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A study of methods of determining the numbers of yeasts and molds in butter

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A STUDY OF METHODS OF DETERMINING THE NUMBERS OF YEASTS AND MOLDS IN BUTTER

BY

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INTRODUCTION

At the present time a great deal of attention is being given to the sanitary quality of the various dairy products. The work along this line has involved principally determinations of the numbers of bacteria in milk but has also included determinations of the numbers of bacteria in market cream, ice cream, etc.; and, in some instances, the presence of certain types of bacteria has been investigated.

In the case of butter, total bacterial counts can not logically be used in studying the general conditions of manufacture and handling because butter culture is often added to the cream and the bacterial content of the finished butter is strikingly influenced in this way. Yeast and mold counts on butter have, accordingly, been suggested because these organisms should be present in only very small numbers, if at all, in butter that is efficiently made from pasteurized cream and then properly handled in thoroughly cleansed equipment.

The yeast and mold count of butter at the present time is used chiefly by the manufacturers and wholesale buyers of butter. Its use in the United States by control and health officials has been limited. In Canada it is used extensively in educational contests to improve the conditions under which butter is manufactured.

Recently within the United States a great deal of emphasis has been placed upon the extraneous biological matter in butter by certain control officials. There is every reason to believe that in the control of this extraneous biological matter yeast and mold counts will be used in aiding in the interpretation of the results secured.

STATEMENT OF PROBLEM

The work herein reported involves a study of methods of determining the number of yeasts and molds in butter as to; (a) the influence of composition of media upon the yeast and mold count, (b) the influence of variations in composition and in methods of preparation of potato dextrose agar upon the yeast and mold count, (c) the influence of the kind of acid used to acidulate the medium and of the degree of acidulation upon the yeast and mold count, (d) the influence of incubation time upon the yeast and mold count, (e) the influence of the oxidation-reduction potential of the medium upon the growth of yeasts and molds, (f) attempts to develop special methods for the yeast and mold count of butter, and (g) some factors influencing the number of yeasts in butter.

GENERAL HISTORICAL

Bouska and Brown [4] found that butter with less than 10 yeasts and molds per ml. was safer for long shipments and storage than butter with a higher yeast and mold content. These investigators considered that a count of 30 or more yeasts and molds per ml. of butter indicated one or more of the following defects: failure to pasteurize; lack of cleanliness and of sterility of utensils, pipe lines and churns; or contaminated starter.

Hood and White[13] reported that there was no test so valuable and so full of information as the yeast and mold count in attempting to control the mold in butter. They found that it furnished a dependable check on the efficiency of pasteurization and sterilization of equipment. These investigators recommended the following standards in judging the sanitary efficiency of creamery operation: below 10 yeasts and molds per ml., excellent; 11 - 50, good; 51 - 100, fair; and over 101, poor.

The standards for satisfactory butter plant operation used by some of the centralizer creameries in the United States vary from less than 10 to less than 50 yeasts and molds per ml. of butter. In some of the Canadian provinces where yeast and mold counts have been used for several years as part of the butter score for samples of exhibition butter,

a perfect score is not given unless the butter contains no yeasts and molds per ml.

In 1928 the American Dairy Science Association appointed a committee to suggest methods for the microbiological analysis of butter. This committee has made two reports[20,21] suggesting methods for yeast and mold counts with the idea that these methods would be studied and used in order that in the future there may be prepared standard methods for the microbiological analysis of butter.

GENERAL METHODS

Samples of butter were secured from tubs and prints in the manner outlined in "Suggested Methods for the Microbiological Analysis of Butter "(20). Approximately 25 grams of butter were taken and placed in 4 oz. sterile glass stoppered bottles and stored at about 0° C. until analyzed.

Butter to be analyzed was softened in a 45° C. water bath. After the butter had reached the consistency of thin cream (usually in about 10 minutes) it was thoroughly shaken and 10 ml. removed with a warm pipette and placed in a 90 ml. water blank, the water in the blank being at 42 - 45° C. The pipettes used were calibrated to contain 10 ml., and after the melted butter had drained from the pipette, warm dilution water was sucked up into the pipette three times in order to rinse out the butter adhering to the walls of the pipette. After shaking 25 times through an arc of approximately 1 foot, a quantity of 2.2 ml. of the dilution was removed by means of a pipette graduated to deliver two 0.1 ml. quantities and two 1.0 ml. quantities; by means of such pipettes dilutions in duplicate could be rapidly prepared.

After liquefying, the hot media were acidulated to pH 3.5 ± 0.1, using tartaric acid unless otherwise specified. The accuracy of acidulation was determined electrometrically by means of the quinhydrone electrode system. After the agar

had solidified, the plates were inverted and incubated for five days at 21° - 25° C. Counting was done with a specially constructed illuminator which aided in differentiating yeast and mold colonies from fat globules.

The media used were prepared as follows:

Potato dextrose agar. An infusion of potatoes was prepared by boiling 200 grams of peeled and sliced potatoes for 1 hour in 1000 ml. of distilled water. It was strained through a double thickness of cheesecloth and if less than 1000 ml. was obtained, it was brought up to this volume with distilled water. To this were added 2.0 per cent of commercial dextrose and 1.5 per cent of agar agar. The reaction of the medium after sterilization for 20 minutes at 15 pounds steam pressure was approximately pH 6.0.

Potato dextrose agar suggested by Weiser[26] is the same as the above with the exception that 0.5 per cent peptone and 0.1 per cent ammonium sulphate were added. The reaction of this medium after sterilization was approximately pH 6.2.

Bacto potato dextrose agar is a dehydrated product prepared by Digestive Ferments Company, Detroit, Michigan. Its composition was as follows: 0.5 per cent infusion of dried potatoes, 2.0 per cent dextrose, 1.5 per cent agar agar and 1 liter of distilled water. After sterilization the

reaction was approximately pH 5.5.

Whey agars made in the laboratory were prepared from fresh skimmed milk. The milk was heated to 38 - 40° C. and 2.0 ml. of rennet extract added per liter. The milk was allowed to curdle firmly, the curd thoroughly broken up to facilitate rapid contraction, and the whey separated by straining through cheesecloth. The whey was divided into two equal parts. To part one was added 0.5 per cent peptone and 1.5 per cent agar agar, calculated on the basis of total volume of whey; it was then heated to dissolve the peptone and agar agar, the water lost restored; and after cooling to 55° C. it was combined with part two which had been heated to 55° C. The pH was adjusted to 7.5, 6.8, or 5.5. Egg was added in the proportion of one whole egg per 2 liters of medium and distributed thoroughly by pouring the medium from one container to another. The medium was then autoclaved, in order to coagulate the albumins, and filtered through cotton, after which it was flasked in definite quantities and sterilized. The resulting pH was approximately the same as the pH to which the whey had been adjusted prior to filtering.

Bacto whey agar was prepared from the dehydrated product manufactured by Digestive Ferments Company, Detroit, Michigan, and had the following composition: 1.3 per cent

dry whey, 1.0 per cent peptone, 0.5 per cent sodium chloride, 1.2 per cent agar agar and 1 liter of distilled water. This medium after sterilization was at a pH of approximately 6.5.

Tomato juice agar had the following composition: Tomato juice procured by straining canned tomatoes through cheesecloth. To 400 ml. of tomato juice was added 600 ml. of distilled water which contained 10 grams of peptone, 20 grams of dextrose and 15 grams of agar agar. The tomato juice was added after the peptone, dextrose and agar agar had been dissolved in the distilled water. The pH of this medium after sterilization was 6.8.

Bacto tomato juice agar was composed of 1 liter of distilled water, 2.0 per cent dried tomato juice, 1.0 per cent peptone, 1.0 per cent peptonized milk, 1.1 per cent agar agar and 2.0 per cent dextrose. The approximate pH of this medium after sterilization was 6.1.

The composition of 5 per cent dextrose nutrient agar was 0.5 per cent peptone, 0.3 per cent beef extract, 5.0 per cent dextrose, 1.5 per cent agar agar and 1 liter of distilled water. The final pH of this medium after sterilization was 6.8.

Two per cent dextrose beef infusion agar was prepared by infusing one pound of lean chopped beef in 500 ml. of distilled water for 18 hours at 0° - 10° C., then draining

through cheesecloth; and the liquid obtained, if less than 500 ml., was brought to this volume with distilled water, and then warmed to 50° C. In 500 ml. of distilled water 5 grams of peptone and 15 grams of agar agar were dissolved by steaming, then cooled to 50° C., the two 500 ml. quantities were mixed and adjusted to pH 6.8. The mixture was autoclaved for 45 minutes at 15 pounds, filtered through cotton, and flasked and sterilized. The pH of the medium after sterilization was approximately 6.8.

Bacto wort agar is composed of the following ingredients: 1.275 per cent maltose, 1.5 per cent malt extract, 0.275 per cent dextrin, 0.235 per cent glycerol, 0.1 per cent dipotassium phosphate, 0.1 per cent ammonium chloride, 0.078 per cent peptone, 1.5 per cent agar agar, and distilled water to make 100.00 per cent. The pH of this medium was approximately 4.8.

Bacto malt agar was composed of malt extract 3.0 per cent, agar agar 1.5 per cent in 1 liter of distilled water. The pH of this medium was approximately 5.5.

Bacto yeast dextrose agar had the following composition: 0.3 per cent beef extract, 0.1 per cent yeast extract, 0.5 per cent peptone, 0.25 per cent tryptone, 0.1 per cent dextrose, 1.5 per cent agar agar and 1 liter of distilled water. After sterilization the pH of the medium was approxi-

mately 7.0.

The pure cultures of yeasts used in some of the experiments were: Torula cremoris, Torula sphaerica, Pink B (Torula glutinis), Liquefier B, Liquefier 106 and Common white P.

These yeasts had been isolated from dairy products by Dr. B. W. Hammer and associates at the Iowa State College. Other yeasts, designated by number, were isolated from samples of butter and used in certain trials; they were of the common white type and differed from each other in colony appearance.

Part 1

INFLUENCE OF COMPOSITION OF MEDIA UPON THE YEAST AND MOLD COUNT OF BUTTER.

Historical

A review of the literature on the procedures employed to determine the yeast and mold counts of butter reveals considerable variation in the composition and methods of preparation of the media used.

Lund[17] employed a medium prepared from unhopped beer wort. Hood and White[14] used a similar medium. Hunziker[15], Macy[18], Bouska and Brown[4], Spitzer and Parfitt[24], and Grimes[9] employed whey as a nutrient base while Stiritz[25] and Abbott[1] used "near beer". Hood and White[14] compared six media for the determination of yeasts and molds in butter. The media used were (a) Bacto wort agar, (b) Bacto malt extract agar, (c) Bacto whey agar, (d) Bacto potato agar, (e) beer wort agar, and (f) whey agar; the beer wort agar and the whey agar were prepared in the laboratory. The conclusion of these investigators was that all six media used were equally suitable for determining the yeast and mold count of butter.

Parfitt[22] compared the suitability of 10 media for the determination of the yeast and mold counts, using 73 samples of butter. The media used were (a) Bacto wort agar, (b) Bacto malt agar, (c) Bacto whey agar, batch A, (d) Bacto whey agar, batch B, (e) whey agar, the whey being

obtained by rennet coagulation, (f) whey agar, the whey being obtained by acid coagulation, (g) 1 per cent lactose beef infusion agar, (h) standard nutrient agar, (i) 1 per cent lactose standard nutrient agar, and (j) 5 per cent dextrose standard nutrient agar. The Bacto agars (wort, malt and whey) gave the highest yeast and mold counts of the 10 media studied; whey agar prepared by rennet coagulation gave the lowest counts.

The committee appointed by the American Dairy Science Association has recommended several media in its reports on methods to be used in counting the numbers of yeasts and molds in butter. In its first report[20] four media were suggested; these were (a) Bacto malt agar, (b) Bacto whey agar, (c) whey agar prepared by rennet coagulation, and (d) whey agar prepared by acid coagulation. The second report of this committee[21] recommended the use of (a) Bacto malt agar, (b) Bacto wort agar, and (c) potato dextrose agar.

A. Influence of media of various composition upon the yeast and mold count of butter.

In order to determine the relative values of some of the various media which have been used for the enumeration of yeasts and molds in butter, samples of butter were secured from 39 tubs, from 39 Iowa creameries. The samples were analyzed for the numbers of yeasts and molds present, using

12 media.

The media employed were as follows:

1. Potato dextrose agar
2. Potato dextrose agar (Weiser)
3. Bacto potato dextrose agar
4. Whey agar secured from whey filtered at pH 7.5
5. Whey agar secured from whey filtered at pH 6.8
6. Whey agar secured from whey filtered at pH 5.5
7. Bacto whey agar
8. Tomato juice agar
9. 5 per cent dextrose nutrient agar
10. 2 per cent dextrose beef infusion agar
11. Bacto wort agar
12. Bacto malt agar

Four samples of butter were analyzed at a time. The time required to prepare dilutions of the four samples of butter and complete pouring of the plates with the 12 media was about 45 minutes. In order to distribute any variation that might be due to the length of time the melted butter was held in the plates before adding the agar, the order in which the media were poured was varied on a regular basis. An analysis of the data showed there was no relationship between the counts obtained on the various media and the order in which the media were poured, which indicates that signifi-

cant growth or destruction of organisms had not taken place during the plating period.

The yeast and mold counts obtained on the samples of butter are given in Table 1. Six samples of butter which showed no yeasts and molds on any of the media, and three samples in which the numbers of yeast and mold colonies were too numerous to count on the 1 to 100 dilution, were omitted from Table 1.

There were wide variations in the yeast and mold counts obtained with the 12 media studied. The means ranged from 21.0 upon whey agar prepared from whey filtered at pH 7.5 to 110.8 upon potato dextrose agar.

Potato dextrose agar gave a higher mean yeast and mold count than did potato dextrose agar prepared according to the formula suggested by Weiser, the means being 110.8 and 100.6 respectively. The potato dextrose agar gave higher counts on 18 samples of butter than did the medium suggested by Weiser. The difference between these media is slight and is within the probable limits of error of plating, but the counts indicate that the addition of 0.5 per cent of peptone and 0.1 per cent ammonium sulphate, which are contained in the Weiser formula and not in the potato dextrose agar, are of no value in stimulating or supporting the growth of yeasts and molds in plates. Bacto potato dextrose agar gave a mean

count of 94.7 and gave a higher plate count with seven samples of butter than did potato dextrose agar prepared in the laboratory; the remaining samples of butter gave higher counts on potato dextrose agar. The Bacto potato dextrose agar is a medium especially prepared for work in plant pathology and mycology. It is prepared from potatoes grown under special conditions. From the data there is no evidence that potatoes grown under such conditions are advantageous in the counting of yeasts and molds in butter.

The media prepared from whey did not give so high yeast and mold counts as the media prepared from potatoes. Whey agar prepared from whey which was adjusted to pH 5.5 before filtering gave the highest mean count of the whey media, the mean being 78.6. This medium gave higher counts for six samples of butter than did potato dextrose agar, while the remaining 24 samples of butter gave higher counts upon potato dextrose agar.

Within the whey agars there existed a correlation between the growth of yeasts and molds and the pH at which the whey obtained by rennet coagulation was filtered. Whey agar prepared from whey filtered at pH 5.5 gave a higher mean count than the whey agar prepared from whey filtered at pH 6.8; the lowest mean count of the whey agars prepared in the laboratory was secured with the whey agar in which the whey

Res

Sample of Butter	1	2	3	4	5	6	7	8
Medium								
Potato dextrose agar	4	18	8	17	6	4	18	19
Potato dextrose agar (Weiser)	7	20	7	10	6	2	5	6
Bacto potato dextrose agar	2	16	5	13	6	4	8	9
Whey agar (whey pH 7.5)	0	2	1	10	2	4	1	9
Whey agar (whey pH 6.8)	2	7	0	14	3	2	4	1
Whey agar (whey pH 5.5)	4	16	1	16	7	4	4	7
Bacto whey agar	1	6	2	11	5	4	1	7
Tomato juice agar	2	17	1	13	7	5	9	9
5% dextrose nutrient agar	1	6	5	14	5	1	10	12
2% dextrose beef infusion agar	2	6	0	9	4	0	0	1
Bacto wort agar	2	6	3	12	4	2	18	12
Bacto malt agar	2	10	3	11	4	1	8	5

Table 1

Influence of the medium upon the yeast and mold counts of butter.

