FACTORS INFLUENCING BACTERIAL GROWTH IN BUTTER[1]

B. W. HAMMER AND H. F. LONG

Iowa State College, Ames, Iowa

From the standpoint of suitability for bacterial growth, butter differs from other dairy products. In butter the water is not the continuous phase, as it is in milk and cream, but is dispersed throughout the fat as small droplets. The fat, which makes up approximately 80 per cent of butter, is relatively resistant to bacterial action, although certain species may attack it. With the water droplets walled off by fat, bacteria cannot migrate from one droplet to another, and for the most part growth is restricted to the droplets originally infected. The water in butter contains different food materials, including proteins, lactose and salts, that are satisfactory for growth of various bacteria. However, in the case of salted butter the water also contains added sodium chloride which delays or prevents growth of many organisms. With salted butter the salt content varies widely, and actually there is less difference in salt content between unsalted and lightly salted butter than between lightly and heavily salted butter.

The presence of water and materials in solution or suspension in it makes butter much more susceptible to bacterial action than are various edible fats and oils that contain very little water and practically no food elements other than fat. Even with its susceptibility to bacterial action, butter commonly is expected to keep well, although at times it is subjected to comparatively high temperatures for rather extended periods.

Butter regularly contains bacteria arising from various sources, such as the cream, equipment, water used to wash the butter, air, employees, cultures added to give the butter a desirable flavor

[1] Journal Paper No. J-263 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 119.

and such miscellaneous sources as salt, packaging materials, etc.; with rare exceptions the cream is pasteurized, but some survival of bacteria is to be expected with the usual pasteurization procedures. Many of the bacteria from these various sources grow in butter unless prevented by the holding temperature, salt concentration or some comparable factor. Growth of certain of the organisms results in flavor defects in the butter. For the most part the organisms responsible for these defects are easily destroyed by heat, and they come commonly from contaminated equipment, with which the cream or butter comes in contact following the pasteurization, or from defective water supplies.

PHYSICAL CONDITION

The water in butter is in the form of small droplets and it has been recognized that this is a significant factor in bacterial growth in butter. In all probability the situation is different with bacteria than with yeasts and molds.

In an extensive study of mottled butter, Storch (104) found that the water therein consists of a very large number of finely divided droplets. Hunziker and Hosman (42) confirmed this report and noted that addition of salt causes a reduction in number and an increase in size of the water droplets, thus giving the butter a deeper yellow color than it had before salting. Boysen (4) reported that the numbers of water droplets in butter usually range from about 11 to 18 billion per ml.; working the butter increases the number of small droplets and decreases their tendency to aggregate, while salting decreases the number of small droplets and increases their tendency to aggregate.

The effect of the distribution of water in butter on growth of bacteria was considered by Rahn and Boysen (82). Since the numbers of water droplets in butter approximate 10 to 18 billion per g., while the numbers of bacteria are always much less than these values, many of the droplets must be sterile. The volume of water that is free of bacteria depends on two factors, the number of bacteria in the cream at the time of churning and the degree of water dispersion in the butter. As evidence of lessened bacterial activity with increased working, it was found that acid

production in butter serum was less with over-worked than with normally worked butter. It was suggested that the thorough working of butter improves the keeping quality.

Hammer and Hussong (32) noted that all the nutrients in butter are not available for bacterial growth. When the serum of unsalted butter was separated from the fat, development of bacteria was more rapid at either 7 or 21 C. than when the product was in the normal physical condition. Collins and Hammer (8) reported that migration of bacteria through butter is not common, and when it does occur poorly worked butter is usually involved.

Using an adaptation of Burri's smear-culture technic, Long and Hammer (54) examined very small portions of butter for their bacterial contents. An irregular distribution of the bacteria was noted, and in many instances the variations were extreme. This was considered additional evidence that bacterial development in butter is largely limited to certain focal points, probably infected water droplets.

Knudsen and Jensen (48) demonstrated that bacterial activity in butter decreases as the working increases; they believed that working tends to decrease the size of the water droplets, thus reducing the nutrients available for growth. Later, they (49) noted that after storage butter with many small water droplets was more satisfactory than butter with large droplets. Conditions which tended to give large droplets, such as addition of salt and churning of high-acid cream, resulted in butter of poor keeping quality. For estimating the number and size of free water droplets in butter, a rapid method employing indicator paper has been proposed by Knudsen and Sörensen (50).

Scharp (94) concluded that, as the working of commercial unsalted butter is increased, the number of organisms after any holding period decreases; this was attributed to finer dispersion of the water. As judged by rate of growth, production of defects, production of acid by butter culture organisms and production of fatty acids by lipolytic organisms, Long and Hammer (55) found that bacteria were more active in under-worked than in thoroughly worked butter. Pont (74) also emphasized that the

physical structure exerts considerable influence on bacterial activity in butter.

The effect of the physical condition of butter on development of the putrid defect has been investigated in some detail. Cullity and Griffin (9) and also Pont (75) noted that thorough working of butter retarded the appearance of the defect. Itzerott (43) observed that the rabbito defect occurred most frequently and developed most rapidly in butter showing an open texture and free moisture. Conversely, Totman, *et al.* (105) found no convincing evidence that the amount of working which butter receives has an appreciable effect on the keeping quality; they stated that very seldom is butter worked so little that the water and salt are not sufficiently distributed to provide the benefit of the antiseptic action of salt.

Since the amount of working that butter receives at the time of manufacture has an effect on growth of bacteria in it, working subsequent to manufacture also would be expected to affect the growth. Prucha and Brannon (78) found that butter which had gone through a printing machine having a crushing action did not keep as well as butter not so treated; in one case a decided off flavor quickly developed in the printed butter, while the unprinted butter did not develop the defect. Gratz (24) observed that bacterial growth was greater in the outer layers of butter than in the interior and emphasized the importance of this in the lipolysis of reworked butter. In several comparisons involving butter reworked with and without the outer layer removed, it was found that fat hydrolysis was more pronounced without the removal. Probably redistribution of organisms through the butter by the reworking was a factor of significance. Cullity and Griffin (9) reported cases in which the reworking of apparently sound butter rapidly resulted in the putrid defect. Turgasen (106) cited instances in which butter printed in a machine having a reworking effect became cheesy, while unprinted butter from the same churning kept satisfactorily.

Long and Hammer (56) concluded that the reworking of unsalted butter, made from pasteurized cream inoculated with various organisms, frequently increased the activity of the organisms at 10 C., as evidenced by growth rates, production of defects,

decreases in pH values of the butter serum when butter cultures were added to the cream and increases in acidities of the fat when lipolytic organisms were used. Apparently redistribution of organisms and aggregation of water and the nutrients contained in it were primarily responsible for the increased activity. In some instances the reworking of salted butter containing organisms capable of producing defects has tended to favor development of the defects, while in other instances reworking has had no effect on deterioration or even tended to inhibit bacterial action (36).

Results of various investigations indicate that the physical condition of butter has a marked influence on growth of bacteria in it. With unsalted butter there appears to be a definite relationship between the amount of working that the butter receives and bacterial growth; with salted butter the same general relationship probably obtains, although the dispersion of the water may be affected somewhat by the presence of salt. The reworking of butter under certain conditions tends to accelerate bacterial growth at favorable temperatures.

TEMPERATURE

Just as it influences the action of bacteria in various food products, the temperature at which butter is held would be expected to influence the activity of bacteria therein. Low temperature constitutes one of the important factors in the control of both bacterial and chemical deterioration of butter.

Bacterial changes in butter held below 0 C., where little change would be expected, have been repeatedly investigated. Sayer, *et al.* (93) found that low temperatures entirely checked the growth of many species, while others grew only slowly. It was noted that salt lowers the freezing point considerably and thus may interfere somewhat with the effect of low temperatures. In certain samples the freezing point of the brine was −22 C. Later Rahn, *et al.* (83) reported that some microörganisms multiplied in salted butter at −6 C.; moreover, salted butter kept better than unsalted above the freezing point as well as below.

In salted butter made from raw and pasteurized cream plus

butter culture, Rogers and Gray (89) found that the numbers of bacteria gradually decreased at both −23.3 and −12.2 C., the decrease being slightly more rapid at the higher temperature. Deterioration of high-acid butter at low temperatures was not attributed to microbiological action.

Brown, *et al.* (6) investigated changes in bacterial numbers in salted butter held at −17.8 C. for 428 days and found that relatively high bacterial counts persisted in butter over 1 year old. However, during the first 9 days of storage there was a rapid decrease in bacteria after which the decrease was more gradual. Lactic acid organisms persisted as long as 275 days and in one case for 426 days. It was suggested that low temperatures and salt decreased the rate of metabolism.

Grimes (25) stored salted butter, made from pasteurized sweet cream plus 10 per cent butter culture with and without ripening, at −21.1 C. for 6 to 7 months. In the butter made from ripened cream, 95 to 99 per cent of the bacteria died during the holding. In the sweet-cream butter, the average decrease in numbers of bacteria was 20 per cent, the maximum being 65 per cent; some lots showed no decrease. When proteolytic bacteria were added to either type of cream before churning, they were not a significant factor in the deterioration of the butter.

Arup and Gilmour (2) held salted butter made from unripened pasteurized cream at −7 C. for 6 months. Bacterial counts every 4 weeks indicated that little or no bacterial growth took place, nor was there any considerable reduction in counts. The temperature of storage was sufficient to stop bacterial action regardless of the extent of infection or of the moisture, acid, curd, iron or copper contents. Later, Arup and Gilmour (3) found that bacterial counts decreased in each of three lots of salted butter held at −12, −6, and −2 C.

