Quality and safety of milk from farm to dairy product

B. O'Brien[1], B. Gallagher[1, 2], P. Joyce[2], W.J. Meaney[1] and A. Kelly[3]
[1]Teagasc, Moorepark Research Centre, Fermoy, Co. Cork;
[2]Department of Zoology, University College Dublin, Ireland.
[3]Department of Food Science and Technology, University College, Cork, Ireland
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Summary

Neutrophils (PMN cells) constitute one of the main cell types in milk. Increased PMN level is an indication of mastitis. An ELISA method has been developed to determine PMN levels in milk. This may allow (in addition to somatic cell count [SCC]) selection of infected quarters at drying off, thereby allowing antibiotic therapy to be limited to those quarters. PMN counts may also be used to select milk for processing. Little information is available on the contribution of different somatic cells in milk to cheese-making efficiency. The overall objective of this study was to establish the influence of the quality of raw milk, as determined by somatic cell level and type, on milk biochemistry and cheese quality. The work firstly included modification to a method for an enzyme immunoassay, which could enumerate milk PMN. Subsequently, the impact of somatic cell and PMN content on biochemistry of individual udder quarter milks and simulated bulk cow milks, and quality of cheese manufactured from such milks was investigated. The somatic cell and PMN content of bulk herd milks was also investigated.

The modification to the test of O'Sullivan *et al* (1992) allowed the accurate measurement of PMN levels in milk. The strong relationship or correlation between SCC and PMN of 92% in the individual quarter milks has confirmed previous preliminary data. This is important since PMN in conjunction with SCC may now provide a more reliable method of selecting milks for processing. The reduction in casein at elevated SCC and PMN levels may have resulted in the trend towards deteriorated milk coagulation properties. A very heterogeneous selection of proteolysis patterns was observed in the miniature cheeses. This substantial difference in proteolytic activity in milk from different quarters had not been observed previously. Enzymes associated with the cells in high SCC milk were retained in the cheese curd and thus, contributed to proteolysis during ripening. Addition of low volumes of high SCC milk had an obvious impact on proteolysis patterns and cheese ripening. However, such trends were generally less clear with increasing PMN milk than those observed for addition of high SCC milk. The poor correlation between SCC and PMN obtained in both cow and herd bulk milks, compared to the correlation in quarter milks was probably due to the mixing of high and low SCC milks from either quarters or cows. Thus, the true effect of PMN may not be observed in bulk herd milk but may still have an adverse effect on milk quality. Whether elevated bulk milk SCC and PMN level is due to milk from a smaller number of cows with extremely high SCC/PMN being included with milk from a predominantly healthy herd, or, to large numbers of cows with sub-clinical infections, probably contributes to variation in the effects of SCC/PMN on dairy products.

Introduction

Mastitis is an inflammatory immune reaction of the mammary tissue, which occurs in response to the introduction, and multiplication of pathogenic microorganisms. Such a reaction is characterised by an influx of white blood cells (somatic cells) into the milk. The primary characteristic change that occurs in milk during mastitis inflammation is a significant increase in somatic cell count (SCC). Milk contains three main somatic cell types, neutrophils (PMN cells), lymphocytes and macrophages. PMN cells in milk are derived from blood. Under inflammatory conditions, the PMN cells infiltrate tissue in large numbers. The main function of macrophages and lymphocytes is to recognise bacteria and then trigger alarm systems which induce a more vigorous host response, eventually leading to very large numbers of PMN cells entering the milk. PMN cells account for approximately 10% of total cells in normal milk, but account for greater than 90% of cells in mastitic milk.

Mastitis may be associated with significant economic losses to dairy farmers, due to reduced milk yields, elevated SCC and altered composition of milk resulting in poor quality dairy products in many cases. Mastitic milk also has elevated levels of plasmin activity, due in part to an influx of plasmin from blood and also due to the activation of plasminogen by somatic cell associated plasminogen activators (Politis *et al*., 1989; Zachos *et al*., 1992). Somatic cells themselves are associated with a range of proteolytic enzymes; including the acid proteinase cathepsin D (Verdi and Barbano, 1991). In addition, levels of the antibacterial peptide lactoferrin are also increased significantly in mastitic milk (Kawai *et al*., 1999). One of the major compositional changes associated with mastitis is a decrease in milk casein level, largely due to the post-secretory degradation by proteinases in the udder (Senyk *et al*., 1985). Consequently, mastitic milk has elevated protein levels, impaired rennet coagulation properties and reduced levels of β-casein (Politis and N-Kwai-Hang, 1988). This has significant implications for the processing properties of the milk, and in particular, reduced yield and quality of Cheddar cheese (Auldist *et al*., 1996a). Recently, high SCC has also been linked to early gelation of UHT milk (Auldist *et al*., 1996b).

It has been suggested that there is a continuous heterometrical change from the physiological to the pathological state, which influences the efficiency of absolute SCC limits in the diagnosis of mastitis, and suggests that cell counts, while being a good indicator of hygienic quality, may not give an accurate indication of herd udder health (Heeschen and Reichmut, 1995).

The total number of cells in milk from udder quarters is commonly used (somatic cell count) as an indicator of mastitis, but little attention is 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, measurement of these cells using a reliable test may allow the early diagnosis of subclinical mastitis. Such a test using a specific monoclonal antibody might provide a more specific indicator of mastitis and inflammation than measuring total somatic cells in milk. O' Sullivan *et al*., (1992) developed a rapid ELISA test to measure PMN antigens using horse radish peroxidase conjugated rabbit polyclonal anti-PMN antisera and a mononclonal antibody specific for PMN cells.

Bulk milk somatic cell counts (BMSCC) are continually used by co-operatives and dairies to identify herds with elevated somatic cell counts and thus, herds with potential mastitis problems. Somatic cell counts are an indication of the udder health of individual cows, but may not be an indication of the general udder health of the herd as a whole. Milk from a cow with a high somatic cell count may enter the bulk milk of the herd but may not be detected in the bulk herd SCC. On the other hand, milk from a cow with a

high SCC may be added to the bulk herd milk and result in a high SCC for the bulk herd milk. However, it may be possible to use PMN level for the selection of milk for processing, such as, cheese making by eliminating cows with high PMN quarters from the bulk milk. Also, if PMN levels could be applied to select infected udder quarters at drying off then antibiotic therapy could be limited to those quarters.
Thus the objectives of this study were firstly to apply the PMN ELISA test of O' Sullivan *et al*., (1992) to determine PMN levels in individual cow milk, bulk cow milks and bulk herd milks. This would allow the relationship between SCC and PMN levels to be established in these milks. Secondly, the effect of SCC and PMN levels on the processing characteristics of quarter milks and bulk cow milks were measured. Thirdly miniature cheeses were manufactured from these milks in order to establish the effects of SCC and PMN levels on cheese quality and ripening characteristics.

1. PMN analysis

Enzyme linked immunosorbant assay (ELISA) (O' Sullivan et al., 1992)
The principle of this assay is that a monoclonal antibody specific for bovine neutrophils is placed in the wells of a plastic microtitre plate. The antibody binds to the plastic. When milk samples are added to the wells then any neutrophils or neutrophil fragments will bind to the antibody i.e. the neutrophils in the milk are captured by the bound antibody. Then a second antibody, which has been produced against bovine neutrophils, is added to the wells. This secondary antibody has the enzyme horseradish peroxidase attached to it. This antibody with the enzyme attached now binds to the neutrophils, which have been captured by the first antibody. Then a substrate for the enzyme is added and any enzyme present will convert the substrate to a coloured product. The amount of colour produced, which can be measured, is proportional to the amount of neutrophils present. The ELISA test measured elevated PMN antigens using horse-radish peroxidase conjugated rabbit polyclonal anti-PMN antisera and a monoclonal antibody specific for PMN cells. Optical densities obtained in the ELISA were used to predict the PMN cell numbers of the milk samples. However, a methodology problem arose at an early stage of the study. A lack of colour development after the addition of chromogen prevented the correct measurement of PMN cell numbers. Secondly, the original monoclonal antibody (derived from ascites fluid) used in the methodology of O' Sullivan et al., (1992) could not be used in this study due to a manufacturing and supply problem. Thus, a monoclonal antibody derived from cell culture was required. This necessitated a recalibration of the standard curve.
1 (a) Enzyme conjugations of the secondary antibody
A series of experiments were conducted to establish which variable was responsible for the lack of colour development. Three enzyme conjugations of the secondary antibody were carried out. Each enzyme conjugated antibody was titrated, with normal and mastitic milk samples, to ascertain the optimum working dilution of the conjugated antibody. Elution profiles of the third enzyme conjugation are shown in Figures 1 and 2. The third enzyme conjugated secondary antibody showed the best results and was used for subsequent tests.
Absorbence readings of protein (IgG) sample fractions from the third conjugation are shown in Figure 1. IgG was separated from rabbit polyclonal antiserum by DEAE cellulose ion-exchange chromatography. 2 ml of dialysed rabbit polyclonal antiserum was applied to the column and eluted with 20mM Phosphate Buffer (PB). 1 ml fractions were collected and the absorbency monitored at 280nm. Sample fractions with an absorbency reading of >0.1 containing protein (IgG) were pooled together (sample fractions 2-12) and concentrated to 2 ml in dialysis tubing on a bed of sucrose.

