

# Kansas Agricultural Experiment Station Research Reports

Volume 0  
Issue 2 *Dairy Research (1984-2014)*

Article 301

2001

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### Recommended Citation

Zimmerman, S.; Jeon, I.J.; McVay, L.; and Ferdinand, E. (2001) "Bacterial degradation of milk components is affected by storage temperature and time," *Kansas Agricultural Experiment Station Research Reports*: Vol. 0: Iss. 2. <https://doi.org/10.4148/2378-5977.3226>

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# Bacterial degradation of milk components is affected by storage temperature and time

## Abstract

Raw milk is an excellent medium for bacterial growth. The objective of this study was to evaluate the number of microbes and component degradation in raw milk. Milk fat content did not affect bacteria counts. As storage temperature or time increased, greater numbers of bacteria were present. In this study, milk protein was degraded preferentially over lactose or milk fat. As the milk storage temperature increased from 39 to 45°F, protein degradation became more pronounced. Milk fat remained relatively stable, though some degradation products were observed, especially after 4 days of storage at 39°F. Both milk fat and protein degradation can produce small, volatile compounds that negatively affect the flavor and odor of milk. Thus, to maintain high quality fluid milk in the market, milk must be available to the consumer soon after its processing.; Dairy Day, 2001, Kansas State University, Manhattan, KS, 2001;

## Keywords

Dairy Day, 2001; Kansas Agricultural Experiment Station contribution; no. 02-133-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 881; Dairy; Raw milk quality; Proteolysis; Lipolysis

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## **BACTERIAL DEGRADATION OF MILK COMPONENTS IS AFFECTED BY STORAGE TEMPERATURE AND TIME**

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### **Summary**

Raw milk is an excellent medium for bacterial growth. The objective of this study was to evaluate the number of microbes and component degradation in raw milk. Milk fat content did not affect bacteria counts. As storage temperature or time increased, greater numbers of bacteria were present. In this study, milk protein was degraded preferentially over lactose or milk fat. As the milk storage temperature increased from 39 to 45°F, protein degradation became more pronounced. Milk fat remained relatively stable, though some degradation products were observed, especially after 4 days of storage at 39°F. Both milk fat and protein degradation can produce small, volatile compounds that negatively affect the flavor and odor of milk. Thus, to maintain high quality fluid milk in the market, milk must be available to the consumer soon after its processing.

(Key Words: Raw Milk Quality, Proteolysis, Lipolysis.)

### **Introduction**

Milk production and processing facilities have become fewer in number and larger in size. These changes have forced raw milk to be transported further before processing and have prolonged the time until milk is consumed. Refrigerated conditions are mandated from on-farm milk storage until retail purchase, but microorganisms are able to replicate in both raw and pasteurized products that are refrigerated.

For many years, one of the greatest concerns of poor milk flavor quality was

“acid” flavors. These acid flavors were the result of lactic acid bacteria that degraded lactose, eventually producing lactic acid. The “soured” milk could be smelled, tasted, and sometimes seen as clotted milk. Refrigeration has minimized the growth of lactic acid bacteria, but enhanced growth of microbes that tolerate colder temperatures. These cold-tolerant microbes (psychrotrophs) grow in raw and pasteurized milk, producing various enzymes and by-products that cause milk to have an off-flavor or odor at the processing facility or the consumers’ home. Generally, these enzymes do not act on lactose to produce acid, but rather they act on fats and proteins, producing other compounds that generate off-flavors that may be just as undesirable as “sour milk.” Thus, this study was undertaken to monitor the number of microbes and component degradation products in raw milk stored at 39° or 45°F for 1 week.

### **Procedures**

Raw milk was obtained from the Kansas Dairy Research and Teaching Facility in Manhattan, KS. Two different milk samples were obtained, milk from a select group of cows that produced high fat milk; and milk from a group of cows that produced milk with normal fat percentages. Immediately after milking, milk was transferred to the K-State Dairy Processing Facility, filtered, sampled, then divided into whirl-pack bags, and placed at 39 or 45°F. Samples were removed for analyses every 2 days for up to 8 days.

