PROTEOLYTIC ORGANISMS IN BUTTER

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SUMMARY

Colonies producing zones on milk agar plates of routine butter samples were examined and it was found that in 55 per cent. of the zones, casein was not precipitated by tannic acid.

Organisms showing zone formation were isolated and examined for proteolytic properties. Approximately 20 per cent. of cultures showing proteolysis on milk agar were not caseolytic on casein agar, in casein broth or in milk.

Many organisms which were proteolytic were found to be heat-resistant.

In this series of cultures there was no relationship between proteolysis and lipolysis.

Only three cultures of Gram-negative rods were isolated among the proteolytic types. The remaining 47 cultures studied in detail were Gram-positive cocci and Gram-positive rods.

Statistical analysis of results for a large number of routine butter samples showed a relatively high correlation between total bacterial count and casein digester count and also between casein digester count and the presumptive coliform test.

It was concluded that there was no advantage in including a test for proteolysis on milk agar in the routine bacteriological tests for butter.

I. INTRODUCTION

Many workers (e.g. Hood and White 1928; Claydon and Hammer 1939; Pont 1941) have shown that certain flavour defects in butter, particularly those described as rabbito, putrefactive or surface taint, are caused by proteolytic organisms. Bacteria belonging to the genus *Pseudomonas* were found to cause these taints and the importance of water supplies as the source of these types has been emphasized by several investigators (Sorensen 1940; Turgasen 1940; Itzerott 1941). In addition to the above particular butter defects, some workers found that proteolysis by certain bacteria was responsible for off-flavours and lowered flavour scores (Spitzer, Parfitt, Manhart, and Epple 1927; Spitzer and Parfitt 1929; Elliker 1945). The presence of large numbers of lipolytic bacteria is also undesirable and can lead to the development of rancidity. When the effects of certain factors on the keeping quality of butter were studied by Guthrie, Schieb, and Stark (1936), they found that, in the absence of other factors, a direct correlation seemed to exist between the numbers of fat-splitting and casein-digesting bacteria present and spoilage of butter held at temperatures which permit bacterial growth.

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Stark and Schieb (1936) made an extensive investigation of fat-splitting and casein-digesting bacteria isolated from butter. These workers found that 96 per cent. of their cultures split tributyrin, 77 per cent. split fat and 63 per cent. were proteolytic on milk. These results suggest that determination of the numbers of lipolytic bacteria would give the best indication of the presence of undesirable lipolytic and proteolytic types. However, because of the toxicity of tributyrin and Nile blue sulphate, Stark and Schieb suggested that the use of milk agar to determine casein-digesters was important as an indication of keeping quality of butter.

A count of casein-digesting bacteria, intended to give an indication of the presence of potential fault-producing types, has been made regularly in the bacteriological analysis of samples for the Queensland Butter Improvement Service (Muller and Nichols 1950). The present investigation was undertaken to determine the types of bacteria which are found to produce zone formation on milk agar plates of butter samples, and to obtain information about their proteolytic and lipolytic properties.

II. DERIVATION OF ORGANISMS

Determination of the type of zone formation on milk agar was made initially on plates of samples of commercial salted butter which had been examined bacteriologically under the Queensland Butter Improvement Service. The zones produced were of two types: those which did not become cloudy when the agar was acidified by flooding the surface with 1 per cent. tannic acid, and those in which the casein was precipitated by the tannic acid. Colonies showing a zone not precipitated by a protein precipitant are normally considered to be proteolytic types.

In a series of 256 samples from 35 factories, 1,112 colonies producing zones were counted and the type of zone determined. In order to study in detail the properties of cultures producing zones, 74 such cultures were isolated from samples of butter from 22 factories. Fifty of these cultures produced zones not affected by tannic acid, and 24 produced zones precipitated by the acid.

In addition to the above types, 23 colonies which did not show zones on milk agar were isolated and purified. These cultures were obtained to compare the physiological properties of bacteria present in butter which did not affect the casein on milk agar plates.

III. METHODS

(a) Reaction on Milk Agar

In the routine bacteriological analysis of butter under the Queensland Butter Improvement Service, the total bacterial count has been made on milk agar, i.e. nutrient agar to which 5 per cent. sterile skim-milk has been added. A count

of casein-digesting organisms has been obtained by counting those colonies showing zone formation on the milk agar. Yeast and mould counts have been made on a synthetic yeast and mould medium, and a presumptive coliform test performed on 1/10 g butter in McConkey broth.