Results expressed as the average count per plate containing 0.1 ml. of butter

8	9	10	11	12	13	14	15	16	17	18	19	20	21
19	53	2	10	7	30	59	23	139	76	30	36	29	2
6	49	0	18	8	46	49	24	57	78	20	47	32	3
9	23	10	12	4	25	54	24	64	90	25	30	24	2
9	0	0	0	2	9	41	13	1	3	5	33	10	1
1	5	2	5	13	30	37	16	12	18	12	37	27	1
7	21	0	7	12	6	50	25	20	56	14	31	38	2
7	2	0	4	9	23	48	24	2	26	17	33	21	1
9	23	10	12	4	46	57	27	43	68	27	37	30	2
12	11	4	24	9	25	53	28	73	32	19	51	23	1
1	5	0	15	4	27	59	8	72	6	3	20	13	1
12	21	0	10	0	27	59	8	72	7	16	64	26	1
5	22	1	4	7	18	54	23	20	64	21	27	27	2

r.

butter

20	21	22	23	24	25	26	27	28	29	30	Mean
29	29	83	48	565	114	89	154	593	789	274	110.8
32	32	61	47	645	85	151	108	451	680	267	100.6
24	27	83	60	574	105	140	103	373	680	248	94.7
10	10	12	16	138	39	25	5	63	89	88	21.0
27	12	27	34	302	57	55	34	114	458	210	51.7
38	25	60	54	532	82	125	113	186	593	248	78.6
21	12	61	27	159	72	55	20	51	346	194	41.4
30	22	78	44	489	105	87	141	230	728	247	87.4
23	14	81	45	505	94	89	140	478	416	240	83.6
13	17	7	5	409	99	27	2	44	145	68	35.9
26	18	60	45	409	99	84	100	634	674	243	91.4
27	28	58	46	521	85	138	114	246	511	251	77.7

prior to filtering was adjusted to pH 7.5. These results are in agreement with those secured by Parfitt[22], who found that whey agar prepared from whey which was secured by acid coagulation of the milk at pH 4.6 gave higher yeast and mold counts than whey agar prepared from whey which was obtained by rennet coagulation, the pH being adjusted at filtering to pH 6.8. Bacto whey agar gave a mean count of 41.4, which approaches the mean yeast and mold count obtained on whey agar prepared from whey filtered at pH 6.8. Bacto whey agar gave higher counts on two samples of butter than potato dextrose agar. The differences between the counts obtained on samples of butter when plated on Bacto whey agar and on potato dextrose agar vary widely. With samples 14 and 19 the differences in yeast and mold counts upon the whey agars and upon potato dextrose agars are slight, while with samples 16 and 27 the differences are large. These results indicate that whey does not make a favorable base for a medium to be used in counting yeast and molds in butter.

The mean yeast and mold count of tomato juice agar was 87.4. Eight samples of butter gave higher yeast and mold counts upon tomato juice agar than upon potato dextrose agar.

The mean yeast and mold count that was obtained for samples of butter plated upon 5 per cent dextrose nutrient agar was 83.6, and there were five samples of butter which

gave higher counts upon this medium than upon potato dextrose agar.

Of the media studied beef infusion was next to the poorest in supporting yeast and mold growth, the mean count being 35.9. One sample of butter gave a higher yeast and mold count upon 2 per cent dextrose beef infusion agar than upon potato dextrose agar. Four samples of butter gave no growth upon the medium prepared from beef infusion, while upon potato dextrose agar the counts on the same samples ranged from 2 to 18. With butter sample number 27 the yeast and mold count per 0.1 ml. of butter was 2, on beef infusion medium upon potato dextrose agar it was 154, and upon tomato juice agar it was 141; in contrast to this, butter sample number 14 gave a count of 59 upon beef infusion medium which equals the count on potato dextrose agar.

The mean count upon Bacto wort agar was 91.4 and upon Bacto malt agar it was 77.7. On 14 of the 30 samples of butter, Bacto wort agar gave the higher count of the two media, and Bacto malt agar on 13 samples, while with 3 there was no difference. Samples of butter 17 and 28 show that not all yeasts and molds grow equally well on these two media. In comparison with potato dextrose agar, Bacto wort agar gave higher counts on but 3 of the 30 samples of butter analyzed.

In order further to test the significance of the effect

of medium upon yeast and mold count, the 30 samples of butter were divided into three groups as follows: Group 1, in which there are 11 low count samples; Group 2, in which there are 12 samples whose mean counts on all media fell approximately midway between the low and high count samples; and Group 3, in which there are 7 high count samples. The results of these groups are shown in Table 2.

The range in the mean yeast and mold counts per 0.1 ml. of the low count samples of butter was from 3.0 upon whey agar prepared from whey filtered at pH 7.5 to 10.0 upon potato dextrose agar. The mean yeast and mold counts per 0.1 ml. in median count samples of butter ranged from 14.0 upon whey agar prepared from whey filtered at pH 7.5 to 53.1 upon potato dextrose agar. The mean yeast and mold counts per 0.1 ml. on the high count samples of butter varied from 63.9 upon whey agar prepared from whey filtered at pH 7.5 to 368.3 upon potato dextrose agar.

The grouping of the 30 samples of butter into low, median and high count groups shows that the various media rank in about the same order within each group. This grouping is shown in Table 3 in which the media are ranked in the order from high to low count.

Table 2

Influence of the medium upon the yeast and mold count of butter.

Samples of butter grouped according to mean count

Medium	11 Low Count Samples	12 Median Count Samples	7 High Count Samples
Potato dextrose agar	10.0	53.1	368.3
Potato dextrose agar (Weiser)	8.1	40.8	341.0
Bacto potato dextrose agar	8.2	43.0	317.6
Whey agar (whey pH 7.5)	5.0	14.0	65.9
Whey agar (whey pH 6.8)	4.6	21.6	175.7
Whey agar (whey pH 5.5)	7.1	29.4	268.4
Bacto whey agar	4.5	22.8	181.4
Tomato juice agar	9.3	43.7	289.6
5% dextrose nutrient agar	9.2	43.2	280.3
2% dextrose beef infusion agar	3.5	20.2	113.4
Bacto wort agar	7.3	37.4	320.4
Bacto malt agar	4.6	34.0	266.6

Table 5

Influence of medium upon the yeast and mold count of butter.

Ranking of medium according to mean count within each group

Medium	Low Count Samples	Median Count Samples	High Count Samples
Potato dextrose agar	1	1	1
Potato dextrose agar (Weiser)	5	5	2
Bacto potato dextrose agar	4	4	4
Whey agar (whey pH 7.5)	12	12	12
Whey agar (whey pH 6.8)	9	10	10
Whey agar (whey pH 5.5)	7	8	7
Bacto whey agar	10	9	9
Tomato juice agar	3	2	5
5% dextrose nutrient agar	2	3	6
2% dextrose beef infusion agar	11	11	11
Bacto wort agar	6	6	3
Bacto malt agar	8	7	8

B. Comparison of Bacto dextrose nutrient agar and dextrose nutrient agar upon the yeast and mold count of butter.

White[27] found that 2 per cent dextrose nutrient agar was equal to Bacto malt agar in supporting the growth of the yeasts and molds found in butter. These results are not in agreement with those of Parfitt[22] who reported that 5 per cent dextrose nutrient agar was not equal to Bacto malt agar in the enumeration of the yeasts and molds in butter. White in his work used Bacto dextrose nutrient agar, while Parfitt used dextrose nutrient agar prepared in the laboratory from Bacto products.

In order to compare 2 per cent dextrose Bacto nutrient agar with 2 per cent dextrose nutrient agar made in the laboratory from Bacto products for the counting of yeasts and molds in butter, 23 samples of Indiana butter were plated on these media and also as controls upon Bacto malt agar and potato dextrose agar. The counts secured are given in Table 4.

The mean yeast and mold count secured on the Bacto dextrose nutrient agar was 235.6; on dextrose nutrient agar prepared in the laboratory it was 228.3. Of the 23 samples of butter analyzed, 11 gave higher counts when the dehydrated product was used as the medium and 9 when the medium prepared

Table 4

Comparison of Bacto nutrient agar and nutrient agar prepared in the laboratory upon the yeast and mold count of butter using as controls Bacto malt agar and potato dextrose agar.

Results expressed as the average count per ml. of butter

Sample of Butter	2% dextrose Bacto nutrient agar	2% dextrose nutrient agar	Bacto malt agar	Potato dextrose agar
1	70	60	70	70
2	40	50	80	70
3	130	90	50	160
4	1530	1560	1070	1800
5	110	450	540	340
6	60	50	30	260
7	280	292	130	160
8	110	40	140	60
9	80	60	50	50
10	120	30	60	70
11	250	60	280	340
12	110	10	40	110
13	50	91	70	100
14	20	30	30	50
15	160	160	310	260
16	0	0	10	10
17	330	350	420	280
18	180	160	220	130
19	0	0	40	50
20	1010	1060	1370	1250
21	80	40	40	30
22	220	260	250	290
23	480	350	510	450
Mean	235.6	228.3	252.6	277.8

in the laboratory was used, while the remaining three samples showed no difference.

The mean yeast and mold count secured on potato dextrose agar was 277.8; on Bacto malt agar it was 252.6. Potato dextrose agar gave the higher count on 11 samples, and Bacto malt agar gave the higher count on 9 samples; in the remaining three samples there was no difference in count. In comparing potato dextrose agar with Bacto dextrose nutrient agar, higher counts were obtained on 13 samples of butter when potato dextrose was used as the medium, and on eight samples the higher count was secured on Bacto dextrose nutrient agar, the remaining two samples showing no difference in count.

C. Influence of the addition of peptone to whey agar upon the yeast and mold count of butter.

In order to compare whey agar made with peptone and whey agar made without peptone for yeast and mold counts on butter, six samples of butter from six Indiana centralizer creameries were plated on these media, the whey used being secured by rennet coagulation and the pH of the whey at filtering being 6.8. Potato dextrose agar and Bacto malt agar were used as controls. The counts obtained are given in Table 5.

The mean yeast and mold count on whey agar containing 0.5 per cent peptone was 51.3; on whey agar containing no

Table 5

The influence of the addition of 0.5 per cent peptone to whey agar upon the yeast and mold count of butter. Results expressed as the average count per plate containing 0.1 ml. of butter.

Sample of Butter	Medium			
	Whey agar + 0.5% peptone	Whey agar	Bacto malt agar	Potato dextrose agar
1	168	212	296	346
2	6	8	17	86
3	0	1	0	9
4	4	8	8	9
5	10	14	18	52
6	120	170	140	250
Mean	51.3	68.9	79.7	125.2

peptone it was 68.9. The addition of 0.5 per cent peptone resulted in a lower yeast and mold count on all the samples of butter studied. The greatest difference between the two media was 50 yeast and mold colonies per plate and the smallest difference was one colony. Potato dextrose agar and Bacto malt agar gave means of 125.2 and 79.7 respectively. The counts obtained with potato dextrose agar were always, and those with the Bacto malt agar usually, higher than the counts obtained with either of the whey media.

To procure more information on the effect of peptone in a whey base medium on the growth of yeasts, whey broths were prepared containing no peptone, 0.5 per cent peptone, and 1.0 per cent peptone. Potato dextrose broth was used as a control. The sterile broths in flasks were acidulated to pH 3.5 $\pm$ 0.1 and tubed under aseptic conditions. Dilutions of T. cremoris, T. sphaerica, Pink B and Liquefier B were prepared in water and inoculated into the broths. Growth was determined after five days' incubation at 21 - 25° C. by streaking approximately 0.005 ml. of the broth from a well shaken tube upon potato dextrose agar. The yeast Pink B failed to grow in the whey broths even when inoculated heavily with cells, but grew in potato dextrose broth even when inoculated lightly. The addition of peptone in 0.5 and 1.0 per cent quantities did not increase the suitability of the

whey broths for the growth of T. cremoris or T. sphaerica, but did increase the suitability for the growth of Liquefier B. In none of the trials did the growth of the yeasts in the whey broths with or without peptone exceed the growth in potato dextrose broth.

D. Influence of low concentration of whey in whey agar upon the growth of yeasts.

Whey agar prepared by rennet coagulation, as given in the first report of the committee on methods for the microbiological analysis of butter[20], did not give so high a yeast and mold count as did Bacto whey agar[22]. The buffer capacity of the Bacto product was found to be less than that of whey agar prepared in the laboratory. Since the buffer capacity of the medium may be a limiting factor of growth, it was deemed advisable to determine the effect of using only small amounts of whey in the preparation of whey agar on the growth of yeasts.

Two batches of whey agar, the pH of which at filtering was 6.8, were prepared; in one, whey(as obtained by rennet coagulation of fresh skimmed milk) was the only liquid used, while the other contained 20 per cent whey and 80 per cent distilled water. Peptone was added to each batch in the proportion of 0.5 per cent.

The buffer capacity of the normal whey agar was 2.67 times that of the diluted whey agar, normal whey agar requiring 16.0 ml. of 5 per cent tartaric acid to reduce 100 ml. of medium from pH 6.6 to pH 3.5, as compared with 6.0 ml. for the diluted whey agar.

Dilutions were prepared from pure cultures of 10 common white yeasts which had been isolated from samples of butter. Definite amounts (0.01 ml.) of the dilutions were smeared over a definite area of the two media, which prior to pouring had been acidulated to pH 3.5± 0.1 with tartaric acid and with lactic acid. The plates were then incubated and the growth upon the normal whey agar compared with that upon the diluted whey agar.

Table 6 gives the extent of growth obtained. None of the 10 yeasts studied grew as well on the diluted whey agar as on the undiluted. Upon the diluted whey agar the growth was slightly better when lactic acid was used as an acidulant than when tartaric acid was used, but on the normal whey agar the acid used had no apparent influence on the growth.

E. Growth of yeasts and molds in media of known composition.

A synthetic medium that would support a good growth of yeasts and molds would be very useful because it could be prepared more uniformly than one requiring unknown plant or

Table 6

Influence of low concentration of whey in whey agar upon the growth of yeasts.

Yeast	Extent of growth			
	Tartaric acid as acidulant		Lactic acid as acidulant	
	100% whey agar	20% whey agar	100% whey agar	20% whey agar
1	++++	++	++++	+++
2	++++	++	++++	++
3	++++	++	++++	+++
4	++++	++++	++++	+++
5	++++	+++	++++	+++
6	++++	++	++++	+++
7	++++	+++	++++	+++
8	++++	+++	++++	+++
9	++++	+++	++++	+++
10	++++	++	++++	+++

++++ = excellent growth +++ = good growth
++ = fair growth + = poor growth

animal extracts.

Fulmer and Grimes[7] suggested a synthetic medium for the growth of yeasts. This medium consists of distilled water containing 0.188 per cent ammonium chloride, 0.1 per cent dipotassium phosphate, 0.1 per cent calcium chloride, 0.1 per cent dextrose, and 1.5 per cent agar agar. Preliminary platings having been made with this medium, it was found after an incubation period of 5 days that the yeast and molds colonies were extremely small. In order to attempt to increase the colony size of yeasts and molds when growing on this medium, the medium was modified by the following additions: (a) 0.1 per cent asparagin, (b) 0.5 per cent hydrolyzed casein, and (c) 0.5 per cent soluble potato starch. The media were acidulated to pH 3.5. Twelve samples of butter were plated using the Fulmer-Grimes medium and the three modifications; potato dextrose agar was used as a control. The counts secured are presented in Table 7.

