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DISTRIBUTION OF SALT IN BUTTER AND ITS EFFECT ON BACTERIAL ACTION

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Dairy Bacteriology

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DISTRIBUTION OF SALT IN BUTTER AND ITS EFFECT ON BACTERIAL ACTION

The distribution of salt in butter is of importance from the standpoints of composition, color, distribution of moisture and deterioration due to the action of micro-organisms. Previous investigators studied the distribution of salt in butter from the chemical angle, while the studies reported herein were undertaken chiefly to obtain information on the effect of salt distribution on bacterial action in butter.

The retarding effect of salt on the activity of many micro-organisms is generally recognized, and various investigators have presented evidence to show that the addition of salt to butter improves the keeping qualities. However, salted butter sometimes shows spoilage due to bacterial action.

Butter is not a homogeneous mass but consists of an emulsion of water in fat, the moisture being present in droplets which vary from 3 microns to more than 100 microns in diameter (5, 30). The nutrients important for bacterial growth in butter, as well as the salt and bacterial cells, are largely included in the serum, whereas the fat is relatively resistant to bacterial action. Growth of bacteria in butter depends to a large extent on the chemical composition of each infected water droplet so that the effectiveness of

salt in preventing bacterial action depends on the extent of the distribution of salt to all infected water droplets.

Since the average concentration of salt in the serum of a churning of butter often is sufficient to inhibit growth of most bacteria producing spoilage in the product, the ability of micro-organisms to grow and produce defects in salted butter suggests that possibly not all of the serum in a churning contains the same concentration of salt. This would permit growth of bacteria in micro portions of butter containing little or no salt, while in other portions, containing higher concentrations, growth would be inhibited. Because of the spoilage of salted butter due to bacterial action, studies on the distribution of salt in butter and its effect on bacterial action were made, using a micro technique for the salt determinations. The investigation involved the development of a micro method for determination of salt in butter, studies on salt distribution in butter and studies on the effect of salt distribution on bacterial action.

EXPERIMENTAL

DEVELOPMENT OF A MICRO METHOD FOR DETERMINATION OF SALT IN BUTTER

The macro methods for the determination of salt in butter are adequate for obtaining the average salt content of an entire churning, but from the bacteriological standpoint such general information is inadequate. Since butter is not a homogeneous mass and organisms are not uniformly distributed through it, the bacteriological changes in a lot of butter are the combined results of the changes in numerous small portions. Accordingly, in studying the effect of salt distribution in butter on bacterial action, salt contents of as small portions as possible are desired, and, for this reason a micro procedure for the determination of salt in butter was developed.

General Method

The micro method is carried out as follows*: an approxi-

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*The method of titrating small amounts of sodium chloride with a very dilute silver nitrate solution was developed by Mr. Clyde L. Ogg who used the method on a number of samples of butter. The procedure followed by Mr. Ogg has been modified by reducing the size of the sample analyzed, by picking the sample under a low power microscope and in other minor respects.

mately 0.2 mg. portion of butter is picked under a low power microscope and weighed on a microbalance. The butter is then ashed, and the ash and salt are taken up in a water-alcohol solution and transferred to a spot plate. A known amount of standard sodium chloride is added, and the mixture is titrated with a standard silver nitrate solution under a fluorescent type lamp, using dichlorofluorescein as the indicator.

General Considerations

The butter to be studied was tempered and held at approximately 13°C. for sampling. This temperature was low enough so that the original body and texture of the butter were not changed and yet high enough to avoid any great tendency for moisture from the air to condense on the sample. A 0.15 mg. sample was considered to be the smallest sample that could be analyzed without danger of the percentage error for the analysis being excessive. To minimize the chances of weighing condensate from the air, a freshly exposed surface was used for each micro sample, and the butter was broken instead of cut so that the original texture at the freshly exposed surface was not changed. The weight of a sample was determined to within plus or minus 0.002 mg.

Each day during the weighing of the first few samples of butter the zero point on the balance fluctuated considerably. Upon investigating the cause of this, the observation was made

that when the balance was allowed to stand without use for a long period, possibly over night, and then the zero point was checked at frequent intervals, it remained constant as long as the balance case door was not opened. However, opening and closing the door of the balance case caused the zero point to change considerably. The shifting of the zero point apparently was caused by the differences in temperature and humidity between the air inside and outside the balance case. The difficulty was eliminated by leaving the door open for 30 minutes before attempting to weigh; this allowed the air inside and outside the balance case to equalize and the balance to come to equilibrium under the new air conditions.

In macro analyses of butter, salt is separated from the fat by washing the butter with successive portions of warm distilled water and recovering the washings containing the salt. This procedure is not adapted to micro analyses since it is impossible to transfer quantitatively such small amounts of liquid as are necessary to avoid greatly reducing the chloride concentration in the titrating solution; therefore, a procedure was used in which the butter was ashed and the ash and salt transferred to a spot plate depression. Blank determinations showed that the salt was transferred quantitatively by the rinsing process employed. Apparently the small amount of salt present was readily dissolved in the proportionally large volume of water used for the rinsing.

Potassium chromate, the indicator commonly used for the argentometric titration of sodium chloride in macro samples of butter, is unsatisfactory for the analysis of very small quantities of chlorides since the color change at the end point can not readily be detected. However, various adsorption indicators have been suggested for the determination of halides.

In 1923, Fajans and Hassel (9) published a method for the argentometric titration of halides, using sodium fluorescein and sodium eosin as adsorption indicators. Other investigators (10,4,20,21,1) confirmed the work of Fajans and Hassel by obtaining accurate results with sodium fluorescein; however, the titration must be carried out in a neutral or slightly alkaline solution, and the chloride concentration must be greater than 0.005 N.

Later, Kolthoff, Lauer and Sunde (22) published a method for the argentometric titration of chlorides using dichlorofluorescein as the indicator. They stated that "dichlorofluorescein is a very suitable indicator for the argentometric titration of chlorides in very dilute as well as in weakly acid solutions." In a semi-micro method for the determination of chlorides, Hõischer (14) obtained good results with dichlorofluorescein as the indicator. Bamback and Rider (2), using this indicator, found that the analytical results on chlorides were within the experimental error of the theoretical values on pure chemicals. Feldman and Powell (11) also reported

favorable results when dichlorofluorescein was used in the determinations of halides in organic compounds.

Bryant (3), in 1937, used dichlorofluorescein for the determination of salt in macro samples of butter. Skelton and Bryant (33) and Weckel (35) compared dichlorofluorescein and potassium chromate as indicators in determining the salt content of butter. Dichlorofluorescein was preferred as it has the advantage of producing a distinct and sudden change in color at the end-point.

Since various investigations show that dichlorofluorescein is a suitable indicator for the argentometric titration of chlorides, especially in very dilute as well as in slightly acid solutions, this indicator was used for the micro determination of salt in butter. It proved to be very satisfactory.

The color change at the equivalence point is due to the adsorption of the indicator on the surface of the precipitate. Accordingly, increasing the surface of the precipitate also increases the intensity of the color (23). The surface of the silver chloride precipitate was increased in the determination by adding a known amount of sodium chloride to the solution before titrating with silver nitrate. The amount of silver nitrate in excess of the amount necessary to react with the added sodium chloride was equivalent to the silver nitrate which reacted with the sodium chloride from the butter.

With proper precautions, the color change of dichlorofluorescein at the equivalence point was distinct. The color

change is from a yellowish-brown or white to a pink. The addition of too large a quantity of indicator gave the solution a rather pronounced brown color which persisted during the entire titration, making the end-point difficult to detect. However, with the proper amount of indicator only a very slight yellow color was present, and this disappeared as the cloudy to white silver chloride precipitate formed during the titration. At this point a localized pink color could be developed at the tip of the burette by discontinuing the stirring. At the equivalence point the solution turned a light shade of pink throughout, and this remained for a short time. If the volume of the solution was allowed to become too large, the pink color was very light and difficult to detect.

When the solution being titrated was allowed to stand for a short time after reaching the end-point, a dark purple color developed, due to the decomposition of silver chloride by light. This reaction is catalyzed by the indicator. The purple color also was observed if an excessively long period elapsed during the titration. Titrating too slowly with insufficient stirring caused the silver chloride precipitate to floculate and settle out, and since the color is produced by the adsorption of the indicator to the precipitate, a poor end-point was obtained. For satisfactory results the solution should be stirred continuously and the titration completed in a reasonable time.

Titrations were made at first with daylight as the only source of light, but this was not satisfactory since the intensity varied considerably from time to time. In an effort to control the intensity of light, titrations were made in a dark room using various sources of artificial light. Most of the lights were not satisfactory since they contained too much yellow which partially masked the pink color at the end-point. However, the fluorescent light was very satisfactory since the light was very nearly colorless and the end-point in the titrations was easily detected.

Apparatus and Reagents

Microscope: A Spencer, wide-field, low-power, binocular microscope, having a 6X magnification, was used in picking the portions of butter.

Microbalance: An Ainsworth microbalance (type FDJ) was employed.

Micro spoons: The micro spoons in which the butter samples were weighed and ashed were made by fusing pieces of platinum foil about 7 mm. in diameter to 24 gauge platinum wires 2.5 cm. in length. The centers of the spoons were made slightly concave, and the wires were bent so that the spoons were level when hanging.

Spot plate: The titrations were made in a white porcelain spot plate with depressions 20 mm. in diameter and 5 mm. deep.

Capillary burettes: Capillary burettes were constructed and calibrated as described by Johns (17). The volumes contained in the burettes over a length of 1 cm. corresponded to approximately 0.004 ml., and the flow of the burettes was controlled to within 1 mm. To eliminate difficulties encountered in transferring liquids quantitatively due to the surface tension, the tips of the capillary burettes were immersed in the liquid being titrated. The titrations were made under a 15 watt fluorescent Cenco Lablite.

Sodium chloride solution: A 0.01 N. sodium chloride solution was prepared from reagent quality sodium chloride.

Silver nitrate solution: An approximately 0.01 N. silver nitrate solution was prepared and standardized against the standard sodium chloride solution, using the micro titration that was later employed for the analyses. The silver nitrate solution was kept in a dark bottle covered with aluminum foil and the concentration checked at frequent intervals.

Dichlorofluorescein solution: One-tenth gram of dichlorofluorescein was dissolved in 60 ml. of ethyl alcohol (95 per cent) and the volume made up to 100 ml. with distilled water. One ml. of this solution was then diluted with 9 ml. of ethyl alcohol.

Procedure

From a freshly broken surface an approximately 0.2 mg.

(± 0.05 mg.) portion of butter was picked under the microscope with a dissecting needle and placed in a weighed micro spoon. The spoon containing the butter was hung on the pan of the microbalance and the weight determined to within 0.002 mg. No difficulty was experienced in weighing the micro samples except on warm, humid days; on such days moisture condensed on the cool sample, and then when the sample was placed on a relatively warm platinum spoon the temperature rather quickly increased and the moisture slowly evaporated.

The micro spoon containing the sample of butter was hung on a nichrome wire drawn across the top of a 15 ml. porcelain crucible. As the crucible was heated slowly with a micro burner, the butter melted and spread in a thin film over the entire surface of the spoon; no spattering of the fat could be observed. After the butter had charred, the heat was increased, the crucible being heated just to redness until all the carbon had oxidized and only the ash and salt remained. With proper spacing of the spoon in the crucible, the heating could be completed in 5 minutes without loss of sodium chloride.

The ash and salt were transferred to the spot plate in which the titration was to be made by placing the spoon in a drop of water in a depression and covering the spoon with another drop of water. After several minutes the salt solution was washed from the spoon with 3 or 4 drops of water followed by 3 or 4 drops of 95 per cent ethyl alcohol. The

addition of alcohol sharpened the end-point and facilitated removal of the salt solution. The volume of the solution was approximately 0.5 ml. and was not allowed to become much greater than this since the color change in the titration was more distinct in a relatively concentrated solution.

A volume of 0.01 N. sodium chloride solution equivalent to 10 to 12 cm. on the burette scale was added from a capillary burette. A small drop of dichlorofluorescein was added with a capillary pipette, and the solution was titrated with the silver nitrate solution. The solution was stirred continuously during the titration, and the first permanent color change was taken as the end-point. The titrations were made in a dark room under a fluorescent light.

From the results of the titration and the weight of the sample, the percentage of salt in the sample was calculated.

Accuracy of Titration

The accuracy of the titration could not be determined by analyses on duplicate samples since butter is not a homogeneous mass and duplicate micro samples could not be obtained. However, the accuracy of the titrations was checked by titrating a 0.01 N. sodium chloride solution, using the micro procedure, and expressing the volumes of the solutions as centimeter divisions on the burette scale.

Table 1 gives the results of eight titrations on the 0.01 N. sodium chloride solution. The titration values

Table 1. MICRO TITRATIONS ON 0.01 N. SODIUM CHLORIDE SOLUTION

Trial no.	Cm. divisions on burette scale corresponding to vol. of solu.	
	NaCl solu.	AgNO3 solu.
1	14.0	14.1
2	14.0	14.2
3	14.0	14.1
4	14.0	14.1
5	14.0	14.1
6	14.0	14.2
7	13.0	13.1
8	13.0	13.1

checked to within 1 mm. on the burette scale; this represents a volume of 0.0004355 ml. of an approximately 0.01 N. silver nitrate solution. Since the water-alcohol solution of the ash and salt was clear, it is probable that the titrations made on the butter samples were as accurate as those made on the salt solution.