Purified cultures were stored on nutrient agar slopes, and nutrient broth was used for carrying cultures being tested.

Milk agar plates were poured, as in the routine testing, by adding 0·5 ml sterile skim-milk and 10 ml nutrient agar to the plate. Plates were incubated at 32°C and incubation for all tests with the exception of gelatin liquefaction was at this temperature.

Gram stains were made on 18 hr broth cultures of the organisms, using the Hucker modification of the Gram staining technique. Motility was determined from hanging-drop preparations of young cultures and the morphology from smears of skim-milk cultures, stained by Newman's stain.

Cultures were tested for heat resistance by holding at 65°C for 30 min, 1 ml of a young broth culture being sealed in a glass ampoule and completely immersed in water in a thermostatically controlled water-bath. The contents of the tube were plated on nutrient agar to determine survival after the heat treatment.

(b) Tests for Proteolysis

In addition to their reaction on milk agar, cultures were examined for hydrolysis of gelatin, proteolysis on casein agar, putrefaction of litmus milk, and casein breakdown in skim-milk, milk broth and liquid casein medium.

Gelatin liquefaction was determined by incubation of stab cultures at 22°C for 21 days. Gelatin medium was originally made up with and without peptone. However, the presence or absence of peptone made no difference to the reactions of the 60 cultures tested on both media, and subsequently nutrient gelatin including peptone was used.

Casein agar was prepared in a manner similar to that described by Frazier and Rupp (1928*a*), using nutrient broth instead of beef infusion. The casein solution and 3 per cent. agar were tubed in 5 ml amounts and mixed immediately before pouring the plates.

Putrefaction of litmus milk was determined by smell after 21 days at 32°C.

The determination of proteolysis in liquid media was done by the method described by Hull (1947) to estimate tyrosine and tryptophane, except that 2 ml of culture and 3 ml of distilled water were used with the original concentration of trichloroacetic acid. It has been shown that small amounts of protein hydrolysis, not detected by conventional methods, can be detected in this way.

The blue colour developed was measured in a direct reading photoelectric colorimeter using Ilford No. 607 orange filter. Control tests on uninoculated media were made in all series of tests. A standard tyrosine curve was prepared, using solutions of various tyrosine concentrations so that the colorimeter readings could be converted to tyrosine equivalent.

Casein medium was prepared containing 2 per cent. casein, which was dissolved in alkaline solution. The pH was adjusted to 7, phosphate buffer added in 0·02 M concentration, then 0·3 per cent. yeast extract and 0·5 per cent. peptone added. Initially tests were also made on media without peptone, but as in the case of gelatin liquefaction, the presence or absence of peptone did not affect the proteolysis, and subsequently all tests were made on media containing peptone.

Tests were also made for casein breakdown in milk broth. To 5 ml nutrient broth, 0·25 ml skim-milk was added, thus giving the same concentration of milk as was used in milk agar.

Determination of tyrosine production in milk, casein medium, and milk broth was made after growth of the cultures in these media for 7 days at 32°C.

(c) Tests for Lipolysis

Hydrolysis of fat was at first determined by using nutrient agar containing butterfat stained with Nile blue sulphate (Knaysi 1941). However, some cultures, especially the Gram-positive cocci, showed little or no growth on this medium. This is in agreement with the results reported by Jones and Richards (1952) concerning the inhibitory effects of Nile blue agar. Cultures were subsequently tested for lipolysis by streaking on nutrient agar containing 0.5 ml of a 3 per cent. fat emulsion in 10 ml agar. The incubated plates were flooded with a 1 in 1500 aqueous Nile blue sulphate solution to detect hydrolysed fat.

Hydrolysis of tributyrin was determined on nutrient agar containing 0·02 per cent. tributyrin. The tributyrin was toxic to many of the cultures and even at the above concentration growth of some was very poor.

IV. RESULTS

Details of the results of all tests on the 97 cultures isolated are shown in Tables 1, 2 and 3, in which the cultures are grouped according to their reaction on milk agar.

(a) Zone Formation on Milk Agar

The type of zone formation was examined on routine plates of butter tested in the Butter Improvement Service. In this way 1,112 colonies showing zones on milk agar were taken. Of these, 610, or 55 per cent., had zones which were not precipitated when the plates were flooded with tannic acid.