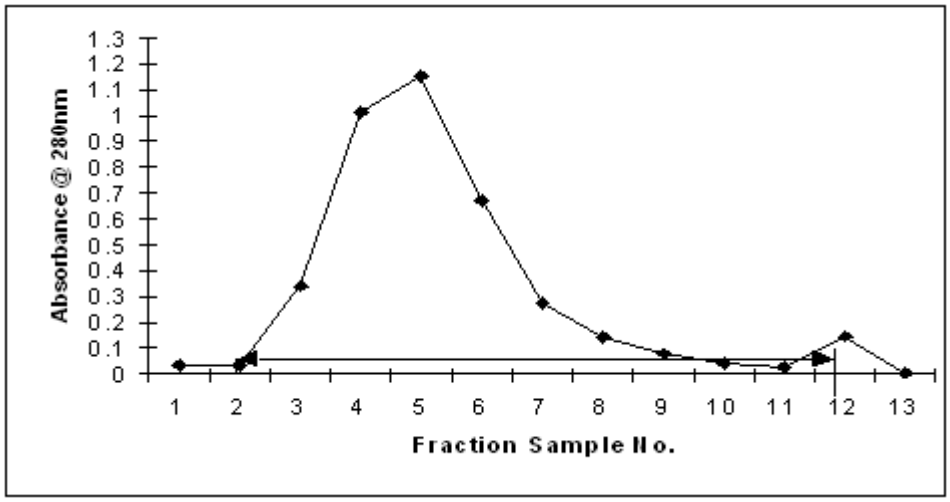


Figure 1: Chromatogram separation of IgG protein on DEAE cellulose column (from the third enzyme conjugation): elution of protein detected by absorbance at 280nm (sample pooled together fractions No. 2-12)

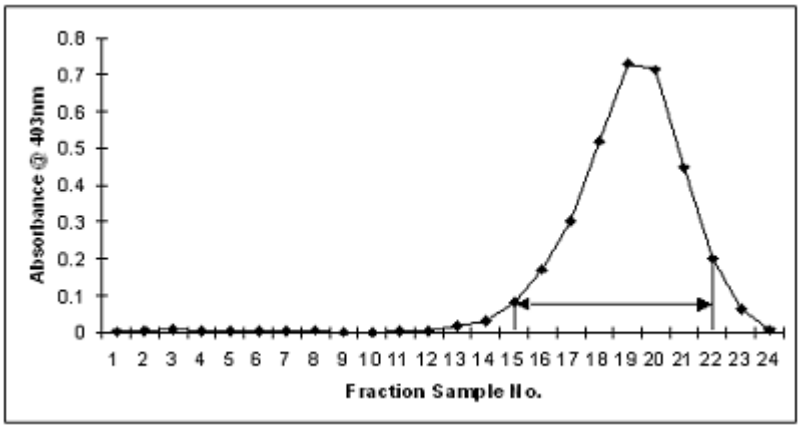


Figure 2: Elution profile of the third conjugated secondary antibody from gel filtration column (Sepharose 6B) (sample fractions pooled together No. 15-22)

Absorbence readings of the third purified conjugated secondary antibody are shown in Figure 2. The conjugate was purified by a Sepharose 6B gel filtration column. 2 ml sample fractions were collected and absorbency read at 280nm and 403nm. Fractions 15-22 were pooled together. These fractions were dialysed and concentrated down to approximately 2 ml. An equal volume of glycerol was added to the concentrated conjugated secondary antibody and stored at –20 ºC.

The original and new secondary antibodies were titrated to evaluate their effectiveness at different concentrations. The effectiveness of the original and new secondary antibodies at dilutions of 1:500 and 1:1,000 are shown in Table 1. The original secondary antibody at a dilution of 1:500 was more effective than the new secondary antibody at the same dilution. The original secondary antibody could identify high PMN from low PMN levels in milk samples and gave a low background reading for the milk standard which contained $<5x10^3$ cells per ml. At a dilution of 1:1,000 the original secondary antibody was slightly more effective than the new secondary antibody at the same dilution. This suggests that the original secondary antibody was effective and the new secondary antibody was of low yield and needed to be used at a higher concentration.

Table 1: Comparison of original and new secondary antibodies at concentrations of 1:500 and 1:1,000

Comparison of original and new secondary antibodies at concentrations of 1:500

Milk PMN ($x10^3$/ ml)	**O.D.* Original 1:500**	**O.D.* New 1:50**	**Milk PMN ($x10^3$/ ml)**	**O.D.* Original 1:1,000**	**O.D.* New 1:1,000**

		0			
142	0.79	0.36	252	0.34	0.18
61	0.09	0.06	118	0.14	0.08
Blood PMN ($x10^3$/ ml)			Blood PMN ($x10^3$/ ml)		
3000	2.18	2.45	3000	2.21	2.33
3000	2.30	2.42	3000	2.26	2.34
Standard**	0.035	0.036	Standard**	0.024	0.021

*O.D. = Optical density reading
**Standard = SCC<$5x10^3$ per ml

The effectiveness of the original and new secondary antibodies at dilutions of 1:100 and 1:250 is shown in Table 2. At a dilution of 1:100 both the original and the new secondary antibodies could differentiate between low PMN and high PMN milk samples. At a dilution of 1:250 both the original and new secondary antibodies were able to differentiate between low and high PMN milk samples but not as clearly as at a dilution of 1:100. At a dilution of 1:100 both the original and new secondary antibodies gave better O.D. readings than dilutions of 1:500 and 1:1,000.

Table 2: Comparison of original and new secondary antibodies at concentrations of 1:100 and 1:250

Comparison or original and new secondary antibodies at concentrations of 1:100 and 1:250

Milk PMN ($x10^3$/ ml)	O.D.* Original 1:100	O.D.* New 1:100	Milk PMN ($x10^3$/ ml)	O.D.* Original 1:250	O.D.* New 1:250
142	1.61	1.29	252	0.95	0.88
61	0.46	0.36	118	0.53	0.36
Blood PMN ($x10^3$/ ml)			Blood PMN ($x10^3$/ ml)		
3000	2.4	2.62	3000	2.46	2.55
Blank	0.006	0.006	Blank	0.005	0.007
Standard**	0.17	0.21	Standard**	0.08	0.10

*O.D = Optical density reading
**Standard = SCC<$5x10^3$ per ml

1 (b) Comparison of the original monoclonal antibody (derived from ascites fluid) and the new monoclonal antibody (derived from cell culture)

The original monoclonal antibody derived from ascites fluid was low in stock and could not be re-sourced. Thus, it was necessary to source a new monoclonal antibody, which

would be derived from cell culture. However, the optimum working dilution of the new monoclonal antibody (derived from cell culture) had to be optimised. This was achieved by varying the concentrations of the original monoclonal antibody (derived from ascites fluid) and the new monoclonal antibody (derived from cell culture) and comparing the results for each.
A bovine blood standard using the original monoclonal antibody (derived from ascites fluid) at a concentration of 1:1,000 is shown in Figure 3. The standard curve suggested that the original monoclonal antibody could detect PMN cells at levels between $100x10^3$/ml and $300x10^3$/ml. A bovine blood standard curve using the new monoclonal antibody (derived from cell culture) at a concentration of 1:80 is shown in Figure 4. These data indicated that the new monoclonal antibody was satisfactory for detection of PMN cells at levels between $50x10^3$/ml and $350x10^3$/ml. In order to detect PMN cells at levels between $350x10^3$/ml and $500x10^3$/ml in milk samples, the samples would have to be diluted to $<10x10^3$/ml prior to assaying.