Nelson and Hammer (67) stored butter at −20 C. following 7 days at 21 C. There were decreases in bacterial counts whether the butter was salted or unsalted, the decreases averaging 64 per cent with salt and 74 per cent without salt. In both types of butter the largest decreases in counts occurred with the samples having the highest counts on entering storage.

Changes in the flora of salted sweet-cream butter held at −9.4 C. for 2 to 8 months were investigated by Grimes and Hennerty (26). In general, increases in total counts (bacteria and yeasts) occurred. The increases were attributed to the growth of yeasts rather than of bacteria. There was neither a definite increase nor decrease in numbers of gelatin-liquefying bacteria during the storage.

Loftus-Hills, *et al.* (53) found only slight changes in bacterial numbers during storage of salted butter at −11.1 C. for 3 months. In general, lactose-agar plate-counts decreased, while gelatin plate-counts increased. During the storage *Escherichia-Aerobacter* organisms, acid-producers and gas-formers disappeared in some samples but appeared in others.

Changes in bacterial numbers in salted and unsalted butter held at −25 C. were compared by Jacobsen (45) who reported marked reduction in numbers with both types of butter. Apparently freezing destroyed the small numbers of proteolytic and lipolytic bacteria originally present. Turgasen (106) investigated the effect of extended holding of butter at −28.9 C. on the activity of organisms causing cheesiness; samples of infected print butter held for 6 months developed the defect when removed from storage and placed at 21.1 C.

At 0 C. or above, changes in bacterial numbers depend considerably upon factors other than temperature. Some investigators have reported decreases in this temperature range, while others have reported increases. In salted butter made from raw and pasteurized cream plus butter culture and held at 0 C., Rogers and Gray (89) noted decreases in bacteria. In salted butter held at 0 to 10 C. for 37 days, or at 35 C. for about 1 month Lafar (52) also obtained decreases; at room temperature the bacteria increased rapidly at first and then began to die, leaving the butter rancid and tallowy. Wilson and Prucha (113) found that 3 weeks at 18.3 C. almost completely eliminated acid-forming organisms from salted butter, leaving alkali-formers and an inert group; the flora of butter stored at 4.4 C. tended to remain the same as that of the fresh butter.

Except for two organisms, Fennema (16) found that a tempera-

ture of 0 to 10 C. largely prevented growth of the cultures employed in salted and unsalted butter made from sterilized cream; *Serratia marcescens* and an organism producing fishiness showed some slight growth in salted butter during the first few days of holding. Guthrie, *et al.* (29) investigated the changes in salted and unsalted butter held at 5, 10 and 24 C. and found that all samples deteriorated more rapidly at the higher holding temperatures.

In unsalted butter held at 21 C., Hammer and Hussong (32) noted rapid increases in numbers of bacteria, while at 7 C. there also were increases but they were much less rapid than at 21 C. In unsalted butter stored at 0 C., Macy, *et al.* (61) found that bacterial counts usually were higher after 1 or 9 months than originally. The increases were more frequent and marked in the butter held 1 month; usually the maximum count was reached in about 10 days, after which there was a gradual decrease. In salted butter a large percentage of the samples had decreased in bacterial count after 1 or 9 months. The unsalted butter frequently developed defects, while the quality of the salted butter remained reasonably satisfactory. Shepard (96) reported that bacterial development in unsalted butter held 14 days was more rapid and extensive at 21 C. than at 0 C.

According to Garrison (21), fluorescent bacteria in unsalted butter survived 6 months at 1 to 3 C. Slightly less than half the cultures studied produced flavor defects at these temperatures.

Itzerott (43) suggested that temperature is the greatest factor influencing development of the rabbito defect. At temperatures between 15.6 and 32.2 C. the defect developed in 2 to 4 days, while at temperatures below 12.8 C. development of the defect was retarded.

Changes in flavor of butter under commercial cold-storage conditions (-10 C. or lower) appear entirely uncorrelated with microbiological activity in all types of butter, according to Pont (74). In general, no convincing evidence of biological activity in butter under these conditions has been produced and, from theoretical considerations alone, none would be expected. At temperatures above 0 C. marked degradation of quality may be

brought about by bacterial development. In this respect, particular organisms or groups of organisms appear of more significance than total bacterial populations. Pont emphasized that under commercial conditions, butter commonly is subjected to temperatures which permit bacterial development both before and after periods of cold storage. Normally, after manufacture at least 3 or 4 days elapse before the temperature of the butter falls below 5 C.; often it may be above 0 C. for 10 days or more before the butter is consigned to storage. After storage, temperatures above 0 C. again are used to permit grading, printing and disposal on the retail market.

The data indicate that growth of bacteria in butter is largely prevented by temperatures of 0 C. or below and that some destruction of bacteria may result. However, it is possible that in salted butter growth of certain bacteria may occur at temperatures slightly below 0 C. due to depression of the freezing point by the salt; such growth is limited both by the temperature and the salt. At temperatures above 0 C. bacterial development may be rapid, especially in unsalted butter.

SALT

The retarding effect of salt on the activity of bacteria in butter is generally recognized and extensively used in butter manufacture, although some butter is made without salt. Amounts of salt used in butter vary widely (from a fraction of 1 per cent to about 4 per cent) depending on the market for which the butter is intended. Unsalted butter is of two principal types, that made with low churning acidities for use in reconstituting cream, etc. and that made with high churning acidities so that it will have considerable flavor.

General observations. Lafar (52) reported that salt had a definite bactericidal action in butter held at 0 C. for about 30 days; light salting (0.5 to 1.0 per cent) seemed as effective as heavier salting at both 0 and 35 C.

In two lots of butter, one salted and one unsalted, held at 0 to 3.3 C., Loveland and Watson (58) found that the numbers of

bacteria gradually diminished as the butter aged, the decrease being much greater in the salted butter than in the unsalted. There was a more rapid decrease during the first few hours of holding than later. Bacterial counts on the fresh butter were extremely high, being 115,302,000 per g. for the unsalted butter and 54,170,000 per g. for the salted butter.

Fettick (17) stored salted and unsalted butter in a refrigerator, which varied in temperature from 0 to 18 C., for 5 months. During this period the numbers of bacteria in the unsalted butter were higher than in the salted at each examination. In the unsalted butter the numbers were still increasing at the end of the holding, while in the salted butter the numbers increased for about 1 month and then did not change materially although some fluctuation was noted. Fettick believed that butter with 2 or 3 per cent salt keeps better than that with higher percentages, the reason being that the lactic acid bacteria can tolerate the lower amounts but not the higher; if the lactic acid bacteria are killed by high salt content, other types which are not salt-resistant may grow and produce undesirable changes.

Rahn, *et al.* (83) found that salted butter kept better than unsalted, both above and below 0 C. They also noted that there are microörganisms in butter which can multiply in salted butter at −6 C.; whether these organisms could cause deterioration was not determined.

McKay and Larsen (63) concluded that salt improves the keeping quality of butter. Later, they (65) stated that, so far as the keeping quality is concerned, it would be advisable to salt butter as heavily as 6 per cent. Such salting would tend to check deterioration due to bacteria. Fennema (16) concluded that salt is a very important factor in butter preservation; he found that salt not only checks bacterial growth but quickly decreases the numbers of organisms in the butter.

The Minnesota Agricultural Experiment Station (66) reported that salt affects butter by both the direct chemical and the indirect biological routes. At temperatures limiting bacterial growth, the effect of salt is to deteriorate butter; under conditions favoring bacterial growth, salt tends to inhibit such growth and

preserve butter. Unsalted butter kept better than salted at −12.2 C.

Bacteriological changes in salted and in unsalted butter held at −26 C. were compared by Washburn and Dahlberg (108). After 113 days every sample of unsalted butter contained more bacteria than its salted duplicate, and similar, though less uniform results, were obtained after 284 days. Low temperature appeared to be so important in preventing bacterial development that the antiseptic property of the salt played a minor role. *Streptococcus lactis* withstood the adverse conditions better than other organisms.

Weigmann (109) recognized that salt is a common, but by no means powerful, preservative for butter and butter-like substances. He observed that unsalted butter, when not made with special precautions, contains more microörganisms than salted butter and that the latter possesses the better keeping quality. Spitzer, *et al.* (103) studied changes in the flora of stored butter; the number of butter samples showing bacterial increases during storage decreased as the salt concentration in the brine increased. Later, Spitzer and Parfitt (102) reported the same results and also that proteolytic bacteria were inhibited least by the salt in butter.

Macy (60) noted a general tendency for salted butter to decrease in bacterial count during storage at 0 to 1.7 C. for 1 to 9 months, while unsalted butter showed an increase. There was no greater tendency for the more highly salted samples to decrease in count than for those of lower salt content; this was explained by the fact that the majority of organisms inhibited by salt are inhibited by relatively small amounts. When the data were considered from the standpoint of concentration of salt in the brine, instead of in the butter, essentially identical results were obtained, although there was a slight indication that more concentrated brines were somewhat more inhibitory.

Hammer and Hussong (32) reported that salted butter held at either 7 or 21 C. tended to decrease in bacterial content. Horovitz-Vlasova, *et al.* (39) concluded that salting efficiently prevents bacterial growth in butter if the cream is salted before churning, but its power is limited by the low solubility of salt in butterfat.

Loftus-Hills, *et al.* (53) stored butter at −11.1 C. for 3 months and found no relationship between salt content or brine concentration and keeping quality of butter, or between brine concentration and bacterial increase or decrease.

Changes in the flora of salted and unsalted butters made from sweet cream plus 8 per cent butter culture and held at 0 C. for 5 to 19 weeks were studied by Shepard (96). In the salted butter a gradual decrease in bacteria occurred, while in the unsalted there was an extensive increase.

