

Milk SCC and PMN as indicators of milk processability and subsequent cheese quality

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Abstract

This study investigated the effect of milk somatic cell count (SCC) and polymorphonuclear leucocyte (PMN, or neutrophil) content on the processability of milks from individual udder quarters. Two milk samples of high and low SCC (6×10^3 and $920 \times 10^3/\text{ml}$, respectively) and two of high and low PMN content ($< 1 \times 10^3$ and $700 \times 10^3/\text{ml}$, respectively) were selected. The milk samples were mixed in appropriate proportions such as to produce four artificial mixed milks of SCC or PMN content $\sim 200 \times 10^3/\text{ml}$ and $\sim 400 \times 10^3/\text{ml}$. All milks were analysed for gross composition, renneting properties and N-fractions. Miniature Cheddar-type cheeses were manufactured and analysed for composition after 30 d and for proteolysis after 30 d and 90 d of ripening. Fat and protein contents of the milks generally decreased with increasing SCC and PMN and there was no effect on N-fractions in the milks or on total solids, moisture, NaCl content or pH of the cheeses. Increasing SCC and PMN had a negative effect on rennet coagulation of milk. Urea-PAGE electrophoretograms indicated that the patterns of proteolysis changed quantitatively and qualitatively with increasing milk SCC and PMN during cheese ripening. High SCC and PMN milk resulted in reduced levels of residual intact α_{s1} - and β -caseins, indicating cell-associated proteinase activity. In conclusion, milks with high SCC and PMN had inferior composition and processing characteristics.

Introduction

The primary characteristic change that occurs in milk during mastitic inflammation is a significant influx of white blood cells (somatic cells). Milk contains three main somatic cell types: neutrophils (polymorphonuclear leucocytes, or PMN cells); lymphocytes; and macrophages. PMN cells account for approximately 10% of total cells in normal milk, but account for greater than 90% of cells in mastitic milk. The total number of cells in milk is commonly used (somatic cell count, SCC) as an indicator of mastitis, but little attention is usually given to the type of cells and their particular function (Concha *et al.*, 1978). Since the increase in the number of cells in mastitic milk has been shown to be predominantly PMN, these cells may have an impact on the chemical composition of milk and subsequent dairy products. Elevated bulk milk SCC has been shown to have significant implications for the processing properties of such milk, and in particular, reduced yield and quality of Cheddar cheese (Auld *et al.*, 1996a). High SCC has also been linked to early gelation of UHT milk

(Auldist *et al.*, 1996b). However, little information is available on the contribution of different somatic cells in milk to cheese-making properties. Additionally, bulk milk SCC is used by co-operatives and dairies to identify milk as being unsuitable for processing. However, a bulk milk SCC reflects the SCC of different udder quarter milks from a number of cows contributing to that milk pool. Thus, the objective of this study was to investigate the effect of SCC and PMN levels on the processing characteristics of quarter milks and on the quality and ripening characteristics of miniature cheeses manufactured from such milks.

Material and methods

Two cows with individual quarter milk of SCC $6 \times 10^3/\text{ml}$ and $920 \times 10^3/\text{ml}$ were identified. Milk samples (1 L) were collected from each of these udder quarters and mixed in proportions calculated to give final SCC of $\sim 200 \times 10^3/\text{ml}$ and $\sim 400 \times 10^3/\text{ml}$. Two further cows with individual quarter milks of PMN $< 1 \times 10^3/\text{ml}$ and $700 \times 10^3/\text{ml}$ were also identified, milk samples were collected from these udder quarters and again, mixed so as to give two artificially-mixed milks of PMN $\sim 200 \times 10^3/\text{ml}$ and $\sim 400 \times 10^3/\text{ml}$.

A Bentley Somacount 300 somatic cell counter (Agri York 400 Ltd, York YO4 2QW, UK) was used to measure SCC in milk. PMN levels were determined using the ELISA method of O'Sullivan *et al.* (1992). The four original milks, the two SCC artificial mixes and the two PMN artificial mixes were analysed for gross composition (Milkoscan 605; Foss Electric, DK-3400 Hillerød, Denmark), rennet coagulation characteristics (McMahon & Brown, 1982) and N-fractions of total protein (International Dairy Federation, 1993) and casein (International Dairy Federation, 1964). The renneting characteristics of the milks determined were: rennet coagulation time in min (RCT), rate of curd aggregation in min (K20) and curd firmness at 60 min in mm of amplitude (A60).

Miniature cheeses were manufactured from each of the eight milks according to the method of Shakeel-Ur-Rehman *et al.* (1998). Cheese composition was analysed by standard methods 30 d after manufacture and proteolysis was studied by urea-polyacrylamide gel electrophoresis after 30 d and 90 d of ripening.