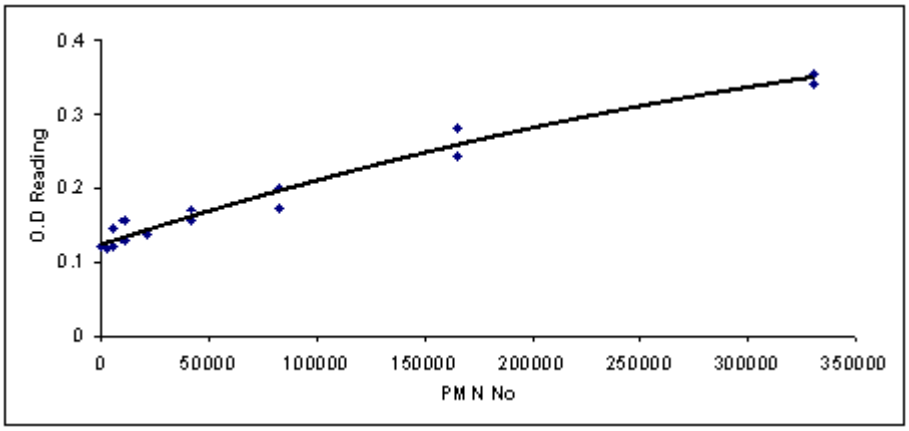


Figure 3: Bovine blood standard curve using original monoclonal antibody (derived from ascites fluid) at a concentration of 1:1,000

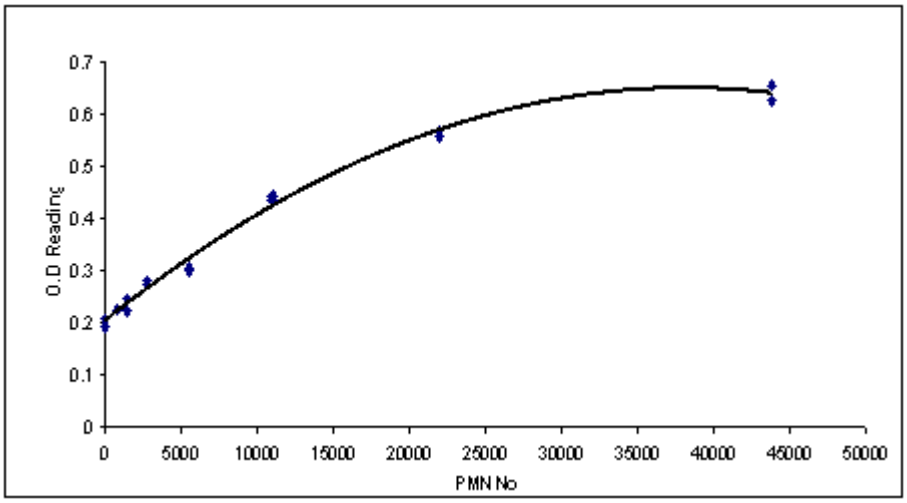


Figure 4: Bovine blood standard curve using new monoclonal antibody (derived from cell culture) at a concentration of 1:80

Discussion

The outlined modification to the test of O'Sullivan *et al* (1992) allowed the measurement of PMN levels in milk. Such a measurement may have important implications in future milk quality programmes in terms of selection of infected udder quarters and product quality.

Previous studies have suggested that milk somatic cells and PMN in particular, may influence the manufacture and ripening of Swiss-type cheese, possibly due to incorporation of enzymes derived from these cells into the curd (Cooney *et al,* 1999).

2. SCC and PMN levels in milks from individual udder quarters

Objective

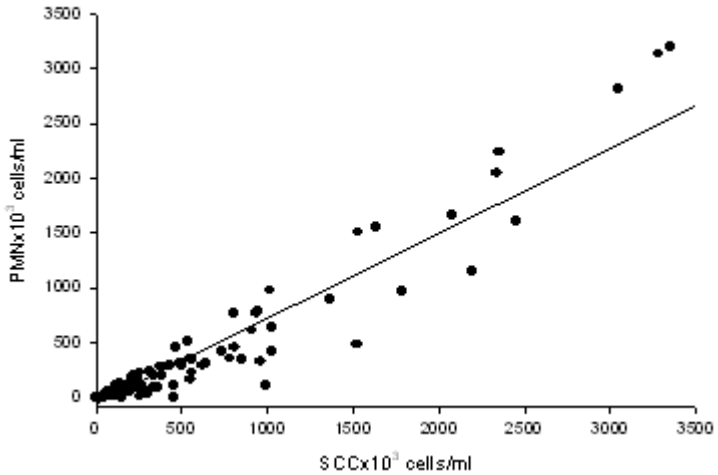
The objective of this study was to determine the correlation between SCC and PMN levels in milks from individual udder quarters.

Materials and methods

Milk from individual udder quarters was collected from 126 Holstein-Friesian cows from the Moorepark herd. Milk samples of 30 ml volumes were collected aseptically from individual cow quarters into sterile universal bottles and stored at 4°C. Samples were collected after foremilk was removed (directly before cluster application) (pre-milk). A 5 ml subsample was taken for SCC measurement. The SCC was measured using a Bentley Somacount 300 somatic cell counter (Agri York 400 Ltd, York YO4 2QW,UK), after calibration and standardization as set out in the International Dairy Federation (1984) standard methods. A further 1 ml subsample was taken from each of the quarter milks, and these were lysed and frozen for subsequent PMN analysis, using the modified version of the ELISA method of O' Sullivan *et al*., (1992).

Results

A scatter plot of SCC and PMN level in 126 milks from individual udder quarters is shown in Figure 5. The SCC and PMN level had a correlation of 92%. Analysis of



variance showed that SCC was significantly correlated to PMN ($p<0.001$).
Figure 5: Scatter plot of SCC and PMN levels in 126 milks from individual udder quarters

A scatter plot of individual cow quarter milks with SCC<1000x10^3/ml and associated PMN level is shown in Figure 6. The SCC and PMN levels had a correlation of 73%. Analysis of variance showed that SCC <1000x10^3/ml was significantly correlated to PMN ($p=<0.001$).

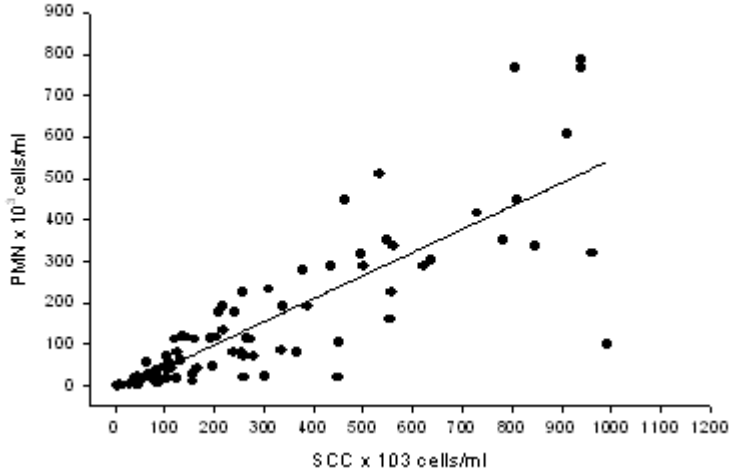


Figure 6: Scatter plot of SCC (<1000x10^3/ml) and PMN levels for milks from individual udder quarters

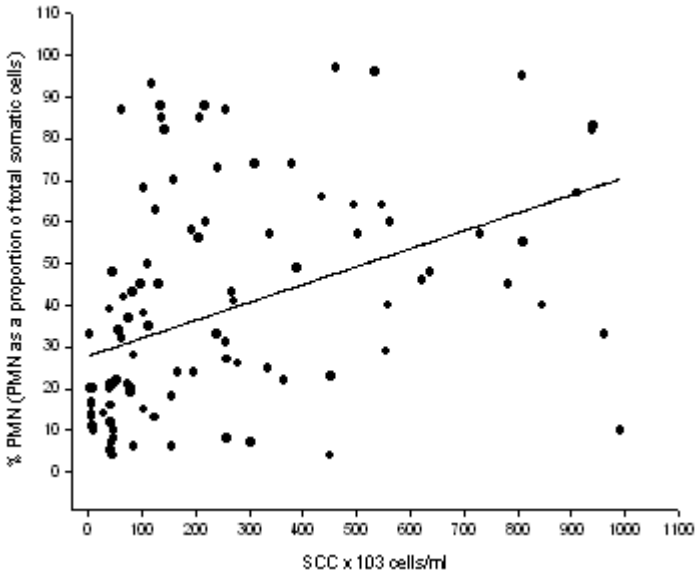


Figure 7: Scatter plot of SCC level (<1000x10^3/ml) and PMN as a proportion of total cell numbers (% PMN) in individual cow quarter milks

A scatter plot of absolute SCC level (<1000x10^3/ml) and PMN as a proportion of total cell numbers in individual cow quarter milks is shown in Figure 7. The level of SCC and PMN as a proportion of total cells were significantly correlated (p=<0.001) with an R^2 value of 19%.

A scatter plot of absolute PMN level (<1000x10^3/ml) and PMN as a proportion of total cell numbers in individual cow quarter milks is shown in Figure 8. The level of PMN and PMN as a proportion of total cells were significantly correlated (p=<0.001) with an R^2 value of 46%.

