



## Research Article

# Effect of somatic cells count of raw cow milk on the fermentation process and quality of ayran

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## Abstract

The present study aimed to provide scientific information for the effect of somatic cells count (SCC) of raw cow milk obtained from different regions of Turkey on the fermentation process and quality characteristics of ayran. Test ayran samples were produced from three different batches of cow milk with low (<400 000 cells.cm<sup>-3</sup>), medium (between 500 000 and 600 000 cells.cm<sup>-3</sup>) and high (1000 000 cells.cm<sup>-3</sup>) SCC, respectively. The main physicochemical parameters as pH, total solids, milk fat, protein, lactose, lactic acid content and microbiological parameters as somatic cells count (SCC) and total viable count (TVC) of raw milk and ayran samples were studied. During the first two hours of coagulation, the higher SCC in the milk seems to have an inhibiting effect on the development of the lactic acid bacteria from the starter culture and on the fermentation process, respectively. Nevertheless, SCC of the raw milk did not have a significant effect on the composition of the ayran. The increase in the SCC values up to 1000 000 cells.cm<sup>-3</sup> in the raw cow's milk affected negatively the organoleptic characteristics of the ayran made from it. Practical applications: The knowledge for the effect of SCC of raw cow milk on the fermentation process allows us to optimize the technology parameters for production of ayran and to improve its quality characteristics. The data from sensory analysis of ayran samples produced by milks with different SCC will provide additional information for the importance of the implementation of the monitoring of somatic cells in raw cow milk quality control.

**Keywords:** somatic cells, ayran, fermentation, sensory quality

## Abbreviations:

ANOVA - analysis of variance; CFU - colony forming units; GLM - general linear models; SCC - somatic cell count; TVC - total viable count

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## Introduction

The somatic cell count (SCC) is considered to be an important criterion for evaluating milk quality and overall udder health. SCC is often used to distinguish between infected and uninfected quarters. There is a general agreement between infection status and the inflammatory response to this infection as measured by an increased SCC (Bytyqi et al. 2010). Normally, in milk from a healthy mammary gland, the SCC is lower than 100 000 cells per mL, while bacterial infection can cause it to increase to above 1 000 000 cells per mL (Bytyqi et al. 2010). Bulk tank SCC values are routinely used to define the national and international regulatory standards that govern hygienic milk production. In Europe, the EEC directive 92/46 in April 1992 stated that milk with a somatic cell count over 400 000 cells per mL may not be used for fluid milk and starting in 1998 not even for human consumption. In North America limits at 750 000 (USA) and 500 000 cells (Canada) are in place (Sargeant et al. 1998). There are plenty of factors that influence milk SCC at individual and herd level apart from intramammary infection. According to Mundan et al. (2015) such factors are nutrition, inadequate biosecurity rules, milk hygiene and farm capacity. The ability to correctly interpret somatic cell counts depends on an understanding of the factors which may affect the number of somatic cells. It was found that the high presence of SCC in milk affects the activity of fermentation process (Tamime and Robinson 1999) and can even stop it. Fernandes et al. (2007) studied the effect of SCC in raw milk on the chemical and physical properties of yogurt. Rogers et al. (1994) stated that the sensory qualities of yogurt produced from milk with low SCC were superior to yogurts made from milk with high SCC. There are not many studies on the influence of SCC on the quality of ayran. Moreover, the information for the effects of SCC in milk on dynamics of the fermentation process is insufficient. Therefore, the aim of the present work was to determine the effects of SCC levels in raw cow milk obtained from different regions of Turkey on the fermentation process and quality characteristics of ayran.

## Materials and Methods

**Milk samples.** Raw bulk milk samples were collected from small-scale dairy farms affiliated with Dairy Producer Associations in Biga district of Çanakkale province, (Turkey). More than 100 samples were brought to the laboratory of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) at 4°C every week. SCC, TVC and composition of milk samples were measured. All analyses of raw milk were carried out in triplicate. For experimental ayran samples preparation were selected three different batches of raw milk with low (<400 000 cells.cm<sup>-3</sup>), medium (between 500 000 and 600 000 cells.cm<sup>-3</sup>) and high (1000 000 cells.cm<sup>-3</sup>) SCC, respectively.