SALT DISTRIBUTION IN BUTTER

Different investigators have studied the distribution of salt in butter, using macro analytical methods, in connection with controlling the composition of butter and preventing certain color defects. While the information obtained with the macro methods is very useful from a number of angles, it is not adequate from the bacteriological standpoint. In considering the effect of salt distribution on bacterial changes in butter, the salt contents of as small portions as possible are desired.

Historical

Guthrie and Ross (13) and Guthrie (12) found that the composition of butter from some churnings varied considerably with respect to moisture, salt and fat and concluded that butter which is thoroughly worked has a more uniform composition than butter which is moderately worked. In connection with studies on the variability in composition of butter

from the same churning, Manhart (28) stated, "of the three non-fatty constituents considered in this investigation, the curd was the most variable, the salt next, and the water least." A more even distribution of these constituents was obtained as the working process was prolonged. McDowall and MacDonald (26) noted that the extent of salt variation largely depends on the method of salting, the temperature and time of working and the type of churn used. As a means of helping control the composition of butter in a churning, Mortensen et al. (29) recommended weighing the salt in two or three lots and distributing each lot over one-half or one-third, respectively, of the butter in the churn. Hood and White (15) found that the salt contents of Canadian butter from the same churning varied as much as 0.90 per cent. The factors responsible for the lack of uniformity in composition were (a) type, slope and mechanical condition of the churn, (b) lack of uniform and standard methods of manufacture and (c) lack of attention to details.

Early investigators reported that mottles, a color defect, occurred only in salted butter. As a means of preventing mottles in butter, Doane (8) recommended working the butter sufficiently to thoroughly distribute the salt. McKay and Larsen (27) found that salt could be present in butter in an undissolved condition without causing mottles, provided the water in the butter was saturated with salt and the salt was evenly distributed in the butter.

Van Slyke and Hart (34) noted that butter, free of buttermilk adhering to the outer surfaces of the granules, does not produce mottles when salted, irrespective of the salt distribution. They stated, "Mottles in butter are due, primarily, to the presence and uneven distribution of buttermilk adhering to the outer surface of the small granules; and, secondarily, to the hardening and localizing effect of salt brine upon the proteid of the buttermilk thus retained in butter. The light portions of mottled butter owe their lighter color to the presence of localized proteid (usually casein lactate)." Working with butterfat free of casein, Sammis and Lee (31) produced mottles when the salt was unevenly distributed in an emulsion obtained by churning the butterfat and water. They emphasized the importance of thoroughly working butter.

Hunziker and Hosman (16) found that mottles appeared in salted butter in which the working was incomplete and that the opaque or light portions contained large numbers of very minute water droplets, while the deeper yellow or clearer portions contained fewer and larger water droplets. The addition of salt to butter tended to reduce the number and increase the size of water droplets; this was due to the tendency of small moisture droplets to migrate to moisture droplets which contained relatively high concentrations of salt.

Procedure

The distribution of salt in butter was studied by analyzing ten samples of approximately 0.20 mg. each from a 15 g. portion of butter, using the micro procedure.

The incorporation of the moisture in butter was observed by cutting the cold butter with a fine nichrome wire and, after several minutes, examining the freshly cut surfaces for the formation of moisture droplets. Butter classed as very good showed no visible moisture on the freshly cut surfaces; butter classed as good showed only a few small water droplets; butter classed as fair and poor showed a considerable number of large water droplets; while butter classed as very poor showed numerous large water droplets which appeared quickly.

The moisture contents of approximately 0.3 to 0.5 mg. portions of butter were determined by picking and weighing the samples in the usual manner, drying them in a 100°C. oven and allowing the dried samples to cool under room conditions for 30 minutes before weighing; the losses in weight during drying represent the moisture contents.

The salt contents of moisture droplets on freshly cut surfaces of butter were determined as follows: A portion of each droplet was removed with a very small, weighed capillary tube; the ends of the tube were then sealed with a small flame, and the tube was weighed. The material was removed from the

capillary tube by crushing it with a small stirring rod in a spot plate depression containing five drops of water. The salt concentration of the solution was high enough so that no additional sodium chloride was necessary. The solution was titrated with an approximately 0.025 N. silver nitrate solution, using dichlorofluorescein as the indicator.

The sodium chloride contents of macro samples of butter were determined by weighing 5 to 10 g. of butter in a 50 ml. beaker; 20 ml. of hot distilled water was added, and after the butter had melted the mixture was transferred to a separatory funnel. The mixture was shaken for several minutes and allowed to stand until the fat and water had separated; the water was then drained into an evaporating dish. The extraction was repeated six times, using 20 ml. of hot water each time. The salt solution was titrated with an approximately 0.1 N. standard solution of silver nitrate, using dichlorofluorescein as the indicator.

Results

Normal Commercial Butter

The samples used in studying the salt distribution in normal commercial butter were obtained from the usual run of production of the Iowa State College butter laboratory and from commercial creameries. They were kept cool so that the original body and texture were not changed.

The results on thirteen churnings of butter are presented in Table 2. The distribution of salt varied considerably in the different samples. Churnings 1 and 2, obtained from the same creamery, showed the best incorporation of moisture of any of those analyzed. No free moisture was observed on the freshly cut surfaces, and the salt distribution was very uniform. With churning 1 the salt contents of the micro samples varied from 2.21 to 2.68 per cent and with churning 2 from 2.30 to 2.68 per cent while the variances of the salt contents were 0.0255 and 0.0176, respectively. Churnings 3 and 4 showed only a few small moisture droplets on the cut surfaces, and with one exception the salt contents of the micro samples were uniform. With churning 3 the salt contents ranged from 1.77 to 2.50 per cent and with churning 4, except for one value of 4.11 per cent, they ranged from 1.77 to 2.22 per cent; the variances of the salt contents were 0.0461 and 0.4975, respectively. Churnings 5, 6 and 7 showed numerous small water droplets, but the salt distribution was fairly uniform, the salt contents varying from 1.87 to 2.39 per cent, from 2.05 to 3.26 per cent and from 1.47 to 2.47 per cent, respectively. The variances of the salt contents were 0.0346, 0.1206 and 0.1410, respectively. Churning 8 was mottled and contained numerous small water droplets. The salt contents of the micro samples ranged from 1.56 to 8.52 per cent and the variance of the salt contents was 3.8594. Churnings 9, 10 and 11 showed large moisture droplets and

Table 2. SALT DISTRIBUTION IN NORMAL COMMERCIAL BUTTER

Churning no.	Condition of butter: H_2O distribution	Color	% NaCl, macro method	% NaCl in micro sample 1	2	3	4	5	6	
1	very good		2.53	2.40	2.21	2.27	2.48	2.53	2.26	2
2	very good		2.55	2.48	2.63	2.36	2.30	2.42	2.40	2
3	good		2.35	2.50	1.77	2.18	1.83	2.14	2.17	2
4	good		2.20	1.77	1.94	1.96	2.22	1.81	1.77	4
5*	fair		1.93	2.09	2.27	2,27	2.06	2.39	1.89	2
6	fair	sl.**mottled	2.21	2.46	2.26	2.36	2.36	2.17	2.19	1
7*	fair		2.43	2.47	1.73	1.59	1.47	2.44	1.51	2
8	fair	mottled	2.81	2.76	1.97	1.56	2.93	8.52	3.50	2
9	poor		2.04	0.90	1.40	1.17	1.21	1.90	0.80	1
10	poor		2.18	2.54	2.62	1.74	2.37	2.02	2.19	2
11	poor		2.35	2.20	2.16	2.48	2.19	2.79	3.26	1
12	very poor		1.74	1.24	1.48	2.16	1.69	4.32	1.86	2
13	very poor		2.10	3.49	2.07	4.05	1.87	2.77	2.32	1

* = Printed butter. ** sl. = slight.

DISTRIBUTION IN NORMAL COMMERCIAL BUTTER

NaCl, % of mod	% NaCl in micro sample no. 1	2	3	4	5	6	7	8	9	10	Variance
53	2.40	2.21	2.27	2.48	2.53	2.26	2.48	2.46	2.65	2.68	0.0255
55	2.48	2.63	2.36	2.30	2.42	2.40	2.55	2.67	2.53	2.68	0.0176
35	2.50	1.77	2.18	1.83	2.14	2.17	2.09	1.90	1.92	1.98	0.0461
20	1.77	1.94	1.96	2.22	1.81	1.77	4.11	1.98	2.11	1.81	0.4975
03	2.09	2.27	2.27	2.06	2.39	1.89	2.30	2.01	2.31	1.87	0.0346
21	2.46	2.26	2.36	2.36	2.17	2.19	3.26	2.05	2.06	2.28	0.1206
43	2.47	1.73	1.59	1.47	2.44	1.51	2.13	1.80	2.16	2.19	0.1410
31	2.76	1.97	1.56	2.93	8.52	3.50	2.48	2.62	2.51	2.44	3.8594
04	0.90	1.40	1.17	1.21	1.90	0.80	1.53	1.64	0.70	1.22	0.1458
18	2.54	2.62	1.74	2.37	2.02	2.19	2.58	3.42	3.17	2.97	0.2690
35	2.20	2.16	2.48	2.19	2.79	3.26	3.01	2.97	2.13	1.68	0.2503
74	1.24	1.48	2.16	1.69	4.32	1.86	2.82	1.99	1.82	2.16	0.7628
10	3.49	2.07	4.05	1.87	2.77	2.32	3.80	2.64	3.46	2.44	0.5751

h.

the salt distribution was not as uniform as in the more thoroughly worked butter. The percentage of salt in the micro samples varied from 0.70 to 1.90, from 1.74 to 3.42 and from 1.68 to 3.26, respectively; the variances of the salt contents were 0.1458, 0.2690 and 0.2503, respectively. Churnings 12 and 13 were very leaky and numerous large water droplets quickly appeared immediately on the cut surfaces; the salt contents of the micro samples ranged from 1.24 to 4.32 per cent and from 1.87 to 4.05 per cent respectively. The variances of the salt contents were 0.7628 and 0.5751, respectively.

Normal Commercial Butter Before and After Printing

Studies on the salt distribution in butter both before printing and after printing with a printer which tended to rework the butter were made on normal commercial butter obtained from creameries in Iowa. The moisture regularly was more thoroughly incorporated in the butter before printing than after printing.

Data on nine churnings of butter are given in Table 3. In churnings 3, 5, 8 and 9 there were practically no differences in the distribution of salt in the butter before printing and after printing; before printing the percentage of salt in the micro samples varied from 2.04 to 3.09, from 2.00 to 3.52, from 1.87 to 2.88 and from 1.92 to 3.69, respectively,

Table 3. SALT DISTRIBUTION IN NORMAL COMMERCIAL BUTTER BEFORE ANI

Churning no.	Sample	Moisture distribution in butter	% NaCl, macro method	% NaCl in micro sample no. 1	2	3	4	5	6	
	B*	very good	1.84	1.71	2.02	1.90	2.15	2.03	1.88	2.
1	A*	fair	1.77	1.96	2.15	1.71	2.50	1.71	1.61	2.
	B	good	2.64	2.44	2.10	2.05	2.15	2.52	2.20	2.
2	A	fair	2.40	2.03	2.29	2.41	1.52	2.32	1.50	2.
	B	good	2.76	2.62	2.04	2.41	2.17	2.18	2.21	3.
3	A	very poor	2.58	2.43	2.53	2.98	2.49	2.68	3.35	2.
	B	fair	2.09	1.90	2.02	2.35	1.99	1.80	1.90	1.
4	A	poor	2.28	3.54	2.60	3.04	2.32	2.55	2.05	3.
	B	fair	2.55	2.61	2.06	2.22	2.29	2.61	2.94	2.
5	A	poor	2.56	2.77	2.52	2.52	1.94	2.28	1.93	3.
	B	fair	2.65	3.27	3.50	2.57	3.86	2.20	2.25	2.
6	A	poor	2.58	2.49	2.39	2.89	2.63	2.50	2.80	2.
	B	fair	2.58	2.40	2.62	2.64	2.72	2.56	1.53	4.
7	A	poor	2.36	2.66	2.44	2.41	2.00	1.89	2.55	2.
	B	poor	2.41	1.87	2.08	2.30	1.88	2.88	1.91	2.
8	A	poor	2.21	1.63	2.46	1.74	1.65	1.89	1.57	1.
	B	poor	2.53	2.48	1.92	2.48	2.58	2.52	2.64	2.
9	A	very poor	2.39	2.40	2.53	2.38	2.20	2.37	1.98	3.

*B = Before printing; *A = After printing.

