Effects of Somatic Cell Count on Quality and Shelf-Life of Pasteurized Fluid Milk[1]

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ABSTRACT

Milk was collected from eight Holstein cows four times before and four times after intramammary infection with *Streptococcus agalactiae*. Postinfection milk had significantly higher somatic cell count (SCC) (849,000 cells/ml) than preinfection milk (45,000 cells/ml). High SCC raw milk had more lipolysis and proteolysis than low SCC raw milk. Pasteurized, homogenized, 2% fat milks from pre- and postinfection periods were stored at 5°C and analyzed for lipolysis, proteolysis, microbial quality, and sensory attributes at 1, 7, 14, and 21 d post processing. During refrigerated storage, the average rates of free fatty acid increase (i.e., lipolysis) and casein hydrolysis in high SCC milk were, respectively, three and two times faster than those in low SCC milk. In general, standard plate counts, coliform counts, and psychrotrophic bacterial counts of both the high and low SCC milks remained low (<100,000 cfu/ml) during 5°C storage. Low SCC milk maintained high organoleptic quality for the entire 21-d shelf-life period. However, for high SCC milk, between 14 and 21 d, sensory defects were detected, which resulted in low overall quality ratings. The sensory defects mainly included rancidity and bitterness and were consistent with higher levels of lipolysis and proteolysis. Hence, mastitis adversely affected the quality of pasteurized fluid milk. It is recommended that the fluid milk industry consider implementation of premium quality payment programs for low SCC milks.

(**Key words:** somatic cell counts, pasteurized fluid milk quality, shelf-life study)

Abbreviation key: ADV = acid degree value, **CC** = coliform count, **CN/CP** = casein as a percentage of crude protein, **CN/TP** = casein as a percentage of true protein, **OQR** = overall quality rating, **PBC** = psychrotrophic bacterial count, **SPC** = standard plate count, **TP** = percentage of true protein.

INTRODUCTION

Mastitis is an inflammatory reaction within the udder of a cow, commonly resulting from bacterial infection. Milk from infected cows is characterized by increased raw milk SCC. Almost all of the major and minor components in milk are affected by mastitis (12, 19). Increased proteolytic (35) and lipolytic (3, 4) activities have been reported. The focus of most studies has been on raw milk quality, and only limited work has been conducted to investigate the effect of SCC on quality of pasteurized milk during refrigerated storage (11, 25).

Adverse effects of using high SCC milk for cheese making include reduced curd firmness (21), decreased cheese yield (6, 13, 22), increased fat and casein loss in whey (22), and compromised sensory quality (19). For these reasons, the cheese industry has provided dairy farmers premium quality payments to encourage reduced raw milk SCC.

The fluid milk industry has been reluctant to initiate a similar quality bonus payment program. Compared with research on cheese, there are almost no published quantitative data regarding the impact of raw milk SCC on the quality and shelf life of pasteurized fluid milk. It has been shown (11, 25) that mastitis decreased the shelf life and sensory quality of pasteurized milk, mostly by higher concentrations of FFA. However, the sensory results of these early shelf-life studies of high SCC milk were only based on sensory evaluation by a very small number of dairy judges. In addition, no data on microbial growth in milk samples were presented in these studies (11, 25).

The specific objectives of this study were to determine the effect of raw milk SCC on lipolysis, proteolysis, sensory attributes, and microbiological quality of pasteurized fluid milk during 21 d of shelf life.

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MATERIALS AND METHODS

Experimental Design

Determination of the effect of milk SCC on the sensory quality of milk, separate from other cow to cow, stage of lactation, and cow environmental conditions is a difficult task. The present study attempts to control for cow to cow and stage of lactation influence by using one group of eight cows over a short period of time (i.e., 5 wk). In the preinfection period, four milk collections, twice per week, were carried out. Milk collected in this period had low SCC. After the preinfection period, each cow was infused with the mastitis-causing pathogen *Streptococcus agalactiae*. Milk collection was resumed 1 wk after infection and was collected twice a week for 2 wk during the postinfection period. The raw milks from individual cows were commingled, standardized to 2% fat, pasteurized, homogenized, packaged, and stored at 5°C for each of the 4 sampling d in the pre- and postinfection periods. Milk quality assessments were made on d 1, 7, 14, and 21 after pasteurization.

Cow Selection and Infection Procedures

The procedures of cow selection, culture infusion, and subsequent cow management were similar to those described by Klei et al. (13). Eight Holstein cows from the Cornell Teaching and Research Farm (Ithaca, NY) were selected based on their low milk SCC and absence of mastitis-causing pathogens and off-flavors in their milks. Cows were milked twice a day throughout the study. In the beginning of the study, cows averaged 182 d in lactation (range: 113 to 234 d) and three, two, one, and two cows were in 2nd, 3rd, 4th, and 5th lactation, respectively.

Streptococcus agalactiae (Cornell 48 strain, ATCC 27956) culture (1000 cfu/ml of saline solution) was infused into the right rear and left front quarters of the udder of each cow. Body temperature, milk production, and milk SCC levels were monitored daily for each cow after the infusion.

Immediately after the infusion of *S. agalactiae*, milk SCC of six cows increased to near or above 1 million cells/ml. Clinical signs of mastitis were apparent 2 to 3 d after infusion in six cows. The other two cows showed no signs of mastitis, and milk SCC increased only slightly and then decreased to preinfection levels. Milk collection postinfection was initiated 1 wk after infusion, when milk SCC decreased to between 600,000 and 1 million cells/ml. After two milk collections, milk SCC from all of the eight cows except one dropped below 500,000 cells/ml, and four cows produced milk with SCC <100,000/ml. A second infusion of *S. agalactiae* was administrated to all eight cows to increase milk SCC. After the second infusion, milk SCC increased faster and remained high for a longer time than after the first infusion. Three out of eight cows showed clinical signs of mastitis. One week after the second infusion, another two milk collections were conducted. After the study, all cows were treated with antibiotic to eliminate infection.

