Journal of Food Protection Vol. 40, No. 12, Pages 848-853 (December, 1977)
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Keeping Quality of Pasteurized Milk for Retail Sale Related to Code Date, Storage Temperature, and Microbial Counts

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(Received for publication May 2, 1977)

ABSTRACT

Keeping quality of milk samples collected in original containers from fillers and stored at 1.7, 5.6, and 10.0 C remained organoleptically acceptable, on the average, 17.5, 12.1, and 6.9 days, respectively. Samples were tested for specific groups of bacteria at collection and when the milk became unacceptable (flavor score < 36). In addition to a total aerobic count the specific groups included pseudomonads, lipolytic, proteolytic, acid-producing, and coliform bacteria, and lipolytic and proteolytic pseudomonads. Keeping quality at any storage temperature was unrelated to the manufacturer's code date (last day product is to be sold). There was a significant correlation between keeping quality at 10.0-C storage and the other two storage temperatures, suggesting a practical test to measure keeping quality at the lower temperatures. Microbial counts, made at bottling and when the sample became unacceptable, were not consistently related to the time required for milk to become unacceptable at any storage temperature. When samples were stratified by flavor defect, certain microbial tests were significantly related to keeping quality.

In Connecticut, milk offered for retail sale must be marked with the last date it can be sold, the "code date." Each processor establishes the length of the code period. We have shown that, on the average, dairies in Connecticut overestimated code periods by about 2 days, but this overestimate varied from 2 to 8 days on code periods that ranged from 7 to 14 days (*9*). Processors point out that milk stored at 1.7 C will keep longer than if stored at a higher temperature, but they do not state what storage temperature reflects the choice of code period. However, not all milk is likely maintained at 1.7 C either in the retail store or in the home. For example, of 864 samples collected in retail stores in 1975 only 41% were below 4.4 C when collected, while 53% were between 4.4 and 7.2 C (*9*). What then is the effect of higher storage temperature on the shelf life of the same batch of milk, and is the possibility of storage at supra-optimal temperatures taken into account when establishing the length of the code period?

We therefore investigated the following: (*a*) Is the code period actually related to the length of time milk will remain acceptable when stored at either 1.7, 5.6, or 10 C? (*b*) Are microbial counts made at the time of bottling related to the length of time milk will remain acceptable? (*c*) Are specific types of microbial counts (e.g. proteolytic or lipolytic bacteria) related to the length of time milk remains acceptable? (*d*) Are microbial counts made on freshly bottled milk related to microbial counts made when the milk is no longer acceptable? (*e*) Are microbial counts made on the same milk stored at different temperatures statistically related?

MATERIALS AND METHODS

Sampling

At intervals from June, 1975, through November, 1976, 54 samples of pasteurized whole milk were obtained from 23 dairies in Connecticut. No dairy was sampled more frequently than once in 2 months. A third of the dairies were sampled once, another third twice, and the remainder three to five times. Each sample consisted of six subsamples collected in original containers taken directly from the filler, and in almost all instances from the same filling valve. Each sample, in its original container, was packed in ice during transport to the laboratory.

One set of three subsamples was used for microbiological analysis and the other set for organoleptic analysis. Seventy percent of the samples were in quart containers and 22% in half-gallon containers. The remainder were either gallons or half-pints. Paper containers comprised 72%, and glass bottles 28%, of the samples.

Organoleptic analysis

Subsamples were stored at 1.7, 5.6, and 10.0 C (35, 42, and 50 F), respectively. Upon arrival at the laboratory and at least every 2 days thereafter, an aseptically removed portion of each subsample was judged organoleptically for flavor score and defects by two persons according to procedures recommended by the American Dairy Science Association as modified for use in the Connecticut Milk Flavor Improvement Program (*8,10*). Subsamples attaining a flavor score of less than 36 were judged unacceptable and flavor judging was discontinued.

Microbiological analysis

Microbial tests were made on each sample initially and when the flavor score at each storage temperature dropped to less than 36. To determine microbial numbers, all inoculations were made by a spread

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plate technique on previously poured and hardened media (11) except that a pour plate technique was used with Violet Red Bile agar. After inoculations, plates were incubated at 30 C for the times designated in the next section.