TION IN NORMAL COMMERCIAL BUTTER BEFORE AND AFTER PRINTING

Cl, : o : od	% NaCl in micro sample no. 1	2	3	4	5	6	7	8	9	10	Variance
4	1.71	2.02	1.90	2.15	2.03	1.88	2.08	1.88	1.82	1.88	0.0175
7	1.96	2.15	1.71	2.50	1.71	1.61	2.26	1.88	1.96	2.81	0.1449
4	2.44	2.10	2.05	2.15	2.52	2.20	2.66	2.32	2.25	2.36	0.0377
0	2.03	2.29	2.41	1.52	2.32	1.50	2.29	2.02	1.79	2.07	0.1071
6	2.62	2.04	2.41	2.17	2.18	2.21	3.09	2.44	2.26	2.37	0.0899
8	2.43	2.53	2.98	2.49	2.68	3.35	2.89	2.21	2.83	2.87	0.1069
9	1.90	2.02	2.35	1.99	1.80	1.90	1.98	2.09	2.02	2.27	0.0283
8	3.54	2.60	3.04	2.32	2.55	2.05	3.30	2.54	3.80	2.43	0.3280
5	2.61	2.06	2.22	2.29	2.61	2.94	2.45	3.52	2.39	2.00	0.2042
6	2.77	2.52	2.52	1.94	2.28	1.93	3.76	2.55	3.23	2.59	0.3081
5	3.27	3.50	2.57	3.86	2.20	2.25	2.82	2.47	2.63	3.58	0.3516
8	2.49	2.39	2.89	2.63	2.50	2.80	2.03	2.08	2.25	2.35	0.0799
8	2.40	2.62	2.64	2.72	2.56	1.53	4.36	2.85	2.38	2.47	0.4889
6	2.66	2.44	2.41	2.00	1.89	2.55	2.06	2.46	2.25	1.95	0.0755
1	1.87	2.08	2.30	1.88	2.88	1.91	2.05	2.58	2.18	2.64	0.1255
1	1.63	2.46	1.74	1.65	1.89	1.57	1.85	2.42	2.55	1.90	0.1373
3	2.48	1.92	2.48	2.58	2.52	2.64	2.05	3.06	3.69	2.90	0.2535
9	2.40	2.53	2.38	2.20	2.37	1.98	3.76	2.11	2.32	2.26	0.2427

printing.

while after printing the percentages varied from 2.21 to 3.35, from 1.93 to 3.76, from 1.57 to 2.55 and from 1.98 to 3.76, respectively. The variances of the salt contents of the butter before printing were 0.0899, 0.2042, 0.1255 and 0.2535, respectively, while after printing the variances were 0.1069, 0.3081, 0.1373 and 0.2427, respectively.

In churnings 1, 2 and 4 the salt distribution was slightly more uniform in the butter before printing than after printing; before printing the percentages of salt in the micro samples ranged from 1.71 to 2.15, from 2.05 to 2.66 and from 1.80 to 2.35, respectively, while after printing the percentages ranged from 1.61 to 2.81, from 1.50 to 2.41 and from 2.05 to 3.80, respectively. The variances of the salt contents before printing were 0.0175, 0.0377 and 0.0283, respectively, and after printing they were 0.1449, 0.1071 and 0.3280, respectively.

In churnings 6 and 7 the distribution of salt was less uniform in the butter before printing than after printing; before printing the salt contents of the micro samples ranged from 2.20 to 3.86 per cent and from 1.53 to 4.36 per cent, respectively, while after printing the salt contents ranged from 2.03 to 2.89 per cent and from 1.89 to 2.66 per cent, respectively. The variances of the salt contents before printing were 0.3516 and 0.4889, respectively, and after printing the variances were 0.0799 and 0.0755, respectively.

In all churnings except 4 and 5 the salt content, as determined by the macro method, was higher in the butter

before printing than after printing.

Butter Worked Various Amounts in a Commercial Churn

The relationship between the amount of working and the salt distribution in butter was studied by removing samples at intervals during the working of commercial-size churnings. Three samples were taken from each of churnings 1, 2 and 3; sample 1 was removed early in the working process when the moisture was very poorly incorporated; sample 2 was removed when the butter was moderately worked and still contained numerous large water droplets; while sample 3 was taken after the working was completed. With churning 3, the working of the sample removed when butter was finished commercially was continued by hand until the butter was dry and sticky. In the finished butter from churning 1 the moisture was poorly incorporated, large moisture droplets being present, while in the finished butter from churning 2 the moisture was fairly well incorporated. Two samples were removed from each of churnings 4, 5 and 6. With churnings 4 and 5, sample 1 was removed early in the working process when the moisture was very poorly incorporated, while with churning 6 the first sample was not removed until the moisture was fairly well incorporated. Sample 2 from each churning was taken after the working was completed. In the finished butter from churnings 4 and 6 the moisture was well distributed,

while the finished butter from churning 5 was mottled and contained large moisture droplets. All the samples were held at 4°C. for 2 to 6 days before analysis.

Data on the six churnings are presented in Table 4. In sample 1 from each of churnings 1, 2 and 3, the salt was very poorly distributed and the percentage of salt in the micro samples varied from 0 to 2.53, from 1.65 to 4.61 and from 0.31 to 1.97, respectively. The variances of the salt contents were 0.8625, 1.1661 and 0.3518, respectively. In sample 2 from each of churnings 1, 2 and 3, the salt contents of the micro samples still varied considerably; the percentages of salt ranged from 0 to 1.65, from 1.49 to 5.83 and from 0.54 to 3.56, respectively. The variances of the salt contents were 0.3150, 1.6218 and 0.6876, respectively. In sample 3 from each of churnings 1 and 2 the salt distribution was not very uniform, while in sample 3 from churning 3 the salt was very uniformly distributed; for the three churnings the percentages of salt in the micro samples of butter ranged from 1.97 to 3.81, from 1.39 to 3.12 and from 1.79 to 2.22, respectively. The variances of the salt contents were 0.3126, 0.2979 and 0.0180, respectively.

In sample 1 from each of churnings 4 and 5, conspicuous differences occurred in the salt contents of the micro samples, the percentages of salt varying from 1.91 to 5.50 and from 0 to 5.25, respectively. The variances of the salt contents were 1.3757 and 3.5885, respectively. Sample 1 from churning

Table 4. SALT DISTRIBUTION IN BUTTER WORKED VARIOUS AMOUNTS IN

Churning no.	Sample no.	Degree of working	Condition of butter: H_2O distribution	Color	% NaCl: macro method	% 1	% 2	% 3
1	1	poor	very poor	streaked	0.41	2.02	0.00	0.00
	2	moderate	poor	wavy	0.86	1.47	1.65	1.26
	3	finished*	fair		2.30	3.25	2.11	3.81
2	1	poor	very poor	streaked	1.60	2.04	3.61	3.74
	2	moderate	poor		2.15	1.49	3.18	1.69
	3	finished	fair		2.41	1.79	2.88	2.06
3	1	poor	very poor	streaked	1.24	1.97	0.34	0.98
	2	finished	fair		2.25	1.98	0.54	1.10
	3	thorough**	very dry	sticky	2.22	1.97	2.06	2.12
4	1	poor	very poor	streaked	2.11	2.36	5.50	2.48
	2	finished	dry		2.30	2.42	1.46	2.90
5	1	poor	very poor	streaked	1.56	0.00	0.00	3.11
	2	finished	poor	mottled	2.22	2.15	1.31	2.81
6	1	moderate	fair		2.40	2.80	2.76	3.23
	2	finished	dry		2.40	2.68	2.23	2.09

* = The working was considered complete from a commercial standpoint; **

D VARIOUS AMOUNTS IN A COMMERCIAL CHURN

Oil, : p : od	% NaCl in micro sample no. 1	2	3	4	5	6	7	8	9	10	Variance
l	2.02	0.00	0.00	0.17	0.24	0.00	1.41	0.35	2.53	0.41	0.8625
5	1.47	1.65	1.26	1.47	0.59	0.67	1.12	0.00	0.31	0.53	0.3150
0	3.25	2.11	3.81	2.73	2.79	2.76	2.89	1.97	2.34	2.23	0.3126
0	2.04	3.61	3.74	3.39	4.61	4.33	1.97	2.49	2.00	1.65	1.1661
5	1.49	3.18	1.69	2.73	4.01	3.17	5.83	2.31	2.37	2.18	1.6218
1	1.79	2.88	2.06	3.12	1.71	1.93	2.38	1.39	2.62	2.12	0.2979
4	1.97	0.34	0.98	0.31	1.26	1.24	0.47	0.36	1.64	1.30	0.3518
5	1.98	0.54	1.10	2.22	2.14	1.22	1.70	3.56	1.46	2.28	0.6876
2	1.97	2.06	2.12	1.95	2.11	2.06	2.22	1.79	2.17	1.88	0.0180
l	2.36	5.50	2.48	4.78	3.70	3.37	2.04	1.91	2.94	3.07	1.3757
0	2.42	1.46	2.90	2.91	2.53	2.35	2.54	1.59	1.77	2.46	0.2639
5	0.00	0.00	3.11	5.25	4.02	0,49	2.64	0.45	1.18	0.16	3.5885
2	2.15	1.31	2.81	1.60	4.26	1.80	3.22	3.05	2.56	2.57	0.7612
0	2.80	2.76	3.23	2.33	2.83	2.43	2.69	2.63	2.38	2.38	0.0780
0	2.68	2.23	2.09	2.24	2.30	2.36	2.41	2.79	2.42	2.23	0.0462

lal standpoint; ** = Working was continued by hand.

6 was removed very late in the working process and the salt was fairly uniformly distributed; the salt contents of the micro samples ranged from 2.38 to 2.83 per cent and the variance of the salt contents was 0.0780. In sample 2 from each of churnings 4, 5 and 6, the salt was more uniformly distributed than in sample 1 from each of the churnings; the percentages of salt in the micro samples of butter ranged from 1.46 to 2.91, from 1.31 to 4.26 and from 2.09 to 2.79, respectively, and the variances of the salt contents were 0.2639, and 0.7612 and 0.0462, respectively.

Abnormal Commercial Butter

The distribution of salt in butter that had developed abnormal flavors in commercial channels was investigated with defective butter from different sources. In many of the churnings the variations in salt distribution were greater than the usual variations encountered in normal commercial butter, while in other churnings the salt distribution was fairly uniform.

Table 5 presents data on fourteen churnings of putrid butter and on one churning of canned butter which had developed a gassy condition. Churnings 1 and 2 showed the greatest variations in salt distribution; the salt contents of the micro samples ranging from 0.35 to 5.33 per cent and from 0.52 to 3.82 per cent, respectively; the variances of the salt contents were 1.9990 and 1.1150, respectively.

Table 5. SALT DISTRIBUTION IN ABNORMAL COMMERCIAL BUT

Churning no.	Defects in butter	% NaCl, macro method	% NaCl in micro sample n					
			1	2	3	4	5	6
1	putrid	1.18	0.35	1.08	1.30	1.32	2.31	5.33
2	putrid	1.40	0.86	0.84	0.52	1.25	2.02	3.18
3	putrid	1.27	1.52	0.45	1.25	0.85	0.96	0.36
4	putrid, mottled	2.41	1.82	1.45	1.11	0.50	0.00	2.30
5	putrid, mottled	1.76	0.34	1.16	1.35	0.47	1.58	1.12
6	putrid	1.61	0.30	0.87	1.14	0.44	3.10	1.29
7	putrid		1.96	1.36	1.84	1.87	1.94	4.28
8	putrid		1.95	0.89	1.50	2.55	1.60	1.38
9	putrid		2.12	1.14	1.49	1.27	1.85	1.23
10	putrid		1.73	1.19	3.17	1.15	2.39	1.69
11	putrid		3.25	2.04	1.69	2.45	1.97	1.73
12	putrid		2.28	2.28	1.75	1.82	2.17	1.60
13	putrid	1.89	1.84	1.84	1.65	1.34	2.59	1.57
14	putrid, dark areas	0.65	0.46	0.42	0.53	0.57	1.06	0.44
15*	unclean, gassy	1.59	2.06	1.65	1.49	1.41	1.49	1.47

* = Canned butter.

BTRIBUTION IN ABNORMAL COMMERCIAL BUTTER

% NaCl in micro sample no.										Variance
1	2	3	4	5	6	7	8	9	10	
0.35	1.08	1.30	1.32	2.31	5.33	2.24	1.00	0.70	2.16	1.9990
0.86	0.84	0.52	1.25	2.02	3.18	1.62	3.82	1.27	1.57	1.1150
1.52	0.45	1.25	0.85	0.96	0.36	1.93	3.22	0.75	0.78	0.7272
1.82	1.45	1.11	0.50	0.00	2.30	1.21	0.34	0.30	1.97	0.6264
0.34	1.16	1.35	0.47	1.58	1.12	0.23	0.38	1.42	0.15	0.3075
0.30	0.87	1.14	0.44	3.10	1.29	1.82	0.90	1.08	0.65	0.6547
1.96	1.36	1.84	1.87	1.94	4.28	2.31	1.44	1.55	2.00	0.6931
1.95	0.89	1.50	2.55	1.60	1.38	3.26	1.79	2.68	1.43	0.5174
2.12	1.14	1.49	1.27	1.85	1.23	1.51	1.99	1.75	1.92	0.1200
1.73	1.19	3.17	1.15	2.39	1.69	1.57	1.95	1.97	1.86	0.3444
3.25	2.04	1.69	2.45	1.97	1.73	2.19	1.39	1.87	1.89	0.2613
2.28	2.28	1.75	1.82	2.17	1.60	2.05	1.96	1.80	2.15	0.0561
1.84	1.84	1.65	1.34	2.59	1.57	1.93	1.60	1.76	1.31	0.1312
0.46	0.42	0.53	0.57	1.06	0.44	0.51	0.37	0.76	0.66	0.0423
2.06	1.65	1.49	1.41	1.49	1.47	1.34	1.43	1.46	1.73	0.0447

Churnings 3, 4, 5, 6 and 8 showed relatively large variations in salt distribution, and with each churning one or more micro samples had salt contents of less than 1 per cent. The percentages of salt in the micro samples varied from 0.36 to 3.22, from 0 to 2.30, from 0.23 to 1.58, from 0.30 to 3.10, and from 0.89 to 3.26, respectively, while the variances of the salt contents were 0.7272, 0.6264, 0.3075, 0.6547 and 0.5174, respectively. With churning 7 the salt contents of the micro samples were fairly uniform with the exception of one high value of 4.28 per cent. The salt contents of the remaining nine samples ranged from 1.36 to 2.00 per cent, and the variance of the salt contents, as determined on the ten micro samples, was 0.6931.

