J. Milk Food Technol. Vol. 38, No. 12, Pages 738-744 (December, 1975)

Quality Control Significance of Special Media for Enumeration of Microbial Groups in Cottage-type Cheese

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(Received for publication April 15, 1975)

ABSTRACT

Cottage-type cheese samples were examined using specialized media. Nine media were used to provide 21 different types of information and to enumerate bacteria able to excrete lipases, proteases, phosphatases, and acids. Further, these media allow subdivision of the bacteria with specific enzymic attributes into gram negative and positive categories and pseudomonads. A yeast and mold count and a test of those able to grow on violet red bile agar also were made. Tests most useful as predictors of keeping quality were primarily those able to differentiate proteolytic and lipolytic gram negative bacteria and pseudomonads. Microbial tests were also correlated with organoleptic analysis. Manufacturers' code dates (last day of sale) overestimated shelf life about 33% of the time.

Microbial tests on cottage-type cheese usually include a test for the coliform group of bacteria and one for yeasts and molds (*1*). For a coliform count, a solid medium such as violet red bile agar or a liquid medium such as brilliant green lactose bile broth (*1*) is usually used. Acidified potato dextrose agar is commonly used for a yeast and mold count (*1*). All of these tests theoretically help to assess the sanitary conditions under which the product was manufactured and presumably give some evidence of post-pasteurization contamination. None of the tests just mentioned provide data on the degradative abilities of the contaminating microorganisms and these tests do not help assess whether they are mesophiles or psychrotrophs. Although an unusually large count might be related to loss in keeping quality, it is at best only indirect evidence, since clearcut correlations between microbial counts and keeping quality have not been estabished. Further, these media do not measure perhaps the largest population of contaminators in dairy products; i.e., the gram negative bacteria and specifically the psychrotrophic pseudomonads which are considered to be among the most important contaminators in dairy products (*17*).

One measure of the utility of any medium used in quality control testing procedures is the amount of useful information that can be obtained from the use of that medium. Media that provide more than a single set of data would be the most useful. In this paper we report on the suitability of media designed to provide at least two sets, and in some instances three sets, of data. The media described allow enumeration of broad segments of microbial contaminators and furnish information on some important extracellular degradative enzymes produced by these contaminators. The data provided by use of test media in the examination of cottage-type cheese are correlated with keeping quality, i.e., the number of days required for the sample to become unacceptable to the consumer, either because of flavor and aroma deterioration or visible change (e.g. surface growth and discoloration). In some instances several of the test media theoretically allow enumeration of the same groups of microorganisms. Thus, data provided by use of these media were examined to ascertain if comparable counts were obtained.

Additionally, information is provided on the quality of cottage-type cheese, what flavor defects are usually encountered, and how well manufacturers' code dates (last day the product is offered for sale) correlates with the actual time for the sample to reach an unacceptable state.

MATERIALS AND METHODS

Samples

Duplicate samples of 74 cottage-type cheeses were collected during 1973 and 1974 from dairy plants and retail outlets in Connecticut. The samples were refrigerated during transport to the laboratory. One sample was used for microbiological analyses, the other for organoleptic analysis. We collected 55 samples of cottage cheese, 15 of ricotta cheese, nine of baker's cheese and three of mozzarella cheese.

Organoleptic analysis

Samples were refrigerated (2-3 C). Portions removed aseptically were judged at least every other day by a minimum of two persons according to procedures recommended by the American Dairy Science Association and modified for use in the Connecticut Milk Flavor Improvement Program (*4-7*). Samples attaining a score of 36 or less were judged unacceptable.

Microbiological analysis

An 11-g sample in a dilution bottle was shaken with 100 ml of sterile 2% sodium citrate solution. Dilutions for plating were made in sterile distilled water. All inoculations were made by a spread plate technique

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on previously poured and hardened media except that a pour plate technique was used with violet red bile agar. After inoculation plates were incubated at 30 C. Violet red bile agar plates were counted after 24 h. All others, except media designed to detect lipase production, were counted after 48 h. The lipase detection medium was counted after 5 days.