**Ayran samples.** Ayran samples were produced according to traditional method for Turkey from cow milks with different somatic cell counts according to the following procedure: the raw milks from three batches (L, M and H) with three different SCC were accepted into the pilot dairy processing plant of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) and the platform tests (dry matter, fat, acidity and antibiotics) were carried out, clarification and fat ratio standardization procedures were performed at 55-60°C. Homogenization was carried out at 55°C under 180 kg/cm<sup>2</sup> pressure. Standardization procedure (6%) of dry matter is realized by adding water and pasteurization process is performed at 95°C for 5 minutes. Milk was cooled down to a fermentation temperature of 42-43°C. Starter culture (1 %) consisting of *Str. thermophilus* (30%) and *Lb. bulgaricus* (70%) bacteria is added in a process tank. After mixing inoculated milk is incubated at 41-43°C until pH value reaches 4.50. After incubation, the curd was broken by stirring and rapid cooling to 4°C in the processing tank. Simultaneously a 0.6% salt was added. After cooling ayran was poured in plastic caps and stored at refrigerated temperatures for 15 days.

**Determination of SCC and chemical composition of raw milk.** Bactocount IBCm (Bentley Instrument, USA) device was used for SCC determination. The milk fat, protein, lactose and total solids content of studied milk samples were measured by using Infrared Milk Analyzer 150 (Bentley Instrument, USA). The instrument was calibrated with certified reference milk samples from Italy Accredited Dairy Laboratories A.I.

**Microbiological analysis.** Total viable count (TVC) was determined by using Plate Count Agar medium according to ISO 4833-2:2013. Inoculated petri dishes were subjected to incubation at 30 °C for 48 to 72 hours and colony forming units (CFU) were counted on petri dishes.

**Physicochemical analysis of ayran.** The milk fat, protein, lactose and dry matter content of studied ayran samples were measured by using Infrared Milk Analyzer 150 (Bentley Instrument, USA). pH values were measured by pH meter. Lactic acid content of milk and ayran samples was calculated on the basis of the results for titratable acidity determined by titration method according to BNS 1111-80.

**Sensory analysis.** The evaluation of the sensory quality of ayran samples was performed with 25 consumers, randomly selected by age, gender and social status. The consumers expressed their opinion by means of hedonic scale. The color, thickness (by spoon), thickness (by taste of mouth), aroma and taste of the ayran samples were determined using a five point hedonic scale from 1 = dislike a lot to 5 = like a lot. Tests were repeated three times. Preferred characteristics were visual and natural thickness, mild sour taste, smooth structure, strength and resistance of the coagulum and clear creamy color. Non-preferred characteristics were stickiness, serum separation, gas indication, high thickness, rough structure, very sour, sweet and/or salty metallic flavour, weak aroma, neutral taste.

**Statistical analysis.** Computer processing of the results was performed by using the program Microsoft Excel 2010. All determinations were carried out in triplicate and data were subjected to analysis of variance (ANOVA). ANOVA was carried out with the General Linear Models (GLM)

with a significant level of  $P < 0.05$  (Draper and Smith 1998). The Fischer's test with a significant difference set at  $P < 0.05$  was used to compare sample values (Kenward 1987).

## **Results and Discussion**

### **Physicochemical and microbiological analysis of raw milk and ayran samples with different SCC.**

The results of the physicochemical and microbiological analysis of the raw milk used for the production of the test samples of ayran have been presented in Table 1. The three raw milk batches used, marked as L, M, and H, were characterized by a low, medium and high somatic cell count respectively. A similar dependence was observed with the TVC values. It can be seen (Table 1) that regardless of the differences in SCC and TVC, the main milk components such as milk fat, proteins and dry matter, were similar in content. According to Altay et al. (2013) the main components of ayran varies as follow: total dry matter (1.67–11%), protein (1.44–3.48%), salt (0.17–1.75%) and fat (0.1–3%).

The milks with a higher SCC (batch H) exhibited slightly lower lactose content correspondent to the weak negative correlation between these two indicators. The active acidity (pH) values and the lactic acid concentration were within the admissible limits for raw cow's milk in the three batches. The higher acidity of the batch H raw milk expressed as lower pH values, and the higher lactic acid concentration in comparison to the other batches were another point of interest. They can be attributed to the occurrence of more intensive microbiological processes in these samples, also demonstrated by the high TVC of the batch H raw milk which reached  $1.2 \times 10^6$  CFU.cm<sup>-3</sup>. The results of the physicochemical analysis of the ayran test samples have been presented in Table 2. It can be seen that the three ayran batches were characterized by similar dry matter, fat, protein and lactose content. No statistically significant ( $P < 0.05$ ) differences were established in the active acidity values and the lactic acid content between samples L, M, and H.

