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Contents

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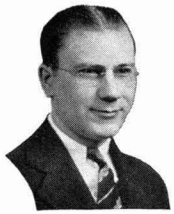

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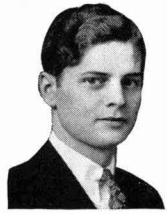

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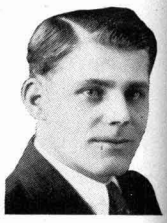

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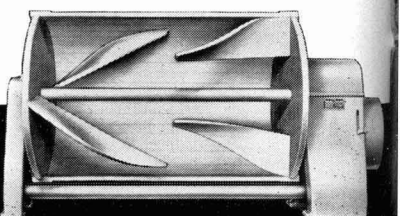

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BACTERIOLOGY OF BUTTER CULTURES: A REVIEW[1]

B. W. HAMMER AND F. J. BABEL

Iowa State College, Ames, Iowa

Previous to 1919[2] it commonly was believed that butter cultures are pure cultures of lactic acid streptococci, although there had been various suggestions that the desirable flavor of butter made from ripened cream is not produced by the lactic acid bacteria growing in the cream. In that year three laboratories established the basis for an understanding of the bacteriology of butter cultures by reporting almost simultaneously that such cultures normally include two distinct types of bacteria. The reports indicated that butter cultures contain flavor-producing organisms associated with the much more conspicuous lactic acid streptococci and that combined action of the two types gives the desired flavor to the cultures and to butter in which the cultures are used.

EARLY EVIDENCE OF ASSOCIATIVE ACTION IN BUTTER CULTURES

The 1919 studies, which showed that butter cultures are not pure cultures of lactic acid streptococci, are:

(*a*) Boekhout and Ott de Vries (23) isolated from sour milk and cream an organism which produced the characteristic and desirable butter culture aroma when grown with an organism of the *Streptococcus lactis*[3] type. It resembled *S. lactis* in morphology but produced no visible change in milk.

(*b*) After noting that butter cultures developed much higher volatile acidities in milk than pure cultures of *S. lactis,* Hammer and Bailey (96) found that butter cultures contained organisms, associated with *S. lactis,* which commonly did not curdle milk but which in combination with *S. lactis* gave high volatile acidities.

(*c*) Storch (260) considered two types of organisms necessary in butter cultures, a lactic acid type and a flavor type. The latter did not coagulate milk or form much acid but produced more volatile acid than the former.

Additional studies have confirmed the presence in butter cultures of

[1] Journal Paper No. J-1073 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 127.

[2] Most of the papers reviewed appeared after January 1, 1919, but some earlier publications are included because of their importance to the general problem of butter cultures.

[3] The name used for an organism often is the one now commonly accepted rather than the one employed in the publication being considered.

organisms other than lactic acid streptococci, and various relationships show that butter cultures definitely differ from pure cultures of the lactic acid bacteria (89). Some of the more recent investigations have involved the specific chemical compounds developed by each of the two types of organisms.

GENERAL TYPES OF ORGANISMS IN BUTTER CULTURES

The two types of organisms normally in butter cultures are:

(*a*) Lactic acid type which attacks lactose and forms relatively large amounts of lactic acid together with small amounts of secondary products; ordinarily, it also effects some proteolysis. Growth of the organisms in milk does not result in a product having a butter culture flavor, but changes are brought about which both directly and through their influence on the action of the flavor organisms greatly affect the flavor of butter cultures.

(*b*) Flavor type which in milk commonly produces acid slowly if at all; it is characterized by the ability to break down citric acid with formation of various compounds, some of which contribute greatly to the flavor of butter cultures, certain types of butter, artificial buttermilk and certain cheeses. Early studies showed that the organisms produce relatively large amounts of volatile acid and that citric acid is the primary source of such acid. These observations have been repeatedly confirmed, and it now is generally recognized that citric acid also is the source of carbon dioxide and a series of neutral compounds of which diacetyl is of particular importance from the flavor standpoint. The ratios of the various compounds are influenced greatly by the conditions under which the organisms grow.

DESIGNATIONS OF THE TYPES OF ORGANISMS IN BUTTER CULTURES

Designation of the lactic acid organisms

The organism now known as *S. lactis* was first isolated by Lister (153), in 1878. It was studied under various names (22, 227) until given the present designation by Löhnis (154), in 1909. Orla-Jensen (190), in 1919, described *Streptococcus cremoris* as a species distinct from *S. lactis*. He considered that it usually is a more typical chain-forming *Streptococcus* than *S. lactis;* frequently fails to grow at 37° C.; usually produces less acid in milk; and generally has less fermenting power, especially on maltose and dextrin. Orla-Jensen and Hansen (188) stated that *S. cremoris* ferments only lactose among the di- and polysaccharides, while *S. lactis* ferments lactose and also maltose and dextrin to a variable extent.

From a pure culture of *S. cremoris* which fermented neither maltose nor sucrose, Sherman and Hussong (242) isolated 458 substrains, using agar plates. Of these, 217 were maltose – and sucrose –, 229 were maltose + and sucrose –, 11 were maltose – and sucrose +, and 1 was maltose + and sucrose +. A culture of *S. lactis* (maltose +, sucrose –) was more stable, yielding 756 substrains which were maltose + and sucrose – and 1 which was maltose + and sucrose +.

According to Sherman (238) *S. cremoris* commonly is more typically chain-forming than *S. lactis;* however, some strains occur normally as diplococci, while some strains of *S. lactis* produce chains, thus preventing a clear distinction on this basis. He also noted that in many cases cells of *S. cremoris* are distinctly larger than those of *S. lactis*, but again there are numerous exceptions. He considered that lactic acid streptococci which are characterized both by large cells and by formation of long chains in milk cultures offer presumptive evidence of being *S. cremoris* rather than *S. lactis.* Generally, *S. cremoris* did not grow as well in artificial media as *S. lactis* and was slightly less acid-tolerant, as shown by a lower average production of acid in milk and by usually not developing quite as low a pH in glucose broth.

Yawger and Sherman (332) give the following distinctions: *S. cremoris* produces no ammonia from peptone and does not grow at 40° C., in the presence of 4 per cent sodium chloride or in a medium of pH 9.2; *S. lactis* produces ammonia and is not inhibited by 40° C., 4 per cent sodium chloride or pH of 9.2. Palladina (199) reported that *S. lactis* attacks alcohol, while *S. cremoris* does not. Niven *et al.* (181) stated that *S. lactis* hydrolyzes arginine, whereas the closely related *S. cremoris* does not or attacks it very slightly.

Smith and Sherman (251) suspended washed cells of *S. lactis* and of *S. cremoris* in a phosphate-buffered glucose solution. With *S. lactis,* 96.6 per cent of the glucose fermented appeared as lactic acid and with *S. cremoris,* 93.7 per cent.

The literature shows a difference of opinion as to the comparative importance of *S. lactis* and *S. cremoris* in butter cultures. Ayers *et al.* (5) isolated many lactic acid streptococci from commercial butter cultures; the most numerous variety was the one most common in normal sour milk. Knudsen (140) considered that the streptococci which develop during the first stages of milk souring (*S. lactis*) are scarcely normal inhabitants of butter cultures; *S. cremoris* was believed to be the organism in good butter cultures, and when *S. lactis* was present it was regarded as a harmless impurity. According to Kelly (131) good butter cultures must contain strains of *S. cremoris;* other lactic acid streptococci (*S. lactis*) normally present in cream and milk were thought to play a part in production of the desired butter aroma. From detailed studies on spontaneously soured milk and butter cultures, Orla-Jensen and Hansen (188) concluded that *S. lactis* is the principal organism in soured milk and that *S. cremoris* is the principal organism in cream ripening.

Farmer and Hammer (73) developed satisfactory butter cultures with various *S. lactis* strains; however, an *S. lactis* culture which combined satisfactorily with one flavor organism often did not combine satisfactorily with another. van Beynum and Pette (280, 281) found that a butter culture consisting of flavor bacteria and *S. lactis* was as good as one consisting of flavor

bacteria and *S. cremoris* and concluded there is no reason to prefer *S. cremoris*. Some excellent butter cultures examined by Yawger and Sherman (332) contained *S. lactis* and not *S. cremoris*. Pont (207) stated that the characteristic organism in a butter culture usually is *S. cremoris* but that *S. lactis* may be, and frequently is, present. Gibshman (78) noted that either *S. lactis* or *S. cremoris* may be used in butter cultures.

In general, it appears that lactic acid streptococci which certain investigators consider as typical of spontaneously soured milk rather than butter cultures often yield satisfactory butter cultures when combined with a suitable flavor organism. There is the possibility that long continued propagation under the rather constant conditions provided in butter cultures tends to fix certain morphologic characters, etc., in a way they are not fixed under less constant conditions.

Variation among strains of lactic acid organisms. Different strains of lactic acid streptococci show considerable variation. In some cases the variation is of significance from the standpoint of butter cultures, while in others it is not.

Division of the organisms on the basis of fermentation of compounds other than lactose (126, 188, 190) has not been of practical value in dairy bacteriology and may lead to an excessive number of species and varieties. Cultures which are like *S. lactis* in all respects except the fermentation of lactose have been reported as variants of this species (71, 121, 183, 288, 331).

Richet *et al.* (220) noted that a lactic acid organism grown on an arsenate medium differed from the original culture. After 30 successive inoculations on the normal medium, it still differed slightly from the original culture.

Ayers *et al.* (8) found two groups of streptococci in butter cultures; one produced carbon dioxide and ammonia from peptone, while the other did not. Ayers and Johnson (4) noted that the most prevalent strain of human fecal streptococci closely resembled a strain of *S. lactis* obtained from sour milk and suggested that *S. lactis* was either closely related to *Streptococcus fecalis* or identical with it. Since *S. lactis* is consumed in fairly large numbers and can withstand action of bile and low surface tension, the authors concluded that it is likely to be found in the intestine where, in the new environment, it may grow and its physiologic characters be slightly modified.

Because of the variation in cultures of *S. lactis*, Hammer and Baker (98) suggested a division of the *S. lactis* group into the following varieties and species: (*a*) Typical *S. lactis* showing only the common characters of the species; (*b*) *S. lactis* var. *maltigenes* showing a malt-like odor in milk, cream, etc.; (*c*) *S. lactis* var. *hollandicus* showing a ropy condition in milk cultures, especially when the cultures are young; (*d*) *S. lactis* var. *anoxyphilus* showing comparatively slow reduction of litmus; (*e*) *S. lactis* var. *tardus* showing comparatively slow coagulation of milk; and (*f*) *Streptococcus thermophilus*, a closely related species showing considerable resistance to heat and growing poorly at 21° C.

Stocker (259) stated that the malt odor which may appear in butter cultures and other dairy products is caused by degenerated types of *S. lactis*. Regular *S. lactis* cultures could not be transformed into cultures which produced the malt odor on propagation. A malt odor was not produced in sugar bouillons but was produced in media containing casein.

In plating ropy cultures of *S. lactis* on whey agar and picking colonies into litmus milk, Hammer (90) found that non-ropy strains sometimes were obtained. Of 36 ropy cultures from two sources, 17 (47.2%) yielded one or more non-ropy strains; the 1,228 colonies from plates poured with ropy cultures yielded 31 (2.5%) non-ropy strains. In plating 23 non-ropy cultures from the two sources, ropy strains were obtained in only one instance and then only to the extent of 5 per cent of the colonies picked. Cultures from plates poured with ropy cultures from a third source yielded, in a small number of trials, only ropy strains; the cultures differed from those of the first two sources in that they failed to show clumps of pairs of cells. Sudden variation in cells could be detected much better by plating and picking colonies than by making a series of transfers in some medium. The sudden variation that occurred in the ropy character of certain *S. lactis* cultures suggests that sudden variations also may occur with other types of organisms.

Hammer (92) isolated cultures of *S. lactis* which were unusual in that they began growth in tubes of litmus milk at the tops instead of the bottoms. They could be split into two types, one of which differed definitely from the original cultures by beginning growth at the bottoms of tubes of litmus milk. Variation obtained by selective inoculation (from bottoms of tubes by means of capillary pipettes before very much growth had taken place) resulted in a culture that was more like the usual *S. lactis* cultures than was the original; it apparently was unstable and, after a number of transfers, reverted to the original type. This suggested that each type of organism encountered could be split from the other. Eventual dominance of the type developing first at the surface of milk undoubtedly was due to its comparatively rapid growth so that the other type tended to remain submerged to such an extent that it was not commonly evident without selective inoculations.

By plating and picking colonies, Harriman and Hammer (103) split certain cultures of *S. lactis* that rapidly coagulated milk into rapid and slow strains. In general, the rapid strains produced considerable proteolysis in milk, while the slow strains brought about little or no proteolysis. General correlation between rapid coagulation and pronounced proteolysis with *S. lactis* cultures appeared to persist when variation occurred in the coagulation rates of strains picked from a plate poured with a pure culture. The strains obtained by plating a slow culture were all slow in their coagulation rates. Variation in the rates of coagulation and the extents of proteolysis among the strains obtained when plating what would ordinarily be regarded as a

pure culture of *S. lactis* makes these characters of little value in the classification of the species.

Davis (56) noted that cultures of *S. lactis* carried for a time became attenuated. Addition of 1 per cent glucose to the attenuated cultures, which he considered produced lactase with difficulty, speeded up coagulation. One strain requiring over 72 days to coagulate litmus milk, coagulated it in 3 days when glucose was added. When both yeast and glucose were added, coagulation occurred in 1 day.

Vas and Csiszar (288) isolated more than 100 strains of streptococci and selected 35 for an exhaustive study with regard to their variability during further inoculations into different nutrient media and also under partially unfavorable biologic conditions. Weakening of acid production and complete degeneration frequently were observed with some strains, while activation was noted with others. The lactic acid strains lost their strong acid-forming ability when grown in milk for long periods or when held at 37° C.

Eagles *et al.* (70) divided a regular powdered culture into two parts, one part being transferred daily for 20 transfers in plain milk and the other in yeast extract milk. The cultures then were plated and colonies picked. The culture carried in plain milk was characterized by a large percentage of organisms which ordinarily would be classed as strains of *S. cremoris.* If those organisms which fermented salicin in addition to lactose, as well as those that fermented maltose or sucrose slightly, are included as strains of *S. cremoris,* 85 of 94 organisms (90.4%) from the culture carried in plain milk and only 64 of 99 organisms (64.6%) from the culture carried in yeast extract milk would be classed as strains of *S. cremoris.* While only 9 of 94 organisms (9.6%) from the culture carried in plain milk fermented the more complex carbon sources other than salicin, 35 of 99 cultures (35.4%) from the culture carried in yeast extract milk exhibited this ability, 23 of the 99 cultures (23.2%) fermenting lactose, salicin, maltose and starch. A greater proportion of slow acid-forming strains was found in the culture carried in yeast extract milk.

Variability in strains of *S. cremoris* was investigated by Hunter (121). In one strain, changes in acid-producing power were evident as well as changes in colony form. One variant which failed to ferment lactose was still susceptible to the specific race of bacteriophage and exhibited the same morphology as the parent culture. Marked variation among strains was noted with regard to acid production, response to high temperatures, power to produce ropiness in milk and degree of resistance to phage attack.

Okulitch (183) induced dissociation and accompanying inhibition of lactose-fermenting ability by serial cultivation of a vigorous culture of the strain under investigation in casein digest broth and in peptonized milk broth, each broth containing 2 per cent glucose. Dissociation among lactic acid streptococci resulted in development of various colony types. The most

common initial change was from a round, smooth colony to a slightly lobate form. When these intermediate types were picked, they never gave broth cultures characteristic of a pure smooth (S) or rough (R) strain. On several occasions very small pin-point colonies also were seen. Morphologic appearance of the pure S-form of lactic acid streptococci was characterized by regularity in size and shape of the cells which usually were in pairs or short chains. On dissociating to the stable R-form, the culture became decidedly long-chained, the cells at times being very irregular in shape. Formation of a true R-dissociant resulted in complete loss by the organism of the ability to ferment lactose, either in milk or broth. On no occasion did an R-form ever acquire lactose-fermenting ability. Induction of dissociation and accompanying inhibition of lactose-fermenting ability took place most readily in peptonized milk broth containing 2 per cent glucose. Strains of both *S. lactis* and *S. cremoris* underwent dissociative changes. Variations in cultural and colony characters were not constant for different strains of the same species, nor did the same strain follow a definite sequence of dissociative changes at different times.

Designation of the flavor organisms

The flavor (or citric acid-fermenting) organisms of butter cultures were divided into two species by Hammer (85) and named *Streptococcus citrovorus* and *Streptococcus paracitrovorus*. The primary difference between them is that *S. paracitrovorus* produces significant amounts of lactic acid in milk, while *S. citrovorus* does not; there also are secondary differences, such as reddening of litmus milk and relatively high volatile acidities in milk with *S. paracitrovorus*.

Knudsen and Sorensen (142) suggested the name *Betacoccus cremoris* for the flavor bacteria and described two types. Type *x* forms very little acid in plain milk, but if autolysed yeast is added, considerable acid is produced; it ferments fructose very slightly, ferments sucrose under favorable conditions and does not ferment both mannose and maltose. Type *a* is more active than type *x;* it forms much more acid in milk and acid production is stimulated by yeast autolysate; it ferments various sugars; in some cases pentoses are fermented and in other cases also fructose and mannose. Maltose is fermented by all cultures of type *a* but not by all cultures of type *x*. Type *a* resembled *S. paracitrovorus* from which it differed through fermentation of sugars. The authors noted that cells of *B. cremoris* were 0.5 to 1.0 micron in diameter, while those of *S. cremoris* usually were 1.0 micron. *B. cremoris* formed lactic acid and also carbon dioxide, acetic acid and probably small amounts of other acids, alcohols and ethers.

In their classification of the *Coccaceae,* Hucker and Pederson (120) placed *S. citrovorus, S. paracitrovorus* and *B. cremoris* in the genus *Leuconostoc. S. paracitrovorus* was designated *Leuconostoc dextranicus;* it

ferments sucrose but not pentoses and produces slime from sucrose. *S. citrovorus* and *B. cremoris* were designated *Leuconostoc citrovorus;* it fails to ferment sucrose or the pentoses and does not produce slime from sucrose.

Knudsen (140) described the flavor organisms as chain-forming cocci, similar to *S. cremoris* but usually somewhat more slender. When growing in milk they do not alter it for a long time and acid which is produced later is largely acetic; among the other compounds formed by the organisms are substances responsible for the characteristic flavor of butter.

The flavor organisms from butter cultures and similar types from other materials show rather wide variations. These involve amounts of volatile and non-volatile acids produced, action on certain fermentable materials, growth temperatures, etc. However, none of the variations are of special significance from the standpoint of butter cultures in which the breakdown of citric acid by the organisms is the important consideration.

ISOLATION OF THE TWO TYPES OF ORGANISMS FROM BUTTER CULTURES

The two types of butter culture organisms grow fairly well on such media as tomato agar, yeast extract agar and tryptone-glucose-extract-milk agar and show the same general colony characteristics. Normally, the lactic acid type is much the more numerous and is readily obtained when colonies are picked; ordinarily, it is easily recognized by the rather characteristic reaction in litmus milk. A series of colonies sometimes yields the flavor type and sometimes does not.

Various attempts have been made to develop differential media for enumeration and isolation of the flavor organisms. Baker (9) plated butter cultures on agar to which an indicator showing pH changes had been added. The acid produced diffused through the medium so that on plates having large numbers of colonies the color indicated acid over the entire plate; with small numbers, all colonies usually appeared to have produced acid and were assumed to be *S. lactis,* the flavor organisms probably having been diluted out. Smit (250) could not differentiate the lactic acid and flavor organisms by microscopic examination or by common culture media. On milk powder agar (formula A) of Ayers and Mudge (6) *S. lactis* colonies were surrounded by a turbid halo, whereas colonies of flavor organisms did not change the medium. Better results were obtained with Orla-Jensen's casein peptone (190) than with commercial peptone and also with the agar concentration relatively low; the medium should not have a pH above 7.0 before sterilization; it should not be over 3 or 4 weeks old; and plates should be incubated at 25° C. for several days.

Benchetrit (21) noted that addition of α bromopropionic acid in certain concentrations to tomato agar used for plating butter cultures restrained *S. lactis* and thus facilitated isolation of the flavor organisms; the concentration that restrained *S. lactis* and still permitted growth of the flavor

organisms appeared to vary with different lots of the agar. Olson (185) also used α bromopropionic acid in tomato agar and the flavor organisms grew well with amounts of the acid that inhibited *S. lactis;* the concentration required to inhibit *S. lactis* varied with different lots of tomato agar but usually ranged from 0.2 to 0.6 ml. N/10 acid per 100 ml. of agar. Bromoacetic acid was less useful than α bromopropionic acid, the range over which it was effective being sharply limited. Other α bromo fatty acids did not give selective inhibition; low solubilities of the α bromo derivatives of the higher fatty acids are important in this connection.

Use of basic fuchsin, acid fuchsin, methylene blue, crystal violet, eosin or mercurochrome in whey agar was not satisfactory for isolation of flavor organisms from fresh butter cultures in trials carried out by Benchetrit (21); also addition of acetylmethylcarbinol or diacetyl to tomato agar was of no advantage. Some of the attempts with agar plates containing thioglycolic acid were encouraging, but in general results with this acid were inconsistent.

The flavor organisms often can be obtained by procedures other than plating active butter cultures. They are relatively acid-tolerant and may be present in a viable condition in cultures that have been allowed to stand until they no longer contain living *S. lactis* cells (85, 89, 140); they then can be recovered by culturing on agar or in milk. Benchetrit (21) readily isolated them by holding butter cultures about 2 weeks at 21° C. and plating and picking colonies. *S. lactis* rarely grew on such plates, while the flavor organisms often grew on plates poured with butter cultures that had been held 3 weeks or more. Boekhout and Ott de Vries (24) obtained the flavor bacteria by inoculating from cultures grown 1 week on whey gelatin. Hammer (85) recovered them by repeated transfer of butter cultures on whey agar slopes, where *S. lactis* may be gradually eliminated.

DISTRIBUTION OF THE BUTTER CULTURE ORGANISMS

In addition to being present in butter cultures of satisfactory quality, both the lactic acid and the flavor organisms are rather widely distributed in various other materials.

Lactic acid organisms

When milk leaves the stable it commonly contains *S. lactis,* but the percentage of the total organisms made up of this species is rather small. Utensils and dairy farm surroundings are important sources of *S. lactis* under ordinary conditions, although with adequate precautions contamination from utensils is not significant. The organism also is widely distributed in dairy plant surroundings.

Stark and Sherman (257) isolated 200 cultures of *S. lactis* from various plants, as follows: Corn 82, navy beans 48, cabbage 20, wheat 19, garden peas 18 and head lettuce 13. Also, 35 cultures were isolated from milk. All 235

cultures agreed perfectly with standard descriptions of *S. lactis*. The organism was not found in mouths or throats of cows, bovine feces, human feces or soil, which were previously reported as sources. The authors suggested that plants may represent the natural habitat of *S. lactis* and noted there is ample evidence that *S. lactis* is not a normal inhabitant of bovine udders.

Flavor organisms

S. paracitrovorus has been repeatedly obtained from sour milk and cream; *S. citrovorus* appears to be much less common in these materials. Both species frequently can be recovered from butter, where they often represent organisms from the butter culture used. They also are encountered in various cheeses.

Hammer (87) noted that when cultures of *S. lactis* were carried in butter plants they sometimes developed into satisfactory butter cultures due to contamination with the flavor organisms; this suggests a wide distribution of the organisms in butter plants. Vas and Csiszar (292) isolated flavor organisms from spontaneously soured milk which showed a positive aroma reaction (Voges-Proskauer test and determination of volatile acid) by plating on whey agar.

Orla-Jensen (190) reported that the organisms which he includes in the genus *Betacoccus* are found on green vegetable matter and juicy roots and are widely distributed on beets. Hucker and Pederson (120) isolated organisms which they include in the genus *Leuconostoc* from slimy sugar solutions and fermenting vegetables as well as from milk and milk products. They stated that earlier investigators studying fermenting vegetables (sauerkraut, pickles, tomato products, etc.), those working with milk and milk products (butter cultures, ripening cheeses, etc.) and those studying organisms from sugar factories were dealing with bacteria which are identical or closely related.

Apparently both the lactic acid and flavor organisms are widely distributed on dairy farms and in the surroundings of dairy plants.

GENERAL FACTORS INFLUENCING GROWTH OF BUTTER CULTURE SPECIES

Various factors that influence growth of the butter culture organisms are of significance in considering the bacteriology of the cultures.

Lactic acid organisms

Growth requirements. Knudsen (138) reported that the lactic acid bacteria do not require a medium with a high buffer action. Yeast autolysate was a good source of nitrogen, but peptone was not; milk was considered a poor source, especially for non-proteolytic types. Davis and Mattick (61) stated that the true lactic acid bacteria do not grow in a medium in which the sole source of nitrogen is an amino acid or an ammonium salt. Presence

of yeast extract, marmite or peptone did not permit utilization of such simple nitrogen. The streptococci exhibited most rapid growth with those peptones containing the largest amounts of the higher fractions; they thrived best on peptic casein digest which was about 15 days old, further digestion impoverishing the growth.

Kluyver (135) also noted that the lactic acid bacteria demand a complex mixture of protein decomposition products and that their nitrogen requirements are not fully satisfied by inorganic ammonium salts. The degree to which a substrate was fermented depended on the concentration of the nitrogen source in the medium. Addition of yeast protein decomposition products often was essential to complete the nutritive values of casein media. Crossley (47) reported that activity of lactic acid streptococci is largely dependent on the nitrogen source available. Palladina (199) stated that the nitrogen requirements are uniform for all lactic acid bacteria but that their accessory food requirements differ. The homofermentative group grew well in milk and poorly on an inorganic medium with peptone and glucose.

Braz and Allen (28) found that cultures of *S. lactis* and *S. cremoris* which curdle plain milk rapidly do not form appreciably more acid in milk to which yeast extract is added. Acid continued to be produced long after the cultures were dead.

According to Orla-Jensen (193) lactic acid bacteria require an activator similar to lactoflavin and bios for their development; peptone was found to contain lactoflavin and small quantities of bios.

Orla-Jensen and Snog-Kjaer (198) treated milk with carbon to remove the bios substances and tested various substitutes for their effect on growth of lactic acid organisms. The best substitute was nicotinamide plus nucleic acid; these did not complete a synthetic medium even with addition of pantothenic acid, and the authors concluded that milk bios contains still other unknown substances. The investigators (196) also found that bios of milk could be partially replaced with nicotinamide and adinosinephosphoric acid in cultivation of streptococci but not in cultivation of rod-shaped lactic acid bacteria. Vitamins B_1 and B_6 had no influence on the lactic acid bacteria. Growth stimulating actions of various plant and animal extracts also were studied (197). Action was due to riboflavin contents rather than to bios contents. Extracts of alfalfa were most active, followed by those of tomato, malt, liver and pancreas. Activation by riboflavin and bios was enhanced by the presence of certain amino acids, especially cysteine, asparagine and lysine. Besides the known thermolabile bactericidal substances in milk, it appeared there are at least two thermostable substances which depress growth of lactic acid bacteria.

Davis (59) found that serum and blood did not have a marked effect on growth of lactic acid bacteria in milk, although certain types appeared

to be specifically stimulated or inhibited. Heated serum, and to a greater extent heated blood, usually stimulated growth. He (58) also noted that sucrose and maltose behave like glucose in accelerating the growth rate of lactic acid bacteria in milk. Sucrose was greatly superior to maltose but never superior to glucose. Citrate was without significant effect. Sterilization of yeast and glucose in milk produced a medium superior to milk plus separately heated yeast and glucose. On the basis of ability to accelerate growth, plant extracts rated as follows: Yeast autolysed for 6 days, malt beer wort, potato, alfalfa, carrot, tomato, clover, yeast autolysed for 2 days and bean. Normal variation in the contents of vitamins B_1 and B_2 and co-carboxylase in milk did not appear to affect the growth rate of butter culture organisms.

Snell and Mitchell (252) noted that adenine stimulated growth and appeared essential for *S. lactis;* thymine was essential. In general, naturally occurring amino derivatives of the purine or pyrimidine bases were replaceable by the corresponding oxy-derivatives, but the latter never gave as good growth as the preferred purine.

Growth temperatures. Various investigators have reported that the optimum growth temperature of the lactic acid streptococci of butter cultures is 25° to 30° C. (140, 142, 192). Knudsen (140) found the organisms grow well at 10° C. Jones (126) stated that the lactic acid group which sours milk grows best at 20° to 22° C., while udder streptococci prefer 38° C. None of the *S. lactis* cultures studied by Sherman and Stark (244) grew at 45° C., the maximum temperatures ranging from 41° to 43° C. Matuszewski (160) found the generation times for *S. cremoris* and *S. lactis* at 28° to 30° C. were 1 hour 38 minutes and 1 hour 14 minutes, respectively. They were less at 38° to 40° C. than at 28° to 30° C. or 18° to 20° C. Rice (219) reported that *S. lactis* formed lactic acid fastest at 37° C. in the early stages of fermentation; 32° C. was most favorable in the later stages.