Milk Collection and Processing

Milk collection and commingling. All of the milk produced by each cow was collected separately. Freshly collected individual cow milks were immediately transported to the Cornell Food Science Pilot Plant (Ithaca, NY) and cooled in ice water. Subsamples of milk from each cow were preserved with Bornopol tablets (Preservative Systems Limited, San Francisco, CA), and SCC was measured [(2); AOAC method number 17.13.01, 978.26].

The commingled milk had a target SCC of <100,000 cells/ml preinfection and 600,000 to 1,000,000 cells/ml postinfection. In the preinfection period, only individual milks with SCC <100,000/ml were commingled. For the 4 d of milk collections during this period, milks from the same seven of eight cows were used. During the postinfection period, individual milk SCC and milk weights were used to estimate SCC of composite milk prior to commingling to achieve a final SCC level close to 750,000 cells/ml, which is currently the upper legal limit for raw Grade A milk in the US (20).

Milk Processing. Composite raw milk was separated at 5°C into skim and cream with a Delaval Tri-Process cream separator (model 340-A; Delaval, Poughkeepsie, NY) at 1800 rpm. Fat contents of separated cream [(2); AOAC method number 995.18] and skim milk [(17); Method number 15.8] were determined by the Babcock method. Milk was standardized to 2% fat, and the fat content was confirmed after pasteurization using the Mojonnier method [(2); AOAC method number 989.05]. The average fat content for the four processing runs in the preinfection period (2.07 ± 0.035%) was similar to that in the postinfection period (2.05 ± 0.042%).

Raw 2% milk was HTST pasteurized (74°C/34 s) (PMS; Processing Machinery and Supply Co., Philadelphia, PA), two-stage homogenized (Gaulin Model 75E, Everett, MA), and cooled to 5°C. Pasteurization was confirmed by the Phosphatase Test (Scharer Modified Phosphatase Test Model D Kit; Applied Research Institute, Perth Amboy, NJ). All processed milks were phosphatase negative. Efficiency of homogenization was verified by measuring fat globule size distribution (32) using a laser light-scattering particle size analyzer (Malvern MasterSizer E; Worcestershire, United Kingdom). All processed milks were efficiently homogenized,

and no differences in the average volume mean diameter of fat globules were observed between milks processed pre- (0.815 ± 0.025 μm) and postinfection (0.825 ± 0.013 μm).

Pasteurized, homogenized, 2% fat milk was hand sealed in new, commercial, opaque half-gallon plastic milk containers, covered with black plastic bags, and stored at 5°C in a cooler. Prior to filling, milk containers were sanitized, drained, and rinsed twice with pasteurized milk. Opaque containers and black plastic bags were used to prevent light oxidation of milk.

Milk Quality Evaluation

Chemical and microbiological tests. All chemical and microbiological tests were done on each of the commingled raw milks and the pasteurized milks after 1, 7, 14, and 21 d of storage at 5°C. At each of the storage dates, milk from one container was used for all the chemical, microbial, and sensory tests; samples for microbial tests were aseptically drawn first. The other containers from the same processing were left unopened for testing at latter storage days to avoid microbial contamination.

The FFA content [expressed as acid degree value (**ADV**) meq FFA/100 g of fat] was determined by the copper soap method (31). The ADV was used as an index of lipolysis. Total nitrogen (**TN**) [(2); AOAC method number 991.20], nonprotein nitrogen (**NPN**) [(2); AOAC method number 991.21], and noncasein nitrogen (**NCN**) (16) were determined by the Kjeldahl method. All nitrogen results were expressed as a protein equivalent using a conversion factor of 6.38. Calculations for content of true protein (**TP**) and CN were respectively (TN – NPN) × 6.38 and (TN – NCN) × 6.38. Casein as a percentage of TP (**CN/TP**) was calculated as (CN/TP) × 100%. All analyses were performed in duplicate. Casein as a percentage of TP was used as an index of proteolysis. Microbiological tests included standard plate count (**SPC**), coliform count (**CC**), and psychrotrophic bacterial count (**PBC**) [(17); Method numbers 6.2, 7.8, and 8.1, respectively].

Sensory evaluation. A trained panel of 12 individuals evaluated the processed milks. Panelists were trained (six sessions, two per week for 3 wk) to score intensity of various descriptors of aroma, taste, and aftertaste on a scale from 1 to 9 (1 = none to 9 = pronounced). Defect training samples were prepared as described by Bodyfelt et al. (7), using freshly processed Cornell 2% milk as starting material. Panelists were instructed to be as consistent as possible in their intensity ratings throughout the study. The same 12 individuals tasted all of the processed milks during the study.

Descriptors used in this study were selected from those recommended by Bodyfelt et al. (7), because milk with high SCC was not available during training. Efforts were made to select descriptors that had a possible association with expected compositional changes in high SCC milk, such as saltiness, rancidity, and bitterness. Milk samples were evaluated for the following attributes: aroma (7 descriptors), taste (10 descriptors), and aftertaste (4 descriptors), consecutively. Within each attribute group, an "other" descriptor was also added for panelists to provide additional descriptive information on any other characteristics of the milks that were not listed on the ballot. At the end of the evaluation, panelists were asked to score the overall quality of each milk sample on a 10-point scale, where 10 = excellent, 8 to 9 = good, 6 to 7 = fair, and <6 = poor (7).

Milk samples (50 ml in a 5-oz. plastic cup with lid) were presented to each panelist in a closed cardboard box to prevent light oxidation. Samples were tasted at 20°C to make the detection of volatile compounds easier and to improve the discrimination power of the trained panelists. Depending on the week of tasting, panelists had to evaluate one, two, or three milk samples in a session. When there was more than one sample for a session, the order of tasting was randomized within a session. Sample evaluations were directly recorded with Compusense® Five System (Compusense Inc., Guelph, Ontario, Canada). Each panelist was required to register their identification on the computer so that ratings from each panelist could be identified for statistical analysis.