**Table 1.** Microbiological and physicochemical characteristics of raw milk used for production of ayran samples

Characteristics Samples	SCC, cells.cm <sup>-3</sup>	TVC, CFU.cm <sup>-3</sup>	Dry matter, %	Fat, %	Proteins, %	Lactose, %	pH	Lactic acid, %
<i>Batch L</i>	210 000	3,4±0,4.10 <sup>5</sup>	12,36±0,45	3,70±0,27	3,29±0,25	4,47±0,33	6,64±0,05	0,162±0,034
<i>Batch M</i>	495 000	5,5±0,3.10 <sup>5</sup>	12,27±0,38	3,58±0,33	3,31±0,19	4,28±0,27	6,62±0,07	0,163±0,028
<i>Batch H</i>	970 000	1,2±0,3.10 <sup>6</sup>	12,17±0,52	3,63±0,21	3,22±0,21	4,17±0,29	6,55±0,08	0,175±0,025

**Table 2.** Physicochemical characteristics of ayran samples

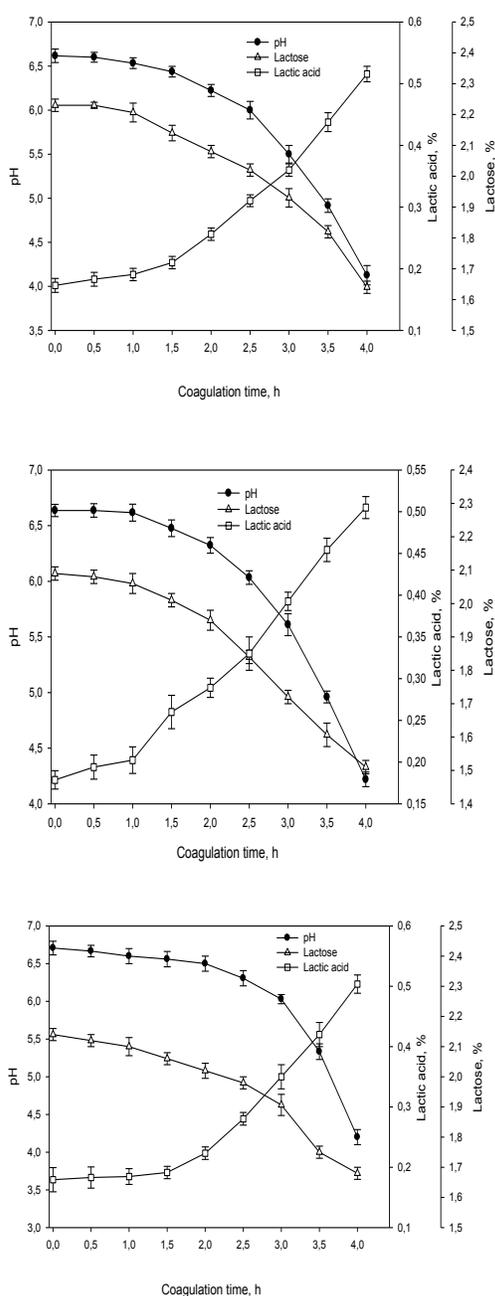
Characteristics Samples	Dry matter, %	Fat, %	Proteins, %	Lactose, %	pH	Lactic acid, %
<i>Batch L</i>	13.20±0,38	3.8±0,24	3.28±0,20	3.77±0,25	4.40±0,06	0.680±0,024
<i>Batch M</i>	13.26±0,40	3.7±0,19	3.27±0,18	3.87±0,28	4.35±0,05	0.682±0,032
<i>Batch H</i>	13.25±0,41	3.8±0,17	3.35±0,22	3.75±0,21	4.38±0,07	0.684±0,029

The results showed that the SCC values in the raw milk did not affect significantly the content of the main components, the active acidity and the lactic acid concentration in the ayran produced.

#### **Fermentation process development during production and storage of ayran samples produced from raw milk with different SCC.**

The changes in the pH values, the lactic acid and residual lactose concentration during the coagulation of the test samples have been presented in Figure 1. A significant decrease in the active acidity ( $P<0.05$ ) values from  $6.59\pm 0.04$  to  $4.20\pm 0.02$  on average and a respective increase in the lactic acid ( $P<0.05$ ) concentration from  $0.18\pm 0.01\%$  to  $0.50\pm 0.01\%$  on average was