Effect of salt on development of defects and on growth of organisms. The ability of salt to retard appearance of butter defects caused by microörganisms has been studied by various investigators. The effect of salt on organisms encountered in butter, but not normally capable of producing defects, also has been considered.

Schmidt (95) found that salt retarded the development of rancidity in butter but was less effective than pasteurization of the cream used for the butter. The best keeping quality was obtained through a combination of salting and pasteurization. Orla-Jensen (68) washed butter containing *Pseudomonas fluorescens*[2] with 25 per cent aqueous salt solution, so that the salt content of the butter was 2.9 per cent, and found the organism almost completely inhibited. He noted that the butter contained 13.4 per cent water, which resulted in a 21.6 per cent brine, a concentration sufficient to prevent growth of almost all bacteria.

According to Giltner and Baker (23), microörganisms which liquefy casein and gelatin are more easily affected by salt than some non-liquefiers. Virtanen (107) reported that fermented, cheesy, putrid and rank flavors in butter generally are caused by organisms that are often of the water type and generally inhibited by salt.

The percentages of salt in surface taint butter agree very closely with the percentages in normal butter, according to Hood and White (38). Surface taint was noted in butter with as high

[2] The name "*Bacterium fluorescens liquefaciens*" has been used by different investigators. It is assumed to refer to the organism now commonly designated *Pseudomonas fluorescens* although additional classification studies on the fluorescent *Pseudomonas* organisms may change this idea.

as 2.67 per cent salt, equal to a brine concentration of 15.37 per cent. They questioned the control of the responsible organisms by high salting since many markets demand butter with less than 2.67 per cent salt.

The effect of salt on development of surface taint at 15.6 C. was investigated by Derby and Hammer (10) who found that unsalted and low-salted butter made from cream inoculated with defective butter developed surface taint in a short time, while medium-salted butter remained normal during the 7-day holding period. Plate and direct counts after 2 and 7 days were higher on the defective butter than on the normal butter. When *Pseudomonas putrefaciens* was used for inoculation, unsalted butter and low-salted butter were putrid in 4 days, while medium-salted butter was still normal after 20 days. Claydon and Hammer (7) indicated that salt tends to prevent the putrid defect in butter but noted that many lots of commercial salted butter become putrid. In studies on cheesy butter, Turgasen (106) found that as much as 4 per cent salt sometimes did not prevent the condition. Pont (75) emphasized the value of heavy salting in controlling the putrid defect.

Itzerott (43) reported that salt had a greater effect than acidity in checking growth of organisms causing the putrid condition in butter; a salt content of 1.7 per cent gave some protection. Long and Hammer (57) noted that in experimental butter containing 2.5 per cent salt and held at 3 and 21 C. growth of *P. putrefaciens* was inhibited; after a slight increase in numbers early in the incubation period a decrease occurred. Salting and working prevented appearance of a definite defect. In unsalted butter growth was rapid at 3 and 21 C., and the putrid defect developed rapidly.

In butter containing 2 per cent salt and held at 21 C., Nelson and Hammer (67) found that butter-culture streptococci developed little or not at all, while in unsalted butter the organisms developed extensively. Jacobsen (44) reported that 2.5 per cent salt in butter prevented growth of lipolytic and proteolytic bacteria at −25, 21 C. and temperatures between.

Rice (85) noted that in salted butter at −10 C. *Escherichia-*

Aerobacter organisms persisted for 8 weeks. At 15.6 C. the organisms lived for considerable periods, but their reproduction was almost suspended; after 21 days there was a slight increase in some samples and a slight decrease in others. However, other microörganisms, especially chromogenic micrococci, increased in numbers. In unsalted butter at 15.6 C., *Escherichia-Aerobacter* organisms increased very rapidly.

White (111) investigated a black discoloration of salted butter and isolated the causative organism which he named *Pseudomonas nigrifaciens*; it is probably identical with an organism described by Hiscox (35). The salting of butter with at least 1.25 per cent salt, to give approximately 7 per cent salt in the serum, was suggested as a control measure.

Hammer and Olson (33) reported that organisms which actively produced phosphatase in milk also rapidly produced it in unsalted butter; these included *P. putrefaciens*, *P. nigrifaciens*, *Pseudomonas mephitica* and *Flavobacterium fecale*. In general, when the butter was salted, production of phosphatase was less rapid and less extensive than in the corresponding unsalted butter but was still definite with various organisms.

Effect of salt on activity of organisms in bacteriological media. Investigators have determined the salt tolerance of different organisms by growing them in liquid media or on solid media containing known salt percentages. While many of the organisms studied are not of importance in butter, a rather exact knowledge of the salt tolerance of various organisms or groups of organisms is useful in considering the inhibitory effect of different brine concentrations in butter.

McKay and Larsen (64) studied the effect of salt on a spore-forming organism and a gas-forming organism isolated from butter. In a medium containing 4 per cent salt both grew, while in a medium containing 6 per cent salt neither grew.

Pettersson (71) reported that the rod-shaped bacteria investigated did not grow in bouillon containing more than 10 to 12 per cent salt, while most cocci grew very well with 15 per cent salt present. Putrefying bacteria were more sensitive to salt than other types. The inhibitory action of salt was especially pronounced at concentrations of 20 to 25 per cent.

The effects of salt on various organisms often found in butter were studied by Fettick (17) who indicated that the following types were quite sensitive to salt: Lactic acid bacteria, *P. fluorescens*, *Bacterium fragariae* (probably *Pseudomonas fragi*), *Alcaligenes viscosus* and *S. marcescens*. Organisms that were quite resistant to salt included *Escherichia coli*, *Aerobacter aerogenes* and certain spore-formers.

Brown (5) studied the salt tolerances of organisms isolated from butter held in storage at −19.4 to −16.1 C. Twenty-four of 57 cultures of bacteria grew on a 12 per cent salt medium at 20 C., and four of these grew well on the medium at 6 C. The ratio of liquefying to non-liquefying cultures was much the same whether the bacteria were isolated on ordinary agar or on agar containing 12 per cent salt.

Giltner and Baker (23) investigated the effect of salt on the flora of butter and found that 8 per cent retarded the physiological processes of most organisms, although concentrations of 12 to 20 per cent did not inhibit all growth; streptococci were sensitive to salt, while micrococci and staphylococci tolerated high percentages.

Gubitz (27) noted that *P. fluorescens* grew poorly in media containing 5 per cent salt. Of two gram negative, fluorescent, gelatin-liquefying bacteria studied by Henneberg (34), one tolerated 7.5 per cent salt, while the other tolerated only 5 per cent. Henneberg indicated that salt is one of the factors tending to protect the fat and protein of butter from the action of *P. fluorescens*. Of twelve species of the *Alcaligenes* group, one tolerated 15 per cent salt, four 10 per cent, five 5 per cent and two 2.5 per cent; of five species of streptococci, three tolerated 5 per cent salt and two 2.5 per cent; of eight species of micrococci, seven tolerated 15 per cent salt and one 2.5 per cent; and of three species of the *Proteus* group, one tolerated 15 per cent salt and two 10 per cent.

The salt tolerances of some lipolytic bacteria in bouillons containing various percentages of salt were investigated by Hammer and Collins (31). Twenty-three cultures representing the following species were studied: *Achromobacter connii*, *Achromobacter lipolyticum*, *Pseudomonas acidiconcoquens*, *P. fluorescens*, *P. fragi*,

Pseudomonas mucidolens, Pseudomonas schuylkilliensis, Pseudomonas synxantha and an unidentified species of *Micrococcus*. Nineteen of the cultures grew in 5 per cent salt bouillon, twelve in 6.25 per cent, two in 7.5 per cent, while one (the micrococcus) grew in 12 per cent.

Hof (37) studied the salt tolerances of organisms by inoculating garden soil into enrichment media containing various percentages of salt. In this manner cultures of lactic acid and *Escherichia-Aerobacter* organisms were obtained in media containing 6 per cent salt, while butyric acid, urea and proteolytic bacteria were obtained in media containing 24 per cent salt. Cultures from the enrichment media tolerated higher percentages of salt than laboratory cultures of the same species.

Kanunnikowa (47) reported that 1 per cent salt in a medium containing 4 per cent butter favored growth of *S. marcescens* and inhibited growth of *P. fluorescens*. Higher salt concentrations retarded the decomposition of butter. Salt retarded the formation and activity of bacterial proteases and lipases.

The organism which Hiscox (35) found responsible for dark discoloration in salted butter required 1 to 2 per cent salt for growth in laboratory media but was inhibited by 5 per cent. White (111) reported that *P. nigrifaciens* grew well in laboratory media containing 1.5 per cent salt but not at all in media containing no salt; growth was decreased by 7.5 per cent salt and was extremely slight with 10 per cent.

Garrard and Lochhead (20) indicated that gram negative micrococci were less salt tolerant than gram positive micrococci. There was little difference between gram negative and gram positive rods, considered as groups. Many organisms isolated on 5 per cent salt agar from a salt free environment tolerated relatively high salt concentrations, over one-half growing in media containing 0 to 15 per cent salt and one-fifth growing in media containing up to 20 per cent; 25 per cent salt was definitely inhibitory to organisms isolated on a 5 per cent salt medium.

Garrison (21) found that addition of 6 per cent salt to beef-extract peptone broth prevented growth of some cultures of fluorescent bacteria but not of others; only a few cultures grew

in broth containing 8 per cent salt. In experimental unsalted butter made without butter culture nearly all the organisms studied produced off flavors at 21 C. Addition of 2 per cent salt to the butter prevented development of off flavors by some cultures but not by others.