The mean yeast and mold count on the Fulmer-Grimes synthetic medium was 139.4. The addition of 0.1 per cent asparagin to this medium gave a mean yeast and mold count of 148.3; the addition of 0.5 per cent hydrolyzed casein gave a mean count of 204.5; the addition of 0.5 per cent soluble starch increased the mean count to 214.2. The mean yeast and mold count on potato dextrose agar was 259.6.

Table 7

Yeast and mold counts secured on Fulmer-Grimes agar and its modifications. Results expressed as the average number of yeasts and molds per 0.1 ml. of butter

Sample of Butter	Medium				
	Fulmer-Grimes	Fulmer-Grimes + 0.1% asparagin	Fulmer-Grimes + 0.5% hydrolyzed casein	Fulmer-Grimes + 0.5% soluble starch	Potato dextrose agar
1	251	184	251	263	332
2	802	940	976	984	1150
3	0	0	511	523	603
4	0	1	0	26	47
5	0	0	0	3	3
6	0	0	0	6	6
7	423	441	462	424	602
8	3	9	6	15	11
9	27	31	42	76	61
10	0	0	0	2	0
11	0	0	0	19	23
12	167	173	186	229	278
Mean	139.4	148.3	204.5	214.2	259.6

The yeast and mold colonies on the Fulmer-Grimes medium and its modifications were small, the colonies appearing not large enough to be counted with the naked eye. When only a few colonies were on a plate and the plate was of low butter dilution, so that it contained numerous fat globules, considerable difficulty was experienced in differentiating between fat globules and yeast and mold colonies; the difficulty was so great that the use of the Fulmer-Grimes medium for determining the yeast and mold count of butter was not practical.

Butter sample number 5 contained only pink yeasts. These yeasts failed to grow in the Fulmer-Grimes medium and also when 0.1 per cent asparagin was added, but on the addition of 0.5 per cent hydrolyzed casein or 0.5 per cent soluble starch, the number of colonies approached the count secured on potato dextrose agar.

In order to secure information as to the effect of acidity and the kind of acid used to acidulate on the growth of yeasts on Fulmer-Grimes medium, ten yeasts isolated from butter were streaked upon the surface of this medium, which had been adjusted to three pH levels with each of five acids; the acids were tartaric, lactic, citric, sulphuric and hydrochloric. After an incubation period of 5 days at 21 - 25° C., the yeast growth on the surface of the agar was compared; the results secured are given in Table 8.

Table 8

Growth of yeasts on Fulmer-Grimes synthetic agar as influenced by the acidity of the medium and the acidulant.

Acidulant	Comparative growth of the 10 yeasts		
Tartaric acid	pH 6.72 Growth scant	pH 4.20 Growth scant, but slightly better than pH 6.72	pH 3.5 Same as at pH 4.20
Lactic acid	pH 6.72 Growth scant	pH 4.10 Growth scant, but slightly better than pH 6.72	pH 3.5 Same as at pH 4.10
Citric acid	pH 6.72 Growth scant	pH 4.30 Growth scant, but slightly better than pH 6.72	pH 3.5 Same as at pH 4.30
Sulphuric acid	pH 6.72 Growth scant	pH 3.8 Growth scant, but slightly better than pH 6.72	pH 3.5 Same as at pH 3.8
Hydrochloric acid	pH 6.72 Growth scant	pH 3.8 Growth scant, but slightly better than pH 6.72	pH 3.3 Same as at pH 3.8

Slightly better growth was secured when the pH was in the vicinity of 4.0 to 3.5 than when at 6.72, but in all cases the growth was scant and the largest colonies were about 0.5 mm. in diameter. The acids used did not affect the growth or colony size of the yeasts. The yeast Pink B failed to develop pigment when grown on the Fulmer-Grimes medium or its modifications.

The Digestive Ferments Company prepared a "synthetic malt agar" which had the following composition: 1.275 per cent technical maltose, 0.275 per cent dextrin, 0.235 per cent glycerol C.P., 0.078 per cent Bacto peptone, 1.500 per cent agar agar, and distilled water. This medium is supplied as a substitute for Bacto malt agar; according to its makers it has the essential nutritive ingredients of malt extract agar. In order to determine its value as a medium for the enumeration of the yeasts and molds in butter, 12 samples of butter secured from three Indiana creameries were plated on it. Potato dextrose agar and Bacto malt agar were used as controls. Table 9 gives the yeast and mold count per ml. of butter.

The mean yeast and mold count on Bacto synthetic agar was 28.0, on potato dextrose agar it was 21.9, and on Bacto malt agar it was 22.8. Seven samples of butter gave higher counts upon the Bacto synthetic malt agar than upon the potato dextrose

Table 9

Yeast and mold counts secured on Bacto synthetic malt agar, potato dextrose agar and Bacto malt agar. Results expressed as the average number of yeasts and molds per ml. of butter

Sample of Butter	Medium		
	Bacto synthetic malt agar	Potato dextrose agar	Bacto malt agar
1	78	59	61
2	43	26	27
3	65	53	64
4	87	63	63
5	4	16	3
6	11	5	11
7	4	8	7
8	0	0	0
9	7	5	3
10	24	5	19
11	8	15	12
12	5	6	4
Mean	28.0	21.8	22.8

agar, and eight samples gave higher counts upon the Bacto synthetic agar than upon Bacto malt agar. The colonies on the Bacto synthetic agar were small, and magnifications of 20 times were necessary to distinguish the colonies from the fat globules. The yeast and mold counts secured upon potato dextrose agar and upon Bacto malt agar were obtained without magnification. The magnification used with Bacto synthetic malt agar may account in part for the higher counts secured upon this medium.

Part 2

INFLUENCE OF VARIATIONS IN COMPOSITION AND IN METHODS OF PREPARATION OF POTATO DEXTROSE AGAR UPON THE YEAST AND MOLD COUNT OF BUTTER.

Introduction

The influence of variations in the composition and in the method of preparing potato dextrose agar upon the yeast and mold count of butter was studied in a series of trials. Potato dextrose agar was investigated for, in the work reported in Part I, it was found to be the most suitable medium for determining the yeast and mold count of butter. Before a medium containing plant extracts can be recommended for the routine analysis of butter for its yeast and mold count, the effect of variations in composition, due to various factors, should be studied.

Experimental

A. Influence of the source of the potatoes on the yeast count upon potato dextrose agar.

In order to secure information on the effect of the source of the potatoes used in preparing potato dextrose agar on the growth of yeasts, two batches of the medium were pre-

pared using the regular formula. Batch 1 and Batch 2 contained an infusion of potatoes grown in Indiana and in Idaho, respectively. The Indiana potatoes were of medium size and egg-shaped; the Idaho potatoes were large and oblong. After baking, the interior of the Indiana potatoes was dark in color and not mealy, while that of the Idaho potatoes remained white in color and was mealy. These two lots of potatoes were selected because they represented extreme types. The media were acidulated and used in plating suspensions in water of various yeasts that are more or less common in butter. After incubation, the plates were counted; the data obtained are presented in Table 10.

The mean yeast and mold count per ml. of suspensions of the seven yeasts was 394.9 upon potato dextrose agar prepared from potatoes grown in Indiana and 394.6 upon potato dextrose agar prepared from potatoes grown in Idaho. In four of the dilutions of yeasts there were no significant differences in the counts secured with the two media; two yeasts, Sacc. cerevisiae and T. cremoris, gave higher counts on the agar prepared from Idaho potatoes and Common white P gave a higher count on the medium prepared from Indiana potatoes.

The kind and original source of potato had no appreciable effect on the number of yeast colonies developing upon potato dextrose agar. Colony size was recorded and no differ-

ence found, indicating that the essential ingredients necessary to initiate and support yeast growth was contained in both types of potatoes.

B. Influence of amount of potato from which the infusion is prepared upon the yeast and mold count obtained on potato dextrose agar.

In order to determine the effect of variations in the amounts of potatoes used in preparing the infusion upon the ability of potato dextrose agar to initiate and support the growth of yeasts in butter, comparisons of media prepared with different amounts of potato were made.

Three batches of potato dextrose agar were prepared. In Batch 1, an infusion from 100 grams of potatoes per liter was used, in Batch 2, an infusion from 200 grams of potatoes per liter, and in Batch 3, an infusion from 400 grams of potatoes per liter. These media were then acidulated and suspensions in water of cultures of seven yeasts were plated. The counts secured are tabulated in Table 11.

Potato dextrose agar prepared from an infusion of 400 grams of potatoes per liter gave a mean yeast count of 401.1; when prepared from an infusion of 200 grams of potatoes the mean count was 394.3 and when prepared from an infusion of 100 grams of potatoes the mean count was 350.7.

Table 10

Influence of the source of the potatoes used in the preparation of potato dextrose agar upon the yeast count.

Yeast used	Count per ml. of suspension upon potato dextrose agar prepared from Indiana potatoes	Count per ml. of suspension upon potato dextrose agar prepared from Idaho potatoes
Liquefier B	650	667
T. sphaerica	370	387
Pink B	770	745
Common white S	186	182
Sacc. cerevisiae	185	202
T. cremoris	178	206
Common white P	425	375
Mean	394.9	394.6

Table 11

Influence of the amount of potatoes used in the preparation of potato dextrose agarupon the yeast count.

Yeast used	Counts per ml. of suspensions upon potato dextrose agar prepared from infusions containing: grams of potatoes per liter		
	100	200	400
Liquefier B	682	666	715
T. sphaerica	212	387	426
Pink B	673	754	814
Common white P	132	182	168
Sacc. cerevisiae	220	201	215
T. cremoris	153	206	176
Common white S	383	373	294
Mean	350.7	394.3	401.1

There was no consistent trend within individual suspensions of yeast to give lower counts upon the medium containing lower than normal infusions of potatoes (100 grams), or to give higher counts upon the medium containing greater than normal (400 grams).

The colony size was larger on the medium containing an infusion of 400 grams of potatoes than upon the media containing an infusion of 100 or 200 grams of potatoes. Upon the medium containing an infusion of 100 grams of potatoes the colonies were so small that it was difficult to count them without the aid of a lens. The difference in colony size on the media containing 100 and 200 grams of potatoes was much greater than the difference on the media containing 200 and 400 grams of potatoes.

Fifty-one samples of butter from four Indiana centralizer creameries were plated on potato dextrose agar prepared by infusing 200 grams of potatoes per liter and also on potato dextrose agar prepared by infusing 400 grams of potatoes per liter. The yeast and mold counts obtained are presented in Table 12.

The mean yeast and mold count secured when the 51 samples were plated upon potato dextrose agar prepared from an infusion of 200 grams of potatoes per liter was 319.2 and when plated upon potato dextrose agar prepared from an infusion of 400

grams of potatoes it was 337.8; this difference in mean counts is not great enough to be of significance. Thirty-three samples gave higher counts on the medium containing an infusion of 400 grams of potatoes, 16 gave higher counts on the medium containing an infusion of 200 grams of potatoes, and with two there was no difference in the counts.

The difference between counts obtained upon infusions of 400 and 200 grams of potato were greater when butter samples were plated than when pure cultures of yeasts were used; the colonies were larger on the medium containing the infusion of 400 grams of potatoes than on that containing the infusion of 200 grams of potatoes.

C. Influence of the addition of salts to potato dextrose agar upon the yeast and mold count.

Fulmer and Grimes[7] have suggested a medium containing inorganic salts for the growth of yeasts. In order to determine the effect of the addition of these salts to potato dextrose agar the following study was undertaken.

Two batches of potato dextrose were prepared, one by infusing 200 grams of potatoes per liter and the other by infusing 40 grams of potatoes per liter. These media were used with and without the salts suggested by Fulmer and Grimes

Table 12

Influence of amount of potato used in preparing potato dextrose agar upon the yeast and mold count.

Sample of Butter	Yeast and mold count per ml. on agar prepared with	
	200 grs. potatoes per l.	400 grs. potatoes per l.
1	0	20
2	150	280
3	300	460
4	170	310
5	160	190
6	90	60
7	40	30
8	40	110
9	50	110
10	250	120
11	20	20
12	90	20
13	10	20
14	20	50
15	440	510
16	430	530
17	350	430
18	420	480
19	270	330
20	390	350
21	460	510
22	370	520
23	1390	1180
24	40	50
25	370	360
26	340	280
27	20	90
28	100	110
29	840	780
30	420	440
31	560	380

12	90	20
13	10	20
14	20	50
15	440	510
16	430	530
17	350	430
18	420	480
19	270	330
20	390	350
21	460	510
22	370	520
23	1390	1180
24	40	50
25	370	360
26	340	280
27	20	90
28	100	110
29	840	780
30	420	440
31	560	380
32	1180	1330
33	210	120
34	240	380
35	710	980
36	250	230
37	430	490
38	720	720
39	520	530
40	530	810
41	1260	1070
42	290	330
43	540	610
44	300	220
45	60	10
46	140	130
47	30	50
48	130	110
49	50	70
50	40	50
51	50	110
Mean	319.2	337.8

(0.188 per cent ammonium chloride, 0.10 per cent dipotassium phosphate, and 0.10 per cent calcium chloride).

The addition of the salts increase the buffer capacity of the media. The medium prepared by infusing 200 grams of potatoes required 2.6 ml. of 5 per cent tartaric acid per 100 ml. of medium to acidulate to pH 3.5 0.1; the same medium with the salts added required 6 ml. of 5 per cent tartaric acid to acidulate. The medium prepared by infusing 40 grams of potatoes required 1.0 ml. of 5 per cent tartaric acid per 100 ml. of medium to acidulate, and when the medium contained the salts, 4.0 ml. of 5 per cent tartaric acid were required.

The yeast and mold counts secured on butter with the various media are given in Table 13.

The mean yeast and mold count per ml. of butter upon potato dextrose prepared from an infusion of 200 grams of potatoes was 98.1; when the salts suggested by Fulmer and Grimes were added it was 99.8. The addition of the salts increased the count in four samples of butter, while six samples of butter had higher yeast and mold counts on the medium to which no salts were added. The mean yeast and mold count per ml. of butter with the medium prepared from an infusion of 40 grams of potatoes was 95.2; when the salts were added it was 95.3. Seven samples gave higher yeast and mold counts when the salts were added to the medium and

Table 13

Yeast and mold counts of butter, using potato dextrose agar with and without the addition of salts.

Sample of Butter	Yeast and mold count per ml. of butter on potato dextrose agar containing per liter:			
	200 grams potato	200 grams potato + salts	40 grams potato	40 grams potato + salts
1	184	172	194	162
2	199	256	192	195
3	2	1	0	1
4	221	220	202	227
5	37	42	39	41
6	46	40	41	43
7	17	19	18	16
8	43	48	40	41
9	121	106	125	112
10	111	94	101	115
Mean	98.1	99.8	95.2	95.3

and three gave higher counts without the salts.

The increase in buffer capacity, due to the addition of the salts, did not affect the growth of yeasts and molds on potato dextrose agar in the trials carried out, nor was any advantage secured in count by the addition of the salts suggested by Fulmer and Grimes.

D. Influence of the addition of brom phenol blue to potato dextrose agar upon the yeast and mold count.

The addition of brom phenol blue to the medium used for plating has two principal advantages. (a) It determines accuracy of acidulation to pH 3.5 $\pm$ 0.1; at pH 3.5 brom phenol blue is a yellow green and in potato dextrose this shade of color is easily recognized. (b) Some yeast colonies absorb the dye, making counting easier, especially on plates showing heavy mold growth.

Because of these advantages and in order to determine whether or not the addition of brom phenol blue inhibited the development of yeasts and molds, dilutions of cultures of yeasts (in water) and also samples of butter from Indiana centralizer creameries were plated, using potato dextrose agar and potato dextrose agar with the addition of 1 ml. of

a 0.5 per cent aqueous solution of brom phenol blue to 100 ml. of medium. The counts obtained are given in Tables 14 and 15.