Dorn and Rahn (68) obtained the most rapid multiplication of *S. lactis* at approximately 34° C., which was at least 4° C. higher than the optimum temperature for maximum population or acidity. Plate counts were slightly higher at 25° than 30° C., but when the results were corrected for the numbers of cells per chain, 30° C. gave the highest number of individual cells.

Rahn and Bigwood (216) noted that when milk cultures of *S. lactis* were held near 0° C. the number of living cells decreased rather slowly for about 1 month. Viable cells were still present after 3 months, but numbers were reduced to a few thousands per ml. It appeared death was partly due to accumulation of acid but mainly to oxygen. Neutralization and replacement of the free oxygen by nitrogen in the cultures prolonged the viability.

In the trials of Sherman and Naylor (243) cells of *S. lactis* remained young for less than 1 week at 1° C. and then progressively aged, losing

entirely the ability to grow without lag. The authors concluded that young cells of *S. lactis* may become mature without reproduction. Young cells of *S. lactis* did not die as rapidly as mature cells, possibly because of their ability to age at 1° C.

Effect of acid. van Dam (284) stated that action of hydrogen ions or of undissociated lactic acid can check the lactic acid fermentation. This was demonstrated by adding soluble lactate to milk, whereby dissociation of lactic acid was depressed and concentration of undissociated molecules increased. Under these conditions the final hydrogen ion concentration remained lower than that necessary to check the fermentation (10×10^{-5}N). When hydrochloric acid was added to milk before inoculation so that only a certain amount of lactic acid would be formed, the final hydrogen ion concentration reached 10 to 20×10^{-5}N, and the concentration of undissociated molecules remained below that necessary to check the fermentation. van Dam pointed out that in studying the biologic properties of lactic acid bacteria, composition of the culture media, principally the buffer action, must be considered.

Svanberg (266) noted that *S. lactis* is sensitive to lactic and acetic acids, lactic acid checking growth in milk at pH 4.4 to 4.7 and acetic acid at pH 4.8 to 5.1. Hydrochloric and phosphoric acids restricted growth at pH values of 3.1 and 3.4, respectively. The organism was not alkali-tolerant and growth was subdued by pH 7.9. Undissociated lactic acid was believed to have an important influence in checking fermentation by all lactic acid bacteria. With *S. lactis* the enzyme-protoplasm complex was said to resist a hydrogen ion concentration of 0.01 N. Later, Svanberg (267) found that optimum growth of *S. lactis* was between pH 5.5 and 6.4; the rate decreased markedly when the pH was lower.

According to Le Gallic (149) the natural lactic fermentation due to *S. lactis* is stopped by an excess of acid; if the acid is neutralized fermentation continues. van Beynum (278) noted that the effect of lactic acid on growth of lactic acid bacteria in milk depends on the concentration. No growth took place above a certain acid concentration which varied with different strains. The concentration inhibiting growth was not as high as the final concentration, the last phase of the acid fermentation apparently being due to enzymatic action.

Sherman and Stark (244) found *S. lactis* cultures were entirely inhibited by pH 9.6.

Character of milk. In general, milk is a satisfactory medium for growth of the lactic acid streptococci, but variations in the product are of significance in this connection.

Heinemann and Glenn (109) noted that *S. lactis* was unlike certain other organisms studied in that it did not decrease in numbers when first inoculated into raw milk but increased at once. Chambers (37) did not

detect a difference between raw milk and milk heated at 85° to 90° C. for 2 minutes in the rate of multiplication of *S. lactis* at 37° C. Sherman and Curran (240) inoculated freshly drawn, aseptic milk with young cultures of *S. lactis* in the period of rapid growth. Controls, using autoclaved milk, also were inoculated. The fresh raw milk showed a slight but definite inhibitory effect on growth of the organism, while no lag phase occurred in the controls. Drewes (69) noted a distinct lag when *S. lactis* and *S. cremoris* were inoculated into raw milk. However, one *S. lactis* strain which showed a particularly high resistance to formalin was entirely resistant to the bactericidal action. Curran (50) found growth of *S. lactis* quite similar in raw Holstein and raw Jersey milk; there appeared to be a slight restraining action during the first 4 hours.

Knudsen and Sorensen (141) reported that different lots of milk had different nutritive properties as evidenced by numbers of organisms and amounts of acid produced by various lactic acid strains. In one comparison Jersey milk showed the highest number of organisms followed by diluted Jersey milk and low testing milk; on addition of 0.005 per cent yeast autolysate, low testing milk showed higher numbers than Jersey milk. Knudsen (139) noted that various lots of milk differed in their ability to grow bacteria; in general, *S. cremoris* grew well in milk, but some cultures were sensitive.

In the trials of Knudsen and Sorensen (142) the lactic acid bacteria grew better the more the milk had been heated. The effect of heating at low temperatures was thought to be due to destruction of bactericidal substances, while the effect at higher temperatures was ascribed to formation of decomposition products which were more easily utilized by the bacteria. Effect of bactericidal substances seemed to vary with different strains of lactic acid bacteria of the same genus. Some strains of bacteria normal to butter cultures grew very poorly in aseptic milk even after it had been sterilized, so there was no effect of bactericidal substances; other strains of the same genera grew very well in the milk. One culture of *S. cremoris* (No. 23) grew very poorly in milk in which another culture (No. 14) grew very well. Small additions of autolysed yeast, hydrolyzed milk, milk digested with molds, etc., caused culture 23 to grow as well as culture 14. Not only were bacterial growth and formation of acid flexible in such milk, but the shape of the cells also deviated from normal. The quantities of autolysed yeast, hydrolyzed milk, etc., which permitted the bacteria to thrive normally were so small the authors thought the action was due to vitamins.

Kelly (131) stated that not all strains of *S. cremoris* grow satisfactorily in high quality milk.

According to Whitehead and Cox (310) milk containing leucocytes in excess of 5 million per ml. gave rise to a rennet curd in which lactic acid

bacteria did not develop normal acidities. Influence on the cells probably was due to phagocytosis. Inhibitory action of the cells was eliminated by heating the milk at 49° to 52° C. for 30 seconds.

Prouty (214) studied the ability of milk from normal and diseased quarters of the udders of three cows to support growth and acid production by *S. lactis*. Milk with an initial pH higher than 6.9 usually failed to support active growth, thus resulting in delayed acid production. Numerous samples with pH values higher than 7.0 showed no appreciable growth of *S. lactis*, whereas normal samples from other quarters showed normal acid development. Adjusting the pH of mastitis milk to that of normal milk only partially prevented delayed acid development. Addition of mastitis milk to normal milk in concentrations as low as 10 per cent had a restraining effect on *S. lactis*. Pasteurizing some samples at 65.6° or 68.3° C. for 30 minutes partially eliminated the retarding influence, but with most samples there was little or no effect.

Rice (219) noted that acid development by *S. lactis* was strongly checked in mastitis milk and even after pasteurization such milk sometimes did not permit normal acid formation. Acid development also was slow in milk from cows late in lactation, but it was slightly increased by addition of 10 to 25 per cent colostrum milk to normal milk.

General resistance. Richet *et al.* (220) found that lactic acid bacteria could be accustomed to potassium arsenate by growing them in bouillon containing it. Copper sulfate tended to shock the organisms and they did not become accustomed to this salt but showed diminishing activity. The organisms became accustomed to cadmium sulfate and cultivation in a medium containing it increased their activity. In general, the toxic salts weakened or activated the organisms, depending on the nature of the salt, but the effect was not permanent. Fouassier (75) noted that presence of copper retarded rate of the lactic acid fermentation in milk.

Barthel (16) stated that bacteria of the *S. lactis* group could be kept alive for at least 9 years without transfer in skimmilk to which calcium carbonate had been added. In sterile garden soil their vitality also was maintained for a long period; cultures which were more than 5 years old and completely dry still showed active bacteria. The morphologic features of the organisms and their ability to ferment lactose did not undergo changes during preservation in calcium carbonate milk or in soil.

In the trials of Farmer and Hammer (73) the maximum periods *S. lactis* cultures in litmus milk could be held at various temperatures and remain as satisfactory for development of butter cultures as cultures regularly transferred at 21° C. were: 1 day at 37° C.; 3 days at 30° C.; 6 days at 21° C.; at least 3 months at 7° C.; and at least 5 months at about – 10° C. Addition of calcium carbonate to the milk greatly increased the period that an *S. lactis* strain remained in a satisfactory condition.

Sherman and Stark (244) found that all of 27 *S. lactis* cultures studied were completely inhibited by 6 per cent sodium chloride in lactose nutrient agar and only 3 (11.1%) grew in the presence of 5.5 per cent.

Nine cultures of lactic acid streptococci were studied by Demeter and Pfundt (67) after cultivation in milk for over 8 years. Their characters were not appreciably changed during that period as indicated by various biochemical reactions.

Tarassuk and Smith (269, 270) noted that rancid milk had an inhibitory effect on growth and acid production by *S. lactis*. Both non-sterilized and sterilized rancid milk inoculated with *S. lactis* showed a lag period somewhat longer than normal. Media with a surface tension of about 35 dynes appeared to be critical for growth of *S. lactis*.

Heat resistance. The lactic acid organisms of butter cultures are easily destroyed by heat. However, certain lactic acid streptococci, of which *S. thermophilus* is an example, are rather heat resistant so that the literature on heat resistance of lactic acid streptococci is somewhat confused.

Peiser (202) found a number of strains of *S. lactis* in milk pasteurized at 63° C. for 20 minutes. They had average thermal death points 5° C. higher in whole milk, 2.5° C. higher in separated milk and 0.5° C. higher in whey than in bouillon. *S. lactis* cultures were isolated from pasteurized milk by Brown and Peiser (34) and the thermal resistance of 12 strains was determined. A variation of 22° C. was obtained; with 10-minute heating the range was from 56° to 78° C. and with 20 minute heating it was from 52° to 74° C. Milk had a protective influence on the organisms and the temperature required to kill them in milk was 3.5° to 4.0° C. higher than in bouillon. Acidifying the milk had no appreciable influence on the thermal resistance.

Slanetz (248) found that five stock cultures of *S. lactis* were killed at 61° C. in 10 minutes. The thermal death point of freshly inoculated cultures varied from 62° to 70° C. for 10 minutes. All the *S. lactis* cultures studied by Sherman and Stark (244) were destroyed by heating at 65° C. for 30 minutes.

Hucker (116) investigated 180 strains of cocci resisting pasteurization. He was unable to find *S. lactis* in any sample of freshly pasteurized milk and stated that this organism did not survive pasteurization.

According to Claydon (39) heating occasionally impaired the ability of *S. lactis* to produce acid in milk, so that cultures reduced litmus milk but did not coagulate it; with such cultures addition of 1 per cent glucose to the milk resulted in coagulation. At 61.7° C. Claydon found the average thermal resistance of five cultures of *S. lactis* grown at 21° C. for 2 days was 5.0 minutes as evidenced by coagulation of milk and 5.4 minutes as shown by reduction of litmus; the resistance of five cultures grown at 37° C. for 2 days was 4.6 minutes and 6.0 minutes, respectively. *S. lactis* cultures grown at 10° C. were more heat resistant than cultures grown at higher

temperatures. The highest resistance obtained with an organism grown at 10° C. was 13 minutes (at 61.7° C.). Thermal resistance of *S. lactis* cultures increased with age to a maximum and then decreased. Rates of increase or decrease were influenced by growth temperatures, being slower at lower temperatures. Thermal resistance of *S. lactis* in milk was decreased by lowering the pH with added lactic acid. Sudden changes in growth temperature of *S. lactis* (from 37° to 10° C. and from 10° to 37° C.), while sometimes affecting the rate of development, had no significant influence on thermal resistance.

On the basis of the present ideas with reference to classification of the group of lactic acid streptococci, it appears that the heat resistant cultures isolated from pasteurized milk by certain investigators were not *S. lactis* but rather *S. thermophilus* or some related species.

Flavor organisms

Growth requirements. Boekhout and Ott de Vries (24) noted that simple compounds are not suitable as nitrogen sources for the flavor organisms. Maddock (157) found beer wort increased the rate of growth of *S. paracitrovorus;* good growth also was obtained on sugar bean agar. Braz and Allen (28) reported that viability of *S. paracitrovorus* was not greatly affected by the presence of yeast extract although increase in acid production was relatively large. According to Palladina (199) the heterofermentative lactic acid streptococci do not grow on inorganic media without activators such as wort, liver extract, etc. Wood *et al.* (329) studied the nutritive requirements of certain heterofermentative lactic acid bacteria and found riboflavin or thiamin and factors occurring in ether extract of yeast extract necessary for maximum growth in an amino acid medium.

Although citric acid is actively broken down by the flavor organisms, van Beynum (278) noted that they grow well in the absence of this acid but that sugar is essential. Previously, Knudsen (139) had reported that the organisms grow poorly on citric acid alone.

Growth temperatures. Hammer (86) found that *S. citrovorus* grew at 21° C. but not at 37° C.; *S. paracitrovorus* grew at 21° and 37° C. According to Boekhout and Ott de Vries (24) the optimum temperature for the flavor organisms was about 21° C.; some varieties did not grow at 31° C. Knudsen (139) and also Knudsen and Sorensen (142) stated that the organisms grew best at 25° to 30° C. Orla-Jensen (192) noted that they developed at temperatures as low as 5° C.; some species grew better at room temperature than at 30° C.

Effect of acid. Hammer (89) found the flavor organisms very acid-tolerant; they were most active late in the ripening period of butter cultures and frequently were present in culture that had been allowed to stand until it no longer contained living *S. lactis.* Knudsen (140) stated that in butter

cultures allowed to stand the flavor organisms were found long after the lactic acid organisms had died.

According to Boekhout and Ott de Vries (24) the flavor bacteria tolerate small quantities of lactic acid but are sensitive to such amounts as 0.35 per cent. Knudsen and Sorensen (142) believed that they could tolerate only certain amounts of acid and that lactic acid organisms may, by formation of acid, limit the growth. Flavor organisms appeared to thrive best in symbiosis with lactic acid organisms which formed acid slowly. That the rate of acid formation by various strains of lactic acid bacteria may influence the growth rate of the flavor bacteria also was suggested by van Beynum and Pette (280, 281).

van Beynum (279) stated that the flavor bacteria show some growth in butter culture of high acidity. The growth limit for them was said to be the final acidity of butter culture.

Davis and Thiel (64) found the optimum pH for growth of *S. paracitrovorus* was between 5.5 and 7.5 and for *S. citrovorus* between 4.5 and 7.0. In bouillon Orla-Jensen and Faulenborg (194) noted that the optimum, maximum and minimum pH values for growth of flavor organisms were 6.5, 7.5 and 4.75, respectively; in milk the optimum was 5.7.

General resistance. Farmer and Hammer (73) found that at room temperature litmus milk cultures of flavor organisms remained satisfactory for development of butter cultures at least 8 months in sealed test tubes and about 4 months, or until shortly before the cultures were completely dry, in test tubes permitting evaporation. With calcium carbonate added to the milk, the cultures still were satisfactory after 1 year.

Grimes (84) noted that *S. paracitrovorus* resisted cold storage conditions.

Heat resistance. Boekhout and Ott de Vries (24) stated that the flavor organisms are killed at 53.5° to 57° C. in 10 minutes. According to Grimes (84) *S. paracitrovorus* resisted 62.8° C. for 25 minutes.

Isolation of *S. paracitrovorus* from pasteurized material, such as sweet cream, suggested to Hammer and Baker (97) that at least certain organisms of this group are rather resistant to heat. Fifty-four cultures of *S. paracitrovorus* were heated 30 minutes at various temperatures; 33 cultures (61.1%) survived 60° C. and 16 (29.6%), 3 (5.6%), and 1 (1.9%) survived 65°, 70° and 75° C., respectively.

GENERAL RELATIONSHIPS OF THE TWO TYPES OF ORGANISMS IN BUTTER CULTURES

The numbers of bacteria in active butter cultures, as determined by plate counts under favorable growth conditions, commonly are in the hundreds of millions and may be over 1 billion per ml. In general, lactic acid organisms are more numerous than flavor organisms; this is evident from the early

report of Hammer and Bailey (96) that gradual dilution of a butter culture yielded cultures which readily coagulated milk but which produced little volatile acid in it, essentially the same as that produced by *S. lactis*. In the cultures studied by Hammer (85), *S. lactis* often made up 90 per cent of the flora and only occasionally fell under 75 per cent; in certain cases the flavor type made up only 1 to 3 per cent of the flora. Orla-Jensen *et al.* (195) reported that flavor organisms occur very scantily in butter cultures in proportion to *S. cremoris;* Knudsen (140) noted that they constitute only 10 per cent of the bacteria in butter cultures. Palladina *et al.* (200) divided the organisms in butter cultures into diplo-streptococci and streptococci; the former made up 70 to 90 per cent of the total number and the latter 10 to 30 per cent.

Farmer and Hammer (73) found that, regardless of the comparative numbers of the two types of bacteria in the original mixture of organisms, combinations of *S. lactis* and the flavor type established the same general relationship so that the same flavor was developed.

Baker (9) determined the approximate numbers of each type of organism in butter cultures by inoculating varying amounts into flasks of sterile milk and, after incubation at 21° C., observing for coagulation to detect *S. lactis* and determining volatile acid to detect flavor organisms. The approximate numbers of lactic acid bacteria were higher than the approximate numbers of flavor bacteria. In general, the numbers of flavor organisms were higher in satisfactory than in unsatisfactory cultures, but there were wide variations in numbers, as well as ratios, of the two types of organisms in both satisfactory and unsatisfactory cultures.

When the lactic acid and flavor organisms grow in combination in butter cultures, each type undoubtedly has an influence on the other. From the standpoint of flavor of the cultures, the most striking influence probably is the effect of acidity changes caused by the lactic acid type on the compounds produced from citric acid by the flavor type.

Results of Ayers and Mudge (7) indicate that *S. lactis* converts the nitrogenous material of milk into a form more available for the flavor organisms and thus increases their growth. Knudsen (140) suggested that since butter cultures are cultivated in ordinary milk in which the flavor organisms do not display any noteworthy activity, their great effect can be explained only by assuming a symbiosis between the flavor and lactic acid organisms in which the latter produces degradation products without which the former cannot develop vigorously. Various strains of the two types do not all thrive together equally well. Influence of the lactic acid type on the flavor type is greater than the converse; action of the former was believed to be very complex.

Hammer (85) reported that inoculation of a flavor organism into milk some time before *S. lactis* may delay coagulation of the milk by *S. lactis;*

such a relationship suggests that the flavor type may form products which tend to inhibit *S. lactis*.

FLAVOR OF BUTTER CULTURES

Properly prepared butter cultures have a conspicuous, characteristic flavor[4] that is difficult to describe and very distinct from the flavor of the usual spontaneously soured milk. The aroma obviously is due to a combination of odors among which the experienced observer can detect one that is mildly acid and another which is now recognized as due to the highly volatile diketone, diacetyl; still other less conspicuous odors also may be involved. The taste is conspicuously acid.

VARIATIONS IN BUTTER CULTURES

While the usual butter cultures are all essentially the same in types of organisms present and in products formed during the ripening, there are differences between them which are of practical significance. Some cultures produce acid relatively slowly and, when ripe, commonly have a delicate, pleasing flavor. Such cultures are especially useful for flavor production in sweet cream butter, and certain consumers prefer culture buttermilk made with them. In general, they require considerable care in propagation if irregularities from day to day are to be avoided. Other cultures produce acid relatively rapidly and, when ripe, tend to have a flavor that is coarse compared to the delicate flavor of the slower cultures. Such cultures often are desired for various cheeses, since the active acid production speeds up the making process, and for butter of certain types; some consumers prefer culture buttermilk made with them. In general, the cultures are carried with less difficulty than the slower cultures.

CITRIC ACID CONTENT OF MILK

Milk contains more lactose (about 4.8 per cent) than can be used by the lactic acid organisms, but the content of citric acid, which is the important constituent attacked by the flavor organisms of butter cultures, is rather limited. Thus the citric acid content of milk is of significance from the standpoint of butter cultures. Commonly, the acid is assumed to exist in milk as salts.

Henkel (110) isolated and identified citric acid as a normal constituent of cows' milk. Identification was based on elementary composition of the crystals, titration value, calcium content of the calcium salt, water of crystallization, melting point, solubility in ordinary solvents and positive test with the Sabanin-Laskowski reaction.

Following studies with goats, Scheibe (232) stated that citric acid in the milk was not obtained from citric acid or other organic acids in the feed

[4] Flavor is used to include both aroma and taste.

for the following reasons: Citric acid is found in human milk and this can hardly have its origin in hay or roots; doses of citric acid up to 40 g. per day did not increase the amount in the milk; feeding wheat or bread, which do not contain citric acid, resulted in the normal amount of the acid in the milk; and starvation or limited feeding also gave the normal amount.

Sommer and Hart (253) found approximately 0.2 per cent citric acid in milk. The acid was not destroyed or changed to an insoluble form by heating at 15 pounds pressure for 1 hour.

Hess *et al.* (111) noted that cows on pasture fodder gave milk with a citric acid content over 50 per cent greater than cows on dry fodder, the values being 0.13 and 0.08 per cent, respectively; the higher citric acid content was explained by the greater amount of citrates in green fodder.

From 0.121 to 0.182 per cent citric acid was found by Supplee and Bellis (262) in milk from individual cows receiving the same feed. The average citric acid content of milk from all cows on a winter ration was 0.142 per cent and of that from all cows on pasture was 0.148 per cent. The citric acid content of milk was not affected during manufacture of evaporated, condensed or dry milk.

Kickinger (133) reported that the citric acid content of pasteurized or boiled milk was the same as that of fresh milk. In milk intermittently sterilized, there was a noticeable decrease in the content compared to fresh milk; after the third heating the citric acid content remained constant. Mussill (178) stated that the citric acid content of germ-free milk is altered by either sterilization or holding.

Kieferle *et al.* (134) compared the citric acid contents of milk from individual quarters of the udders of 36 cows. With one exception, values ranged from 0.20 to 0.40 per cent; there appeared to be less variation between samples from different quarters of the same cow than between samples from different cows.

Results of 335 citric acid determinations on milk by Sherwood and Hammer (245) showed that individual animals of various breeds gave milk with citric acid contents ranging from 0.07 to 0.33 per cent and averaging 0.18 per cent. Breed, time of milking, stage of lactation and season of year had no significant effect. Cream from two sources failed to show a seasonal variation in citric acid content. Hartman and Hillig (105) presented data which suggest that the citric acid content of fluid whole milk is fairly uniform; the average value was 0.16 per cent.

In the tests of Rumments (230) the average citric acid content of milk was 0.213 per cent; milk from the same cows toward the end of lactation averaged 0.134 per cent citric acid and varied from 0.038 to 0.180 per cent. Rumments considered that the citric acid in milk is in combination with one or more organic compounds rather than in the form of salts.

Allen (2) reviewed the literature with reference to the mineral constitu-

ents and citric acid contents of milk. Methods of analysis, occurrence in milk and importance of citric acid in dairy problems were included.

While the citric acid content of milk shows some variation, it appears that herd milk commonly contains from 0.16 to 0.18 per cent.

SPECIFIC CHEMICAL COMPOUNDS OF SIGNIFICANCE IN BUTTER CULTURES

Studies on butter cultures indicate that a number of specific chemical compounds are of significance from the standpoint of flavor development in such cultures. Additional studies may extend the list. The more important of the compounds now recognized are:

(*a*) Lactic acid ($CH_3 \cdot CHOH \cdot COOH$): A non-volatile acid which is odorless so that it cannot affect the aroma of butter cultures although it is largely responsible for the acid taste.

(*b*) Acetic and propionic acids ($CH_3 \cdot COOH$, $CH_3 \cdot CH_2 \cdot COOH$): Volatile acids that affect both the aroma and taste of butter cultures.

(*c*) Carbon dioxide (CO_2): A gas which affects the taste of butter cultures just as it affects the taste of various carbonated beverages.

(*d*) Diacetyl ($CH_3 \cdot CO \cdot CO \cdot CH_3$): A diketone which has a high aroma and affects the aroma of butter cultures even when present in very small amounts. In low concentrations it is pleasing and definitely suggests the aroma of butter cultures; in high concentrations the odor is pungent and rather objectionable (108, 201, 205, 209, 268).

(*e*) Acetylmethylcarbinol ($CH_3 \cdot CHOH \cdot CO \cdot CH_3$): Properly purified, this compound has no odor. It is important in butter cultures because presumably it is the substance from which diacetyl is produced through biologic oxidation. It probably is never present in butter cultures in amounts that significantly affect the taste (108, 156, 209).

(*f*) 2,3-butylene glycol ($CH_3 \cdot CHOH \cdot CHOH \cdot CH_3$): Reduction of acetylmethylcarbinol leads to the formation of 2,3-butylene glycol. Properly purified, it has no odor, and it probably is never present in amounts sufficient to affect the taste (102, 108).

As with the acid compounds in butter cultures, the neutral compounds—diacetyl (ac_2), acetylmethylcarbinol (amc) and 2,3-butylene glycol (2,3-bg)—are not characteristic of butter cultures but are present in various materials; the following brief summary illustrates this: Artichokes—ac_2, amc (38); beer, dark—ac_2 (235); bread—ac_2, amc (144, 145, 276, 277, 300); butyl alcohol fermentation—amc (326); coffee, roasted—ac_2 (235); grains, germinating—amc, 2,3-bg (151); honey—ac_2 (235); molasses, cane—ac_2 (325); pea pulp—ac_2, amc (38); products of pepsin action on milk—ac_2 (38); raspberry juice—ac_2 (40); rennet—ac_2 (221); strawberry juice—ac_2 (41); sugar cane juice, fermented—amc (36); tobacco smoke—ac_2 (233, 235); vinegar—amc (11, 35). One or more of the compounds are produced by many different bacterial species (77, 114, 115).

PRODUCTION OF VARIOUS COMPOUNDS BY BUTTER CULTURES GROWING IN MILK

Production of acid

At favorable temperatures butter cultures normally produce acid in milk relatively rapidly, the acid being largely lactic acid which is formed from lactose through activity of the lactic acid organisms. When sufficient acid has developed, usually from 0.5 to 0.65 per cent[5] with different lots of milk, coagulation occurs. Acid production continues until well-ripened cultures commonly contain from 0.8 to 1.0 per cent or more acid and have pH values of 4.3 to 4.7. Butter cultures vary considerably in their rates of acid production because of differences in the strains of organisms present.

Role of lactic acid organisms. The lactic acid organisms usually produce from 0.7 to 1.0 per cent acid in milk (208), with the maximum about 1.2 per cent; most of the acid is lactic. Unless cultures are transferred frequently, they may decrease in activity, probably as a result of continued exposure to relatively high acid. From butter culture, sour cream, etc., it is common to obtain organisms that apparently are slow acid-producing strains of *S. lactis;* some of these never coagulate milk at ordinary temperatures.

Suzuki *et al.* (265) stated that the group of bacteria represented by *S. lactis* produces from 90 to 98 per cent of the theoretic yield of lactic acid from the sugar fermented, the remainder of the sugar going to alcohols, aldehydes and esters.

Van Slyke and Bosworth (287) "pasteurized and separated fresh milk" and inoculated it with *S. lactis.* The milk was kept at 32.2° C., and at intervals samples were taken and at once chloroformed. Fermentation of lactose was slow during the initial period, most of the change taking place between the 10th and 25th hours. The percentage of fermented lactose that was changed to lactic acid varied from 70 to 93 per cent. After 96 hours 20 per cent of the original lactose was fermented.

With 50 cultures of lactic acid streptococci, Sherman and Albus (239) found the acid produced in milk (10 days at 35° C.) ranged from 0.60 to 0.95 per cent, the value most frequently encountered being 0.80 per cent. Evans (71) noted considerable variation in final pH values in different broths with any one strain of *S. lactis* and also in final values in any one broth with different strains; the values ranged from 4.0 to 5.0. Svanberg (266) reported that the pH produced by *S. lactis* in milk or whey was 4.0. When milk was acidified with hydrochloric, sulfuric or phosphoric acid before inoculation, values down to 3.6 were obtained.

Jersey milk, diluted Jersey milk and low testing milk were inoculated with lactic acid streptococci by Knudsen and Sorensen (141) and portions incubated at 20° and 30° C. for 1 day. Most acid was produced in the

[5] Total acidities in milk commonly are calculated as lactic acid.

Jersey milk and for acid production diluted Jersey milk was superior to the low testing milk. Amounts of acid produced by various cultures differed considerably. Hydrolyzed milk added to regular milk in concentrations of 0.1 to 1.0 per cent slightly increased acid production.