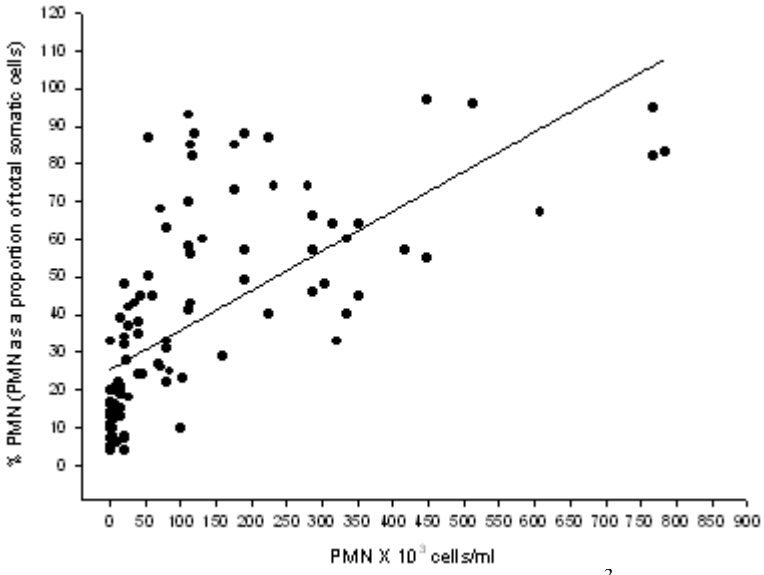


Figure 8: Scatter plot of PMN level (<1000x10^3/ml) and PMN as a proportion of total cell numbers (% PMN) in individual cow quarter milks

Discussion

The strong relationship or correlation between SCC and PMN of 92% in the individual quarter milks has confirmed previous preliminary data. This is important since PMN in conjunction with SCC may now provide a more reliable method of selecting milks for processing. O'Brien *et al* (1999) also indicated that the best correlation between SCC and PMN was shown in milks from individual udder quarters. The poorer correlation obtained in both cow and herd bulk milks was probably due to the mixing of high and low SCC milks from either udder quarters or cows. The higher correlation between SCC and PMN in all quarter milks compared to quarter milks of SCC <1,000 x 10^3/ml may indicate a greater response in increasing PMN at high SCC levels.
The data indicated a very weak relationship between SCC <1,000 x 10^3/ml and PMN as a proportion of total cell numbers. This has also confirmed previous preliminary data of O'Brien *et al* (1999) which showed that PMN levels were variable, even within the SCC range of 80x10^3/ml to 100x10^3/ml. A weak relationship between absolute PMN levels and PMN as a proportion of total cells was also evident. Thus, milk PMN levels may be controlled by factors other than those controlling SCC. These factors need to be elucidated.

3. Milk composition and miniature cheese manufacture of milks from individual udder quarters

Objective

The objective of this study was to determine the effect of SCC and PMN in individual quarter cow milks on milk composition and processing characteristics.

Materials and methods

Twenty individual cow udder quarters from Holstein-Friesian cows were selected based on preliminary SCC screening of the herd. Individual cow quarter milk was collected from these 20 udder quarters. A 5 ml subsample was screened for SCC, and a 1 ml subsample was screened for PMN. Seven of the 20 individual cow udder quarters were

then identified as having milk of SCC and PMN ranging from < $100x10^3$/ml to between $800x10^3$/ml and $1,000x10^3$/ml. Milk was collected from these 7 udder quarters using a milking unit designed to collect milk in separate containers from each of the four udder quarters. Milk fat, protein and lactose of the quarter milk sample were determined by the Infra-red Milk Analyser (Milkoscan 605; Foss Electric, DK-3400 Hillerød, Denmark) which was calibrated according to International Dairy Federation (1990). A Formagraph instrument (Model 11700 Foss Electric) was used to assess the rennet coagulation properties (McMahon and Brown, 1982) of the quarter milks. These characteristics were measured at the natural pH of milk. The milk was heated to 36^{o}C, equilibrated for 20 min and chymosin (double strength chymax; Pfizer Inc., Milwaukee, WI 53214 - 4298, USA), diluted 1:100 with de-ionized water, was added at a rate of 77.7 μl/10 ml. The following rennet coagulation characteristics were measured: rennet coagulation time (RCT) in min, rate of curd aggregation (K20) in min and curd firmness at 60 min (A60) in mm of amplitude. The milks were also analysed for total protein (IDF, 1993), non-protein nitrogen (NPN) and whey protein as outlined by Guinee *et al*., (1995). Miniature cheeses were manufactured by the method of Shakeel-Ur-Rehman *et al*., (1998) from the selected quarter milks, based on SCC and PMN levels. Compositional analysis (total solids, moisture and chloride contents and pH) of cheese was carried out 1 month after manufacture. Proteolysis of miniature cheeses was measured by urea-polyacrylamide gel electrophoresis after 1 and 3 months of ripening.

Results

The effect of SCC and PMN on gross composition, rennet coagulation properties and nitrogen fractions of the 7 selected quarter milks for miniature cheese manufacture are shown in Table 3. Regression analysis of the seven quarter milks showed that SCC and PMN had a significant effect on fat ($p<0.05$) and protein ($p< 0.01$) contents. As SCC and PMN increased the fat content also increased. Protein content significantly decreased as SCC and PMN increased ($p<0.01$). The RCT, K20 and A60 were significantly affected by an increase in SCC and PMN ($p<0.001$), ($p< 0.01$) and ($p<0.01$), respectively. There was an increase in both the RCT and K20 as the SCC and PMN increased. The A60 decreased as the SCC and PMN increased. The total protein content was significantly affected ($p<0.001$) by an increase in SCC and PMN. SCC and PMN also had a significant effect ($p<0.05$) on the casein and NPN contents and on casein number.

Table 3: Effect of SCC and PMN level on gross composition, rennet coagulation properties and N fractions of selected quarter milks for miniature cheese manufacture

Effect of SCC and PMN level on gross composition, rennet coagulation properties and N fractions of selected milks for miniature cheese manufacture

	SCC ($x10^3$ cells/ml)	PMN ($x10^3$ cells/ml)	Gross composition			Renneting properties			Nitrogen fractions			
			Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	RCT (min)	K20 (min)	A60 (mm)	Total protein (g/kg)	Casein (g/kg)	NPN (g/kg)	Casein no. (%)
	54	14	42.3	36.4	47.2	21.3	6.9	47.0	37.2	29.5	0.22	79.1
	61	22	46.2	34.7	46.8	25.8	7.5	42.0	35.7	26.0	0.21	78.3
	85	10	41.1	35.2	47.1	19.5	6.0	49.5	35.5	29.1	0.25	79.9
	581	470	54.4	33.1	44.8	34.2	22.0	25.4	32.1	24.7	0.18	77.0
	735	628	58.8	31.5	47.2	40.1	26.2	10.0	32.5	24.1	0.19	77.3
	510	405	58.7	32.2	45.8	28.0	16	33.6	32.8	25.6	0.18	78.5
	956	842	65.1	31.8	46.9	47.5	20.5	22.0	31.2	25.1	0.17	77.1
R^2SCC		+0.99***	+0.90*	-0.80**	0.0^{NS}	+0.88***	+0.78**	-0.75**	-0.88***	-0.53*	-0.64*	-0.64*
R^2PMN	+0.99***		+0.90*	-0.79**	0.0^{NS}	+0.90***	+0.77**	-0.75**	-0.85***	-0.53*	-0.65*	-0.66*

Significance: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

The effect of SCC and PMN on total solids, H_2O, NaCl and pH of miniature cheeses manufactured from selected quarter milks in shown in Table 4. Results showed no overall trend.

Table 4: Composition of miniature cheese manufactured from milks from selected udder quarters

Composition of miniature cheese manufactured from milks from selected udder quarters

	SCC (x10^3 cells/ml)	**PMN (x10^3 cells/ml)**	**Total solids (g/100g)**	**Moisture (g/100g)**	**NaCl (g/100g)**	**pH**
	54	14	55.0	45.0	1.2	5.1
	61	22	61.0	39.2	1.3	5.0
	85	10	67.3	32.7	0.9	5.1
	581	470	56.2	43.8	1.0	5.1
	735	628	59.7	40.3	1.7	5.1
	510	405	55.2	44.8	1.3	5.0
	956	842	58.8	41.2	1.2	4.9
R^2 SCC		+0.99***	0.0^{NS}	0.0^{NS}	$+1.4^{NS}$	0.0^{NS}
R^2 PMN	+0.99***		0.0^{NS}	0.0^{NS}	$+0.7^{NS}$	0.0^{NS}

Significance: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

The Urea-PAGE electophoretogram (Figure 9) shows the effect of SCC and PMN levels on individual casein fraction levels in miniature cheeses manufactured from quarter

milks at 30 days (lanes 1-7) and 90 days (lanes 8-14) of ripening. The urea-PAGE electophoretogram indicated a very heterogeneous selection of proteolysis patterns in the miniature cheeses. The lanes corresponding to different SCC milk samples differed quantitatively and qualitatively in terms of band patterns; however, there was no clear relationship with milk SCC.