Table 1

RESULTS OF TESTS ON CULTURES SHOWING ZONES ON MILK AGAR NOT PRECIPITATED BY TANNIC ACID

No. of Cultures	Gram Stain	Morphology	C.D. on Milk Agar	C.D. on Casein Agar	Lipolysis		Spore Production	Heat Resistance	Motility	Hydrolysis of Gelatin	Proteolysis of—		
					Fat	Tributyrin					Milk Broth	Milk	Casein
2	+	cocci	+	+	−	−	−	+	−	+	+	+	sl
4	+	cocci	+	−	−	−	−	+	−	+	+	−	−
1	+	cocci	+	+	−	−	−	−	−	+	+	−	−
1	+	cocci	+	−	−	−	−	−	−	+	+	−	−
2	+	cocci	+	+	−	+	−	+	--	+	+	−	−
3	+	cocci	+	+	−	+	−	−	−	+	+	+	+
9	+	cocci	+	+	+	+	−	−	−	+	+	+	+
2	+	cocci	+	−	+	+	−	+	−	+	+	−	−
1	+	cocci	+	−	+	+	−	+	−	−	+	−	−
5	+	rods	+	+	−	−	−	−	−	+	+	+	+
4	+	rods	+	+	−	−	−	−	−	+	+	sl	+
2	+	rods	+	+	−	−	−	−	+	+	+	−	−
1	+	rods	+	−	−	−	−	−	+	+	+	+	−
2	+	rods	+	+	−	−	−	+	+	+	+	−	+
5	+	rods	+	+	−	+	+	+	+	+	+	+	+
2	+	rods	+	+	−	+	−	−	+	+	+	+	+
1	+	rods	+	−	−	+	−	+	+	+	+	sl	−
3	−	rods	+	+	−	−	−	−	−	+	+	+	+
50			50	40	12	25		19		49	50	35	35

Table 2

RESULTS OF TESTS ON CULTURES SHOWING ZONES ON MILK AGAR PRECIPITATED BY TANNIC ACID

No. of Cultures	Gram Stain	Morphology	C.D. on Milk Agar	C.D. on Casein Agar	Lipolysis		Spore Production	Heat Resistance	Motility	Hydrolysis of Gelatin	Proteolysis of—		
					Fat	Tributyrin					Milk Broth	Milk	Casein
1	+	cocci	p	−	−	−	−	+	−	+	−	−	−
2	+	cocci	p	−	−	−	−	+	−	−	−	−	−
5	+	cocci	p	−	+	+	−	−	−	+	−	−	−
1	+	cocci	p	−	+	+	−	−	−	+	−	sl	sl
2	+	cocci	p	+	+	+	−	−	−	+	−	−	+
1	+	rods	p	−	−	−	−	−	+	+	−	−	−
3	+	rods	p	+	−	−	−	+	+	+	+	−	sl
3	+	rods	p	−	−	+	+	+	−	−	−	−	−
3	+	rods	p	−	−	+	+	+	+	+	−	−	−
2	+	rods	p	−	−	+	+	+	+	+	sl	−	−
24				6	8	16		14		19	6	1	7

Table 3

RESULTS OF TESTS ON CULTURES WHICH DID NOT SHOW ZONE FORMATION ON MILK AGAR

No. of Cultures	Gram Stain	Morphology	C.D. on Milk Agar	C.D. on Casein Agar	Lipolysis		Spore Production	Heat Resistance	Motility	Hydrolysis of Gelatin	Proteolysis of—		
					Fat	Tributyrin					Milk Broth	Milk	Casein
3	+	cocci	—	—	—	—	—	—	—	—	—	—	—
4	+	cocci	—	—	—	—	—	+	—	—	—	—	—
3	+	cocci	—	—	—	+	—	—	—	—	—	—	—
4	+	cocci	—	—	—	+	—	+	—	—	—	—	—
1	+	cocci	—	—	+	+	—	—	—	—	—	—	—
3	+	rods	—	—	—	—	—	+	—	—	—	—	—
1	+	rods	—	—	—	—	+	+	—	—	—	—	—
1	+	rods	—	—	—	+	—	—	—	—	—	—	—
3	—	rods	—	—	—	+	—	—	—	—	—	—	—
23			—	—	1	12		12		—	—	—	—

Seventy-four cultures showing zones on milk agar were isolated for further study. It was found that 50, or 68 per cent., of these gave zones not precipitated by tannic acid.