A relationship between rate of growth and total acid production (acid tolerance) of *S. lactis* in milk was noted by Sherman and Hodge (241). When flasks were inoculated with a milk culture in varying amounts, more acid was produced in the flasks receiving the largest inoculations; the suggested explanation is that high dilutions of the original culture contained only the most rapidly growing strains which could not endure high acid. A culture of *S. lactis* grown for a time in milk with very frequent transfer had lower acid-producing power than the original culture, and acidities produced with varying inoculations were more nearly the same; the suggested reason is that slow-growing strains which produced the most acid were eliminated by frequent transfer, and the resulting culture contained only fast growing strains which produced relatively little acid.

Okulitch and Eagles (184) studied the effect of certain fermentable materials on rate and amount of acid production in milk by a vigorous *S. cremoris* culture. Glucose and salicin exhibited the strongest inhibitory effect, the organism completely losing its ability to ferment lactose in milk or broth after 11 serial transfers. With mannose and fructose, 15 and 18 transfers, respectively, were required for complete inhibition of lactose fermentation. Galactose and lactose had no effect. The authors suggested that loss of lactose-fermenting ability was due to inhibition of lactase by a product formed when the organism was grown on a specific material or that this product served to so alter metabolism of the organism that lactase was not elaborated.

Foter and Rahn (74) noted that when large numbers of *Streptococcus* cells were placed in milk at low temperatures, lactic acid fermentation occurred, even at 0° C., with all species tested. Rate of fermentation was greatly reduced at 0° C., more so with species which did not grow there than with those which did. With *S. lactis*, which does not grow at 0° C., the enzyme content was distinctly decreased by holding 1 week at 0° C. When held 4 to 8 weeks, the cells required a number of generations before they came back to their original fermenting capacity. Total acid produced by each species was lowest at 0° C. and increased with temperature; the difference was smaller with those species which grew at 0° C. but was noticeable in all cases and could not be explained by deterioration of the enzyme. No explanation could be given for the fact that *S. fecalis* and *Streptococcus glycerinaceus* grew at 0° C., while *S. lactis* and *Lactobacillus acidophilus* died at this temperature and multiplied very slowly at 5° C. The amount of lactose consumed during the doubling of one cell was constant at low and medium temperatures but increased toward the optimum temperature. *S.*

lactis required twice as much lactose as *S. fecalis,* and *L. acidophilus* needed ten times as much, to double one cell.

In studies by Rahn *et al.* (217) rate of lactic fermentation was measured by titration, using cells obtained by centrifuging and suspending in buffered glucose solution. Fermentation was most active at pH 7.0. Deviation to the alkaline side retarded fermentation less than deviation to the acid side. With more than 0.2 per cent glucose present, the fermentation rate was not affected by sugar concentration. One per cent sodium lactate slightly retarded it and 4 per cent reduced it to less than half the normal. Continued agitation by a current of air or oxygen retarded fermentation, while nitrogen increased it. Little difference was found between cells of different ages (2 hours to 2 days) when they were kept young by frequent transfer. However, if old cells were transferred to fresh medium, fermenting capacity was doubled when the cells came out of lag and decreased again after the number of cells had doubled. Centrifuged cells could be stored in phosphate buffer at 2° C. for 4 days without loss of fermenting capacity and with very little loss in viability.

Dorn and Rahn (68) noted that with two strains of *S. lactis* the maximum acidity was reached at 30° C. When cells were grown in broth, centrifuged and suspended in buffer solution with 2 per cent glucose, the enzyme producing the lactic acid behaved like all other enzymes with respect to temperature. Fermentation was very rapid at temperatures far above the maximum for growth, but decreased rapidly because of deterioration of the enzyme. At lower temperatures the rate remained constant until the acid affected the pH of the buffer. The authors stated that the results obtained are not in agreement with those of growing milk cultures. It appeared that cells grown at different temperatures contained different amounts of enzyme and therefore produced different amounts of acid per cell per hour.

Rahn and Bigwood (216) reported that early cessation of fermentation when large numbers of cells are kept in milk at 0° C. (or any temperature below the minimum for growth) is due to some damaging effect of oxygen which the inactivated growth mechanism cannot prevent or repair. Enzyme content of the cells, as measured by their fermenting capacity, slowly decreased at temperatures below the growth minimum. After restoration to higher temperatures, the cells recovered their enzyme content very slowly and required several generations for complete recovery.

Role of flavor organisms. The flavor organisms produce variable quantities of acid in milk, but even strains forming the largest amounts develop it slowly compared to *S. lactis;* all strains produce some volatile acid, while certain strains also form some lactic acid from lactose.

Boekhout and Ott de Vries (24) reported that acid production in milk by the flavor organisms was very limited; the acid consisted of acetic and small quantities of non-volatile acid. Hammer (86) noted that *S. citro-*

vorus formed little acid in milk; *S. paracitrovorus* produced from 0.39 to 0.77 per cent with most cultures failing to coagulate milk. Orla-Jensen *et al.* (195) also found that the flavor bacteria produced small amounts of lactic acid. Hucker (119) stated that the organisms generally formed small amounts of acid and that lactose was hydrolyzed faster than lactic acid was formed.

Six strains of *S. paracitrovorus* which had been repeatedly grown in sterile milk were inoculated into pasteurized milk by Maddock (157). Acidities after 24 hours at 22° C. ranged from 0.26 to 0.82 per cent and after 48 hours from 0.86 to 0.94 per cent. The average acidity produced in cream was 0.7 per cent for the cultures and 0.9 per cent for butter cultures.

Palladina *et al.* (200) found that 23 cultures of *S. paracitrovorus* grown in plain milk for 24 hours produced[6] from 0.19 to 0.37 per cent acid with an average of 0.31 per cent; in milk plus yeast autolysate acidities ranged from 0.31 to 0.55 per cent and averaged 0.44 per cent. Eighteen cultures of *S. citrovorus* in plain milk produced acidities ranging from 0.19 to 0.58 per cent and averaging 0.35 per cent; in milk plus yeast autolysate acidities ranged from 0.19 to 0.77 per cent and averaged 0.53 per cent.

When 10 to 50 per cent yeast extract was added to milk, Orla-Jensen *et al.* (195) found the flavor organisms formed as much lactic acid as other streptococci. Some freshly isolated strains coagulated milk when 1 per cent yeast extract was added. Effect of the extract was believed to be due to vitamins. Vas and Csiszar (288) obtained an increase in acid production by growth of the flavor organisms in carefully heated yeast extract milk or by repeated inoculations of large quantities of culture. Braz and Allen (28) noted that yeast extract added to milk increased lactic acid production by *S. paracitrovorus.*

Thiel (274) reported that *S. citrovorus* and *S. paracitrovorus* formed more lactic acid at 19° than at 32° C. under both aerobic and anaerobic conditions.

Isomeric form of lactic acid produced

The lactic acid in butter cultures commonly includes considerable *d* and some *i* acid.

Hammer (86) noted that butter cultures having relatively high acidities always contained some *i* lactic acid and proposed this as proof that such cultures are not pure cultures of *S. lactis.* At low acidities the cultures sometimes contained only *d* acid. Mixtures of *S. lactis* and a flavor organism regularly developed some *i* acid; when action of the latter type was increased by inoculating it some time before *S. lactis,* more *i* acid was formed. Certain of the data suggested, but did not prove, that the flavor organisms can change part of the *d* acid produced by *S. lactis* to *l* acid. Knudsen (140)

[6] Original data recalculated to per cent lactic acid, using 1.032 as sp. gr. of original milk.

found the lactic acid in butter cultures was largely *d* but included some *l;* accordingly, *i* acid was present.

Role of individual species. Both *S. lactis* (86, 87, 119, 139, 191) and *S. cremoris* (140) produce *d* lactic acid.

The flavor organisms which yield appreciable quantities of lactic acid produce the *l* form (86, 119, 142). Organisms of the genus *Betacoccus,* in which the flavor bacteria sometimes are included, generally produce *l* lactic acid, *i* acid being rare (191, 192, 195).

Production of volatile acid

The important observation leading to detection of the flavor organisms in butter cultures was the high volatile acidities of such cultures compared to *S. lactis* cultures. The high volatile acidities of butter cultures are evident from the odor and are readily detected by chemical procedures.

Hammer and Bailey (96) found the volatile acidities of butter cultures ranged from 31.2 to 37.6 (ml. N/10 NaOH to neutralize 1 l. steam distillate from 250 g. culture); the total acidities varied from 0.87 to 1.08 per cent. With a low volatile acidity flavor was lacking, but some cultures had a high volatile acidity and poor flavor. At 37° C. volatile acid production was low; butter cultures carried there for a number of transfers and then returned to 20° C. continued to show low volatile acidities. When *S. lactis* and a flavor organism were combined, the volatile acidities ranged from 28.0 to 39.2.

Cordes and Hammer (42) noted that the volatile acidity of a butter culture increased as the total acidity increased until, in general, it reached 10 to 15 per cent of the total acidity. The increase in percentage of total acid that was made up of volatile acid indicated that activity of the flavor organisms was greater late in the ripening period; colonies picked from plates showed that the organisms made up a larger percentage of the total flora late in the ripening.

Total and volatile acidities of a culture incubated at 21° C. were determined at periods from 15 to 39 hours by Lind (152). Both increased as the incubation period increased. A certain total acidity was required before a high volatile acidity was produced. When butter cultures were steam distilled without addition of sulfuric acid, the volatile acid values were lower than with the sulfuric acid.

With 183 butter cultures grown in pasteurized milk without addition of citric acid or citrate, Templeton and Sommer (272) found the volatile acidities averaged 15.43 per cent of the total acidities; with 32 cultures grown in sterilized milk plus 0.2 per cent citric acid the value was 22.42 per cent; with 28 cultures grown in pasteurized milk plus 0.2 per cent citric acid the value was 23.10 per cent; and with 60 cultures grown in pasteurized milk plus 0.2 per cent citric acid added as sodium citrate the value was 22.21 per cent.

Ritter and Christen (224) determined the volatile acidities produced by four cultures after incubation for 8 to 66 hours. The values increased for 32, 66, 66 and 27 hours, and the final volatile acidities were 0.75, 1.00, 1.00 and 0.80 (ml. N/20 NaOH to neutralize 10 ml. steam distillate from 50 ml. culture). With each culture an increase in volatile acid occurred with addition of citric acid and also for each time interval at which determinations were made. The authors noted that occasionally the volatile acid in a butter culture increased without apparent effect on the flavor.

Cultures were transferred for long periods by Ritter and Nussbaumer (225) without them undergoing appreciable changes. However, an alteration might suddenly take place which was characterized by an increase in volatile acid and a simultaneous decrease in diacetyl and acetylmethylcarbinol. Attempts to bring about the change artificially were not successful. Vas and Csiszar (289) noted that high acidity was coupled with larger amounts of volatile acid than low acidity. Ritter (222) stated that a volatile acidity equivalent to 0.8 (ml. N/20 NaOH to neutralize 10 ml. steam distillate from 50 ml. culture) may be regarded as satisfactory; a value of 1.2 was taken as a criterion that acetylmethylcarbinol and diacetyl were declining in favor of volatile acid and butylene glycol. Brewer *et al.* (31) found that, under various conditions, stirring a culture gave higher volatile acidities than aeration. Gibshman (78) reported that cultures 10 to 20 hours old produced the most volatile acid. No correlation was observed between production of volatile acid and formation of acetylmethylcarbinol plus diacetyl. Volatile acid accumulated in large quantities at temperatures lower than 25° to 30° C.

Boekhout and Ott de Vries (24) found that symbiosis of the two types of butter culture organisms not only yielded flavor but also considerable volatile fatty acid which was acetic; the quantity of the acid was considerably greater than with cultures of a single species. The data of Hammer and Sherwood (101) suggest that the volatile acid of butter cultures is largely acetic with small amounts of propionic. Knudsen (140) noted that good butter cultures contained acetic and propionic acids, with the latter present in very small quantities.

Role of lactic acid organisms. The lactic acid organisms produce relatively little volatile acid in milk, definitely less than a satisfactory butter culture; the acid apparently is acetic with some propionic.

Suzuki *et al.* (265) inoculated a medium containing lactose, peptone and barium carbonate with *S. lactis* and incubated it at 35° C. Analyses made after 3 days showed 0.43 ml. N/10 formic acid, 3.88 ml. N/10 acetic acid and 0.00 ml. N/10 propionic acid in 100 ml.; after 56 days the values were 1.68, 8.06 and 0.00, respectively. No butyric or caproic acid was found. When a medium containing calcium carbonate instead of barium carbonate was employed, *S. lactis* formed propionic acid. It also formed

a small amount in another trial in which barium carbonate was used. Barium carbonate interfered with production of volatile fatty acids; it exerted a depressing influence on the metabolism of the organism.

Evans (71) determined the volatile acidities in milk cultures of five strains of *S. lactis*. Four strains showed between 9.90 and 11.27 ml. N/10 acetic acid in 500 ml. of milk and the other strain showed 7.25 ml. Evans concluded that *S. lactis* produces a small and fairly constant quantity of acetic acid in milk cultures, equivalent to about 0.12 g. per l. of milk. With 35 *S. lactis* strains from good butter cultures, Hammer and Bailey (96) obtained volatile acidities (ml. N/10 NaOH to neutralize 1 l. steam distillate from 250 g. culture) from 4.3 to 8.8 (av. 6.6) and with 15 strains from milk and cheese the values were 4.7 to 18.2 (av. 9.2), only a few being over 10.0; for the 50 cultures the average was 7.4. Results with combinations of the strains indicated that the high volatile acidities of butter cultures are not due to combined action of *S. lactis* strains.

Cordes and Hammer (42) found the percentage of total acid that was volatile was more or less constant with *S. lactis* at various acidities; the volatile acidity never reached the high value of a butter culture.

According to Hammer and Sherwood (101) cultures of *S. lactis* produce volatile acid which is made up of acetic with considerable propionic. Hucker (119) noted that *S. lactis*, as well as most other streptococci associated with milk and dairy products, form volatile acid composed of acetic with traces of propionic or some acid giving distillation constants higher than acetic acid; with *S. lactis* less than 10 per cent of the total acid was volatile.

Palladina *et al.* (200) could not increase production of volatile acid by addition of citric acid to cultures of *S. lactis* or *S. cremoris*.

Role of flavor organisms. At a favorable pH the flavor organisms develop relatively large amounts of volatile acid in milk, and they are primarily responsible for the volatile acid in butter cultures; the acid is largely or entirely acetic.

Hammer (85) reported that *S. citrovorus* and *S. paracitrovorus* produce volatile acid from the citric acid normally present in milk. From the standpoint of volatile acid production, the importance of citric acid normally present in milk, or added during manufacture of butter culture, has been repeatedly confirmed.

Farmer and Hammer (73) found that the volatile acidities (ml. N/10 NaOH to neutralize 1 l. steam distillate from 250 g. culture) produced in milk by 71 strains of flavor organisms varied from 7.1 to 30.0 (av. 20.3), while the values in milk plus 0.4 per cent citric acid ranged from 25.0 to 84.8 (av. 57.3).

At one time it appeared (87) that lactic acid also was a minor source of volatile acid under certain conditions. However, Michaelian and Hammer (167) noted that addition of sulfuric or phosphoric acid to sterile milk

inoculated with a flavor organism gave essentially the same amount of volatile acid as addition of lactic or tartaric acid. Since it is improbable that the inorganic acids can be changed to volatile acid, it appears that citric acid is the actual source of volatile acid and that various acids, including lactic, may change the citrates naturally present in milk to a form from which volatile acid is readily produced. This would explain why *S. paracitrovorus,* which produces some lactic acid, gives more volatile acid in milk than *S. citrovorus,* which does not produce lactic acid. The authors found that the effects of acids in increasing volatile acid production in milk varied greatly with different cultures and was greatest with cultures that formed comparatively small amounts of titrable acid and least with cultures that formed comparatively large amounts.

Stine (258) noted that when sulfuric acid was used to acidulate milk cultures, volatile acid production of the flavor organisms was fairly constant at pH values from 3.8 to 4.2 and then decreased rapidly as the pH decreased. He assumed that the decrease was due to toxicity of the added acid.

Hucker (119) reported that the flavor streptococci produced relatively large amounts of volatile acid, as much as 40 per cent of the total acid being volatile. Percentages of volatile and non-volatile acids remained about the same regardless of the total acid produced. Templeton (271) found that on the average *S. paracitrovorus* in milk produced 34.88 per cent of the total acid as volatile acid, while *S. citrovorus* produced 28.23 per cent.

Volatile acid production of the flavor organisms in milk could not be increased by Michaelian and Hammer (168) through addition of diacetyl, acetylmethylcarbinol or 2,3-butylene glycol. This indicates that when acetylmethylcarbinol plus diacetyl disappears in a butter culture the compounds are not changed to volatile acid.

Gibshman (79) reported that additions of citric, acetic and pyrotartaric acids and, especially diacetyl oxide, increased the formation of volatile acid in milk by *S. citrovorus.*

Hammer and Sherwood (101) noted that the volatile acid produced by *S. citrovorus* or *S. paracitrovorus* in milk or in milk plus citric acid was largely acetic acid. van Beynum (278) found that acetic acid is the only volatile acid produced in cultures of the flavor organisms. The increase in acetic acid paralleled growth of the organisms. The acid did not decrease when aroma was destroyed by the bacteria. A larger amount of acetic acid was obtained in cultures with citric acid added and the amount of acetic acid produced was independent of the presence of lactic acid or lactic acid bacteria. van Beynum and Pette (282) stated that acetic acid is the only volatile acid formed by the flavor organisms when growing in plain or acidified milk.

According to Thiel (274) *S. citrovorus* and *S. paracitrovorus* form more

acetic acid at 19° than at 32° C., under both aerobic and anaerobic conditions.

Production of carbon dioxide

Butter cultures contain carbon dioxide (140), but except in very unusual instances the amounts are too small to result in gas bubbles in the coagulated milk. With certain gas-forming contaminants (*Torula cremoris* and *Torula sphaerica, Escherichia-Aerobacter* species, etc.) present, gas bubbles may be very conspicuous.

Role of individual species. Although the lactic acid organisms develop small amounts of carbon dioxide, most of the gas in butter cultures is formed by the flavor organisms, citric acid being the important source. When citric acid is added to milk from which culture is made, stirring the ripened culture frequently results in vigorous gas evolution.

Orla-Jensen *et al.* (195) noted that freshly isolated cultures of flavor organisms caused vigorous evolution of carbon dioxide in milk containing added citrates; after extended propagation, the cultures lost the ability to produce the gas.

Hucker (118) studied various streptococci and noted that with the exception of *S. citrovorus* and *Streptococcus kefir* they showed an increase in carbon dioxide production as the peptone in the medium was increased. *S. citrovorus* and *S. kefir* produced no carbon dioxide from peptone but formed relatively large amounts from glucose; *S. cremoris* produced only small amounts from peptone and only slightly more on addition of glucose.

According to van Beynum (278) sour cream generally contains carbon dioxide which is formed by the flavor bacteria. The amount produced in milk did not depend on the acidity of the milk; however, velocity of production was changed since addition of lactic acid caused slower bacterial growth. Carbon dioxide production began sooner in plain milk than in acidified milk but required a longer time for completion.

Stine (258) noted that flavor streptococci varied in the production of carbon dioxide in milk with added citric acid. Palladina *et al.* (200) obtained increases in carbon dioxide production with various strains of *S. paracitrovorus* and *S. citrovorus* on addition of citric acid to the milk. van Beynum and Pette (282) stated that carbon dioxide is one of the fermentation products of the flavor organisms, both in plain and acidified milk; it also was noted in mixtures of the organisms and lactic acid streptococci.

With the capillary tube method Hassouna and Allen (106) found that organisms commonly used in butter cultures (*S. lactis, S. cremoris, S. paracitrovorus*), whether in pure or mixed culture, gave no evidence of gas formation in skimmilk, in skimmilk plus yeast extract or in evaporated milk. Small amounts could be detected by a titration method in which carbon dioxide was absorbed by alkali. When *S. paracitrovorus* was inoculated into tins of evaporated milk it produced sufficient gas to bulge the ends of

the tins; on one occasion it produced enough gas in a flask of separated milk to blow the bung.

Davis and Thiel (64) found the optimum initial pH for gas production by *S. paracitrovorus* was 6.0; for *S. citrovorus* it was 5.5.

Production of acetylmethylcarbinol (amc) *and diacetyl* (ac_2) *in butter cultures*

The recognition by van Niel *et al.* (286) that ac_2 is either responsible for the aroma of butter or is the principal component of the aroma material soon led to studies on the production of amc and ac_2 in butter cultures. In the early studies the compounds commonly were determined together (amc + ac_2) gravimetrically as nickel dimethyl glyoximate, while more recently they have been determined separately, usually with colorimetric procedures.[7]

Production of amc + ac_2 frequently is followed by a decrease, often a large one on a percentage basis. Apparently, the decrease is due to reduction of the compounds to 2,3-butylene glycol. Thus the situation definitely is different than with certain compounds produced in butter cultures which evidently are not attacked by the normal butter culture organisms.

Relationship of amc + ac_2 to flavor. Michaelian *et al.* (166) determined the amounts of amc + ac_2 in satisfactory cultures and also in cultures lacking flavor. The results showed that considerable amc + ac_2 was present in satisfactory cultures, while a smaller amount or none was present in cultures lacking flavor. Each of the satisfactory cultures yielded 10.0 mg. or more nickel dimethyl glyoximate per 200 g., the maximum being 39.5 mg.; the maximum for cultures lacking flavor was 7.4 mg.

The relationship between a satisfactory flavor in butter culture and the presence of comparatively large quantities of amc + ac_2 has been repeatedly confirmed. Various commercial plants have used determinations of amc + ac_2 in checking flavor development in butter cultures.

Correlation between acidity and amc + ac_2. From general observations it is evident that butter cultures have very little flavor until considerable acid has been developed. In agreement with this, Michaelian *et al.* (166) found that cultures contained only small amounts of amc + ac_2 during the early stages of ripening, while later conspicuous increases occurred. Early in the ripening pronounced changes in titrable acid or pH had little effect on the amount of amc + ac_2 present, but later striking increases occurred with little or no change in acidity. Vas and Csiszar (289) noted that high acidity in a culture was coupled with larger amounts of aroma (amc + ac_2) than low acidity. Barnicoat (13) reported that with a commercial culture the amount of amc + ac_2 at 0.75 per cent acid was only about one-third that at 0.81 per cent.

[7] The Appendix, Part A, gives a general statement on methods of determining amc and ac_2.

Mohr and Wellm (174) determined the ac_2 contents of various butter cultures grown in skimmilk and found fluctuations on various days even when acidulated to the same degree. Eight cultures treated exactly the same showed ac_2 contents from 1.53 to 2.5 mg. per l.; contents of amc + ac_2 varied from 24.8 to 54.2 mg. per l.[8]

Prill and Hammer (211) noted that addition of sulfuric acid to a ripened culture held at 21.1° C. resulted in increases in ac_2 and in amc, but the increases were considerably less than those in cultures having similar initial pH values as a result of adding citric acid. However, when held in ice the samples with added sulfuric acid often were higher in ac_2 than the corresponding samples with added citric acid. The results with sulfuric acid emphasize the importance of pH from the standpoint of production of ac_2 and amc since sulfuric acid cannot be attacked by the organisms. It was apparent that there is a definite optimum pH for production of ac_2 with sulfuric or citric acid which seems to be lower than the pH developed in a normal butter culture; possibly, the value varies with the temperature.

Destruction of amc + ac_2. Michaelian *et al.* (166) studied destruction of amc + ac_2 using a medium composed of skimmilk and butter culture that had been given a high heat treatment. When the medium was allowed to stand for 20 days at 6° C. the amount of amc + ac_2 remained constant, but when it was inoculated with a butter culture or a flavor organism and similarly held there regularly was a pronounced decrease. Some flavor organisms were less active in the destruction than others. Destruction of amc + ac_2 also was studied with a mixture of milk and butter culture (20%) that had been held at 21° C. until various desired acidities were reached; portions were removed, pasteurized at high temperatures, inoculated with flavor organisms and amc + ac_2 determinations made after 9 days at 21° C. Results showed that there was a pronounced destruction of amc + ac_2 which was especially conspicuous and uniform with the different organisms at the lower acidities. At higher acidities there were variations in the destruction with the different organisms, due presumably to variations in acid tolerance; with all organisms small differences in the higher acidities had a pronounced effect on the destruction. In neutralized butter culture held at 21° C. a rapid decrease in amc + ac_2 occurred; at 0° C. the decrease was very small, due undoubtedly to inability of the organisms to grow actively. A decrease in amc + ac_2 after neutralization commonly was followed by an increase because of reestablishment, through development of acid, of conditions favorable for production. Repeated neutralization resulted in complete disappearance of amc + ac_2.

Davies (51) stated that ac_2 is not stable but is subject to reduction to amc through absorption of oxygen by bacteria or fat and to loss by evapora-

[8] Because of variation in the analytical procedures used and in the results obtained, relatively few actual values for amc + /or ac_2 are included and these are given primarily for purposes of comparison.

tion. Pien *et al.* (205) suggested that the volatility of ac_2 is sufficient to cause a rapid disappearance in cultures. One culture was examined for ac_2 after 24, 48 and 72 hours; contents were 1.0, 0.7 and 0.5 mg. per l., respectively.

Wiley *et al.* (323) found the content of amc in a culture grown at 21° C. reached a maximum a few hours after the content of ac_2 had reached its maximum. After the maximum concentration was reached, destruction of amc was rapid for some hours, but after 60 hours the concentration remained constant for an additional 80 hours. Wiley *et al.* (321) noted that mixed cultures of *S. cremoris* and flavor organisms produced ac_2 rapidly during the period of logarithmic growth rate, but after reaching a peak value the amount fell rapidly and approximately 90 per cent was destroyed in 12 hours.

It was noted by Williams and Morrow (324) that amc is destroyed by certain strains of *Escherichia-Aerobacter* organisms, by the green fluorescent bacteria and by all the aerobic spore formers tested. It was not destroyed by certain representatives of the *Salmonella, Eberthella, Proteus* and *Serratia* groups. Amc was believed to serve as a source of carbon for certain organisms.

Virtanen and Kontio (295) added ac_2 or amc to portions of autoclaved milk, inoculated the milk with various organisms isolated from butter and incubated at 19° to 21° C. for 70 or 160 hours. *Bacillus punctatum* destroyed about 90 per cent of both compounds, while *Bacillus vulgatus* destroyed the same amount of amc but only 40 to 60 per cent of the ac_2. A non-proteolytic coccus and *Pseudomonas fluorescens* destroyed only 9 per cent of the amc but 30 to 50 per cent of the ac_2. A mixture of yeasts destroyed up to 30 per cent of the amc and 30 to 40 per cent of the ac_2.

Source of amc and ac_2. The early studies on production of amc and ac_2 in butter culture indicated the importance of citric acid as a source of the compounds (166) and various later investigations have confirmed the relationship (207, 211, 223, 283, 291). Commonly, addition of citric acid or one of its salts, such as sodium citrate, to the milk used for butter culture greatly increases production of amc and ac_2.

In various instances Ritter and Christen (224) found that the amount of amc + ac_2 was more than 100 per cent greater in a culture with added citric acid than in a culture without the added acid. When sodium citrate was added to one culture, there was an increase of over 300 per cent in 64 hours.

Ruehe (228) obtained the largest amount of amc + ac_2 by adding citric acid when the culture was 24 hours old and then incubating an additional 24 hours.

Prill and Hammer (211) reported that 0.15 per cent citric acid added to the milk intended for butter culture appeared to be a practical amount.

Larger percentages, such as 0.30 per cent, did not give correspondingly higher ac_2 contents unless the ripened culture was held for extended periods with sufficient aeration.

Before the presence of flavor organisms in butter cultures was recognized, production of flavor in the cultures was attributed to a breakdown of the lactose and also to a breakdown of the protein. Rather recently certain investigators have contended that amc and ac_2 in a butter culture are formed from the lactose, presumably because various bacterial species produce amc from sugar. In general, these species are not present in butter cultures, and if they were, growth of most of them would be very limited because of the rapid acid production.

Effect of temperature and time of incubation. Studies involving the influence of incubation temperature and time on production of amc and ac_2 in butter culture are difficult to control because of the great effect of acidity and also because production is rapid with very little change in acidity after a suitable pH is once established.

Pien *et al.* (205) found the amount of ac_2 formed in butter culture at 30° C. was less than at 20° C. for the same period. Prolonged incubation resulted in a still greater difference between the two temperatures. The authors attributed the results to volatility of ac_2 at the higher temperature. Cultures examined immediately after coagulation at 20° C. showed less ac_2 than cultures grown at 30° C. for the same period.