Long and Hammer (57) reported that *P. putrefaciens* grew in milk containing 4 per cent salt but not in milk containing 10 per cent; with 6 or 8 per cent salt, some cultures of the organism grew and some did not.

Salt distribution. Rahn (81) observed that not all water droplets in butter have the same salt content since salt is added after washing when many small droplets are already enclosed in fat. He suggested that this probably is of great importance from the standpoint of the growth of microorganisms in salted butter. Weigmann (110) emphasized the same ideas.

Claydon and Hammer (7) concluded that salt is not entirely effective in inhibiting the putrid defect in butter unless combined with thorough working of the butter. When butter containing a pure culture of *P. putrefaciens* was worked only slightly, neither 1 nor 2 per cent salt prevented the defect; with thorough working either amount controlled it.

Hoecker (36) used a micro procedure for determining the salt in approximately 0.2 mg. portions of butter. With both normal and abnormal commerical butter, some churnings had the salt very uniformly distributed while others had it poorly distributed. With most churnings there was a correlation between salt distribution and incorporation of water. As the working process continued, the salt became more uniformly distributed. Printing butter in equipment having a reworking action did not significantly affect the salt distribution.

Salt concentration necessary to inhibit bacteria. Orla-Jensen (68) reported that the development of microörganisms in butter is completely inhibited only when the concentration of salt in the brine reaches 25 per cent, for example with 13 per cent water and 3.3 per cent salt. Later, he (70) observed that the preserving action of salt is the more pronounced the lower the percentage of water in the butter, and he again pointed out that a relatively

high concentration of salt in the brine is essential for preventing growth of all bacteria. Weigmann (109) likewise indicated that the percentage of salt necessary to preserve butter depends on the water content. He (110) also noted that research on the effect of salt on microörganisms is not abundant and does not always agree. He suggested that salt percentages which are toxic for some species may be stimulatory for others; thus lactic acid bacteria might be killed by a salt percentage which would stimulate undesirable species. Kretchmar (51) stated that, according to its concentration in solution, the action of salt on bacteria may vary, a 0.5 molar concentration being stimulatory and a 3.0 molar concentration being inhibitory. In addition, salt widens the pH range of media within which bacteria will grow.

Pont (74) indicated that the concentration of salt in the moisture of butter may play a large part in controlling the activity of microörganisms; he also noted that in many experiments these concentrations have not been given and their effect on microbiological changes has not been emphasized.

Hammer (30) pointed out that the influence of salt varies with the species present in butter and suggested that this is an important factor in determining whether bacterial increases occur.

Relationship of initial contamination to salt effect. It is probable that bacterial growth in salted butter depends to some extent on the numbers of organisms initially present. Winslow, *et al.* (114) reported that in broth large numbers of organisms tend to neutralize the inhibitory effect of salt and stated that there always is a mass effect caused by large numbers of living or dead cells which tends to neutralize any inhibitory action. Various concentrations of *E. coli*, *Micrococcus albus* and *Bacillus mesentericus* were inoculated by Slemmons (99) into bouillons containing from 1 to 12 per cent salt. The larger inoculations of the organisms showed greater salt tolerance than the smaller inoculations. With *M. albus* an inoculation of 50 cells failed to grow in 4 per cent salt bouillon, whereas an inoculation of 50,000 cells grew in 11 per cent salt bouillon.

Adaptation to salt. In studying the effect of salt on the bacteria

in butter, Giltner and Baker (23) noted that the salt tolerance of some organisms could be increased by continued cultivation on salt-agar. Garrard and Lochhead (20), in working with pickle brine, found that various species of bacteria can adapt themselves somewhat to changes in salt concentration and suggested that some adjustment to the high salt content of pickle brine is possible. However, of 23 species investigated, all of which grew on a 5 per cent salt medium, none grew on a 10 per cent salt medium. From this it was concluded that the action of 30 per cent salt involved a toxic effect, and there was no adjustment to the high salt concentration. It was noted that various organisms displayed more resistance to salt in pickle brine than in broth of similar salt content. Of 15 species inhibited after 5 days in 30 per cent salt broth and 22 species inhibited after 10 days, only 5 and 6 species, respectively, were inhibited in pickle brine of almost identical salt content.

The available data show that salt tends to retard bacterial growth in butter and thus to delay or prevent bacterial deterioration. The important factors influencing the action of salt in butter appear to be concentration in the water and uniformity of distribution throughout the water droplets. Even when considerable salt is added to butter the concentration in the water may not be sufficient to inhibit all bacterial growth, and with light salting the inhibition is much less. Many instances have been reported in which organisms known to be relatively sensitive to salt have produced conspicuous defects in salted butter. Probably there are various causes for this, the chief one being failure to thoroughly incorporate the salt; as a result, salt-sensitive bacteria can develop in those droplets of water which contain little or no salt. The present demand for lightly salted butter may account for some of the outbreaks of bacterial spoilage in butter, especially when combined with poor salt distribution. The extent of contamination doubtless is of considerable practical importance in this general connection, and the ability of certain organisms to adapt themselves to higher concentrations of salt than they normally encounter also may be a factor.

ACIDITY

The acid contents of different types of butter vary widely. Salted butter commonly has a low acidity because of the danger of serious chemical deterioration from a combination of salt and high acid; the danger is particularly great in butters with relatively high copper content. Unsalted butter made for reconstitution of cream, etc. has a low acidity because of the objectionable effect of high acidity on the flavor of the reconstituted products. On the other hand, unsalted butter that is intended to have a high flavor must contain considerable acid for proper flavor development. Since some bacteria are known to be sensitive to acid, the acidities of certain lots of butter would be expected to influence bacterial growth therein.

Orla-Jensen (68) inoculated butter with *P. fluorescens* and *S. lactis* and noted that the lactic acid formed by the latter organism inhibited fat hydrolysis. There was little free volatile acid produced, and the odor and taste were not particularly objectionable. The effect of acid production by *S. lactis* on the growth of *P. fluorescens* in milk was studied by Luxwolda (59) who found that both species appeared to profit by the association at 10, 13, or 15 C. Since the fluorescent organism lived in the acid medium, it was believed that the milk-souring bacteria produce something besides acid which hinders the growth of fluorescent organisms in sour milk.

Shutt (98) reported that churning cream at an acidity of not less than 0.35 per cent was beneficial in avoiding defects caused by *P. fluorescens* since the organism grows only feebly at a pH as low as 6.6. He indicated that surface taint, which he believed could be caused by *P. fluorescens*, occurs only in sweet-cream or neutralized-cream butter and never develops in sour-cream butter. However, Gubitz (27) found that in bacteriological media *P. fluorescens* grew at pH values as low as 5.4 to 5.8. Rahn (81) noted that the acidity of butter serum has an effect on bacterial growth in butter, but that in butter made from sour cream some organisms can grow which cannot grow in sour milk. For example, sour-cream butter often becomes rancid due to growth of *P. fluorescens* although this organism cannot grow in sour milk.

Rahn believed that removal of some of the acid from butter during washing of the granules lowers the acidity to a point where *P. fluorescens* can grow. He reported that other species, such as *E. coli*, *A. aerogenes*, yeasts and molds, tolerate a rather highly acid medium.

The pH tolerances of 505 cultures of fluorescent bacteria were investigated by Garrison (21). In beef-extract peptone broth they all grew from pH 5.5 to pH 10.0, while many grew at pH 4.5 and four grew at pH 4.0. In skim milk adjusted to pH 5.0, the 10 cultures tested grew rather rapidly. The effect of butter culture on the ability of the organisms to produce defects in experimental butter was studied with 52 cultures; addition of 10 per cent butter culture to the cream prevented development of off flavors in unsalted butter by 16 of the cultures and in salted butter by 27 of them.

Rogers (86) reported that during cream ripening the lactose is partly fermented to lactic and similar acids which protect the butter from fermentation by less acid-tolerant bacteria. He noted that putrefactive bacteria, which often attack the curd of butter, are usually checked by acid.

Mazé (62) pointed out that the bacteria causing deterioration in butter attack the casein and lactose and that these organisms are retarded by lactic acid. Addition of lactic acid to the finished butter in the proportion of 0.5 to 1 g. per liter was advocated as a control measure, but this was not believed to completely prevent growth.

In a study of butter obtained in the Boston market, Rosenau, *et al.* (90) found no relationship between numbers of bacteria and reactions of the butter. Gilmour and Cruess-Callaghan (22) could detect no relationship between acidities of Irish Free State creamery butter and rates of growth of microörganisms as a group. Pont and Sutton (76) reported that the majority of samples of New South Wales butter examined had pH values between 7.0 and 7.7, the modal value being 7.4. Within experimental limits, no correlation was observed between pH values and bacterial counts. The data did not suggest that increased alkalinity of the serum favors development of proteolytic species.

Fennema (16) found in salted sour-cream butter stored at 0 to 10 C. a decrease in numbers of bacteria from the beginning of storage. The decrease was rapid for the first few days, then more gradual, until at the end of several months only a few organisms remained. More rapid decreases were encountered in samples originally containing large numbers of bacteria than in those originally containing small numbers. In salted sweet-cream butter there was a rapid increase during the first few days of storage and then a decrease.

Virtanen (107) indicated that bacteria of the water type, which often cause cheesy and putrid defects in butter, are inhibited by the acidity of sour-cream butter. In studies on butter spoilage Horovitz-Vlasova, *et al.* (39) noted that acidification inhibits the growth of putrefactive bacteria but may favor other organisms.