Results

The effect of SCC and PMN level on gross composition, rennet coagulation properties and nitrogen fractions of low and high SCC or PMN content milks, together with the artificial mixes of $\sim 200 \times 10^3/\text{ml}$ or $\sim 400 \times 10^3/\text{ml}$ SCC and PMN, are shown in Table 1. As SCC and PMN content of milk increased, the fat and protein contents generally decreased. Analysis of variance (one-way ANOVA) indicated that RCT increased as both SCC and PMN increased ($p < 0.01$). A60 decreased as both SCC and PMN increased ($p < 0.05$). SCC and PMN content had no significant effect either on the K20 or on the N-fractions of milk.

The effect of SCC and PMN level on total solids, moisture, NaCl contents and pH of miniature cheeses manufactured from quarter milks and artificial mixes of quarter milks are shown in Table 2. SCC and PMN had no clear effect on the composition of miniature cheeses. Analysis of variance indicated that the SCC and PMN levels investigated had no significant effect on total solids, moisture and NaCl contents or pH of miniature cheeses.

Table 1. Effect of SCC and PMN level on gross composition, rennet coagulation properties and N fractions of high and low SCC and PMN milks together with artificial mixes of SCC or PMN content approximately 200×10^3 /ml or 400×10^3 /ml.

Milk type	SCC ($\times 10^3$ cells/ ml)	PMN ($\times 10^3$ cells/ ml)	Gross composition		Renneting properties			N fractions	
			Fat (g/ 100 g)	Protein (g/ 100 g)	RCT (min)	K20 (min)	A60 (mm)	Total protein (g/100 g)	Casein (g/100 g)
Low SCC	6	<1	4.20	3.80	20.7	6.5	48.0	2.00	1.13
Mix SCC 200×10^3	236	130	4.50	3.60	30.7	10.2	40.0	3.66	2.80
Mix SCC 400×10^3	488	350	4.40	3.50	35.2	12.5	34.0	2.75	1.89
High SCC	920	830	3.50	3.20	51.5	17.5	10.0	3.30	2.46
Low PMN	4	<1	4.40	3.40	21.0	6.7	46.0	3.53	2.70
Mix PMN 200×10^3	380	205	4.20	3.40	28.7	10.7	44.5	3.52	2.72
Mix PMN 400×10^3	545	430	4.10	3.40	32.7	15.5	32.5	3.44	2.56
High PMN	850	700	3.80	3.30	41.5	18.2	17.0	3.40	2.48

Table 2. Effect of SCC and PMN content of milk on composition of miniature cheeses manufactured from milks from individual udder quarters or artificial mixes of quarter milks.

Milk type	SCC ($\times 10^3$ cells/ml)	PMN ($\times 10^3$ cells/ml)	Total solids (g/100 g)	Moisture (g/100 g)	NaCl (g/100 g)	pH
Low SCC	6	<1	48	52	1.51	5.2
Mix SCC 200×10^3	236	130	47	53	1.31	5.2
Mix SCC 400×10^3	488	350	47	53	1.66	5.1
High SCC	920	830	51	49	1.48	5.0
Low PMN	4	<1	51	49	1.19	5.2
Mix PMN 200×10^3	380	205	56	44	1.41	5.1
Mix PMN 400×10^3	545	430	57	43	1.38	5.0
High PMN	850	700	50	50	1.69	5.1

The Urea-PAGE electrophoretogram (Figure 1) show the effects of milk SCC on proteolysis of caseins during ripening of miniature cheeses manufactured from low and high SCC milks, together with the artificial mixes, of SCC $\sim 200 \times 10^3$ /ml and $\sim 400 \times 10^3$ /ml at 30 d (lanes 1-4) and at 90 d (lanes 5-8) of ripening. There was a clear effect of milk SCC on proteolysis during ripening of the Cheddar-type cheese; the patterns of proteolysis differed both quantitatively and qualitatively with SCC.

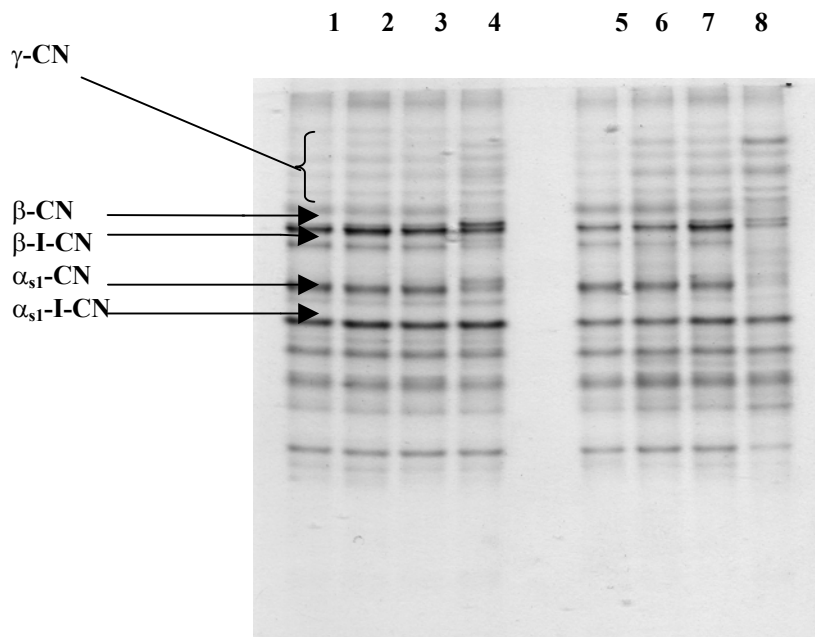


Figure 1. Effect of SCC and PMN level on proteolysis of casein during ripening of miniature cheeses manufactured from low and high SCC milks together with the artificial mixes of approximately $200 \times 10^3/\text{ml}$ and $400 \times 10^3/\text{ml}$ SCC at 30 days (lanes 1-4) and 90 days (lanes 5-8) of ripening.