The mean count from plating dilutions of seven yeasts, isolated from dairy products, on potato dextrose agar containing 0.005 per cent of brom phenol blue, was 355.2; when the medium contained no brom phenol blue the mean count was 401.7. Two yeasts, Common white yeasts S and P, gave higher counts when the medium contained brom phenol blue, while the remaining yeasts gave higher counts on the medium containing no brom phenol blue.

The mean yeast and mold count per 0.1 ml. of butter when the 20 samples were plated on potato dextrose agar containing 0.005 per cent of brom phenol blue was 37.7, and on the medium without brom phenol blue it was 37.5. Higher yeast and mold counts were secured with 14 samples on the potato dextrose agar containing brom phenol blue than on the medium containing no brom phenol blue, but the difference is within the probable limits of error of plating. The data obtained indicate that 0.005 per cent of brom phenol blue did not retard or inhibit the growth of the yeasts and molds.

In some cases counting was simplified when the dye had been added to the medium, especially if molds were present, for by inverting the plate it was possible to count the yeast

Table 14

Influence of the addition of brom phenol blue on the growth of yeasts in potato dextrose agar.

Yeast Used	Yeast count per 0.001 ml. of suspension on medium containing	
	0.005% brom phenol blue	No brom phenol blue
Common white S	495	466
Common white YB	634	688
Common white B	436	518
Pink B	408	519
Common white P	226	207
T. sphaerica	165	285
Liquefier B	122	129
Mean	355.2	401.7

Table 15

Influence of the addition of brom phenol blue on the growth of yeasts and molds in potato dextrose agar.

Sample of Butter	Yeast and mold count per ml. of butter on medium containing	
	0.005% brom phenol blue	No brom phenol blue
1	52	44
2	49	43
3	36	35
4	43	42
5	35	27
6	24	39
7	60	46
8	19	37
9	149	139
10	3	4
11	29	37
12	36	34
13	3	2
14	11	10
15	79	84
16	12	30
17	29	23
18	9	9
19	24	23
20	52	42
Mean	37.7	37.5

colonies under the mold colonies, because of the dye absorption by the yeasts. On plates which contained considerable fat there was no distinct advantage in the use of the dye; in the presence of fat the absorption of the dye by the yeast colony was apparently retarded and the colonies did not appear so distinct as when no brom phenol blue was used, although they could easily be distinguished from the fat.

E. Influence of the method of preparing the potato infusion upon the yeast count.

Two methods of preparing potato dextrose agar were studied. Method A was the usual method as described in general methods. In method B, the sliced potatoes, distilled water, dextrose and agar agar were placed in a flask in the same proportions as in method A and autoclaved for 30 minutes at 15 pounds steam pressure, filtered through cotton and then dispensed into flasks and sterilized by autoclaving. The latter method is easier and less time-consuming than the former and is better suited to routine laboratory procedure.

A suspension in water of each of 14 yeasts, which had been isolated from butter, was prepared and plated, using potato dextrose agar prepared by the two methods described. The counts obtained are given in Table 16.

Table 16

Influence of the method of preparing the potato infusion upon the yeast count.

Yeast Used	Counts per ml. of suspension upon potato dextrose agar prepared by	
	Method A	Method B
1	650	541
2	370	416
3	770	568
4	186	180
5	185	175
6	178	158
7	425	380
8	667	609
9	387	454
10	745	832
11	182	172
12	202	225
13	206	186
14	373	315
Mean	394.7	372.2

The mean yeast count per ml. of suspension upon potato agar prepared according to Method A, the recommended method, was higher than upon the potato dextrose agar prepared according to Method B; the mean counts were 394.7 and 372.2 respectively. Potato dextrose agar prepared according to Method A gave higher counts with 10 yeasts than did Method B, and the reverse was true for the medium prepared by Method B with four yeasts. The effect of the method of preparation of potato dextrose agar, as exhibited by the results secured, is slight and probably within the limits of error of the technique, but favors the method which was used in all of the previous work.

F. Influence of heating potato infusion in the preparation of potato dextrose agar upon the yeast and mold count of butter.

An infusion of 400 grams of potatoes in 1 liter of distilled water was prepared by permitting the sliced potatoes to remain in contact with the water for 24 hours at about 5° C. The potato infusion was filtered through a Mandler filter (control plates showed that yeasts and molds were removed). A 3 per cent agar solution containing 4 per cent dextrose was prepared and sterilized, cooled to 50° C. and mixed aseptically in equal proportions with the unheated

potato infusion. This medium was then acidulated and yeast and mold counts were made on 10 samples of Indiana butter. The same samples were plated using potato infusion prepared in the usual manner. The yeast and mold counts secured are presented in Table 17.

The mean yeast and mold count obtained upon the potato dextrose agar prepared from potato infusion which was not heated was 87.4 per ml. while upon the medium prepared by boiling the infusion it was 98.1. Seven samples of butter gave higher yeast and mold counts upon the medium prepared from the infusion which was heated than upon the medium prepared from the unheated infusion, while for two samples the reverse was true. This difference is slight, although in favor of heating the infusion, which is the routine method of preparing potato dextrose. These data indicate the boiling of the potatoes for one hour to obtain the infusion, the autoclaving for 30 minutes in order to suspend the agar, the filtering and the sterilizing, does not destroy substances which enhance the growth of yeasts and molds. Colony size on the medium containing the unheated potato infusion was the same as on the medium containing the heated potato infusion.

Table 17

Influence of heating potato infusion in the preparation of potato dextrose agar upon the yeast and mold count of butter.

Sample of Butter	Count per ml. on potato dextrose prepared from infusion which was	
	Unheated	Heated
1	148	184
2	174	199
3	1	2
4	187	221
5	35	37
6	45	46
7	17	17
8	46	43
9	106	121
10	115	111
Mean	87.4	98.1

Part 3

INFLUENCE OF THE KIND OF ACID USED TO ACIDULATE THE MEDIUM AND THE DEGREE OF ACIDULATION UPON THE YEAST AND MOLD COUNT OF BUTTER.

Historical

White and Hood[30] presented data which show that a pH of from 3.4 to 3.8 in the medium effectively inhibits the growth of bacterial colonies, and does not affect significantly the yeast and mold count of butter. These investigators recommended that the medium to be used for the routine analysis of butter for yeasts and molds be acidulated with lactic acid to pH 3.5± 0.1. Hammer[10], following the method of Bouska and Brown[4], used 1 ml. of a 1 per cent tartaric acid solution per plate for acidulation. The first report of the committee[20] recommended that lactic acid be used as the acidulant; its second report[21] recommended tartaric acid. Lund[17], one of the early workers in this field, used tartaric acid to acidulate the medium which he employed. Hastings[11] suggested the use of citric acid, and Davis[6] found that citric acid was the most convenient acid to use for adjusting media for yeast and mold counts. Parfitt[22], in studies on the influence of the medium upon the yeast and mold count of butter, used lactic acid.

Introduction

A study of the acids used to acidulate the medium was carried out because of the variation in the acids employed by the different investigators. Moreover, it had been noted that on Bacto malt agar, which prior to pouring has been accurately acidulated to pH 3.5 with lactic acid, bacterial colonies occasionally were present. This occurred most often when the medium contained more than 150 yeast and mold colonies per plate, and commonly the bacteria developed in close proximity to a yeast or mold colony. When the medium was tested for acidity by scraping the contents of the petri dish into a small beaker, adding about 15 ml of distilled water and allowing the medium and water to remain in contact for about an hour, the pH of the medium, in some cases, was found to be above pH 4.0. The suggested procedure for the determination of yeasts and molds [14,20,21] recommends incubating the plates 5 days at 21 - 25° C. This period of incubation would readily permit the development of bacterial colonies upon the plates if the acidity had decreased to a point which was satisfactory for bacterial growth. In the counting of yeasts and molds in butter it is usually assumed, because of the work of Hood and White[14] and White and Hood[28], that when the medium is acidulated to pH 3.5 only

yeast and mold colonies develop, so that it is not necessary to verify colonies by microscopic examination.

The studies on the acidulation of media for yeast and mold counts on butter included: (1) the effect of the kind of acid used upon the yeast and mold count of butter, (2) neutralization of acids by yeasts and molds, and (3) the influence of the pH of the medium upon the growth of pure cultures of yeasts.

Experimental

A. Effect of the kind of acid used to adjust the medium to pH 3.5 upon the yeast and mold count of butter.

Twelve random samples of salted butter manufactured in Indiana were plated upon (a) potato dextrose agar, (b) Bacto whey agar, (c) Bacto malt agar, and (d) 2 per cent dextrose nutrient agar. Each medium was acidulated to pH 3.5 $\pm$ 0.1 with tartaric acid, with lactic acid and with citric acid. The results secured are given in Table 18.

The mean yeast and mold counts obtained when tartaric acid was used to acidulate potato dextrose agar, Bacto whey agar, Bacto malt agar and 2 per cent dextrose nutrient agar were 444.5, 390.1, 334.5 and 423.8 respectively; the mean

count for the four media when acidulated with tartaric acid was 398.2. The mean yeast and mold counts obtained with lactic acid were 401.6 for potato dextrose agar, 308.3 for Bacto whey agar, 315.9 for Bacto malt agar and 350.1 for 2 per cent dextrose nutrient agar; the mean count for the four media acidulated with lactic acid was 343.9. The mean yeast and mold counts when citric acid was used to acidulate potato dextrose agar, Bacto whey agar, Bacto malt agar and 2 per cent dextrose nutrient agar were 442.1, 343.0, 387.2 and 409.6 respectively, and the mean count obtained upon the four media when acidulated with citric acid was 395.4.

With each of the four media studied, the lowest mean yeast and mold count was secured when the medium was acidulated with lactic acid. The difference between tartaric acid and citric acid as acidulants for media was slight. Higher mean counts were secured upon potato dextrose agar, Bacto whey agar and 2 per cent dextrose nutrient agar when these media were acidulated with tartaric acid than when they were acidulated with citric acid. A higher mean count was obtained when citric acid was used as the acidulant for Bacto malt agar than when tartaric acid was used. Of the media studied, Bacto whey agar was the most sensitive to the acidulant, the difference between the mean counts secured with lactic acid and with tartaric acid being 81.8 colonies. Potato dextrose

agar was the least senstive to the acidulants in that the mean counts ranged from 401.6 to 444.5 with the four media studied.

Dilutions of pure cultures of six yeasts which had been isolated from Indiana butter were streaked on Bacto whey agar acidified with each of five acids (tartaric, lactic, citric, sulphuric, hydrochloric) to pH 5.5 ± 0.1 (with hydrochloric acid a pH of 3.0 was also employed). In the preparation of the streaks from a given dilution a uniform count of inoculating material was used. The plates were incubated for 5 days at 21 - 25° C. and the growth then compared. The results of the comparisons are recorded in Table 19.

Growth was not so abundant when the medium was acidulated to pH 3.5 ± 0.1 with lactic acid as when it was acidulated with sulphuric, hydrochloric, citric or tartaric acids, and there was less growth with sulphuric acid than with hydrochloric, citric or tartaric. No difference in the luxuriance of growth was apparent on the medium when acidulated to pH 3.5 with hydrochloric, citric or tartaric acids. Good growth was secured when the medium was acidulated to pH 3.0 with hydrochloric acid.

Table 18

The effect of the acid used to adjust the medium to pH 3.5 ± 0.1 upon the yeast and mold count of butter.

Results expressed as the average number of yeasts and molds per ml. of butter.

Sample of Butter	Potato dextrose agar acidulated with			Bacto whey agar acidulated with		
	tartaric acid	lactic acid	citric acid	tartaric acid	lactic acid	citric acid
1	10	20	10	10	10	0
2	95	70	100	55	115	85
3	85	70	150	200	125	180
4	200	235	295	320	310	315
5	40	30	30	10	0	10
6	520	455	490	472	435	426
7	951	951	742	749	864	881
8	190	237	250	244	110	209
9	915	808	1001	505	543	529
10	1166	909	1152	1230	572	819
11	189	122	207	192	164	202
12	974	913	899	695	452	460
Mean	444.5	401.6	442.1	390.1	308.3	343.0

Table 18 (Continued)

Sample of Butter	Bacto malt agar acidulated with			2% dextrose nutrient agar acidulated with		
	tartaric acid	lactic acid	citric acid	tartaric acid	lactic acid	citric acid
1	9	10	9	10	10	10
2	65	80	90	65	65	75
3	115	145	160	195	105	215
4	230	250	275	280	280	320
5	0	0	15	3	8	30
6	314	530	425	570	417	452
7	749	864	881	1110	855	970
8	244	110	209	244	158	241
9	505	543	529	783	516	703
10	1033	699	1027	998	777	949
11	195	156	219	191	131	209
12	556	604	810	638	881	741
Mean	334.5	315.9	387.2	423.8	350.1	409.6

Table 19

Influence of the acid used to acidulate Bacto whey agar to pH 3.5 ± 0.1 upon the growth of yeasts.

Yeast used	Degree of Growth: Acids used to secure pH 3.5 ± 0.1					
	tartaric acid	lactic acid	citric acid	sulphuric acid	hydrochloric acid	hydrochloric acid to pH 3.0
1	✓ ✓ ✓	✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓	✓ ✓
2	✓ ✓ ✓	✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓	✓ ✓
3	✓ ✓ ✓	✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓	✓ ✓
4	✓ ✓ ✓	✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓	✓ ✓
5	✓ ✓ ✓	✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓	✓ ✓
6	✓ ✓ ✓	✓	✓ ✓ ✓	✓ ✓	✓ ✓ ✓	✓ ✓

✓ fair growth
✓ ✓ good growth
✓ ✓ ✓ excellent growth

B. Change in pH of medium by the growth of yeasts.

In order to determine the amount of neutralization that takes place in a medium in which yeasts are growing, the following procedure was used. Suspension in water of pure cultures of yeasts isolated from dairy products were prepared and plated upon potato dextrose agar which had been acidulated (prior to pouring) to pH 3.5 ± 0.1 with tartaric, with lactic, with citric and with sulphuric acids. After 5 days' incubation the numbers of yeast cells on the plate were counted. The medium in each plate was then removed to a 50 ml. beaker and thoroughly mixed with 15 ml. of distilled water; after standing for one hour the pH was determined electrometrically. Plates which showed contamination with molds were discarded. Table 20 gives the numbers of yeast colonies on the plates after 5 days incubation and the final pH values of the agar.

The decrease in acidity, because of the growth of yeasts, was less when potato dextrose was acidulated with tartaric or sulphuric acids than when acidulated with lactic or citric acids. The mean final pH values obtained from all plates acidulated with an acid are as follows: tartaric acid 3.64, lactic acid 5.03, citric acid 4.14, and sulphuric acid 3.65. When tartaric acid was used, only those yeasts which are capable of proteolyzing casein were able to increase the pH

Table 20

Change in pH of potato dextrose agar because of the growth of yeasts.

Plates incubated 5 days at 21 - 25° C..