Milk samples were analyzed for compositional analyses, total plate counts, psychrotrophic counts, pH, titratable acidity, proteol-

ysis, and acid degree value following published, standardized methods. Compositional analyses were made to confirm the difference in milk composition and these tests were completed on day 1 only. Bacteria counts were monitored throughout storage. Total plate counts (TPC) were used as a quality index for fluid milk and as a decision tool for accepting raw milk into a fluid processing plant. Raw milk is not accepted into the fluid milk processing facility if TPC are  $>100,000$  cfu/ml for a single producer and  $300,000$  cfu/ml for commingled milk. Psychrotroph counts provided an indication of the shelf life of pasteurized milk. Generally when counts were close to  $1,000,000$  cfu/ml, the milk has reached the end of its shelf life. Although the psychrotrophic bacteria are not considered to be harmful, their various enzymes catalyze the degradation of milk fat, protein, and lactose to such an extent as to render the milk to be "poor quality."

Throughout storage, titratable acidity (TA %) and pH were measured as an indication of lactose degradation. Proteolysis was monitored to determine if the protein was being degraded in the milk and acid degree value was measured to determine the extent of fat degradation in the milk. Because the milk was refrigerated, the lactose, protein, and fat degradation resulted from enzymes associated with the metabolic activities of the bacteria in the milk.

## Results and Discussion

Table 1 shows the overall average composition and somatic cell counts of the two milk samples of different fat content (high vs. normal). Lactose contents of the two milk samples were similar as were protein contents. Higher fat content might provide greater amounts of substrate for lipolytic enzymes excreted from the bacteria. Somatic cell counts indicated that the normal fat milk sample had much higher SCC than did the higher fat milk sample.

Data for microbial counts of the two milk samples stored at the two temperatures are

shown in Tables 2 and 3. Both milk samples stored at the higher temperature ( $45^{\circ}\text{F}$ ) had greater bacteria counts than those stored at  $39^{\circ}\text{F}$ . The composition of milk did not seem to affect the microbial growth. Because single-herd, raw milk with  $>100,000$  cfu/ml is not accepted into a fluid milk plant, milk stored at  $45^{\circ}\text{F}$  would not be accepted on or after day 2. However, the milks stored at  $39^{\circ}\text{F}$  would have been accepted on day 2, but not on day 4 using the TPC standard only. The psychrotrophic bacteria counts showed similar trends -- higher counts for both milk samples stored at the higher temperature. However, a sharp decrease in counts was observed on day 8 for both milk samples and both storage temperatures. Overall, bacteria counts of the  $45^{\circ}\text{F}$  milk samples on day 4 and the  $39^{\circ}\text{F}$  milk samples on day 6 exceed the bacterial limits for even manufactured grade milk.

Data of the component degradation analyses showed that storage temperature and time affected the rate of biochemical reactions. Proteolysis results (Table 4) indicated that the milk stored at  $45^{\circ}\text{F}$  had almost twice the amount of protein breakdown products than milk stored at  $39^{\circ}\text{F}$  on day 8, with proteolysis starting to increase sharply by day 6. However, acid degree value data (ADV; Table 5) indicated that lipolysis or lipid degradation occurred at a faster rate in milk stored at  $39^{\circ}\text{F}$  than that stored at  $45^{\circ}\text{F}$ . Generally, an ADV  $>0.7$  is an indication of lipid breakdown. Although milk did not reach that threshold during this study, the trend showed that lipid degradation did occur during the storage of these raw milk samples.

TA and pH values (Tables 6 and 7) showed little change during the 8-day storage period at either storage temperature, indicating that the lactose probably was not a substrate during these test conditions. Although complete degradation of milk lipids and proteins would generate some acids, it seemed that the generation of acids by these degradation pathways were not sufficient to cause a change in the TA or pH values in this study.

**Table 1. Average Percentage of Fat, Protein, Lactose, and Solids-Not-Fat and Somatic Cell Counts (SCC) of Two Milk Samples of Different Fat Content**

Fat content	Fat	Protein	Lactose	SNF <sup>a</sup>	SCC <sup>b</sup> (×1000)
High (n = 3)	3.85	3.15	4.75	8.84	247.8
Normal (n = 3)	3.46	3.03	4.79	8.75	749.4

<sup>a</sup>SNF = solids not fat. <sup>b</sup>SCC = somatic cell count.