Statistical Models and Analysis

The ANOVA models used for statistical analyses of the lipolysis and proteolysis data and the sensory data are listed in Table 1. Split-plot designs were used for analyses of lipolysis, proteolysis, and sensory data with infection (I) (pre- and post-) as whole plot and storage time (T) as subplot. In the sensory data analysis, the panelist effect was treated as random. The slopes of the regression lines for the changes in lipolysis and proteolysis as a function of days of refrigerated storage were used to compare the rate of change of these parameters during shelf life in pre- versus postinfection periods. Analyses were done using SAS® (27).

RESULTS

Milk SCC and Milk Yield

The SCC of commingled raw whole milks collected in the preinfection period were 42,000, 34,000, 44,000, and 58,000 cells/ml for the 4 d of collection. For the 4

Table 1. The ANOVA models used for data analysis.

Data	Model terms[1]	df	Analyzed as	Error term
Lipolysis and proteolysis	I	1	Whole plot (fixed)	E (a)
	E (a)	6	Whole plot error	
	T	3	Subplot (fixed)	E (b)
	I × T	3	Interaction (fixed)	E (b)
	E (b)	18	Subplot error	
	Model equation: Y = I + E (a) + T + I × T + E (b)			
Descriptive sensory analysis	PN	11	Block (random)	
	I	1	Whole plot (fixed)	PN × I
	PN × I	11	Interaction (random)	
	E (c)	72	Whole plot error	
	T	3	Subplot (fixed)	PN × T
	I × T	3	Interaction (fixed)	PN × I × T
	PN × T	33	Interaction (random)	
	PN × I × T	33	Interaction (random)	
	E (d)	216	Subplot error	
	Model Equation: Y = PN + I + PN × I + E (c) + T + I × T + PN × T + PN × I × T + E (d)			

[1]I = infection (pre and post), E = error, T = time of refrigerated storage, and PN = panelist.

d of collection during the postinfection period, SCC were 771,000, 732,000, 978,000, and 916,000/ml. The mean milk SCC of the milks in the postinfection period (849,000 cells/ml) was significantly higher ($P < 0.05$) than that in the preinfection period (45,000 cells/ml) (Table 2). After infection, the average daily milk weight for the eight cows decreased significantly ($P < 0.05$) from a preinfection level of 39.50 ± 3.64 to 27.76 ± 3.34 kg/d postinfection.

Milk Fat and Lipolysis

Average fat content of commingled fresh raw milk was significantly higher for milk with high SCC (4.04%) than for milk with low SCC (3.43%) (Table 2). However, when considering the much-decreased daily milk output volume, per day synthesis of milk fat was substantially less in the postinfection period (1.22 kg/d) as compared with that in the preinfection period (1.36 kg/d). No difference in fat globule size distributions (i.e., volume mean diameter and d 0.9) was detected between composite raw whole milk of low and high SCC (Table 2). The particle size distribution was typical of raw whole milk (18).

Mean ADV of commingled fresh raw milks was significantly lower ($P < 0.05$) in preinfection milks (0.27 meq/100 g fat) than in postinfection milks (0.43 meq/100 g fat) (Table 2). The ADV changes in pasteurized,

Table 2. Mean composition, somatic cell count (SCC), and particle size distribution for raw milks collected pre- and postinfection.

Parameter	Pre-infection	Post-infection	SEM[1]	LSD[2]
SCC, cells/ml	45,000[b]	849,000[a]	42,613	147,464
Fat, %	3.43[b]	4.04[a]	0.148	0.517
ADV[3], meq FFA/100 g of fat	0.27[b]	0.43[a]	0.043	0.148
Crude protein, %	3.19[b]	3.28[a]	0.019	0.065
True protein, %	2.96[b]	3.08[a]	0.016	0.056
Casein, %	2.44[b]	2.49[a]	0.012	0.040
CN/TP[4], %	82.32[a]	80.75[b]	0.311	1.075
VMD[5], μm	2.91[a]	2.99[a]	0.027	0.099
d(0.9)[6], μm	5.28[a]	5.44[a]	0.059	0.214

[a,b]Means in the same row with no common superscript differ ($P < 0.05$).

[1]SEM = Standard error of mean, calculated as square root (error mean square/replicate) (33).

[2]LSD = Fisher's least significant difference. Critical values to compare means using the LSD test were calculated as [square root (2 × error mean square/replicate)] times the critical Studentized t value for the $\alpha = 0.05$ confidence level (33).

[3]ADV = Acid degree value.

[4]CN/TP = Casein as a percentage of true protein.

[5]VMD = Fat globule volume mean diameter.

[6] d (0.9) = 90% of the volume of fat is contained in fat globules with diameter less than this value.

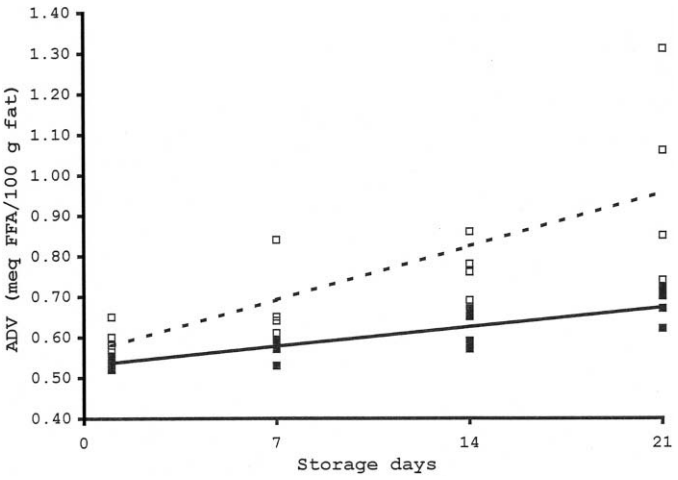

Figure 1. Acid degree value (ADV) increase in pasteurized, homogenized, 2% fat milks collected during pre- (-■-) and postinfection (--□--) periods (4 processing d in each period) and stored at 5°C for 1, 7, 14, and 21 d. The linear regression line predicting ADV increase for milk with low SCC (i.e., preinfection, solid line) was 0.53 + 0.0068X (R^2 = 0.713) and was 0.56 + 0.0189X (R^2 = 0.552) for milk with high SCC (i.e., postinfection, dotted line), where X = days of cold storage.

