The importance of somatic cell count in dairy technology

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Abstract

Mastitis is an important infection that affects herd health, udder health and milk yield of individual animals. Somatic cells (SCs) naturally present in milk are used as an indicator of subclinical mastitis, but also to assess milk quality and safety. Somatic cell count is the main monitoring variable used in milk quality and safety assessment. This review especially focuses on the effect of SCs on milk as food and also their effect on the dairy technology. The most significant effect of SCC on dairy technology is provided by the enzymes released from these cells. Alternatively, a limited number of conclusions were reported that SCs may assist dairy technology. SCs pass to milk and continue inhibitory effect on bacteria.

Key words: somatic cell count; raw milk; quality; milk safety; dairy technology

Introduction

Milk is a high-value biological fluid used for human nutrition. On the other hand, milk itself is a highly perishable food, containing suitable nutrients, moisture and optimum pH for microbiological growth, resulting in subsequent spoilage. From that point of view, spoilage prevention, together with monitoring safety and quality of raw milk are highly important with regards to public health and commercial benefits. Somatic cells (SCs) are naturally present in milk. The somatic cell count (SCC) represents the acceptable number of SCs, as a sign for monitoring subclinic mastitis in herd and also to evaluate quality and safety parameters. An increase in SCC above the defined limit is an indicative parameter for udder inflammation. Routine diagnosis of mastitis is based on the indicators of inflammation, and consequently the milk SCC has been used longly in the history and still being used (Leitner et al., 2016; Rainard et al., 2018). Mastitis is an inflammation of the mammary gland that can be caused by chemical, physical or traumatic accidents as well as by microorganisms, usually bacteria, which results in tissue damage (Bae et al., 2017; Carvalho-Sombra et al., 2021; Pegolo et al., 2021). Subclinical mastitis is 15 to 40 times more prevalent than clinical mastitis and causes high economic losses (Malik et al., 2018). In this regard, SCC with the standard plate count analysis are the main monitoring variables used for the assessment the quality of milk (Moradi et al., 2021; Naing et al., 2019). Increase of SCC in milk is related with changes in milk components, properties and microbiological contamination (Barlowska et al. 2009; Musayeve et al., 2016). Due to the huge importance of monitor the quality and safety aspects of raw milk, this review specially focuses on the role of SCs for tracking the guality parameters of milk and meaning of SCC in terms of milk technology.

Definition of somatic cells and meaning of somatic cell count in raw milk

The mammary gland has its own immune defence system for fighting against infections. Leukocytes and other immune system cells pass from blood into udder to fight pathogens. They also play a role on repairing the damaged tissues, thus causing an increase in the number of SCs that are released into the milk (Albenzio et al., 2019; Moradi et al., 2021; Petzer et al., 2017). The number of SCs are called somatic cell count (SCC) and accepted as a sign of udder health and is also used as a parameter to indicate milk quality and the products produced from these milks. Increased SCC is related with udder inflammation, which leads to pathogen contamination and a change in the sensorial and physicochemical parameters of milk (Paape et al., 2002; Talukder and Ahmed, 2017). When the pathogenic bacteria invade the mammary gland, an infection occurs. This infection causes inflammation and therefore white cells migrate from blood to mammary gland to fight against infection agents. The sheep and the goats have higher SCC than cows in normal conditions even if there is no infection in udders, which increases throughout the duration of the lactation stage. Milk secretion in cows is mainly via the merocrine way but in goats and sheeps the milk is secreted in apocrine pathway. This is the reason why the milk contains noncellular cytoplasmic particles which can be confused with SCs in milk from infected udders (Alhussien and Dang, 2018; Moradi et al., 2021; Paape et al., 2001; Stocco et al., 2019).