Barnicoat (13) obtained an unusually low production of amc + ac_2 when cultures were incubated at 18° C.; larger amounts were developed at 30° C. Of the temperatures (37°, 21° and 17° C.) used by Mohr and Wellm (174), 37° C. was the most unfavorable for production of amc and ac_2; 21° C. was the most favorable. Vas and Csiszar (291) reported 12° and 16° C. were more favorable than 24° C. for formation of ac_2. Gibshman (78) obtained the highest production of amc + ac_2 with cultures 35 to 48 hours old; 25° to 30° C. was the most favorable temperature for the production by both single strain and mixed cultures.

At 7° C. Wiley *et al.* (323) found that cultures showed a steady increase in ac_2. At 21° C. production was rapid during the first 12 hours, destruction taking place during the next few hours. In 24 hours there was considerably more ac_2 in the cultures grown at 21° C. than in those grown at 7° C.; after 36 hours the curves crossed and from then on the 21° C. cultures contained less ac_2 than the 7° C. cultures. In the cultures at 7° C. ac_2 concentration increased for 70 hours and then decreased; amc concentration followed a similar course. After 40 hours concentration of ac_2 in the cultures grown at 7° C. was equal to that of the cultures grown at 21° C. and after 70 hours it was twice as great; after 140 hours it had fallen to a value below that of the 21° C. cultures. At 7° C. there was the regular development of ac_2 in unacidified milk without a corresponding development of acid; a regular

but greater production of ac_2 occurred in acidified milk. At 21° C. the course of ac_2 production and destruction was similar in the control and in acidified milk; the maximum production of ac_2 was considerably greater in the acidified milk. Production of acid in a culture was believed to favor formation of ac_2 by the flavor organisms.

Effect of amount of inoculation. Because of the increase and decrease in amc and ac_2 in a butter culture, the effect of amount of inoculation is related to the time of examination.

Stine (258) found the amount of inoculation of a culture into skim-milk or cream affected the rate of amc + ac_2 formation but not the total production of the compounds; the production seemed to depend primarily on the pH established.

Although amc and ac_2 were not determined, the results of Vas and Csiszar (291) show how flavor may be destroyed in a culture when the inoculation is heavy and the incubation period is long. Only a trace of aroma was observed when 4 per cent culture was inoculated into milk and the milk incubated 24 hours at 24° C., but with 1 or 2 per cent inoculation aroma was distinct.

Effect of oxygen supply. Since oxygen apparently is needed for production of ac_2 in butter cultures, the air supply would be expected to influence the quality of a culture.

Horowitz-Wlassowa and Rodinowa (115) stated that under certain conditions involving an abundance of air some bacterial species oxidize amc to ac_2.

Michaelian and Hammer (169) obtained variations in yields of amc + ac_2 in certain trials when carbon dioxide, hydrogen, nitrogen, oxygen and air were bubbled for a short time through different portions of a freshly inoculated culture and the containers sealed. There also were variations in yields of ac_2, with the portions through which oxygen had been bubbled regularly showing the highest values. Portions with which carbon dioxide, hydrogen, nitrogen or air had been used frequently did not show appreciable quantities of ac_2, but when they did air gave higher yields than the other gases. Variation in yields of ac_2 could not be attributed to differences in the cultures employed since one of the trials with a culture gave appreciable quantities of ac_2 only when oxygen was used, while another trial with the culture gave appreciable quantities under all the conditions, although oxygen yielded much more ac_2 than the other gases. Addition of increasing amounts of citric acid gave a progressive increase in yields of amc + ac_2 either with or without oxygen; without oxygen no appreciable production of ac_2 occurred, while with oxygen appreciable quantities were obtained in all cases except the control to which no citric acid had been added, and the yields of ac_2 increased as the yields of amc increased. The data indicate that oxidation of amc to ac_2 in a butter culture is due to activity of the flavor organisms rather than to direct chemical oxidation.

Virtanen and Tarnanen (299) found ac_2 in a butter culture only when oxygen had been bubbled through it or when air had access. Treatment with carbon dioxide or nitrogen prevented formation of ac_2. Virtanen *et al.* (298) stated that the presence of oxygen is not only necessary for formation of ac_2 from amc but also enhances production of the latter. Cultures acting on thick layers of milk produced little amc but when acting on thin layers formed it abundantly. Mohr and Wellm (174) noted that production of ac_2 is increased in cultures by access of air during the ripening. Virtanen (294) reported that when butter cultures were allowed to ripen in thin (1 to 2 cm.) layers a vigorous production of amc took place even without addition of citrates. According to van Beynum and Pette (283) ac_2 is formed only when an oxidation with atmospheric oxygen can take place. Wiley *et al.* (323) found that aeration delayed the rapid destruction of ac_2.

Amc was found in cultures by van Beynum and Pette (282) when grown either aerobically or anaerobically. Ac_2 was formed only as a result of an oxidation when the oxygen of the air had access. When cultivated anaerobically, ac_2 and aroma were not observed.

Brewer *et al.* (30, 31) found the ac_2 contents of cultures aerated under pressure were two or more times as great as of unaerated control cultures. Pressure without aeration did not result in significantly increased yields of ac_2. Cultures saturated with oxygen failed to give increased yields of ac_2 when ripened under pressure. In most cases aeration under pressure produced larger amounts of ac_2 than did saturation with oxygen, although oxygen treated cultures uniformly contained higher quantities of the compound than controls. An aerated, a stirred and an unagitated culture were run at three pressures (15, 30 and 60 lbs.); 15 lbs. was little more effective than atmospheric pressure, but higher pressures gave substantially larger amounts of ac_2; a pressure above 30 lbs. gave little additional increase in ac_2. In later trials with use of larger equipment, more effective stirring led to yields of ac_2, in cultures stirred under considerable pressure, that usually exceeded those of aerated cultures. A possible explanation given was the volatility of ac_2. Without pressure ac_2 values were uniformly higher in agitated than in unagitated cultures. Stirring at room pressure was not effective in oxidizing amc to ac_2. No significant changes were noted in either the amc or ac_2 contents when unaerated butter cultures were ripened under pressure. In cultures aerated under pressure the yield of amc sometimes was larger in the controls and sometimes smaller.

Prill and Hammer (211) found that one of the factors tending to give relatively high ac_2 contents in butter cultures was the oxygen supply. Greater percentages of citric acid, such as 0.30 per cent, did not give correspondingly higher ac_2 contents than 0.15 per cent unless the ripened cultures were held for extended periods with sufficient aeration. Amc

contents of cultures also were influenced by the oxygen supply. The authors stated that oxygen presumably tends to prevent reduction to 2,3-butylene glycol. Other experiments by the authors (212) showed that cultures grown with aeration under pressure for 12 to 13 hours had extremely high ac_2 contents (up to 37.4 mg. per kg.); the amc contents also were much higher than those of regular cultures. Cultures which were aerated under pressure for 3 hours after the usual ripening period contained relatively large amounts of ac_2, and the amc contents were somewhat higher than those of regular cultures.

Davies (55) stated that the two most important organisms for the formation of ac_2 are *S. cremoris* and *S. paracitrovorus* which ordinarily can be propagated along with the common *S. lactis* quite successfully for long periods. These two types, in the presence of free oxygen in solution, form considerable ac_2, but they also form amc in much larger amounts, especially when all the oxygen has been used by the bacteria. The ability to form ac_2, however, is never lost, even under conditions of low oxygen tension.

Fabricius (72) suggested that butter culture should be cooled quickly and with vigorous stirring to oxidize the flavorless and odorless amc to ac_2. The culture should be cooled to a low temperature to prevent reduction of ac_2 to 2,3-butylene glycol.

Davis (60) found that aeration slightly inhibited the growth of butter culture organisms.

Preservation of amc and ac_2. The destruction of amc and ac_2 in butter culture suggests the importance of using procedures in the handling of culture which will limit or prevent such changes. This is especially true when the method of employing the culture depends on the carrying over of ac_2 from the culture to the butter, rather than on production of ac_2 in the cream or butter.

The common practice of cooling a ripened culture definitely limits the destruction of amc and ac_2 and there may even be increases in the compounds. However, in some instances there is a relatively rapid decrease; this may be in part due to the difficulty of rapid cooling to low temperatures. Addition of small amounts of citric acid, such as 0.05 per cent, to the ripened culture definitely limits the destruction and in certain instances has resulted in significant increases in amc and ac_2, even when the culture was cooled considerably below the normal ripening temperature.

Mohr and Wellm (174) noted that the ac_2 content of a high acid butter culture was preserved by cooling to 10° C. Temperatures of 0° to −10° C. had a more favorable effect on maintenance of the amc and ac_2 during storage for 10 to 12 days. At 10° C. the ac_2 content showed an increase after 4 days and a decrease after 12 days. When the acidity of butter culture was very high, the amc and ac_2 contents reached a certain limit and then decreased.

van Beynum (279) suggested that the final acidity produced by the lactic acid bacteria in butter culture preserves the aroma. Prill and Hammer (211) considered that oxygen tends to prevent reduction of ac_2 to 2,3-butylene glycol.

It appears that one of the important factors influencing preservation of the amc and ac_2 contents of a ripened culture is a reserve of citric acid; without this, destruction of amc and ac_2 may be relatively rapid when the temperature permits bacterial action. There is some evidence that the destruction varies with different cultures.

Comparative amounts of amc and ac_2. In general, a butter culture contains much more amc than ac_2. With relatively low amc contents, ac_2 may be difficult to detect. The ratio of ac_2 to amc is not constant and undoubtedly is influenced by various factors.

Davies (52) stated that in normal butter cultures the ratio of ac_2 to amc is about 1 to 20; in cheese cultures the ratio is about 1 to 30. Davies (53) noted that the main factor influencing the ratio of ac_2 to amc appeared to be the dissolved oxygen content of the medium. When free oxygen was present ac_2 was formed together with some amc, but with increased development of acid and exhaustion of dissolved oxygen ac_2 was formed only in traces and amc in greater quantity. The rate of production of amc + ac_2 for one type of organism growing in milk or cream was found to be proportional to the titrable acid. Although the total amc + ac_2 increased with the titrable acid, the ratio of ac_2 to amc varied according to the type of organism or culture. The ratios of ac_2 to amc in a cheese culture, a commercial butter culture and an *S. cremoris* culture were 1 to 40, 1 to 20 and 1 to 18, respectively, when incubated for 6 hours. The final ac_2 to amc ratios at titrable acidities of 0.9 to 1.1 per cent were only slightly wider than these. Most commercial butter cultures showed an ac_2 to amc ratio of 1 to 20. Wiley *et al.* (323) noted that at the maximum ac_2 concentrations cultures grown at 21° C. showed a higher amc to ac_2 ratio than a culture grown at 7° C.

Prill and Hammer (211) indicated that a high ac_2 content would accompany a high amc content, but that various factors, such as oxygen supply, temperature, absence of citric acid, etc., prevented the establishment of any close relationships. The amc contents were influenced by the same general factors as the ac_2 contents.

Importance of ac_2 as a flavor constituent. The importance of ac_2 as a flavor constituent of butter culture is evident from analyses showing that a desirable flavor is accompanied by relatively large amounts of ac_2 and also from trials in which flavor was improved by adding small amounts of ac_2. However, a relatively high ac_2 content is no assurance of a good flavor because definite off flavors due to a number of causes may be present.

Various investigators have found the ac_2 contents of satisfactory cul-

tures higher than those of unsatisfactory cultures. Palladina *et al.* (200) reported that cultures which contained no ac_2 were very devoid of flavor, while those containing from 3.4 to 7.2 mg. per 100 ml. were pleasing in flavor. Production of ac_2 did not always mean a good flavored butter culture; however, an active culture of flavor organisms always produced a high volatile acidity as well as ac_2 and the best cultures always developed ac_2. Rumments (230) noted that the ac_2 number (mg. nickel dimethyl glyoximate per 500 g.) of good aromatic butter cultures varied from 8.0 and 14.0; with poorer and less aromatic cultures values varied from 4.0 to 7.9.

According to Wiley *et al.* (322) the assumption, hitherto unquestioned, that the ac_2 content or aroma of a butter culture is a sure guide to its value as a flavor producer in butter is fallacious. There is no necessary relationship between ac_2 production at high temperatures and low temperatures since the metabolism of *S. cremoris* is different at different temperatures and the flavor organisms do not function at low temperatures. Under low temperature conditions a culture consisting of a single strain of *S. cremoris,* usually considered quite unsuitable for butter manufacture, may impart as much aroma to butter as a good butter culture.

Identification of amc and ac_2. Ac_2 in butter culture has been identified on the basis of the color reactions used in various methods of determination and amc by the same general reactions following oxidation of the amc to ac_2.

Hammer (95) determined the nickel contents of the salts obtained when cultures were steam distilled, after adding ferric chloride, and the distillate treated with hydroxylamine hydrochloride, sodium acetate and nickel chloride. Results indicated that the diketone steam distilled from butter cultures after addition of ferric chloride was ac_2 rather than one of the homologs, and that if homologs were present they were limited to relatively non-significant amounts.

In the studies of Prill *et al.* (209) distillates from ordinary butter cultures gave no evidence of the presence of either higher homologs of ac_2 or methylglyoxal; also, homologs of amc were absent.

Role of lactic acid organisms. Some strains of *S. lactis* and *S. cremoris* apparently produce small amounts of amc and/or ac_2.

Schmalfuss and Barthmeyer (234) grew cultures of *S. lactis* plus *S. cremoris* in sterile milk and recognized ac_2 by the odor; it was isolated and identified as the dioxime, but the amount was so small that careful analyses were impossible. van Niel *et al.* (286) identified amc in a 2-day culture of an organism considered to be *S. cremoris* but not in a comparable culture of an organism considered to be *S. lactis.* Of 34 *S. lactis* strains tested by Michaelian *et al.* (166), 4 produced amc + ac_2 in milk and these gave only traces. When grown in milk plus 0.1 or 0.3 per cent acetic acid or 0.03 per cent acetaldehyde, more of the cultures produced small amounts of the compounds. Some strains of *S. lactis* actually destroyed amc + ac_2. With 380

strains of *S. lactis,* Palladina *et al.* (200) found no ac_2 production, but some of the 150 strains of *S. cremoris* produced ac_2. van Beynum and Pette (280, 281) examined 63 strains of lactic acid streptococci from various sources and concluded that not only does *S. cremoris* form amc, but a number of strains of *S. lactis* also produce it. Davies (52) stated that pure cultures of *S. cremoris* have an ac_2 to amc ratio of 1 to 15 to 1 to 18.

A culture of *S. lactis* studied by Joshi and Ram Ayyar (127) produced no amc + ac_2 in regular milk and only a trace in milk plus 0.5 per cent citric acid. Pien *et al.* (205) concluded that the lactic acid organisms which produced the most ac_2 also were the most active in lactic acid production. Rumments (231) stated that *S. cremoris* does not attack citric acid of milk at pH 4.2 in 9 days; nor was any amc formed. *S. lactis* did not decompose detectable quantities of citric acid in the same period and only a trace of amc was found. Yawger and Sherman (332) noted that *S. cremoris* produces amc more frequently and in larger amounts than *S. lactis;* however, the individual culture which produced the largest amount was *S. lactis.*

In milk inoculated with *S. lactis* after addition of amc, Michaelian and Hammer (169) found no evidence of oxidation to ac_2.

Davis *et al.* (63) investigated the metabolism of resting and growing cells of lactic acid bacteria and found that only the fecal streptococci (*S. fecalis* and *Streptococcus liquefaciens*) produced considerable quantities of ac_2. It was produced only in the presence of oxygen. Since distillation with ferric chloride greatly increased the yield of ac_2, relatively little of the amc formed in the fermentation had been oxidized to ac_2. Long and Hammer (155) reported cultures of *S. liquefaciens* varied widely in their production of amc in skimmilk.

According to Gibshman (79) pyrotartaric acid is the main source of amc and ac_2 with the lactic acid streptococci.

Metabolism of *S. cremoris* in milk or cream was found by Wiley *et al.* (322) to vary with the temperature of incubation. After 18 hours at 20° C. there was marked acid production and low ac_2 production. At 7° C. acid production was greatly restrained, but ac_2 production was scarcely affected and occasionally was greater than at 20° C. The authors (323) stated that the function of *S. cremoris* in a butter culture probably is to form acid which favors production of ac_2 by the flavor organisms.

Role of flavor organisms. The flavor organisms are primarily responsible for the amc and ac_2 in buter cultures. The compounds are actively produced only at favorable acidities.

van Niel *et al.* (286) isolated cultures of flavor organisms from butter cultures and noted that they produced amc in 3 days in a glucose-chalk-yeast water medium.

Michaelian *et al.* (166) studied 35 strains of flavor organisms with regard to production of amc + ac_2. With milk cultures grown 7 days at 21°

C., 29 strains produced no amc + ac_2; the others yielded variable amounts. In general, amc + ac_2 was not produced by the organisms when a small amount of sulfuric acid was added to inoculated milk, while with more acid amounts comparable to those of butter cultures were formed; with excessive acidification, no amc + ac_2 was produced, due presumably to failure of the organisms to develop at a high acidity. Addition of any one of a number of acids, in suitable concentrations, to milk containing considerable numbers of flavor organisms resulted in production of amc + ac_2. With some acids (acetic, propionic, butyric) no amc + ac_2 was obtained; these acids may have been toxic for the organisms. When citric acid was added, more amc + ac_2 was produced than with other acids. Trials with added lactic acid showed that in milk the flavor organisms produced amc + ac_2 only over a certain acid range, both higher and lower concentrations apparently being unsatisfactory. With citric acid amc + ac_2 again was produced over a wide acid range. As the acid increased there was an increase and then a decrease in yield of amc + ac_2. Yields after 48 hours commonly were higher than after 24 hours. Although addition of small amounts of citric acid to milk inoculated with a flavor organism did not result in an increase in amc + ac_2, at higher acidities amc + ac_2 values were much in excess of those of butter cultures. In general, the highest amc + ac_2 values were correlated with the highest volatile acidities (also 163).

Stine (258) found that the pH range over which the flavor organisms produced amc + ac_2 in milk acidulated with sulfuric acid varied considerably but in general was between 5.0 and 2.3. Usually the maximum amount of amc + ac_2 was produced between pH 4.2 and 3.8; however, with two organisms the maximum was produced at pH 3.4.

According to van Beynum (278) flavor can be produced by the flavor organisms without the presence of lactic acid bacteria if the milk is acidified with lactic acid. Other acids (organic and inorganic) also can be used. Casein and albumin in milk were not sources of flavor constituents. Beef infusion with glucose or lactose added showed good growth of flavor organisms, but aroma compounds were not formed unless citric acid was added. Tests indicated that aroma was produced only during the period of growth of the flavor organisms. The author suggested that more aroma might be expected with flavor organisms which resist high acidities because the interval of possible aroma production would be longer than with organisms whose activity is stopped at low acidities. Cultures of flavor bacteria in milk could be made to keep the aroma they had produced by adding a suitable quantity of acid at the moment aroma was observed (also 279).

With both *S. citrovorus* and *S. paracitrovorus* Palladina *et al.* (200) found that some strains produced ac_2 in milk, while some did not. Rodenkirchen (227) stated aroma is first formed by the flavor bacteria when the lactic acid content of the milk reaches 0.40 to 0.80 per cent. In milk acidi-

fied with lactic acid and inoculated with a flavor organism, Wiley *et al.* (323) found production of ac_2 was similar to that of a butter culture. Wiley *et al.* (321) reported that pure cultures of flavor organisms, when grown in milk at 21° C., did not produce appreciable quantities of ac_2 over long periods.

Rumments (230) noted that *S. citrovorus* formed amc and ac_2 in large amounts only when the milk had a pH below 5.0. With a higher pH the citric acid in the milk gradually disappeared, but amc could not be detected. When citric acid was added to a milk culture of *S. citrovorus,* the amount of amc + ac_2 could be increased considerably; the increase was not as great with sodium or potassium citrate. Accumulation of amc occurred at a pH below 5.0 and the best pH for its formation was 4.3.

In the trials of Michaelian *et al.* (170) the yield of amc + ac_2 was always much higher when citric acid was used to acidify milk cultures of the flavor organisms than when other acids were employed. A significant production occurred over a wide pH range; however, maximum yields were obtained at pH values from 3.9 to 3.7. When lactic acid was the acidulant, yields of amc + ac_2 were comparatively low and a significant production occurred over a rather low pH range; maximum yields were obtained at pH values from 4.4 to 4.1 which approximate the values commonly found in butter cultures. Sulfuric acid gave higher yields of amc + ac_2 than lactic acid and a significant production over a wider pH range, maximum yields being obtained from pH 3.6 to 3.2. A mixture of 0.15 per cent lactic acid and variable amounts of sulfuric acid, depending upon the pH desired, gave yields comparable to those obtained with sulfuric acid alone. Maximum yields occurred at pH 3.9 to 3.5, these values being between the corresponding values for lactic and sulfuric acids.

Michaelian and Hammer (168) found that milk cultures of the flavor organisms which had been killed with heat, formalin or chloroform failed to produce amc + ac_2 when treated with 0.15 per cent citric acid and 0.30 per cent sulfuric acid, amounts which give good production with living organisms. Suspensions of the organisms washed from whey agar plates with sterile water and killed with chloroform produced no amc + ac_2 when added to milk containing 0.50 per cent citric acid. A rather close relationship existed between numbers of flavor organisms added to milk having a low pH, as the result of addition of citric acid or citric and sulfuric acids, and amounts of the usual fermentation products. Ninety-six hours after adding 0.60 per cent citric acid to a 48-hour milk culture of a flavor organism, the amc + ac_2 value was very high; when 0.15 per cent acid was added 24 hours after the original addition, the value was increased somewhat.

When purified amc was added to sterile skimmilk adjusted to an acidity and temperature satisfactory for rapid production of amc by the flavor organisms, Michaelian and Hammer (169) did not obtain appreciable

amounts of ac_2 in 48 to 72 hours. Results were the same when carbon dioxide, hydrogen, nitrogen or oxygen was bubbled through the milk. Production of ac_2 was definitely influenced by bubbling various gases through freshly acidified milk cultures of flavor organisms. Oxygen regularly increased the yield, while carbon dioxide, hydrogen or nitrogen decreased it. With all the gases there commonly was a greater production of ac_2, as well as amc + ac_2, when the cultures were acidified with a mixture of citric and sulfuric acids than when acidified with sulfuric acid alone.

Brewer *et al.* (30) obtained increases up to several hundred per cent in the ac_2 contents of cultures of flavor organisms by bubbling air through the cultures under pressure. There also were slight increases in amc. The effect of pressure alone without aeration was negligible. Virtanen (294) noted that the flavor organisms produced more amc in the presence of sufficient air than when grown in thick layers.

Vas and Csiszar (292) stimulated various cultures of flavor organisms which produced only a slightly positive aroma reaction by adding 0.5 to 2.0 per cent yeast extract and 0.25 per cent disodium citrate. To maintain their full capacity of forming amc and ac_2, it was necessary to grow the organisms in pasteurized or slightly boiled whole milk; they lost their aroma, as well as their capacity of producing volatile and other acids, when grown in whey agar, bouillon or sterilized milk.

Abbott (1) found that tomato juice greatly increased production of amc + ac_2 in milk cultures of flavor organisms. Cultures containing tomato juice produced the highest yields, which were almost twice those of the controls, at a pH of 3.18; the maximum yield in the control culture occurred at pH 4.01.

Production of 2,3-butylene glycol (2,3-bg)

Reduction of amc to 2,3-bg can be brought about by various bacteria and apparently occurs in butter cultures through action of the flavor organisms.[9] The reduction is influenced by a number of factors.

Hammer *et al.* (102) noted that the decrease in amc in butter cultures was accompanied by an increase in 2,3-bg and that commonly there was an increase, from one examination to the next, in total molarity of the two compounds. When ripened culture was neutralized to a low acidity there was a rapid decrease in the amc and in some trials this was followed by an increase as the acidity again increased. Decrease in amc was accompanied by a rapid increase in 2,3-bg and there also was an increase in total molarity of the two compounds. Hydrogen peroxide, in certain concentrations, delayed reduction of the amc to the glycol, as did 1 per cent sodium fumarate or 12 per cent sodium chloride. Ice water temperatures also delayed reduction in either neutralized or unneutralized cultures, but reduction was more rapid with neutralization than without (also 255).

[9] The Appendix, Part B, gives a general statement on methods of determining 2,3-bg.

2,3-bg was not detected in butter cultures by Rumments (230), who assumed that it did not occur as an intermediate product in amc production or as a reduction product of amc. van Beynum and Pette (282, 283) found that reduction of amc to the glycol was more complete at a low acidity but also depended on the strain of flavor organism used.

Role of flavor organisms. In the trials of Hammer *et al.* (102) addition of amc or ac_2 to a tomato bouillon culture of a flavor organism resulted in a rapid disappearance of amc or ac_2 and an increase in 2,3-bg. With amc the change to the glycol was delayed when sulfuric acid was added in amounts to give a pH of 4.0 to 3.8. Conversion of amc or ac_2 to 2,3-bg also took place when the compounds were added to a milk culture. With ac_2 there was an increase in amc as well as in glycol; the increase was greater in the carbinol. With various amounts of sulfuric acid added to milk cultures of the organisms, amc was not produced at higher pH values but was produced at lower values, while 2,3-bg was produced at both higher and lower values. Less glycol was formed at lower pH values than at higher ones, which indicates that a higher acidity interferes with reduction of amc to 2,3-bg. Addition of 0.65 per cent citric acid to a milk culture increased the amc and this increase was especially rapid during the early part of the holding period. There also was a striking increase in 2,3-bg, but this began somewhat later than the increase in amc. Potassium nitrate did not delay reduction of amc to 2,3-bg. Sufficient hydrogen peroxide definitely delayed the reduction, while smaller quantities did not. In cultures of flavor organisms which had been acidified with sulfuric acid to a pH of about 3.9, addition of acetaldehyde or propionaldehyde increased the amc present after 96 hours at 21° C. but decreased the 2,3-bg and also commonly decreased the total molarity of the two compounds. Results suggested that the increase in amc is accounted for by a decrease in reduction of amc, rather than to an aldehyde condensation, involving, in part, the added aldehyde (also 255).

van Beynum and Pette (282) stated that 2,3-bg is one of the fermentation products produced by the flavor bacteria in acidified milk.

Proteolysis

Proteolysis occurs in butter cultures, the breakdown apparently going to the amino acid stage, and is due primarily to the lactic acid organisms. It is not evident from appearance of the coagulated milk but is readily detected chemically. The proteolysis presumably favors growth of the flavor organisms and the same general type of change probably plays a part in the ripening of various cheeses. Hammer and Patil (100) found that proteolysis in cultures did not require long incubation and was evident in freshly coagulated cultures.

Barthel and Sadler (18) noted that the same amounts of soluble nitrogen were produced from casein by various cultures of streptococci and by butter

cultures, but that amounts of amino nitrogen produced by the streptococci were lower. Kelly (130) reported that *S. cremoris* and a butter culture produced approximately the same amounts of amino nitrogen, but that with *S. lactis* the amount was lower.

Role of lactic acid organisms. Many cultures of *S. lactis* and *S. cremoris* have definite proteolytic properties, while some apparently do not. Among the proteolytic strains there are wide variations in the activity.

In 1904, Orla-Jensen (189) noted considerable proteolysis with two strains of *S. lactis* grown 3 months in milk with added calcium carbonate. One strain fermented more sugar and decomposed more protein at 35° C. than at room temperature. A year later von Freudenreich and Thöni (303) found as high as 30.35 per cent of the total nitrogen in a soluble form in old cheese (6.5 months) made from carefully selected milk to which *S. lactis* had been added.

Barthel (15) reported that in milk with added calcium carbonate *S. lactis* strains from various sources decomposed more protein at 14° to 20° than at 36° C. during 2 to 4 months. Using the Sörensen formol titration method, Itano (124) found that *S. lactis* is capable of breaking down protein. Barthel and Sandberg (19) classified the decomposition products formed from casein by *S. lactis* as soluble, trialbumin, peptone and amino nitrogen. Amounts of soluble nitrogen formed in milk by 22 newly isolated strains varied from 0 to 23.21 per cent of the total nitrogen. Both weak and strong proteolyzers were found in a butter culture. Protein-splitting ability was constant for a strain under varied unfavorable conditions and accumulation of soluble nitrogen had no effect on the inherent casein-decomposing ability.

Orla-Jensen (190) studied the proteolytic action of *S. lactis* and *S. cremoris* strains from various sources. After inoculating them into milk with added calcium carbonate and incubating at 30° C. for 1 month, from 0 to 20.4 per cent of the total nitrogen was in a soluble form and, as determined by the phosphotungstic acid method, amino nitrogen varied from 0.9 to 10.1 per cent. No relationship was found between proteolytic powers of the strains and rates of curdling milk at 30° C.; many low-proteolyzing strains curdled milk very rapidly.