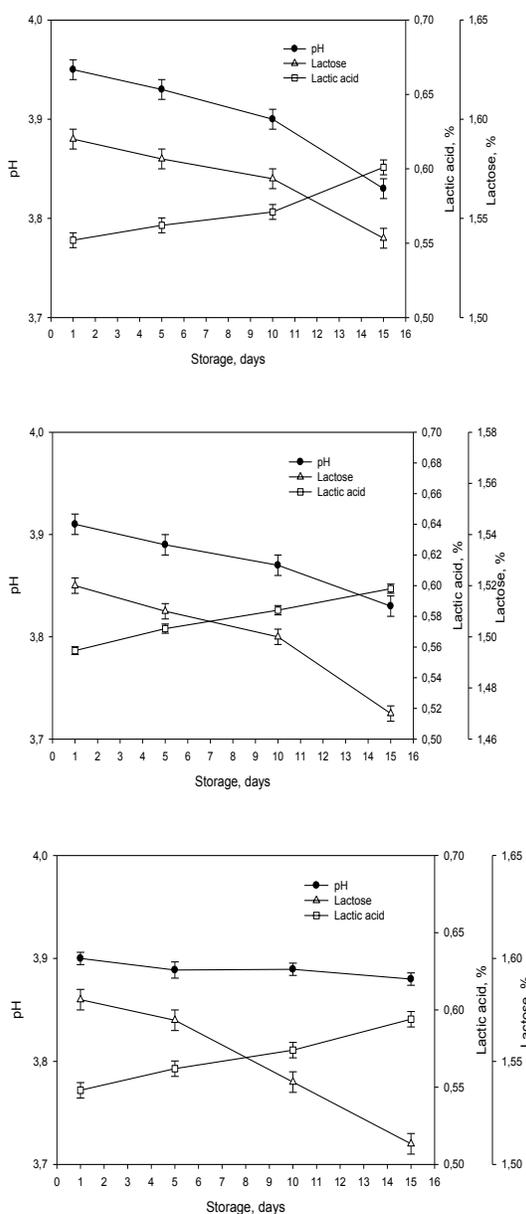
observed. No statistically significant differences ( $P<0.05$ ) were established in the values of these indicators at the end of the coagulation of the three milk batches. In the production of ayran certain differences were observed in the acidification rate between the milks with a high SCC (samples H) and the other two batches (samples L and M). The milks with a high SCC demonstrated a slower rate of acidification until the second hour of coagulation compared to samples L and M. These data showed that during the first two hours of coagulation, the higher somatic cell count in the milk could have an inhibiting effect on the development of the lactic acid bacteria from the starter culture.



**Figure 1.** Changes in the pH values, lactic acid and residual lactose concentrations during fermentation of ayran samples from batch L (a), batch M (b) and batch H (c).

This could be attributed to the higher sensitivity of *Lb. bulgaricus* to the inhibiting factors in the environment. The share of *Lb. bulgaricus* in the