The SCs profile in milk varies depending on a number of factors such as species, breed type, lactation period, genetic factors, diurnal variation, milking intervals, sampling procedures/intervals, daily stress and/or trauma, herd management strategies, seasonality and storage conditions (Li et al., 2014). In this section, members of SCs and the roles they play are described. SCs include epithelial cells, leucocytes, macrophages, polymorphonuclear neutrophils cells (PMNs), and lymphocytes and they are generally the predominant cell types. Generally, macrophages are also predominant in healthy cows' milk. In the beginning of infection, the macrophages secretes some chemical compounds such as messengers or chemoattractants, and these chemicals increase the number of PMNs in the local infection area (Burvenich et al., 2003; Li et al., 2014; Pham, 2006). In milk of cows which are free from mastitis, SCC is typically up to 100,000 cells/mL (nearly, 50 % epithelial cells, 50 % WBCs). If SCC is more than 200,000 cells/mL this generally indicates udder infection and the milk that in normal forms (Alhussien and Dang, 2018; Kelly et al., 2018; Panthi et al., 2017). Additionally, it is reported that the milk of goats and sheep without infection, includes typically higher SCC at about 200,000 cells/mL for sheep milk and 300,000 cells/mL for goat milk) (Hernandez-Ramos et al., 2019). Also, SCC may be as high as 2,000,000 cells/mL in the last period of the lactation with no infection (Moradi et al., 2021; Quintas et al., 2017). Another important point to highlight is the SCC is the total number of cells does not give any idea for distribution of cell types and cell profile. Hence, milk with different SC profiles has a particular fingerprint which may be related to the different final characteristics of the dairy product (Li et al., 2014).

The international limits for SCC in raw milk

International and national legislations have been established to provide the maximum acceptable limits of SCC in different countries (Table 1) (Moradi et al., 2021). The upper limit according to EU Regulation no. 853/2004 is 400,000 cells/mL in cows' milk (EU, 2004), goat and sheep milk acceptable limits are not currently defined in the EU (Ducková et al., 2019; Li et al., 2014).
 Table 1. The acceptable limits for SCs in bovine raw

 milk in different countries (Moradi et al., 2021)

Legal limit (cell/mL)	Countries
350,000	Switzerland
400,000	Australia
	New-Zealand
	Canada
	European Union
	Sweden
	Finland
450,000	Iceland
	Denmark
500,000	Brazil
	China
	Iran
	Turkey
	Tunisia
700,000	Russia
750,000	USA
No limit	Chile
	India

Bovine milk tank somatic cell count (BMTSCC) values are routinely analysed to define if the milk production meets the national and international regulatory standards. The national limits for BMTSCC vary from <400,000 cells/mL (EU, Australia, New Zealand and Canada) to <1,000,000 cells/mL (Brazil) (Ruegg and Pantoja, 2013). The BMTSCC is used for monitoring cow udder health and mastitis level. The optimal BMSCC is not definitively described, but it is generally considered to be <250,000 cells/mL for milk premium (De Vliegher et al.; 2012).

The mean of high SCC in terms of milk safety and quality

The meaning of SCs in raw milk in terms of milk safety and milk quality is associated with two important issues: (1) the direct effect of the released SC endogenous enzymes, (2) indicatin the presence of contaminating bacteria which induce compositional and physicochemical changes in the milk (Albenzio et al., 2011). In many studies, SCs can be the sources of enzymes which change milk composition and cause defects in the end products. Numerous enzymes can be released into milk after the cell lysis and lipases (e.g., lipoprotein lipase), oxidases (e.g., catalase, lactoperoxidase), glycosidases (e.g., lysozyme) and proteases (e.g. cathepsins, elastase, collagenase) are those moft widely reported groups of enzymes (Ducková et al., 2019; Li et al., 2014; Murphy et al., 2019). The number of somatic cells gives an idea for both, individual animals' milk and for BMTSCC as well, as a measure of general intramammary health status of the herd (Moradi et al., 2021; Leitner et al., 2016)

Taking SCC under loop in terms of dairy technology

Qualified raw milk for manufacturing high quality and nutritious dairy products should (1) be complete in terms of composition (e.g. protein, fat, lactose amount); (2) be free from off- flavours; (3) be free from any residues and other adulterants; (4) have an acceptable microbiological load (5) have acceptable level of SCC (Murphy et al., 2019). If the main effects of SCC in milk and dairy products are summarized; lower cheese yield, fat and protein loss, high humidity-low total solids, shortened shelf-life and changes in sensory characteristics, more free fatty acids due to greater lipolysis are the most important statements regarding high SCC (Bezerre et al., 2020; Deshapriya et al., 2019; Fernandes et al., 2007; Ivanov et al., 2020; Ma et al., 2000).