Test media

Total counts of aerobic and proteolytic bacteria were made on the medium of Martley et al. (15). After 48 h of incubation both the total number of bacterial colonies as well as the number producing a protease were counted. The agar surface was then flooded with an oxidase reagent (7) (composed of a-naphthol and p-aminodimethylaniline oxalate). Those colonies which turned blue were classified as pseudomonads (7). Thus, from a single plate of medium, total aerobic, total proteolytic, total pseudomonads, and proteolytic pseudomonads were enumerated (11).

Lipase production was detected on the medium described by Sierra (16), with Tween 20 (Fisher Chem. Co., Fairlawn, N.J.) as the lipid source. After incubation for 5 days, the lipase producers were counted and after flooding the agar surface with the oxidase reagent, the lipolytic pseudomonads were enumerated.

Acid-producing bacteria were determined with the medium of Fabian et al. (3). Colfiorm bacteria were detected using Violet Red Bile agar (Difco).

Statistical analysis

All microbial counts were transformed as previously described (7). Data were statistically analyzed using Data-Text statistical computer programs (2).

Definitions

"Code date" is the date marked on the container designating the last day on which the milk may be sold or offered for sale. "Code period" is the number of days between the date of bottling and the code date. "Days to go bad" is the number of days from bottling until the flavor score dropped below 36. Similarly, "keeping quality" is the number of days a sample remained acceptable; that is, with a flavor score of 36 or higher.

RESULTS AND DISCUSSION

Relation of code period and temperature of storage to keeping quality

The code period among the 54 samples ranged from 7 to 15 days and averaged 10.8 days (Table 1). As expected, if the flavor defect was of microbial origin, the days to go bad decreased as the storage temperature increased. At 1.7 C the average number of days to go bad was 17.5, exceeding even the longest code period of 15 days. Nevertheless, 9% of the samples stored at this temperature were judged unacceptable before the expiration of their code period. About 5% of the samples had defects which were probably not of microbial origin; such as oxidized and chemical flavor.

At 5.6 C, the average days to go bad, 12.1, exceeded the code period of 70% of the samples, and nearly 43% of the samples became unacceptable before the expiration of their assigned code period. At 10.0 C only 4% of the samples remained acceptable beyond their code period, the average being 7 days. The time of year of sampling did not affect the length of time required for samples to become unacceptable.

It is clear from data in Table 1 that the lower the storage temperature the longer the milk retains an acceptable flavor and this agrees with the observations of others (1,4). At any of the three storage temperatures it is also clear that there is no relation between the assigned code period and the length of time the samples remained acceptable. Therefore, among the 23 dairies sampled, code period appears to be arbitrarily assigned without regard to the actual keeping quality of the milk.

TABLE 1. *Keeping quality (days to go bad) of 54 pasteurized milk samples in relation to code period (days) and storage temperature*

Code period (Days)	No. samples	Storage temperature (C)		
		1.7	5.6	10.0
7	4	24.8[1]	16.0	8.3
9	3	15.0	11.0	7.0
10	25	15.8	10.6	6.4
11	6	18.7	14.0	7.7
12	8	18.9	14.1	7.1
14	6	18.0	10.8	6.7
15	2	18.5	13.0	7.5
10.8 (Avg)		17.5 ± 5.2[2]	12.1 ± 3.9	6.9 ± 1.6

[1]Average number of days to attain a flavor score of < 36 for the number of samples shown.
[2]Mean ± standard deviation.

Among the 54 samples, the number of days required to go bad at one temperature was closely related to the length of time required to go bad at the other storage temperatures. Although samples stored at 10.0 C quickly became unacceptable (Table 1), the brief time they remained acceptable correlated well with their longer acceptable storage life at 1.7 and 5.6 C (Fig. 1). This