Gorini (82) stated that with the lactic acid ferments the acid-proteolytic property is important; the action occurs in normal milk that is acid. Later, he (83) noted that cultures of the *S. lactis* group split casein at cheese ripening temperatures but were not proteolytic at higher temperatures. Certain of the acid-proteolytic bacteria survived rather high temperatures.

In milk cultures of *S. lactis* (from milk and cheese) incubated 1 week at room temperature, Virtanen (293) observed that the maximum acidity developed by different strains varied considerably. With calcium carbonate added to the milk, there was no correlation between maximum acidity and extent of casein cleavage; many strong acid-producing strains did not decompose casein in appreciable amounts.

Spitzer *et al.* (254) reported that an *S. lactis* culture caused a slight increase in various groups of protein decomposition products when grown in milk or in an unsalted synthetic butter made by adding skimmilk to butterfat. With various pure cultures, Barthel and Sadler (18) obtained the highest soluble nitrogen content from a strain of *S. cremoris* (also 17). According to Hucker (117) certain *S. lactis* strains have the ability to increase materially the soluble nitrogen in milk.

In studying proteolytic action of certain lactic acid bacteria, including a strain of *S. lactis,* Peterson *et al.* (203) noted decomposition of protein in three of the media used. When cultures of *S. lactis* were incubated for various periods, an increase in amino nitrogen was noted in the majority of cases.

Anderegg and Hammer (3) isolated *S. lactis* strains from various materials and determined their proteolytic activity in milk, with and without added calcium carbonate; usually 14 days at room temperature were employed. In general, there was definite proteolysis with cultures coagulating milk rapidly, while slow-coagulating cultures apparently did not decompose the protein. Commonly, protein cleavage was more pronounced with calcium carbonate than without. Addition of peptone seemed to retard proteolytic activity rather than accelerate it. Protein degradation was carried to the amino acid stage, there being a corresponding increase in amino nitrogen with an increase in soluble nitrogen. Sterile lactic acid added to milk which was then held at room temperature failed to increase the soluble nitrogen.

Proteolysis in butter cultures was attributed to *S. lactis* by Hammer and Patil (100). Failure of lactic acid to increase the soluble or amino nitrogen when added to milk at the rate of 1 or 2 per cent indicated that breakdown of milk protein is not due to lactic acid developed by *S. lactis* but to an enzyme.

Kelly (130) compared proteolytic action of *S. lactis* and *S. cremoris* in milk, with and without added calcium carbonate. The carbonate increased hydrolysis of both protein and sugar. Between the species little difference was noted in rates of production or in the final amounts of nitrogen soluble in acetic acid, trichloroacetic acid or phosphotungstic acid. Orla-Jensen (192) stated that both *S. lactis* and *S. cremoris* attack casein. Crossley (47) noted that lactic acid bacteria possess proteolytic powers in addition to their ability to produce lactic acid.

In a study of various lactic acid bacteria, Braz and Allen (28) found that all cultures except two strains of *S. cremoris* showed appreciable increases in protein after 6 weeks in milk not containing calcium carbonate and this appeared to have occurred at the expense of the proteose peptone fraction. Cultures containing carbonate showed a breakdown of protein in every instance; with both *S. lactis* and *S. cremoris* it was very large after 12 weeks. Loss of protein was accompanied by corresponding increase in the proteose peptone fraction. There was a tendency for greater protein breakdown in

cultures containing yeast extract than in plain milk and for amino acids to increase more in plain milk than in milk containing yeast extract.

Role of flavor organisms. The flavor organisms are unimportant from the standpoint of proteolytic action in milk.

Anderegg and Hammer (3) and also Hammer and Patil (100) reported that *S. citrovorus* and *S. paracitrovorus* were not proteolytic. Braz and Allen (28) found them slightly proteolytic.

FERMENTATION OF LACTOSE AND CITRIC ACID

In the preparation of butter culture, the constituents of the milk which yield important decomposition products through bacterial action are lactose and citric acid. Mechanisms by which these compounds are broken down are only incompletely understood, but there have been various suggestions.

Fermentation of lactose

Undoubtedly, the first step in the breakdown of lactose is hydrolysis to glucose and galactose. Further reactions apparently include phosphorylations, hydrolyses, oxidations and reductions.

Hucker (119) reported that *S. lactis* does not hydrolyze lactose faster than lactic acid is produced. According to Tapernaux (268) the two molecules of hexose formed from one molecule of lactose under the influence of lactase are each split into two portions with formation of methylglyoxal in the hydrated form. Lactic acid then is produced by intramolecular oxidation-reduction of part of the methylglyoxal. Tapernaux noted that the lactic acid fermentation is characterized by inability of the lactic acid bacteria to form a product other than lactic acid from methylglyoxal. Pien (204) stated that lactic acid bacteria change lactose to glucose and galactose, lactic acid then being produced with the intermediate formation of methylglyoxal (also 227).

According to certain recent investigations, methylglyoxal is not a cardinal intermediate and plays no essential role in the breakdown of lactose. The suggested mechanism is that glyceraldehyde, in the phosphorylated condition, is changed to phosphoglyceric acid and not methylglyoxal, the phosphoglyceric acid then being converted to pyruvic acid which is reduced to lactic acid (306, 307).

Fermentation of citric acid

General action of bacteria. Action of bacteria on the citric acid in milk was recognized, in 1910, by Bosworth and Prucha (27) who observed that during spontaneous souring of milk the citric acid was changed to acetic acid and carbon dioxide. Butter culture and buttermilk also contained some agent capable of bringing about this change. Of the various common dairy bacteria tried, *Aerobacter aerogenes* was the only one that fermented citric

acid; it produced two molecules of acetic acid from each molecule of citric acid. *S. lactis* had no action on citric acid. The results of the authors were explained by discovery that the flavor organisms in butter cultures attack citric acid.

Supplee and Bellis (262) noted that when raw milk was held, the citric acid content decreased as the titrable acid increased; milk with an initial content of 0.134 per cent contained 0.042 per cent after 28 days. Pasteurized milk did not show a change in its citric acid content until after 14 days during which the acidity increased slightly; however, during 28 days the acidity increased appreciably and the citric acid decreased.

According to Kickinger (133) *Bacillus subtilis, Bacillus mesentericus* and *Proteus vulgaris* caused a decrease in citric acid content of milk, while lactic acid organisms did not.

Hastings *et al.* (107) reported that fermentation of citric acid by bacteria depends on the nature of the medium. Organisms fermenting citric acid in milk did not necessarily ferment it in another organic medium, and those which fermented it in an inorganic medium in which sodium citrate was the only source of carbon did not necessarily ferment it in milk. Nature of the available carbon was considered the determining factor in citric acid fermentation. Organisms found to ferment citric acid in milk were: *Escherichia coli, Aerobacter cloacae, S. citrovorus* and *S. paracitrovorus;* those which did not ferment it included *S. lactis, Lactobacillus casei, L. acidophilus, A. aerogenes,* casein digesters and lactose-fermenting yeasts.

Mussill (178) noted that the citric acid content of milk could be reduced by bacterial action, *E. coli* and spore formers being important. There was no correlation between breakdown of citric acid and of protein.

Bacteria of very different types are now recognized as fermenting citric acid and action on citric acid is used in the classification of certain groups (22).

Specific action of flavor bacteria. The studies on the flavor organisms of butter cultures show that they form a series of compounds from the citric acid normally in the milk or that added to the milk. For a time only general observations indicated the trend of the reactions, but more recently attempts have been made to outline them in some detail.

Knudsen (139) observed a decrease in the titration value of butter culture when citric acid was decomposed and carbon dioxide driven off.

Various investigators (166, 168, 278) have noted a decrease in citric acid content of milk in which the flavor organisms were active. In a detailed study Rumments (231) found organisms of the *S. citrovorus* group were the most active in decomposing citric acid. *S. citrovorus* reduced the amount of citric acid in milk from 0.161 per cent to 0.040 per cent in 8 days at a pH up to 5.92; only traces of amc were detected. The lower the pH, within the range of 5.8 to 4.8, the more actively *S. citrovorus* decomposed the acid.

Milk having 0.216 per cent citric acid was reduced to 0.002 per cent in 24 hours at pH 4.95. It made no difference whether citric acid or lactic acid was used to acidify the milk. Citric acid was decomposed to the same extent regardless of the quantity present. Amc began to accumulate in cultures when the pH was between 5.8 and 4.8. In 24 hours at pH values of 5.81, 5.60, 5.36, 5.10 and 4.77, the percentages of citric acid decomposed were 58, 69, 69, 90 and 99 per cent, respectively. In milk at pH 5.44 no amc was detected after 48 hours, while at pH 4.34 as much as 100 mg. per l. was produced (also 271).

Knudsen and Sorensen (142) suggested that the flavor organisms bring about two fermentations: (*a*) Lactic acid fermentation whereby sugar is converted to lactic acid and (*b*) fermentation by which citric acid and perhaps other organic acids are converted to carbon dioxide, acetic acid and small amounts of additional substances and whereby the acidity is reduced. To what extent one or the other of the fermentations occurs depends upon the pH. Later, Knudsen (140) stated that the flavor bacteria do not appear to utilize citric acid primarily; they grow poorly on citric acid alone and give the greatest evolution of gas when both sugar and citric acid are present. He believed that it was only when large numbers of the bacteria had developed and they were unable to tolerate more acid that they resorted to the second fermentation (production of carbon dioxide, acetic acid, etc.) in which the citric acid in particular is attacked. Since the second reaction proceeds best in an acid medium, it begins late.

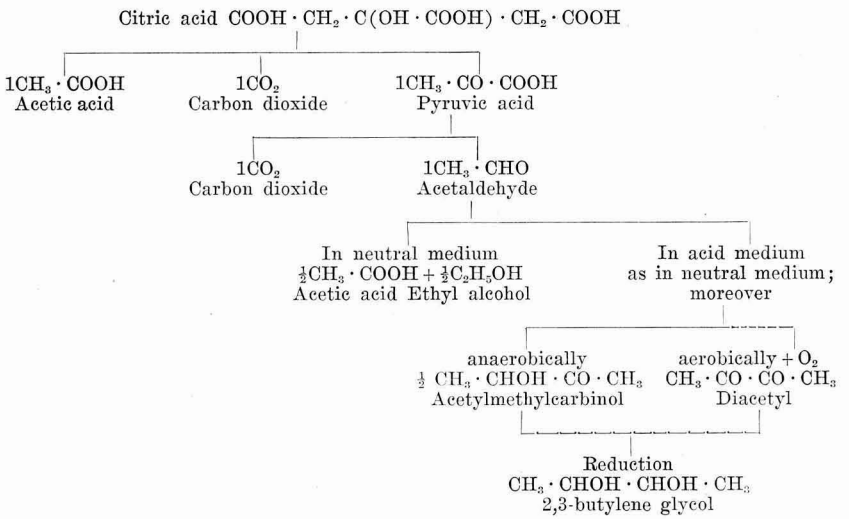
Because whey and Chamberland filtrates from milk contained only slightly less citric acid than the original milk, van Beynum (278) concluded that citric acid was not absorbed by the milk proteins. In butter cultures, citric acid disappeared in 2 to 3 days. Fermentation of citric acid in milk by cultures of flavor bacteria depended upon the individual properties of the strains. In artificially acidified milk inoculated with the organisms, flavor was noted during or shortly after beginning decomposition of the citric acid. Flavor bacteria could ferment large quantities of citric acid, the amount depending largely on the initial concentration and ratio of citric acid to citrate. Decomposition of citric acid in normal milk proceeded gradually, while in acid milk it was explosive in character; decomposition velocity was especially high at acidities which caused good flavor formation. Carbon dioxide production during the citric acid fermentation was proportional to the concentration of the acid. Two molecules of citric acid yielded about 4 molecules of carbon dioxide and 3 molecules of acetic acid.

Rumments (230) isolated 100 cultures from export butter, sour cream, sour milk and butter cultures; of these only *S. citrovorus* fermented citric acid. An *S. citrovorus* culture containing 65 million organisms per ml. decomposed 27 per cent of the original citric acid in the milk in 24 hours at 22° C. (pH 6.38), 19 per cent during the next 24 hours (pH 6.2), 16 per

cent during the next 24 hours, etc., until on the ninth day only 0.01 per cent of the acid remained. The higher the acidity of the milk, the more actively *S. citrovorus* decomposed the citric acid. With a pH of 5.8, 58 per cent of the citric acid was decomposed in 24 hours, with a pH of 5.36, 89 per cent and with a pH of 4.77, 99 per cent. *S. citrovorus* was believed to decompose the citric acid of milk with its endo-enzymes. Acetic acid and carbon dioxide in butter cultures were determined. In view of the results, the author assumed that the amc fermentation proceeded as follows, with acetaldehyde playing a role as an intermediate product:

$$2COOH \cdot CH_2 \cdot C(OH \cdot COOH) \cdot CH_2 \cdot COOH \cdot H_2O \rightarrow CH_3 \cdot CHOH \cdot CO \cdot CH_3 + 2CH_3 \cdot COOH + 4CO_2 + 2H_2O.$$

van Beynum and Pette (282) stated that although citric acid is the important source of all flavor substances, some acetic acid is produced from sugar by the flavor bacteria. From quantitative estimation of products formed by *Streptococcus citrophilus,* flavor bacteria or lactic acid and flavor bacteria, it was found that 1 molecule of citric acid yielded nearly 2 molecules of carbon dioxide, from 1 to 1.5 molecules of acetic acid and from 0 to 0.5 molecule of C_4 compounds (amc, ac_2 and 2,3-bg). The higher the acidity the more C_4 compounds and the less acetic acid were obtained. This reciprocal relation indicates that the C_4 compounds and a part of the acetic acid are produced from the same intermediate. It appeared highly probable that pyruvic acid was an intermediate in the fermentation; production of flavor, C_4 compounds, acetic acid and carbon dioxide from this substance was demonstrated. The citric acid molecule was decomposed to 1 molecule of pyruvic acid, 1 of acetic acid and 1 of carbon dioxide. Pyruvic acid then was split to carbon dioxide and acetaldehyde. The acetaldehyde reacted in two ways: (*a*) Condensation to amc ($2CH_3 \cdot CHO \rightarrow CH_3 \cdot CHOH \cdot CO \cdot CH_3$); with oxidation or reduction, a part of the aldehyde was in the form of other C_4 compounds, ac_2 or 2,3-bg. (*b*) A Cannizaro reaction resulting in ethyl alcohol and acetic acid ($2CH_3 \cdot CHO + H_2O \rightarrow CH_3 \cdot CH_2OH + CH_3 \cdot COOH$). In a neutral medium only reaction *b* occurs, while in an acid medium reaction *a* also occurs. With higher acidity a higher percentage of acetaldehyde is transformed to C_4 compounds. From the two reactions it was noted that a direct correlation exists between the amounts of C_4 compounds and acetic acid. Presence of ethyl alcohol in cultures was demonstrated. The suggested system of citric acid decomposition accounts for the varying molecular amounts of acetic acid and C_4 compounds and for production of 2 molecules of carbon dioxide from 1 molecule of citric acid regardless of reaction. Ac_2 was not considered an oxidation product of amc but of an intermediate substance, probably acetaldehyde ($2CH_3 \cdot CHO + 1/2O_2 \rightarrow CH_3 \cdot CO \cdot CO \cdot CH_3 + H_2O$). The citric acid fermentation is represented in the following scheme:



If all the fermentation products mentioned are produced from citric acid, the amount of carbon in the reaction products must be independent of the circumstances of the experiment. This was demonstrated with cultures at different acidities. The amount of carbon was constant when ethyl alcohol was taken into account, although not determined, and was the same as the carbon in the citric acid present in normal milk (also 279, 283).

According to Brewer (29) *S. paracitrovorus* does not readily dissimilate citric acid in the absence of sugar but attacks it rather vigorously with small quantities of glucose or lactose present. The effect of the sugars in initiating the dissimilation was believed to be catalytic. Sugars which acted catalytically were fermented to approximately equimolar quantities of carbon dioxide, ethyl alcohol and lactic acid; in addition to these compounds, dissimilation of a combined substrate of citrate and glucose yielded acetic acid, ame, 2,3-bg and, under certain conditions, pyruvic acid which acted as an intermediate. Fermentation of relatively little glucose in a combined substrate often permitted decomposition of a several-fold quantity of citric acid. Pyruvate was dissimilated by *S. paracitrovorus* to products similar to those formed from a combined substrate. Dissimilation of citric acid and glucose by the flavor organisms was essentially an anaerobic process; little oxygen was consumed in aerobic trials. Arsenite, which inhibits dissimilation of citric acid by *Escherichia-Aerobacter* organisms and animal cells, had no effect on the fermentation by *S. paracitrovorus*. Addition of several common biologic hydrogen donators and acceptors to suspensions of *S. paracitrovorus* in citrate failed to stimulate the dissimilation in a manner comparable to glucose or lactose so that the catalytic action of sugars in the fermentation apparently is not due entirely to hydrogen transfer. In milk the lactose probably catalyzes fermentation of the citric acid.

Virtanen *et al.* (296) stated that separate fermentation of glucose and citric acid by the flavor organisms does not yield amc, while fermentation of the compounds together does. Amc was formed in the fermentation of glucose if a suitable hydrogen acceptor, such as methylene blue or quinone, was added. Under similar conditions citric acid did not yield amc; this indicated that citric acid acts only as a hydrogen acceptor in the flavor-producing process. The authors suggested that by action of a suitable hydrogen acceptor, the glycerophosphoric acid formed as a by-product in the fermentation is oxidized to triosephosphoric acid which results in inhibition of reduction of pyruvic acid to lactic acid. Amc was thought to be formed from pyruvic acid. Later, Virtanen *et al.* (297) grew flavor bacteria on various media. Amc was produced from glucose in the presence of methylene blue or quinone but not in the absence of a suitable hydrogen acceptor. It was not formed from citric acid, even in the presence of methylene blue.

The reaction by which amc is produced by flavor bacteria was investigated by Storgards (261). Buffer solutions containing the test substances were inoculated with washed bacterial cells. Neither glucose nor citric acid alone or in combination gave amc. Calcium or barium salts were necessary to initiate the reaction; the pH was maintained at or below 5.0. There was no evidence that acetaldehyde is an intermediate product in the reaction. The results suggest the possibility that the true source of amc is pyruvic acid, an intermediate product in the fermentation of sugars, and that citric acid plays the secondary role of hydrogen acceptor, in this way promoting breakdown of the sugar.

Slade and Werkman (246) suspended cells of *S. paracitrovorus,* grown in the presence of citrate and lactose, in a nitrogen-free medium and found citrate, oxaloacetate and pyruvate were fermented. From their studies they concluded that dissimilation of citric acid proceeds through oxaloacetic and pyruvic acids as intermediates.

According to Slade *et al.* (247) *S. paracitrovorus* assimilates carbon dioxide. Heavy carbon, used as a tracer in the fermentation, was present in succinic acid. The fixed carbon dioxide in succinic acid was believed to arise by 3 and 1 carbon addition. With *S. lactis* there was no indication of carbon dioxide fixation.

There have been various suggestions with reference to the relationship between amc and ac_2 in the citric acid fermentation. Commonly, it has been assumed ac_2 is formed from amc through biologic oxidation. Virtanen and Tarnanen (299) concluded that ac_2 is not formed through an oxidation-reduction process between two molecules of amc with simultaneous production of 2,3-bg but by oxygen as a hydrogen acceptor. Pien *et al.* (205) stated that amc is produced by the reduction of ac_2, or more exactly during the passage between 2,3-bg and ac_2. Barnicoat (13) considered that possi-

bly ac_2 was derived from amc, the conversion being due to bacterial dehydrogenation. Certain simple tests did not prove that amc was converted to ac_2, but most of the evidence is consistent with this theory. The alternate theory, that ac_2 is derived from amc by oxidation with oxygen, was not in accord with observations of the author. van Beynum and Pette (282) believed that ac_2 is formed by oxidation of an intermediate product in the fermentation of citric acid.

FACTORS INFLUENCING QUALITY OF BUTTER CULTURE

While the strain of butter culture employed for inoculation is an important factor in determining the quality of culture produced in a dairy plant, other factors also have an effect and some of them are of considerable significance.

Milk used. Milk for butter culture should have certain obvious qualities, such as satisfactory bacteriologic condition and freedom from serious off flavors due to feeds, etc., but other considerations also appear to be involved.

Knudsen (138) noted that in milk which yielded good butter culture, the culture showed higher volatile acidity, pH and titration value than poorer cultures in other milk. The greater titration value was explained by the higher content of acetic acid. A poor butter culture could not always be made a good one by growing it in suitable milk. Later, Knudsen (139) reported that milk from two cows fed the same feeds gave different results in the preparation of culture. The decisive factor in producing culture with a mild, rich flavor was considered by Knudsen and Sorensen (142) to be the buffer action of the milk. Addition of 6 ml. N/1 hydrochloric acid to 100 ml. of milk usually changed the pH from 6.6 to 4.5, but with milk yielding poor culture reduction was to pH 4.2. The authors believed that in abnormal milk factors other than buffer action would inhibit the flavor organisms.

A number of commercial butter cultures studied by Kelly (129) developed a flat, yeasty flavor when grown in milk from a certain herd but produced a typical, clean, acid flavor in milk from another herd. Later, he (131) stated that milk for culture should be well buffered so that acid produced by *S. cremoris* will not inhibit growth of the flavor bacteria.

According to Knudsen (140) milk for culture should be without off flavors, high in total solids and of good bacteriologic quality. Slatter (249) stated that use of whole milk gives best results in the preparation of mother cultures. Jersey and Guernsey milk were preferred because of their higher contents of citrates which are involved in the elaboration of flavor materials; milk from cows early or late in lactation should not be used because of composition changes during those periods. Other investigators have preferred whole milk to skimmilk. For example, Weiser (305) stated that whole milk

is best for preparation of culture since culture grown in skimmilk seems to lack delicate flavor; the whole milk should be high in total solids. Bogdanow (25) obtained better flavor production with cream than with whole milk or skimmilk.

Barnicoat (13) investigated the effect of the period of ripening and of the medium on development of amc and ac_2. Experiments were made with milk or cream steamed for 30 to 40 minutes, allowed to cool and inoculated with 0.5 per cent culture. Results indicated that there was no essential difference, when due allowance was made for the fat content of the cream, in the action of the culture, whether grown in skimmilk, whole milk or cream.

Larsen and White (148) found that butter culture made with milk powder produced as good results as culture prepared from fresh milk. Cost of the powder, however, was greater than fresh milk under normal creamery conditions. Other investigators (187) also have found skimmilk powder practical and convenient to use. However, some plant operators consider that it is not satisfactory.

Knudsen (139) noted that milk from infected udders which had a high chlorine-sugar ratio did not make good culture. Moir (176) stated that milk of low acidity due to mammitis should not be used for culture. According to Davis (60) mastitis milk has an inhibitory effect on culture organisms; pasteurization of the milk partially neutralizes this. Milk of late lactation had an effect similar to mastitis milk.

A culture carried in the usual manner was reported by Eagles *et al.* (70) to be much superior to the same culture carried in yeast enriched milk; in the modified milk the culture decreased in vitality. Abbott (1) found that addition of tomato juice to butter cultures accelerated production of amc + ac_2 very considerably, but the final amounts of the compounds were not significantly increased. In a representative trial in which 0.15 per cent citric acid was added to skimmilk inoculated with a butter culture, 1 per cent juice increased the yield of amc + ac_2 after 24 hours at 21° C. by 31.1 per cent, 10 per cent juice doubled the yield, while 40 per cent juice reduced the yield 41.2 per cent below the control. Tomato juices from various sources showed great differences in their effects on the yield. Some brands were as potent as juices from fresh tomatoes, but none exceeded them.

Addition of citric acid to milk. Since citric acid is the primary source of flavoring materials in butter culture and is present in milk in only limited amounts, its addition to milk intended for culture should result in flavor improvement. Addition of citric acid is the only method of modifying milk for culture that has been widely accepted, although additions of other milk constituents have been investigated (10).

Hammer (89) found that citric acid added to milk employed for culture frequently increased the volatile acidity over that produced in milk alone, even when all the citric acid in the unmodified milk was not used. In some

trials it appeared that there was more aroma after adding the citric acid, while in others there seemed to be little difference.

Templeton and Sommer (272) stated that addition of citric acid to milk for culture tends to give a rather weak curd which wheys off when the culture is allowed to incubate more than 24 hours. Addition of sodium citrate, however, tended to give just as good a curd as no addition. Cultures grown in sterile milk with added citric acid showed a greater tendency to whey off than when pasteurized milk was used. With removal of cultures containing added citric acid from the incubator as soon as possible after curdling and thorough chilling, there was little if any variation in curd formation from the control. The flavor of culture containing added citric acid or sodium citrate was preferred in most cases to that of untreated culture. As satisfactory results were obtained when citric acid or its equivalent of sodium citrate was added to the milk at the time of inoculation as when the culture was carried in milk having these reagents added at each transfer.

Vas and Csiszar (291) considered citric acid the basic substance for formation of ame in culture. Addition of citric acid gave a greater and more uniform production of aroma. Langton (147) stated that since citric acid or citrates help the virility of the culture organisms, citrates are added to improve the yields of ac_2. Fabricius (72) recommended addition of citric acid to milk used in preparing culture.

Under practical conditions citric acid frequently has been added to milk to be made into culture. About 0.15 per cent of the acid (containing 1 molecule water of crystallization) commonly is employed although larger amounts have been used. The acid is dissolved in a small quantity of water, using heat to aid in the solution and to destroy most of the organisms present; a glass container is advisable since concentrated citric acid attacks various metals. After cooling, the solution is poured into the milk which has been pasteurized and cooled to the incubation temperature. The solution should be added slowly with considerable agitation to prevent curdling of the milk at the point where the acid strikes it. After the addition, the milk is inoculated and ripened in the usual way.

The effect of added citric acid usually is evident from the increased flavor of culture made with it. Determinations of volatile acid and ac_2 also indicate a higher flavor.

Pasteurization exposure. The primary purpose of pasteurizing milk for butter culture is to destroy most of the microorganisms present; another effect is to limit or eliminate the normal germicidal property of milk.

Hammer and Baker (99) prepared culture from milk pasteurized with different exposures. Milk heated at 62.8° C. for 30 minutes developed acid and coagulated more slowly than milk heated at considerably higher temperatures. The growth rate of the culture organisms was essentially the same after heating at 71.1° C. for 30 minutes as when a higher temperature

was used for this period. With heating for 30 minutes, the influence of heat seemed to be gradual and spread over a considerable temperature range, instead of occurring completely at a definite temperature. Variation in the coagulation rates following different pasteurization exposures was thought to be due to effect of heat on the germicidal property of milk. The common practise of heating milk intended for butter culture to at least 82.2° C. for at least 30 minutes was considered very desirable.

Orla-Jensen (192) found that higher temperature pasteurization of milk intended for culture destroyed the foreign bacteria more effectively than low temperature heating and also destroyed the bactericidal constituents. Exclusion of foreign lactic acid bacteria from the milk was not certain unless it was heated to at least 80° C.; the lowest temperature at which bactericidal substances were completely destroyed was 75° C. Slatter (249) noted that milk pasteurized at high temperatures coagulated more quickly on inoculation with culture than milk heated at low temperatures. When raw milk was inoculated with culture, Davis (60) obtained less acid development than when milk heated at 73.9° C. for 30 minutes was employed. Milk heated to 82.2° C. by the flash method formed more acid than milk heated at 73.9° C. for 30 minutes, and milk boiled for 24 hours formed more acid than milk heated to 82.2° C. by the flash method.

In the trials of Farmer and Hammer (73) some cultures appeared to be greatly affected by the germicidal property of milk, while others were not.

Ripening temperature. Butter cultures commonly are ripened at 21.1° to 22.2° C., experience having shown that such temperatures are very satisfactory.

Toens and Hammer (275) developed good cultures over a wide temperature range, at least from 18° to 32° C. Although rapid acid development took place at 37° C., good cultures were not obtained, and the fermented material could hardly be called butter culture because of lack of normal flavor. When cultures were set at 32° C., a temperature known to yield good cultures, those developed at a constant temperature were more satisfactory than those developed at an irregular temperature involving a drop of 7° to 9° C. The constant temperature gave the better culture in 70 per cent of 87 comparisons.

Farmer and Hammer (73) found that better cultures developed at 25.5° or 21° C. than at 37°, 19° or 15° C.; 37° C. favored growth of the organisms surviving pasteurization, while 19° and 15° C. greatly decreased the rate of coagulation. Moir (176) stated that cultures grow best at 20° to 22.2° C. Gibshman (80) suggested that optimum temperature conditions for development of lactic acid bacteria may not be the same as those most suitable for producing flavor. When butter culture was prepared at 20° to 22° C. it retained its original properties most completely. Davis (60) noted that temperature exerts its greatest effect on cultures between 20° and 30° C.; this emphasizes the importance of not allowing cultures to fall below 22° C.

Degree of ripening. Since the flavor compounds of a butter culture are not produced in significant amounts until considerable acid has developed, little flavor is evident until rather late in the ripening period.