The types of cultures showing proteolysis can be seen from Table 1. It is to be noted that only 3 of 50 isolates were Gram-negative rods, and that 25 were Gram-positive cocci.

(b) Digestion on Casein Agar

The reactions of all cultures on the casein agar of Frazier and Rupp (1928*a*) are included in Tables 1, 2 and 3.

Only 40 of the 50 cultures which gave zones on milk agar not precipitated by tannic acid showed proteolysis on casein agar. The other 10 cultures were not caseolytic on the casein agar. On the other hand, 6 of the 24 cultures giving precipitated zones on milk agar attacked the casein agar. The cultures which did not affect milk agar also showed no caseolysis on casein agar.

(c) Liquefaction of Gelatin

Forty-nine of the 50 cultures which produced zones on milk agar not precipitated by tannic acid liquefied gelatin. In addition, 18 cultures which showed zones which were precipitated also liquefied gelatin. All cultures which had no effect on milk agar did not attack gelatin. It would therefore appear that cultures showing other proteolytic powers were able to liquefy gelatin, but that many cultures able to attack gelatin did not hydrolyse other protein substrates.

(d) Proteolysis of Milk, Milk Broth and Casein Medium

Cultures which were actively proteolytic produced large amounts of tyrosine from milk and casein medium. Amounts of over 200 μg per ml in the cultures were considered to be positive, and the levels were generally much higher than this. Values between 80 and 200 μg per ml were recorded as slight and less than this amount as negative. In testing for proteolysis by this method in milk broth, the amounts of tyrosine produced were always of a much lower order. The most actively proteolytic cultures gave values in the range of 100 to 150 μg per ml. The reason for this difference in levels was not apparent. In this series of tests, values of over 90 μg per ml were considered to be positive, between 60 and 90 μg slight, and less than 60 μg negative.

A summary of the number of cultures attacking the above substrates is shown in Tables 1, 2 and 3. From Table 1 it can be seen that all cultures which gave zones not precipitated by tannic acid showed proteolysis in milk broth, but only 35 of the 50 attacked milk and 35 attacked liquid casein medium. In the second group of 24 cultures which showed precipitated zones on milk agar, 7 attacked milk broth, 1 attacked milk and 7 attacked casein. The 23 cultures which did not show zone formation on milk agar did not attack the liquid substrates.

(e) Peptonization of Litmus Milk

Reactions of the cultures in litmus milk at 32°C were noted after 7 days, and the cultures were checked again for off-flavours after 21 days. Twelve of the 25 cultures of casein-digesting rods, and two of the 25 cultures of casein-digesting cocci, peptonized milk. These cultures were strongly proteolytic in all other tests. The other strongly proteolytic cultures rapidly reduced and clotted the litmus milk and no peptonization could be detected.

(f) Relationship Between the Various Tests for Proteolysis

Gelatin appeared to be the substrate most easily attacked by organisms having any proteolytic powers. With one exception, cultures which did not attack gelatin did not attack any other substrate. There was generally good agreement between results in liquid and on solid media. Cultures which showed digestion on milk agar also showed proteolysis in the milk broth medium. However, 10 of these cultures were not truly caseolytic, as they did not attack casein agar or casein broth. There was also good agreement between the results of tests on casein agar and production of tyrosine and tryptophane from casein broth. Only three cultures giving a positive reaction on agar did not show caseolysis in broth medium. Five cultures which showed caseolysis in casein broth and on casein agar did not produce detectable amounts of tyrosine and tryptophane from milk and two cultures showed proteolysis in milk but not in the casein media.

From the results of tests on the 24 cultures which gave zones on milk agar precipitated by tannic acid, it appeared that 6 were caseolytic, and 3 others attacked milk or milk broth slightly. The remainder did not show any proteolytic powers on these substrates though some attacked gelatin. It would appear that the zones produced by these 15 cultures initially on milk agar were due to solution of the casein and not to digestion.

Cultures which did not produce zones on milk agar did not show any proteolytic properties.

(g) Lipolysis

Lipolytic properties were tested, using butterfat and tributyrin as substrates. Twenty-two cultures split fat and tributyrin and another 32 split tributyrin but not fat; 55 per cent. of the fat-splitting cultures and 48 per cent. of the cultures splitting tributyrin were also proteolytic. Therefore in this group of cultures there was no marked relationship between lipolytic and proteolytic properties.