As described above SCs' endogenous enzymes are the most important components which directly effect dairy processing. They are active in the udder and are kept at optimal conditions with the body temperature, however their activity continues even during the refrigerated storage and ultimately leads to changes in the unique properties of processed products (Kellv and Fox 2006). However, the enzymes released from SCs are difficult to measure and it is still not clear (Li et al., 2014), these enzymes can be classified as lipases, oxidases, glycosidases and proteases. Proteases are the most studied of these enzymes and are active on caseins. Therefore, proteases can modify the casein degradation of milk and their products and can change the textural and organoleptic properties of the final products, additionally, they may reults in a reduction in the total cheese yield. Cathepsin B, cathepsins G and elastase, which are important proteases from SCs, are responsible for the hydrolysis of as1- and B-caseins. Another protease cathepsin D can hydrolyze all caseins (αs1-, αs2-, β- and κ-caseins) (Hurley et al. 2000). Plasmin which is a heat-stable protease, is a predominant enzyme for proteolysis in milk from healthy and infected udders (Leitner et al., 2006). High plasmin activity is associated with increased clotting time, decreased moisture in cheese, reduction in curd stability and yield (Kelly and Fox, 2006). Plasminogen activators related to SCs, may cause differences in plasmin activity (Albenzio et al., 2004). On the other hand, although, the profile of SC types present in milk is not fully identified in general, enzymatic activities may be considered as heterogeneous (Santos et al., 2003). Milk with different levels of SC counts including different endogenous enzymes reflect as different characteristics of the end product.

Another important SC endogenous enzyme is lipoprotein lipase. Lipoprotein lipases are generally considered responsible for "rancid" flavour in some dairy products. The real effect of lipase from SCs is unclear due to different sources of lipolytic enzymes in milk (Dherbécourt et al., 2010). Similar conclusions could be drawn for other SC enzymes. Added enzymes during the dairy process such as rennet and pepsin or fungal rennet and enzymes produced by natural or contaminant microbiota of milk can also contribute to this issue (Li et al., 2014). Moradi et al. (2021) reviewed all enzymes associated with SCs in their paper, which is presented in Table 2.

Correlation between SCC and nutritional components

Research has shown that elevated SCC is a factor which changes the composition of the milk (Coelho et al., 2017). Geary et al. (2013) reviewed many of these publications in their meta-analysis study. Table 3 which is modified from the study of Geary et al. (2013), represents the summary of the correlation between the SCC and nutritional components of milk.

As Le Roux et al. (2003) concluded in their review article, during the infection, 3 mechanisms may be involved in milk composition change: reduction in synthesis of milk components, an increase in the permeability of the milk barrier and also in the proteolytic activities. As the conclusion of the study performed by Guariglia et al. (2015), the lactose levels decreased with increased SCC. On the other hand, defatted dry extract (DDE) is minimally influenced by increased SCC. It has been shown that the percentage of fat increased with increased SCC (Guariglia et al., 2015). This increase in fat content can be explained with the reduced milk production. Guariglia et al. (2015) and Noro et al. (2006) also observed that the milk protein concentrations increased with elevated levels of SCC. However, the casein level decreased with the high levels of SCC, whereby the plasma proteins migrate to inflammation site and increase the milk protein concentration. Reduced lactose content might be associated to the decreased lactose amount transferred from blood to the mammary gland due to the differences in membrane permeability (Pereira et al., 1999).

Effect of SCC on cheese technology

SC enzyme cathepsin D which has a similar amino acid sequence to chymosin and pepsin, is one of the most researched enzymes in milk and cheeses. Similarity to chymosin and pepsin allows this enzyme to contribute to the curd forming mechanism in the cheese making process (Lee et al., 1998: Li et al., 2014: Moatsou et al., 2008), On the other hand, cathepsin B which has similar cleavage sites to cathepsin D and chymosin and is active after heat treatment at about 55 °C /30 min or 72 °C/30 s (Magboul et al., 2001). Elastase which is another enzyme from SCs, influences coagulation like cathepsins and effective on cheese maturation. High SCC also increases plasmin activity and plasmin significantly active on breaking down of casein. In conclusion, all of the aforementioned mechanisms can promote more proteolysis in cheeses made with high SCC (Le Maréchal et al., 2011).