Initial pH of agar 3.5 ± 0.1

Yeast used	Amount of dilution per plate	Average count	Final pH of potato dextrose agar acidified with			
			tartaric acid	lactic acid	citric acid	sulphuric acid
T. cremoris	1-10		3.60	7.60	3.40	
	1-100		3.59	7.15	3.60	
	1-1000	164	3.50	5.14	3.55	
Liquefier 106	1-10		3.73	5.83	4.60	
	1-100		3.42	4.45	3.50	
	1-1000	121	3.40	4.23	3.40	
Liquefier B	1-10		4.00	7.55	6.93	
	1-100		3.80	7.45	6.86	
	1-1000	152	3.80	7.32	broken	
T. sphaerica	1-10		3.55	4.12	3.75	3.55
	1-100		3.56	3.81	3.80	3.55
	1-1000	190	3.68	3.70	3.57	3.55
Liquefier C	1-10		4.00	7.10	7.02	3.70
	1-100		4.00	5.95	6.05	3.60
	1-1000	216	4.00	4.40	4.33	3.70
Common white P	1-10		3.82	5.03	4.00	3.59
	1-100		3.45	3.80	3.65	3.40
	1-1000	219	3.45	3.80	3.70	3.50
Pink B	1-10		3.80	6.36	3.82	3.40
	1-100		3.80	4.20	3.80	3.51
	1-1000	154	3.60	3.92	3.65	3.40

	1-100					
	1-1000	152	3.80	7.32	broken	
T. sphaerica	1-10		3.55	4.12	3.75	3.55
	1-100		3.58	3.81	3.80	3.55
	1-1000	190	3.68	3.70	3.57	3.55
Liquefier C	1-10		4.00	7.10	7.02	3.70
	1-100		4.00	5.95	6.05	3.60
	1-1000	216	4.00	4.40	4.33	3.70
Common white P	1-10		3.82	5.03	4.00	3.59
	1-100		3.45	3.80	3.65	3.40
	1-1000	219	3.45	3.80	3.70	3.50
Pink B	1-10		3.80	6.36	3.82	3.40
	1-100		3.80	4.20	3.80	3.51
	1-1000	154	3.60	3.92	3.65	3.40
T. cremoris / mycoderma	1-1000	200	3.60	3.92	3.60	3.60
Common white P / Pink B / Liquefier B	1-100	203	3.56	3.90	3.80	3.60
Common white P / Common white S	1-100	78	3.50	3.70	3.60	3.50
T. sphaerica / Common white P	1-100	78	3.50	3.71	3.60	3.50
Pink S	1-100	400	3.50	4.52	3.75	4.80
Common white P / T. sphaerica	1-100	410	3.48	5.20	3.66	3.50
Mean of all pH determinations			3.64	5.03	4.14	3.65

to a point at which bacterial growth can take place. Sulphuric acid was neutralized above pH 4.0 by two yeasts which are designated Common white P and Pink B.

The number of yeast colonies on a plate influenced the degree of neutralization that took place in the medium. These yeasts designated as liquefier, which were capable of proteolyzing casein, decreased the acidity when lactic or citric acid was used, to above pH 4.0 when there were as few as 78 colonies on the plate. Each of the yeasts studied, with the exception of Liquefier C, was capable of reducing the acidity to a greater degree with lactic than with citric acid.

The auxanographic method was used to study the change in pH by adding 1 ml. of 0.5 per cent brom phenol blue to 100 ml. of (a) potato dextrose agar (b) Bacto malt agar and (c) Bacto whey agar and adjusting these media to pH 3.5 ± 0.1 with tartaric, with lactic, with sulphuric and with hydrochloric acids. Ten yeasts which had been isolated from butter were streaked on the surface of the media. After 5 days' incubation at 21 - 25° C., the plates were examined for the extent of growth and also for the degree of neutralization as exhibited by the color change in the media. A summary of the data is given in Table 21; in general the results are in agreement with those given in Table 20.

Table 21

Degree of neutralization caused by the growth of yeasts, as exhibited by color change of brom phenol blue, upon three media acidulated with each of four acids.

Summary of reaction change* caused by 10 yeasts

Medium	Acids used to acidulate media to pH 3.5 ± 0.1			
	tartaric acid	lactic acid	sulphuric acid	hydrochloric acid
Potato dextrose agar	Growth good, slight neutralization	Growth good, completely neutralized	Growth good, slight neutralization	Growth good, slight neutralization.
Bacto malt agar	Growth good, slight neutralization	Growth good, nearly complete neutralization	Growth good, slight neutralization	Growth good, slight neutralization
Bacto whey agar	Growth good, slight neutralization	Growth good, complete neutralization	Growth good, complete neutralization	Growth good, complete neutralization

* Neutralization was measured by the amount of the area of the plate which had turned from yellow green to blue. The pH range of brom phenol blue is 3.0 - 4.6

Neutralization occurred on all plates but not to so great a degree on potato dextrose agar as it did on Bacto malt or Bacto whey agars; even when lactic acid was used, the degree of neutralization was not so great upon potato dextrose agar as it was upon Bacto malt agar or Bacto whey agar.

C. Influence of pH of the medium upon growth of yeasts.

In order to determine the effect of acidity of the medium upon the growth of yeasts, five media were acidulated with tartaric, with lactic and with citric acids to various degrees of acidity. The media used were (a) potato dextrose agar, (b) Bacto whey agar, (c) Bacto malt agar, (d) Bacto wort agar, and (e) Bacto peptonized milk agar. Eight yeasts which were selected as representative of the common Torula found in butter, were suspended in water and definite amounts smeared on the surfaces of the various media. The numbers of each yeast inoculated were nearly constant for a uniform loopful was taken from a dilution blank containing the suspension, which was frequently agitated, and smeared over a given area of the surface of each medium. The growth was examined after 5 days incubation at 21 - 25° C. The results obtained are given in Table 22.

The growth of yeasts on potato dextrose agar and Bacto whey

Table 22

Influence of pH of the medium upon the growth of yeasts

Acid used	Medium	Initial pH	Culture of yeast 1	2	3	4	5	6	7	8	9	10
			Growth after 5 days at 21 - 25° C.									
Tartaric	Potato dextrose agar	5.8	E	E	E	E	E	E	E	E	E	E
		3.4	E	E	E	E	E	E	E	E	E	E
		3.1	E	E	E	E	E	E	E	E	E	E
Tartaric	Bacto whey agar	6.1	E	E	E	E	E	E	E	E	E	E
		4.1	E	E	E	E	E	E	E	E	E	E
		3.5	E	E	E	E	E	E	E	E	E	E
Tartaric	Bacto malt agar	5.3	E	G	E	G	G	E	G	E		
		3.8	E	G	G	E	G	E	E	G		
		3.3	E	E	G	E	G	G	G	P		
Tartaric	Bacto wort agar	5.2	G	G	E	E	G	G	P	P		
		3.6	E	E	E	E	G	G	G	P		
		3.2	E	G	E	G	G	G	G	P		
Tartaric	Bacto peptonized milk agar	6.4	N	N	N	N	N	N	N	N	N	N
		3.7	P	P	P	P	P	P	P	P	P	P
		3.2	N	N	N	N	P	P	N	N	N	N
Lactic	Potato dextrose agar	5.8	E	E	E	E	E	E	E	E	E	E
		4.1	E	E	E	E	E	E	E	E	E	E
		3.4	E	E	E	E	E	E	E	G	E	E
Lactic	Bacto whey agar	6.1	E	E	E	E	E	E	E	E	E	E
		4.2	E	E	E	E	E	E	E	E	E	E
		3.6	E	E	E	E	E	E	E	E	E	E
Lactic	Bacto malt agar	5.3	E	G	E	G	G	E	G	E		
		3.9	E	E	E	E	E	E	E	E		
		3.5	E	E	E	E	G	E	E	P		
Lactic	Bacto wort	5.2	G	G	E	E	G	G	P	P		
		3.4	E	E	E	E	G	E	E	P		

Lactic	Potato dextrose agar	5.8	E	E	E	E	E	E	E	E	E	E								
		4.1	E	E	E	E	E	E	E	E	E	E								
		3.4	E	E	E	E	E	E	E	G	E	E								
Lactic	Bacto whey agar	6.1	E	E	E	E	E	E	E	E	E	E								
		4.2	E	E	E	E	E	E	E	E	E	E								
		3.6	E	E	E	E	E	E	E	E	E	E								
Lactic	Bacto malt agar	5.3	E	G	E	G	G	E	G	E										
		3.9	E	E	E	E	E	E	E	E										
		3.5	E	E	E	E	G	E	E	P										
Lactic	Bacto wort agar	5.2	G	G	E	E	G	G	P	P										
		3.4	E	E	E	E	G	E	E	P										
		3.0	E	G	E	E	G	E	E	N										
Lactic	Bacto peptonized milk agar	6.4	N	N	N	N	N	N	N	N	N	N								
		3.5	P	P	P	P	P	P	P	P	P	P								
		3.3	P	P	P	P	P	P	P	P	P	P								
Citric	Potato dextrose agar	5.8	E	E	E	E	E	E	E	E	E	E								
		3.9	E	E	E	E	E	E	E	E	E	E								
		3.3	E	E	E	E	E	G	E	E	E	E								
Citric	Bacto whey agar	6.1	E	E	E	E	E	E	E	E	E	E								
		4.1	E	E	E	E	E	E	E	E	E	E								
		3.4	E	E	E	E	E	E	E	E	E	E								
Citric	Bacto malt agar	5.3	E	G	E	G	G	E	G	E										
		3.7	E	E	G	G	G	E	G	P										
		2.7	E	P	E	N	N	E	P	P										
Citric	Bacto wort agar	5.2	G	G	E	E	G	G	P	P										
		5.9	E	G	E	E	E	E	G	P										
		2.9	G	G	G	P	G	G	G	N										
Citric	Bacto peptonized milk agar	6.4	N	N	N	N	N	N	N	N	N	N								
		3.6	P	P	P	P	P	P	P	P	P	P								
		2.9	N	N	N	N	N	N	N	N	N	N								

E = excellent growth
P = poor growth
G = good growth
N = no growth

agar which had been acidulated with tartaric, with lactic, and with citric acids was excellent over a pH range of 3.1 to 6.1; Bacto malt agar gave excellent to good growth when acidulated with each of the three acids over a pH range of 3.3 to 5.3 with the exception of yeast No. 8. At pH 2.7 which was obtained with citric acid, two yeasts failed to grow; three gave poor growth; and the remaining three gave excellent growth. Bacto wort agar in general gave better growth when acidulated with each of the three acids to 3.4 to 3.7 than when not so acidulated. Growth was excellent to good at pH 3.0 and 3.2 with the exception of yeast No. 8; at pH 2.9 yeast No. 8 failed to grow, yeast No. 4 gave poor growth, and the remaining yeasts gave good growth.

A striking difference was noted when Bacto peptonized milk agar was compared with the above media as a medium for the growth of yeasts at various pH values. Upon this medium none of the yeasts grew when it was at pH 6.4; growth was poor at pH 3.5 to 3.7, and became negative for all yeasts when the pH was decreased with citric acid to pH 2.9.

Part 4

INFLUENCE OF INCUBATION TIME UPON THE YEAST AND MOLD COUNT OF BUTTER.

Introduction

White and Hood[29] compared the yeast and mold counts obtained on butter with Bacto malt agar after 2 and 5 days' incubation at 25° C. and concluded that the length of the incubation time affected the count obtained. They stated: "If mould and yeast counts of butter analyzed at different laboratories are to be comparable, a uniform temperature and incubation period should be adopted, and 25° C. for 5 days gave the most satisfactory results in this study". In view of the fact that this was the only work reported on a comparison of incubation periods, the committee on the microbiological analysis of butter, in its reports[20, 21], suggested an incubation of 5 days at 21 - 25° C. for plates used in determining the yeast and mold count of butter.

Experimental

A. Influence of incubation time on the yeast and mold count of butter as determined with potato dextrose agar.

In order to secure information on the effect of the incubation time on the yeast and mold count of butter, 51 samples of butter were plated upon potato dextrose agar containing an infusion of 200 grams of potatoes and also upon potato dextrose agar containing an infusion of 400 grams of potatoes. The butter was from Indiana creameries. Yeast and mold counts were made at the conclusion of 3, 4, 5 and 8 days' incubation at 21 - 25° C. The data obtained are presented in Table 23.

The mean yeast and mold counts secured on potato dextrose agar prepared from an infusion of 200 grams of potatoes per liter of medium were 285.7 after 3 days, 309.0 after 4 days, 319.2 after 5 days, and 319.2 after 8 days. The mean yeast and mold counts secured on potato dextrose agar prepared from an infusion of 400 grams of potatoes per liter were 308.6 after 3 days, 329.4 after 4 days, 342.7 after 5 days, and 342.7 after 8 days.

A detailed study of Table 23 shows that, using potato dextrose agar prepared from an infusion of 200 grams of potatoes per liter of medium, 17 samples of butter had the same yeast and mold count after 4 days as for 3 days, and 28 samples of butter had the same yeast and mold count after 4 days as after 5 days. There were 38 samples of butter in which the increase in colony count from the third to the

Table 23

Influence of incubation time on yeast and mold count of butter as determined with potato dextrose agar.

Sample of Butter	Agar containing infusion of 200 grams of potatoes per l.				Agar containing infusion of 400 grams of potatoes per l.			
	Count per ml. after incubation of				Count per ml. after incubation of			
	3 days	4 days	5 days	8 days	3 days	4 days	5 days	8 days
1	10	10	10	10	10	20	20	20
2	50	140	150	150	260	260	280	280
3	220	290	300	300	410	460	460	460
4	160	160	170	170	280	310	310	310
5	60	140	160	160	150	160	190	190
6	40	40	90	90	40	50	60	60
7	10	30	40	40	30	30	30	30
8	0	40	40	40	40	90	110	110
9	0	50	50	50	40	70	110	110
10	120	250	250	250	50	120	120	120
11	0	20	20	20	0	10	20	20
12	90	90	90	90	20	20	20	20
13	10	10	10	10	10	20	20	20
14	0	20	20	20	0	30	50	50
15	420	420	440	440	410	450	510	510
16	420	430	430	430	510	530	530	530
17	350	350	350	350	410	410	430	430
18	390	400	420	420	450	460	480	480
19	230	240	270	270	290	290	330	330
20	390	390	390	390	280	320	350	350
21	420	420	460	460	480	510	510	510
22	300	310	370	370	360	450	520	520
23	1380	1380	1390	1390	1180	1180	1180	1180
24	20	30	40	40	50	50	50	50
25	230	300	370	370	320	340	360	360
26	340	340	340	340	270	270	280	280
27	20	20	20	20	30	40	90	90
					110	110	110	110

11	0	20	20	20	0	10	20	20
12	90	90	90	90	20	20	20	20
13	10	10	10	10	10	20	20	20
14	0	20	20	20	0	30	50	50
15	420	420	440	440	410	450	510	510
16	420	430	430	430	510	530	530	530
17	350	350	350	350	410	410	430	430
18	390	400	420	420	450	460	480	480
19	230	240	270	270	290	290	330	330
20	390	390	390	390	280	320	350	350
21	420	420	460	460	480	510	510	510
22	300	310	370	370	360	450	520	520
23	1380	1380	1390	1390	1180	1180	1180	1180
24	20	30	40	40	50	50	50	50
25	230	300	370	370	320	340	360	360
26	340	340	340	340	270	270	280	280
27	20	20	20	20	30	40	90	90
28	90	90	100	100	110	110	110	110
29	840	840	840	840	740	780	780	780
30	400	400	420	420	380	400	440	440
31	490	560	560	560	310	380	380	380
32	1180	1180	1180	1180	1300	1310	1330	1330
33	190	200	210	210	120	120	120	120
34	210	240	240	240	350	380	380	380
35	640	690	710	710	960	980	980	980
36	210	250	250	250	230	230	230	230
37	380	410	430	430	460	460	490	490
38	690	710	720	720	690	720	720	720
39	510	520	520	520	440	520	530	530
40	500	530	530	530	780	810	810	810
41	1200	1260	1260	1260	960	1010	1070	1070
42	270	280	290	290	320	330	330	330
43	520	540	540	540	600	610	610	610
44	290	300	300	300	220	220	220	220
45	0	30	60	60	10	10	10	10
46	50	130	140	140	80	130	130	130
47	10	20	30	30	40	50	50	50
48	90	130	130	130	110	110	110	110
49	30	50	50	50	50	60	70	70
50	40	40	40	40	0	10	50	50
51	30	50	50	50	110	110	110	110
Mean	285.7	309.0	319.2	319.2	308.6	329.4	342.7	342.7

fourth day of incubation was not more than 3 colonies per plate. Of the 51 samples of butter studied there were 47 in which the count secured after 5 days' incubation was not more than 3 colonies greater per plate than the count obtained after 4 days' incubation. Incubating the plates for 8 days did not cause any increase in count over that obtained after 5 days. The size of the mold colonies after 8 days' incubation often made counting difficult.