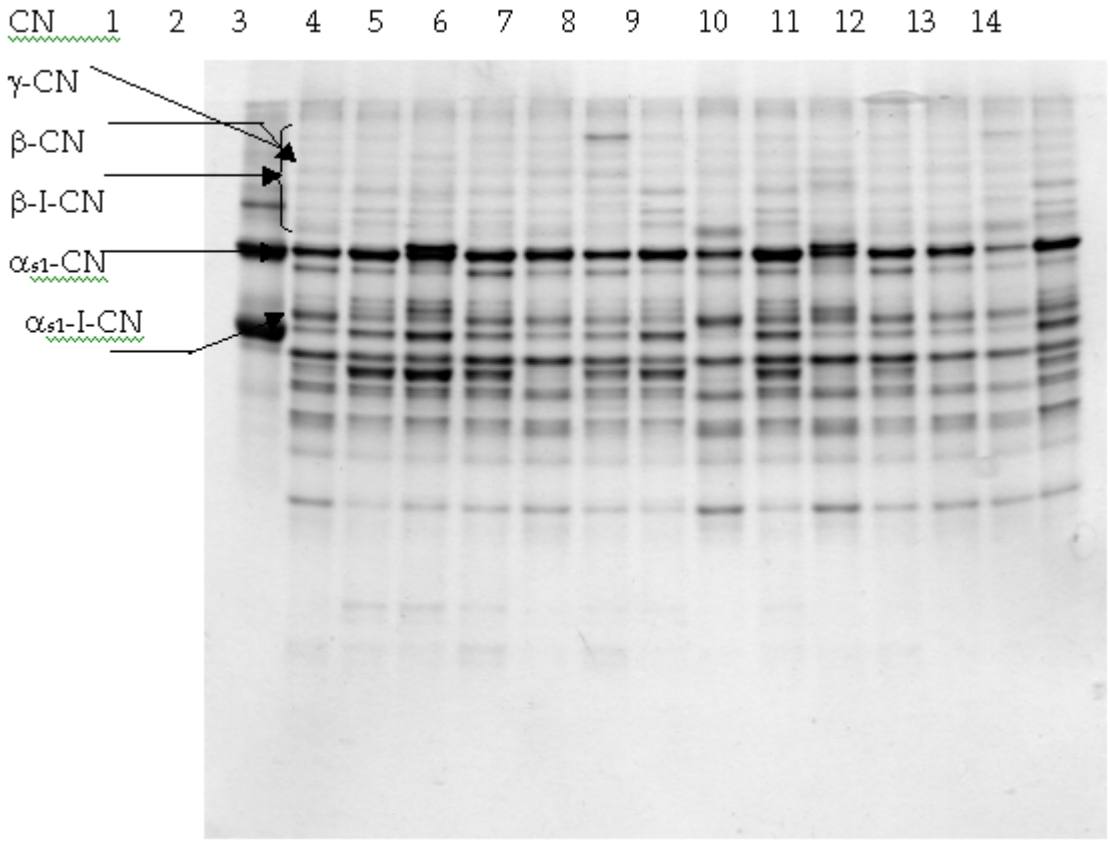


Figure 9: Effect of SCC and PMN level on individual casein fraction levels in miniature cheeses manufactured from udder quarter milks at 30 days (lanes 1-7) and 90 days (lanes 8-14) of ripening

Discussion

Mastitis can cause many changes to the chemical composition of milk. In the current study elevated SCC and PMN levels resulted in increased fat content in milk, reduced protein and had no significant effect on lactose. The literature suggests that the effects of mastitis on milk fat have not been studied nearly as extensively as for protein and the direction of change, if any, remains uncertain. A decline in milkfat concentrations during mammary infection is logical given the reduced synthetic and secretary ability of the mammary gland and this has been reported by some researchers. It is possible however, that in other studies, the concentrating effect of a reduction in milk yield has offset any reduction in the synthesis and secretion of milkfat, to produce a negligible change in overall fat concentration or even an increase (Auldist and Hubble, 1998). This may reflect the occurrence in this study. Reports on the effect of mastitis on concentrations of total protein are conflicting and varied. Overall, it is generally accepted that during mastitis there is a decrease in casein coupled with an increase in whey protein, producing a negligible change in total milk protein. The direction of change, if any, may be influenced by factors such as the severity and type of pathogen

involved. Previous studies (Milles *et al*, 1983; Shuster *et al*, 1991) have shown that elevated SCC (>1,000x10^3/ml) resulted in reduced milk lactose, however, milk SCC levels in this study were <1,000x10^3/ml. Green and Grandison (1993) reported that renneting behaviour of milk is largely affected by the casein concentration in the milk. Thus the reduction in casein at elevated SCC levels in the current study may have resulted in the non-significant trend towards deteriorated milk coagulation properties. The association between elevated SCC and increased milk plasin concentration (Politis *et al*, 1989) may also have had a negative influence on the milk coagulation properties. An elevated SCC can be associated with low recoveries of milk fat and protein in cheese and a decline in the yield of cheese per kilogram of milk. This is largely due to a decrease in casein as a percentage of total protein, since it is mostly casein that is incorporated into the curd, while the whey is expelled during syneresis (Auldist and Hubble, 1998). It is also due to a sub-optimal ratio of casein-to-fat, causing a lower percentage of fat to become entrapped in the curd (Politis and Ng-Kwai-Hang, 1988). This loss of cheese yield has been a key economic driver for companies to reduce the SCC of their bulk supply. In terms of product quality, mastitis is found to cause increases in cheese moisture concentrations. This is one of the most serious cheese quality defects, and can place the product outside product specifications if very high SCC milk is used. Elevated cheese moisture content often causes decreases in curd firmness, leading to a deterioration of the organoleptic properties of cheese. Textural problems, specifically soft, pasty cheese, occur as a result of the high moisture content of the cheese. Flavour defects can also occur, especially during ripening, as a result of the increased activity of hydrolytic enzymes (Auldist and Hubble, 1998). The increase in cheese moisture associated with elevated SCC is caused by a slow, weak coagulation, due largely to alterations in milk protein composition and mineral balance and an increased milk pH. All of these changes in milk composition interfere with the expulsion of water from the curd during syneresis. For example, Auldist *et al* (1996a) found deleterious effects of high milk SCC on the yield and quality of Cheddar cheese in late lactation and concluded that effects of stage of lactation are exaggerated by an elevated BMSCC. In an experiment using milks obtained from commercial dairy farms during late lactation in Victoria, Australia, cheese moisture contents increased (8%) and textural defects were observed in the cheese as BMSCC changed from 252,000 to 1,463,000 cells/ml. Concurrently, actual and moisture-adjusted cheese yields were reduced (by 5% and 10% respectively), when the high BMSCC milk was used.
As far as can be ascertained, miniature cheese has not been manufactured from udder quarter milk previously, and hence interpretation of the results in the current study is very difficult. However, the possibility that samples from different quarters could differ so substantially in proteolytic activity (assuming this would explain the differences seen) is very interesting, and deserves further study. As samples are mixed, whether at individual cow level (4 quarters mixed together) or at farm bulk tank level (dozens or hundreds of quarter samples mixed together) such differences would be averaged out, which is why such heterogeneity may not have been observed in other studies.

4. Milk composition and miniature cheese manufacture of milks from individual udder quarters and artificial mixes of those milks

Objective

This study was undertaken to establish the effects on milk composition and miniature cheese manufacture of mixing high and low SCC and PMN milks to achieve mixes of SCC and PMN of approximately $200x10^3$/ml and $400x10^3$/ml.

Materials and methods

Cows with individual quarter milks of SCC $920x10^3$/ml and $6x10^3$/ml were identified. One litre volumes of milk were collected from each of these udder quarters. The milks were mixed in various proportions to give two artificial mix milks of SCC $200x10^3$/ml and $400x10^3$/ml, approximately. Two further 1L volume milk samples were collected from udder quarters and mixed in various proportions to give two artificial mix milks of PMN $200x10^3$/ml and $400x10^3$/ml approximately. The four original milks, the two SCC artificial mixes and the two PMN artificial mixes were analysed for gross composition, rennet coagulation characteristics and N fractions, as previously described. Miniature cheeses were manufactured from each of the eight milks following the protocol described by Shakeel-Ur-Rehman *et al*., 1998. Cheeses were analysed for composition after 1 month. Proteolysis of miniature cheeses was measured by urea-polyacrylamide gel electrophoresis after 1 and 3 months of ripening.

Results

The effect of SCC and PMN level on gross composition, rennet coagulation properties and nitrogen fractions of low and high SCC and PMN milks together with the artificial mixes of approximately 200 x 10^3/ml and 400 x 10^3/ml SCC and PMN are shown in Table 5. As SCC and PMN increased the fat, protein and lactose contents generally decreased.