Wiley (112) concluded that at 4.4 and 18.3 C. butter deterioration caused by bacterial action was not delayed by the presence of acid. Jacobsen (44) found that at room temperature flavor deteriorations and increases in bacteria were more extensive in unsalted non-culture butter than in unsalted culture butter.

The effect of butter culture on development of surface taint in experimental butter was investigated by Derby and Hammer (10). When 10 per cent butter culture was added to pasteurized cream inoculated just before churning with surface taint butter or *P. putrefaciens*, development of a putrid condition was prevented in either salted or unsalted butter held at 15.6 C. *P. putrefaciens* developed in milk with acidities of 0.27, 0.28 and 0.29 per cent but not in milk with acidities of 0.30 and 0.31 per cent. Claydon and Hammer (7) studied the effect of pH on production of the putrid defect in unsalted butter by *P. putrefaciens* and concluded that the organism can cause the defect over a wide pH range. Butter made from cream with a pH of 4.5 did not spoil; that made from cream with pH values of 5.2 and 6.0 was slightly defective in 1 day at 21 C.; pH values from 6.0 to 7.8 allowed rapid growth of the organism in butter. The inhibitory effect of butter culture in unsalted butter also was investigated. At 21 C., 5 per cent butter culture in the cream prevented the defect and was as

effective as 12 per cent, whether the organism was added to the cream before churning or to the water used to wash the butter.

Pont (75) indicated that within the range of safe limits from the standpoint of chemical changes in butter, high acidities aided in minimizing the putrid defect. In the work of Itzerott (43) cream acidities below 0.15 per cent had little effect on the time required for development of the putrid defect in butter. Above 0.15 per cent, however, acidity appeared to have a retarding influence. High acidities in conjunction with low temperatures definitely inhibited the defect. Unsalted butter from cream with an acidity of 0.20 per cent and inoculated with rabbito organisms showed no evidence of the defect when held between 4.4 and 12.8 C. for 3 weeks. However, at higher temperatures (26.7 to 32.2 C.) the taint developed despite the high acidity.

Long and Hammer (57) investigated the resistance of *P. putrefaciens* to various amounts of lactic acid in skim milk. At a pH of approximately 5.3, the organism survived only a relatively short time, usually less than 48 hours. With pH values appreciably above 5.3, it multiplied in the acidified milk, and with values below 5.3 it was killed in less than 48 hours; in one lot of milk acidified to pH 4.9, *P. putrefaciens* was killed in 8 hours. The authors suggested that use of butter culture in making butter should have a protective action so far as the putrid defect is concerned.

In an investigation of the cheesy defect of butter, Turgasen (106) found that varying the acidity of the cream, so that the pH of the butter serum ranged from 5.4 to 7.8, did not control the spoilage; the source of infection of the butter was the wash water.

Rahn and Boysen (82) showed that in unsalted butter acid-producing bacteria develop more acid in unwashed butter than in washed butter. This could not be demonstrated in salted butter because of lack of acid production in the presence of salt. The authors indicated that the smallest water droplets in butter are entirely enclosed in the butter granules and do not come in contact with the wash water. From the outsides of the granules, however, the wash water removes all milky material since butter usually is washed until the water drains clear. The water in-

corporated during the washing and working processes forms the large droplets found in butter, making in effect two entirely different types of water droplets in butter. Since calculation showed that most bacteria are present in large droplets which are chiefly clear water, as compared to the small droplets, the result of washing is removal of nutrient materials. For this reason washing protects the keeping quality of the butter, provided the wash water is not contaminated. If the water contains lipolytic bacteria, thorough washing might be detrimental for there would not be enough lactose in the water to allow significant acid formation by the lactic acid organisms.

Hunziker (41) reported that formerly it was thought washing butter improved the keeping quality by removing bacterial food. However, this is not borne out in commerical practice. In cream ripening the cream is impregnated with lactic acid bacteria, lactic acid and its salts; these keep undesirable bacteria in check and butter from ripened cream has better keeping quality than that from unripened cream. Lightly washed, unsalted butter has better keeping quality than heavily washed, unsalted butter from the same cream.

Orla-Jensen (69) found that lactic-acid-producing rods multiplied much more rapidly in unwashed than in washed butter, whereas the reverse was true with lactic-acid-producing streptococci. Later, he (70) stated that the best method of preventing harmful organisms from developing to any extent in butter is to wash it so thoroughly that they will not find sufficient nutrients present.

White (111) reported that *P. nigrifaciens* did not grow at pH 5.2 in broth or on agar; good growth occurred at pH values from 6.8 to 8.4.

The effect of butter culture and lactic acid on fat hydrolysis in cream by pure cultures of lipolytic organisms was studied by Fouts (19). When butter culture was added to the cream there was definite inhibition of *Achromobacter lipolyticum*, *Alcaligenes lipolyticus* and *P. fluorescens;* when lactic acid was added to the cream used to culture the organisms, all grew even with enough acid to give a titratable acidity of 1 per cent.

Accumulation of fatty acids in rancid butter has a decidedly bactericidal effect on many organisms. Schmidt (95) reported a rapid increase in bacteria in a sample of rancid butter, then after 20 to 40 days a decrease which continued almost to sterility of the sample. Eichholz (14) observed rancid butter which after a time was free of vegetative forms and contained only a few spores. Rumment (91) noted that *P. fluorescens* does not tolerate the fatty acids in rancid butter.

Hammer and Collins (31) studied changes in numbers of bacteria at 21 C. in 11 lots of unsalted butter made from sterilized cream inoculated with pure cultures of lipolytic organisms. Early in the holding large numbers of organisms were present and rancidity developed; later, the numbers of bacteria declined until at the last examination (after 15 to 20 days) the counts were relatively low. The rapid decreases in numbers of bacteria in the rancid butter were considered due to accumulation of the lower fatty acids. It was pointed out, however, that decreases in numbers of organisms in various dairy products, following increases responsible for defects, are common and are not limited to defects involving fat hydrolysis.

While it should be recognized that acid has an effect on the growth of bacteria in butter, the protective action of acid alone may be overemphasized. Certain of the organisms considered rather sensitive to acid, for example *P. putrefaciens*, tolerate pH levels lower than those normally found in butter. However, addition of butter culture to cream (or wash water) seems to inhibit certain organisms and limit certain defects so that the protective action of butter culture may be due to products of metabolism other than lactic acid. The inhibitory effect of volatile fatty acids should be recognized, but probably with the amounts of the acids normally present in butter this factor is not significant.

AIR SUPPLY

Since most of the bacteria that develop in butter are comparatively aerobic, the air content of butter is a factor affecting

bacterial growth. The relatively large numbers of organisms often found at the surface of butter and the intensity of certain flavor defects there usually are attributed to the greater air supply.

Lafar (52) reported that the bacterial content of the interior of butter is much lower than that of the exterior. Orla-Jensen (68) compared changes in numbers of organisms at the surface and in the interior of sweet-cream butter held at 18 to 20 C. for 6 weeks. At 3 days and each interval thereafter, the numbers of organisms were higher at the surface than in the interior. The surface layer was disagreeable after 3 days and rancid after 7 days, while the interior was not rancid after 6 weeks. Gratz (24) emphasized that bacteria develop most rapidly in the outer layers of butter and, as a consequence, lipolysis is more marked and the acid content is higher there. He noted that various investigators have found air normally present in butter; because of this some bacterial increase would be expected in the inner layers.

Hammer and Collins (31) investigated the growth of lipolytic bacteria in the surface and subsurface portions of unsalted butter held at 21 C. The bacteria commonly grew faster at the surface than in the deeper layers and early in the holding were more numerous at the surface; later, the larger numbers were sometimes found in the interior due to death of organisms at the surface.

Changes in the flora of several lots of canned butter held at room temperature were investigated by Rogers (86). Initially the flora was made up of lactic acid bacteria, yeasts and a few liquefying bacteria. Both the lactic acid bacteria and yeasts decreased rapidly until at the end of 100 days only a few spore forming bacteria remained, most of which were liquefiers.

Rogers (87) noted that the whitening effect of over-working butter is due to the air introduced and also that air is worked into butter in the ordinary process of manufacture. He reported from 5 to 6 ml. of air per 100 g. of butter. Rogers, *et al.* (88) found that about 10 per cent by volume of fresh butter was gas; this consisted, among other things, of 20 per cent oxygen. The oxygen content was materially decreased after 13 months at

−17.9 C. Over-working butter in a churn did not appear to incorporate more air; it was noted that this cannot be compared to over-working small amounts of butter with a spatula. Sommer and Smit (100) stated that over-working butter may increase the air content.

The pore space in fresh butter was found by Pickerill and Guthrie (72) to range from 0.5 to over 6 per cent. Rahn and Mohr (84) reported that the average air content of 290 samples of butter was 4.2 ml. per 100 g., the variations being from 0.97 to 8.38 ml. Individual dairies tended to produce butter of rather uniform air content although the content varied somewhat with the season, being high from June to October. In fairly well worked butter Guthrie (28) found from 2.85 to 6.42 ml. of air per 100 g., with an average of 4.65 ml.; in thoroughly worked butter there was from 4.17 to 6.70 ml., with an average of 5.37 ml.

Dyer (13) studied changes in the gas content of butter during storage and found that after 6 months at −17.8 C. the composition of the gas in a churning of pasteurized sweet-cream butter, known to contain bacteria, showed little or no variation from the original. A portion of the same churning held at 0 C. showed a decided change which was characterized by a decrease in oxygen and an increase in carbon dioxide. The change was further increased by holding at room temperature.

Commonly the numbers of bacteria are greater at the surface of butter than in the interior. This suggests that the somewhat restricted air supply in the interior may affect the growth of certain bacteria; however, growth is not prevented at favorable temperatures since the amounts of air worked into butter at the time of manufacture are sufficient for extended bacterial development.