In churnings 9, 10, 11 and 13 the salt distribution was fairly uniform; the percentages of salt in the micro samples varied from 1.14 to 2.12, from 1.19 to 3.17, from 1.39 to 3.25 and from 1.31 to 2.59, respectively, while the variances of the salt contents were 0.1200, 0.3444, 0.2613 and 0.1312, respectively.

Churnings 12, 14 and 15 showed the smallest variations in salt distribution. The percentages of salt in the micro samples ranged from 1.60 to 2.28, from 0.37 to 1.06 and from 1.34 to 2.06, respectively, while the variances of the salt contents were 0.0561, 0.0423 and 0.0447, respectively.

Churning 14 contained very little salt; as determined with the macro method, the content was 0.65 per cent. Besides

the putrid defect, the butter contained dark areas close to the surface due to the growth of a black pigment producing organism which White (36) named Pseudomonas nigrifaciens. Churning 15 was packaged in 5 pound tins; gas produced in the butter caused the cans to swell, and the flavor was very unclean.

Mottled Commercial Butter

Light and dark portions of mottled butter, obtained from various commercial sources, were analyzed for salt,

Data on four churnings are presented in Table 6. The light portions generally contained less salt than the dark portions. Churnings 1 and 2 showed very pronounced mottling. The percentages of salt in micro samples picked from the light portions ranged from 0 to 0.49 and from 0.38 to 2.92, respectively, and the average salt contents were 0.22 and 1.50 per cent, respectively; for the dark portions the percentages of salt ranged from 1.18 to 5.25 and from 2.36 to 4.05, respectively, and the average salt contents were 3.24 and 3.22 per cent, respectively.

In churnings 3 and 4 the color defect was much less pronounced than in churnings 1 and 2, the butter being only slightly mottled. The percentages of salt in micro samples picked from the light portions varied from 0 to 2.34 and from 1.21 to 2.16, respectively, the average salt

Table 6. SALT DISTRIBUTION IN MOTTLED COMMERCIAL BUTTER

Churning no.	Degree of mottling	% NaCl, macro method	Light portions of butter: % NaCl in micro sample no. 1	2	3	4	5	Average	% NaC 1	
1	pronounced	1.56	0.00	0.00	0.49	0.45	0.16	0.22	3.11	5
2	pronounced	3.12	0.38	2.21	1.66	2.92	1.35	1.50	2.36	3
3	slight	2.75	0.93	0.95	0.00	1.82	2.34	1.21	1.09	2
4	slight	3.03	2.16	1.92	1.91	1.21		1.44	2.27	2

ISTRIBUTION IN MOTTLED COMMERCIAL BUTTER

Light portions of butter						Dark portions of butter					
aCl in micro sample no.					Average	% NaCl in micro sample no.					Average
2	3	4	5			1	2	3	4	5	
0.00	0.49	0.45	0.16	0.22		3.11	5.25	4.02	2.64	1.18	3.24
2.21	1.66	2.92	1.35	1.50		2.36	3.46	3.60	4.05	2.64	3.22
0.95	0.00	1.82	2.34	1.21		1.09	2.92	2.82	2.06	2.47	2.27
1.92	1.91	1.21		1.44		2.27	2.93	2.71	2.96		2.72

contents being 1.21 and 1.44 per cent, respectively; for the dark portions the percentages of salt ranged from 1.09 to 2.92 and from 2.27 to 2.96, respectively, and the average salt contents were 2.27 and 2.72 per cent, respectively. In churning 3 the micro sample containing no salt was picked from a white spot in the butter and presumably was a particle of curd.

A sample of commercial butter that had a color defect somewhat suggestive of mottles was studied for the salt distribution in it. The butter showed dark-colored portions that appeared as streaks or spots throughout the mass of butter; they suggested that at some time the butter had become soft enough so that there was some fat separation. The dark-colored portions of the butter made up only a relatively small part of the entire volume of butter.

The light-colored portions generally contained more salt than the dark-colored portions which is in direct contrast with the results on the typically mottled butter. The salt contents of the micro samples picked from the light-colored portions ranged from 2.31 to 3.75 per cent, the average being 3.10 per cent, while for the dark-colored portions the salt contents ranged from 0.92 to 2.48 per cent, the average being 1.70 per cent.

Distribution of Salt and Moisture in Butter

The distribution of salt and moisture was studied on a mi-

cro basis in four churnings of commercial butter and three churnings of experimental butter churned in a dazey churn. Moisture determinations were made on 0.3 to 0.5 mg. samples of butter and then the salt contents were determined on the dried samples. The percentage of salt in the serum was calculated from the determined weight of moisture and salt in the micro sample. Large moisture droplets that appeared on freshly cut surfaces of samples in which the water was poorly incorporated were analyzed for salt. With each of the experimental churnings two samples were prepared; both these samples received the same amount of working, but in sample 1 the salt was added at the beginning of the working process, while in sample 2 the salt was added when the working was half completed.

Much larger variations occurred in the moisture contents than in the salt contents of the micro samples. Also, there were much larger variations in the percentages of salt in the serum of the micro samples of butter than in the percentages of salt in the large moisture droplets. The salt contents of the serum calculated from the micro analyses usually were slightly lower than those of the serum calculated from the macro analyses, while the salt contents of the large moisture droplets commonly were higher than those of the serum calculated either from the micro or macro analyses.

Table 7 gives the data on the four churnings of commercial butter. Churnings 1 and 2 were samples of normal butter. The salt distribution was very uniform, especially in churning

Table 7. SALT AND MOISTURE DISTRIBUTION IN COMMERCIAL BUTTER

Churning no.	Description of butter	Compounds determined	Macro sample	Mic 1	2	3	4
1	normal	NaCl in butter, %	2.53	2.65	2.64	2.96	2.70
		H_2O in butter, %	16.25	16.5	29.8	16.8	14.5
		NaCl in serum, %	15.55	16.1	8.7	17.6	18.0
2	normal	NaCl in butter, %	2.82	2.85	2.83	2.84	2.95
		H_2O in butter, %	16.40	18.4	16.6	24.4	19.7
		NaCl in serum, %	17.20	15.5	17.0	11.6	14.9
3	putrid	NaCl in butter, %	0.65	0.46	0.42	0.53	0.57
		H_2O in butter, %	16.49	19.4	23.8	23.1	19.6
		NaCl in serum, %	3.95	2.3	1.7	2.2	2.9
4	canned, gassy	NaCl in butter, %	1.59	2.06	1.65	1.49	1.41
		H_2O in butter, %	14.53	25.4	15.4	24.2	17.8
		NaCl in serum, %	10.93	8.1	10.7	6.2	7.9

N IN COMMERCIAL BUTTER

Micro sample no.									:Variance
: 2	: 3	: 4	: 5	: 6	: 7	: 8	: 9	: 10	:
2.64	2.96	2.70	2.78	2.56	2.26	2.27	2.93	2.51	0.0569
29.8	16.8	14.5	15.2	17.2	18.6	23.4	16.9	17.4	21.1979
8.7	17.6	18.0	17.7	14.9	12.1	9.73	17.5	14.5	11.8043
2.83	2.84	2.95	2.84	3.26	2.85	2.86	3.14	3.04	0.0230
16.6	24.4	19.7	17.0	20.8	16.9	18.6	16.1	19.1	6.1582
17.0	11.6	14.9	16.1	15.6	16.8	15.4	19.5	15.9	3.8845
0.42	0.53	0.57	1.06	0.44	0.51	0.37	0.76	0.66	0.0423
23.8	23.1	19.6	16.8	15.9	15.6	19.0	16.9	15.0	9.3765
1.7	2.2	2.9	6.2	2.7	3.2	1.9	4.4	4.3	1.9956
1.65	1.49	1.41	1.49	1.47	1.34	1.43	1.46	1.73	0.0447
15.4	24.2	17.8	14.5	18.7	12.8	21.8	13.4	21.3	20.3845
10.7	6.2	7.9	10.3	7.9	10.4	6.6	10.9	8.1	2.9943

2. The salt contents of the micro samples ranged from 2.26 to 2.96 per cent for churning 1 and from 2.83 to 3.26 per cent for churning 2, and the variances of the salt contents were 0.0569 and 0.0230, respectively. With churning 1 larger variations occurred in the percentages of moisture in the butter and in the percentages of salt in the serum than with churning 2. The moisture contents of the micro samples ranged from 14.5 to 29.8 per cent for churning 1 and from 16.1 to 24.4 per cent for churning 2, the variances of the moisture contents being 21.1979 and 6.1582, respectively. The salt contents of the serum ranged from 8.7 to 18.0 per cent for churning 1 and from 11.6 to 19.5 per cent for churning 2; the variances of the salt contents were 11.8043 and 3.8845, respectively.

Churnings 3 and 4 were samples of commercial butter that developed abnormal flavors; part of the data on these churnings also are included in Table 5. The salt distribution in the butter was fairly uniform; the salt contents of the micro samples varied from 0.37 to 1.06 per cent and from 1.34 to 2.06 per cent, respectively, and the variances of the salt contents were 0.0423 and 0.0447, respectively. The variations in the moisture contents of the churnings were within the range of the variations occurring in the normal commercial butter. The moisture contents of the micro samples of butter ranged from 15.0 to 23.8 per cent with churning 3 and from 12.8 to 25.4 per cent with churning 4; the variances of

the moisture contents were 9.3765 and 20.3845, respectively. The salt contents of the serum in the micro samples were relatively uniform for both churnings; the values ranged from 1.7 to 6.2 per cent with churning 3 and from 6.2 to 10.9 per cent with churning 4 and the variances of the salt contents were 1.9956 and 2.9943, respectively.

Table 8 contains the data on the three churnings of experimental butter. The salt distribution was fairly uniform in sample 1 from each of churnings 1, 2 and 3; the percentages of salt in the micro samples ranged from 1.54 to 2.25, from 1.21 to 2.34 and from 1.48 to 2.19, respectively, the variances of the salt contents being 0.0443, 0.1075 and 0.0422, respectively. In sample 2 from each of churnings 1, 2 and 3 the salt was poorly distributed; the percentages of salt in the micro samples of butter varied from 0.62 to 2.12, from 0.65 to 3.89 and from 0.70 to 3.18, respectively, and the variances of the salt contents were 0.2385, 0.7547 and 0.6999, respectively.

The moisture contents of the micro samples from churning 1 varied more when the salt was well distributed (Sample 1) than when it was poorly distributed (Sample 2). With churning 2 the largest variations in the moisture contents of the micro samples occurred when the salt was poorly distributed (Sample 2), the moisture content of one sample being only 2.5 per cent. With churning 3 no appreciable difference was noted between the moisture distribution in samples 1 and

Table 8. SALT AND MOISTURE DISTRIBUTION IN EXPERIMENTAL BUTTER

Churning no.	Sample no.	Salt distribution in butter	Compounds determined	Macro sample	1	2	3
1	1	good	NaCl in butter, %	1.70	1.86	1.54	1.74
			H_2O in butter, %	13.58	9.4	18.6	26.2
			NaCl in serum, %	12.52	19.7	8.3	6.6
	2	poor	NaCl in butter, %	1.47	1.69	1.12	0.65
			H_2O in butter, %	10.82	16.4	14.3	16.8
			NaCl in serum, %	13.58	10.3	7.9	3.8
			NaCl in H_2O droplets %		20.9	21.2	19.4
2	1	good	NaCl in butter, %	1.43	1.54	2.34	1.64
			H_2O in butter, %	13.75	15.7	26.2	19.0
			NaCl in serum, %	10.40	10.0	8.9	8.6
	2	poor	NaCl in butter, %	1.33	3.89	1.86	1.21
			H_2O in butter, %	11.48	22.5	18.8	22.3
			NaCl in serum, %	11.59	17.6	9.9	5.5
			NaCl in H_2O droplets %		20.3	19.7	17.6
3	1	good	NaCl in butter, %	1.66	2.19	1.84	1.48
			H_2O in butter, %	14.82	14.1	20.0	22.1
			NaCl in serum, %	11.20	15.5	9.3	6.7
	2	poor	NaCl in butter, %	1.79	2.94	3.18	1.23
			H_2O in butter, %	13.45	18.2	11.8	18.0
			NaCl in serum, %	13.31	16.1	26.8	6.7

IMENTAL BUTTER

Micro sample no.									Varience
2	3	4	5	6	7	8	9	10	
1.54	1.74	1.60	1.69	1.75	1.89	1.83	1.54	2.25	0.0443
18.6	26.2	10.3	13.3	27.7	23.4	12.8	16.3	33.3	66.3823
8.3	6.6	9.3	12.7	6.3	8.1	14.3	9.4	6.8	18.0538
1.12	0.65	1.50	0.62	1.29	2.12	1.18	1.74	0.90	0.2385
14.3	16.8	10.0	10.9	12.7	11.1	20.5	13.0	9.8	12.0294
7.9	3.8	15.0	5.6	10.2	19.0	5.8	13.4	11.8	22.2218
21.2	19.4	21.7	21.1	19.0	20.1	20.8	18.8	19.9	0.9966
2.34	1.64	1.36	1.55	1.35	1.89	1.74	1.21	1.41	0.1075
26.2	19.0	13.3	16.4	18.3	14.6	26.0	16.1	23.7	22.3646
8.9	8.6	10.3	9.5	7.4	12.9	6.7	7.7	5.9	4.1143
1.86	1.21	1.81	1.36	2.27	0.65	2.47	1.82	2.11	0.7547
18.8	22.3	2.5	12.7	12.4	13.2	24.5	12.4	20.9	45.8284
9.9	5.5	71.4	10.7	18.9	4.9	10.1	14.7	12.0	378.5268
19.7	17.6	19.0	15.7	16.5	15.0	18.3	17.5	18.5	2.9010
1.84	1.48	1.55	1.90	1.70	1.94	1.86	1.90	1.93	0.0422
20.0	22.1	17.7	15.6	19.5	15.4	16.5	13.6	14.6	8.0188
9.3	6.7	8.7	12.2	8.7	12.6	11.3	14.0	13.2	7.7396
3.18	1.23	1.00	1.18	1.79	11.24	1.92	1.09	0.70	0.6999
11.8	18.0	13.7	12.9	16.5	15.1	15.7	12.0	16.0	5.3699
26.8	6.7	7.2	9.1	6.2	8.2	12.9	9.1	4.4	43.6401

2. In sample 1 from each of churnings 1, 2 and 3, the percentages of moisture in the micro samples varied from 9.4 to 33.3, from 13.3 to 26.2 and from 13.6 to 22.1, respectively, the variances of the moisture contents being 66.3823, 22.3646 and 8.0188 , respectively; in sample 2 from each of the churnings the percentages ranged from 9.8 to 20.5, from 2.5 to 24.5 and from 11.8 to 18.2, respectively, and the variances of the moisture contents were 12.0294, 45.8284 and 5.3699, respectively.