Test media

Lipase production by bacterial colonies was detected with the medium described by Sierra (*15*) with sorbitan monolaurate (Tween 20, Fisher Chemical Co., Fairlawn, NJ) as the lipid source. Colonies which produce a lipase are either surrounded by a precipitate of the calcium salt of the liberated fatty acid, or a clear zone when the lipid is completely degraded. The medium described by Martley et al. (*10*) was employed to detect protease production, except that plate count agar (Difco) was used instead of standard methods agar. Colonies which produce a protease are surrounded by a ring of precipitated para-casein. Plate count agar, potato dextrose agar, and violet red bile agar were all commercial preparations (Difco). The potato dextrose agar was acidified to pH 3.5 with tartaric acid.

To indicate acid phosphatase production, the disodium salt of phenolphthalein diphosphate (K and K Laboratories, Plainview, NJ) was added to plate count agar (*2*) to provide a final concentration of 0.1 mg/ml in the medium. The phenolphthalein diphosphate is warmed gently on a hot plate (to avoid hydrolysis) with stirring to effect solution and while still warm, filter sterilized. It was added while warm (rewarmed if necessary) to the previously cooled and tempered (48 C) plate count agar. After incubation of inoculated plates and growth of colonies, the open plate was inverted over a beaker of ammonium hydroxide and colonies which turned pink to red within 60 sec were phosphatase producers. No antibiotics were used in this medium.

Acid production was determined with the medium of Fabian et al. (*3*) to which antibiotics were added. To determine if colonies, on any medium, were oxidase positive, plates were flooded with an oxidase reagent composed of α-napthol and p-aminodimethylanaline oxalate as previously described (*8*), and the characteristic development of a blue color was noted.

Antibiotics used

To exclude gram positive bacteria, chloramphenicol (Chloromycetin, Sigma Chemical Co., St. Louis, MO) and erythromycin (Sigma) were added to media to provide a final concentration of 2.5 and 5.0 μg/ml, respectively (*11*) (Mixture A). Sterilization by autoclaving affects solution. Both antibiotics were prepared in aqueous suspension at the rate of 50 mg/100 ml. The former was added to sterile and tempered (48 C) media at the rate of 0.5 ml/100 ml and the latter at 1 ml/100 ml of media.

A more selective antibiotic mixture was used to allow only pseudomonads to grow (*13, 14*). This mixture (Mixture B) contained penicillin G, novobiocin (Albamycin, Upjohn, Kalamazoo, MI) and cycloheximide (Actidione, Upjohn). To prepare these antibiotics 45 mg penicillin G (75,000 units), 45 mg novobiocin and 75 mg cycloheximide were mixed in a sterile container and 1 ml of ethanol was added. After 30 min, 9 ml sterile distilled water was added. One milliliter of this sterile antibiotic suspension was added to each 100 ml of sterile and tempered media, just before pouring the plates.

Statistical analysis

To facilitate statistical analysis, all microbial counts were transformed as follows. 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 $10^n \leq X$ but $<5 \times 10^n$, or $2n + 1$ if $5 \times 10^n \leq X$ but $<10 \times 10^n$. For example a count of 40/g is transformed to 2; a count of 75 is transformed to 3. Such similar transformations on logarithmic intervals have been described previously (*7*). Results from different media theoretically enumerating the same bacterial groups were compared by correlation and linear regression analysis. The relation of microbial counts to keeping quality (i.e., days to go bad from manufacture or collection) was determined by multiple regression analysis (*16*). Prof. G. M. Furnival, Yale University, kindly provided a computer program for rapid screening of independent variables.