Neethling (179) found an acidity of 0.80 per cent in culture was the most desirable from the standpoint of flavor. Orla-Jensen (192) stated that best results are obtained with culture having an acidity of 0.78 to 0.95 per cent. According to Price *et al.* (208) a flat flavor in culture may result if too little acid is developed, while a sharp, acid flavor may result if the acid is allowed to increase much above 0.80 per cent. Gibshman (80) noted that when a culture has an acidity of 0.80 to 0.85 per cent, the important flavor components (volatile acid, ac_2, etc.) are present in sufficient amounts.

Results obtained by Prill and Hammer (211) on ripened culture held cold showed that changes in amc and ac_2 contents can occur at temperatures considerably below those used for ripening culture. Addition of citric acid to the culture held cold tended to give increases in both amc and ac_2. The results are in agreement with practices in plants which regularly hold ripened culture cold at least 24 hours before using it in cream. Addition of as little as 0.08 per cent citric acid was effective; agitation incident to distribution presumably also was a factor.

Recently, there has been a definite tendency to ripen culture to higher acidities than formerly when it is to be added to cream for butter. This is a logical procedure since up to a certain point contents of flavor constituents definitely are increased with an increase in acid. Commonly, ripened culture held at relatively low temperatures (1.7° to 7.2° C.) increases in ac_2 content for a time, especially when a small amount of citric acid is added to the ripened culture even if acid was added to the original milk. In certain cases ac_2 disappears rapidly from ripened culture; commonly, this can be prevented by addition of citric acid to it. Barnicoat (13) stated that butter culture is not always at its maximum flavor development when used and suggested that the practice of keeping ripened culture for a day at chilled temperatures appeared to be a sound procedure.

Rate of ripening. The rate of ripening of a culture is controlled primarily by varying the amount of inoculum, the time and temperature of incubation being kept constant. With relatively long incubation, such as 14 to 18 hours, considerable change in the inoculation is required to significantly affect the degree of ripening.

In a comparison of rapid coagulation (6 to 8 hours) and slow coagulation (16 to 20 hours) Toens and Hammer (275) noted that the rapid coagulation more frequently gave the better culture. Moir (176) stated that the amount of inoculum to use in preparing culture depends on the time and temperature of incubation.

According to Fabricius (72) most operators and commercial laboratories that are successful in the manufacture of fine culture emphasize the impor-

tance of varying the inoculation to suit the growth rate of the particular culture. Usually an inoculation of 0.25 to 1.0 per cent is desirable.

Air supply. Before the recognition of the significance of ac_2 in flavor production in butter culture, Neethling (179) had noted that culture grown in an abundant air supply had better flavor than culture grown in a restricted air supply; also Toens and Hammer (275) had found that air supply influenced flavor development in culture, the effect apparently being dependent on conditions under which the culture was carried. Such general observations are in agreement with results on production of ac_2 in butter culture.

Holding culture for inoculation. The organisms in butter culture used for inoculation must be reasonably active, and accordingly the conditions of holding the culture are important. In general, a normally ripened culture can be held at such temperatures as 4° to 8° C. for as long as several days without serious changes in activity of the organisms. At such temperatures as 37° to 40° C. deterioration may be rapid.

Knudsen (140) stated that the longer a culture was held after coagulation, the weaker it became; however, even after several weeks, enough bacteria were still alive so that a serviceable culture could be obtained by repeated inoculation.

In the trials of Farmer and Hammer (73) the maximum periods ripened cultures could be held at various temperatures and still yield a satisfactory culture on transfer were as follows: 1 day at 37° C., 3 days at 30° C., about 7 days at 21° C., about 1 month at 7° C. and about 2 months at approximately −10° C. In general, culture withstood freezing and thawing from 5 to 7 times without destroying its ability to produce a satisfactory culture; when frozen and thawed from 7 to 10 times there was delayed coagulation on transferring, but the flavor was quite satisfactory; when frozen and thawed from 10 to 15 times the culture failed to coagulate milk into which it was inoculated.

Baker (9) noted a small increase in keeping quality of butter culture when calcium carbonate was added (2 g. to 150 ml.) and the culture held at 25° to 32° C. Advantage due to the carbonate was greater when culture was held for longer periods than when held for shorter periods. In short periods (5 days or less) advantage due to carbonate was slight. When culture was held at 21° and 37° C. with carbonate added, that held at 21° C. had increased keeping quality, but no increase was noted at 37° C. Only 1 of 28 trials in which culture was held with added carbonate at room temperature for long periods (154 to 272 days) yielded a satisfactory culture when transferred to pasteurized milk.

Gibshman (80) stated that temperatures of 4° to 6° C. are best suited for storage of butter culture.

Various plants have found it advantageous to rapidly freeze a small

amount of culture whenever the culture appears to be unusually good so that it can be thawed and used for inoculation when culture carried in the regular way is unsatisfactory.

DEFECTS OF BUTTER CULTURES

Various defects occur in butter cultures. They involve primarily flavor, body and texture and rate of acid development.

Defects in flavor

The most common defect of butter cultures is lack of flavor, and relatively few cultures have as much flavor as is desired.

Hammer (91) noted that in handling cultures it is difficult to maintain the proper relationship between the two normal bacterial types and thus between the products formed by them. An abnormal relationship results in lack of flavor or even in off flavors. Common causes suggested for loss of the proper relationship between the organisms are over-ripening, unsatisfactory ripening temperatures and contamination. Ordinarily, the organisms surviving pasteurization are few in number and are held in check by the acid development. Foreign organisms which tolerate acid are the ones which may cause harmful results.

In studies on a culture that lacked flavor and failed to develop normal amounts of amc + ac_2 and volatile acid, Michaelian *et al.* (166) found flavor organisms were present in 1/100 ml. but not in 1/1000 ml., while *S. lactis* was present in 1/100,000,000 ml., the smallest volume tested. Since a satisfactory culture ordinarily contains flavor organisms in 1/1,000,000 or 1/10,000,000 ml., it is evident that they were present in unusually small numbers. Baker (9) also noted larger numbers of flavor organisms in satisfactory cultures than in unsatisfactory ones.

Ritter and Christen (223) reported that the flavor organisms may disappear from a culture with successive transfers and a harsh acid or malt flavor appear. According to Davies (52) the main difficulty with cultures is the decrease in numbers of flavor organisms with successive propagations. Weiser (305) suggested that over-ripe cultures may show a decrease in amc + ac_2 and usually lack flavor; lack of flavor also may be due to failure of the flavor organisms to develop.

Definite off flavors in butter cultures are of different types and are due to various causes. Baker and Hammer (10) noted that milk having such defects as saltiness and rancidity was not suitable for cultures since the flavors were readily carried over to them; certain feed flavors were not readily carried over.

Farmer and Hammer (73) found that combinations of various strains of *S. lactis* var. *maltigenes* and flavor organisms developed a malty flavor through a series of transfers. *S. lactis* strains showing a flavor suggesting cabbage when grown alone produced this same defect in combination with

certain flavor organisms, while with others satisfactory cultures were obtained. Rice (218) stated that a malty flavor in butter culture is due to a variety of *S. lactis*.

Some butter cultures contain contaminating organisms without the flavor being appreciably affected, while in other cases it is influenced. Yeasts and molds especially may develop extensively in cultures because of their acid tolerance; in some instances they have been responsible for conspicuous off flavors.

Davis (57) listed the common contaminants of butter cultures as: (*a*) Coliform types, due largely to utensils and occasionally found in dry commercial cultures; (*b*) yeasts, due to careless propagation; (*c*) molds, due to air contamination; (*d*) aerobic spore formers; and (*e*) anaerobic spore formers.

Various flavor defects of cultures encountered in practise have been explained on the basis of general observations rather than on extensive detailed studies. For example, Wilster and Price (327) have listed various flavor defects of cultures and their causes as follows: Sharp acid flavor due to over-ripening, bitter flavor due to use of milk having a bitter flavor or growth in the milk of bacteria producing a bitter flavor, cheesy flavor due to growth of certain undesirable bacteria in the milk and metallic flavor due to over-ripening the culture and keeping it in poorly tinned containers (also 165).

Defects in body and texture

While body and texture of a butter culture are relatively unimportant when the culture is used in making butter or certain cheeses, it is of significance in making culture buttermilk, cottage cheese, etc.

Cultures which have been normal in consistency for extended periods may develop ropiness gradually or suddenly; the defect may disappear and then reappear. Frequently ropy cultures yield *S. lactis* var. *hollandicus* on plating.

Hammer (93) isolated both non-ropy and ropy *S. lactis* cultures from a ropy butter culture and found that in litmus milk the non-ropy cultures began reduction at the bottom, while the ropy cultures began reduction at the top. With a butter culture that had lost its ropiness after a period in which the character was regularly evident, selective inoculations from the surface resulted in recovery of ropy cultures. Rice (218) stated that ropiness in a butter culture may appear and then suddenly disappear. The condition seemed to be associated with absorption of oxygen from the air during cooling of the milk after pasteurization. Continuous inoculation from the surface of the culture also was thought to induce the defect.

According to Weiser (305) poor texture in butter culture is due to high incubation temperature or poor quality milk. Weak body in a culture was said to be due to low acidity, low solids in the milk or contamination. Brewer

et al. (31) noted that the body of culture prepared with aeration under pressure was very weak because the agitation during development of acid prevented formation of the firm curd necessary for a normal body.

Fabricius (72) stated that it is advisable to keep mother cultures slightly under-ripe and to transfer frequently in order to keep the organisms in a viable condition. He recommended over-ripening a culture in attempting to overcome such abnormal conditions as ropiness.

Slow acid production

Slow acid production is one of the very serious defects of butter cultures. In some instances it appears suddenly, while in others the cultures become less and less active over a period of several days or even weeks. Various causes may be involved, the most important apparently being presence of bacteriophage in the cultures.

Milk used. Baker and Hammer (10) noted that milk low in total solids should be avoided in the preparation of butter culture because of the probability of slow acid development. According to Rice (218) sudden failure or slow acid development with a culture may be due to various causes, including mastitis milk; he considered that milk for cultures should be herd milk because certain cows give milk which hinders the organisms. Crossley (47) stated that when cultures become slow, the lactic acid streptococci suddenly lose their power to ferment lactose, resulting in slow acid development or even failure to produce acid. Slow cultures were confined to milk from certain districts at certain seasons of the year. Davis and McClemont (62) found that many samples of mastitis milk gave slow growth of cultures; deficiencies in bacterial growth factors such as vitamins B_1, B_2 and C were believed responsible. Most important cause of slow acid production in milk was considered to be abnormal chemical composition. A high correlation existed between slow rennet action and slow growth of culture in various lots of milk. Such samples had high globulin, low casein and low calcium contents.

In the studies of Nelson *et al.* (180) the source of milk was not important as a cause of the explosive type of slow acid production under the usual circumstances. Harrison and Dearden (104) investigated a case of slow cultures in which abnormal milk had no effect on rate of acid production compared to normal milk.

Davis (59) noted that unheated blood serum inhibited *S. lactis* when a small inoculum of washed cells was used, but no effect was noticeable under conditions similar to those employed in practice. He concluded that it is unlikely slow cultures are attributable to infiltration of serum or constituents of serum into the milk.

The following procedure was suggested by Cox (43) for determining the ability of various samples of milk to support growth of butter culture organ-

isms: Twenty ml. of each sample to be tested is placed in a test tube and pasteurized by immersing the tube in water at 62.5° C. for 10 to 15 minutes. After cooling, 3 drops of culture and 1 ml. methylene blue solution are added to each tube. The tubes are incubated at 36.6° C. and the reduction time noted. The author stated that milk which is reduced most rapidly is most suitable for growth.

Contaminating organisms. Various organisms influence the rate of growth of *S. lactis,* some accelerating it and some delaying it (44, 158, 182).

Marshall (159) noted that *B. subtilis* in milk influenced the lactic acid fermentation. The higher the numbers in the original milk, the greater the stimulus in the earlier stages of the fermentation. The stimulating action of *B. subtilis* was more evident at low temperatures than at high temperatures. Morgan and Curle (177) found that organisms of the *B. subtilis* type sometimes caused slow acid development in butter cultures. Trials showed that *B. subtilis* could grow in combination with an active culture of *S. lactis. B. subtilis* was capable of producing a marked alkalinity in milk followed by peptonization of casein with production of ammonia.

Whitehead and Riddet (319) isolated from a typical case of "non-acid" milk a *Streptococcus* which reproduced the phenomenon on inoculation into normal milk. The non-acid phenomenon was said to be due to a growth product of the organism; it was not destroyed even when the milk was heated at 100° C. for 30 minutes.

Whitehead (308) isolated two organisms which retarded acid production by lactic acid streptococci. One of them was inhibitory because of some heat stable substance formed during growth. Milk inoculated with it still was inhibitory after heating at 100° C. for 30 minutes to destroy the organism. Relatively small quantities of inhibitory substance had a marked influence on acid development; as little as 3 per cent inoculated milk added to normal milk almost completely inhibited acid production. The substance was believed to be a protein derivative, probably a polypeptide, which was readily destroyed by trypsin but was resistant to pepsin. The other organism did not appear inhibitory to normal lactic acid streptococci unless it first was destroyed by pasteurization.

Streptococci which produced a substance inhibitory to normal lactic acid streptococci were rather frequently detected in small numbers in normal milk by Cox and Whitehead (45). Presence of the substance was demonstrated by a special enrichment technic. Apparently the organism existed irregularly and in small numbers in the udders of some cows, in cow dung and silage and on the coats of the animals.

According to Rice (218) contaminating organisms are one of the causes of slow acid development in cultures. Davis (57) noted that aerobic spore formers could stimulate or slow down cultures; anaerobic spore formers did not thrive under the conditions of culture propagation. He stated that all

these organisms are killed by heating milk at 98.9° C. for 1 hour. Nelson *et al.* (180) found that extraneous bacteria, either those present in milk or those gaining entrance from plant equipment, appeared unimportant as a cause of retarded acid development in cultures. In investigating a case of slow cultures Harrison and Dearden (104) failed to isolate "non-acid" organisms. According to Davis (60) coliform organisms have little effect on butter cultures in the early stages but definitely inhibit the culture organisms in the later stages of growth.

Davis (57) suggested that butter cultures can be tested for purity by adding 1 part of 20 volume hydrogen peroxide to 1 part of culture in 10 parts of water in a glass tube or bottle. The contents are mixed and allowed to stand. A continuous evolution of gas indicates that the culture is contaminated. He also suggested that the culture be smeared on a milk agar slope to test for foreign bacteria.

Preparation of milk. Whitehead and Wards (320) noted that preparation of culture in large open vessels had an adverse influence on certain culture bacteria within two or three generations; the difficulty was prevented by pasteurizing and cooling the milk without agitation in small closed vessels, contamination being more easily avoided in this way.

In an investigation by Whitehead and Cox (311) sudden failure in growth of cultures was due to the method of pasteurizing the milk for the cultures. With certain cultures, aeration of the milk immediately before inoculation had an inhibitory effect on the organisms. Normal cultures were not affected by aeration of the milk; however, they could suddenly develop a sensitivity to aeration and also could suddenly revert to the normal state. Mere absorption of atmospheric oxygen while the milk was being cooled after pasteurization in a wide mouth cylindrical vessel could aerate sufficiently to inhibit sensitive cultures of lactic acid streptococci. Milk pasteurized and cooled in conical flasks plugged with cotton did not reabsorb sufficient oxygen to lead to inhibition, provided the flasks were not violently agitated. Evidence was presented which suggested that aeration is related to the oxidation-reduction reactions by which the organisms normally obtain their growth energy.

Bacteriophage. In 1933, studies at the Iowa Agri. Expt. Sta. (123) showed that bacteria free filtrates from slow butter cultures inhibited acid production when added to certain normal cultures.

Whitehead and Cox (313) isolated bacteriophage by the usual methods from an aerated culture which failed to develop acid. It was propagated in serial culture for several weeks and was obtained in such strength in a bacteria free filtrate that one part of the filtrate could be diluted with several hundred thousand million parts of milk and still lyse a susceptible organism. With the organism on a solid medium, the phage gave the classic appearance of plaques, lytic action spreading from the hypothetical phage

corpuscles to form circular, clear spaces in a mass of confluent growth. The phage appeared to be specific in its action on one strain of *Streptococcus*. Under usual growth conditions it gave little evidence of its presence in a culture; however, aeration seemed to activate it. Later, Whitehead and Cox (314) described a method for purifying suspensions of phage. The dilution at which evidence of phage action just failed was taken as the titre; titres of 10^{-8} to 10^{-9} commonly were obtained. Phage suspensions were very specific. Their optimum temperature was about 30° C., but they acted at both 20° and 37° C. They were stable at 4° C. for several months but showed slight loss of activity at room temperature in 1 week; they were almost completely destroyed at 70° C. in 30 minutes.

Matuszewski *et al.* (162) found that occasionally butter culture developed 0.27 to 0.45 per cent lactic acid and then failed to develop more acid; at the same time the bacteria suddenly disappeared from the milk.

Whitehead and Hunter (315) noted that phages appear to arise spontaneously in milk cultures of lactic acid streptococci under certain growth conditions. A resistant culture obtained by action of a primary phage on a sensitive strain of *Streptococcus* ultimately was attacked by a phage quite distinct from the original one. Series of resistant cultures and secondary phages thus were obtained for each type of *Streptococcus*. Contamination of culture with phage was considered unlikely in view of the specific relationships between phages and organisms and the consequent multiplicity of phages which would necessarily exist in the surroundings of the cultures. Results were believed to support the theory that phage is a product of the organism.

According to Crossley (47) the explanation that slow cultures are due to phage seems to fit more of the known facts than any other, but in England attempts to isolate phage from culture failed.

Whitehead and Hunter (316) found that single strain cultures of streptococci failed to produce acid due to spontaneous appearance of phage. This was remedied by increasing the inoculum from about 0.2 to 1.0 to 1.6 per cent. The authors suggested that the effect of heavy inoculation in eliminating spontaneous appearance of phage was due to more rapid growth of the streptococci when transferred to each new batch of milk, that is, to shortening the lag period. Phage tended to appear whenever the lag period was prolonged as a result of light inoculation, low incubation temperature or aeration or aging of the milk. With some milk supplies the heavy inoculation technic was not completely effective in preventing appearance of phage.

In the trials of Nelson *et al.* (180) marked retardation of acid production often resulted when a slow butter culture was added to a normal culture at the time of inoculation. Plate counts showed that slow acid development was due to comparatively slow growth of the culture organisms. When bacteria free filtrates from butter cultures were added to freshly inoculated

S. lactis cultures or butter cultures, a majority of the filtrates from slow cultures and a smaller percentage of those from apparently normal cultures markedly restrained acid production and increase in numbers of organisms. Bacteria free filtrates from slow and normal butter cultures varied widely in their abilities to inhibit different strains of *S. lactis*. Almost every filtrate from a slow culture prevented normal acid development by at least one strain of *S. lactis,* and most filtrates were active against several strains. Filtrates from normal cultures often contained inhibitory principle, but in most cases they were active against only one or two closely related organisms. It appeared that at least part of the difference between slow and normal butter cultures was that slow cultures contained enough strains of principle to inhibit a substantial fraction of the *S. lactis* types present, while normal cultures either contained no inhibitory principle or only one or two strains of limited activity. When the principle became dominant, growth of sensitive organisms was retarded and organisms not sensitive became very numerous in the culture. The principle then was unable to multiply for lack of sensitive organisms and was diluted out, the culture becoming normal. Such a general sequence of events also partially explains the changes in types of inhibitory principle and of sensitive organisms which were observed in various transfers of a butter culture. Adaptation of a strain of principle to activity against an organism not previously inhibited was observed but once and then under conditions which were not well controlled. Characteristics of the principle active against *S. lactis* were those commonly reported for bacteriophages. It was capable of passing an ultrafilter. Propagation on sensitive organisms was possible under a variety of conditions. Lysis of sensitive organisms was accomplished by addition of filtrates containing the principle to suspensions of the organisms in various broths. The principle was not affected by freezing and, at the proper pH, only slightly by drying. At approximately neutral reactions some strains of principle survived 70° C. for 10 minutes but not for 15 minutes; other strains were inactivated by less heat. The principle was more resistant to heat than its homologous organism. It was inactivated by crystal violet, methylene blue, hydrogen peroxide and potassium permanganate. Strains of principle studied were most active at neutral or slightly acid reactions. Virulence of the principle apparently was somewhat less than that of phages active against certain species but was equal to that of phages active against some pathogenic streptococci. No definite procedure for controlling or preventing outbreaks of retarded acid development was indicated by the results. Methods which destroyed the phage also destroyed the organisms with which it was associated. None of the non-sensitive butter cultures were studied sufficiently to demonstrate complete immunity to all strains of phage. Observations indicated that some butter cultures had more of a tendency to become slow than others. Selection of strains was suggested as a method of arriving at comparative freedom

from outbreaks of slow acid production. Cultures rather susceptible to delayed acid development generally were those which gave the most desirable flavors, especially in butter.

Normal cultures of an organism which Sutton (263) used in phage enrichment trials formed fairly long chains of box-shaped cells, while phage treated cultures formed much shorter chains which could break down to diplococci or even single cells. Another phage effect was formation of aggregates of 20 or more cells having a distorted appearance; the cells seemed to be tightly packed into irregular bundles. Two strains of phage destroyed or distorted the majority of cells in a culture, but they seldom delayed coagulation more than 24 hours after inoculation.

Yakovlev (330) found phage retarded and prevented coagulation of milk. Phage was isolated from a strain of *S. cremoris*. In milk it survived 80° C. for 10 minutes or 90° C. for 5 minutes; in a dry state it resisted 95° C. for 2 hours. It induced lysis at pH 6.5 to 5.0 and was resistant to lactic acid. Spontaneous lysis during fermentation of pasteurized or sterilized milk by lactic acid bacteria was preceded by agglutination of all bacterial cells. Shaking and stirring stimulated lysis and retarded growth in secondary cultures. When grown on solid media the latter were temporarily resistant; on liquid media their resistance was more lasting, but they remained carriers of phage. Methylene blue was a good indicator for detection of lysis in fermenting milk. Substitution of resistant bacteria was the only remedy against phage under industrial conditions.

Whitehead and Hunter (317) outlined a procedure for isolation and purification of phage. Preparations could be stored for many months in a refrigerator without much loss of activity. Nine phage strains were all very similar in general properties: Plaques had diameters of 0.25 to 0.5 mm.; thermal death points approximated 70° to 75° C. with 30 minute exposure at pH 6.0; and optimum temperatures were between 30° and 37° C. The outstanding difference between them was in their specific action on the various organisms. Phages showed a tendency to be specific for bacterial strains, but some races attacked up to four strains. Cross resistance tests, in cases where two or more phages acted on one strain, indicated that relationships between the phages did not follow any simple rule.

Hunter and Whitehead (122) found active chlorine the most effective and convenient agent for destruction of phage. Hydrogen and hydroxyl ions inactivated phage when they were present in sufficient concentration, but their effects between pH values of 4 and 7 were negligible during several days at room temperature.

According to Sutton (264) phage in milk cultures of *S. cremoris* was not affected by 1 hour irradiation, but when present in thin films or droplets of whey or in whey dust it was completely inactivated by irradiation. He suggested incidence of culture failure might be decreased by use of effectively designed irradiation hoods.

Johns and Katznelson (125) found sudden stoppage of acid development in a culture was due to activity of polyvalent streptococcal phage. Presence of phage in the culture could not be demonstrated which suggested outside contamination. Tests on milk from individual cows and from herds and on stable and laboratory air failed to indicate the origin of the agent.

Harrison and Dearden (104) investigated a case in which slow cultures were observed annually; they began in early May and continued until late July or August. The flora of the cultures was largely *S. lactis* which the authors considered unusual. Trouble was remedied by changing the source of the cultures. Attempts to detect phage failed.

Phage active against lactic acid streptococci was found in the atmosphere of a plant by Whitehead and Hunter (318). Concentration of air borne phage sometimes was so great that with normal bacteriologic technique it was impossible to prevent infection of cultures for more than a few propagations. Protection of the cultures from air borne phage eliminated culture failures.

Delayed coagulation of butter cultures due to bacteriophage constitutes a very serious problem in the preparation of cultures. Outbreaks of slow cultures apparently are becoming more common. Use of mother cultures from new sources often does not solve the difficulty and commonly when an outbreak is over the reason is not evident.

Activities of butter cultures

There are wide variations in the activities of different butter cultures, due undoubtedly to the particular organisms making up the cultures, the presence of bacteriophage, etc. Within certain limits, differences in activities can be compensated for by varying the inoculation, but when the activity is greatly decreased this is not possible. Cultures that are especially rapid sometimes are desired, particularly when used in certain cheeses.

Whitehead and Cox (309) developed the following procedure for determining the relative activities of various cultures from the standpoint of cheese making: A pint of milk is placed in a wide mouth jar holding about 0.75 quart, the temperature is adjusted to 37.7° C. and 5 ml. culture is added. After 0.5 hour, 1 ml. of rennet is added and the mixture returned to the water bath for 1 hour. The resulting curd is cut into 0.25 inch cubes and the bottle returned to the water bath for 2 hours. The whey is drawn off and a 9 ml. portion titrated with N/10 sodium hydroxide. The curd is incubated an additional hour and the whey which has exuded is once more titrated. The values obtained with different cultures are compared.

DEVELOPMENT OF BUTTER CULTURES

At one time cultures used in ripening cream for butter were obtained from natural sources, such as sour milk or cream, buttermilk, butter, etc.

In general, they were not satisfactory; objectionable flavors often were present and irregularities occurred from day to day because of contaminating organisms.

Neethling (179) found that cultures prepared from natural sources, such as feeds and butter, usually contained organisms which produced objectionable flavors and which could not be readily eliminated. Vas and Csiszar (292) attempted to produce self-prepared cultures for cream ripening from naturally fermented raw milk by propagation in boiled skimmilk. The cultures were not satisfactory, the raw milk being contaminated with organisms which soon predominated in the transfers. Varieties of lactic acid streptococci which caused objectionable flavors sometimes were present.

When it was recognized that butter cultures contain two distinct types of bacteria, each with a definite function, development of butter cultures by mixing cultures of the necessary organisms became possible. The selected strains commonly are mixed in sterile milk, and a mixture may be carried through a number of transfers there before being propagated in pasteurized milk. Certain investigators have preferred to inoculate one of the species, which differs with different investigators, some time before the other in an attempt quickly to establish a satisfactory relationship between them.

Boekhout and Ott de Vries (24) reported that not all strains of lactic acid and flavor bacteria are suited to each other, some combinations producing no flavor. Hammer (87) prepared butter cultures of good quality by mixing *S. lactis* and a flavor organism; the best procedure seemed to be to grow the flavor organism in sterile milk at least 1 day before adding *S. lactis* so that it would have ample opportunity for development. Both *S. citrovorus* and *S. paracitrovorus* gave good butter cultures in some of the trials. Later, Hammer (89) noted that preparation of butter cultures by mixing organisms permits selection of the cultures used. Malty *S. lactis* cultures should be avoided. By selection it was possible to develop butter cultures showing little tendency to high acidities. In the trials of van Beynum (278) the best flavor was produced when the two types of bacteria used to prepare the culture were inoculated simultaneously. Satisfactory flavor also was produced when the flavor bacteria were inoculated after the lactic acid bacteria had developed more or less in the milk, but no flavor was detected when they were inoculated 24 hours before the lactic acid organisms.

Farmer and Hammer (73) found that *S. lactis* var. *maltigenes, S. lactis* var. *hollandicus, S. lactis* var. *tardus* and *S. thermophilus* have characters which make them unsatisfactory for development of butter cultures. *S. lactis* var. *anoxyphilus* in combination with flavor organisms sometimes yielded fairly satisfactory butter cultures. Typical *S. lactis* strains which produced a sharp, high-acid flavor when grown alone generally gave this same character in combinations with flavor organisms. Typical strains showing no objectionable flavor when grown alone gave satisfactory butter cultures with

certain flavor organisms but not with others. Among unselected *S. lactis* and flavor organisms, an *S. lactis* strain which combined satisfactorily with one flavor organism often did not combine satisfactorily with another, and a flavor organism which combined satisfactorily with one *S. lactis* strain often did not combine satisfactorily with another. Flavor organisms that improved the flavor of *S. lactis* strains produced the most improvement with strains that alone gave a clean, mild flavor. A flavor organism which in milk gave an odor suggesting a good butter culture did not always develop a good culture when combined with an *S. lactis* strain producing a clean, mild flavor. Each combination of lactic acid and flavor organisms was very consistent in its ability or inability to develop satisfactory butter cultures. Some combinations of organisms produced flavor in the first transfer in pasteurized milk, while with others several transfers lacked flavor and the desired flavor then appeared. Some combinations produced flavor in the first few transfers, while later transfers lacked flavor.