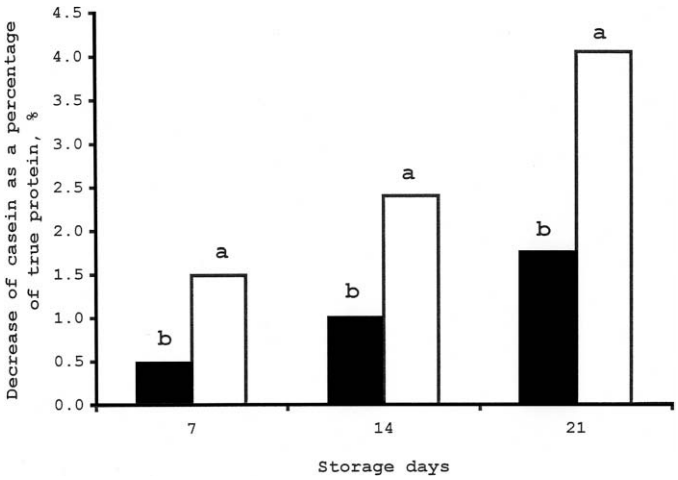

Figure 2. Average decreases in casein as a percentage of true protein (CN/TP) in pasteurized milk during a 21-d refrigerated storage period. Decreases of CN/TP at d 7, 14, and 21 were calculated by subtracting CN/TP at d 7, 14, and 21 from that at d 1, respectively, for milk from both pre- (filled bar) and postinfection (empty bar) periods. Means within the same category (i.e., d 7, 14, and 21) with no common letters differ.

homogenized, 2% fat milks during storage for 21 d at 5°C for the pre- and postinfection periods are shown in Figure 1. There was a significant influence ($P < 0.05$) of infection and time of refrigerated storage on milk lipolysis (i.e., ADV) and a tendency ($P = 0.063$) for an interaction of infection and time (Table 3). The linear regression equation predicting ADV increase for milk with low SCC was 0.53 + 0.0068X (R^2 = 0.713) and was 0.56 + 0.0189X (R^2 = 0.552) for milk with high SCC (where X = days of cold storage). The rate of ADV increase in high SCC milk (i.e., postinfection) was significantly higher ($P < 0.05$) than that in low SCC milk (i.e., preinfection).

Table 3. Sum squares (SS) probabilities (*P*) for lipolysis and proteolysis analysis for pasteurized, homogenized, 2% fat milk stored at 5°C for 21 d.

	Lipolysis		Proteolysis	
Factors[1]	SS	*P*	SS	*P*
Whole-plot factor				
I	0.2016	0.016	43.92	0.001
Error (a)	0.1080		7.80	
Subplot factor				
T	0.3066	<0.001	35.37	<0.001
I * T	0.0720	0.063	5.61	<0.001
Error (b)	0.1494		1.39	
R^2	0.69		0.90	

[1]I = infection (pre- and post-), T = time of refrigerated storage of pasteurized, homogenized, 2% fat milk, and R^2 = (sum square of all main and interaction effects)/(total sum square).

Milk Protein and Proteolysis

Percentages of crude protein, TP, and CN in commingled raw milk collected postinfection were significantly higher than in the preinfection period (Table 2). However, when taking into account the 30% decrease in average daily milk volume, per day output of these components, in kilograms, were substantially less in the postinfection period as compared with the preinfeciton period. Average daily output of crude protein, TP, and CN, respectively, decreased from 1.26, 1.17, 0.96 kg/d in the preinfection period to 0.91, 0.86, and 0.69 kg/d in the postinfection period. In addition, CN/TP was significantly ($P < 0.05$) lower in the postinfection period (Table 2).

Pasteurization causes heat denaturation of serum proteins. Some of the serum proteins bind to casein micelles during heating and increase observed CN/TP. After pasteurization, the extent of CN/TP increase was not significantly different ($P > 0.05$) between low (2.89% increase) and high (3.31% increase) SCC milks, suggesting comparable heat treatment conditions. Because of a lower level of CN/TP in the high SCC raw milk (Table 2), pasteurized high SCC milk also had a significantly ($P < 0.05$) lower level of CN/TP (84.06%) than pasteurized low SCC milk (85.21%).

Decrease of CN/TP during 21 d of refrigerated storage in pasteurized milks is shown in Figure 2. There was a significant effect ($P < 0.001$) of infection and storage time on milk proteolysis (i.e., casein breakdown) (Table 3). The interaction between infection and time was also

significant ($P < 0.001$) (Table 3). The linear regression equation predicting the decrease of CN/TP for low SCC milk was 85.34 – 0.086X ($R^2 = 0.684$), whereas that for high SCC milk was 84.20 – 0.194X ($R^2 = 0.837$) (where X = days of refrigerated storage). During postpasteurization cold storage, casein hydrolysis occurred in milks of both high and low SCC. However, the rate of CN/TP decrease in high SCC milks was significantly greater ($P < 0.01$) than that in the low SCC milks. At each sampling time during storage, proteolysis was higher in high SCC milk (Figure 2).

Descriptive Sensory Analysis

Aroma, taste, and aftertaste. Comparison of aroma, taste, and aftertaste attributes in pasteurized milk with high or low SCC at each storage time (Tables 4 and 5) provided the following observations. Up to 14 d of cold storage, no obvious sensory defects were detected by panelists in milks of either low or high SCC (Table 5). Ratings for most of the attributes were similar across days and between samples (Table 5). Therefore, panelists were not able to detect significant differences in the flavor attributes between low and high SCC milks. One exception was cooked aroma, which was slightly higher in low SCC milk than high SCC milk throughout the shelf-life study. Significant differences in cooked aroma were detected at 7 and 14 d of storage. It is unclear why high SCC had lower cooked aroma scores.