RESULTS AND DISCUSSION

Detection of microbial groups and enzymes

What each medium specifically measures is detailed in

TABLE 1. *Test media and groups of organisms enumerated*

Media number	Media description	Antibiotic mixture used in medium	Test applied[1]	Groups detected
1A	Plate count agar with	—[2]	total growth	total gram negative and positive bacteria
1B	phenolphthalein diphosphate	—	reaction of colonies to ammonium hydroxide	bacteria producing phosphatases
2A	Plate count agar	A[3]	total growth	total number of gram negative bacteria
2B		A	oxidase test	oxidase positive gram negative bacteria (presumably pseudomonads)
3A	Sierra's medium with	B	total growth	total number of pseudomonads
3B	Tween 20	B	lipase excretion	pseudomonads excreting lipases
3C		B	oxidase test	oxidase positive pseudomonads excreting lipases
4A	Sierra's medium with	A	total growth	total number of gram negative bacteria
4B	Tween 20	A	lipase excretion	total number of gram negative bacteria excreting lipases
4C		A	oxidase test	oxidase positive lipase excreters (presumably pseudomonads)
5A	Martley's medium	B	total growth	total number of pseudomonads
5B	(Modified)	B	protease excretion	pseudomonads excreting proteases
5C		B	oxidase test	oxidase positive pseudomonads excreting proteases
6A	Martley's medium	A	total growth	total number of gram negative bacteria
6B	(Modified)	A	protease excretion	total number of gram negative bacteria excreting proteases
6C		A	oxidase test	oxidase positive protease excreters (presumably pseudomonads)
7A	Fabian's medium	A	total growth	total number of gram negative bacteria
7B		A	dye reaction (pH change)	total number of gram negative bacteria excreting acids
8A	Violet red bile agar	—	typical colonies	coliform group of bacteria
9A	Acidified potato	—	typical colonies	yeast count
9B	dextrose agar	—	typical colonies	mold count

[1]Tests on each medium are applied sequentially in the order shown.
[2]-Indicates no antibiotics in this medium.
[3]Mixture A contains chloramphenicol and erythromycin.
Mixture B contains penicillin, novobiocin, and cycloheximide.

TABLE 2. *Counts obtained with 21 test media on 74 samples of cottage-type cheese*

Test applied	Media number	Av. transformed count	Numbers per gram (Range)
Total count	1A	8.1	10,000-50,000
Total no. of gram negative bacteria	2A, 4A, 6A, 7A	8.0 ± 0.2	10,000-50,000
Total no. of pseudomonads	2B, 3A, 5A	5.9 ± 0.3	500- 1,000
Proteolytic pseudomonads	5B, 5C, 6C	5.0 ± 0.3	500- 1,000
Lipolytic pseudomonads	3B, 3C, 4C	5.3 ± 0.2	500- 1,000
Proteolytic gram negative bacteria	6B	6.4	1,000- 5,000
Lipolytic gram negative bacteria	4B	6.2	1,000- 5,000
Yeast count	9A	4.6	100- 500
Mold count	9B	3.4	50- 100
Phosphatase producers	1B	6.7	1,000- 5,000
Gram negative acid producers	7B	7.2	5,000-10,000
Coliform bacteria	8A	3.5	50- 100

Table 1. Test media with the same Arabic numeral are the same media and plate. The letter designation following the numeral indicates tests which are applied sequentially to the medium to obtain differential counts of selected microbial groups, either on a biochemical basis (enzyme excretion), gross differentiation (gram negative and gram postive bacteria), or differential count (yeasts and molds). Medium 1 in Table 1 does not contain any antibiotics and is plate count agar to which phenolphthalein diphosphate was added. Thus, this medium provides both a count of gram negative and gram positive bacteria as well as those colonies able to produce a phosphatase. Media 2, 4, and 6 contain antibiotic mixture A which essentially allows most gram negative bacteria to grow. Tests with pure cultures of representative gram negative bacteria that may be present in dairy products have confirmed this. These antibiotics were originally proposed to be used in media to enumerate pseudomonads selectively (*11*), but we have found it to be less selective than suggested. Media 3 and 5 contain antibiotic mixture B which essentially allows only growth of pseudomonads (*13, 14*). Media 8 and 9 are commercial preparations in common use.