MISCELLANEOUS FACTORS

The effects of certain miscellaneous factors have been considered in the studies on bacterial changes in butter.

Gases. Hunziker (40) reported that carbonating does not destroy the bacteria present in cream which are harmful to the

flavor and keeping quality of butter made from it; carbonated butter from unpasteurized cream developed the usual bacterial flavor defects. Commercial carbon dioxide was found by Prescott and Parker (77) to be practically sterile. Butter churned in an atmosphere of carbon dioxide had a lower bacterial content than butter churned in air; however, buttermilk from the carbonated churning contained larger numbers of bacteria than buttermilk from the control churning.

The effects of oxygen, hydrogen, nitrogen and carbon dioxide on growth of organisms were investigated by Prucha, *et al.* (79). After a few trials all the gases except carbon dioxide were discontinued because of unfavorable results. Carbonation of sweet cream tended to suppress certain types of bacteria but did not hinder others; the effect was to delay souring of the cream for a few hours at room temperature and several days at 1.7 C. When carbon dioxide was applied at churning time, by charging the cream in the churn and by replacing the air above the cream in the churn, no significant benefit resulted, and the fresh butter tasted sour. In the butter, molds were not inhibited and bacteria were not measurably affected. However, storing butter in an atmosphere of carbon dioxide inhibited mold growth and prolonged keeping quality. In additional studies, the same investigators (80) arrived at essentially the same conclusions.

Sherwood and Martin (97) reported that bacterial counts on butter made from cream treated with carbon dioxide before churning did not indicate that the gas reduced the numbers of organisms present. In no case was there any great difference between bacterial counts on carbonated and uncarbonated butter, although various modifications in the manufacturing procedure were used. Erbacher and Schoppmeyer (15) found that butter stored in an atmosphere of carbon dioxide developed an off flavor due to absorption of the gas, while storage in atmospheres of hydrogen or nitrogen accelerated putrefaction.

Preservatives. Although use of special preservatives in butter is now rare, they were employed extensively at one time. The compounds varied in nature; it appears that some of them were inferior to salt.

Fischer and Gruenert (18) compared various conservation materials used to increase the keeping quality of butter. These included salt, benzoic acid, hydrin (a preservative analyzing 13.67 per cent free benzoic acid, 9.16 per cent sodium oxide, 7.52 per cent phosphorus pentoxide, 35.14 per cent salt, 9.26 per cent lactic acid and 24.89 per cent water), salicylic acid and boric acid. Salt was considered to be much superior to the other compounds investigated.

Orla-Jensen (70) reported that, where permissible by law, it is advisable to add to butter 0.75 per cent benzoic acid, 2 per cent sodium benzoate or a mixture of 0.5 per cent benzoic acid and 0.5 per cent sodium benzoate. Benzoic acid was regarded as one of the less objectionable preservatives since it is transformed in the human body to hippuric acid.

Use of boron preservatives was investigated in New Zealand (1). In seven experiments in which the amounts of preservatives ranged from 0.08 to 0.99 per cent, the average quality of the butter was only slightly in favor of the preserved product. It was concluded that the preservatives had little effect in sustaining keeping quality, either in cold storage or afterwards at room temperature.

Weigmann (110) reported that in various countries it formerly was the practice to treat butter with different conservation materials, such as boric acid preparations, sugar, saltpeter and gum arabic; he also reported that fluorides have been detected in French butter.

Treatment of wash water. Since contaminated wash water has been the cause of many outbreaks of defective butter, various treatments have been proposed for creamery water. With some of these, such as pasteurization, no inhibitory effect would be expected to carry over into the butter, but with others involving addition of some bactericidal material to the water the substance may influence bacterial growth in the butter itself.

Salmon (92) recommended that water used to wash butter be purified with ozone and reported that this treatment preserved the natural flavor of butter and retarded rancidity. Dornic and Daire (12) sterilized wash water for butter with ultraviolet light and considered the process a practical one.

Demeter and Haase (11) investigated the effect of washing butter with water treated by the katadyn process. In fresh butter there was considerable reduction in numbers of organisms which grew on lactose agar and on casein agar and also of acid-forming organisms; no effect was noted on proteolytic or *Escherichia-Aerobacter* species. When the washed butter was held in cold storage, there was no action on proteolytic organisms. Butter washed with water treated too strongly developed a metallic taste.

Sorensen (101) pointed out that creameries located in large cities constantly use water which contains 0.1 to 0.5 p.p.m. of available chlorine; when water containing 5 p.p.m. of available chlorine was used to wash butter no chlorine could be detected in the water as it left the churn. Hunziker (41) reported that wash water for butter may be sterilized by treatment with 25 to 35 p.p.m. of available chlorine and indicated that this amount has no objectionable effect on the flavor of butter.

Various outbreaks of bacterial spoilage in butter have been controlled by treatment of the wash water. Jensen (46) described an unusual condition which was effectively prevented by chlorination of water and heat treatment of utensils. It involved variations in the quality of butter from one part to another of the same box. The property chiefly affected was the flavor and, in well-marked cases, areas having a distinct off flavor were found interspersed with areas of normal flavor. The defect was not noted in freshly made butter, but in butter held at 4.4 to 10 C. or higher it developed rapidly, becoming noticeable in 3 to 4 days. It was attributed to localized action of microörganisms carried to surfaces of the butter by wash water and utensils used in the packing operation.

Ultraviolet light and x-rays. Dornic and Daire (12) stated that butter cannot be sterilized by ultraviolet light on account of its opacity and because of the production of a tallowy taste and odor by the ozone generated by the lamps. A method for increasing the keeping quality of butter by irradiating with x-rays for 10 minutes and storing in an atmosphere of carbon dioxide was proposed by Pimenov (73). Irradiation with ultraviolet light for

5 minutes permitted even longer keeping. Contrary to the results of Dornic and Daire (12), the latter treatment did not affect the organoleptic properties of the butter.

TABLE 1

Factors limiting bacterial growth in butter and suggested counterbalancing influences

FACTORS LIMITING GROWTH	SUGGESTED COUNTERBALANCING INFLUENCES
Fine dispersion of water	High initial contamination resulting in many infected water droplets Printing butter under conditions tending to aggregate water droplets Action of salt in tending to aggregate water droplets
Low temperature	Presence of psychrophilic organisms which grow just above freezing point of water Exposure of butter for short periods (during handling, etc.) to temperatures allowing rapid growth Depression of freezing point of water in butter by salt
Addition of salt	Poor distribution of salt Relatively little salt added High initial contamination Presence of salt-tolerant species Adaptation of certain species to relatively high salt concentration
Use of butter culture; acidity	*S. lactis* less salt-tolerant than many other species Inhibition of *S. lactis* but not psychrophilic species by holding temperatures Acid content of salted butter and some unsalted butter commonly too low to inhibit organisms
Air supply	Air incorporated in butter during manufacture sufficient for many species
Special preservatives	Some that have been suggested not as effective as salt
Treatment of wash water	Outbreaks of defective butter attributed to contaminated wash water actually due to some other cause

GENERAL CONSIDERATIONS

From the studies reported in the literature it is evident that there are various factors which tend to restrain the growth of

bacteria in butter. The data suggest that some of these are very effective if they are completely operative, and the combined action of two or more of them would be expected to control bacterial action. However, under commercial conditions bacterial spoilage of butter occurs rather frequently, even when protective measures are employed in the manufacture. This spoilage indicates that there are influences tending to counterbalance the restraining actions. When bacterial growth occurs in butter it is probable that more than one of these counterbalancing influences are involved. In general the growth of bacteria in butter must be considered from the standpoint of the balance between the factors restraining growth and those favoring growth; in this balance the effect of the extent of contamination is frequently overlooked.

The factors limiting bacterial growth in butter and suggested counterbalancing influences are listed in table 1.

REFERENCES

1. Anon. 1925 Preservatized and non-preservatized butter. New Zealand J. Agr., **31**, 157–158.
2. Arup, P. S., and Gilmour, G. Van B. 1932 Investigations to test bacteriologically and chemically the effect of cold storage on the keeping qualities of Irish Free State creamery butter. Irish Free State Dept. Agr. J., **31**, 179–189.
3. Arup, P. S., and Gilmour, G. Van B. 1933 Investigation to test chemically and bacteriologically the effect of cold storage on the keeping qualities of Irish Free State creamery butter. Irish Free State Dept. Agr. J., **32**, 257–272.
4. Boysen, H. 1927 Die Wasserverteilung in der Butter. Milchw. Forsch., **4**, 221–248.
5. Brown, C. W. 1912 Some actions of microorganisms upon the constituents of butter. Zentr. Bakt. Parasitenk. II, **34**, 69–70.
6. Brown, C. W., Smith, L. M., and Ruehle, G. L. A. 1920 A bacteriological and biochemical study of experimental butters. J. Dairy Sci., **3**, 375–405.
7. Claydon, T. J., and Hammer, B. W. 1939 Bacteriology of butter. VIII. Relationship of *Achromobacter putrefaciens* to the putrid defect of butter. Iowa Agr. Expt. Sta., Research Bull. 267.
8. Collins, M. A., and Hammer, B. W. 1933 Migration of bacteria through butter. Iowa State Coll. J. Sci., **7**, 453–460.
9. Cullity, M., and Griffin, D. G. 1938 Rabbito or surface taint in butter. J. Dept. Agr., W. Australia. Ser. 2., **15**, 137–147.
10. Derby, H. A., and Hammer, B. W. 1931 Bacteriology of butter. IV. Bacteriological studies on surface taint butter. Iowa Agr. Expt. Sta., Research Bull. 145.