(h) Heat-resistance

All cultures were tested for their ability to withstand heating to 65°C for 30 min. A total of 45 cultures was heat-resistant, these comprising 19 showing permanent zones on milk agar, 14 showing zones precipitated by tannic acid, and 12 which did not affect milk agar. The heat-resistant types included 22 cultures of cocci and 10 Gram-positive non-sporing rods in addition to the 13 cultures of Gram-positive sporing rods.

The percentage of heat-resistant types among the cultures studied here was much greater than that found by Stark and Schieb (1936). From 486 isolations, these workers obtained only 34 sporing rods and 7 Gram-negative non-sporing rods which were found to be heat-resistant. These organisms were obtained, however, from sweet cream butter, the cream apparently not being pasteurized. The cultures investigated here were all obtained from butters made from heat-treated cream. Therefore, it was to be expected that the total flora should contain a higher percentage of heat-resistant types. It was found that an appreciable number of the proteolytic and lipolytic bacteria were among the heat-resistant forms.

(i) Relationship between Casein Digester Count and other Tests on Routine Butter Samples

A summary of the distribution of total bacterial count and casein-digester count in 1971 samples examined under the Butter Improvement Service over a period of 12 months is shown in Table 4.

Table 4

RELATIONSHIP BETWEEN TOTAL BACTERIAL COUNT AND CASEIN DIGESTER COUNT IN ROUTINE ANALYSES OF BUTTER SAMPLES

Casein Digesters per ml ('000)	No. of Samples with Total Bacterial Counts in Various Categories ('000 per ml)					Total
	< 20	> 20 and < 50	> 50 and < 100	> 100 and < 300	> 300	
< 1	244	75	45	25	4	393
1 to 3	325	143	56	28	9	561
4 to 5	85	72	24	13	2	196
> 5 and < 10	48	106	39	18	..	211
> 10 and < 20	10	48	41	22	5	126
> 20	..	42	97	218	127	484
Total ..	712	486	302	324	147	1971

These figures have been analysed statistically to determine the relationship between the two counts. Class intervals for bacterial counts were taken to be 20, 40, 80, 160, 450, or in terms of logarithms to the base 2 at relative values of 1, 2, 3, 4, 5·5. On this scale the variance within classes for number of casein digesters was reasonably constant, the average value being 0·996. The mean bacterial counts are summarized in Table 5. If the casein-digester count classes are taken to be centred at equally spaced log. values, 1, 2, 4, 8, 16, and 32, the correlation coefficient between the two counts is 0·6513.

Table 5

STATISTICAL ANALYSIS OF THE RELATIONSHIP BETWEEN TOTAL BACTERIAL COUNT AND NUMBER OF CASEIN DIGESTERS

	No. of Casein Digesters per ml ('000)	Bacterial Count		Percentage of Samples > 100,000 per ml
		log.	Equivalent Count per ml	
a	<1	1·66	31·5	7·4
b	1–3	1·68	32·0	6·6
c	4–5	1·86	36·2	7·7
d	>5–<10	2·13	43·7	8·5
e	>10–<20	2·73	66·5	21·4
f	>20	4·02	162·1	71·3

Significant differences f >> e >> d >> c > b, a

The relationship between casein-digester count and results of the presumptive coliform test has also been determined for the same 1971 samples. The results of tests are shown in Table 6. A X^2 test gave a value of 130·0 (5 D.F.), indicating that these percentages were not homogeneous. This reflects the obvious increase in positive tests with increasing casein-digester counts.

Table 6

RELATIONSHIP BETWEEN CASEIN DIGESTER COUNT AND PRESUMPTIVE COLIFORM TEST

Casein Digester Count	No. of Samples	Percentage of Positive Coliform Tests
<1	412	16·3
1–3	577	18·9
4–5	202	21·3
6–10	216	29·6
11–20	117	32·5
>20	491	48·5

V. DISCUSSION

In this investigation, the zone formation on milk agar plates by cultures isolated from butter has been compared with their ability to hydrolyse gelatin and break down casein in liquid media. It appeared that proteolysis on milk agar was not directly related to casein breakdown in milk, in casein broth or on casein agar. Hammer (1948, p. 101) similarly noted that some organisms which are actively proteolytic on milk agar have only limited action when inoculated into milk. He suggested that this may be due to the abundance of sugar in the milk which the organisms use in preference to casein. However, such organisms in this study were not proteolytic in casein media in the absence of sugar. A similar result was obtained by Frazier and Rupp (1928 *a*), who found that cultures

which were not caseolytic broke down lactalbumin in milk and failed to give a postive test for tryptophane. Frazier and Rupp (1928 *b*) also found that some cultures which were caseolytic were able to decompose lactalbumin as well as casein and these workers suggested that such cultures may attack lactalbumin preferentially in milk.