Several examples serve to illustrate how the media were used and the information provided by sequential analysis. Counting all colonies on medium 6 (Table 1) provides a total count of gram negative bacteria. Colonies on the same plate that show the typical reaction for proteases are called proteolytic (medium 6B). Colonies that produce a protease were marked on the underside of the plate and the plate was then flooded with the oxidase reagent. Marked colonies which turn blue were called proteolytic pseudomonads (medium 6C). The rationale of designating oxidase positive organisms in dairy products as pseudomonads as well as their potential as psychrotrophs has been discussed previously (*8*). A second example is use of medium 3 which is selective for pseudomonads. Medium 3A (Table 1) provides a total countof pseudomonads. Colonies giving the typical lipase reaction were counted, and thus medium 3B provides a count of lipolytic pseudomonads. These colonies were marked and the plate flooded with the oxidase reagent. Colonies that were oxidase positive provide a confirmed count of lipolytic pseudomonads (medium 3C).

Microbial counts

The actual range of counts provided by the various test media used is shown in Table 2. Since counts were transformed, the average transformed value is shown. Further, since in some instances several test media measured the same group of microorganisms they were grouped together and the standard deviations shown. The range of counts per gram of sample are shown as indicated by the transformed value. The transformed value also shows whether the average count is close to the top or the bottom of the range. For example, for total pseudomonads the transform is 5.9 which indicates a value near the top of the range while a value of 5.0 for proteolytic pseudomonads indicates the lower end of the range. Generally, most counts are fairly low, if one considers 50,000 as not being excessive for cottage-type cheese. It is interesting that the total count of all bacteria is essentially the same as for the total number of gram negative bacteria. Overall we conclude that lipolytic and proteolytic gram negative bacteria account for between 5 and 10% of the total number of gram negative bacteria. Also, most of the pseudomonads detected were able to produce both lipases and proteases. Of considerable interest is the number of phosphatase producers detected. It could be important to determine how these phosphatase producers could affect the phosphatase test which is used to determine proper pasteurization of dairy products.

Correlations among media

If our conclusions that certain test media enumerate the same micro-organisms are correct, then tests numbered 2A, 4A, 6A, and 7A should all give the same measure of the gram negative bacterial flora. Similarly, media 2B, 3A, and 5A should all measure total number of pseudomonads; 3B, 3C, and 4C measure lipolytic pseudomonads and 5B, 5C, and 6C proteolytic pseudomonads. The means of the results obtained from media within these groups agree well (Table 2). As a further test, correlations among results of media within these same groups were calculated (Table 3).

The correlation coefficients, r, in Table 3, are highly significant, indicating high correlation between test media. Medium 8A, violet red bile agar, a "standard"

TABLE 3. *Correlation coefficients*[1] *(r) between media used to enumerate the same groups of bacteria in cottage type cheese (n = 74)*

Total number of gram negative bacteria					Total number of pseudomonads		
	media number[2]					media number[2]	
	4A	6A	7A	8A		3A	5A
2A	.87	.96	.76	.66	2B	.81	.81
4A	—	.85	.74	.69	3A	—	.97
6A	—	—	.76	.62			
7A	—	—	—	.58			

Lipolytic pseudomonads			Proteolytic pseudomonads		
	media number[2]			media number[2]	
	3C	4C		5C	6C
3B	.93	.85	5B	.92	.83
3C	—	.90	5C	—	.81

[1]All correlation coefficients (r) in this table are highly significant.
[2]Refer to Table 1 for media descriptions.

medium used to enumerate the coliform group of bacteria is also highly correlated with our test media, but the r values were lower. It was not unexpected to note that a high correlation exists for this medium since the coliform group undoubtedly comprises a portion of the count obtained on the other media. The highest correlation (r = 0.96) was obtained between media 2A and 6A. Both have the same components except that medium 6A contains added sodium caseinate. Media 3A and 5A, used to enumerate the total number of pseudomonads, contain a different antibiotic mixture than medium 2A. Even so there is a highly significant correlation between these tests. The same situation prevails for lipolytic pseudomonads in that media 3C and 4C contain different antibiotic mixtures and yet the tests are still highly correlated. It is not surprising that media 3B and 3C are significantly correlated since the data for 3C are obtained from 3B, i.e., the same test plate is used. An examination of data for tests for proteolytic pseudomonads shows the same situation to exist. From these analyses, shown in Tables 2 and 3, we conclude that our initial assumptions, that media designed to provide the same information, were correct.