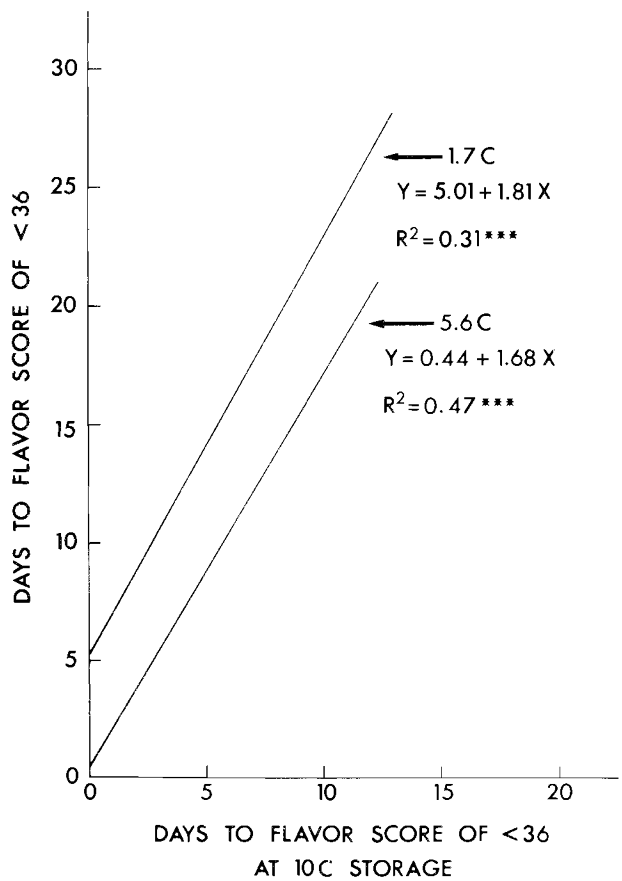

Figure 1. *Relation of days required to attain an unacceptable flavor score (< 36) for samples stored at 1.7 and 5.6 C compared to days required at 10.0 C. *** significant at P ⩽ 0.001.*

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suggests that practical, rapid estimates of storage life, hence code period, at lower and more reasonable storage temperatures could be easily obtained from results of storage at higher temperatures. The experimental evidence suggests that such a procedure could give rise to more meaningful code periods than are presently used.

Of considerable importance in the present study is that only 9% of the samples stored at 1.7 C failed to reach the end of their assigned code period. For the samples collected from retail stores in 1974 and 1975, 15% were unacceptable before the end of the code period (*9*). This indicates that processors lose control of the product after it enters the retail store. For example, the temperature of 53% of samples collected from stores in 1974-75 was in the 4.4 to 7.2 C range (*9*). Similarly, in the present study 43% of the samples stored at 5.6 C became unacceptable on or before the last day of the code period. This appears to show that if all milk at the retail market were allowed to reach the end of the code period before sale, almost half the samples would be unacceptable. These results emphasize that a major share of maintaining milk quality rests with the retail market and reaffirms the need for lower storage temperatures in store refrigerators, preferably below 4.4 C. These data also indicate that the flavor of milk stored between 4.4 and 7.2 in home refrigerators will likely also deteriorate to unacceptable levels before the code period expires.

Relation of initial microbial counts to keeping quality

Microbial counts made on the day of bottling (called initial counts) were generally low (Table 2) and negatively correlated with length of time for the sample to become unacceptable at any storage temperature. That is, the lower the initial count, the longer it took for the sample to become unacceptable. However, at all storage temperatures only acid producers were significantly correlated with days to go bad (Table 3). For samples stored at 5.6 and 10.0 C, the total count of proteolytic bacteria was also significantly negatively correlated with days to go bad.

TABLE 2. *Transformed bacterial counts[1] (means of 54 samples) of pasteurized milk on the day of bottling (initial) and when the flavor score dropped below 36 at different storage temperatures*

Microbial test	Initial	Storage temperature (C) 1.7	5.6	10.0
Total aerobic count	5.1[a2]	11.4[b]	14.1[c]	14.8[c]
Proteolytic bacteria	3.9[a]	11.0[b]	13.6[c]	14.4[c]
Pseudomonads	3.7[a]	10.8[b]	13.5[c]	13.9[c]
Proteolytic pseudomonads	3.5[a]	10.7[b]	13.3[c]	13.7[c]
Lipolytic bacteria	4.3[a]	10.3[b]	12.8[c]	13.5[c]
Lipolytic pseudomonads	3.6[a]	10.2[b]	12.4[c]	12.8[c]
Acid producers	1.9[a]	5.3[b]	8.4[c]	11.2[d]
Coliform bacteria	1.2[a]	1.4[a]	4.3[b]	8.5[c]