11. DEMETER, K. J. AND HAASE, E. 1933 Über Butterwaschversuche mit Katadynwasser. Milchw. Forsch., **15**, 546–572.
12. DORNIC AND DAIRE. 1909 Contribution à l'étude de la stérilisation par les rayon ultra-violets. Application à l'industrie beurriére. Compt. rend. **149**, 354–356.
13. DYER, D. C. 1916 Progressive oxidation of cold-storage butter. J. Agr. Research, **6**, 927–951.
14. EICHHOLZ, W. 1901 Untersuchungen über die Ursachen des Ranzigwerdens der Butter. Inaugural Dissertation. Friedrich-Wilhelms-Universität zu Berlin.
15. ERBACHER, E., AND SCHOPPMEYER, W. 1939 Investigations of quality changes in packaged butter. Deut. Molkerei-Ztg., **60**, 468–469, 504–506, 536–538, 609–611 (orig. not seen). Chem. Abstracts, **33**, 8838. 1939.
16. FENNEMA, N. 1916 The bacteriology of butter with special reference to the number of organisms and the change in the numbers during the storage of the butter. Thesis. Iowa State College Library. Ames, Iowa.
17. FETTICK, O. 1909 Quantitative und qualitative Untersuchungen über die Bakterien, Hefen und Pilze der Butter und über den Einfluss des Kochsalzes auf dieselben. Welcher Kochsalzgehalt ist für Dauer oder Exportbutter zulässig? Zentr. Bakt. Parasitenk., II, **22**, 32–44.
18. FISCHER, K., AND GRUENERT, O. 1911 Über den Einfluss einiger Konservierungsmittel auf Haltbarkeit und Zusammensetzung von Butter und Margarine. Z. Untersuch. Nahr. u. Genussm., **22**, 553–582.
19. FOUTS, E. L. 1940 Effect of lactic acid on the hydrolysis of fat in cream by pure cultures of lipolytic microorganisms. J. Dairy Sci., **23**, 303–306.
20. GARRARD, E. H., AND LOCHHEAD, A. G. 1939 A study of bacteria contaminating sides for Wiltshire bacon with special consideration of their behaviour in concentrated salt solutions. Can. J. Research, **17**, 45–58.
21. GARRISON, E. R. 1940 The fluorescent bacteria in dairy products. Thesis. Iowa State College Library. Ames, Iowa.
22. GILMOUR, G. VAN B., AND CRUESS-CALLAGHAN, G. 1932 Rate of growth of micro-organisms in Irish Free State creamery butter. Irish Free State Dept. Agr. J., **31**, 226–231.
23. GILTNER, W., AND BAKER, J. D. 1915 Effect of salt on butter flora. Ann. Rept. Mich. Agr. Expt. Sta., p. 209.
24. GRATZ, O. 1928 The lipolysis of worked butter, several days after preparation. Proc. 8th. World's Dairy Congr.
25. GRIMES, M. 1923 A study of the action of certain bacteria, yeasts and molds on the keeping quality of butter in cold storage. J. Dairy Sci., **6**, 427–445.
26. GRIMES, M., AND HENNERTY, A. J. 1934 A study of the quantitative changes in the microbiological flora of sweet-cream salted butter of good keeping quality when held at 15°F. for a period of two to eight months. J. Dairy Research, **5**, 137–143.
27. GUBITZ, H. 1928 Kältebakterien, mit besonderer Berücksichtigung ihrer Bedeutung für die Milchwirtschaft. Milchw. Forsch., **5**, 407–456.
28. GUTHRIE, E. S. 1930 Air in butter. J. Dairy Sci., **13**, 461–470.
29. GUTHRIE, E. S., SCHEIB, B. J., AND STARK, C. N. 1936 The effect of certain factors upon the keeping quality of butter. J. Dairy Sci., **19**, 267–278.

30. HAMMER, B. W. 1938 Dairy Bacteriology. John Wiley and Sons Inc., New York, pp. 358–359, 2nd ed.
31. HAMMER, B. W., AND COLLINS, M. A. 1934 The numbers of lipolytic bacteria in various dairy products, as determined with nile-blue sulfate. Iowa Agr. Expt. Sta., Research Bull. 169.
32. HAMMER, B. W., AND HUSSONG, R. V. 1930 Bacteriology of butter. 1. Influence of the distribution of the non-fatty constituents on the changes in bacterial content during holding. Iowa Agr. Expt. Sta., Research Bull. 134.
33. HAMMER, B. W., AND OLSON, H. C. 1941. Phosphatase production in dairy products by microorganisms. J. Milk Tech., **4**, 83–85.
34. HENNEBERG. 1931 Zur Kenntnis der Alkalibildner in der Milch. Milchw. Forsch., **12**, 222–235.
35. HISCOX, E. R. 1936 A pigment-producing organism (*Pseudomonas* sp.) isolated from discoloured butter. J. Dairy Research, **7**, 238–243.
36. HOECKER, W. H. 1941 Distribution of salt in butter and its effect on bacterial action. Thesis. Iowa State College Library. Ames, Iowa.
37. HOF, T. 1935 Investigations concerning bacterial life in strong brines. Amsterdam, Drukkerij Mulder and Zoon.
38. HOOD, E. G., AND WHITE, A. H. 1928 Surface taint butter. Can. Dept. Agr., Pamph. 91, New Ser.
39. HOROVITZ-VLASOVA, L. M., LIVSHITZ, M. I., KACHANOVA, E. E., OBERHARD, I. A., AND SHALBEROVA, A. B. 1932 Experimental studies of butter spoilage. Schriften zentral. biochem. Forsch. Inst. Nahr- u. Genussmittelind. (Moscow), **2**, 315–338. (orig. not seen) Chem. Abstr., **27**, 5435. 1933.
40. HUNZIKER, O. F. 1924 Facts about carbonated butter. J. Dairy Sci., **7**, 484–496.
41. HUNZIKER, O. F. 1938 Handling of cream between pasteurizer and churn. Creamery J., **50**, no. 3, p. 5, p. 28.
42. HUNZIKER, O. F., AND HOSMAN, D. F. 1920 Mottles in butter—their causes and prevention. J. Dairy Sci., **3**, 77–106.
43. ITZEROTT, A. G. F. 1941 The "rabbito" defect in butter. J. Dept. Agr., Victoria, **39**, 39–42.
44. JACOBSEN, D. H. 1937 A holding test at room temperature as an indication of the keeping quality of butter in storage. S. Dakota Agr. Expt. Sta., Bull. 308.
45. JACOBSEN, D. H. 1938 Bacterial content and keeping quality of butter after removal from storage. J. Dairy Sci., **21**, 187–193.
46. JENSEN, T. M. 1941 The "sectional contamination" defect in butter. J. Dept. Agr., Victoria, **39**, 283–288.
47. KANUNNIKOWA, Z. A. 1936 The influence of different NaCl concentrations on the decomposition of butter by microorganisms and the formation of some ferments. Arch. Sci. Biol. (U.S.S.R.), **43**, nos. 2-3, 117–127.
48. KNUDSEN, S., AND JENSEN, M. 1930 Nogle Undersøgelser over Smørrets Holdbarhed. Maelkeritidende, **43**, 1016–1039.
49. KNUDSEN, S., AND JENSEN, M. 1933 Quelques recherches sur la conservation du beurre. Lait., **13**, 885–904.

50. KNUDSEN, S., AND SÖRENSEN, A. 1938 Über eine Methode zur Beurteilung der Grösse der Wassertröpfchen in Butter. Fette u. Seifen, **45**, 669–670.
51. KRETCHMAR, H. H. 1940 Butter: Its composition and deterioration. J. Dept. Agr., W. Australia. Ser. 2., **17**, 93–134, 141–152.
52. LAFAR, F. 1891 Bacteriologische Studien über Butter. Arch. Hyg., **13**, 1–39.
53. LOFTUS-HILLS, G., SCHARP, L. R., AND BELLAIR, T. S. 1934 A study of factors influencing the keeping quality of some Victorian salted butters in cold storage. J. Dairy Research, **5**, 124–136.
54. LONG, H. F., AND HAMMER, B. W. 1938 Examination of butter with the Burri smear culture technic. Iowa State Coll. J. Sci., **12**, 441–450.
55. LONG, H. F., AND HAMMER, B. W. 1938 Bacteriology of butter. VI. Effect of moisture dispersion in butter on growth of bacteria. Iowa Agr. Expt. Sta., Research Bull. 246.
56. LONG, H. F., AND HAMMER, B. W. 1939 Bacteriology of butter. VII. Effect of reworking butter on growth of bacteria. Iowa Agr. Expt. Sta., Research Bull. 263.
57. LONG, H. F., AND HAMMER, B. W. 1941 Classification of the organisms important in dairy products. III. *Pseudomonas putrefaciens*. Iowa Agr. Expt. Sta., Research Bull. 285.
58. LOVELAND, A. E., AND WATSON, W. S. 1894 Bacteria in the dairy. VII. Some observations of the number of bacteria in dairy products. Ann. Rept. Storrs Agr. Expt. Sta., pp. 73–76.
59. LUXWOLDA, W. B. 1912 Wachstum und Wirkung einiger Milch-bakterien bei verschiedenen Temperaturen. Zentr. Bakt. Parasitenk. II, **31**, 129–175.
60. MACY, H. 1930 Quantitative changes in the microflora of butter during storage. J. Dairy Sci., **13**, 266–272.
61. MACY, H., COULTER, S. T., AND COMBS, W. B. 1932 Observations on the quantitative changes in the microflora during the manufacture and storage of butter. Minn. Agr. Expt. Sta., Tech. Bull. 82.
62. MAZÉ, M. 1906 Causes d'altération des beurres. Contrôle bactériologique de la fabrication. Compt. rend., **143**, 1198–1201.
63. MCKAY, G. L., AND LARSEN, C. 1903 The keeping quality of butter. Iowa Agr. Expt. Sta., Bull. 71.
64. MCKAY, G. L., AND LARSEN, C. 1904 Report of the Iowa educational butter contest. Iowa Agr. Expt. Sta., Bull. 80.
65. MCKAY, G. L., AND LARSEN, C. 1911 Principles and Practice of Buttermaking. John Wiley and Sons Inc., New York, p. 259, 2nd ed.
66. Minn. Agr. Expt. Sta. 1917 Cold storage of butter. Ann. Rept., **25**, 48–49.
67. NELSON, J. A., AND HAMMER, B. W. 1933 Studies on the butter culture organisms in butter. J. Dairy Sci., **16**, 375–385.
68. ORLA JENSEN, (S.) 1902 Studien über das Ranzigwerden der Butter. Zentr. Bakt. Parasitenk., II, **8**, 11–16, 42–46, 74–80, 107–114, 140–144, 171–174, 211–216, 248–252, 309–312, 342–346, 367–369, 406–409.
69. ORLA-JENSEN, S. 1910 Étude bactériologique sur le beurre danois. Rev. gén. lait, **8**, 409–417.
70. ORLA-JENSEN, S. 1921 Dairy bacteriology. P. Blakiston's Son and Co., Philadelphia, pp. 120–121.