Predicting keeping quality from microbial tests

The ability to predict keeping quality of cottage type cheese from the results of microbial tests would be most desirable. Unfortunately, single microbial tests in common use generally fail to predict adequately, and this failure may be in part due to lack of specificity of the tests. In this study we have enumerated broad groups such as pseudomonads and total numbers of gram negative bacteria. Further, we have utilized tests designed to enumerate, within the broad categories, specific groups of microorganisms that excrete extracellular degradative enzymes.

In all, 21 tests described previously, were performed. Transformed microbial counts from the 21 tests were used singly or in combination to find which test best estimated the time to go bad from manufacture or from collection. The best results from combinations of one or two variables (i.e., test media) are shown in Table 4.

The two best single estimators of days to go bad from manufacture were measures of proteolytic gram negative

TABLE 4. *Relation of the 2 best single or pairs of 21 microbiological tests to the time required for cottage type cheese samples to become unacceptable (n = 74)*

Variables (groups detected and media no.)[1] Time to become unacceptable from manufacture	R^2
Proteolytic gram negative bacteria (6B)	.144
Lipolytic pseudomonads (4C)	.124
Lipolytic pseudomonads (3B) and lipolytic pseudomonads (4C)	.235
Lipolytic pseudomonads (3C) and total gram negative bacteria (4A)	.210
Total on 21 variables	.465
Time to become unacceptable from collection	
Total gram negative bacteria (7A)	.206
Total gram negative and positive bacteria (1A)	.147
Total gram negative and positive bacteria (1A) and total gram negative bacteria (6A)	.262
Total gram negative bacteria (6A) and total gram negative bacteria (7A)	.259
Total on 21 variables	.490

[1]Refer to Table 1 for media descriptions.

bacteria (medium 6B) and lipolytic pseudomonads (medium 4C). The best 2-variable combinations measured lipolytic pseudomonads (media 3B and 4C) whereas, the next best 2-variable combination enumerated lipolytic pseudomonads and total numbers of gram negative bacteria (media 3C and 4A). The combination of all 21 variables accounted for less than half of the observed variability in days to go bad and combinations of two or more variables did not significantly improve the overall R^2 value for the combinations. Nevertheless, it is interesting that an assay of lipolytic pseudomonads (media 3B, 3C, and 4C) appeared most frequently in the best combinations of variables. This suggests that lipolytic pseudomonads have an important effect on keeping quality of cottage-type cheeses held under refrigeration conditions.

On the other hand, variables best related to days to go bad from collection were measures of the total number of gram negative bacteria (media 7A and 6A) and total number of gram negative and positive bacteria (medium 1A).

It is obvious that no quality control or regulatory laboratory routinely would do 21 analyses on a single product. Therefore, based on data obtained from 21 tests on 74 samples as described above, we selected 10 tests (or variables) to use to ascertain the best single and pair of estimators. Selection of variables considered both statistical performance, as determined previously, as well as practical utility. The 10 tests selected were measures of lipolytic gram negative bacteria (medium 4B), lipolytic pseudomonads (medium 4C), total number of gram negative bacteria and positive bacteria (medium 1A), total number of gram negative bacteria (medium 2A), total pseudomonads (medium 2B), proteolytic gram negative bacteria (medium 6B), proteolytic pseudomonads (medium 6C), yeast count (medium 9A), mold count (medium 9B), and a count of the coliform group of bacteria (medium 8A). The latter four were selected because they are "standard" tests for dairy products (*1*); rather than for any potential for estimating keeping quality. For the 10 tests indicated above (media 1, 2, 4, 6,

8, 9) only six test plates are needed since many of the media provide information in two categories as shown in Table 1. Further, media 4B and 4C and 6B and 6C would also provide a total count of gram negative bacteria and medium 1A could also be used to indicate bacteria able to produce a phosphatase. Because the time from manufacture to collection of cheese varied considerably, the data were stratified into two groups, 0 to 3 days and 0 to 7 days from manufacture to collection.