[1]For counts less than 10 the assigned code was 1. For any count-x, equal to or greater than 10, the transform was 2n, if $10^n \leqslant x$ but $< 5 \times 10^n$, or $2n + 1$ if $5 \times 10^n \leqslant x$ but $< 10 \times 10^n$. Such transformations on logarithm intervals have been described (*6,11*). The range of the transformed counts shown in the table can be calculated as follows: for odd-numbered transforms, subtract 1 from the transformed value and divide the integer portion by 2. The result is the exponent, n, where the value, x, has the range $5 \times 10^n \leqslant x < 10 \times 10^n$. For example, the transformed count 5.1 indicates that the microbial count lies in the range 500 but less than 1,000. For even-numbered transforms, divide the integer portion by 2; the result is the exponent, n, where x lies in the range $10^n \leqslant x < 5 \times 10^n$. For example, a tranform of 14.1 indicates that the microbial count lies in the range 10 million but less than 50 million.
[2]Means in any row followed by the same letter do not differ significantly at $P \leqslant 0.05$.

TABLE 3. *Relation (R^2) of microbial counts on the day of bottling with keeping quality (days to go bad) of 54 pasteurized milk samples stored at different temperatures*

Microbial test	Storage temperature (C) 1.7	5.6	10.0
Acid producers	0.144**	0.076*	0.159**
Proteolytic bacteria	0.020	0.072*	0.086*
All 8 microbial tests	0.211	0.143	0.308*

*Significant at P = 0.05.
**Significant at P = 0.01.

However, despite the statistical significance these correlations accounted for 16% or less of the variablility in days to go bad. Indeed, all eight initial bacterial counts combined accounted for only 14 to 31% of the observed variability in days to go bad at any of the storage temperatures. Watrous et al. (*17*) have also shown that initial bacterial counts including Standard Plate, coliform, and psychrotrophic counts on commercially pasteurized samples were of little value in predicting keeping quality. Randolph et al. (*14*) found no correlation between keeping quality and a Standard Plate Count of samples obtained directly from dairies. Further, Patel and Blankenagel (*13*) also clearly point out that milk with low bacterial counts does not necessarily have a long shelf life. Keeping quality, they suggest, is affected primarily by the type of organism and not necessarily by the total number present. Our earlier reports showed all of this statistically (*6,10*). In none of the statistical analyses in the present study did the initial total count appear as an indicator of keeping quality. Thus, initial bacterial counts have little apparent value by themselves for predicting keeping quality of milk at any storage temperature.

We did observe, however, that samples in glass containers became unacceptable sooner than those in paper cartons (Table 4). Initial microbial counts, except for coliform bacteria, were slightly but insignificantly higher in glass than in paper containers. When the samples became unacceptable, nearly all bacterial counts were slightly, but insignificantly, lower in glass containers compared to paper. In essence, although

TABLE 4. *Keeping quality (days to go bad) of pasteurized milk in glass and paper containers stored at different temperatures*

Container	No. samples	Storage temperature (C) 1.7	5.6	10.0
Paper	39	18.8	12.9	7.4
Glass	15	14.2	9.8	5.7
Difference		4.6	3.1	1.7
t-test (d.f. = 52)		3.13**	2.80**	3.75***

**Significant at P = 0.01.
***Significant at P = 0.001.

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samples became unacceptable sooner in glass than in paper containers, at the time they became unacceptable there was no difference in the microbial counts.

Relation of final microbial counts to keeping quality

Final microbial counts at all storage temperatures were obviously higher than the initial counts (Table 2). However, unlike the initial counts, all final counts were positively correlated with the number of days to go bad at all storage temperatures. That is, the longer the time required to become unacceptable, the greater the number of bacteria. This apparently reflects the effect of incubation period. It also suggests that no particular group of organisms can be singled out as the cause of unacceptable flavor.