The salt contents of the serum in churnings 1, 2 and 3, varied less when the salt was well distributed (Sample 1) than when the salt was poorly distributed (Sample 2). The percentages of salt in the serum in sample 1 from each of the churnings ranged from 6.3 to 19.7, from 5.9 to 12.9 and from 6.7 to 15.5, respectively, the variances of the moisture contents being 18.0538, 4.1143 and 7.7396, respectively; in sample 2 from each of the churnings, the percentages ranged from 3.8 to 19.0, from 4.9 to 71.4 and from 4.4 to 26.8, respectively, and the variances were 22.2218, 378.5268 and 43.6401, respectively. The excessively high salt content of 71.4 per cent in sample 2 of churning 2 was probably due to an undissolved salt crystal in the micro sample.

The salt contents of the large water droplets in churnings 1 and 2 when the salt was poorly distributed were relatively uniform; the salt contents ranged from 18.8 to

21.7 per cent with churning 1 and from 15.0 to 20.3 per cent with churning 2, and the variances of the salt contents were 0.9966 and 2.9010, respectively.

EFFECT OF SALT DISTRIBUTION ON BACTERIAL ACTION

Salt is known to have an inhibitory effect on the growth of bacteria in butter and to improve the keeping qualities of the product. However, bacteriological defects develop in salted butter in which the average salt content presumably is high enough to inhibit the growth of bacteria. This suggests that possibly the salt may not be uniformly distributed in butter showing defects due to bacterial action, and that sufficient growth of organisms to produce a defect may occur at points having relatively low salt contents.

Historical

Scharp (32) and Long and Hammer (24) investigated the growth of bacteria in unsalted butter worked various amounts and concluded that the growth was influenced by the extent of the working. They attributed the comparatively low bacterial activity in the thoroughly worked butter to the finer dispersion of the moisture. Other investigators (18, 19) found that butter containing many small water droplets did not deteriorate as rapidly as butter containing large water droplets and suggested that the addition of salt may be respon-

sible for the formation of larger water droplets and thus, in some instances, may impair the keeping qualities of the butter.

Hunziker and Hosman (16) and Boysen (5) presented evidence showing that the addition of salt tends to free the moisture in butter. The brine solution must be incorporated during the working of the butter. The extent to which the brine is distributed throughout the butter may greatly influence the activity of micro-organisms.

Cullity and Griffin (7) studied samples of Australian salted butter in which rabbito, or surface taint, had developed. They noted that in some defective samples the texture of the butter was poor and later found by investigating the manufacturing records that butter from 70 per cent of the churnings developing rabbito was poorly worked. They tentatively concluded that a high salt content and thoroughly working of the butter retard the development of the defect.

Working with experimental butter churned from cream inoculated with Ps. putrefaciens, Claydon and Hammer (6) showed that, although salt tended to inhibit the defect, it was not completely effective unless combined with thorough working. They suggested that with an irregular distribution of salt the organism can grow sufficiently at the points having relatively low salt contents to produce the defect.

Procedure

The effect of salt distribution on bacterial action in butter was studied with small laboratory churnings. Pasteurized, sweet cream was obtained from the butter laboratory, held over night at 5°C. and churned in a dazey churn that had been chemically sterilized. A culture of an organism capable of producing a change in butter was added either to the cream immediately before churning, to the wash water or to the butter. The cream was churned cold enough so that the butter granules were firm. The butter was washed with cold, sterile water and worked in sterile equipment; it was worked in one lot until the free moisture was drained, after which the butter was divided and treated as described.

Since various investigations show that the dispersion of moisture is an important factor in the growth of organisms in butter, an effort was made to control the moisture distribution by working all samples in a churning the same length of time, except the sample of poorly worked unsalted butter. The extent of the distribution of salt was determined by the time at which the salt was added to the butter during the working process. In butter having the salt well distributed the salt was added at the beginning of the working process and the working continued until the butter was dry, while in butter having the salt poorly distributed

the salt was added when the working process was half completed and the butter then worked to dryness. In all salted samples 1.5 per cent salt was added.

Results

Changes in Numbers of Organisms in Butter

The effect of salt and moisture distribution on the changes in numbers of organisms in butter was studied by making plate counts, using beef infusion agar and an incubation of 4 days at 21°C. Counts were made on six churnings of butter. Pasteurized cream was inoculated immediately before churning with 0.3 per cent of various milk cultures as follows: With churning 1, *Pseudomonas fragi*; with churning 2, an unidentified *Micrococcus*; with churning 3, *Achromobacter lipolyticum*; with churning 4, *Mycotorula lipolytica*; and with churnings 5 and 6, *Pseudomonas putrefaciens*. Churnings 1, 2, 3, 5 and 6 each contained two salted and two unsalted samples of butter, while churning 4 contained three salted and one unsalted sample. The samples of butter were held at 15.5°C. and plate counts were made at intervals of 4 days over a period of 16 days.

The results on four of the churnings are presented in Table 9. In general, salt retarded the growth of organisms in the butter, the counts being consistently higher in the

Table 9. EFFECT OF SALT AND MOISTURE DISTRIBUTION ON CHANGES I

Churning no.	Cream inoculated with	Sample no.	Description of butter: Salt distribution	Degree of working	Numbers of organism 0 days	4 days
1	Ps. fragi	1	good	thorough	8,000	9,000
		2	poor	thorough	8,000	60,000
		3	no salt	thorough	8,000	9,000,000
		4	no salt	poor	8,000	32,000,000
2	unidentified Micrococcus	1	good	thorough	87,000	84,000
		2	poor	thorough	87,000	18,000,000
		3	no salt	thorough	87,000	55,000,000
		4	no salt	poor	87,000	109,000,000
3	Ach. lipolyticum	1	good	thorough	37,000	4,000
		2	poor	thorough	37,000	670,000
		3	no salt	thorough	37,000	89,000,000
		4	no salt	poor	37,000	250,000,000
4	Myc. lipolytica	1	good	thorough	7,000	5,000
		2	poor	thorough	7,000	65,000
		3	poor	poor	7,000	82,000
		4	no salt	poor	7,000	590,000

STURE DISTRIBUTION ON CHANGES IN NUMBERS OF ORGANISMS IN BUTTER

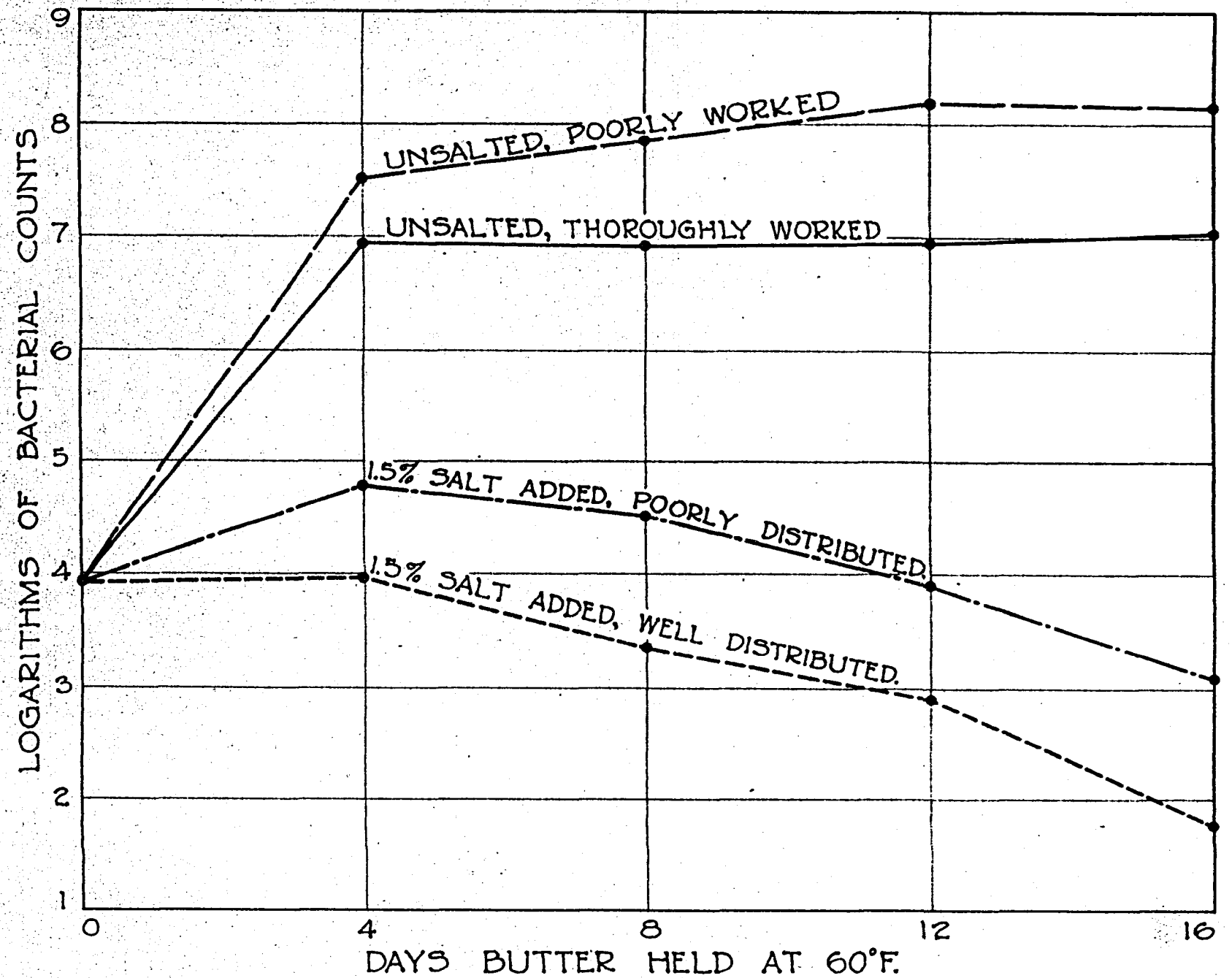
of butter : Degree of working	Numbers of organisms per ml. in butter held at 15.5°C. for 0 days	4 days	8 days	12 days	16 days
thorough	8,000	9,000	2,000	800	less than 100
thorough	8,000	60,000	32,000	8,000	1,000
thorough	8,000	9,000,000	8,700,000	8,900,000	10,300,000
poor	8,000	32,000,000	68,000,000	149,000,000	136,000,000
thorough	87,000	84,000	85,000	120,000	150,000
thorough	87,000	18,000,000	26,000,000	21,000,000	21,000,000
thorough	87,000	55,000,000	73,000,000	65,000,000	61,000,000
poor	87,000	109,000,000	116,000,000	79,000,000	84,000,000
thorough	37,000	4,000	12,000	14,000	11,000
thorough	37,000	670,000	1,700,000	1,700,000	1,700,000
thorough	37,000	89,000,000	118,000,000	92,000,000	103,000,000
poor	37,000	250,000,000	134,000,000	140,000,000	131,000,000
thorough	7,000	5,000	3,000	4,000	7,000
thorough	7,000	65,000	54,000	132,000	88,000
poor	7,000	82,000	86,000	144,000	250,000
poor	7,000	590,000	1,600,000	3,400,000	1,300,000

samples of unsalted butter than in the samples of salted butter.

With churning 1 the butter having the salt well distributed showed a decrease in numbers of organisms during holding, while the butter having the salt poorly distributed showed a small increase in numbers of organisms at 4 days followed by a decrease during the remainder of the holding. In the samples of unsalted butter there were large increases in numbers of organisms at 4 days followed by small increases during the remainder of the holding; the largest increases occurred in the poorly worked butter. The data on churning 1 are also presented in Graph 1.