For 28 samples collected 0 to 3 days after manufacture, the test best related to the number of days from manufacture for a sample to become unacceptable was a test for lipolytic gram negative bacteria (medium 4B, Table 5). This test alone accounted for 22% of the observed variability, compared to 41% for all 10 tests. The second best single estimator was the yeast count (medium 9A). Although adding an additional test (the 2-variable combination) accounted for a greater proportion of the observed variability, the increase was not statistically significant. With the exception of the yeast count, no other test generally used in dairy product testing was closely related to the time required from manufacture for the samples to become unacceptable.

TABLE 5. *Relation of 2 best single or pairs of 10 microbiological tests to time required for cottage type cheese samples to become unacceptable*

Variables (group detected and media no.)[1]	R^2
28 samples collected 0 to 3 days after manufacture (dependent variable = days to go bad from manufacture)	
Lipolytic gram negative bacteria (4B)	.216
Yeasts (9A)	.190
Lipolytic gram negative bacteria (4B) and total pseudomonads (2B)	.319
Lipolytic gram negative bacteria (4B) and proteolytic pseudomonads (6C)	.255
Total on 10 variables	.414
51 samples collected 0 to 7 days after manufacture (dependent variable = days to go bad from collection)	
Total gram negative and gram positive bacteria (1A)	.194
Lipolytic gram negative bacteria (4B)	.193
Total gram negative bacteria (2A) and total gram negative and positive bacteria (1A)	.298
Lipolytic gram negative bacteria (4B) and total pseudomonads (2B)	.256
Total on 10 variables	.451

[1]Refer to Table 1 for media description.

For 51 samples collected 0 to 7 days after manufacture, the number of days to go bad from collection was most closely related to tests for the total number of gram negative and gram positive bacteria (medium 1A) and lipolytic gram negative bacteria (medium 4B, Table 5). Although addition of another test (the 2-variable combination) accounted for a larger proportion of the observed variability, the increase was not statistically significant. All of the sets of variables most closely related to days from collection to become unacceptable contained a test that indicated the presence of gram negative bacteria.

Even though a test for lipolytic gram negative bacteria (medium 4B) was a good estimator of time required to become unacceptable, it may not be the test of choice since this test requires 5 days to complete (although 3 days can be used, but with less certainty). Thus, it may not be the most useful test for a manufacturer to use to establish a meaningful code life for his product. However, it does appear that it would be a useful test for monitoring the manufacturing process, since the 5-day period needed to complete the test is well within the usual code period for most samples (Table 6). On the other hand, either the test for lipolytic gram negative or gram negative and positive bacteria may be of more immediate use for the analysis of samples collected at retail outlets, to estimate their remaining shelf life. Nevertheless, this latter test (medium 1A) does not indicate the biochemical abilities of the organisms in the samples as does a test for lipolytic bacteria.

TABLE 6. *Relation of number of days for cottage type cheese to go bad from manufacture or collection and manufacturer's estimate of keeping quality*

Category	Number of samples	Days	
		Average	Range
1. Actual time from manufacture to unacceptability (all samples)	74	17.8	1-41
2. Actual time from collection to unacceptability (all samples)	74	12.4	1-38
3. Manufacturers estimate of keeping quality (code date; last day offered for sale)	56	18.8	7-35
4. Actual time from manufacture to unacceptability (only coded samples)	56	21.3	1-41
5. Actual time from manufacture to unacceptability (only uncoded samples)	18	12.3	3-30