Studies by Baker (9) showed that, in mixing organisms for development of butter cultures, addition of flavor organisms in large numbers compared to *S. lactis* tended to increase the amount and rate of volatile acid production. However, results with the procedure were not constant and indicated that the flavor organisms sometimes grow well in combinations even when added in small numbers at the time of preparing the mixture. Ability of combinations of flavor organisms and *S. lactis* to produce high volatile acidities was not a satisfactory basis on which to select them for developing butter cultures. Freshly isolated cultures of *S. lactis* were no better than old cultures for combining with flavor organisms.

Stine (258) noted that flavor streptococci which markedly increased production of carbon dioxide in milk on addition of 0.5 per cent citric acid were much more likely to give good flavor when combined with *S. lactis* than those failing to give such an increase.

van Beynum and Pette (280, 281) found that, when mixed with flavor organisms, lactic acid streptococci which did not form amc could produce as good a flavor as amc positive strains. Gibshman (78) reported that *S. lactis* and *S. cremoris* strains which form amc + ac_2 and also strains which do not may be used in butter cultures.

Variations in flavor production in butter cultures were attributed by Vas and Csiszar (291) partly to the strains of streptococci used and partly to the fact that the cultures contained certain organisms which destroyed the flavor so that finally destruction of flavor exceeded production. Davies (54) noted that the flavor-producing efficiency of a butter culture depends on the distribution of bacterial strains in it, especially with regard to the number of special flavor bacteria (*S. paracitrovorus* and *S. cremoris*) and their persistence in repeated propagations.

For developing marked flavor in margarine, Palladina *et al.* (200) recom-

mended selected strains of *S. cremoris* plus *S. lactis*. To improve flavor of storage goods without high acidity, flavor organisms were suggested.

SPECIAL CULTURES FOR BUTTER MANUFACTURE

Various special cultures have been suggested for development of flavor in butter. These include pure cultures of flavor organisms present in the usual butter cultures and also pure cultures that differ distinctly from them. Some of the latter appear to combine the characters of the two types of organisms normally in butter cultures in that they actively attack both lactose and citric acid and form the same general decomposition products.

Pure cultures of the usual flavor organisms

Hammer (88) stated that use in pasteurized cream of a culture of *S. citrovorus* or *S. paracitrovorus* with either citric or lactic acid added cannot be expected to yield butter of better flavor than use of butter culture. The proper balance between *S. lactis* and flavor organisms apparently results in elaboration of compounds that are excellent for production of flavor in butter. Acid development by *S. lactis* was believed to prevent growth of undesirable bacteria that are present in cream even when it has been carefully pasteurized; products formed by *S. citrovorus* and *S. paracitrovorus* probably have no such effect. This suggests that control of the fermentation would be more difficult with cultures of the flavor organisms than with butter cultures.

In the studies of Maddock (157) cultures of *S. paracitrovorus* produced butter of exceptionally fine flavor under controlled conditions, but the uncertainties under practical conditions were too great to warrant their general use. When *S. paracitrovorus* was employed in a commercial butter plant, butter made with the first sub-culture was excellent but thereafter it was very inferior to butter made with regular butter culture. Addition of the organism to a commercial butter culture gave disappointing results. Both *S. citrovorus* and *S. paracitrovorus* were unable to compete successfully with the inevitable contaminants encountered in practice. Maddock concluded that *S. paracitrovorus* alone is not suitable for use in butter manufacture because of the time required to improve the vigor and virulence of acid production, unequal results in the butter produced and failure to withstand vigorously growing contaminants.

Michaelian *et al.* (166) prepared a special butter culture by inoculating pasteurized milk with a flavor organism, holding at 21° C. for 15 to 24 hours, adding 0.15 per cent citric acid and 0.3 per cent sulfuric acid (sp. gr. 1.83–1.84) and incubating an additional 24 hours. After adding the acids, production of amc + ac_2 was very rapid, and finally the amounts of these compounds and of volatile acid were greater than with regular butter culture. During rapid production of amc + ac_2 there was an increase in pH and a

decrease in titrable acid in the cultures; rather large amounts of the compounds were produced at pH values from 4.6 to 3.1, but production was most rapid at pH 4.3 and 4.0 with the strain of organism used.

Other species of bacteria

Wolff (328) described an organism which produced both *l* and *d* lactic acid and also acids of the aliphatic series. In milk it gave a strong aroma which apparently was due to ac_2 formed from sugar. The organism was named *Bacterium diacetylicum* (Voss).

Two organisms were isolated by van Beynum and Pette (280, 281) and as pure cultures were used in butter manufacture. Descriptions are as follows:

Streptococcus aromaticus, which was found in cheese and whey, produced *d* lactic acid and did not attack citric acid. In milk, no acetic acid or carbon dioxide was formed, but small amounts of amc and ac_2 were produced. The organism formed acid slowly and curdled milk after 5 days or more at 21° C. It grew well in milk and whey, but peptone and yeast autolysate did not meet the nitrogen requirements. Fermentation tests in beef broth showed that only glucose, lactose and mannose were fermented. Since ac_2 was produced, milk cultures possessed an aromatic odor, but it was not quite the same as that of good butter culture. With small amounts of acetic acid and bicarbonate added, the odor of milk cultures perfectly resembled that of butter culture.

S. citrophilus, which was isolated from market milk, acidified milk within a short time, formed *d* lactic acid and fermented maltose and dextrin. It also vigorously fermented citric acid and produced carbon dioxide, acetic acid, amc and ac_2. Quantities of the last three compounds were the same as with butter culture. Milk cultures did not smell like butter culture but had a disagreeable, acid odor like so many lactic acid cultures. Only when growing in thin layers with free access to air was an aromatic odor noted.

Vas and Csiszar (289) found certain types of *S. lactis* which, in pure culture, behaved exactly like butter cultures. They stated that these species should be considered in the preparation of cultures for butter.

Matuszewski *et al.* (161) isolated, from sour potato mash and kefir, five strains of an organism which produced lactic acid as well as amc; they named it *Streptococcus diacetilactis.* In milk *d* lactic acid was produced to the extent of 0.77 to 0.81 per cent, acetic acid in amounts ranging from 0.055 to 0.072 per cent and carbon dioxide in amounts varying from 0.022 to 0.034 per cent. The optimum, maximum and minimum growth temperatures were 26°, 40° and 5° C., respectively. Rate of acid development corresponded to that of *S. lactis.* After frequent transfers in sterile milk the organism produced sliminess. Considerable amc was present in a 24-hour milk culture, but ac_2 was not present in detectable amounts. Amc was formed when the

total acid was 0.45 per cent and reached a maximum at 0.79 to 0.90 per cent. Addition of 0.5 per cent sodium citrate greatly increased production of amc. Proteolytic action of the organism was slight. Butter manufactured from cream ripened with the organism had a delicate and pleasing flavor after 2 or 3 days. This was explained by the gradual oxidation of amc to ac_2 in the early stages of holding. With *S. diacetilactis,* Davis and Thiel (64) found the optimum initial pH for gas production was 5.5; optimum pH for growth was 6.5.

Joshi and Ram Ayyar (127) isolated from butter an organism which gave a better flavor than the usual organisms likely to be used in Indian dairies. It was similar to *S. citrovorus* and *S. paracitrovorus,* but because of its ability to produce lactic acid and aroma it was named *Streptococcus lactis aromaticus.* The organism produced considerable volatile acid when grown for 1 week in plain milk and still more when grown in milk plus 0.4 per cent citric acid. Optimum growth temperature was 30° C. In plain milk only a trace of amc + ac_2 was produced, but in milk plus 0.5 per cent citric acid the amount was much larger.

An intermediate type of lactic acid *Streptococcus,* having the properties of both *S. lactis* and *S. citrovorus,* also was isolated by Bogdanow and Efimchenko (26).

Matuszewski *et al.* (162) obtained highest production of amc with a mixture of *S. diacetilactis, S. cremoris* and *S. citrovorus.* Lowest production was obtained with mixtures of *S. cremoris* and *S. citrovorus.* Cultures involving mixtures of *S. diacetilactis, S. cremoris* and *S. citrovorus,* as well as *S. diacetilactis* alone, produced considerable amc.

Gibshman and Bannikova (81) studied the biochemical properties of 460 cultures of sour milk streptococci obtained from various milk products. Some of them formed both acid and flavor substances, but the instability of these activities prevented replacing the usual mixed cultures with the single strain cultures.

Hoecker and Hammer (112) found cultures of various streptococci produced relatively large amounts of ac_2 and amc in milk containing added citric acid. These included *S. citrovorus, S. paracitrovorus, S. diacetilactis, S. citrophilus* and an unidentified organism. *S. aromaticus,* which does not ferment citric acid, produced ac_2 and small amounts of amc in milk. With each of the species, the ratios of ac_2 to amc varied in the different trials; frequently, the ac_2 was much higher in proportion to the amc than with butter cultures.

APPENDIX

General statements on the methods of determining diacetyl, acetylmethylcarbinol and 2,3-butylene glycol are presented in an attempt to show the variations in the procedures used with each of the compounds and some of the difficulties involved. In the actual determinations the standardized procedures should be followed in detail.

PART A. STATEMENT ON METHODS OF DETERMINING DIACETYL (ac_2) AND ACETYLMETHYLCARBINOL (amc)

Since amc is readily oxidized in large part to ac_2, using ferric chloride or some comparable reagent, methods of determining ac_2 also are applicable to amc or amc + ac_2.

Gravimetric and colorimetric methods. Prill *et al.* (209) noted that certain generalizations can be made with regard to the analytically important reactions of the α dicarbonyl compounds. With few exceptions these compounds form dioximes which, in many cases, react with certain metal ions to produce undissociated complex salts, the nickel salts being the best known.

van Niel (285) stated that the method of Lemoigne (150) does not give quantitative results. He described a method based on the Lemoigne reaction which permits a quantitative estimation of ac_2 and amc as nickel dimethyl glyoximate (also 168).

Conditions affecting the completeness of precipitation of ac_2 as nickel dimethyl glyoximate and the separation of amc + ac_2 and ac_2 from butter by distillation were studied by Barnicoat (12). He proposed a colorimetric method for the determination of traces of nickel dimethyl glyoximate. It is based on solution of the compound in chloroform and comparison with solutions of known amounts of the salt.

Mohler and Herzfeld (172) described a method for the determination of ac_2 as nickel dimethyl glyoximate. Various qualitative tests for ac_2, such as the salicylaldehyde test, the iodoform test and the phenylhydrazine test, also were given (also 215).

Stahly and Werkman (256) stated that the Lemoigne-van Niel method (285) of determining amc gives constant but not quantitative results. They found that it accounted for approximately 84 per cent of the amc.

Various factors which influence the determination of amc and ac_2 as nickel dimethyl glyoximate were studied by Michaelian (164).

Mohr and Wellm (175) suggested that the method of Barnicoat (12) be employed as the standard procedure for determining amc and ac_2. However, certain modifications in the method were proposed. Recovery of amc was said to be from 80 to 90 per cent.

Schmalfuss and Werner (237) described a simplified apparatus for use with a modification of the nickel dimethyl glyoximate method. They determined the smallest size sample which consistently gave a positive test for ac_2 or amc and on this basis estimated the amounts of the compounds. They could detect 0.000012 g. in water, paraffin oil, peanut oil and margarine made with milk and 0.000016 and 0.000024 g., respectively, in slightly rancid and highly rancid margarine made with water.

Dehove and Dessirier (66) suggested distillation of ac_2 from a sample and treatment of the distillate with hydroxylamine hydrochloride and sodium hydroxide. After agitation, nickel sulfate and acetic acid were

added. The precipitate was washed, purified and compared with standards. The authors were able to evaluate ac_2 with an accuracy of 0.5 mg. per kg.

Various other investigators have reported on the determination of ac_2 as nickel dimethyl glyoximate and on modifications of the method (51, 113, 128, 136, 171, 213, 236, 268, 301).

Kunze (146) described a micromodification of the gravimetric method for determining amc and ac_2 as nickel dimethyl glyoximate. He recommended colorimetric determinations for amounts less than 0.3 mg.

Ruehe and Corbett (229) developed a rapid volumetric method for determining amc + ac_2 in butter cultures. It is based on the oxidation of one molecule of ac_2 to two molecules of acetic acid with hydrogen peroxide.

A colorimetric method for the determination of ac_2 was suggested by Testoni and Ciusa (273). They oxidized the nickel dimethyl glyoximate and obtained a soluble red complex in which the nickel had a higher valence number.

Pien *et al.* (205) developed a colorimetric procedure by reacting ac_2 with m-p-toluilenediamine and making use of the yellow color which the resulting quinoxaline derivative exhibits in the presence of strong acid. No color was developed in the absence of ac_2 and the test was sensitive to 1 part ac_2 in 100,000 parts of solution. Later, they (204, 206) used diaminobenzidine and obtained a stronger yellow color which they assumed resulted through formation of a bisquinoxaline derivative and which they considered useful for measuring as little as 0.05 mg. ac_2 in 10 ml. of distillate. The method was said to be 10 to 20 times more sensitive than the toluilenediamine method. Ritter and Nussbaumer (226) did not obtain the pure yellow color with the method of Pien *et al.* (205) but always obtained a yellow-brown color. The difficulty was traced to the sulfuric acid employed and the authors suggested use of both pure concentrated sulfuric acid and a fresh solution of m-p-toluilenediamine. Dehove and Dessirier (66) noted that the method of Pien *et al.* (205) necessitates too great a quantity of butter, considering the sensitivity. It did not permit an accuracy of 4 mg. per kg. when 50 g. of butter was used. Brioux and Jouis (32) determined the amc and ac_2 contents of butter samples by the method of Pien *et al.* (205). Determination of amc was accomplished by placing 100 g. butter in a 300 to 400 ml. flask, adding 20 ml. of iron perchloride and distilling slowly with steam until 50 ml. of distillate was obtained. Ten ml. of distillate was then used in the colorimetric determination of ac_2.

The method of Pien *et al.* (206) was extended by Cox and Wiley (46). The apparatus, amount of sample, method of distillation and estimation of ac_2 in the distillate were standardized. The authors concluded that, for small amounts, the colorimetric method may be as accurate as the nickel method, but the nickel precipitation method is probably more accurate for measuring large quantities.

Prill and Hammer (210) developed a colorimetric method for the microdetermination of ac_2 which is based on the formation of the intensely colored ammono-ferrous dimethyl glyoximate. With this method it was possible to detect a difference between 0.001 mg. and no ac_2 in 5 ml. water. The color produced is proportional to the amount of ac_2.

Rapid tests. O'Meara (186) described a qualitative method for determining amc in bacterial cultures by use of creatine and sodium hydroxide. Development of a red color is indicative of amc; the color forms in a few minutes. Dicyanodiamide was suggested as a substitute for creatine. The method was considered more accurate and sensitive than the Voges-Proskauer reaction (302). Toth (276) recommended the colorimetric method using dicyanodiamide for the rapid and reliable determination of ac_2. He suggested that a dilute aqueous solution of acid colored with methyl orange and methyl red be used for comparison.

Hammer (94) applied the method of O'Meara (186) to the rapid testing of butter cultures for amc + ac_2. Results indicated that the method could be used to advantage since it gave general information on comparative amounts of amc + ac_2 in butter cultures. Mohr and Wellm (174) stated that the results obtained by tasting did not always correspond to the ac_2 and amc contents, nor to the creatine test. Davies (55) recommended the Voges-Proskauer reaction and use of creatine as a qualitative test for ac_2 but stated that the color obtained in the test is not quantitative, due to polymerization of ac_2 in alkaline solution to benzene derivatives (also 51).

Barritt (14) reported on intensification of the Voges-Proskauer reaction by addition of α naphthol. Use was made of creatine as described by O'Meara (186) plus addition of 0.5 ml. of an alcoholic solution of α naphthol. With the method the author detected 1 part ac_2 in 2,500,000 parts. The test was said to be specific for amc or ac_2. Batty-Smith (20) compared the method of O'Meara (186) with that of Barritt (14). He found the latter did not give false positives.

The various rapid methods for estimation of amc and ac_2 were summarized by Ritter and Nussbaumer (225). The most simple and positive was the method of Hammer (94) with the addition of α naphthol. Ritter (222) reported on use of the Voges-Proskauer reaction on a portion of the distillate from a volatile acid determination.

Vas and Csiszar (290) and Csiszar (49) determined the presence of aroma substances by adding 2.5 ml. of culture and 2.5 ml. of 30 per cent potassium hydroxide in a white porcelain vessel and shaking for 0.5 hour. A red color indicated aroma substances. The same test was used on butter serum for the detection of flavor substances (48) (also 132).

The literature on the determination of amc and ac_2 has been reviewed by various investigators (65, 76, 209, 225).

PART B. STATEMENT ON METHODS OF DETERMINING 2,3-BUTYLENE GLYCOL (2,3-bg)

Brockmann and Werkman (33) described a method for determination of 2,3-bg which consists of steam distillation and oxidation of the glycol in the distillate to ac_2 by means of potassium periodate and sulfuric acid. The ac_2 is passed into an absorption tower containing hydroxylamine hydrochloride. An excess of acetone is added to liberate hydrochloric acid and the volume of standard sodium hydroxide required to titrate the hydrochloric acid liberated from the excess hydroxylamine hydrochloride is measured. The method has been used (102) to determine 2,3-bg in butter cultures.

Results of Stahly and Werkman (256) in treating an amc solution in the manner described by Brockmann and Werkman (33) for the determination of 2,3-bg indicated that amc reacts in the procedure and must therefore be determined separately and subtracted from the total. The authors stated that Brockmann and Werkman assumed that 1 molecule of amc yielded 2 molecules of acetaldehyde and that their results were not adjusted to the purity of the carbinol which they used.

van Beynum and Pette (282) modified the method of Brockmann and Werkman (33) as used with butter cultures (102). They employed sodium chloride to decrease the solubility of 2,3-bg in water and neutralized the liquid with sodium hydroxide to a slightly alkaline reaction to prevent distillation of acid. Previously sodium carbonate had been used for both purposes (33, 102).

Kniphorst and Kruisheer (136, 137) described methods for determination of 2,3-bg in wine and other fermentation products and Komm and Flugel (143) outlined a method for use with bread; the procedures involve oxidation of 2,3-bg to ac_2 by means of bromine.

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THE RELATION BETWEEN THE DEGREE OF SOLIDIFICATION OF FAT IN CREAM AND ITS CHURNING TIME. I. MEASUREMENT OF THE DEGREE OF SOLIDIFICATION

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The churning time of cream is markedly affected by the temperature of churning and also by the length of time the cream has been held at any particular temperature before churning. These effects are thought to result from changes in the degree of solidification of the fat. Qualitative evidence favors this supposition: as the temperature of cream is lowered, within the churning limits, the churning time increases. Unquestionably as the temperature is lowered within these limits more fat becomes solidified. It is also a fact that small changes in churning temperature have a great effect on churning time; that is, raising the churning temperature by two degrees may shorten the churning time from 40 minutes to 20 minutes. Conceivably, these marked effects may result from proportionately large changes in the degree of solidification of the fat, or there may be a critical zone of solidification governing churning. To explore these possibilities requires quantitative data regarding the proportions of solid and liquid fat in cream at usual churning temperatures and regarding the effects of temperature changes on these proportions.

That milk fat exists partially in the solid and partially in the liquid state at the usual churning temperatures is well supported by the work of van Dam (8), King (5), Rishoi and Sharp (6), van Dam and Burgers (9), Bowen (1), Hammer and Johnson (3), Jack (4), and others. These investigations include dilatometer studies, x-ray diffraction technique, calorimetric data, and melting-rate observations. A method must be devised whereby the proportions of solid and liquid fat in cream can be determined before their influence on churning time can be shown.

There are many possible ways for measuring the proportions of solid and liquid fat. A thermal method based upon the heat capacity and upon the heat of fusion of fat seemed the most direct. Although no records giving these values were available, related data indicated that the heat of fusion is probably many times greater than the heat capacity. If this is true, a calorimetric study observing the heat input and the temperature rise of a cream sample of known fat content should indicate rather accurately how much fat changes from the solid to the liquid state, once the heat capacity and the heat of fusion of the fat are known. Consequently,[1] a calorimeter

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was devised suitable for determining the heat of fusion and the heat capacity of milk fat in cream.

EXPERIMENTAL PROCEDURE

Figure 1 shows the calorimeter assembly. The calorimeter vessel is a wide-mouth silvered vacuum flask of one-liter capacity, wrapped with sheet

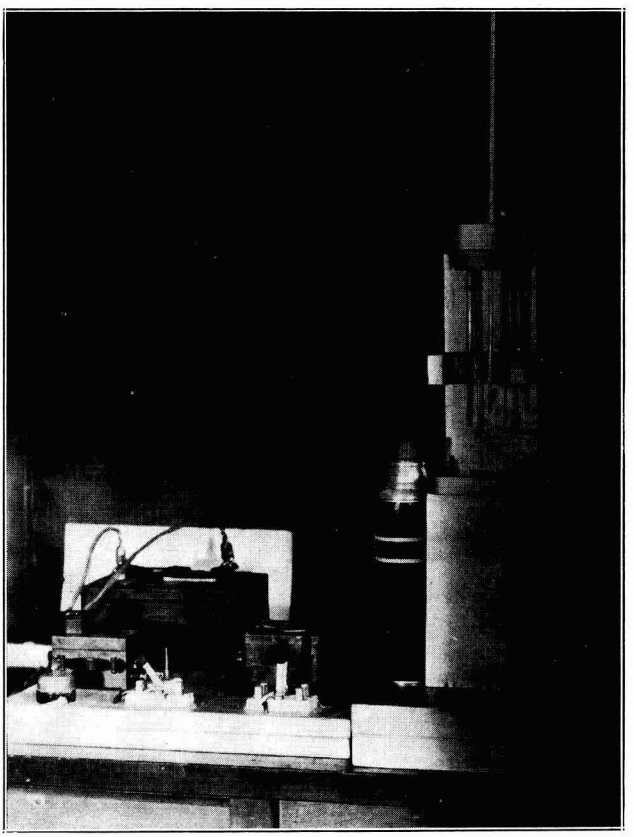

FIG. 1. Calorimetric assembly used in this study.

asbestos and tape for additional insulation. The heating element is a 20-watt, nickel-plated, knife-edge heater. Temperature was measured to 0.02° C. by means of a thermometer with a range from −10° C. to +60° C. Twin copper-constantan thermocouples suspended at different depths in the vessel were tried but because of difficulties in electrical shielding, proved inaccurate. These can be seen in the figure together with the cold junction. Heat was supplied from a three-cell storage battery, and the voltage was

measured with a cabinet-type volt meter to the nearest 0.01 volts. The liquid in the vessel was stirred by a propeller-type agitator at 700 r.p.m. The agitator, heater, thermometer, and other equipment are suspended through a rubber stopper that serves to close the calorimeter vessel: According to White (10), precision greater than 1 per cent can be attained only with very elaborate equipment; but precision of 2 per cent is attainable with elementary assemblies such as that used here, provided the principal errors are determined experimentally and compensated for in the calculations.

When electrical current is used as a heating medium, the heat generated is derived from Joule's Law as follows:

$$H = \frac{E\ I\ t}{4.186}$$

where H = calories generated,
E = the potential difference between terminals of the heating element,
I = current passing through the heating element,
t = time in seconds,
and 4.186 = Joule's constant.

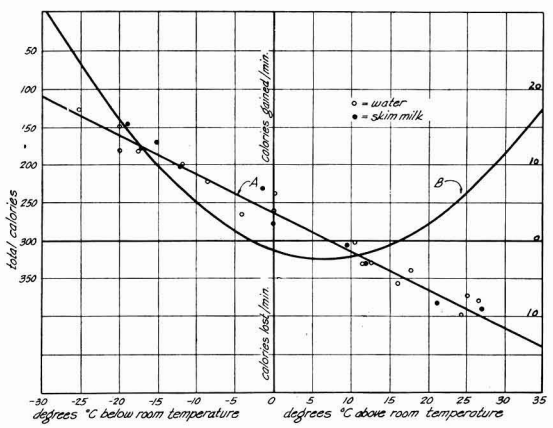

FIG. 2. Graphical representation of correction values used to correct the calorimeter for heat gained or lost as a result of thermal head, friction, etc. Curve A—Heat gained or lost per minute by a 500-gram sample at observed temperature. Curve B—Summation curve showing heat gained or lost per minute for observed temperature interval.

The resistances were measured with a Wheatstone bridge, and suitable adjustments were made to compensate for the resistance of the leads.

A calorimeter for this purpose is best calibrated experimentally with water, the heat capacity of which is considered to be unity over the temperature range studied—from 0 to 60° C. With this method not all the sources of heat loss or gain are evaluated individually, but an accurate overall correction can be obtained. This correction will include thermal leakage because of thermal head from the surroundings, losses from evaporation,

and the effects of agitational friction. Other sources of error such as fluctuations in heat input because of unsteady source of electrical energy, timing, and the like, are kept at a minimum through careful attention to operational technique. Figure 2 shows the correction data for both water and skim milk. These values were used to adjust each determination, thus compensating for environmental influences.

Besides the correction values discussed above, a portion of the heat added will be used to raise the temperature of the calorimeter. This portion is represented by the difference between the total heat input and the theoretical heat required for the observed rise in temperature when water is used as the calibration material. The amount might be called the heat capacity of the calorimeter, although it more correctly comprises also other factors that escape detection by the experimental calibration correction values shown in figure 2. It was found to be represented by the equation

$$Y = 0.44X - 47.2$$

where Y = the heat capacity of the calorimeter in calories per degree and
X = the temperature for which the heat capacity is desired.

Because of the physical character of fat, the heat values for milk fat in this temperature range must be determined indirectly. In consequence, the determinations were made on cream, which was considered to be composed of fat and skim milk—an assumption probably involving no significant error. The heat capacity of skim milk had therefore to be determined so that the heat required for raising the temperature of this portion of the cream could be subtracted from the total effective heat. The values determined were calculated as specific heats comparing to water at a heat capacity of 1 gram-calorie per degree and will be designated thus.

Five hundred grams of skim milk were weighed into the calorimeter, which was then placed in operating position. A temperature-adjustment period of 15 minutes was allowed before starting the determination, which consisted of successive five-minute heating periods followed by two-minute holding periods until the desired temperature range was covered. The voltage was read two minutes after heating was initiated in each heating period, and the temperature was read at the end of the holding period. This is the length of heating period recommended by White (10). Since a maximum temperature lag of one minute was observed, the two-minute holding period was chosen to eliminate it. The heating rate was approximately 0.6° C. per minute. Table 1 shows specific heat values for skim milk.

The column in the table for skim milk plus sodium chloride was necessary because the cream studies were extended to temperatures below the freezing point. A 17 per cent sodium chloride solution on the water basis was chosen to prevent freezing.

The values shown in the table compare with 0.9388 reported by Fleischmann (2) at a temperature of 14–16° C. Rishoi and Sharp (6) used an

TABLE 1

Specific heat values for skim milk and skim milk plus NaCl at temperatures ranging from −10° C. to 60° C.

Temperature, °C.	Specific heat values	
	Skim milk	Skim milk plus NaCl*
−10	.....	0.805
− 5	.....	0.807
0	0.943	0.812
5	0.944	0.815
10	0.944	0.815
15	0.945	0.816
20	0.946	0.817
25	0.948	0.817
30	0.949	0.818
35	0.951	0.819
40	0.954	0.819
45	0.957	0.821
50	0.960	0.823
55	0.963	0.825
60	0.966	0.827

* NaCl added at the rate of 0.17 grams per gram of water.

average value of 0.943. Hammer and Johnson (3) report specific heat values for skim milk ranging from 0.940 at 0° C. to 0.963 at 60° C., but the figures here reported are 0.003 higher over the same temperature range than theirs.

Cream samples ranging in composition from 15 per cent to 35 per cent fat were chosen to evaluate the thermal characteristics of milk fat. These were cooled in ice water and held at 0° C. from 14 to 16 hours. As Rishoi and Sharp (7) have shown, such a procedure produces a condition closely approaching physical equilibrium within the fat. These samples were studied calorimetrically over a temperature range from −10° C. to 60° C., using the technique described above for skim milk.

Table 2 gives the data showing the values obtained from a typical experiment.

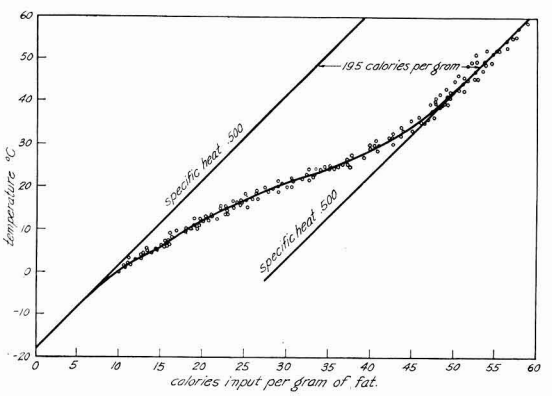

FIG. 3. Average melting curve for one gram of fat.