As Geary et al. (2013) mentioned, there is a significantly negative relation between SCC and recoveries of protein and fat for variety of cheeses. SCC cause lower yield

Somatic cell type	Enzymes
Macrophages	Cathepsin B
	Cathepsin D
	Cathepsin H
	Cathepsin L
	Cathepsin G
	Cathepsin S
	Elastase
	Lipoprotein lipase
	Collagenase
	Myeloperoxidase
	Cathepsin B
PMNs	Cathepsin C
	Cathepsin D
	Cathepsin L
	Cathepsin G
	Cathepsin S
	Elastase
	Lipoprotein lipase
	Collagenase
	Myeloperoxidase
Lymphocytes	Elastase
	Cathensin B

Cathepsin D

Cathepsin L

Cathepsin K

Catalase

Table 2. SCs and their enzymes found in raw milk
and dairy products (Moradi et al., 2021)

Table 3. Effect of SCC on the milk components
(Geary et al., 2013)

Epithelial cells

Unknown

Components (%)	Effect
Crude protein	Not consistent
True protein	Not consistent
Total nitrogen	Increase
Non-protein nitrogen	Not consistent
Non-casein nitrogen	Not consistent
Casein	Not consistent
Casein as a percentage of true protein	Decrease
Whey protein	Increase
Whey fat	Not consistent
Fat	Not consistent
Lactose	Decrease
Total Solid	Not consistent
Solids non fat	Not consistent

as a consequence of the decreased casein content and decreased level of albumins such as a-lactalbumin and B-lactoglobulin. On the other hand, high SCC increases lactoferrin and lysozyme levels and serum albumine. SCC also prolongs rennet coagulation time, which delays cheese manufacturing, decreased curd firmness, increased cheese

moisture and reduced cheese quality. Many quality defects were observed by researchers in cheeses produced from high SCC milk. Auldist et al. (1996) experimentally produced cheddar cheese with milk including 252,000 and 1,400,000 cells/mL. They found that cheeses produced from milk with higher SCC had a higher moisture content. These authors also observed textural and flavour defects due to high moisture and fat oxidation by lipolysis. Vianna et al. (2008) studied on Prato cheese manufactured with milk with SCC lower than 200,000 cells/mL and higher than 700,000 cells/mL. They found a 3 % higher moisture in the cheeses with high SCC. The level of SCs also affects the pH of the end product (Hachana et al., 2018)

Another important issue for cheeses is the level of biogenic amines (BAs). SCC is also considered as one of the factors related to BA formation in cheeses (Costa et al., 2018). Ubaldo et al. (2015) detected tyramine and tryptamine in Mozzarella cheese which is produced with milk including high SCC. However, these two BAs are not naturally found in this type of cheese. Ivanova et al. (2021) reported BA concentration of cheeses from milk with low SCC (<400,000 cells/mL) and Medium SCC (between 500,000 and 600,000 cells/mL) remained below 10 mg/kg, while they reported an increase in the BA content during the maturation period and cold storage in the cheese produced from high SCC milk (>1,000,000 cells/mL). The main BAs reported were tyramine, putrescine and cadaverine at the end of storage.

Effect of SCC on yoghurt technology

Higher SCC affects the pH and the lactose content of milk, and accordingly changes the acidifying dynamics during the yoghurt fermentation and leads to unsuitable texture and sensory scores (Vivar-Quintana et al., 2006). Oliveira et al. (2002) reported lower sensorial scores for yoghurt produced from milk containing more than 800,000 cells/mL. Hachana and Paape (2012) investigated the high SCC on the physico-chemical characteristics of stirred yoghurt under cold chain. For this study, yoghurts with low, intermediate and high amount of SCs (95,000 cells/mL, 398,000 cells/mL and 1,150,000 cells/mL, respectively) were experimentally produced. In conclusion, SCC did not significantly affect acidity or the pH of the yoghurt after 1 day of storing, but significant differences were observed after 14 and 28 days of storage. Yoghurt samples made from intermediate and high SCC milk showed higher viscosity and lower casein content on days 14th and 28th of storage. High FFA concentrations were detected only in yoghurt made from high SCC milk. As expected, high number of SCs increased both proteolysis and lipolysis in yoghurt while storing under cold chain and this leads to differences in its sensorial properties. Najaf Najafi et al. (2010) produced yoghurt with sheep milk and investigated the effect of SCC on yoghurt quality. They concluded that SCC did not affect the total solids and fat content of yoghurt produced from sheep milk. They also reported no effect on the acidity of the yoghurt after 24 h, but a significant effect was observed after 168 h of storage. Vivar-Quintana et al. (2006) showed that the pH values of yoghurt produced from sheep milk with high SCC were lower than yoghurt produced from low or medium SCC milk at the 15th day of storage under cold chain. Fernandes et al. (2007) reported that the casein amount in high SCC milk yoghurt was higher than that in low SCC milk yoghurt. However, Roger and Mitchell (1994) reported a negative correlation between SCC and yoghurt viscosity.