The organoleptic quality of low SCC milk remained high during cold storage, even 21 d after pasteurization (Table 5). Ratings for all of the defect-oriented terms, such as rancid and bitter, remained low and did not change significantly ($P > 0.05$) within the 21 d shelf-life period for the low SCC milk (i.e., preinfection) (Table 5).

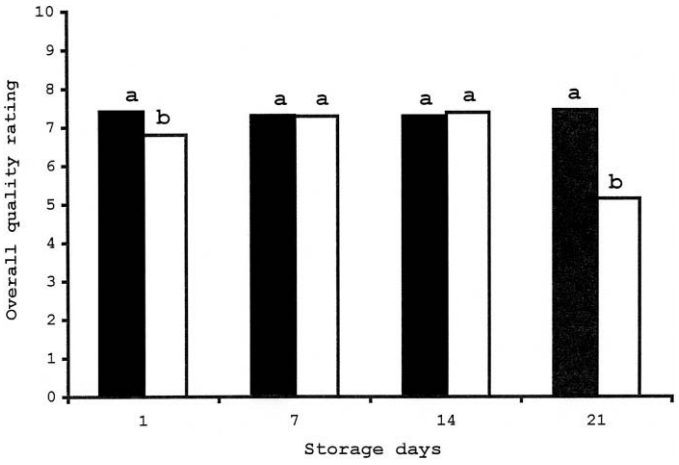

Figure 3. Mean overall quality rating for pasteurized, homogenized, 2% fat milk collected during pre- (filled bar) and postinfection (empty bar) periods and stored at 5°C for 1, 7, 14, and 21 d. Mean sensory scores within the same storage day with no common letters differ. The LSD (one-sided; $\alpha = 0.05$) used for mean separation is 0.520 (33).

Several defects were perceived by the panelists for d 21 high SCC milks. These defects included rancid, oxidized, and fruity aroma; salty, rancid, bitter, and astringent taste; and bitter and lingering aftertaste (Table 5). Ratings for such defect-oriented descriptors were significantly higher for high SCC milks than low SCC milks at d 21 (Table 5). Among all these defect-oriented descriptors, the extent of average rating increase was highest for rancid aroma and taste, followed by bitter and astringent taste and bitter aftertaste (Table 5). Panelists indicated that rancidity was the predominant flavor defect perceived in d 21 high SCC milks. These results are consistent with the observed increases in lipolysis and proteolysis in the high SCC milks. Although the defects were detected on d 21 after pasteurization, the exact day between 14 and 21 d when significant differences could be detected was not determined. Ratings for microbial-induced flavor defects, such as sour, malty, and fruity, were low for both high and low SCC milks throughout the 21 d shelf life, except for the d 21 high SCC milk, which showed a higher average score for fruity aroma (Table 5).

Overall quality. Overall quality ratings (**OQR**) for low SCC milk were high throughout the study (Figure 3). The average OQR were 7.4, 7.3, 7.3, and 7.4 on d 1, 7, 14, and 21, respectively. For milk with high SCC, at d 1, average OQR was 6.8, significantly lower ($P < 0.05$) than that of low SCC milk (Figure 3). Several panelists noted that the d 1 milk samples smelled "different" as compared with normal milk. Comments such as "without normal milk aroma," "lacks freshness," "unclean," and "less sweet than normal milk" were made by several panelists. Average OQR of high SCC milk were similar to those of low SCC milk at 7 and 14 d of storage (Figure 3). At d 21, average OQR of high SCC milk decreased significantly to 5.15, which was in the range of poor flavor quality (Figure 3), while OQR of low SCC milk remained unchanged. The decrease in OQR for high SCC milk is related to increased ratings of defect-oriented descriptors, especially rancidity and bitterness (Table 5).

Microbial Tests

The bacterial counts for all of the composite raw whole milks collected in both the pre- and postinfection periods were quite low (i.e., <12,000 cfu/ml) (Table 6). The microbiological quality of the pasteurized, homogenized, 2% fat milk is shown in Table 7. Coliform counts were <1 cfu/ml for all of the milk samples except one d

Table 4. The ANOVA for infection, day, and infection by day interaction effects on the aroma, taste, and aftertaste characteristics of 2% fat, pasteurized, homogenized milk from either pre- or postinfection period.

	Model[1]		Infection			Day			Interaction		
Descriptor	SSM	R^2	SSF	*F*	*P*	SSD	*F*	*P*	SSI	*F*	*P*
Aroma											
Cooked	299.3	0.66	9.09	5.59	*	9.14	3.25	*	0.17	0.09	NS
Rancid	91.4	0.37	9.58	19.26	***	24.18	22.05	***	24.28	23.50	***
Oxidized	210.1	0.79	2.45	2.37	NS[2]	2.92	3.77	*	1.96	4.85	**
Fruity	36.9	0.46	1.98	5.16	*	2.94	3.51	*	3.28	3.58	*
Malty	19.1	0.40	0.14	0.54	NS	0.05	0.13	NS	0.22	0.54	NS
Sour	14.0	0.39	0.34	4.17	NS	0.62	2.65	NS	0.65	1.83	NS
Feedy	99.0	0.49	1.37	2.82	NS	2.97	3.60	*	0.63	0.50	NS
Taste											
Sweet	81.2	0.60	0.10	0.37	NS	0.41	0.49	NS	0.27	0.52	NS
Salty	21.8	0.42	0.44	2.75	NS	0.73	2.01	NS	1.64	4.14	*
Cooked	289.2	0.78	3.28	2.35	NS	1.00	0.56	NS	0.18	0.10	NS
Rancid	117.2	0.38	14.68	33.20	***	35.54	35.55	***	35.90	33.03	***
Oxidized	242.4	0.78	0.04	0.05	NS	0.20	0.22	NS	0.70	1.10	NS
Sour	17.5	0.40	0.21	2.56	NS	1.57	3.67	*	0.42	1.52	NS
Malty	23.1	0.41	0.07	0.50	NS	0.20	0.55	NS	0.16	0.39	NS
Feedy	97.8	0.58	1.46	3.13	NS	0.90	1.41	NS	1.60	1.23	NS
Bitter	64.3	0.43	4.00	7.28	*	8.11	7.30	***	10.14	8.34	***
Astringent	71.2	0.47	1.15	1.50	NS	5.89	5.27	**	6.94	6.29	**
Aftertaste											
Bitter	66.3	0.48	3.14	7.34	*	5.87	4.53	***	7.54	6.91	***
Oxidized	163.2	0.73	0.33	0.41	NS	0.01	0.02	NS	1.00	2.30	NS
Drying	218.0	0.57	0.01	0.01	NS	6.76	4.47	**	5.84	2.40	NS
Lingering	395.6	0.59	4.17	0.93	NS	11.36	3.18	*	10.88	3.48	*
Overall quality	622.2	0.47	48.88	25.80	***	67.38	20.09	***	88.40	14.41	***