With churning 2 the butter having the salt well distributed increased in numbers of organisms at 12 and 16 days, while the butter having the salt poorly distributed showed a large increase in numbers of organisms at 4 days with little change during the remainder of the holding. The samples of unsalted butter showed large increases in numbers of organisms at 4 days, a smaller increase at 8 days and little change during the remainder of the holding, the largest increases occurring in the poorly worked butter.

With churning 3 the butter in which the salt was well distributed decreased in numbers of organisms during holding, while the butter in which the salt was poorly distributed increased in numbers of organisms at 4 and 8 days with no further change during the remainder of the holding. In the



Graph 1. EFFECT OF SALT AND MOISTURE DISTRIBUTION ON CHANGES IN NUMBERS OF ORGANISMS IN BUTTER

well worked, unsalted butter increases in numbers of organisms occurred at 4 and 8 days, followed by little or no change during the remainder of the holding period; in the poorly worked, unsalted butter a large increase in numbers of organisms occurred followed by a decrease, the maximum count being obtained at 4 days.

With churning 4 three samples of salted butter and one sample of unsalted butter were prepared. In sample 1 the salt was well distributed, in sample 2 the salt was added as usual when the working process was half completed and the butter then worked to dryness, while in sample 3 the salt was added at the beginning of the working process and the working discontinued when the moisture was still poorly incorporated. With butter having the salt well distributed no significant change in numbers of organisms occurred during holding, while with both samples of butter having the salt poorly distributed, increases in numbers of organisms occurred, the largest increase occurring in sample 3. In sample 2 increases in numbers of organisms were noted at 4, 8 and 12 days followed by a decrease at 16 days, while in sample 3 increases in numbers of organisms occurred during the entire holding period. In the poorly worked, unsalted butter large increases in the numbers of organisms were noted at 4, 8 and 12 days, followed by a decrease at 16 days.

Data on churnings 5 and 6, in which the cream was inoculated with cultures of Ps. putrefaciens are not included in Table 9. Most investigators that have worked with

this organism appreciate the difficulties encountered in determining the numbers of organisms by the plate method or any other procedure, and since the counts that were obtained may be misleading the data are not given. However, the data indicate that in the salted butter only a very few organisms were present while the unsalted butter contained very large numbers of organisms. The maximum counts on unsalted butter were obtained at 4 or 8 days, after which there was a rapid decrease in numbers.

Distribution of Organisms in Salted Butter

The effect of the distribution of salt on the numbers and distribution of organisms in butter was studied with an adaption of the Burri smear culture technique (25) in which 20 portions of butter from each sample were smeared on dry, beef infusion agar slopes. Also, the numbers of organisms were studied in large moisture droplets appearing on the freshly cut surfaces of butter in which the moisture was poorly incorporated, one portion from each of ten moisture droplets from a sample of butter being smeared on an agar slope. The slopes were incubated at 21°C. for 4 days.

The distribution of organisms was studied in six experimental churnings and in one commercial churning of butter. From each of the experimental churnings two samples of salted butter were prepared; sample 1 had the salt well distributed

and sample 2 had the salt poorly distributed. In churnings 1, 2, 4, 5 and 6 the slopes were prepared after the flavor defects had developed in one of the samples from each churning, and in churning 3 the slopes were prepared after the butter had been held for 5 days at 15.5°C. In churnings 1 and 2 milk cultures of Ach. lipolyticum and Ps. putrefaciens, respectively, were added to the pasteurized cream immediately before churning; in churning 3 a milk culture of Ps. putrefaciens was added to the water used to wash the butter; while in churnings 4, 5 and 6 milk cultures of Ps. putrefaciens were added to the butter during the working process. Sample 1 from each experimental churning and sample 2 from churning 3 did not develop abnormal flavors. Sample 2 from churnings 1 and 2 developed off flavors, while sample 2 from each of churnings 4, 5 and 6 developed a putrid flavor. Churning 7 was commercial butter, containing 0.65 per cent salt, which developed a putrid flavor.

Data on the seven churnings are presented in Table 10. In general, the slopes prepared from butter with the salt well distributed contained fewer colonies than the slopes prepared from butter with the salt poorly distributed. With churnings 1, 2 and 3, both the butter having the salt well distributed and that having the salt poorly distributed yielded very few colonies on the slopes prepared with portions of the butter and a large percentage of the slopes showing no colonies; only one slope contained more than four colonies.

Table 10. NUMBERS OF COLONIES ON SLOPES USING BURRI SMEAR CULTURE TECHNI

Slope no.	1. Cream inoculated with Aoh. lipolyticum			2. Cream inoculated with Ps. putrefaciens			3. Wash Ps.
	Churning no.						
	Salt well distributed: Portions of butter	Salt poorly distributed: Portions of butter	H_2O droplets	Salt well distributed: Portions of butter	Salt poorly distributed: Portions of butter	H_2O droplets	Salt well distribut: Portions of butte
1	0	2	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	3	0	0	0	0	0
6	0	0	0	1	0	0	0
7	0	0	0	0	0	0	1
8	0	0	0	0	0	0	0
9	0	1	0	0	0	0	0
10	0	0	0	0	0	0	0
11	1	0		0	1		0
12	0	1		0	1		0
13	0	1		0	0		0
14	1	0		0	0		0
15	1	3		0	33		0
16	2	0		1	0		0
17	0	1		0	4		0
18	0	0		0	0		0
19	0	3		0	1		1
20	0	0		0	0		0

* o.g. = over grown.

TURE TECHNIQUE ON SALTED BUTTER

g no.	3. Wash H_2O inoculated with Ps. putrefaciens			4. Butter inoculated with Ps. putrefaciens				5. Butter Ps. put
	Salt well distributed: Portions of butter	Salt poorly distributed: Portions of butter	H_2O droplets	Salt well distributed: Portions of butter	Salt poorly distributed: Portions of butter	H_2O droplets	Slope no.	Salt well distributed: Portions of butter
	0	1	0	1	50	0	1	0
	0	0	0	3	7	o.g.*	2	0
	0	0	0	2	24	0	3	0
	0	2	0	1	70	0	4	0
	0	1	0	0	19	7	5	0
	0	1	0	1	7	o.g.	6	0
	1	0	0	3	0	7	7	0
	0	0	0	1	0	4	8	0
	0	0	0	2	1	0	9	2
	0	0	0	1	3	125	10	1
	0	1		6	31		11	1
	0	1		4	0		12	3
	0	1		5	8		13	7
	0	2		2	22		14	1
	0	0		5	2		15	2
	0	0		2	1		16	2
	0	0		3	12		17	3
	0	0		0	4		18	0
	1	0		2	0		19	0
	0	1		3	17		20	3

Churning no.							
5. Butter inoculated with Ps. putrefaciens			6. Butter inoculated with Ps. putrefaciens			7. Putrid commercial butter	
Salt well distributed:	Salt poorly distributed		Salt well distributed:	Salt poorly distributed			
Portions of butter	Portions of butter	H_2O droplets	Portions of butter	Portions of butter	H_2O droplets	Portions: of butter	H_2O droplets
0	11	0	0	0	10	o.g.	o.g.
0	30	0	0	0	3	153	o.g.
0	3	0	0	1	4	201	93
0	2	26	0	6	1	o.g.	o.g.
0	18	0	4	27	0	o.g.	o.g.
0	0	0	0	0	2	o.g.	o.g.
0	23	2	0	0	o.g.	142	o.g.
0	6	0	0	4	0		
2	0	0	11	0	11	70	o.g.
1	77	0	5	12	o.g.	o.g.	80
1	0		3	10		27	
3	5		1	5		167	
7	0		2	50		o.g.	
1	1		1	o.g.		o.g.	
2	0		0	21		151	
2	5		0	20		87	
3	0		1	16		o.g.	
0	o.g.		0	11		o.g.	
0	150		0	7		o.g.	
3	0		1	6		o.g.	

The slopes prepared from the moisture droplets showed no growth of organisms.

With churnings 4, 5 and 6, slopes prepared from butter with the salt well distributed showed very few colonies, and a large percentage of the slopes showing no colonies; only one slope showed more than seven colonies. Slopes prepared from butter with the salt poorly distributed showed much larger numbers of organisms and the distribution of the organisms was irregular; a few slopes contained no colonies while two slopes contained so many colonies that the numbers could not be estimated. The slopes prepared from the moisture droplets showed a very irregular distribution of organisms; some slopes contained no colonies while on other slopes the numbers of colonies were too large to estimate.

In churning 7, the slopes prepared from the butter and moisture droplets indicated that extensive growth of organisms had occurred in the butter. Growth developed on all the slopes prepared from both the butter and the moisture droplets, with a large percentage of the slopes containing numbers of colonies that were too large to estimate.

Changes in pH of Butter Serum

The effect of salt and moisture distribution on changes in pH of butter serum was studied with butter made from pasteurized cream inoculated with lactic acid-producing streptococci just before churning. In churnings 1, 2, 3 and

4 the cream was inoculated with a butter culture, while in churning 5 the cream was inoculated with a milk culture of Streptococcus diacetilactis. Churnings 1, 3, 4 and 5 each contained two salted and two unsalted samples of butter, while churning 2 contained three salted samples and one unsalted sample. The samples of butter were held at 15.5°C. pH determinations were made on the butter serum at intervals of 4 days, the serum being recovered by centrifuging the melted butter and drawing off the fat; the pH was determined electrometrically, using a quinhydrone electrode.

Data on the five churnings are given in Table 11. The addition of salt to butter tended to lower the pH of the serum. With churning 1 there were no significant differences in pH of the serum between butter with good and with poor salt distribution; a slight increase in pH occurred in both samples; the pH values increasing from 6.00 to 6.19 and from 6.00 to 6.14, respectively. In the unsalted butter large decreases in pH of the serum occurred, the largest decrease being in the poorly worked butter. The pH of the serum decreased from 6.31 to 5.70 in the thoroughly worked butter and from 6.31 to 5.18 in the poorly worked butter.

With churning 2 small decreases occurred in the pH of the serum from salted butter, the largest decrease occurring in the sample with the salt poorly distributed. In the samples of butter having a good, fair and poor distribution of salt the pH of the serum decreased from 6.36 to 6.25, from 6.36 to

Table 11. EFFECT OF SALT AND MOISTURE DISTRIBUTION ON CHANGES IN pH OF

Churning no.	Cream inoculated with	Sample no.	Description of butter: Salt distribution	Degree of working	pH of serum of: 0 days	4 days
1	5% butter culture H-1	1	good	thorough	6.00	6.05
		2	poor	thorough	6.00	6.06
		3	no salt	thorough	6.31	6.15
		4	no salt	poor	6.31	5.88
2	1% butter culture 15/1	1	good	thorough	6.36	6.35
		2	fair	thorough	6.36	6.33
		3	poor	thorough	6.35	6.30
		4	no salt	thorough	6.90	6.80
3	5% butter culture H-1	1	good	thorough	6.16	6.12
		2	poor	thorough	6.08	5.91
		3	no salt	thorough	6.57	6.01
		4	no salt	poor	6.55	5.45
4	5% butter culture H-2	1	good	thorough	5.66	5.65
		2	poor	thorough	5.59	5.56
		3	no salt	thorough	5.57	5.45
		4	no salt	poor	5.88	5.70
5	5% milk culture of *S. diacetilactis*	1	good	thorough	5.98	5.96
		2	poor	thorough	5.88	5.75
		3	no salt	thorough	6.25	5.82
		4	no salt	poor	6.19	5.09