71. PETTERSSON, A. 1900 Experimentelle Untersuchungen über das Conserviren von Fisch und Fleisch mit Salzen. Arch. Hyg., **37**, 171–238.
72. PICKERILL, H. M., AND GUTHRIE, E. S. 1915 Two factors causing variation in the weight of print butter. N. Y. (Cornell) Agr. Expt. Sta., Bull. 355.
73. PIMENOV, D. M. 1938 A propos de l'augmentation de la stabilité du beurre. Molotchno-Maslod. Prom., 5: no. 3. 4–8 (orig. not seen). Chimie et industrie, **41**, 1188, 1939.
74. PONT, E. G. 1939 Micro-organisms in butter and their relationship to quality. J. Australian Inst. Agr. Sci., **5**, 202–207.
75. PONT, E. G. 1941 The rabbito defect of butter. J. Council Sci. Ind. Research, **14**, 1–10.
76. PONT, E. G., AND SUTTON, W. S. 1938 The pH of N.S.W. butter and its relationship to bacterial and yeast counts. J. Australian Inst. Agr. Sci., **4**, 96–99.
77. PRESCOTT, S. C., AND PARKER, M. E. 1925 Certain aspects of carbonation as applied to buttermaking. N. Y. Prod. Rev. and Amer. Creamery, **60**, no. 1, p. 12, p. 14, pp. 16–18.
78. PRUCHA, M. J., AND BRANNON, J. M. 1926 Prints lower the keeping quality of butter. Ann. Rept. Ill. Agr. Expt. Sta., p. 96.
79. PRUCHA, M. J., BRANNON, J. M., AND RUEHE, H. A. 1925 Carbonation of butter. J. Dairy Sci., **8**, 318–329.
80. PRUCHA, M. J., BRANNON, J. M., AND RUEHE, H. A. 1931 Effect of carbonation on bacterial content and keeping quality of dairy products. Ill. Agr. Expt. Sta., Bull. 368.
81. RAHN, O. 1931 Die Butterbereitung. Handbuch der Milchwirtschaft. W. Winkler, Editor. Verlag von Julius Springer. Wien. **2**, pp. 104–107.
82. RAHN, O., AND BOYSEN, H. H. 1928 Distribution and growth of bacteria in butter. J. Dairy Sci., **11**, 446–470.
83. RAHN, O., BROWN, C. W., AND SMITH, L. M. 1909 Keeping qualities of butter. II. The influence of salt. III. The decomposition of proteins. Mich. Agr. Expt. Sta., Tech. Bull. 2.
84. RAHN, O., AND MOHR, W. 1924 Der Luftgehalt der Butter. Milchw. Forsch., **1**, 213–221.
85. RICE, E. B. 1938 Studies on the coliform bacteria found in butter. Queensland Agr. J., **50**, 708–715.
86. ROGERS, L. A. 1904 Studies upon the keeping quality of butter. 1. Canned butter. U. S. Dept. Agr., Bur. An. Ind., Bull. 57.
87. ROGERS, L. A. 1909 Fishy flavor in butter. U. S. Dept. Agr., Bur. An. Ind., Circ. 146.
88. ROGERS, L. A., BERG, W. N., POTTEIGER, C. R., AND DAVIS, B. J. 1913 Factors influencing the change in flavor in storage butter. U. S. Dept. Agr., Bur. An. Ind., Bull. 162.
89. ROGERS, L. A., AND GRAY, C. E. 1909 The influence of acidity of cream on the flavor of butter. U. S. Dept. Agr., Bur. An. Ind., Bull. 114.
90. ROSENAU, M. J., FROST, W. D., AND BRYANT, R. 1914 A study of the market butter of Boston. J. Med. Research, **30**, 69–85.
91. RUMMENT, J. 1931 The effect of water on the keeping quality of butter. 9th Intern. Dairy Congr. Report of the Delegation of the U. S. to the Sec. of State, p. 62.

92. SALMON. 1924 La contamination des eaux utilisées dans l' industrie laitière et leur stérilisation par l'ozone. Lait, **4**, 848–855.
93. SAYER, W. S., RAHN, O., AND FARRAND, B. 1908 Keeping qualities of butter. 1. General studies. Mich. Agr. Expt. Sta., Tech. Bull. 1.
94. SCHARP, L. R. 1936 Bacteriological infection of butter. Off. Rept. 43rd Ann. Conf. Vict. Dairy Fact. Mgrs. and Sec. Assoc. Melbourne, Australia, 50–52.
95. SCHMIDT, H. 1898 Ueber die Vorgänge beim Ranzigwerden und den Einfluss des Rahm-pasteurisirens auf die Haltbarkeit der Butter. Z. Hyg. Infektionskrankh., **28**, 163–188.
96. SHEPARD, S. 1934 The relationship between changes in the number of bacteria and in the scores of butter held at 0°C. Thesis. Iowa State College Library. Ames, Iowa.
97. SHERWOOD, F. F., AND MARTIN, F. G. 1926 Influence of carbon dioxide upon quality and keeping properties of butter and ice cream. Iowa Agr. Expt. Sta., Research Bul. 95.
98. SHUTT, D. B. 1929 Contaminated water as a source of surface flavor in pasteurized creamery butter. Sci. Agr., **9**, 316–320.
99. SLEMMONS, W. S. 1926 Observations of bacterial inhibition and the quantity of inoculation. J. Bact., **11**, 88–89.
100. SOMMER, H. H., AND SMIT, B. J. 1923 The fishy flavor in butter. Wis. Agr. Expt. Sta., Research Bull. 57.
101. SORENSEN, C. M. 1940 The keeping quality of butter. J. Dairy Sci., **23**, 423–436.
102. SPITZER, G., AND PARFITT, E. H. 1929 A study of the proteolytic action of specific organisms and groups of organisms in butter made from graded cream. J. Dairy Sci., **12**, 1–20.
103. SPITZER, G., PARFITT, E. H., MANHART, V. C., AND EPPLE, W. F. 1927 A study of the proteolytic action of specific organisms and groups of organisms in butter made from graded cream. Ind. Agr. Expt. Sta., Bull. 319.
104. STORCH, V. 1897 Undersøgelser over: Nogle ret hyppig forekommende Konsistensfejl hos Smørret og Aarsagerne til deres Fremkomst, samt over: Maelkekuglernes Bygning. Sex og tredivte Beretning. Kgl. Veterinaer- og Landbohøjskoles. Kjøbenhavn.
105. TOTMAN, C. C., MCKAY, G. L., AND LARSEN, C. 1939 Butter. John Wiley and Sons Inc., New York, p. 263.
106. TURGASEN, V. L. 1939 Trouble shooting for flavor defects. Address 31st Ann. Meeting Am. Butter Inst., Chicago, Ill.
107. VIRTANEN, A. I. 1931 Butter defects caused by water bacteria. 9th Intern. Dairy Congr. Report of the Delegation of the U. S. to the Sec. of State, p. 42.
108. WASHBURN, R. M., AND DAHLBERG, A. C. 1918 The influence of salt on the changes taking place in storage butter. J. Dairy Sci., **1**, 114–126.
109. WEIGMANN, H. 1924 Die Pilzkunde der Milch. Verlagsbuchhandlung Paul Parey, Berlin, p. 277.
110. WEIGMANN, H. 1932 Lehrbuch der Milchwirtschaft. Verlag von Paul Parey, Berlin, pp. 593–596.

111. WHITE, A. H. 1940 A bacterial discoloration of print butter. Sci. Agr., **20**, 638–645.
112. WILEY, W. J. 1937 The effect of acidity on the keeping quality of unsalted butter. J. Council Sci. Ind. Research, **10**, 327–332.
113. WILSON, C. A., AND PRUCHA, M. J. 1940 Changes in the bacterial flora of butter. J. Dairy Sci., **23**, 508–509.
114. WINSLOW, C.-E. A., WALKER, H. H., AND SUTERMEISTER, M. 1932 The influence of aeration and of sodium chloride upon the growth curve of bacteria in various media. J. Bact., **24**, 185–208.