**Table 2. Means of Total Plate Counts (CFU/ml ×1000) of Two Milk Samples of Different Fat Content Stored at 39 or 45°F for 8 Days**

Fat content	Temperature (°F)	Day 0	Day 2	Day 4	Day 6	Day 8
High (n = 3)	45	16.6	812.8	11,220	87,096	TNTC*
	39	6.91	34.8	141.2	109.7	1,023.3
Normal (n = 3)	45	34.7	1,479	15,488	34,673	TNTC*
	39	37.1	25.7	190.5	104.7	1,819

\*To numerous to count.

**Table 3. Means of Psychrotrophic Counts (CFU/ml ×1000) of Two Milk Samples of Different Fat Content Stored at 39 or 45°F for 8 Days**

Fat content	Temperature (°F)	Day 0	Day 2	Day 4	Day 6	Day 8
High (n = 3)	45	9.5	562	5,623	40,783	5,495
	39	8.5	56.2	208	208	0.11
Normal (n = 3)	45	2.75	218.8	5,623	40,738	14,453
	39	9.8	6.9	70.8	323.6	3.23

**Table 4. Means and Standard Deviations of Proteolysis Data (μmole/ml protein) of Two Milk Samples of Different Fat Content Stored at 39 or 45°F for 8 Days**

Fat content	Temperature (°F)	Day 0	Day 2	Day 4	Day 6	Day 8
High (n = 3)	45	459.2	438.0	574.5	838.5	1117.25
		± 82.1	± 27.0	± 39.5	± 56.9	± 23.1
	39	459.2	403.7	464.5	524.5	578.0
		± 82.1	± 26	± 14.7	± 63.6	± 11.1
Normal (n = 3)	45	452.7	432.2	572.5	770.7	1193.5
		± 90.0	± 26.4	± 96.3	± 200	± 459.1
	39	452.7	417.7	457.0	538.7	630.7
		± 90.0	± 23.5	± 20.0	± 44.2	± 141.3

**Table 5. Means and Standard Deviations of Acid Degree Values of Two Milk Samples of Different Fat Content Stored at 39 or 45°F for 8 Days**

Fat content	Temperature (°F)	Day 0	Day 2	Day 4	Day 6	Day 8
High (n = 3)	45	0.24 ± 0.01	0.32 ± .04	0.35 ± 0.05	0.36 ± 0.01	0.29 ± 0.07
	39	0.25 ± 0.01	0.38 ± 0.02	0.49 ± 0.21	0.59 ± 0.09	0.45 ± 0.20
Normal (n = 3)	45	0.25 ± 0.04	0.29 ± 0.13	0.32 ± 0.11	0.39 ± 0.08	0.36 ± 0.14
	39	0.25 ± 0.04	0.38 ± 0.04	0.53 ± 0.27	0.56 ± 0.05	0.45 ± 0.10

**Table 6. Means of pH Values of Two Milk Samples of Different Fat Content Stored at 39 or 45°F for 8 Days**

Fat content	Temperature (°F)	Day 0	Day 2	Day 4	Day 6	Day 8
High (n = 3)	45	6.82	6.81	6.75	6.70	6.79
	39	6.85	6.81	6.77	6.73	6.80
Normal (n = 3)	45	6.82	6.80	6.79	6.75	6.85
	39	6.85	6.79	6.79	6.76	6.87

**Table 7. Means and Standard Deviations of Titratable Acidity (Expressed as % Lactic Acid) of Two Milk Samples of Different Fat Content Stored at 39 or 45°F for 8 Days**

Fat content	Temperature (°F)	Day 0	Day 2	Day 4	Day 6	Day 8
High (n = 3)	45	0.14 ± 0.01	0.13 ± .01	0.13 ± 0.0	0.13 ± 0.01	0.15 ± 0.00
	39	0.13 ± 0.03	0.13 ± 0.01	0.12 ± 0.00	0.13 ± 0.01	0.13 ± 0.01
Normal (n = 3)	45	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	0.15 ± 0.02
	39	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.15 ± 0.00