)ISTURE DISTRIBUTION ON CHANGES IN pH OF BUTTER SERUM

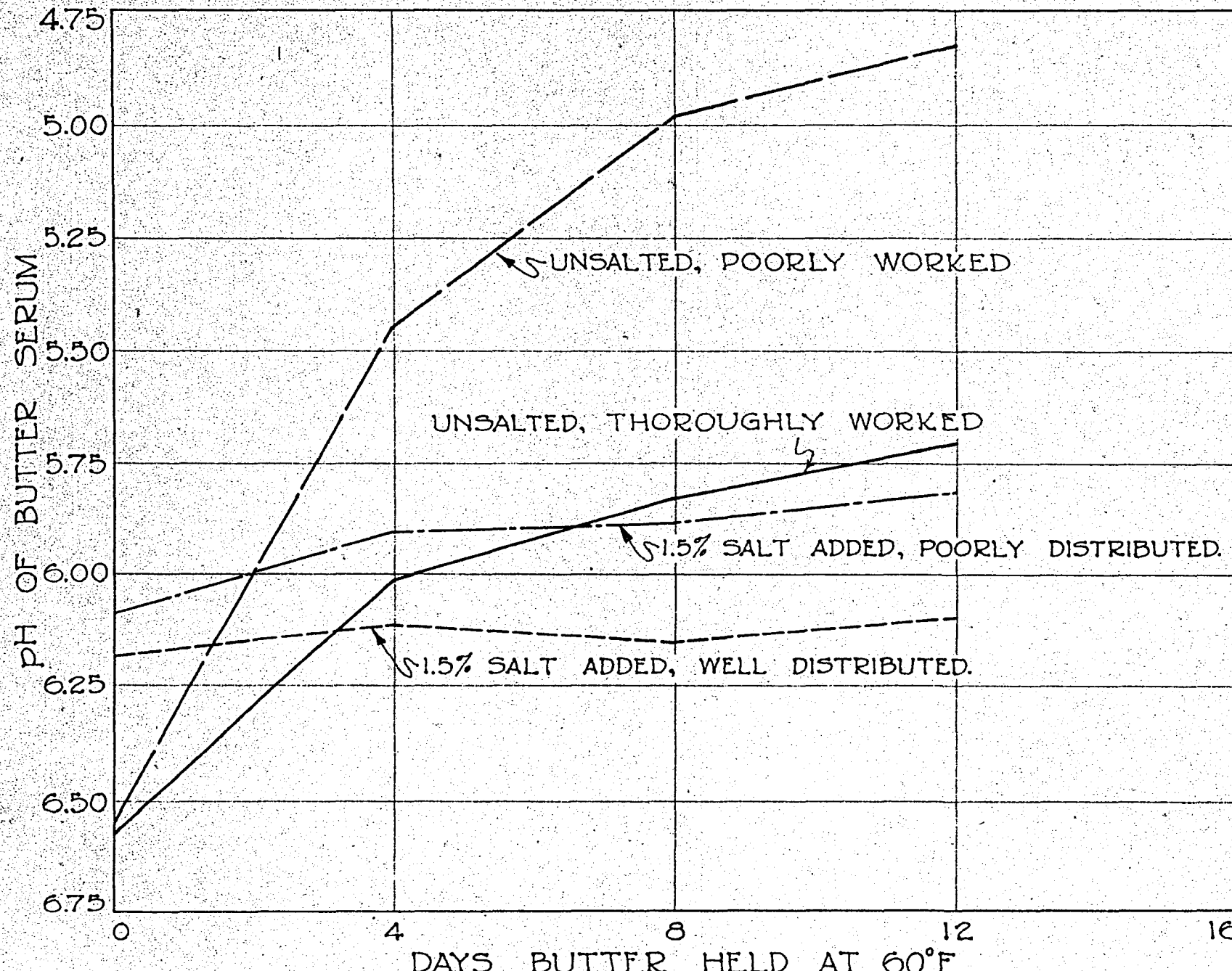
Description of butter : Salt distribution	Degree of working	pH of serum of butter held at 15.5°C. for 0 days	4 days	8 days	12 days	16 days
good	thorough	6.00	6.05	6.05	6.10	6.19
poor	thorough	6.00	6.06	6.05	6.10	6.14
no salt	thorough	6.31	6.15	5.95	5.73	5.70
no salt	poor	6.31	5.88	5.63	5.26	5.18
good	thorough	6.36	6.35	6.25	6.26	
fair	thorough	6.36	6.33	6.30	6.23	
poor	thorough	6.35	6.30	6.25	6.09	
no salt	thorough	6.90	6.80	6.75	6.63	
good	thorough	6.16	6.12	6.16	6.10	
poor	thorough	6.08	5.91	5.89	5.82	
no salt	thorough	6.57	6.01	5.83	5.71	
no salt	poor	6.55	5.45	4.99	4.83	
good	thorough	5.66	5.65	5.65	5.65	
poor	thorough	5.59	5.56	5.58	5.55	
no salt	thorough	5.57	5.45	5.41	5.43	
no salt	poor	5.88	5.70	5.71	5.73	
good	thorough	5.98	5.96	5.86	5.86	5.85
poor	thorough	5.88	5.75	5.67	5.61	5.53
no salt	thorough	6.25	5.82	5.12	5.70	5.70
no salt	poor	6.19	5.09	4.91	5.20	5.16

6.23 and from 6.35 to 6.09, respectively. In the unsalted butter only a small decrease in pH occurred, the values decreasing from 6.90 to 6.63.

With churning 3, no significant change occurred in the pH of serum of butter with the salt well distributed. However, with the salt poorly distributed there was a small decrease in pH, the values decreasing from 6.08 to 5.82. In both the thoroughly worked and poorly worked samples of unsalted butter, large decreases occurred in the pH of the serum, the largest decrease occurring in the poorly worked butter; the pH values decreased from 6.57 to 5.71 and from 6.55 to 4.83, respectively. The data on churning 3 are also presented in Graph 2.

With churning 4, there were no significant changes in the pH of the serum from the salted samples of butter, while small decreases occurred in the serum from the unsalted samples. The pH values decreased from 5.57 to 5.43 in the thoroughly worked unsalted butter and from 5.88 to 5.73 in the poorly worked unsalted butter.

With churning 5, in which the cream was inoculated with a milk culture of S. diacetilactis, a small decrease occurred in the pH of the serum from the butter with the salt well distributed, the pH value decreasing from 5.98 to 5.85; in butter with the salt poorly distributed larger decreases occurred in pH, the pH values decreasing from 5.88 to 5.53.



Graph 2. EFFECT OF SALT AND MOISTURE DISTRIBUTION ON CHANGES IN pH OF BUTTER SERUM

In samples of unsalted butter large decreases, followed by increases, occurred in the pH of the serum. In the thoroughly worked unsalted butter the pH values decreased from 6.25 to 5.12 at 8 days and then increased to 5.70 at 12 days with no further change at 16 days, while for the poorly worked unsalted butter the pH values decreased from 6.19 to 4.91 at 8 days and then increased to 5.20 at 12 days with no significant change at 16 days.

Microscopic examinations were made on the serum of butter from a number of the churnings. In butter having the salt well distributed very few bacterial cells were observed, and the cells appeared singly or in pairs, while in butter having the salt poorly distributed somewhat larger numbers of cells and a few short chains were observed. In the samples of unsalted butter large numbers of bacterial cells were present and they often appeared in very long chains, the largest numbers of cells and longest chains occurring in the poorly worked butter.

Development of Defects in Butter

The effect of salt and moisture distribution on the development of defects in butter was studied by holding samples of experimental butter at 15.5°C.; the samples were examined at frequent intervals for the defect expected on the basis of the organism inoculated into the pasteurized cream, the wash water or the butter.

Organisms Added to Cream.

Eleven churnings of butter were made from pasteurized cream inoculated immediately before churning with a milk culture of an organism capable of producing a defect in butter; except in churning 7 with which only 0.06 per cent inoculum was used, 0.3 per cent of the culture was added. The organisms inoculated were: In churning 1, Ps. fragi; in churnings 2, 3 and 7, Myc. lipolytica; in churning 6, Ach. lipolyticum; in churnings 4, 5, 8 and 9, Ps. putrefaciens; in churning 10, Ps. putrefaciens and Aerobacter aerogenes; and in churning 11, Ps. putrefaciens and Escherichia coli. From each churning two salted and two unsalted samples of butter were prepared.

Results on the eleven churnings are presented in Table 12. In butter with the salt well distributed flavor defects developed in only two churnings, while in butter with the salt poorly distributed defects developed in eight churnings. In the unsalted butter all except one churning developed defects in flavor, the defects developing sooner in the poorly worked butter than in the thoroughly worked butter. Usually the flavor defects were more pronounced in the unsalted butter than in the salted butter.

In churnings 2 and 3 all the samples of butter were very rancid 1 day after churning. In churnings 4 and 11 about the same time was required for the development of the flavor defects in the salted butter with the salt poorly

Table 12. EFFECT OF SALT AND MOISTURE DISTRIBUTION ON DEVELOPMENT OF DEFECTS IN BUTTER

Churning no.	Cream inoculated with	Days required for production of defect in butter held at 15.5°C.			
		Salted butter		Unsalted butter	
		Salt well distributed	Salt poorly distributed	Thoroughly worked	Poorly worked
	Causative Organisms Added to Cream				
1	Ps. fragi	-*	-	6	3
2	Myc. lipolytica	1	1	1	1
3	Myc. lipolytica	1	1	1	1
4	Ps. putrefaciens	-	3	3	2
5	Ps. putrefaciens	-	4	2	1
6	Ach. lipolyticum	-	4	3	2
7	Myc. lipolytica	-	10	8	5
8	Ps. putrefaciens	-	-	4	2
9	Ps. putrefaciens	-	3	-	1
10	Ps. putrefaciens and A. aerogenes	-	-	5	2
11	Ps. putrefaciens and E. coli	-	3	3	2

*- = Indicates no defect developed in the butter during holding period.

distributed than in unsalted butter that was thoroughly worked. In churning 9 salted butter with the salt poorly distributed and the unsalted butter that was poorly worked developed abnormal flavors while the salted butter with the salt well distributed and the unsalted butter that was thoroughly worked did not develop abnormal flavors.

Organisms Added to Cream and Wash Water.

In twelve churnings of butter comparisons were made on the effect of salt and moisture distribution on development of defects in flavor between butter in which cultures of Ps. putrefaciens were added to the cream and butter in which the cultures of Ps. putrefaciens were added to the wash water. The lots of cream were divided in two parts; to one part 0.3 per cent of a milk culture of Ps. putrefaciens was added to the cream immediately before churning and to the other part 10 ml. of the milk culture was added to 1 liter of wash water. Two salted and two unsalted samples of butter were prepared from each churning.

Table 13 presents data on the twelve churnings. In butter with the salt well distributed no defects developed during holding in either the butter made from the inoculated cream or the butter washed with the inoculated water. In butter with the salt poorly distributed defects developed in three samples of butter in which the organisms were added to the cream, whereas no defects developed in butter in which

Table 13. EFFECT OF SALT DISTRIBUTION ON DEVELOPMENT OF DEFECTS IN BUTTER

Cultures of Ps. putrefaciens Added to Cream and Wash H_2O

Churning no.	Organisms added to	Days required for production of defect in butter held at 15.5°C.			
		Salted Butter		Unsalted butter	
		Salt well distributed	Salt poorly distributed	Thoroughly worked	Poorly worked
1	cream	-*	5	-	2
1a	wash H_2O	-	-	5	2
2	cream	-	3	9	2
2a	wash H_2O	-	-	5	2
3	cream	-	-	-	5
3a	wash H_2O	-	-	-	5
4	cream	-	-	5	2
4a	wash H_2O	-	-	5	2
5	cream	-	-	3	2
5a	wash H_2O	-	-	3	2
6	cream	-	3	4	2
6a	wash H_2O	-	-	-	2

*- = Indicates no defect developed in the butter during holding period.

the organisms were added to the wash water. In the unsalted butter apparently there was no difference in the development of defects between the butter made from the inoculated cream or the butter washed with the inoculated water. In the thoroughly worked unsalted butter defects developed in eight churnings while in the poorly worked unsalted butter defects developed in all the churnings; the defects appeared sooner in the poorly worked butter than in the thoroughly worked butter. In the unsalted butter the defects which occurred were more typically putrid than in the salted butter.

In churnings 2 and 6 defects occurred in the salted butter with the salt poorly distributed before defects occurred in the unsalted butter that was thoroughly worked. In churning 1 salted butter with the salt poorly distributed and the unsalted butter that was poorly worked developed defects in flavor, whereas in the unsalted butter that was thoroughly worked no defects developed.

Organisms Added to Butter.

Various amounts of inoculum were added to butter samples prepared from ten churnings of pasteurized cream. Churnings 1 and 2 each contained eight samples of butter, while the remaining churnings each contained six samples. Sample 1 in each churning was butter having the salt well distributed and to which no organisms were added. Sample 2 in each churning was unsalted butter to which 0.5 ml. of a milk culture of an organism was added. The remaining samples from

each churning were salted and were divided into pairs; in one sample from each pair the salt was well distributed, while in the other sample the salt was poorly distributed. To each sample of a pair an equal amount of a milk culture of an organism was added to the butter early in the working process. All samples of butter were thoroughly worked and at the completion of working the moisture appeared to be well incorporated. Except in churning 3, in which a milk culture of an unidentified proteolytic, gram-negative rod was used, a milk culture of Ps. putrefaciens was added to the butter.

The data on the ten churnings of butter are presented in Table 14. In the butter to which no organisms were added, flavor defects did not appear in any of the churnings, while in the unsalted butter from each churning, to which 0.5 ml. of a milk culture of an organism was added, a putrid defect developed. In churning 2 none of the samples of salted butter, to which a culture of an organism was added, developed a putrid defect during holding.

In churnings 3, 6 and 8 the samples of butter with the salt well distributed developed no defects, whereas in the butter with the salt poorly distributed defects appeared only in the samples from each churning containing the largest amounts of inoculum.