The greatest increase in the number of yeast and mold colonies from the third to the fourth day of incubation was with butter sample number 10 in which the number of colonies per plate increased from 12 to 25. The greatest increase in count from the fourth to the fifth day of incubation was with butter sample 25, in which the number of colonies increased from 30 to 37.

When an infusion prepared from 400 grams of potatoes was used, 14 samples of butter had the same count after 4 days' incubation as after 3 days, while 27 had the same count after 5 days' incubation as after 4 days. Of the 51 samples of butter studied, there were 40 in which the number of colonies that were visible to the naked eye after 4 days incubation was not more than 3 per plate greater than the number after 3 days; and the increase in count from the fourth to the fifth day of incubation was not more than 3 colonies with 41 samples

of the butter.

The greatest increase in count from the third to the fourth day of incubation was 9 colonies with butter sample 22, and with 5 days' incubation the count on this sample increased 7 colonies over the count after 4 days; of all the increases from fourth to fifth day of incubation, this was the greatest.

The results secured indicate that when potato dextrose agar was used as a medium the maximum yeast and mold count was obtained after 5 days' incubation at 21 - 25° C. and that the count obtained after 4 days' incubation approached this maximum count which is within the probable limits of error of plating. Greater differences in count were obtained by doubling the amount of potatoes used in preparing the infusion than by reducing the incubation time from 5 days to 3 days.

B. Number of yeasts in broths after 1, 3 and 5 days' incubation.

Pure cultures of three yeasts, Torula sphaerica, Pink B and Liquefier 106, were grown in (a) whey broth containing no peptone, (b) whey broth containing 0.5 per cent peptone, (c) nutrient broth with the addition of 2.0 per cent dextrose, (d) nutrient broth with the addition of 5.0 per

cent dextrose, and (e) potato dextrose broth. The whey broths were prepared by rennet coagulation. All the broths were adjusted to pH 3.5 ± 0.1 with tartaric acid. The broths were dispensed in 100 ml. quantities in screw cap dilution bottles and the cultures studied in duplicate. Counts were made to determine the numbers of cells introduced into the broths and also the numbers present after 24 hours', 3 days' and 5 days' incubation at 21 - 25° C. The data obtained are given in Table 24.

The numbers of yeast cells found in broths of different composition at the end of 1, 3 and 5 days varied with the three yeasts used. The counts secured in broths inoculated with T. sphaerica indicate that this yeast grows more rapidly in potato dextrose agar than in any of the other media. The count secured from potato dextrose broth after 1 day of incubation was equivalent to the count secured from whey broth containing 0.5 per cent peptone after 5 days' incubation, and the count obtained from potato dextrose broth at 3 days was greater than the counts secured at 5 days from the other broths with the exception of whey broth containing no peptone.

The yeast Pink B developed greater numbers of cells in potato dextrose broth after 1 day's incubation than any of the other media. After 3 days' incubation the number of cells found in 5 per cent dextrose nutrient broth approached

Table 24

Number of yeasts in broth media after 1, 3 and 5 days' incubation at 21 - 25° C..

Yeast used	Time of incubation in days	Number of yeasts per ml. of broth				
		A	B	C	D	E
T. sphaerica	1	>10	>10	>10	>10	4,600
	3	2,100,000	1,000	1,000	1,000	4,900,000
	5	9,600,000	4,000	4,000,000	500,000	34,000,000
Pink B	1	>10	>10	>1,400	4,400	45,000
	3	1,000	1,000	3,900,000	8,000,000	8,000,000
	5	600,000	400,000	45,600,000	33,600,000	36,000,000
Liquefier 106	1	36,000	55,000	474,000	330,000	339,000
	3	9,700,000	1,100,000	7,100,000	5,600,000	219,000,000
	5	36,000,000	20,000,000	37,000,000	88,000,000	218,000,000

A = Whey broth 0.0% peptone B = Whey broth + 0.5% peptone
C = 2% dextrose nutrient broth D = 5% dextrose nutrient broth
E = Potato dextrose > = less than

Number of yeast cells contained in 100 ml. of broth before incubation
T. sphaerica 3, Pink B, 55, and Liquefier 106, 51.

the count secured from potato dextrose broth. The number of cells found in potato dextrose broth after 3 days' incubation was greater than the number of cells found after 5 days' incubation in the whey broths.

Yeast Liquefier 106 initiated growth faster in the broths used than did the other two yeasts. The number of cells found in 2 per cent dextrose nutrient broth after 1 day's incubation was greater than secured from potato dextrose broth after the same incubation period. The count secured after 3 days' incubation in potato dextrose agar exceeded the count for the other broths after incubation for the same period, and was also greater than any of the counts secured from the other broths after 5 days' incubation.

Part 5

INFLUENCE OF THE OXIDATION-REDUCTION POTENTIAL OF THE MEDIUM UPON THE GROWTH OF YEASTS AND MOLDS.

Introduction

During the past few years a great many studies have been made on the electromotive properties of biological systems. In general, the studies indicate that bacterial cultures as well as sterile bacteriological media are active oxidation-reduction systems. Allyn and Baldwin[2] reported that marked effects on the growth of an aerobic bacterial species were induced by certain changes in the oxidation-reduction character of the media. Later these same authors[3] found that the oxidation-reduction potential of a medium exerts a decisive influence on the ability of certain aerobic bacteria to initiate growth. Reid and Baldwin[23] reported that the oxidation-reduction potential of the medium exerts a decided influence on the ability of Saccharomyces cerevisiae to initiate growth. These investigators used a mineral salt sugar medium, and with the addition of agents which lowered the potential of the medium were able to initiate growth with an inoculium of 10 cells per ml., while the untreated medium required, to insure growth initiation, an inoculum of 10,000 cells per ml.

No information is available as to the role played by the

oxidation-reduction potential of the medium on the growth of yeasts and molds found in butter. The medium used for determining the yeast and mold count of butter should be at the optimum potential in order to secure maximum growth. Reid and Baldwin [23] have pointed out that in the case of Saccharomyces cerevisiae the potential of the mineral salt sugar medium exerts a decided influence on the ability of this yeast to start to grow. In the counting of yeasts and molds in butter, the dilution of the butter was thoroughly shaken, with the objective of distributing the yeast and mold cells equally throughout the dilution. By such agitation single cells were probably often obtained, and it was assumed in counting the plates that each colony counted at the end of the incubation period had resulted from a single cell. Data are presented in Table 1 (Part 1) which show that 2 per cent dextrose beef infusion agar was an inferior medium, when compared with 5 per cent dextrose nutrient agar, for the growth of yeasts and molds. The ingredients contained in these two media were somewhat similar (for bacterial growth the infusion medium is usually preferred). In view of previous work it was assumed that the potential of the infusion medium was unfavorable, whereas the potential of the 5 per cent dextrose nutrient agar was favorable, for the initiation of growth by small numbers or single cells of the yeasts and molds found in butter.

In order to obtain data on the oxidation-reduction potential of a medium and its influence upon the growth of yeasts and molds found in butter, studies were made as to the potentials of various media and the ability of media of different potentials to support the growth and development of the yeasts and molds found in butter.

Experimental

A. Oxidation-reduction potential of media used for counting yeasts and molds.

Using a simple potentiometer circuit, potential measurements were made of the various media in the broth form. The broth was placed in large test tubes which had been blown out at the base in order to prevent the electrodes from coming in direct contact with the glass during the operations. Two electrodes were inserted into each tube at the same level beneath the surface of the medium.

The electrodes, consisting of bright platinum, were about 0.5 inch long and sealed in the end of soft glass tubing; the circuit contact was made by means of mercury. The electrodes were cleaned in choromic-sulphuric acid, rinsed thoroughly in sterile distilled water and flamed in an alcohol flame before each experiment. The electrodes were not sterilized further, for the broths were at pH 3.5± 0.1, and by careful washing and

handling no yeast and mold contamination was secured.

Potential measurements were obtained upon agar media in the same manner as upon broth media, with the exception that the agar was poured into the electrode vessel at 42 - 45° C., allowed to cool to 21° C., and measurements then made. All the broth and agar media were studied in duplicate.

Considerable time was expended in endeavoring to obtain duplicate results with a vacuum tube potentiometer, designed for glass electrode work by Goodhue, Schwarte, and Fulmer(8), but without success.

In computing the oxidation-reduction potentials of the various media a saturated calomel half cell was used as a standard of comparison in actual operations. The potentials of various broth and agar media at pH 3.5± 0.1 are given in Table 25.

At the start the range in oxidation-reduction potential of the broth and agar media studied varied with 2 per cent dextrose infusion from a low of 0.057 volts to a high of 0.267 volts with potato dextrose broth.

The media studied divide themselves into three groups: Group 1, those media that have voltages of 0.2 volts or greater, includes potato dextrose broth, potato dextrose agar and Bacto malt agar; Group 2, those media that show a voltage of more than 0.1 volts but less than 0.2 volts, includes whey broth, whey broth containing no peptone, and dextrose nutrient

broth; Group 3, those media that show a voltage of 0.1 volts or less, includes dextrose infusion broth, Bacto tomato juice broth, Bacto tomato juice agar, and Bacto yeast dextrose agar.

Of the media used in Table 1 (Part 1) whey agar prepared by rennet coagulation was found to give the lowest mean yeast and mold count. Whey broth prepared by rennet coagulation gave a voltage of 0.127 to 0.130. Bacto tomato juice broth and 2 per cent dextrose beef infusion broth gave voltages which were less than that of whey broth, the voltages being 0.059 and 0.057 respectively. The agars prepared from these broths gave higher mean yeast and mold counts than whey agar. It would appear from these data that the oxidation-reduction potential, within the range studied, is not a limiting factor in the growth of the yeasts and molds found in butter, since growth was secured over the entire potential range studied.

B. Influence of number of yeast cells inoculated into media of various potentials upon their ability to initiate growth.

In order to determine whether or not the potentials of the media used for counting the yeasts and molds in butter are limiting factors in the growth of yeasts when small numbers of yeasts are inoculated into them, four yeasts in

Table 25

Oxidation-reduction potentials of sterile broths and of sterile agars used in counting yeasts and molds in butter.

Media at pH 3.5 ± 0.1 Temperature at 25° C.

Broth medium	Readings in volts				
	Start*	3 hrs.	6 hrs.	24 hrs.	48 hrs.
Whey rennet coag.	.130		.131	.112	.115
Whey rennet coag.	.127		.127	.120	.119
Whey - no peptone	.120		.124	.142	.132
Whey - no peptone	.140	.147	.119	.124	.120
Dextrose nutrient	.135	.125	.124		
Dextrose nutrient	.133	.130	.130		
Dextrose infusion	.057		.048		.046
Dextrose infusion	.057		.048		.046
Bacto tomato juice	.059		.076		.076
Bacto tomato juice	.058		.055		.055
Potato dextrose	.267		.254	.251	.225
Potato dextrose	.230	.227	.235	.230	.231
Agar medi:					
Potato dextrose	.228	.231	.225	.210	.210
Bacto malt	.258	.264	.271	.248	.258
Bacto yeast dextrose	.089	.088	.070	.096	.096
Bacto tomato juice	.058	.046	.035	.034	.009

* Media were steamed in flowing steam for one hour, then cooled to 21° C. The voltage readings were made at 1½ to 2½ hours after removing from the steamer.

various concentrations of cells were inoculated into media of various potentials. The media used were of low voltage (dextrose infusion broth and tomato juice broth), of median voltage (whey broth, with and without peptone and dextrose nutrient broth), and of high voltage (potato dextrose agar). The broths were acidulated to pH 3.5 ± 0.1 with tartaric acid and were dispensed into sterile test tubes under aseptic conditions. The amount of broth in each tube was approximately 10 ml. The yeasts used were Pink B, Liquefier B, T. sphaerica and T. cremoris. Dilutions were prepared of these yeasts and definite quantities inoculated into the broths. The numbers of cells inoculated into the broths were determined by plating similar quantities on potato dextrose agar. The tubes were incubated for 5 days at 21 - 25° C., and growth was measured by removing approximately 0.005 ml. portions of each broth with a wire loop after the tubes had been thoroughly shaken and streaking on potato dextrose agar. The results obtained are presented in Table 26.

The results show tendency for the media of low potential to inhibit or retard yeast growth. With the method of examination used dextrose infusion broth which had a potential below 0.1 volts did not show growth when inoculated with the lowest numbers of cells of Pink B or T. sphaerica, while the Liquefier B and T. cremoris were able to initiate growth in this medium

Table 26

Influence of number of yeast cells inoculated upon their ability to initiate growth in broth media of various oxidation-reduction potentials.

pH of broth media 3.5 ± 0.1

Yeast used	No. of cells inoculated	Growth after 5 days at 21 - 25° C.											
		Volts below 0.1				Volts 0.1 - 0.2						Volts ≠ 0.2	
		dextrose infusion		tomato juice		whey*		whey* no peptone		dextrose nutrient		potato dextrose	
Pink B	377	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	30	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	4	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	1	-	-	≠	≠	≠	≠	≠	≠	≠	-	≠	≠
Liquefier B	198	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	15	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	2	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	≠	≠
	0	-	-	-	-	-	-	-	-	-	-	-	-
T. sphaerica	304	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	28	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	2	-	-	≠	≠	≠	≠	≠	≠	-	-	≠	≠
	0	-	-	-	-	-	-	-	-	-	-	-	-
T. cremoris	543	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	92	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	5	≠	≠	-	≠	≠	≠	≠	≠	≠	≠	≠	≠
	0	-	-	-	-	-	-	-	-	-	-	-	-

* Whey broth prepared by rennet coagulation. ≠ = growth - = no growth

with the smallest inoculations used. Tomato juice broth, which also had a potential of below 0.1 volts, supported growth of Pink B and T. sphaerica with inoculations which did not give growth in dextrose infusion broth. Growth was initiated in all tubes of whey broths with and without peptone, and was also initiated in all tubes of potato dextrose broth which had a higher potential than the whey broths. Pink B, Liquefier B and T. sphaerica failed to grow in one or both tubes with inoculations into dextrose nutrient broth but gave growth in the whey broths. The difference between the oxidation-reduction potentials of the dextrose nutrient broth and whey broths was less than the difference between the whey broths and potato dextrose broth.

The general results indicate that the composition of the medium is of greater significance than its potential.

In order to secure further information on the effect of the oxidation-reduction potential of the medium upon the growth of yeasts found in dairy products, eight yeasts were plated upon three media. The media used were potato dextrose agar, which had a voltage greater than 0.2 volts, and Bacto yeast dextrose agar and Bacto tomato juice agar, which had voltages of less than 0.1 volts. Dilutions of the yeasts were plated upon the three media after acidulating to pH 3.5 ± 0.1. The numbers of colonies per plate were counted after 5 days' incubation at 21 - 25° C., the counts obtained are presented

in Table 27.

Although the potato dextrose agar had a voltage greater than 0.2 volts and Bacto yeast dextrose agar and Bacto tomato juice agar had voltages of less than 0.1 volts, the differences in count per plate were greater between Bacto yeast dextrose agar and Bacto tomato juice agar than between Bacto yeast dextrose agar and potato dextrose agar. In none of the dilutions of the yeasts used was the number of colonies per plate on Bacto yeast dextrose agar or on Bacto tomato juice agar greater than that on potato dextrose agar, and only in one case (Common white YB) was the count per plate greater on Bacto tomato juice agar than on Bacto yeast dextrose agar. The yeast Pink B failed to grow in Bacto tomato juice agar even when inoculated with approximately 5,190,000 cells per plate. This yeast grew on Bacto yeast dextrose agar, but did not produce so many colonies per plate as it did upon potato dextrose agar when a corresponding inoculation was used. With all the yeasts studied the colonies which were produced upon Bacto tomato juice agar were as large as the colonies of the same yeast upon Bacto yeast dextrose agar or upon potato dextrose agar, indicating that when growth was established there was no lack of nutrients in the medium.