Examination of data in Table 2 reveals that the final counts at 5.6 and 10 C do not differ except for acid producers and coliform bacteria, and that all final counts of milk stored at 1.7 C are significantly less than at 5.6 and 10 C. This further indicates that the number of organisms is not the sole determinant of unacceptable flavor, particularly at colder temperatures where they multiply slowly.

At 1.7 C proteolytic pseudomonads made the greatest relative increase over initial count. At 5.6 C the acid producers and proteolytic pseudomonads showed the largest relative increase while at 10.0 C acid producers and coliform bacteria exhibited the largest relative increases over initial counts. Thus, at each temperature it is likely that a different group of organisms may be responsible for deterioration of flavor score.

Relation of individual flavor defects to keeping quality

The final flavor defects were examined to determine what caused the samples to become unacceptable (Table 5). Initially, three flavor defects; feed, cooked, and cooked and feed were assigned to 94% of the samples. When a sample was declared unacceptable the most common flavor defect was old or lacking freshness, accounting for 61 and 54% of samples stored at 1.7 and 5.6 C, respectively. However, at 10.0-C storage, less than a third were designated as lacking freshness. At 10-C storage, the two off-flavors putrid and curdled, accounted for 31% and acid flavor accounted for 9% of the samples. The number of samples with these off-flavors found at 10-C storage was noticeably increased over that of samples stored at 5.6 C. The proportion of samples with a fruity flavor remained nearly constant at all storage temperatures. Bitter flavor , accounting for 17% of the samples at 1.7 C, dropped to 11% at 5.6 and 10.0 C.

The keeping quality of samples stored at different temperatures and with a specific flavor defect is also given in Table 5. For example, samples stored at 1.7 C and judged as lacking freshness lasted an average of 18.1 days, while those with the same flavor criticism but stored at 10.0 C lasted an average of only 7.4 days. The keeping quality of samples stored at 5.6 C is nearly midway between those stored at 1.7 and 10.0 C. At 10.0-C storage no large differences are seen in number of days to go bad between any of the flavor defects.

Relation of keeping quality to initial flavor score and microbial counts

The initial flavor score was only correlated with the number of days to go bad at 1.7 C, and it accounted for only 9% of the observed variation (Table 6). Initial flavor score was poorly correlated with days to go bad for samples stored at 5.6 and 10.0 C. Results of an earlier study of laboratory-pasteurized milk showed that initial flavor score accounted for 38 to 41% of the variability in days to go bad when the milk was stored at 1.1 to 3.3 C (*10*).

Initial microbial counts also did not correlate with days to go bad at any storage temperature. Combining initial flavor score with one or more initial microbial counts improved the prediction of days to go bad for samples stored at 1.7 and 5.6 C, but not at 10.0 C (Table 6). However, at best only 22% of the observed variability in days to go bad was accounted for by these counts. Use of counts from other microbial tests gave no improvement to the prediction of days to go bad. Thus, neither initial flavor score nor microbial counts appear to be useful as reliable predictors of keeping quality of commercially pasteurized milk.

TABLE 5. *Keeping quality (days to go bad) of pasteurized milk samples stored at different temperatures and classed by final flavor defect*

	Storage temperature (C)					
	1.7		5.6		10.0	
Flavor defect	No. samples	Avg days to go bad	No. samples	Avg days to go bad	No. samples	Avg days to go bad
Rancid	1	21.0	0	—	1	4.0
Acid	0	—	1	10.0	5	7.4
Lacks freshness	33	18.1	29	12.6	17	7.4
Putrid and/or curdled	4	14.8	9	13.4	17	6.8
Bitter	9	21.6	6	13.7	6	7.0
Fruity	4	16.5	4	11.0	5	6.8
Malty	1	7.0	1	4.0	1	7.0
Unclear	0	—	1	15.0	1	9.0
Misc.[1]	2	4.0	3	3.4	1	3.0
Avg days to go bad		17.5 ± 5.2		12.1 ± 3.9		6.9 ± 1.6

[1]Misc. includes oxidized and chemical; not considered microbial defects.