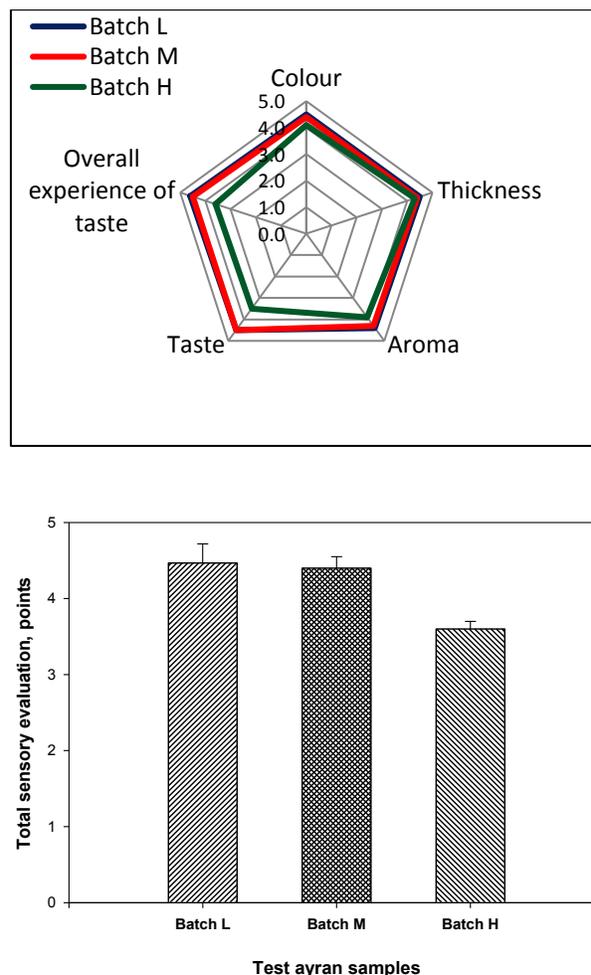
ayran starter culture is higher in comparison with traditional yoghurt starter culture. The *Lb. bulgaricus* to *Str. thermophilus* ratios in the starter cultures for ayran and for yoghurt were 3:1 and 1:3 respectively. This could be the reason for a longer lag phase of the starter microflora of ayran, which determined the slower acidification during the first two hours of coagulation. Despite the observed initial delay in the fermentation process in the milks with high SCCs, the acidification rate after the second hour of coagulation was considerably accelerated and at the end of the process these milks had pH values and lactic acid concentration similar to those of the other test samples. The milk coagulation time for all three test samples was 4 hours. The amount of the lactic acid produced by the yoghurt culture reached  $0.50 \pm 0.02\%$  at the end of the coagulation. The residual lactose concentration in the ayran test samples varied within the  $1.68 \pm 0.04\%$  range. The changes in the active acidity values, the lactic acid and residual lactose concentration during the cold storage of the three ayran batches (L, M, and H) have been presented in Figure 2. During the 15-day storage period, no statistically significant ( $P < 0.05$ ) changes were established in the active acidity values and lactic acid concentration, the values of these indicators being  $3.85 \pm 0.04\%$  and  $0.59 \pm 0.02\%$  respectively at the end of the process. Similar results for pH values and lactic acid concentration of ayran were reported by other authors. According to Gülmez et al. (2003) and Kocak et al. (2006) the titratable acidity of ayran varies between 0.4% and 1.73% and the pH values - between 3.44 and 4.44. In the present study were not found statistically significant ( $P < 0.05$ ) differences in the active acidity values, lactic acid concentration and residual lactose during the different periods of the cold storage of the three ayran batches. These data indicated lack of post-acidification during the cold storage of the ayran test samples. In this study, no significant effect of the SCC was established on the post-acid forming capacity of the lactic acid bacteria from the starter culture. The residual lactose amount at the end of the storage of the ayran test samples varied within the 1.47% to 1.54% range.



**Figure 2.** Changes in the pH values, lactic acid and residual lactose concentrations during cold storage of ayran samples from batch L (a), batch M (b) and batch H (c).

**Sensory analysis.** The results on the individual organoleptic characteristics and the overall sensory evaluation score on the ayran test samples at the end of the cold storage have been presented in Figure 3.

The current study did not establish any statistically significant ( $P < 0.05$ ) differences in the organoleptic scores for colour, thickness, taste, aroma and overall experience of taste of the ayran samples made from raw milk with low and medium SCCs (batches L and M). Similar results were obtained on the total sensory evaluation score of these ayran batches.



**Figure 3.** Sensory evaluation scores for the individual organoleptic characteristics (a) and total sensory evaluation scores (b) of the test ayran samples according to the following hedonistic scale: 1-very bad; 2-bad; 3-not bad nor good; 4-good; 5-very good.

This showed that variations in the somatic cell count in raw milk below 500 000 cells/cm<sup>3</sup> had no significant ( $P < 0.05$ ) effect on the organoleptic characteristics of ayran. The results obtained in the current study (Fig. 3) showed that the majority of

the scores on the individual sensory characteristics as well as the total sensory evaluation score on the ayran samples made from raw milk with a high SCC (batch H) were lower ( $P < 0.05$ ) in comparison to those given to batches L and M. The colour and thickness scores were an exception since no statistically significant ( $P < 0.05$ ) differences were established with all test samples. The most considerable influence of the somatic cell count was observed with the taste, aroma and overall experience of taste indicators. As seen from Figure 3, the batch H samples received the lowest scores for these indicators. The results obtained showed that the increase in the SCC values in the raw cow's milk above 500 000 cells.cm<sup>-3</sup> had a negative effect on the organoleptic characteristics of the ayran made from it.

## Conclusions

The results obtained in the present study showed that the SCC values of the raw milk did not have a significant effect on the ayran composition. The milks with a high SCC demonstrated a slower rate of acidification until the second hour of coagulation. Results obtained indicated for lack of post-acidification during the cold storage of the ayran test samples. In the present study, no statistically significant differences ( $P < 0.05$ ) were established in the pH values, the lactic acid and residual lactose concentration at the different cold storage periods of the three milk batches. The variations in the SCC in the raw milk within the range up to 500 000 cells.cm<sup>-3</sup> had no significant effect on the organoleptic properties of the ayran. The increase in the SCC values up to 1000 000 cells.cm<sup>-3</sup> in the raw cow's milk affected negatively the organoleptic characteristics of the ayran.

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