[1]SSM = Model sum squares; R^2 = SSM/SST = coefficient of multiple determination; SST = total sum squares (33); SSF, SSD, and SSI = mean squares for infection, day, and infection by day interaction; and *F* = statistic for *F*-test.

[2]NS = not significant at $P > 0.05$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

21 sample in the postinfection period (processing day 3). The SPC and PBC were all below 10^6 cfu/ml except two d 21 samples, one in the preinfection period (processing day 2) and the other one in the postinfection period (processing day 3). The d 7 and 14 samples in the preinfection period for processing day 2 also had elevated bacterial counts.

DISCUSSION

Quality of Fresh Raw Milk

During mastitis, the net synthesis of casein and fat in the mammary gland is generally decreased (12). Our results indicated increases in the percentages of fat, crude protein, total protein, and casein in postinfection milks. These increases were caused by a much greater decrease in milk volume (about 30%) after infection than in per day synthesis of these components.

Mean ADV of fresh raw commingled milks collected pre- and postinfection (Table 2) were relatively low (0.27 and 0.43 meq/100 g fat) in our study, mainly because of rapid cooling and careful milk handling procedures used prior to pasteurization. In practice, average ADV for farm bulk tank milks for every other day pickup are in the range of 0.80 to 1.20 meq/100 g of fat (5, 8). The average ADV for high SCC milk was about 1.6 times that of low SCC milk (Table 2). This finding is in agreement with previous reports (23, 34) that indicated that when cows had mastitis, concentrations of FFA in fresh raw milk increased.

After infection, CN/TP was lower than before infection, as observed in previous studies (6, 13). The enzyme that is primarily responsible for this proteolysis is plasmin. Plasmin survives pasteurization (24) and, thus, we expect proteolysis to continue in milk after pasteurization. Relatively low bacterial counts for all of the fresh commingled raw milks (Table 6) suggested minimum proteolysis by enzymes of microbial origin occurred. Therefore, higher ADV and lower CN/TP in raw high SCC milks were likely a consequence of milk compositional changes associated with mastitis.

Quality and Shelf Life of Pasteurized Milk

Heat treatment. After HTST pasteurization, measured casein as a percentage of crude protein increases,

Table 5. Mean scores for aroma, taste, and aftertaste characteristics of 2% fat, pasteurized, homogenized milk stored at 5°C for 1, 7, 14, and 21 d.[1]

Attribute and descriptors	Days of Storage								
	Day 1		Day 7		Day 14		Day 21		
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	LSD[2]
Aroma									
Cooked	2.30	2.04	1.98[a]	1.65[b]	2.11[a]	1.75[b]	1.91	1.64	0.331
Rancid	1.08	1.10	1.04	1.12	1.08	1.06	1.07[b]	2.25[a]	0.245
Oxidized	1.22	1.31	1.26	1.29	1.24	1.36	1.28[b]	1.68[a]	0.153
Fruity	1.05	1.06	1.03	1.10	1.02	1.06	1.02[b]	1.44[a]	0.230
Malty	1.15	1.12	1.19	1.08	1.11	1.12	1.12	1.10	. . .
Sour	1.12	1.12	1.04	1.11	1.10	1.07	1.09	1.26	. . .
Feedy	1.58	1.44	1.54	1.30	1.45	1.42	1.30	1.23	. . .
Taste									
Sweet	2.08	2.09	2.18	2.08	2.16	2.19	2.13	2.07	. . .
Salty	1.15	1.27	1.27	1.24	1.25	1.17	1.19[b]	1.45[a]	0.151
Cooked	2.02	1.91	1.96	1.75	1.93	1.72	1.96	1.76	. . .
Rancid	1.06	1.09	1.03	1.11	1.06	1.07	1.05[b]	2.50[a]	0.209
Oxidized	1.36	1.40	1.45	1.37	1.42	1.38	1.37	1.51	. . .
Sour	1.14	1.19	1.07	1.09	1.12	1.09	1.17	1.32	. . .
Malty	1.09	1.10	1.12	1.10	1.11	1.14	1.11	1.20	. . .
Feedy	1.39	1.39	1.63	1.29	1.51	1.43	1.39	1.32	. . .
Bitter	1.17	1.19	1.09	1.10	1.12	1.07	1.32[b]	1.84[a]	0.265
Astringent	1.23	1.29	1.19	1.15	1.39	1.25	1.23[b]	1.79[a]	0.253
Aftertaste									
Bitter	1.25	1.21	1.11	1.19	1.16	1.18	1.13[b]	1.79[a]	0.252
Oxidized	1.41	1.37	1.45	1.30	1.46	1.30	1.33	1.43	. . .
Drying	1.80	1.75	1.78	1.62	1.90	1.67	1.85	2.25	. . .
Lingering	2.08	2.21	2.04	2.10	2.09	1.97	2.08[b]	2.85[a]	0.426

[a,b]Means in the same row within the same day group with no common superscript differ ($P < 0.05$).