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TABLE 6. *Relation (R^2)[1] of keeping quality (days to go bad) with initial flavor score (made at bottling) and inital microbial counts of 54 samples stored at different temperatures*

Variable	Storage temperature (C) 1.7	5.6	10.0
Initial flavor score (IFS)	0.092*	0.072	0.007
IFS + acid producers	0.208*	—	—
IFS + acid prod. + lipolytic bacteria	0.216*	—	—
IFS + lipolytic pseudomonads	—	0.140*	—
IFS + lip. pseud. + acid prod.	—	0.182*	—

*Significant at P = 0.05.
[1]Only selected R^2 values shown since the same variables did not appear at each storage temperature.

Relation of individual flavor defects to microbial counts

The cause of certain flavor defects can be attributed to particular kinds of organisms (*5*). Bitter, fruity, putrid, and stale (lacking freshness) have been attributed to psychrotrophic organisms (*14*). We have seen that neither initial nor final microbial counts correlated with the keeping quality of all milk samples expressed as the number of days to go bad. Therefore, samples were stratified according to the final flavor defect and again examined for the relation of days to go bad to microbial counts (Table 7). For both initial and final microbial counts we expect that a negative correlation of days to go bad with a microbial count to be important. That is, the lower the count, the longer the milk will remain acceptable. A positive correlation, on the other hand, may be interpreted simply as the effect of incubation period. The longer the milk remains acceptable, the greater the opportunity for bacteria to grow. Unfortunately, results obtained with this analysis were extremely variable.

Examples of the relations of initial or final microbial counts with three different flavor defects are given in Table 7. In the group judged as lacking freshness, initial counts did not correlate with days to go bad for samples stored at 1.7 and 5.6 C. At 10.0-C storage, acid producers were significantly negatively correlated with days to go bad, but accounted for only 19% of the variability. At 1.7-C storage all final counts did not correlate with days to go bad although all tended to be negatively correlated. At 5.6- and 10.0-C storage all correlations were positive, with total count being most highly correlated with days to go bad. Similar results are shown in Table 7 for two other flavor defects, putrid and bitter.

Thus, as we and others have shown (*1,3,4,6,10,14,17*) total counts bear little relation to keeping quality. We show here that even when samples are stratified for flavor defect, there is no clear relation between microbial count, but there is some statistical significance between specific microbial groups and keeping quality.

The information presented here and by others (*13,17*), requires one to reflect on the ultimate value of determining keeping quality based solely on bacterial tests, an example being the Mosely keeping quality test (*12*). This test, like others, is not correlated with actual flavor analysis since it has been shown that milk with a flavor defect may or may not have a high total bacterial count. Thus, more discriminative tests for keeping quality need to be developed, and such tests must be uncomplicated enough to be done routinely in any laboratory.

ACKNOWLEDGMENTS

We thank Margaret Staba for excellent technical assistance and Matthew Meyer for help in collecting samples and organoleptic analyses.

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TABLE 7. *The best correlation (r) of days to go bad with initial or final microbial counts: grouped by final flavor defect*

		Temperature of storage (C)								
		1.7			5.6			10.0		
Final flavor defect	Microbial count	No. samples	Microbial test	r	No. samples	Microbial test	r	No. Samples	Microbial Test	r
Lacks freshness	Initial	33	PrPs[1]	+0.137	29	Lip	−0.204	17	Acid	−0.438*[2]
	Final	33	Acid	−0.197	29	TotCnt	+0.424*	17	TotCnt	+0.765***
Putrid	Initial	4	Acid	−0.802	9	Acid	−0.497	17	Coli	−0.863***
	Final	4	Pr	+0.636	9	Lip	−0.801**	17	PrPs	+0.651**
Bitter	Initial	9	LipPs	+0.436	6	Pr	−0.833	6	Acid	+0.577
	Final	9	Lip	+0.763*	6	TotCnt	+0.986***	6	Ps	+0.845

[1]Abbreviations: PrPs, proteolytic pseudomonads; Lip, lipolytic bacteria; Acid, acid producers; TotCnt, total number of bacteria; Coli, coliform bacteria; Pr, proteolytic bacteria; LipPs, lipolytic pseudomonads; Ps, pseudomonads.
[2]Significance level: *P = 0.05; **P = 0.01; ***P = 0.001.

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