The results secured indicate that the oxidation-reduction potential, within the limits used, was not the limiting factor

Table 27

Influence of media of different oxidation-reduction potentials upon the numbers of yeast colonies developing when dilutions of yeasts were plated.

Yeast used	Dilution	Average counts per plate on potato dextrose agar	Bacto yeast dextrose agar	Bacto tomato juice agar
Common white S	1 - 10,000	466	352	337
Common white YB	1 - 1,000	688	300	564
Common white YB	1 - 10,000	79	24	76
Common white B	1 - 1,000	518	454	304
Common white B	1 - 10,000	74	65	34
Common white C	1 - 10,000	207	164	46
Pink A P	1 - 1,000	TNC *	TNC *	169
Pink A P	1 - 10,000	408	302	16
Pink B	1 - 100	TNC*	TNC*	0
Pink B	1 - 1,000	TNC*	TNC*	0
Pink B	1 - 10,000	TNC*	TNC*	0
Pink B	1 -100,000	519	381	0
T.sphaerica	1 - 1,000	285	241	110
Liquefier B	1 - 1,000	TNC*	TNC*	208
Liquefier B	1 - 10,000	129	59	0

* TNC too numerous to count

in the growth of some of the common yeasts found in dairy products, for there is no close agreement between the potential of the medium and its ability to support yeast growth. The medium with the highest potential, however, supported the greatest number of colonies. This could be interpreted as indicating that a single yeast cell finds a more favorable environment in a medium of a voltage of 0.2 than in a medium of lower potential. As with the data presented in Table 26, there exists as great a difference in yeast counts between media of approximately the same oxidation-reduction potential as between media of different oxidation-reduction potentials.

C. Influence of addition of thioglycollic acid or hydrogen peroxide to potato dextrose agar on the growth of yeasts and molds.

Reid and Baldwin[23] found that the oxidation-reduction potential of the medium they used to study the growth of Saccharomyces cerevisiae could be altered by the addition of small quantities of thioglycollic acid or hydrogen peroxide, which would indicate that the medium was poorly poised. In order to obtain data as to the poising capacity of potato dextrose agar and the effect of adding these materials the following experiment was performed.

Potato dextrose agar was prepared and dispensed in 150 ml. quantities into three screw cap bottles, the medium was acidulated to pH 3.5 ± 0.1 with 5 per cent tartaric acid and varying quantities of thioglycollic acid or hydrogen peroxide added. To insure thorough mixing the contents were agitated and then the medium was permitted to stand for 48 hours at 50° C. Thirty samples of butter made in two Indiana centralizer creameries were plated, 15 samples upon the medium containing thioglycollic acid and 15 upon the medium containing hydrogen peroxide. The counts obtained are presented in Tables 28 and 29.

The addition of thioglycollic acid caused a regular decrease in the mean yeast and mold count of the 15 samples of butter. This decrease is correlated with the amount of thioglycollic acid used, but the differences were well within the limits of error of plating, for there was no regularity with individual samples. When 0.01 ml. of thioglycollic acid was added to 150 ml. of medium, ten of the 15 samples of butter had lower yeast and mold counts than when no thioglycollic acid was added; four had higher counts and with one there was no difference.

The addition of hydrogen peroxide caused a regular increase in the mean yeast and mold count of the 15 samples of butter, the increase correlated with the amount of hydrogen

Table 28

Influence of the addition of thioglycollic acid to potato dextrose agar at pH 3.5 ± 0.1 upon the growth of yeasts and molds.

Sample of Butter	ml. of thioglycollic acid added per 150 ml. of medium			
	0.0	0.002	0.005	0.01
	Count per 0.1 ml. of butter			
1	14	11	10	9
2	7	7	6	8
3	9	6	6	6
4	14	9	8	6
5	8	9	9	10
6	11	4	5	4
7	15	15	12	12
8	22	16	16	15
9	24	18	14	15
10	8	8	10	10
11	16	14	8	10
12	10	8	9	4
13	12	13	13	7
14	1	1	5	4
15	7	10	16	7
Mean	11.9	9.9	9.8	8.5

Table 29

Influence of additon of hydrogen peroxide to potato dextrose agar at pH 3.5 ± 0.1 upon the growth of yeasts and molds.

Sample of Butter	ml. of 3 per cent hydrogen peroxide added per 150 ml. of medium		
	0.0	1.0	2.0
	Counts per 0.1 ml. of butter		
16	5	3	4
17	4	8	5
18	7	9	8
19	14	15	13
20	13	8	9
21	7	8	10
22	9	14	15
23	74	77	110
24	6	9	21
25	10	13	9
26	18	16	23
27	3	6	7
28	3	3	2
29	1	1	0
30	20	25	20
Mean	9.2	10.2	11.6

peroxide added; the differences in mean counts were within limits of error of plating. The addition of 3.0 ml. of 3 per cent hydrogen peroxide to potato dextrose at pH 3.5 ± 0.1 caused the medium to lose its gel properties .

These data would indicate that potato dextrose agar is well poised and that the addition of small amounts of thioglycollic acid or hydrogen peroxide do not significantly affect the formation of yeast and mold colonies on the plates. The addition of 1.0 ml. of hydrogen peroxide to 150 ml. of medium yielded higher counts on 10 samples of butter than the count obtained when no hydrogen peroxide was added; three samples gave higher counts when no hydrogen peroxide was added; and with two there was no difference. Seven samples gave higher counts when 2.0 ml. of hydrogen peroxide was added than when 1.0 ml. was added. The difference in count within individual samples was in the case of some samples significant. Four samples gave lower counts with the addition of 2.0 ml. of hydrogen peroxide than with the addition of 1.0 ml., and in four samples the count was lower with the addition of 2.0 ml. of hydrogen peroxide than when no hydrogen peroxide was added to the medium.

Part 6

SPECIAL METHODS ATTEMPTED FOR COUNTING YEASTS AND MOLDS IN BUTTER.

A. Comparison of the smear technique with the plate method for counting yeasts and molds in butter.

The smear technique, which consists of smearing the inoculating material on the surface of the agar plate rather than distributing through the liquefied medium, presents what appear to be advantages over the plate method for determining the yeast and mold count of butter in that yeast colonies are larger when growing on the surface than when growing beneath the surface of the medium. The smear technique used was somewhat similar to the method suggested by Burri(5) for bacterial counting.

Yeast and mold counts were made on samples of butter using the smear technique and the usual plate method. Potato dextrose agar and 2 per cent dextrose nutrient agar were used. For the smear technique these agars were acidulated to pH 3.5 ± 0.1, poured into sterile petri dishes to a depth of 0.8 cm. and the dishes placed in a 37° C. incubator for 2 days, in order to evaporate any condensed water which may have been

on the surface of the medium. The medium was inoculated with 1.0 ml. of the butter dilution and this material smeared over the surface of the medium by means of a sterile bent glass rod. The plates were then inverted and incubated. Twenty-five samples of Iowa butter were studied; the yeast and mold counts obtained are given in Table 30.

The mean yeast and mold count secured per ml. of butter on potato dextrose agar when using the smear technique was 330.5, while when using the usual plate method it was 383.3. The mean counts obtained on dextrose nutrient agar using the smear technique and the usual plate procedure were 264.5 and 326.0 respectively.

When potato dextrose was used as the medium, five samples of butter gave equal counts with the two methods, seven samples showed higher counts with the smear technique and 13 samples of butter had higher counts with the usual plate procedure. Upon 2 per cent dextrose nutrient agar five samples gave equal counts with both methods, five gave higher counts with the smear technique, and 15 gave higher counts with the usual plate method.

a. Influence of milkfat on the surface of the medium upon the yeast count.

Mold and yeast counts are usually made on low dilutions

Table 30

Comparison of the smear technique with the plate method of determining yeasts and molds in butter.

Sample of Butter	Average yeast and mold count per ml. of butter			
	Potato dextrose agar		Dextrose nutrient agar	
	Smear technique	Plate method	Smear technique	Plate method
1	1181	859	684	808
2	904	808	875	900
3	47	284	178	372
4	12	6	8	3
5	381	611	270	437
6	89	69	52	58
7	252	196	187	99
8	69	57	39	39
9	443	1275	259	777
10	398	408	328	346
11	140	469	115	338
12	651	652	590	491
13	323	689	460	566
14	6	5	3	4
15	549	499	529	502
16	1	3	2	5
17	2	2	1	1
18	5	6	3	8
19	27	32	23	28
20	478	588	495	476
21	651	634	491	608
22	550	533	608	506
23	355	599	215	417
24	209	342	145	293
25	7	72	16	59
Mean	330.5	383.3	264.3	326.0

of butter, and as a result considerable quantities of fat are added to the plates. When the usual plating procedure is used the fat is mixed into the medium with the rotating of the plate; when the smear technique is used, the fat remains upon the surface of the medium and the surfaces of the fat globules are not surrounded by the medium. In order to determine whether or not the lower yeast and mold counts obtained with the smear technique were due to the presence of fat on the surface of the medium (the fat preventing the yeast cells from contacting the medium), the following study was undertaken.

Dilutions of cultures of 15 yeasts which had been isolated from Iowa butter were smeared upon and plated upon potato dextrose agar. The results secured are presented in Table 31.

The mean yeast count when the smear technique was used was 386.1; when the usual plate method was used it was 382.0. Eight dilutions of yeasts gave higher counts when smeared upon the surface of the medium, five gave higher counts when plated, and two showed no difference in count. The difference between the smear technique and the usual plate procedure in the determination of the yeast count on dilutions of yeasts was within the limits of experimental error.

Dilutions of eight yeasts were smeared upon potato

Table 31

Comparison of smear technique with plate procedure for counting yeasts in dilutions.

Yeast used	Count per ml. of dilution on potato dextrose agar using	
	Smear technique	Plate method
1	599	634
2	505	506
3	514	404
4	404	377
5	157	68
6	452	392
7	353	420
8	452	524
9	379	364
10	374	454
11	350	432
12	364	364
13	354	313
14	296	263
15	239	215
Mean	386.1	382.0

dextrose agar and also upon potato dextrose agar upon whose surface sterile fat had been smeared. The amount of fat smeared over the agar was 0.1 ml. (approximately the amount that is contained in 1 ml. of a 1 to 10 dilution). After the fat had been smeared over the surface, dilutions of yeasts were inoculated and smeared. The counts secured are given in Table 32.

The mean yeast count obtained when 0.1 ml. of milk fat had been smeared over the surface of the potato dextrose was 129.4; when no fat had been smeared over the surface of the medium it was 145.1. Seven of the eight dilutions of yeasts gave a higher count when fat was not present on the surface of the medium. The percentage difference between the mean counts of the smear and plate methods, using potato dextrose as a medium after plating samples of butter (Table 32) was 13.8 per cent, and the percentage difference between the mean counts presented indicate that the presence of fat on the surface of the medium tends to prevent the development of some of the yeasts inoculated upon it.

B. Attempts to count the yeasts and molds in butter with a microscopic procedure.

Samples of butter which had yeast and mold counts between 100 and 1000 per ml. were selected for the attempts to count

Table 32

Influence of fat on the surface of medium upon the count secured on dilutions of yeasts with the smear technique.

Yeast used	Count per ml. of dilution on potato dextrose agar with	
	0.1 ml. of fat per plate	No fat per plate
1	93	95
2	69	86
3	78	67
4	279	315
5	274	294
6	160	197
7	70	84
8	14	23
Mean	129.4	145.1

the yeasts and molds with a microscopic procedure. The samples of butter had been made from sour cream and salted.

Using the method which was developed by Hammer and Nelson for the microscopic examination of butter, the fat was separated from the serum and attempts were made to further concentrate the yeasts contained in this serum. Further concentration of the yeasts in the serum seemed necessary because the numbers of yeasts and molds contained in butter are usually less than 500 per ml. and such values made a low factor advisable in calculating the number per ml. of butter.

The methods of concentration attempted were as follows:

A. The serum obtained from 10 ml. of butter was placed in a centrifuge tube and centrifuged for 15 minutes at 2000 r.p.m. (diameter of centrifuge head was 7 inches). The upper portion of liquid was then siphoned off and the sediment remaining was smeared over an area of 1.0 sq. cm. and stained with the Newman-Lampert one-solution stain. The amount of foreign material present was so great that yeasts and molds could not be identified.

B. Method A was used except that the small end of the centrifuge tube was broken off so as to permit inserting a small rubber stopper. After centrifuging, the entire liquid content of the centrifuge tube was poured off, and the sediment adhering to the rubber stopper was smeared over 1.0 sq. cm.

and stained; the foreign material, curd, etc. prevented preparing a satisfactory film, since the material was too concentrated. The sediment adhering to the cork was then smeared over 9.0 sq. cm., but clear fields, in which yeast cells, mold spores and mold mycelium could be identified, were not obtained.

C. Method B was used except that after procuring the serum from the butter it was diluted with equal quantities of each of the following solutions and thoroughly shaken:

1. M/5 lithium hydroxide
2. N/10 sodium hydroxide
3. M/5 sodium bicarbonate
4. M/5 sodium borate

The object in adding the above materials was to endeavor to put the curd-like material, which was being thrown out with the yeast and mold cells, into suspension or solution.

The staining of sediments from butter which according to plate count contained approximately 1000 yeasts per ml. gave results which were of no value in enumerating yeasts and molds, because of inability to obtain films free from large amounts of insoluble material.

In the examination of the sediments obtained from butter it was found that they consisted chiefly of curd, but that sand, rust and particles of metal were also present.

Part 7

SOME FACTORS INFLUENCING THE NUMBER OF YEASTS IN BUTTER.

In enumerating the yeasts and molds in samples of salted butter there is the possibility that sufficient salt may be carried over into the plates to inhibit the growth of the organisms contained in the butter. Also there is little information available as to the influence of the medium upon the salt concentration that is necessary to inhibit the growth of the yeasts found in butter. The studies of Nelson[19] give evidence on the influence of salt in butter on the growth of yeasts. This investigator examined microscopically 448 samples of butter and detected a greater increase after an incubation period of 7 days at 21° C. in the yeast count in unsalted butter than salted; the salt percentage of the samples of butter was not recorded. In order to procure information concerning these factors, the following work was undertaken.

Experimental

A. Influence of the percentage of salt in the media upon the growth of yeasts.

Skimmed milk, potato dextrose broth, and Bacto whey broth were prepared containing from 1.0 to 10.0 per cent of

salt (sodium chloride). These media were dispensed in 10 ml. quantities into test tubes and sterilized. The broths were inoculated with the yeasts T. cremoris, Common white P, and Pink B. The numbers of yeasts inoculated were determined by plating quantities equal to those inoculated upon acidulated potato dextrose agar. The inoculated broths were incubated for 5 days at 21 - 25° C. At the conclusion of the incubation period the tubes were thoroughly shaken, 1.0 ml. was removed and dilutions prepared so that the plates would contain the approximate number of colonies inoculated into the broth at the time of inoculation; these plates were then incubated. Table 33 presents the data obtained, the positive sign indicating that the number of yeasts found in the broth was in excess of the number in the broth before incubation.

A relatively heavy inoculation did not increase the tolerance of the yeasts to salt but slightly decreased it. The yeasts T. cremoris and Common white P, when grown in skimmed milk and potato dextrose broth, showed a greater tolerance for salt than when grown in Bacto whey broth; with the yeast Pink B the reverse was true. T. cremoris was the most sensitive to salt of the three yeasts when grown in Bacto whey broth, growth being inhibited by a salt concentration of 4.0 per cent; the yeast Common white P was the most tolerant of the three yeasts, growth taking place in skim

Table 33

Influence of percentage of salt in media upon the growth of yeasts.