In the tests reported here a few cultures were caseolytic on the casein agar but not in the casein broth. In the latter case the casein was in the form of sodium caseinate, whereas in the agar medium it was present as calcium caseinate. Frazier and Rupp (1928 *b*) found that some cultures attacked the calcium salt more readily and this effect could have been operative here.

A few cultures which produced zones on milk agar precipitated by acid were found to be caseolytic in other tests. This serves to emphasize the difficulty of determining whether or not digestion has taken place on milk agar. On some occasions casein was reprecipitated when the plate was flooded with acid but the zone did not become as dense as the background, suggesting that some caseolysis had taken place, and care had to be taken in reading the result. The interpretation of casein-digestion on poured plates with a mixed flora is complicated because the size of the zone and the clarity depend on the depth of the agar, whether the colony is a surface or a sub-surface one, and the density of colonies on the plate.

The types of organisms isolated in the present study and found to be proteolytic differed markedly from the distribution found by Stark and Schieb (1936). In the present study only 3 of 50 isolates were Gram-negative rods and half the total were Gram-positive cocci. This difference can be attributed to the fact that Stark and Schieb made their isolations from sweet cream, unsalted butter, the cream apparently not being heat-treated, whereas butter examined here was made from neutralized, vacreated cream.

When all isolates were tested for lipolytic properties it was found that 22 per cent. hydrolysed fat and 55 per cent. hydrolysed tributyrin. However, there appeared to be no definite relationship between proteolysis and lipolysis. All cultures hydrolysing fat were Gram-positive cocci. These types were found by Jones and Richards (1952) to be the dominant lipolytic flora in butter and milk.

In Queensland a count of casein-digesting organisms has been utilized for some time in the Butter Improvement Service scheme, its purpose being to give some estimate of the presence of potentially fault-producing types. It was also considered that this count would give an indication of contamination after processing, as many workers have shown that types of organisms frequently occurring in contaminated water supplies are proteolytic. The results obtained here, however, show that a count of casein digesters includes types of organisms which are not caseolytic and do not cause proteolysis in milk. It therefore seems that many types of organisms would be included which would not cause lowering of quality because of proteolysis.

It has also been shown here that a substantial proportion of the proteolytic organisms is heat-resistant. Heat-resistance in these studies was examined by holding in broth at 65°C for 30 min. Treatment of cream for buttermaking is now at much higher temperatures than this. However, because of the protective action of fat in the cream during heating, the short time of exposure of the cream to high temperatures, and the fact that many of the heat-resistant types were spore formers, at least some of these heat-resistant forms could arise from the cream. Therefore the casein-digesting count cannot be expected to give reliable information of post-pasteurization contamination. In any case, the presumptive coliform test has been used successfully to indicate contamination after heat treatment and there is no need to attempt to use another test for this purpose.

It has also been stated (Stark and Schieb 1936) that there is a high correlation between proteolytic and lipolytic properties and that therefore the use of milk agar to determine casein-digesters was important as an indication of butter keeping quality. However, a marked relationship between proteolytic and lipolytic properties was not found to exist for organisms isolated in the present study, so that a count of casein digesters would have little relation to potential deterioration due to lipolytic organisms.

A statistical analysis of the results for total bacterial count and casein digester count in routine examinations of a large number of samples of butter showed that there was a relatively high correlation between these two values. This indicates that samples with high plate counts can be expected to exhibit also high casein digester counts. There was a similar finding with regard to the relationship between the presumptive coliform test and the casein digester count. Butters with positive results in the coliform test were found to have relatively high casein digester counts.

In view of these results and of the other tests reported in this paper it would seem that there is little ground for retaining a casein-digester count on milk agar in bacteriological tests on butter. It should be adequate to take the total bacterial count and the presumptive coliform test, in conjunction with tests for yeasts and moulds to indicate the bacteriological quality, without recourse to a casein-digester count which is of doubtful significance. There does not seem to be any need to perform tests, as a routine procedure, for specific groups of organisms such as lipolytic organisms, proteolytic organisms, casein-digesting Gram-negative rods, and psychrophilic organisms.

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