Comparing lanes 1-4, increasing numbers of bands of slow electrophoretic mobility were apparent towards the top of the gel with progressively increasing SCC. These bands could be either products associated with high SCC milk which became entrapped in the curd during manufacture, or the products of proteolytic enzymes associated with the somatic cells acting during ripening. Comparison of the same samples after 90 d of ripening (lanes 5-8) supports the latter interpretation, as these bands had increased in level of intensity. In addition, after 90 d of ripening the cheese made from the milk of highest SCC (lane 8) had much less residual intact α_{s1} - and β -caseins, again indicating cell-associated proteinase activity. Hence, it appears that enzymes associated with the cells were retained in the cheese curd and contributed to the proteolysis during ripening, and even the addition of small volumes of high SCC milk had an obvious impact. Addition of milk with high PMN levels had approximately similar effects to addition of high SCC milk, with increasing PMN levels resulting in altered patterns of proteolysis (not shown). Cheeses made from milk with the highest PMN level also had the lowest levels of residual α_{s1} - and β -caseins during ripening. High SCC milk is expected to have elevated activities of the somatic cell-derived proteinases, e.g, cathepsins B and D, and the blood-derived proteinase plasmin (Kelly and McSweeney, 2004). The latter enzyme preferentially attacks β -casein, while the former generally affect α_{s1} -casein, and thus the pattern of proteolysis seen is consistent with proteinases associated with high SCC milk being trapped in the curd and contributing to ripening.

Discussion

Reports as to the effect of elevated milk SCC on protein content are conflicting and varied. The decline in milkfat content associated with elevated milk SCC is logical, given the

reduced synthetic and secretory ability of the mammary gland (Auldist, 2000). Meanwhile Verdi and Barbano (1991) indicated associated extended rennet clotting time in making cheese from high SCC milk. The results of the current study are in general agreement with these authors. It is difficult to associate any particular SCC level with the onset of specific defects in dairy products. Some researchers have reported that SCC begins to affect product as it increases above $100 \times 10^3/\text{ml}$ (Barbano *et al.* 1991), while others have suggested that the threshold is closer to $500 \times 10^3/\text{ml}$ (Politis and Ng-Kwai-Hang, 1988). Whether elevated bulk milk SCC is due to milk from a small number of cows with extremely high SCC being included with milk from a predominantly healthy herd, or to large numbers of cows with low-level subclinical infections probably contributes to variation in the effects of SCC on dairy products. Additionally, some pathogens may affect milk composition in different ways, irrespective of SCC level. It is also likely that other factors such as nutritional status and stage of lactation could affect the magnitude of the effects of mastitis on milk composition and dairy product quality, possibly also being influenced by the immune system of the cow. Similarly, some types of somatic cell may have greater effects on milk composition than other cell types. However, SCC and PMN generally had similar effects on milk composition and processability in this study.

Le Roux *et al.* (1995) showed that proteolysis occurred in quarter milk samples with SCC as low as $250 \times 10^3/\text{ml}$. The impact of elevated SCC and PMN milks on proteolysis during ripening of miniature cheeses in the current study was obvious. The enzymes associated with the cells must have been retained in the cheese curd and have contributed to proteolysis during ripening. The patterns of proteolysis were different to those normally associated with cheese ripening, and even the addition of small amounts of high SCC milk had an obvious impact.

In conclusion, variation in SCC of individual udder quarter milk results in variation in composition and processability of milk. Mixing of high and low SCC milk from individual udder quarters generally resulted in intermediate composition and processing characteristics. Addition of high PMN milk and addition of high SCC milks to low PMN and SCC milks, respectively, had similar effects on the composition and processability of the mixes. An SCC standard of $400 \times 10^3/\text{ml}$ for bulk milk is currently used in milk quality schemes world-wide as a result of the European Union requirements which came into force in January 1998. This level of SCC should minimise the effects of mastitis on product quality, although evidence for potential deleterious effects on the quality of dairy products has been suggested by the results in this study. Additionally, Auldist and Hubble (1998) reported that a bulk milk SCC of $400 \times 10^3/\text{ml}$ was indicative of approximately 40% of cows in a herd being mastitically infected. The use of a test for PMN in conjunction with SCC may assist in reducing the incidence of infected milk entering the bulk tank.

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