[1]Milk was collected either pre- or postinfection.

[2]LSD = Fisher's least significant difference. Critical values to compare means using the LSD test (33) at $\alpha = 0.05$ (two-sided test).

because of heat denaturation of milk serum proteins. Proteins such as β-LG can form disulfide bonds with free sulfhydryl groups of κ-CN at the surface of CN micelles. These serum proteins are retained with CN precipitation in the test to measure CN and cause an overestimation of CN (16). Measurement of CN as a percentage of CP (**CN/CP**) before and after pasteurization provides a good index of heat treatment. The magnitude of apparent CN/CP increase depends on the severity of the heat treatment. To determine whether our pilot scale heat treatment (74°C/34 s) was comparable with commercial conditions, we compared CN/CP values in pre- and postpasteurized milk samples obtained from 17 fluid milk-processing plants in New York State. Temperature and time combinations used for commercial pasteurization varied from plant to plant. Typical pasteurization conditions were 76 to 80°C for 20 to 30 s. Increases of CN/CP under these conditions ranged from 2.81 to 5.56% with an average of 3.79 ± 0.76%. In this study, the average increases of CN/CP resulting from pasteurization in low (2.83 ± 0.17%) and high SCC (3.04 ± 0.34%) milk were not significantly different ($P > 0.05$). Therefore, we presume that the pasteurization conditions used in this study gave similar heat denaturation in low and high SCC milks, and these conditions were also comparable with those in commercial fluid milk plants.

Table 6. Standard plate count (SPC), coliform count (CC), and psychrotrophic bacterial count (PBC) (expressed as cfu/ml) of fresh raw milk.

Processing day	Method	Preinfection	Postinfection
1	SPC	MD[1]	200 (E)[2]
	CC	MD	<1
	PBC	MD	<100 (E)
2	SPC	12,000	400 (E)
	CC	310 (E)	<1
	PBC	9700	700 (E)
3	SPC	5000	200 (E)
	CC	63	7 (E)
	PBC	2600	300 (E)
4	SPC	5800	1400 (E)
	CC	550 (E)	44
	PBC	3800	600 (E)

[1]MD = Missing data.

[2]E = Estimated.

Lipolysis. Compared with pasteurized low SCC milk, pasteurized high SCC milk was more susceptible to

Table 7. Standard plate count (SPC), coliform count (CC), and psychrotrophic bacterial count (PBC) (expressed as cfu/ml) of pasteurized, homogenized, 2% fat milk collected at 4 d during pre- and postinfection periods and stored at 5°C for 1, 7, 14 and 21 d.

		Preinfection				Postinfection			
PD[1]	Method	d 1	d 7	d 14	d 21	d 1	d 7	d 14	d 21
1	SPC	440	400	100 (E)[2]	330	5 (E)	5 (E)	14 (E)	350
	CC	<1	<1	<1	<1	<1	<1	<1	<1
	PBC	<1	<10 (E)	14,000	400	<1	5 (E)	13,000	290,000 (E)
2	SPC	4 (E)	97,000	35,000	3,500,000	7 (E)	10 (E)	13 (E)	9 (E)
	CC	<1	<1	<1	<1	<1	<1	<1	<1
	PBC	MD[3]	>60,000 (E)	31,000	4,400,000	<1	1 (E)	2 (E)	44
3	SPC	5 (E)	40 (E)	80 (E)	5200	2 (E)	14,000 (E)	<10 (E)	>60,000,000 (E)
	CC	<1	<1	<1	<1	<1	<1	<1	>6000 (E)
	PBC	10 (E)	<10	80 (E)	5700	2 (E)	18,000 (E)	<10 (E)	>60,000,000 (E)
4	SPC	4 (E)	<10 (E)	30 (E)	<100	13 (E)	6 (E)	10 (E)	32
	CC	<1	<1	<1	<1	<1	<1	<1	<1
	PBC	<1	<10 (E)	30 (E)	<100	<1	2 (E)	3 (E)	10 (E)

[1]PD = processing day.
[2]E = Estimated.
[3]MD = Missing data.

lipolysis during refrigerated storage. Continued lipolysis (Figure 1) in pasteurized milk during cold storage indicated that milk lipases survived pasteurization. Shipe and Senyk (30) reported that sufficient lipase activity remains after minimum pasteurization (72°C/16 s) and can induce rancidity during cold storage of pasteurized milk. They suggested that more severe treatment (79°C/20 s) was needed to completely inactivate lipoprotein lipases. For low SCC milks, at d 21, the average ADV was 0.68 meq/100 g fat, which was a 25% increase from the d-1 level. In the high SCC milks, the average ADV was about 1.00 meq/100 g fat at d 21, a 65% increase from the d-1 level.

Rancidity has been associated with elevated levels of FFA in milk (31). A sensory threshold of about 1.0 meq/100 g fat has been previously reported (7, 9). Our current results are in general agreement. At d 21, when the average ADV of high SCC milks approached the 1.0 meq/100 g fat threshold, ratings of rancid aroma and taste increased significantly (Table 5). Rancid off-flavor has also been described as having a soapy, bitter, unclean taste and lingering aftertaste (7, 29). Increased lipolysis may have also contributed to higher ratings for bitter and lingering aftertastes in high SCC milks at d 21 (Table 6). Bitterness has been associated with high levels of short-chain fatty acids (7, 29).

In the present study, milk handling and processing procedures were quite conservative with respect to minimizing ADV increase in raw milk. In addition, storage temperature was also well controlled at 5°C. However, milk with high SCC still developed rancid off-flavor between d 14 and 21. Milk rancidity has been described as a major quality defect associated with market milk (5). Our results suggest that if milk of low SCC is used, and careful milk handling procedures are implemented, it would be possible to extend the refrigerated shelf life of fluid milk products without developing rancid off-flavors. However, if high SCC milk is used, although FFA concentration is low in raw milk or freshly pasteurized milk, because of high levels of lipolysis, ADV can increase substantially during postpasteurization cold storage, resulting in rancid off-flavor and decreased shelf life of milk.