Correlation of microbial and organoleptic tests

An attempt was made to determine if the reason for rejection of samples by the organoleptic test was correlated with the microbiological analyses. Therefore, the data were stratified according to the defect observed when the sample became unacceptable. The dependent

TABLE 7. *Relation of the 2 best single or pairs of 6 microbiological tests to the time required from date of collection for cottage cheese type samples, rejected for a specific flavor, to become unacceptable*

Variables (groups detected and media no.)[1]	R^2
Bitter flavor (n = 18)	
Mold count (9B)	.151
Lipolytic gram negative bacteria (4B)	.013
Mold count (9B) and lipolytic gram negative bacteria (4B)	.212
Lipolytic pseudomonads (4C) and mold count (9B)	.169
Total on 10 variables	.306
Surface growth (n = 15)	
Lipolytic pseudomonads (4C)	.245
Proteolytic pseudomonads (6C)	.180
Lipolytic pseudomonads (4C) and mold count (9B)	.288
Lipolytic gram negative bacteria (4B) and lipolytic pseudomonads (4C)	.275
Total on 10 variables	.399
Old/Lacks freshness (n = 12)	
Yeast count (9A)	.388
Proteolytic pseudomonads (6C)	.188
Lipolytic pseudomonads (4C) and proteolytic pseudomonads (6C)	.532
Yeast count (9A) and proteolytic pseudomonads (6C)	.479
Total on 10 variables	.604

[1]Refer to Table 1 for media description.

variable, days to go bad from collection was regressed on six microbial tests singly and in combinations. Those considered most likely to provide meaningful data were media 4B, 4C, 6B, 6C, 9A, and 9B. Results for three different types of defects are shown in Table 7.

The best single estimator of keeping quality for samples judged as bitter was the mold count (medium 9B, Table 7) which accounted for 15.1% of the observed variability. A poor second best estimator, lipolytic gram negative bacteria (medium 4B) accounted for only 1.3% of the total variability. The best single estimators for samples rejected because of surface growth was a test for lipolytic pseudomonads (medium 4C) and proteolytic pseudomonads (medium 6C) which accounted for 25% and 18% of the observed variability, respectively. The best single estimators for samples judged as old or lacking freshness were the tests for yeasts (medium 9A) and for proteolytic pseudomonads (medium 6C).

Why certain microbial tests were better estimators than others for specific flavor defects is difficult to explain since much depends on the chemical entities elaborated by microorganisms during growth. For surface growth defects, we attempted to differentiate in our testing between that caused by fungi and that by bacteria. Only 3 of 15 samples rejected because of surface growth could be attributed to molds. Even though a mold count accounted for nearly the same amount ($R^2 = .151$) of the total variability as proteolytic pseudomonads (Table 7), it is obvious that for these samples more bacterial than fungal surface growth was encountered and the regression analysis confirms this. The reason why a yeast count is the best single estimator for the old or lacking freshness defect is unclear. It was expected that a total count of pseudomonads would have been the best single estimator, as has previously been shown for this defect in milk (*4, 7*). In this previous study on pasteurized milks, pseudomonads accounted for 17% of the total variability among samples with this defect. However, yeast counts were not made. Although in the present study yeasts accounted for nearly 39% of the total variability, proteolytic pseudomonads accounted for about 19% and lipolytic pseudomonads, 15%. In combination these latter two tests accounted for 53% of the variability (Table 7).