Yeast used	No. of cells inoculated	Medium	Growth in salt percentages of									
			1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
T. cremoris	50	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	50	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	50	Bacto whey broth	≠	≠	≠	-						
	150	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	-		
	150	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	150	Bacto whey broth	≠	≠	≠	-						
	1200	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	-		
	1200	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	1200	Bacto whey broth	≠	≠	≠	-						
Common white P	1020	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
	1020	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠

	1200	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	
	1200	Bacto whey broth	≠	≠	≠	-							
Common white P	1020	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	≠	≠		≠
	1020	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠		≠
	1020	Bacto whey broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	
	12,000	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	
	12,000	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	-			
	12,000	Bacto whey broth	≠	≠	≠	≠	≠	≠	≠	-			
	65,000	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	
	65,000	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	-			
	65,000	Bacto whey broth	≠	≠	≠	≠	≠	≠	-				
Pink B	1250	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	-			
	1250	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	
	1250	Bacto whey broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	
	10,000	Skimmed milk	≠	≠	≠	≠	≠	-					
	10,000	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	-			

	12,000	Bacto whey broth	≠	≠	≠	≠	≠	≠	≠	-		
	65,000	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	65,000	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	-		
	65,000	Bacto whey broth	≠	≠	≠	≠	≠	≠	-			
Pink B	1250	Skimmed milk	≠	≠	≠	≠	≠	≠	≠	-		
	1250	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	1250	Bacto whey broth	≠	≠	≠	≠	≠	≠	≠	≠	≠	-
	10,000	Skimmed milk	≠	≠	≠	≠	≠	-				
	10,000	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	-		
	10,000	Bacto whey broth	≠	≠	≠	≠	≠	≠	-			
	20,000	Skimmed milk	≠	≠	≠	≠	≠	-				
	20,000	Potato dextrose broth	≠	≠	≠	≠	≠	≠	≠	-		
	20,000	Bacto whey broth	≠	≠	≠	≠	≠	≠	-			

≠ = growth - = no growth

milk with concentrations of 9.0 to 10.0 per cent. The yeast Pink B was slightly more tolerant in potato dextrose broth than in Bacto whey agar, growth being inhibited in salt concentrations of 7.0 to 9.0 per cent.

The data indicate that the salt concentration of butter is not great enought to effect the growth of yeasts upon the medium in which the butter sample is plated.

B. Distribution of yeasts and bacteria during the churning process.

In order to compare the percentage of yeasts and of bacteria that are retained in butter during its manufacture, the following study was undertaken.

Pure cultures of yeasts and bacteria were inoculated into sterile sweet cream. The yeasts used were T. cremoris, Pink B and Common white P, and the bacteria used were Achromobacter lipolyticum and Aerobacter aerogenes. The numbers of organisms inoculated were determined by plating a small quantity of the inoculum and calculating the number per ml. of cream from the volume. The cream was churned in sterile quart mason jars, without the use of butter culture. The churn was stopped when the butter granules were slightly smaller than wheat grains. The amount of cream in all the

churnings was approximately the same, and to secure uniformity, the cream was churned in a multiple churn in which six samples were churned at the same time and in each trial duplicate churnings were made. The volume of water used to wash the butter was the same for all the churnings, and the butter was not salted. Control churnings were made to check the sterility of the method of churning and washing of the butter, and it was found that the number of contaminating organisms was less than 10 per ml.

The counts were made within 2 hours after churning. The yeast counts were made upon potato dextrose agar acidulated to pH 3.5 $\pm$ 0.1 and incubated for 5 days at 21 - 25° C. while the bacterial counts were made on beef infusion agar prepared from Bacto beef and the plates were incubated for 5 days at 21 - 25° C. The counts secured are given in Table 34 and the percentages of the organisms retained in the butter as calculated from Table 34 are presented in Table 35.

The number of yeasts in the cream varied from 4,700 to 800,000 per ml., and the number of bacteria varied from 28,000 to 750,000 per ml.. The number of yeasts found in the buttermilk ranged from 2,600, to 2,000,000 per ml., and the number of bacteria found in the buttermilk ranged from 40,000 to 600,000 per ml. The number of yeasts found in the buttermilk was greater in nine trials than the number of yeasts in the

Table 34

The distribution of numbers of yeasts and bacteria during the churning process.

Organism used	Trial	Number of organisms per ml.				
		Cream	Buttermilk	1st wash water	2nd wash water	Butter
T. cremoris	1	14,000	7,700	400	10	420
	2	9,000	5,600	1,050	110	900
Pink B	1	15,400	10,600	3,000	220	4,200
	2	87,000	90,000	8,500	3,500	1,900
	3	193,000	150,000	30,000	4,100	40,000
	4	170,000	200,000	22,000	1,500	30,000
	5	40,000	129,000	5,600	600	5,300
	6	35,000	62,000	7,300	900	3,900
Common white P	1	4,700	2,600	1,000	380	500
	2	10,000	14,000	1,500	290	180
	3	30,000	70,000	30,000	10,000	5,800
	4	800,000	2,000,000	30,000	7,000	190,000
	5	40,000	172,000	12,000	1,000	5,900
	6	55,000	150,000	27,000	3,200	7,600
Ach. lipolyticum	1	28,000	60,000	8,500	1,160	100
	2	400,000	350,000	125,000	55,000	6,000
	3	90,000	330,000	96,000	10,800	1,400
	4	750,000	480,000	850,000	8,000	38,000
A. aerogenes	1	30,000	40,000	10,000	1,300	100
	2	460,000	600,000	300,000	45,000	42,000
	3	190,000	140,000	140,000	15,000	7,000
	4	300,000	400,000	175,000	41,000	32,000

Table 35

The percentage of organisms retained in the butter. Percentage calculated from number of organisms in cream as 100 per cent.

Organism used	Trial	Butter
T. cremoris	1	3.0
	2	10.0
Pink B	1	27.3
	2	2.2
	3	20.7
	4	17.6
	5	13.3
	6	11.2
Common white P	1	10.6
	2	1.8
	3	19.3
	4	23.7
	5	14.7
	6	13.8
Ach. lipolyticum	1	0.3
	2	1.5
	3	1.5
	4	5.0
A. aerogenes	1	0.3
	2	9.1
	3	3.7
	4	10.5

cream, in five trials greater numbers of bacteria were found in the buttermilk than in the cream, in these cases the unusual relationship was probably due to the failure to break up the groups of organisms when plating the inoculating material as completely as when plating the buttermilk. The number of yeasts removed with the first wash water varied from 400 to 30,000 per ml.; the number of bacteria varied from 8,500 to 850,000 per ml. The second wash water removed fewer organisms than the first wash water. The number of yeasts retained in the butter ranged from 180 to 190,000 per ml., the number of bacteria from 100 to 42,000. The samples of butter made from cream of high count retained a greater number of organisms than samples of butter made from low count cream, but there existed no constant ratio between the number of organisms in the cream and the number of organisms retained in the butter.

The percentage of yeasts retained in the butter, as calculated from the cream as containing 100.0 per cent ranged from 1.8 to 27.3 per cent, the percentage of bacteria retained ranged from 0.3 to 10.5 per cent. The average percentage of yeasts and of bacteria retained in the butter was 13.4 and 3.9 respectively. Six trials were conducted using the yeasts Pink B and Common white P, the percentage of these yeasts retained was 15.4 and 14.0 respectively. Four trials were conducted using Ach. lipolyticum and A. aerogenes and the number of these

organisms retained was 2.1 per cent and 5.9 per cent respectively.

These data show that a higher percentage of yeasts are retained in butter than bacteria.

SUMMARY

Part 1

The medium used for determining the yeast and mold count of butter greatly affected the count secured. The mean counts on 30 samples of butter using 12 media ranged from a low of 21.0 yeast and molds per 0.1 ml. of butter on whey agar, prepared from whey adjusted to pH 7.5 at the time of filtering, to a high of 110.8 yeasts and molds on potato dextrose agar.

Potato dextrose agar gave higher counts than the potato dextrose agar suggested by Weiser or Bacto potato dextrose agar. Potato dextrose agar gave the higher yeast and mold count for a greater number of individual samples than did any other medium. No medium consistently gave high or low counts within individual samples.

Within the whey agar media there were correlations between the growth of yeasts and molds and the pH at which the whey obtained by rennet coagulation was filtered. The whey filtered at the lowest pH (5.5) gave the highest mean count; that filtered at the highest pH (7.5), the lowest mean count.

Tomato juice agar did not give so high a mean yeast and mold count as potato dextrose agar for 22 of the 30 samples of butter, nor did it give as high a count as upon potato

dextrose agar.

Five per cent dextrose nutrient agar gave higher counts than 2 per cent dextrose beef infusion agar, but neither medium gave so high a mean count nor yielded as many high counts with individual samples of butter as potato dextrose agar.

Bacto wort agar gave a higher mean count than Bacto malt agar, but these media did not give so high mean counts as potato dextrose agar.

The difference between Bacto dextrose nutrient agar and dextrose nutrient agar prepared in the laboratory from Bacto products was within the limits of experimental error.

When peptone was used in whey agar, lower counts resulted than when no peptone was added.

The buffer capacity was not the factor limiting the growth of yeasts on whey agar prepared by rennet coagulation, since reducing the buffer capacity by diluting the whey caused a decrease in extent of growth of yeasts.

Media of known composition were found unsatisfactory for yeast and mold counts in butter because the colonies failed to develop sufficiently during the incubation period to be counted without magnification and also because of the great difficulty of differentiating between yeast or mold colonies and fat globules.

Part 2

The yeast counts secured upon potato dextrose agar by plating dilutions of yeasts isolated from dairy products were essentially the same with two lots of the medium prepared from two extreme types of potatoes.

The amount of potato used in the preparation of the infusion to be made into potato dextrose agar affected the yeast and mold count within narrow limits. The mean count was less when an infusion prepared from 100 grams of potatoes per liter was used than when an infusion prepared from 200 grams was used. The highest mean count for the three amounts of potatoes used was secured upon the infusion containing 400 grams of potatoes. Yeast colonies were too small to be readily counted with the naked eye when grown on potato dextrose agar prepared from an infusion of 100 grams of potatoes. Yeast and mold colonies were somewhat larger on the medium containing an infusion of 400 grams of potatoes than on the medium containing an infusion of 200 grams of potatoes. Higher counts were secured on 33 of the 51 samples of butter upon the medium containing 400 grams of potatoes than on the medium containing 200 grams of potatoes; in two samples there was no difference and in the remaining 16 the higher counts were secured on the medium containing 200 grams of potatoes.

The addition to potato dextrose agar of the salts

suggested by Fulmer and Grimes for a synthetic medium for the growth of yeasts did not affect the yeast and mold count of butter when compared with potato dextrose agar not containing the salts. The addition of the salts increased the buffer capacity of the medium.

The yeast and mold count of butter was not affected by the addition of 0.005 grams of brom phenol blue to 100 ml. of potato dextrose agar. In the counting of yeasts in plates which were heavily infected with molds, the dye aided in the counting, but in the presence of considerable fat there was no advantage.

The counts secured upon plating dilutions of yeasts isolated from dairy products on potato dextrose agar were slightly greater, though the difference was not significant, when the infusion was prepared by boiling the potatoes for one hour and then autoclaving the infusion with the remaining ingredients of the medium, than when the potatoes were autoclaved with the other ingredients of the medium. The mean counts obtained were 394.7 when the infusion was prepared prior to autoclaving and 372.2 when the infusion was prepared while autoclaving.

Potato dextrose agar prepared from an infusion of potatoes by allowing the potatoes to remain in contact with water for 24 hours at 5° C. yielded lower mean yeast and

mold counts than an infusion prepared by boiling the potatoes for one hour; the means were 87.4 and 98.1 respectively, indicating that heating the potatoes did not destroy any growth-promoting quality. The unheated infusion was filtered through a Mandler filter and added to the sterile dextrose agar solution; it was not heated higher than 50° C.

Part 3

Lower yeast and mold counts were secured when lactic acid was used as the acidulant for (a) potato dextrose agar, (b) Bacto whey agar, (c) Bacto malt agar, and (d) 2 per cent dextrose nutrient agar than when tartaric or citric acids were used. The difference in the yeast and mold counts secured when tartaric and citric acids were used as acidulants in the four media was slight.

Potato dextrose agar acidulated to pH 3.5 ± 0.1 with each of the following acids, tartaric, lactic, citric and sulphuric, increased in pH during the incubation period as a result of the action of yeasts growing in the medium. The increase in pH was greatest when lactic acid was used as an acidulant and least when tartaric acid was used.

All the yeasts studied grew well on potato dextrose agar and Bacto whey agar acidulated over a pH range of 3.1 to 6.1

Part 4

The maximum yeast and mold count of butter was secured upon potato dextrose agar after 5 days' incubation at 21 - 25° C. The difference in the mean counts secured after 4 days and after 5 days was within the limits of error of plating. Higher counts were secured when the medium was prepared from 400 grams of potatoes per liter than when prepared from 200 grams of potatoes per liter. The mean count secured after 3 days' incubation upon the medium containing 400 grams of potatoes per liter was equivalent to the mean count secured after 4 days incubation upon the medium containing 200 grams of potatoes per liter, and was within the limits of experimental error of the count secured after 5 days' incubation with the latter medium.

The counts secured upon incubation of three yeasts for 1, 3, and 5 days in five broth media show that the counts after 3 days approached the counts after 5 days more closely with potato dextrose broth than with the other media.

Part 5

Using a simple potentiometer circuit with a saturated calomel half cell, the voltages of some of the media (in broth and agar forms) used in determining the yeast and mold

of butter were determined. The range of voltage found was from 0.057 volts in dextrose infusion broth to 0.267 volts in potato dextrose broth. The media used were classified into three groups: Group 1, media having a voltage greater than 0.2 volts, included potato dextrose broth, potato dextrose agar and Bacto malt agar; Group 2, media having a voltage of more than 0.1 volts but less than 0.2 volts included whey broth, whey broth containing no peptone and dextrose nutrient broth; Group 3, media having a voltage of less than 0.1 volts, included dextrose infusion broth, Bacto tomato juice broth, Bacto tomato juice agar and Bacto yeast dextrose agar. Inoculations of varying numbers of cells of four yeasts into broths of different voltages indicated that growth of yeasts could be initiated in broths of high or low voltages within the range studied.

Highest yeast counts were secured on potato dextrose agar, which had the highest voltage of the agar media studied. Bacto yeast dextrose agar and Bacto tomato juice agar had approximately the same voltage, less than 0.1 volts, but Bacto yeast dextrose agar gave higher yeast counts than Bacto tomato juice agar. Dilutions of yeasts were found to yield widely different colony counts when grown on media of different composition but of approximately the same voltage.

The addition of various amounts of thioglycollic acid

to potato dextrose agar resulted in a decrease in the mean yeast and mold count of samples of butter, whereas the addition of various amounts of hydrogen peroxide to potato dextrose agar resulted in an increase. The decrease in mean count resulting from the addition of thioglycollic acid and the increase in mean count resulting from the addition of hydrogen peroxide were within the experimental limits of error of plating; and there was found no consistent regularity with individual samples.

Part 6

The smear technique was compared with the usual plate method for enumerating the yeasts and molds in butter. The smear technique failed to support as high a yeast and mold count as the usual plate method, the reason probably being that the fat on the surface of the medium did not permit all the yeasts to come in contact with the medium.

Attempts were made to count the yeasts and molds in butter with the aid of the microscope; but, on account of the necessity of concentrating these cells in the serum, difficulty was encountered. The other materials present in the butter dominated the microscopic field and made identification of yeast and mold cells impossible.

Part 7

The salt tolerance of the yeasts studied was found to vary with the medium used. Growth of yeasts was apparent at higher concentrations of salt in potato dextrose broth than in skimmed milk or Bacto whey broth. The number of yeast cells present in the medium did not increase the tolerance of the cells to salt but caused a slight decrease in tolerance.

The data secured indicate that a greater percentage of yeasts than of bacteria was retained in the butter and that there existed no constant ratio between the number of organisms in the cream and the number of organisms retained in the butter.

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