Proteolysis. During refrigerated storage, more extensive CN degradation occurred in the high SCC milk than in low SCC milk. For high SCC milk samples, compared with d-1 level, CN/TP decreased by an average of 4.04% after 21 d of cold storage. Actual levels of CN degradation were probably even higher because the Kjeldahl method for nitrogen analysis may underestimate the extent of proteolysis (36).

Decreased CN/TP during cold storage of milk, especially in high SCC milk, indicated that significant levels of proteolytic activity remained after pasteurization. High SCC raw milk has been shown to have high concentrations of plasmin (28), plasminogen (28), and proteases of somatic cell origin (36). Plasmin is heat stable with large percentages surviving minimum pasteurization (72°C/15 s) (24). Even after UHT treatment, 30 to 40% of plasmin activity can still remain (1).

Other researchers have indicated that extensive proteolysis in milk can result in the accumulation of small hydrophobic peptides, causing bitterness (26) and astringency (15). The high SCC milk had significantly higher scores for bitter and astringent flavors at 21 d than low SCC milks. Although bitterness is rarely a problem associated with pasteurized milk, it can be a problem when levels of heat-resistant plasmin are high, as is the case during mastitis (26). Proteolysis will be

a more significant problem in extended shelf life of refrigerated fluid milks.

Overall sensory quality. The slight increase of OQR for high SCC milk after 7 d of storage relative to d 1 (Figure 3) suggested that the description of "smelled different from normal milk" in d 1 high SCC milk was caused by some volatile substances, which were readily perceived in milk immediately after processing but dissipated during storage. The nature of these volatile substances is unknown.

For the d 21 high SCC milk, the decrease in OQR was correlated with increased scores for defect-oriented descriptors, such as "rancid," "bitter,", and "astringent." Lipolysis was probably the most important factor that contributed to lower sensory quality in high SCC milk. The sensory results from the present study were in general agreement with other researchers, who have also shown that pasteurized milk with high SCC has shorter shelf life and poorer flavor quality (11, 25). Compared with previous research (11, 25), the descriptive panel used in this study not only yielded information on overall milk quality, but individual defects were also identified, quantified, and statistically analyzed. Our results indicated that milk SCC <1 million/ml can have a significant negative impact on milk quality and shelf life.

Microbiological quality. No correlation between microbial counts in pasteurized milk and raw milk SCC was observed in the present study. Postpasteurization contamination was likely responsible for trial-to-trial variations observed in microbial numbers (Table 7). In the present study, sanitized plastic containers were filled with pasteurized milk, which were then hand sealed. Thus, it was possible that a particular container was contaminated. At each storage date, a different container of milk was used for all the tests (chemical, microbial, and sensory). Individual container contamination could explain the low counts for the d 14 sample as compared with the microbial counts observed in the d 7 sample for the postinfection processing d 3 (Table 7).

Growth of psychrotrophic bacteria after pasteurization can be an important factor contributing to milk lipolysis and proteolysis (10). However, enzymes of microbial origin are usually not significant unless bacterial counts exceed 10^6 or 10^7 cfu/ml (10). The bacterial loads of most milk samples in our study were low throughout the 21-d shelf life (Table 7).

The relatively high bacterial counts in the d 21 sample of postinfection processing d 3 complicated interpretation of the data. This milk sample had very high SPC, CC, and PBC at 21 d of storage. Quite possibly, milk quality deteriorated because of the growth of microorganisms, especially psychrotrophs (10) and coliform bacteria (14). This sample also had the highest ADV (1.31 meq/100 g fat) among all of the other milk samples tested. Its scores for rancid aroma (4.43), rancid taste (5.06), and bitter taste (2.84) were also the highest, whereas its OQR was the lowest (2.4). However, the extent of CN hydrolysis does not seem to be significantly affected by the high microbial counts in this sample. Casein as a percentage of true protein was 79.40% for this sample, which was similar to the average value (80.02 ± 0.865%) of d 21 milks collected postinfection.

Milk quality decreased in d 21 milk of postinfection on processing d 4, although it had minimal microbial counts (Table 7). For this sample, high ADV (1.06 meq/100 g fat) and high ratings for rancid aroma (2.47), rancid taste (2.84), and bitter taste (2.19) were observed. The OQR was also low (4.58).

In addition to the above observation, we also repeated the statistical analysis of the sensory data excluding those data from postinfection on processing d 3. High SCC milks again had significantly ($P < 0.05$) higher scores for rancid aroma and taste, bitter taste and aftertaste, astringent taste, and lingering aftertaste at d 21. The new statistical analysis showed that the average score for fruity aroma in d 21 high SCC milks was 1.10, which was not significantly different from that of low SCC milks (1.02). This finding suggested that the high bacterial counts in the d 21 sample from postinfection on processing d 3 substantially increased fruity aroma score, which is generally associated with high PBC. Therefore, deterioration of the flavor quality and shelf life of pasteurized high SCC milk was mainly caused by changes associated with mastitis. The presence of high bacterial counts can certainly aggravate the adverse effects that are associated with high milk SCC.

CONCLUSIONS

Mastitis significantly elevated raw milk SCC. Raw milk quality was lower (i.e., significantly higher lipolysis and proteolysis) in high SCC milk than in low SCC milk. Mastitis also adversely affected the quality of pasteurized milk and decreased its shelf life. During postpasteurization cold storage, the rates of FFA increase and CN hydrolysis were significantly higher in high SCC milk than low SCC milk. The organoleptic quality of low SCC milk remained high even 21 d after processing under refrigerated storage. However, 21-d-old pasteurized, high SCC milk showed significant sensory defects, which included rancidity, bitterness, and astringency. The decrease in sensory quality of high SCC milk occurred between d 14 and 21 and was probably caused by high levels of lipolytic and proteolytic enzymes. Quality premium payment programs based on milk SCC in the fluid milk industry are recommended to improve fluid milk quality.

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