In churnings 1, 5 and 7 the samples of butter with the salt well distributed developed no defects during holding,

Table 14. EFFECT OF SALT DISTRIBUTION ON DEVELOPMENT OF DEFECTS IN BUTTER

Causative Organisms Added to the Butter and Half of the Butter Reworked after 4 Days

Churning no.	Butter inoculated with:	Sample no.	Ml. milk culture added to 130 g. butter	Salt distribution in butter	Defects Butter nc
1	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid at
		3	0.5	good	none
		4	0.5	poor	slightly
		5	1.0	good	none
		6	1.0	poor	putrid at
		7	3.0	good	none
		8	3.0	poor	off at 4
2	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid at
		3	0.5	good	none
		4	0.5	poor	none
		5	1.0	good	none
		6	1.0	poor	none
		7	3.0	good	none
		8	3.0	poor	none
3	Unidentified gram-negative rod	1	none	good	none
		2	0.5	no salt	putrid at
		3	1.0	good	none
		4	1.0	poor	none
		5	5.0	good	none
		6	5.0	poor	slightly
		7			
4	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid at
		3	1.0	good	none
		4	1.0	poor	none
		5	3.0	good	slightly of
		6	3.0	poor	putrid at
5	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid at
		3	1.0	good	none
		4	1.0	poor	putrid at
		5	3.0	good	none
		6	3.0	poor	putrid at

)ISTRIBUTION ON DEVELOPMENT OF DEFECTS IN BUTTER

lams Added to the Butter and Half of the Butter

Days

Sample with:	Sample no.	Ml. milk culture added to 130 g. butter	Salt distribution in butter	Defects in butter held at 15.5°C.	
				Butter not reworked	Butter reworked
	1	none	good	none	
	2	0.5	no salt	putrid at 2 days	
	3	0.5	good	none	slightly rancid
	4	0.5	poor	slightly off at 8 days	slightly rancid
	5	1.0	good	none	
	6	1.0	poor	putrid at 2 days	
	7	3.0	good	none	slightly putrid
	8	3.0	poor	off at 4 days	putrid
	1	none	good	none	
	2	0.5	no salt	putrid at 2 days	
	3	0.5	good	none	
	4	0.5	poor	none	
	5	1.0	good	none	
	6	1.0	poor	none	
	7	3.0	good	none	
	8	3.0	poor	none	
	1	none	good	none	
	2	0.5	no salt	putrid at 2 days	
	3	1.0	good	none	none
	4	1.0	poor	none	slightly off
	5	5.0	good	none	mone
	6	5.0	poor	slightly off	putrid
	7				
	1	none	good	none	
	2	0.5	no salt	putrid at 2 days	
	3	1.0	good	none	slightly off
	4	1.0	poor	none	putrid
	5	3.0	good	slightly off at 9 days	slightly off
	6	3.0	poor	putrid at 6 days	putrid
	1	none	good	none	
	2	0.5	no salt	putrid at 2 days	
	3	1.0	good	none	none
	4	1.0	poor	putrid at 7 days	slightly off
	5	3.0	good	none	none
	6	3.0	poor	putrid at 4 days	putrid

Table 14. (contd.) EFFECT OF SALT DISTRIBUTION ON DEVELOPMENT OF DEFECT

Causative Organisms Added to the Butter and Half of Reworked after 4 Days

Churning no.	Butter inoculated with:	Sample no.	Ml. milk culture added to 130 g. butter	Salt distribution in butter	Defect Butter
6	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid
		3	1.0	good	none
		4	1.0	poor	none
		5	3.0	good	none
		6	3.0	poor	putrid
7	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid
		3	1.0	good	none
		4	1.0	poor	putrid
		5	3.0	good	none
		6	3.0	poor	putrid
8	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid
		3	1.0	good	none
		4	1.0	poor	none
		5	3.0	good	none
		6	3.0	poor	putrid
9	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid
		3	1.0	good	none
		4	1.0	poor	off at
		5	3.0	good	off at
		6	3.0	poor	putrid
10	Ps. putrefaciens	1	none	good	none
		2	0.5	no salt	putrid
		3	1.0	good	none
		4	1.0	poor	putrid
		5	3.0	good	putrid
		6	3.0	poor	putrid

F SALT DISTRIBUTION ON DEVELOPMENT OF DEFECTS IN BUTTER

e Organisms Added to the Butter and Half of the Butter after 4 Days

Sample no.	Ml. milk culture added to 130 g. butter	Salt distribution in butter	Defects in butter held at 15.5°C.	
			Butter not reworked	Butter reworked
1	none	good	none	
2	0.5	no salt	putrid at 2 days	
3	1.0	good	none	slightly off
4	1.0	poor	none	off
5	3.0	good	none	none
6	3.0	poor	putrid at 6 days	putrid
1	none	good	none	
2	0.5	no salt	putrid at 2 days	
3	1.0	good	none	slightly off
4	1.0	poor	putrid at 6 days	slightly off
5	3.0	good	none	off
6	3.0	poor	putrid at 2 days	putrid
1	none	good	none	
2	0.5	no salt	putrid at 2 days	
3	1.0	good	none	none
4	1.0	poor	none	none
5	3.0	good	none	none
6	3.0	poor	putrid at 2 days	putrid
1	none	good	none	
2	0.5	no salt	putrid at 2 days	
3	1.0	good	none	
4	1.0	poor	off at 3 days	
5	3.0	good	off at 3 days	
6	3.0	poor	putrid at 3 days	
1	none	good	none	
2	0.5	no salt	putrid at 2 days	
3	1.0	good	none	
4	1.0	poor	putrid at 2 days	
5	3.0	good	putrid at 2 days	
6	3.0	poor	putrid at 2 days	

while in the samples of butter with the salt poorly distributed flavor defects were produced. For churning 1, in butter containing 3 ml. of inoculum the flavor defect appeared sooner and the defect was more intense than in butter containing 3 ml. of inoculum. In butter containing 1 ml. of inoculum the defect was only slight. For churnings 5 and 7 the putrid defect appeared first in the samples of butter containing the largest amounts of inoculum.

In churning 4 the butter with the salt well distributed showed no defect in the sample to which 1 ml. of inoculum was added, whereas a slight defect developed in 9 days in the sample to which 3 ml. of inoculum was added. In the butter with the salt poorly distributed no defect developed in the sample to which 1 ml. of inoculum was added, while a putrid flavor developed in 6 days in the butter to which 3 ml. of inoculum was added.

For churnings 9 and 10, in butter with the salt well distributed defects appeared only in the samples of butter containing 3 ml. of inoculum. In butter with the salt poorly distributed defects developed in both samples of butter from each churning, the defect being more intense in the samples containing 3 ml. of inoculum than samples containing 1 ml. of inoculum.

Reworking of Butter.

Four samples were reworked from each of churnings 1, 3, 4, 5, 6, 7 and 8 described in Table 14. The butter was

cooled to approximately 4°C. and half of each sample reworked by passing it through a small Universal food chopper two times. Before reworking each sample the food chopper was placed in boiling water for a few minutes and then cooled by pouring cold, sterile water over it. The butter was again held at 15.5°C.

The data on the reworking of the butter are also presented in Table 14. With some samples of butter reworking seemed to have no effect on the keeping quality, with other samples reworking tended to increase or produce defects, while with still other samples reworking tended to decrease the intensity of the defect. In churning 8 the reworking of the butter did not affect the keeping quality.

In samples of butter with the salt well distributed, in churnings 3, 5 and 6 little or no differences occurred between the butter reworked and not reworked; whereas in churnings 1, 4 and 7 the reworked butter developed defects during holding, while in butter not reworked no defects developed.

With samples of butter with the salt poorly distributed, in churnings 1, 3, 4 and 6 the reworking of the butter tended to favor the development of defects, while in churnings 5 and 7 the reworking of the butter containing 1 ml. of inoculum tended to inhibit the development of defects.

DISCUSSION OF RESULTS

A reasonable degree of accuracy undoubtedly can be obtained in determining the sodium chloride content of an approximately 0.2 mg. sample of butter. However, the salt content of such a micro sample may not be representative of an entire churning of butter. The value of the method is in connection with the study of the salt contents of very small portions of butter, and it is particularly useful in the study of the effect of salt distribution on bacterial action in butter.

On the basis of micro analyses, considerable variation in the distribution of salt in different samples of commercial butter is to be expected because of the large variations which occur in the incorporation of the moisture in different samples. Since the salt is largely dissolved in the water, the distribution of the salt depends to a great extent on the incorporation of the brine during the working process.

Actually, the distribution of the salt and the incorporation of the moisture are very closely related. When salt is added to butter it apparently first is largely dissolved in a relatively small portion of the moisture, the remaining moisture being more or less distinct from the loosely held moisture in which the salt is dissolved. As the

working of the butter progresses, the brine solution is divided into smaller and smaller droplets. Presumably there is a mixing of these droplets containing a relatively high salt content with other droplets containing little or no salt, and in this way the salt contents of the various water droplets tend to equalize. During holding of butter having the salt poorly dispersed through the moisture there may be a movement of water droplets containing little or no salt to other droplets containing relatively large amounts. With butter having the brine thoroughly incorporated, a relatively complete mixing of the brine and water phases will have occurred, and there will be comparatively little tendency for moisture to migrate, whereas with butter having the brine poorly incorporated, the salt will be dissolved in a relatively small portion of the moisture and there will be a greater tendency for the moisture to migrate and form large water droplets. With butter having the salt very poorly distributed the color may be uneven as a result of the irregular dispersion of salt and water.

Very probably salted butter can contain water droplets that have little or no salt in them. Approximately 0.2 mg. sample of butter contains so many water droplets that some droplets containing no salt may be included without the salt content of the entire sample being appreciably reduced. The ideal type of analysis for various bacterio-

logical studies would be one permitting the determination of the salt contents of individual small water droplets. It is logical to assume that any moisture that is free of salt consists of droplets that were included in the butter granules during the churning process, rather than the moisture added to the butter later. This relationship is suggested by the fact that when organisms causing defects in butter were added to the wash water no defects were produced in the butter in which the salt was either well distributed or poorly distributed, whereas when organisms were added to the cream defects often appeared, especially in the butter in which the salt was poorly distributed.

Although the incorporation of moisture in salted butter undoubtedly has an effect on the bacterial action, it is somewhat different than the effect in unsalted butter. Usually the large moisture droplets in salted butter contain enough salt to prevent the growth of most bacteria. However, if the salt is poorly distributed, the butter probably can contain relatively large moisture droplets having little or no salt and in these sufficient growth of bacteria may occur to produce a defect in the butter. As in unsalted butter, increased working tends to prevent bacterial action due to the finer dispersion of moisture and there is also a better distribution of salt.

While the methods of manufacturing butter should be such that the harmful bacteria in cream are killed and any

serious contamination of the pasteurized cream or butter prevented, the thorough distribution of salt and moisture in the manufacture of butter should be regarded as a precautionary measure against defects caused by bacterial action.

SUMMARY AND CONCLUSIONS

1. A micro procedure was developed for the determination of salt in samples of butter of approximately 0.2 mg. each. The method is especially applicable to the study of the effect of salt distribution on bacterial action in butter. Results obtained with the method on both commercial and experimental churningsled to the following conclusions:

a. In some samples of normal commercial butter the salt was very uniformly distributed, while in other samples the salt was not uniformly distributed. With most samples a correlation was noted between salt distribution and incorporation of the moisture.

b. With normal commercial churning no significant differences in salt distribution were noted between the butter before and after printing with equipment which tended to rework the butter.

c. With commercial churnings it was evident that as the working process continued, the salt became more uniformly distributed.

d. In some samples of putrid commercial butter the salt was very unevenly distributed, while in other samples the salt was very uniformly distributed.

e. In mottled commercial butter the light-colored portions usually contained less salt than the dark-colored

portions. In a sample of commercial butter having a conspicuous color defect that was not typical mottling, the light-colored portions generally contained more salt than the dark-colored portions.

f. Much larger variations occurred in the moisture contents than in the salt contents of the micro samples of butter. Also, much larger variations occurred in the salt contents of the serum in the micro samples than in those of the large moisture droplets. The salt contents of the serum calculated from the micro analyses were slightly lower than the value calculated from the macro analyses. The salt contents of the large moisture droplets usually were higher than those of the serum calculated either from the micro or the macro analyses.

2. The addition of 1.5 per cent salt greatly inhibited bacterial action in butter held at 15.5°C. The greatest bacterial inhibition usually occurred in the butter having the salt well distributed. The greatest activity of microorganisms occurred in the poorly worked unsalted butter.

3. The growth of various organisms in butter churned from inoculated pasteurized cream and held at 15.5°C. was influenced by the distribution of salt in the butter, the lowest bacterial counts being obtained on the butter having the salt well distributed. In the unsalted butter, growth was most rapid and the numbers of organisms were the highest

in the poorly worked butter.

4. As determined by an adaption of the Burri smear culture technique, the numbers of organisms were lower and the organisms were more uniformly distributed in butter in which the salt was well distributed than in butter in which the salt was poorly distributed. Many of the agar slopes prepared by smearing portions of the large moisture droplets appearing on the freshly cut surfaces of butter contained no growth, while on a few slopes the colonies were too numerous to count.

5. In some comparisons the pH of the serum of butter churned from cream inoculated with lactic acid-producing streptococci and held at 15.5°C. decreased more in butter with the salt poorly distributed than in butter with the salt well distributed, but in other comparisons there were no significant differences. In the unsalted butter the largest decreases in the pH of the serum usually occurred in the poorly worked butter.

6. When lots of pasteurized cream, each inoculated with an organism capable of producing a defect in butter, were churned and the butter held at 15.5°C., the butter having the salt well distributed usually did not develop defects, while butter having the salt poorly distributed frequently developed defects. Both the thoroughly and

poorly worked samples of unsalted butter developed defects, the defect commonly appearing earlier in the poorly worked butter.

7. When butter was churned from lots of pasteurized cream and cultures of Ps. putrefaciens added either to the cream or to the wash water, the butter having the salt well distributed did not develop defects, whereas the butter having the salt poorly distributed developed defects in some samples in which the organisms were added to the cream, but not in the samples in which the organisms were added to the wash water. The unsalted butter usually developed a putrid flavor regardless of whether the organisms were added to the cream or to the wash water; no significant differences were noted between the two methods of inoculation.

8. When organisms capable of producing defects were added to butter churned from pasteurized cream, a greater inhibition of the activity of micro-organisms occurred in the butter having the salt well distributed than in the butter having the salt poorly distributed.

9. In some samples the reworking of salted butter containing organisms capable of producing defects tended to favor the development of the defects, while in other samples the reworking had no effect on the deterioration of the butter or even tended to inhibit the bacterial action.

10. The distribution of salt in butter on a micro basis should be considered a factor that definitely influences the action of micro-organisms in butter; the more thoroughly the salt is distributed the greater is the inhibitory effect of the salt.

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