seasons. Besides, as similar with 5d after freezing logSCC values, 8d mean of winter season was different from autumn mean, statistically ($P < 0.01$). It is likely that thawing milk samples and preparing slides for SCC test processes in the room temperature caused to seasonal variation in SCC in the current study. Such that, Barkema *et al.* (1997) emphasized that cooling and thawing were effective on both SCC and pathogens.

LogSCC values were assessed by regarding all seasons in the study (Table 3). It can be seen that logSCC means of fresh milk samples were relatively in high levels, and logSCC values of milk samples decreased with advancing MA. This result was in agreement with the finding of Sanchez-Macias *et al.* (2010). However, logSCC means for 8d and 15d MA at FT were found as significantly ($P < 0.001$) different from that calculated for fresh samples. This case was also found as harmonic with the result of Sánchez *et al.* (2005), who reported that freezing milk samples gave rise to a significantly reduced logSCC values. Also, Schukken *et al.* (1989) reported that intracellular and extracellular ice might have caused the damage and reduction of somatic cells. Besides, this result clearly shows that SCC can accurately be determined up to 5d when raw milk was stored at FT. In this study, logSCC for various MA at RT were observed in a alternative trend. But, no statistical difference was determined between logSCC mean of fresh milk samples and logSCC means of the samples stored for various MA periods at RT. However, means of 1d MA and 15d MA were different from 5d MA, statistically ($P < 0.001$). Thusly, these results indicate that SCC records can reliable be used up to 15d when stored at RT. Notwithstanding, owing to this study investigated raw milk samples until 15d, works carried out on longer storage periods are needed.

Correlations among logSCC for various MA periods are shown in Table 4. As seen that significant ($P < 0.01$) relationships were estimated among all values. This finding clearly points out that SCC records determined in various storage periods could be useful

reflectors for assessing raw milk quality or selecting cows in the herds.

Results here indicate that storing bovine raw milks up to 5d at FT and 15 at RT could be performed to determine SCC for quality evaluation. Besides, SCC values of stored milks were lowest in the winter and

highest in the autumn, approximately. Thus, seasonal changes among various storage periods should be taken into account for obtaining more accurate SCC records. In addition, further investigations focused on longer storage periods should also be carried out.

Table 1. Seasonal distribution of logSCC for different milk age at refrigeration temperature

Season	Fresh	1d**	2d**	5d*	8d	15d***
1	5.85±0.02	5.79±0.02a	5.87±0.02ab	5.8579±0.02a	5.82±0.02	5.72±0.01a
2	5.84±0.01	5.82±0.02ab	5.83±0.01a	5.91±0.02ab	5.87±0.01	5.85±0.01b
3	5.87±0.02	5.89±0.02b	5.89±0.01ab	5.88±0.01a	5.84±0.01	5.82±0.01b
4	5.88±0.02	5.89±0.02b	5.92±0.02b	5.96±0.02b	5.88±0.01	5.88±0.01b
Overall	5.86±0.01	5.84±0.01	5.87±0.01	5.90±0.01	5.86±0.01	5.82±0.01

Within the columns the numbers with different superscripts differ significantly

* P<0.05, ** P<0.01, *** P<0.001

Season 1: winter, 2: spring, 3: summer, 4: autumn

Table 2. Seasonal distribution of logSCC for different milk age at freezing temperature

Season	Fresh	1d**	2d**	5d**	8d	15d**
1	5.85±0.02	5.77±0.01a	5.77±0.02a	5.76±0.02a	5.76±0.02	5.74±0.02a
2	5.84±0.01	5.78±0.02a	5.78±0.02ab	5.80±0.02ab	5.80±0.02	5.81±0.01ab
3	5.87±0.02	5.81±0.01ab	5.86±0.01bc	5.82±0.02ab	5.81±0.01	5.79±0.01ab
4	5.88±0.02	5.87±0.02b	5.87±0.02c	5.88±0.022b	5.83±0.01	5.84±0.01b
Overall	5.86±0.01	5.80±0.01	5.82±0.01	5.81±0.01	5.80±0.01	5.80±0.01

Within the columns the numbers with different superscripts differ significantly

** P<0.01

Season 1: winter, 2: spring, 3: summer, 4: autumn

Table 3. Changes of logSCC values by milk age

MA	n	FT	RT
Fresh	401	5.86±0.01 b	5.86±0.01 ab
1 d	392	5.80±0.01 ab	5.84±0.01 a
2 d	396	5.82±0.01 ab	5.87±0.01 ab
5 d	395	5.81±0.01 ab	5.90±0.01b
8 d	290	5.79±0.01 a	5.85±0.01 ab
15 d	349	5.80±0.01 a	5.82±0.01 a
Overall	2223	5.82±0.01	5.86±0.01

Within the columns the numbers with different superscripts differ significantly (P<0.001)

MA:milk age, RT :+4 °C, FT: -20 °C

Table 4. Correlations among logSCC values at different storage temperature and periods

MA/ST*	1d-RT	1d-FT	2d-RT	2d-FT	5d-RT	5d-FT	8d-RT	8d-FT	15d-RT	15d-FT
Fresh	0.667	0.622	0.646	0.581	0.523	0.577	0.443	0.536	0.428	0.563
1d-RT		0.721	0.710	0.647	0.607	0.652	0.530	0.606	0.466	0.612
1d-FT			0.639	0.633	0.572	0.604	0.456	0.508	0.433	0.576
2d-RT				0.649	0.574	0.581	0.475	0.580	0.426	0.622
2d-FT					0.577	0.610	0.489	0.572	0.423	0.557
5d-RT						1	0.641	0.499	0.448	0.585
5d-FT							0.496	0.576	0.431	0.570
8d-RT								0.512	0.375	0.519
8d-FT									0.433	0.596
15d-RT										0.522

*All correlations statistically significant at the level of P<0.01

MA: milk age (day), ST: storage temperature (RT: +4 °C, FT: -20 °C).

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