Milk of low SCC and PMN had much better renneting properties than milks of high SCC and PMN. As SCC and PMN increased the RCT and K20 increased and the A60 decreased. Milk of SCC $6x10^3$/ml (low SCC) and PMN $<1x10^3$/ml had average RCT, K20 and A60 values of 20.7 min, 6.5 min and 48.0 mm, respectively. Milk of SCC $920x10^3$/ml (High SCC) and PMN $830x10^3$/ml had average RCT, K20 and A60 values of 51.5 min, 17.5 min and 10 mm, respectively. Milk of PMN $1x10^3$/ml (low PMN) and SCC $<4x10^3$/ml had average RCT, K20 and A60 values of 21.0 min, 6.7 min and 46.0 mm, respectively. Milk of PMN $700x10^3$/ml (High PMN) and SCC $850x10^3$/ml had average RCT, K20 and A60 values of 41.5 min, 18.2 min and 17.0 mm, respectively. The SCC and PMN had no clear effect on the nitrogen fractions of the original milks or on the artificial mixes made from the original milks.

The effect of SCC and PMN level on total solids, moisture and NaCl contents and pH values of miniature cheeses manufactured from quarter milks and artificial mixes of quarter milks are shown in Table 6. SCC and PMN had no clear effect on the composition of miniature cheeses.

Table 5: 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 the artificial mixes of approximately 200x10^3/ml and 400x10^3/ml SCC and PMN

Effect of SCC and PMN level on gross composition, rennet coagulation and N fractions of high and low SCC and PMN milks together with the artificial mixes of approximately 200x10^3/ml and 400x10^3/ml SCC and PMN

Milk type	SCC (x10^3 cells/ml)	PMN (x10^3 cells/ml)	Gross composition			Renneting properties			N fractions			
			Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	RCT (min)	K20 (min)	A60 (mm)	Total protein (g/kg)	Casein (g/kg)	NPN (g/kg)	Casein no. (%)
Low SCC	6	<1	42.0	38.0	46.0	20.7	6.5	48.0	20.0	11.3	2.17	57.6
Mix 1 SCC	236	130	45.0	36.0	45.0	30.7	10.2	40.0	36.6	28.0	2.17	77.2
Mix 2 SCC	488	350	44.0	35.0	44.0	35.2	12.5	34.0	27.5	18.9	2.04	69.2
High SCC	920	830	35.0	32.0	43.0	51.5	17.5	10.0	33.0	24.6	1.72	74.0
Low PMN	4	<1	44.0	34.0	46.0	21.0	6.7	46.0	35.3	27.0	2.55	77.2
Mix 1 PMN	380	205	42.0	34.0	45.0	28.7	10.7	44.5	35.2	27.2	2.55	77.6
Mix 2 PMN	545	430	41.0	34.0	44.0	32.7	15.5	32.5	34.4	25.6	2.42	74.4
High PMN	850	700	38.0	33.0	42.0	41.5	18.2	17.0	34.0	24.8	2.30	73.3

Table 6: Composition of miniature cheeses manufactured from milks from individual udder quarters and artificial mixes of quarter milks

Composition of miniature cheeses manufactured from milks from individual udder quarters and artificial mixes of quarter milks

	SCC (x10^3 cells/ml)	**PMN (x10^3 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 1 SCC	236	130	47	53	1.31	5.2
Mix 2 SCC	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 1 PMN	380	205	56	44	1.41	5.1
Mix 2 PMN	545	430	57	43	1.38	5.0
High PMN	850	700	50	50	1.69	5.1

The Urea-PAGE electophoretogram (Figure 10) shows the effect of SCC and PMN level on individual casein fraction levels in miniature cheeses manufactured from low and high SCC and PMN milks, together with the artificial mixes, of approximate SCC 200x10^3/ml and 400x10^3/ml at 30 days (lanes 1-4 and lanes 9-12 [repeat]) and at 90 days (lanes 5-8) of ripening. There was a clear effect of milk SCC on proteolysis during ripening of Cheddar type cheese; the patterns of proteolysis differed quantitatively and qualitatively

with SCC. Comparing lanes 1-4, increasing numbers of bands of slow electrophoretic mobility are 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. The same general trends could be seen in replicate trials (lanes 9-12). 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 (lanes 9 and 10). The implication of these changes on proteolysis due to somatic cell enzymes for cheese quality and flavour warrant further investigation.

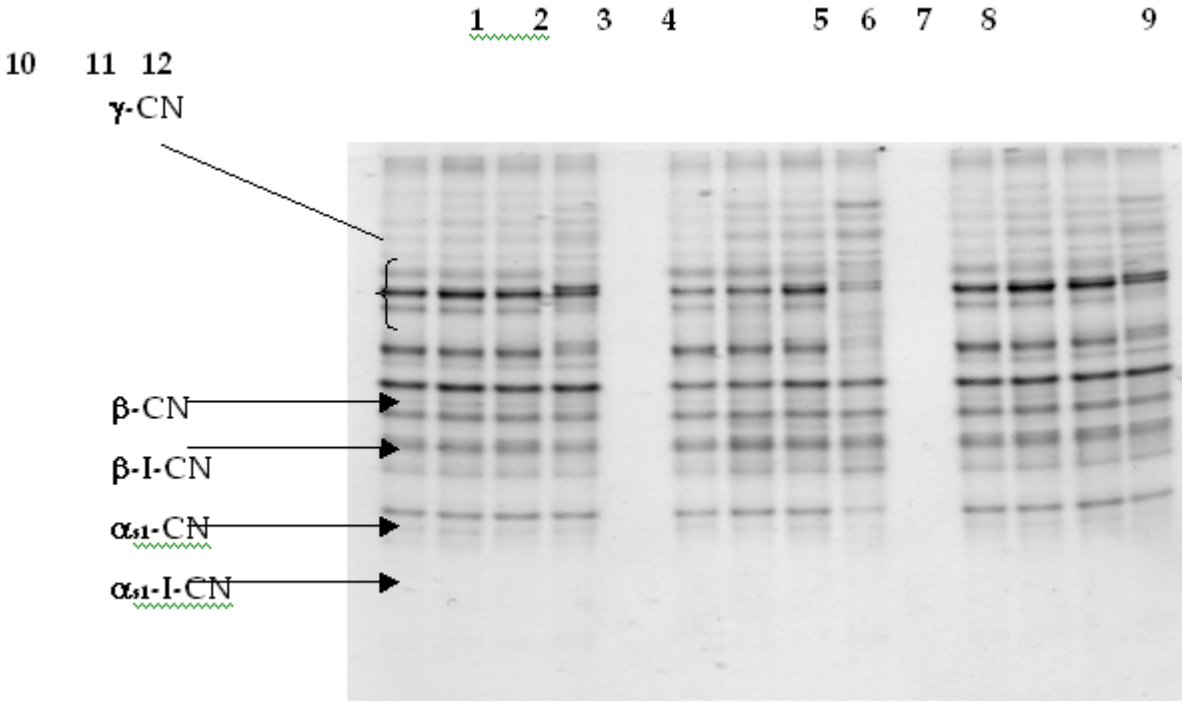


Figure 10: Effect of SCC and PMN level on individual casein fraction levels in miniature cheeses manufactured from low and high SCC and PMN milks together with the artificial mixes of approximately $200x10^3$/ml and $400x10^3$/ml SCC at 30 days (lanes 1-4 and lanes 9-12 [repeat]) and at 90 days (lanes 5-8) of ripening

The effect of PMN and SCC level on individual casein fraction levels in miniature cheeses manufactured from low and high PMN and SCC milks together with the artificial mixes of approximately $200x10^3$/ml and $400x10^3$/ml PMN at 30 days (lanes 1-4 and lanes 9-12 [repeat]) and at 90 days (lanes 5-8) of ripening are shown in Figure 11. It can be seen from Figure 11 that the addition of milk with high PMN levels resulted in altered patterns of proteolysis (lane 8, sample made from milk with the highest PMN level had

the least residual α_{s1}- and β-caseins during ripening). However the trends in patterns of proteolysis were generally less clear than those observed for the addition of high SCC milk.