Relation of keeping quality to manufacturers' code

Of the 74 samples examined, 44 were collected from 10 manufacturing plants in Connecticut. The remaining 30 samples, collected in retail outlets, represented nine out-of-state manufacturers. The average time from manufacture to collection was 3.3 days for in-state and 11.3 for out-of-state samples. All but one sample was judged organoleptically acceptable at collection time. The average time to go bad from manufacture for all 74 samples was 17.8 days (line 1, Table 6) and from collection, (line 2) 12.4 days. Time to go bad from manufacture or collection is defined as the time interval in days from date of manufacture, or collection, to the date when the sample was judged to be unacceptable, that is, a flavor score of 36 or less or visible surface growth. The code date (last day to be offered for sale) and the date of manufacture were known for only 56 of the samples. The longevity of these samples, estimated by the manufacturer, was 18.8 days whereas the actual time to go bad from manufacture averaged 21.3 days (lines 3 and 4). The average interval between manufacture and collection was 8.0 days for the 56 coded samples. This probably represents the average age of the product when purchased by the consumer. The average time for 18 uncoded samples to go bad was 12.3 days (line 5). The 9-day discrepancy between coded and uncoded samples (Table 6, lines 4 and 5) is likely due to the difference in manufacturing operations. The 18 uncoded samples were generally from small manufacturers that produce basket- and baker-type cheeses.

Despite the apparent underestimation of longevity by the manufacturer (Table 6, lines 3 and 4), 26.9% of the samples from six Connecticut manufacturers, in fact, were judged unacceptable before the estimated code date expired. For nine out-of-state manufacturers this value was 43.3%. This shows that for Connecticut consumers, purchases of cottage type cheeses, approximately one-third of the time, cannot be expected to remain acceptable to the specified last date of sale. However, the percentages for in- and out-of-state manufacturers are not significantly different.

TABLE 8. *Flavor criticisms of cottage type cheeses in 1973-74 and 1950, and average number of days to go bad from manufacture for 1973-74 samples*[1]

Flavor criticism	Present study		1950 study
	% of samples	Av. days to go bad from manufacture	% of sample
Bitter	24.3	19.4	18.0
Surface growth	20.3	23.6	NR[2]
Old/lacks freshness	16.2	21.8	NR
Rancid	10.8	7.8	NR
Putrid	8.1	20.7	NR
Yeasty	5.4	10.0	25.4
Fruity	5.4	17.8	8.2
No specific comment (or misc. in 1950 study)	4.1	27.7	15.6
Sour (includes acidy in 1950 study)	2.7	19.0	16.4
Oxidized	1.4	5[3]	NR
Unclean	1.4	16[3]	12.3
OK	0	—	1.6
Unnatural	NU[2]	—	2.5

[1]Flavor defect determined after 7-days-storage. Data adapted from Morgan et al. (*12*).
[2]NR indicates this criticism not recorded in 1950 study. NU = this criticism not used in present study.
[3]Single sample.

The reasons for samples to be judged unacceptable is shown in Table 8. Even though three criticisms, bitter flavor, surface growth, and old or lacking freshness (stale) accounted for 60% of rejections, it is important to note that the time to go bad from manufacture for samples with these three criticisms exceeded the average for all samples; 17.8 days (Table 6). Rancid and yeasty flavors accounted for 16% of rejections and the average time to attain these defects was about half that of all 74 samples.

Comparison with earlier studies

A comparison (Table 8) with an earlier study made in 1950 (*12*) showed that after 7-days storage the flavor defects bitter, yeasty, and sour predominated (60% of the samples). This difference in major flavor defects between the 1950 study and the present one may indicate a change, with time, in types of microbial contaminants. In the 1950 study 98% of the samples were unacceptable after 7 days. In the present study 76% remained acceptable 7 days after collection.

It has been pointed out often that psychrotrophic pseudomonads are important to keeping quality of dairy products (*17*). Recently Juffs (*9*) suggested that proteolytic psychrotrophs are important in raw milk in New Zealand. He found that proteolytic psychrotrophs comprised about 7.6% of the total bacterial count of raw milk and 54% of the total psychrotrophic count. With such a large proportion of psychrotrophs and their ability to excrete a wide variety of substances which might cause off-flavors, it is understandable why these organisms are important in keeping quality. Based on previous data that potential psychrotrophs are oxidase positive (*8*) the use of media for enumeration of proteolytic pseudomonads, and other important groups, as reported in this paper, could prove most useful in quality control testing.

ACKNOWLEDGMENTS

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

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