Ivanov et al. (2020) investigated the effect SCC on the volatile organic compounds of cow milk yoghurt. A lower diacetyl and acetoin content and a higher content of 2-heptanone, and butanoic and hexanoic acids were reported in yoghurt samples from batches with high SCC milk. Significantly lower concentrations of the carbonyl compounds and higher concentrations of butanoic and hexanoic acids were established in the yoghurt of high SCC batch compared to low and medium batches. These data highlight the fact that SCC has an important effect on the chemical composition which provides yoghurt with its unique sensorial properties. Specifically, diacetyl and acetone affect the aroma of fermented milk. From that point of view, high SCC could be considered as the reason of slightly expressed aroma in yoghurts. Additionally, higher butanoic and hexanoic acid contents may cause taste defects. The increased FFA levels originate from lipolytic processes. This may lead to higher butanoic and hexanoic acid contents in high SCC milk yoghurts because of lipolytic enzymes of SCs (Li et al., 2014). Ivanov et al. (2020) also reported the appearance and taste defects in yoghurts obtained from milk with high SCC and linked it to the high levels of butanoic and hexanoic acid. On the other hand, chemical (titratable acidity, pH, fat, total protein) and microbiological parameters were not affected by SCC during the 30-day storage.

Conclusion

Milk obtained from mastitis-bearing udder might contain high numbers of saprophytes that spoil the product and pathogens that cause food infections. In addition, immune system elements and epithelial cell debris in the mammary gland, which tries to protect itself from infection, pass into the milk we consume under the definition of "somatic cells". Those somatic cells give a valuable information about all these aforementioned events, but in fact they are undesirable in terms of dairy technology. For that reason, countries define legal limits for the count of somatic cells in milk and have pricing systems dependent on this count. The most important effect of SCC on dairy technology is the enzymes released from these cells. Limited number of conclusions were reported that SCs may assist dairy technology. However, it should not be ignored that the SCC must remain below a certain limit and the microbial load of the milk must remain at an acceptable level. Consuming milk with a high cell count has no direct risks to public health. But the relationship of milk with high SCC to clinic and subclinic mastitis, low hygienic conditions in farm, antibiotic residues and the presence of pathogen microorganisms can pose risk factors for consumers when high SCC milk is marketed.

Važnost broja somatskih stanica u mljekarskoj tehnologiji

Sažetak

Mastitis je važna infekcija koja utječe na zdravlje stada, zdravlje vimena i mliječnost pojedinih životinja. Somatske stanice prirodno prisutne u mlijeku služe kao pokazatelj supkliničkog mastitisa te za procjenu kvalitete i sigurnosti mlijeka. Broj somatskih stanica glavna je varijabla koja se kontinuirano nadzire i koristi u procjeni kvalitete i sigurnosti mlijeka. Ovaj rad posebno se fokusira na učinak somatskih stanica na mlijeko kao hranu, ali i na njihov učinak u tehnologiji prerade mlijeka. Enzimi koji se oslobađaju iz somatskih stanica imaju najznačajniji učinak u tehnologiji proizvodnje mliječnih proizvoda. Međutim, ograničen broj provedenih istraživanja ukazuje kako somatske stanice mogu biti korisne u tehnologiji mliječnih proizvoda. Somatske stanice prelaze u mlijeko i nastavljaju inhibitorno djelovanje na bakterije.

Ključne riječi: broj somatskih stanica; sirovo mlijeko; kvaliteta; sigurnost mlijeka; tehnologija prerade mlijeka

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