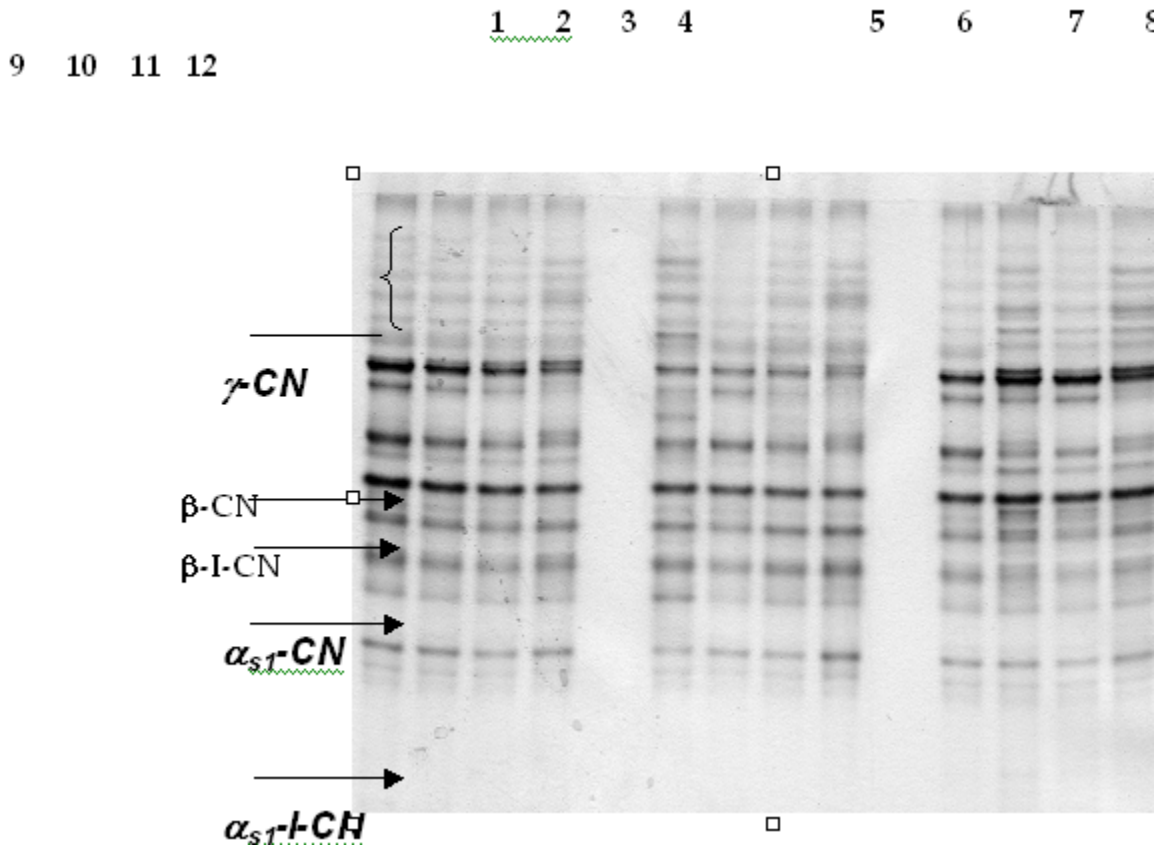


Figure 11: Effect of PMN and SCC level on individual casein fraction levels in miniature cheeses manufactured from low and high PMN and SCC milks together with the artificial mixes of approximately 200x10^3/ml and 400x10^3/ml PMN at 30 days (lanes 1-4 and lanes 9-12 [repeat]) and at 90 days (lanes 5-8) of ripening

Discussion

An SCC standard of 400x10^3/ml for bulk milk is being adopted 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 deleterious effects on the quality of dairy products have been reported for milk with an SCC as low as 100x10^3/ml. Auldist and Hubble, (1998) reported that the major mastitis-causing organisms in Australia and New Zealand were *Streptococcus* and *Staphylococcus spp.* and that a bulk milk SCC of 400x10^3/ml was indicative of approximately 40% of cows in a herd being infected. Data from the current study indicated various influences of SCC and PMN levels on milk and cheese characteristics. The influence of SCC on the composition and quality of Cheddar cheese has been investigated in other studies (Rogers and Mitchell, 1994). The use of milk containing >500x10^3/ml resulted in Cheddar cheese with a high moisture content and proteolysis

breakdown products and poor flavour, texture and body grades. During manufacture, rennet coagulation times were increased by 25% and there were increased losses of fines, fat and protein in the whey. Losses in the whey resulted in reduced cheese yields of approximately 8.9% (Rogers and Mitchell, 1994). Similar studies in which SCC's ranged between $100x10^3$/ml and more than $1000x10^3$/ml showed increases in cheese moisture (5%), decreases in moisture-adjusted cheese yield (9%) and an increase in coagulation time (21%). Increasing the SCC to $500x10^3$/ml decreased cheese yield by 5% and increased coagulation time by 2%. The authors concluded that the composition of milk, enzyme activity, coagulation time and the yield and quality of cheese was adversely affected by an increase in SCC (Politis and Ng-Kwai-Hang 1988 a, b, c). Sub-clinical forms of mastitis (where a specific mastitis pathogen cannot be recovered but the milk SCC is elevated) can cause changes in milk chloride content, decreases in milk fat and lactose and increases in the activity of proteolytic and lipolytic enzymes (Mijacevic *et al.* 1993). These changes in milk quality are known to cause problems in cheese manufacture. Coagulation time of milk has been reported to be directly dependent on SCC, and the decrease in pH during cheese manufacture is slower in cheese curd in high SCC milk. Analysis of whey demonstrated an increased loss of proteins in the whey with higher SCC (Mijacevic *et al.* 1993).

In summary, the large variation in the SCC at which the manufacturing properties of milk are affected is probably due to a number of factors. Whether and elevated bulk milk SCC is due to milk from a smaller 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 sub-clinical infections, probably contributes to variation in the effects of SCC on dairy products. Additionally, some pathogens affect milk composition in different ways, irrespective of SCC level. Similarly, recent evidence suggests that some types of somatic cell can have greater effects on milk composition than others. 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 products, possibly through an influence on the immune system of the cow. 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 between $100x10^3$/ml (Barbano *et al.* 1991), while others have suggested that the threshold is closer to $500x10^3$/ml (Politis and Ng-Kwai-Hang, 1988c).

It appears that enzymes associated with the cells were retained in the cheese curd and contributed to proteolysis during ripening. The patterns of proteolysis were different to those normally associated with cheese ripening, and even the addition of low amounts of high SCC milk had an obvious impact. However, the trends in patterns of proteolysis were generally less clear with increasing PMN milk than those observed for addition of high SCC milk. The implications of these changes in proteolysis due to somatic cell enzymes for cheese quality and flavour warrant further investigation.

5. Bulk herd milks for SCC and PMN screening

Objective

The objective of this study was to examine 49 bulk herd milks for SCC and PMN and determine if bulk herds with similar SCC had similar or different PMN levels.

Materials and methods

Fifty bulk herd milks were supplied by an Irish milk processing outlet and screened for SCC and PMN. A 30ml milk sample was taken from each of the 49 bulk tanks. A subsample of 10ml was taken for PMN analysis from each and stored at –30ºC for subsequent measurement by the modified ELISA method of O' Sullivan *et al*., (1992). SCC was measured using a Bentley Somacount 300.

Results

Data are presented in 4 categories, i.e. bulk milks of SCC <250x10^3/ml, 200x10^3-300x10^3/ml, 300x10^3-450x10^3/ml and 500x10^3-900x10^3/ml. Absolute SCC and PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC <250x10^3/ml are shown in Figure 12. The results indicated that, generally, the PMN level in the milks followed a similar trend to that of SCC level.

Absolute SCC, PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC between 200x10^3/ml and 300x10^3/ml are shown in Figure 13. The results indicated that, generally, the PMN level in the milks followed a similar trend to that of SCC level.

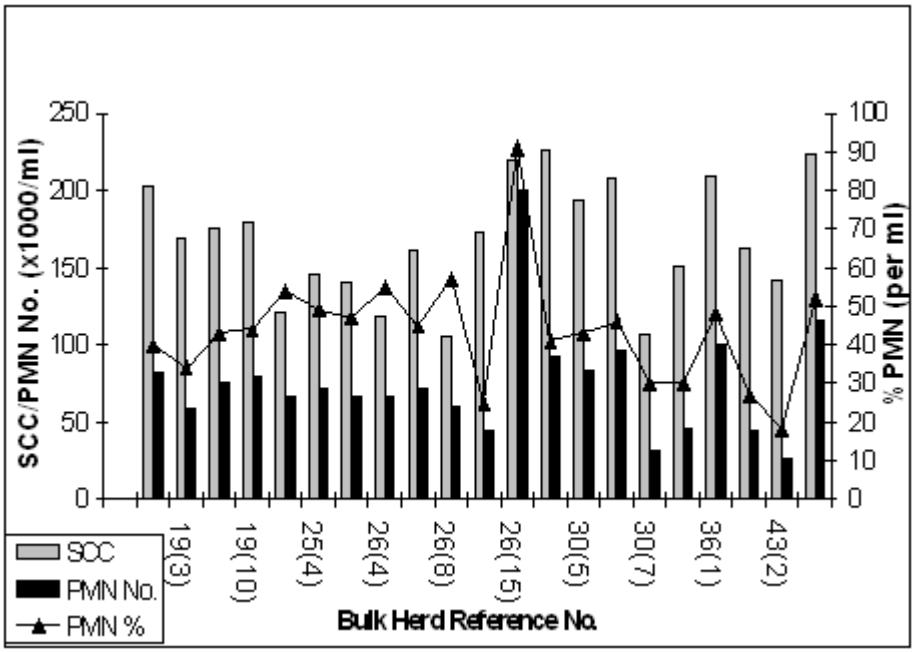


Figure 12: SCC, PMN and PMN as a proportion of total somatic cells within bulk herds with a SCC <250x10^3/ml (n=21)

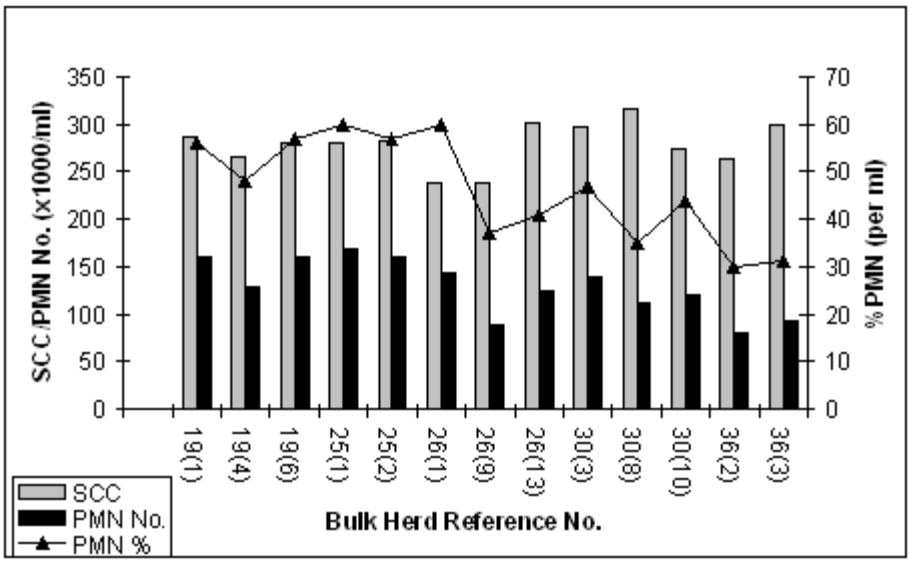


Figure 13: SCC, PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC between 200x10^3/ml and 300x10^3/ml (n=13)

Absolute SCC, PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC between 300x10^3/ml and 450x10^3/ml are shown in Figure 14. The results indicated that there was greater variation in PMN as a proportion of SCC in milks of this cell count range compared to milks of SCC up to 300x10^3/ml.

Absolute SCC, PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC between 500x10^3/ml and 900x10^3/ml are shown in Figure 15. The results indicated that, generally, the PMN level in the milks followed a similar trend to that of SCC level.

Regression analysis of total bulk herd milks (n=49) showed a correlation between SCC and PMN to be equal to 75% and a p value of <0.0001. Correlation between SCC and PMN as a proportion of total somatic cells was not significant. Correlation between PMN and PMN as a proportion of total somatic cells was significant with a p value of <0.0001.

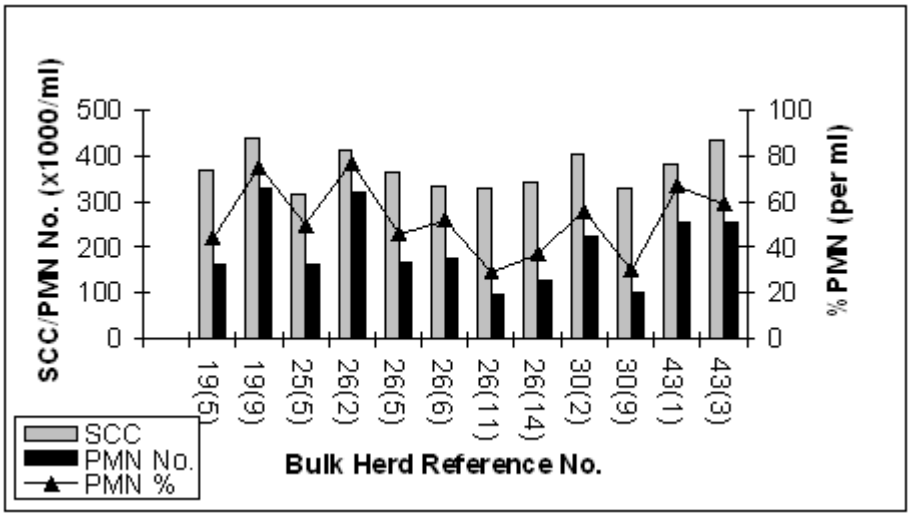


Figure 14: SCC, PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC between 300 x10^3/ml and 450 x10^3/ml (n=12)

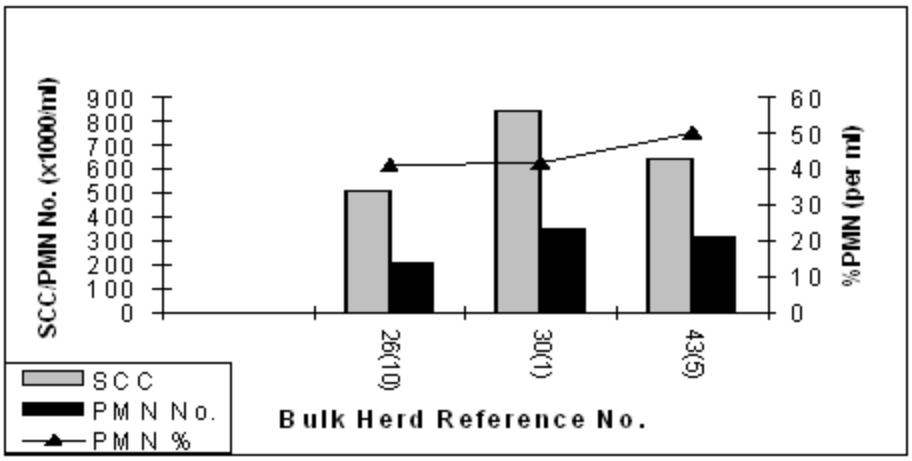


Figure 15: SCC, PMN levels and PMN as a proportion of total somatic cells within the bulk herd milks with an SCC between 500 x10^3/ml and 900 x10^3/ml (n=3)

Discussion

Bulk herd milk samples with an SCC range of <250x10^3/ml generally had between 20% and 60% of cells represented by PMN's. PMN cells accounted for between 30% and 60% of cells in bulk herd milks within the SCC range 200x10^3/ml to 300x10^3/ml. PMN cells accounted for between 30% and 75% of cells in milks of SCC range 300x10^3/ml to 450x10^3/ml. PMN cells accounted for between 40% and 50% of cells in milks of SCC range 500x10^3/ml to 900x10^3/ml. An associated increase in PMN with the increase in SCC was not observed in bulk herd milks. However, such an increase was observed in individual quarter milks. This may be due to the dilution effect of high SCC in bulk herd milk. Thus, the true effect of PMN may not be observed in bulk herd milk but may still have an adverse effect on milk quality.

Conclusions

- The modification to the test of O'Sullivan *et al* (1992) allowed the measurement of PMN levels in milk. Such a measurement may have important implications in future milk quality programmes in terms of selection of infected udder quarters and product quality.
- The strong relationship or correlation between SCC and PMN of 92% in the individual quarter milks has confirmed previous preliminary data. This is important since PMN in conjunction with SCC may now provide a more reliable method of selecting milks for processing.
- The general trend in reduced casein content at high SCC and PMN levels in the current study may have resulted in the trend towards deteriorated milk coagulation properties. A very heterogeneous selection of proteolysis patterns was observed in the miniature cheeses. This substantial difference in proteolytic activity in milk from different quarters had not been observed previously.
- Enzymes associated with the cells in high SCC milk were retained in the cheese curd and thus, contributed to proteolysis during ripening. Addition of low volumes of high SCC milk had an obvious impact on proteolysis patterns and cheese ripening. However, such trends were generally less clear with increasing PMN milk than those

observed for addition of high SCC milk.

- The poor correlation between SCC and PMN obtained in both cow and herd bulk milks, compared to the correlation in quarter milks was probably due to the mixing of high and low SCC milks from either quarters or cows. Thus, the true effect of PMN may not be observed in bulk herd milk but may still have an adverse effect on milk quality.
- Increases in bulk milk SCC and PMN levels may be due to extremely high SCC/PMN milk from a small number of cows being added to milk from a predominantly healthy herd, or to large numbers of cows with sub-clinical infections. Either of those bulk milk SCC scenarios may contribute to variation in the effects of SCC/PMN on dairy products.

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