TABLE 2

Determination made with 500 grams of cream testing 32% fat (room temperature 24° C.)

Heating time	Temperature at end of heating period	Temperature rise during heating period	Voltage	Heat added from battery	Correction for thermal head	Heat added to cream	Heat required by the Calorimeter	Effective heat	Heat requirements of skim	Heat requirements of fat	Heat requirements per gram of fat	Apparent specific heat of fat	Summation of the heat requirements per gram of fat
				calories	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*		*calories*
0	0.71	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	4.10	3.39	5.958	1556	99.9	1656	164	1492	1086	406	2.537	0.548	2.537
10	7.62	3.52	5.958	1555	86.5	1646	175	1467	1130	337	2.104	0.598	4.641
15	10.90	3.28	5.958	1554	75.6	1630	168	1462	1054	410	2.561	0.781	7.202
20	13.96	3.06	5.955	1551	56.6	1608	161	1447	984	463	2.892	0.946	10.094
25	16.80	2.84	5.955	1549	55.1	1604	153	1451	914	537	3.355	1.181	13.449
30	19.47	2.67	5.955	1546	43.8	1590	148	1442	860	582	3.572	1.338	17.021
35	22.00	2.53	5.950	1544	35.1	1579	143	1436	815	620	3.880	1.532	20.901
40	24.60	2.60	5.950	1542	24.1	1566	150	1416	838	578	3.610	1.386	24.511
45	27.40	2.80	5.948	1540	16.5	1557	164	1393	903	490	3.062	1.094	27.573
50	30.20	2.80	5.940	1535	6.3	1541	168	1373	904	469	2.930	1.045	30.503
55	33.10	2.90	5.935	1531	− 7.0	1524	178	1346	938	408	2.550	0.879	33.053
60	36.07	2.97	5.928	1526	−15.0	1511	186	1325	960	365	2.280	0.768	35.333
65	39.18	3.11	5.920	1521	−24.0	1497	199	1298	1008	230	1.811	0.582	37.144
70	42.30	3.12	5.915	1518	−35.0	1483	204	1279	1012	267	1.668	0.534	38.812
75	45.41	3.11	5.910	1515	−49.8	1565	207	1258	1010	248	1.550	0.498	40.362

The values obtained from the cream-calorimetry experiments are shown in figure 3 as a melting curve.

The tangent to the slope of the curve in its upper portion represents the specific heat of liquid milk fat in cream. The best value from the experimental data shows the specific heat in this region to be 0.5. Physical difficulties prevented going below $-10°$ C. at the lower end of the curve. Whether or not this point represents complete solidification of the fat is difficult to say. Probably, since the slope of the experimental data in this region appears uniform, the amount of unsolidified fat present (if any) is very small. This condition did not hold at 0° C., where the slope was noticeably changing. If the fat is all solidified in this lower range, the best slope for the data is 0.5, and thus the specific heat is 0.5. The heat of melting between $-10°$ C. and 60° C. is calculated as 19.5 calories per gram. Whether or not this value represents the complete heat of melting of milk fat is subject to the conjecture concerning the complete solidification of the fat at $-10°$ C. At any rate, the figure is so near the value for the complete heat of melting that it may be used for calculating the proportions of liquid and solid fat in a sample of cream at the churning temperature without undue error.

These data, specific heat of 0.5 and heat of melting of 19.5 calories per gram, can be used to calculate the proportions of solid and liquid fat in an unknown sample at any temperature by measuring the amount of heat necessary to melt completely one gram of fat in the sample and then applying the following formula:

$$\text{Percentage of solid fat} = \frac{\text{Heat input/gm.} - (0.5 \times T_2 - T_1)}{19.5 \text{ calories/gm.}} \times 100$$

Heat input per gram = total amount of heat necessary to raise the temperature from T_1 to T_2.

$(0.5 \times T_2 - T_1)$ = amount of heat necessary to raise the temperature from T_1 to T_2 provided no heat is used for melting fat.

19.5 calories per gram = amount of heat necessary to melt one gram of fat.

Table 3 shows data based on calculations for samples of cream at differ-

TABLE 3

Table showing percentage of solid fat in cream at different temperatures

Temperature of cream in degrees C.	Time held at temperature, in hours	Total heat input/gm. fat, in calories	$(0.5 \times T_2 - T_1)$, in calories	Heat actually used to melt fat, in calories	Per cent solid fat
11.80	0	22.45	18.55	3.90	20.0
12.80	2	26.70	17.40	9.30	47.7
15.20	2	16.34	9.60	6.74	34.5
11.70	4	28.29	16.12	12.17	62.4
16.92	4	19.82	12.58	7.24	37.1
10.91	12	30.27	17.95	12.32	63.3
14.08	12	23.69	12.50	11.19	57.3

ent temperatures. These were cooled from the pasteurizing temperature to the temperatures noted.

The values given here are representative, indicating the differences that may be observed. The significance with respect to churning time will be reported in a subsequent paper.

DISCUSSION

The melting of milk fat constitutes, of course, a solubility phenomenon as well as a phenomenon of fusion and so the heat required for melting is probably a combination of heat of fusion and heat of solution.

The critical consideration for the purpose at hand is the validity of the value 19.5 calories per gram as the heat of melting for milk fat. The effect of variations within the fat is best illustrated by plotting the derivative values as apparent specific heats. Figure 4 shows three separate samples.

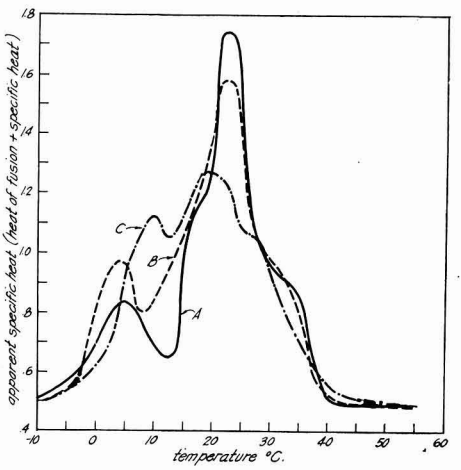

FIG. 4. Apparent specific heat values of milkfat over the temperature range studied. Curves A and B—Apparent specific heat values for milkfat in cream which was cooled in brine and held for 16 hours at −10° C. Curve C—Apparent specific heat values for milkfat in cream which was cooled in ice-water and held for 50 hours at 0° C.

Curves A and B represent samples treated identically and show the same melting characteristics, differing only in intensity; Curve C was prepared differently and has different melting characteristics, yet slopes in the region of what is apparently complete solidification and also where the fat is completely liquid are essentially the same. In addition, the areas subtended by each curve are almost identical, being equal to 19.2 calories for A, 19.8 calories for B, and 19.2 calories for C in the enclosed space above an apparent specific-heat value of 0.5.

It has been suggested that conventional calorimetry is not well suited for studying the thermal characteristics of milk fat because of the length

of time necessary for the fat to come to physical equilibrium. This is true if one desires to know the equilibrium state at intermediate temperatures in going from the solid to the liquid state. In this instance, however, the information desired was the over-all heat value without regard for the intermediates. The essential condition here has been to have the fat in physical equilibrium at the beginning and at the end of the heating period. Apparently that condition has been achieved.

SUMMARY

A calorimetric method is described for measuring the specific heat and heat of melting of milk fat in cream.

The specific heat of skim milk was found when measured to range from 0.943 at 0° C. and 0.963 at 60° C.

Milk fat in cream was found to have a specific heat of 0.5.

The heat of melting one gram of milk fat between −10° C. and 60° C. was measured at 19.5 calories.

Typical values for the percentage of solid fat in cream at various temperatures are given. These vary according to the temperature of the cream and the length of time it was held at that temperature.

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EFFECT OF SPRAYING COWS WITH REPELLENT TYPE SPRAYS AS MEASURED BY MILK PRODUCTION[1,2]

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Because of the seeming annoyance of dairy cows by flies both while the cows are feeding and at milking time, it is not surprising that fly annoyance is often suggested as one of the contributing causes for decline in milk flow during summer months. Regardless of whether the cows were distracted from eating the usual amount of feed or ruminating as usual because of time spent in fighting flies, or whether excess energy was expended in fighting these pests, or whether the loss of blood and the nervous reaction of the cow might be the most important factor, the end result would be caused by flies. If the distress to cows caused by flies could be prevented or mitigated by spraying the cows with repellent materials, it would seem logical to anticipate that the result might be reflected in increased milk production. Such information would be of value to dairymen and spray manufacturers.

Investigators in the fields of dairy husbandry and entomology have conducted experiments designed to measure, in milk production, the effect of spraying cows with repellent type sprays. As early as 1899 it was reported by Carlyle (4) that spraying cows resulted in no increase in milk production as compared with unsprayed cows. Beach and Clark (2) and Eckles (7) arrived at similar conclusions. Bishop (3) cited a loss of from 40 to 60 per cent in milk flow during a severe outbreak of stable flies. Cory (5) divided the herd, spraying one half with an emulsion and using the other half for check. He presented no data, but commented that spraying would result in increased production.

Eaton (6) divided the Alabama station herd equally into two groups, spraying one group with a commercial spray for two weeks and then alternating the groups. Such alternations with groups continued for 168 weeks. He reported that the non-sprayed cows gave 2.79 pounds of milk more per week than those on which spray was used. Although the total milk produced per week was not given, the difference seems insignificant.

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[1] This publication is a report on some phases of a cooperative investigation supported by the establishment of a fellowship at the Kansas Agricultural Experiment Station, June, 1940, by the Hercules Powder Company, Wilmington, Delaware. Associated in the general outline and supervision of the investigation were Friar M. Thompson, Jr., Entomologist, Hercules Powder Company, and Roger C. Smith, Entomologist, F. W. Atkeson and A. O. Shaw, Dairy Husbandmen, Kansas Agricultural Experiment Station. Graduate Assistants appointed to this fellowship were Floyd J. Holmes, Department of Entomology, in 1940, and A. Russell Borgmann, Department of Dairy Husbandry, in 1941.

[2] Contribution No. 145 from the Department of Dairy Husbandry.

Fitch and associates (8, 9, 10) have reported more investigations on the effect of spraying cows on their milk production, using large numbers of cows under herd conditions, than any other investigators. Fitch and Lush (9) reported that the results of three seasons trials with five sprays used on 48 milking cows, showed that the sprayed cows produced less than 0.1 per cent more milk than unsprayed cows. Later Fitch and Cave (10) used 12 cows kept in a barn when comparing three sprays during three 20-day periods. They found that the sprayed cows produced 5,215 pounds while unsprayed cows produced 5,270 pounds, or a difference of slightly more than 1 per cent, which is within experimental error.

Freeborn and Regan (11, 12, 14) of the California station have conducted the most comprehensive and carefully controlled experiments on the effects of flies and of spraying on milk production. Through the design of their experiments, they were able to measure independently and jointly the effect of these two factors on milk production. In their first year's work (11) four pairs of cows were kept in separate screened stalls containing, respectively, no flies, 73,000 horn flies, 71,000 stable flies, and 140,000 house flies. The loss in milk production during a 30-day period attributed to the flies was: horn flies—1.4 per cent, stable flies—9.3 per cent, and house flies—3.3 per cent. All four groups were then sprayed for 30 days, two groups being sprayed with an odorless, non-toxic petroleum oil (Saybolt viscosity of 105), and the remaining two groups being sprayed with a mixture of 2 parts of the same oil and 1 part of an oil used for household spray containing an extract of pyrethrum in distillate. Spraying the check group (no flies) with the mixed spray caused a reduction of 4.3 per cent in milk production, while another group similarly sprayed and exposed to 23,250 stable flies produced 12.4 per cent less than normal expectancy. Thus the difference between the two groups ($12.4\% - 4.3\% = 8.1\%$) was approximately the same reduction caused by stable flies as found in the previous trial (9.3%). The group sprayed with base oil only and exposed to 196,000 horn flies declined in production 13.1 per cent more than normal as compared with 1.4 per cent reduction previously mentioned for horn flies without spray. The fourth group, also sprayed with base oil only, and exposed to 26,800 stable flies had an excess reduction in milk flow of 21 per cent, as compared with 3.3 per cent for the same group when unsprayed and exposed to house flies instead of stable flies. The oil seemed to depress milk production much more than did the mixed spray. The amount of oil applied per animal was 400 cc. in all instances.

During the second year's trials Freeborn and Regan (12) found that sponging cows with water caused no reduction in milk flow, while spraying with water caused a reduction of 5.4 per cent. Spraying for two weeks with pine tar-creosote resulted in a loss of 6.9 per cent, while a loss of 9.7 per cent resulted from spraying with oil (viscosity of 68). Continuation of the

same spraying schedule for two more weeks plus 5,000–10,000 house flies resulted in losses of 12.5 per cent for the pine tar-creosote mixture and 22.8 per cent for the base oil only. The authors attributed all the loss in excess of expected reduction in both instances to be due to continuation of the spraying. The sprays were applied at the rate of 250 cc. per 1,000 pounds of animal. A pair of cows exposed concurrently for six weeks to 5,000 to 10,000 house flies (no spray) produced above the expected normal.

Bartlett (1) reported three experiment trials conducted on a privately owned farm. During one trial two groups of cows, one sprayed and the other unsprayed, were compared in average production by 14 day periods for a total of 56 days. Although the unsprayed cows averaged slightly higher in production, the average decline per cow by periods showed no significant differences between the groups. In the second trial one group of cows was alternately sprayed, unsprayed, sprayed and unsprayed during four consecutive 14 day periods; while another group was treated just the reverse during each period. The production per cow in each group was consistently higher during the spraying periods, than during the unsprayed periods. The groups differed greatly in numbers, however, which makes statistical analysis of the significance of the differences difficult. In the third trial one group of cows were alternately unsprayed, sprayed and unsprayed during three 14 day periods. Average butterfat percentage of the milk was consistently higher during the spraying period. No other experiments have come to the attention of the authors in which the effect of spraying cows was measured by differences in fat percentage of milk.

Nelson (13) refers to the above experiment conducted at the New Jersey Agricultural Experiment Station on the effect of spraying on the butterfat content of milk, and also refers to unpublished data at that station on the effect of spraying on milk production as follows: "The results of this test showed rather definitely that there was a decided decrease in total production of milk per cow during the month of the test in the unsprayed group, while in the case of the sprayed group the shrinkage in milk production per cow was less. There was, of course, normal shrinkage to be expected from month to month and this, of course, was taken into consideration.

"The final results of this test showed that there was an average shrinkage per cow in the untreated group of 22 pounds of milk during the month and this is certainly a big enough difference to make it worth while to treat the animals." He did not state what the average shrink in milk flow was among the cows when not sprayed. Most dairymen would consider an average shrinkage in a month of approximately two-thirds of a pound of milk daily as remarkably persistent production, well above normal decline, and of no experimental significance. It is also doubtful whether at prevailing milk prices 22 pounds of milk would pay for the labor and cost of spray materials involved in 30 sprayings per month.

EXPERIMENTAL

During the summer of 1940 an experiment was conducted at the Kansas Agricultural Experiment Station to measure the effect on milk production of spraying dairy cows. Twenty cows selected from the station herd were divided into two groups of 10 cows. Four breeds—Holstein, Ayrshire, Guernsey, and Jersey,—were represented. The average plane of production was sufficiently high to make the animals sensitive to changes in management. Both groups were fed and managed in the usual manner, except that one group was sprayed while the other was not sprayed, alternations in spraying and not spraying were made on the same day for both groups. The sub-periods of alternating treatment varied from 10 to 12 days, except for the last period which was only four days. The experimental period represented 58 consecutive days from July 9 to September 4, inclusive.

The cows were all milked three times daily, 5:00 to 7:00 A.M., 1:00 to 3:00 P.M. and 9:00 to 11:00 P.M. Except when in the barn to be milked, the cows were kept loose in a paddock where hay, water and some shade were available. Spraying was done with an electric sprayer twice daily at about 7:00 A.M. and 3:00 P.M. Approximately 25 cc. of spray was used on each animal, care being exercised to distribute the spray evenly over the animal, except possibly the head. Thus the spray used per animal was 50 cc. daily, which is more than is used under present-day practice. The animals were not washed during each sub-period except for the udder, any spray residue being allowed to accumulate. Just previous to change from spray to non-spray each cow was thoroughly washed with soap and water. The spray used during sub-periods 1 and 2 was a mixture of $3\frac{3}{4}$ per cent of a 20:1 concentrate of pyrethrum plus 15 per cent of pine oil in a colorless, odorless petroleum oil.[3] During periods 3 and 4 the spray consisted of $2\frac{1}{2}$ per cent of the same pyrethrum concentrate plus 5 per cent of "D.H.S."[4] activator in the same base oil. During periods 5 and 6 the spray used was composed of 3 per cent of Thanite[5] in the same base oil. Thus Groups A and B each were sprayed during one period with each of the sprays. Since each spray

[3] Specifications of base oil used:

Specific gravity	0.8251
Saybolt viscosity	36 min.—41 max.
Pensky Marten flash	250 min.
Fire point	300 min.
Unsulfonatable residue	90%
Distillation end point	600° F.
Saybolt color	+15 min.
Acid test	40+
3-hr. corrosion test at 122° F.	Must pass
Cold test	30 max.

[4] Ethylene glycol ether of Pinene (Reg. Pat.).

[5] Fenchyl thiocymyl acetate (Pat. No. 2209184).

was composed of materials extensively used in present-day commercial sprays, and each mixture had been found to be an effective fly repellent (15), the sprays can be considered representative. A high degree of repellance resulted during the first two hours, with decreasing repellance with each succeeding hourly count, but each of the sprays showed a highly significant difference from the check (unsprayed) group $7\frac{1}{2}$ hours after spraying. Also the fact that three different spray mixtures were used in balanced fashion during the experiment lends credence to the results by not limiting the conclusions to the condition of one spray mixture.

TABLE 1

Effect of spraying dairy cows with fly repellents on milk production

Sub-periods		Kind of spray used* No.	Group A		Group B	
			Not sprayed	Sprayed	Not sprayed	Sprayed
	Days		Average daily milk production (lbs.)	Average daily milk production (lbs.)	Average daily milk production (lbs.)	Average daily milk production (lbs.)
Ante-Experimental Period						
7– 2 to 7– 8	7	...	36.8	.....	36.3	.....
Experimental Period						
7– 9 to 7–20	12	1	.....	36.3	34.3	.....
7–21 to 7–31	11	1	35.5	.....	.....	32.0
8– 1 to 8–10	10	2	.....	34.9	31.1	.....
8–11 to 8–20	10	2	34.3	.....	.....	30.1
8–21 to 8–31	11	3	.....	36.0	29.7	.....
9– 1 to 9– 4	4	3	30.9	.....	.....	28.3
Post-Experimental Period						
9– 5 to 9–14	10	...	30.1	.....	25.9	.....
Total weighted ave. experimental period			34.3	35.2	31.8	30.6

* Spray No. 1 = $3\frac{3}{4}$% of 20: 1 concentrate of pyrethrum, plus 15% of pine oil in a colorless, odorless petroleum oil, viscosity 50 seconds.
Spray No. 2 = $2\frac{1}{2}$% of 20: 1 concentrate of pyrethrum, plus 5% of "D.H.S." activator, in same base oil.
Spray No. 3 = 3% "Thanite" in same base oil.
"D.H.S." activator = Ethylene glycol ether of Pinene (Reg. Pat.).
"Thanite" = Fenchyl thiocymyl acetate (Patent No. 2209184).

The average milk production of each group for each sub-period and for the 58-day experimental period is shown in table 1. Group A averaged 34.6 pounds of milk daily for the three periods totaling 22 days when not sprayed and 35.2 pounds daily for the three periods totaling 30 days when sprayed. Group B averaged 30.8 pounds for three periods totaling 30 days when not sprayed, and 30.6 pounds for the three periods totaling 22 days when sprayed. The true daily average for the two groups combined was

32.4 pounds when not sprayed and 33.2 pounds when sprayed, or a difference of less than 2 per cent. The differences obtained for either group, or the combined groups are too small to indicate any true difference due to spraying or not spraying. Consecutive sub-periods comprising alternation from spraying to non-spraying, or vice versa, are likewise consistently close in average production.

The reliability of the averages is substantiated by the graph of daily average production by groups, with the sprayed and unsprayed periods indicated for each group (fig. 1.). The variations are no more than would be expected from two such groups of cows, even if managed identically. Maximum and minimum daily temperatures are also presented to reflect some of the conditions of the experiment, particularly because of the rela-

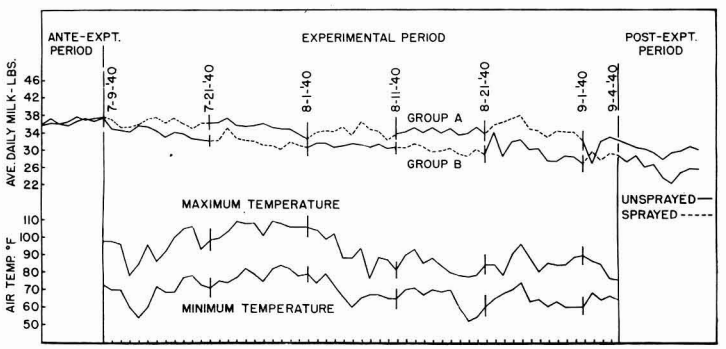

FIG. 1. Daily average production per cow in each group, with spray and non-spray periods indicated for each group. Daily maximum and minimum atmospheric temperatures are shown to indicate environmental conditions.

tionship of temperature to physiological processes of sprayed cows as reported by Regan and Freeborn (14).

No skin injuries or any indications of distress were evident among the cattle being sprayed.

DISCUSSION

The results of this experiment are in agreement with most of the previous investigators reviewed, with the exception of the reports of Freeborn and Regan (11, 12, 14) who conducted the most carefully controlled experiments yet reported. Although their experiments were conducted under climatic conditions, especially atmospheric temperatures, rather similar to the conditions of the experiment herein reported, there are several reasons why the conclusions are at variance. One of the primary reasons is the fact that our experiment was conducted under normal herd management conditions while theirs was under more controlled conditions. The numbers of flies on their animals were not published but certainly the thousands of flies caged with

their cows would appear to be far in excess of the numbers to which the cows in the Kansas experiment were exposed. How many flies the cows in the Kansas experiments were exposed to in the paddocks is not known, but from other experiments being conducted concurrently it was found that the numbers of flies per cow averaged about 40, and that the flies were primarily stable flies with a small proportion of horn flies. The fact that Freeborn and associates obtained practically no decrease in milk production when cows were exposed to such large numbers of horn flies and house flies makes unchallengeable their conclusions that those two species do not materially affect milk production. Their reported loss of 9.3 per cent in milk production caused by stable flies (without spray) supports the belief by most farmers that flies reduce milk production, because stable flies are the species which are most prevalent throughout the summer and seem most annoying to cattle. Whether a loss of 9.3 per cent can be accepted as typical of most field conditions is doubtful, because of the large numbers of flies to which their cows were exposed.

Another important difference between the California and Kansas experiments is the fact that approximately ten times as much sray was used in the California project as in the Kansas project. The amount used in the Kansas experiment was in accord with present-day spray practices, and Freeborn and Regan later reported that the amount which they used was excessive. Their classical experiments on the effect of sprays on body temperatures and respiration rates proved the detrimental effect of excess spray. The fact that some of their cows suffered from the effects of spraying, whereas our cows did not, seems to indicate that their results on the depressing effect of sprays on milk production were magnified beyond present-day field results.

Freeborn and Regan used oil of 105 viscosity in their first year's work and oil of 68 viscosity in their second year's work while our sprays were made up with an oil of 50 viscosity. The fact that they obtained a loss of 21 per cent in milk production when the cows were sprayed with base oil only and exposed to stable flies, while a loss of 12.4 per cent was obtained under the same conditions when the oil was diluted 2:1 with household spray made of light weight oil, indicates that the viscosity of oil was an important factor. They obtained a reduction of only 4.3 per cent when the latter spray mixture was used alone. This reduction was less than the reduction due to water spray the second year. A loss of 9.74 per cent with lighter oil (68 viscosity) was obtained the second year, while pine oil-creosote emulsion caused 6.93 per cent the first two weeks and 12.5 per cent the second two weeks, which seems high for an emulsion type spray but 3 per cent of about 300 cc. of spray makes a rather heavy oil application daily.

The Kansas experiments might be challenged because the subperiods were not long enonugh for sufficient accumulative effect of spraying but

this criticism is hardly valid in the face of results obtained during two-week periods in the California experiment.

The conclusions of the Kansas experiments seem valid because large numbers of cows were used, the types of oils used and the amounts of sprays applied were in line with present-day practices, and because three kinds of sprays were used with replications.

Considering these experiments it seems reasonable to conclude that stable flies in the numbers found during this experiment, do not cause any more reduction in milk flow than does spraying with accepted sprays in accepted amounts. Perhaps the reduction caused by flies may approximate the reduction caused by spray, but certainly good sprays properly applied cannot be condemned more than no spray, under practical herd management.

SUMMARY AND CONCLUSIONS

Twenty cows, divided into two groups of 10 cows, were used in measuring the effect on milk production of spraying versus non-spraying. The experiment covered 58 consecutive days, divided into five sub-periods. During each sub-period one group was sprayed and the other not, alternations of groups being made during each sub-period. The cows were sprayed twice daily with 25 cc. of three different typical sprays of known composition.

Group A averaged 34.3 pounds of milk daily when not sprayed and 35.2 pounds when sprayed, while Group B averaged 31.8 and 30.6 pounds, respectively. The true daily average of the combined groups was 32.9 pounds when not sprayed and 33.2 pounds when sprayed, the difference being well within experimental error. Reliability of these averages was substantiated by a graph of the average daily production of each group with spray and non-spray periods indicated.

It seems safe to conclude that:

1. Stable flies did not depress production any more than spraying with an acceptable spray properly applied.

2. Decrease in production of both sprayed and unsprayed cows showed a normal decline.

3. Good sprays properly applied did not depress milk flow more than normal.

4. No skin injuries or any indications of distress were evident among the sprayed cows.

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AN EXPLANATION OF THE INCREASED EFFICIENCY OF GELATIN IN ICE CREAM MIX WHEN INITIALLY AGED AT 68° F.*

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INTRODUCTION

It has been commonly assumed that the aging of ice cream mix is most effective at 32° to 40° F. However, in an earlier publication (9), the writer has shown that this assumption is not correct for mixes that contain gelatin. It was found that initial aging temperatures between 50° and 99.86° F. increased the gel strength and basic viscosity when compared to aging at the low temperature only, and the maximum effect was produced at 68° F. These effects were also apparent for gelatin-water solutions. Mueller and Frandsen (12) found that when a commercial ice cream mix contained gelatin and was initially aged at 68° F. for 4 hours, the efficiency of the gelatin was increased to such an extent that the gelatin content could be reduced approximately one-fourth without impairing the quality of the ice cream. Also, Mueller and France (11) found that this special method of aging did not increase the bacterial count, provided that the ice cream mix was not recontaminated after pasteurization.

In view of the present national emergency, it seems particularly timely to emphasize the use of this special aging method as a means of saving gelatin. The purpose of this study was to find an explanation for the effects of high initial aging temperature. It was hoped that such information might be helpful in suggesting ways by which the efficiency of gelatin in ice cream could be further increased. It is also conceivable that such information might be applicable to other industrial uses of gelatin.

GENERAL PLAN OF STUDY AND PRESENTATION

An explanation for the high initial aging temperature effects, namely the increased efficiency of gelatin in ice cream, was sought by studying properties of gelatin which are likely to be influenced by the gel structure or which would serve as an index to the colloidal behavior of the gelatin. In addition to the viscosity and gel strength studies, which were referred to, the following studies were made: 1) optical rotation; 2) light scattering (Tyndall phenomenon); 3) electrical conductance; 4) gold number, and 5) effect of agitation.

The writer is cognizant of the vast amount of reported research of the effect of hydrogen-ion concentration on the properties of gelatin and its

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importance in connection with the many industrial uses of this product. In this study the use of gelatin in ice cream is the major consideration. The pH of ice cream mixes made from fresh products of good quality varies only within the range of 6.1 to 6.4. Although the pH of commercial gelatin samples ranges from 3.8 to 6.2, the gelatin assumes the pH of the ice cream mix because the latter is highly buffered. Therefore, in this study pH is not stressed.

The great diversity of experimental procedure used in this study makes it desirable from the standpoint of simplicity and clarity to present each part as a unit by itself. This, in turn, will be followed by a general discussion of all the data as a whole.

EXPERIMENTAL

I. *Optical Rotation*

Optical rotation of gelatin in solution is generally considered an important index to the colloidal behavior of the system. Kraemer and Franselow (4) concluded that the optical activity of gelatin is a function of such colloid features as degree and state of dispersion, in addition to the intramolecular stereo-chemical relations. Trunkel (16) reported that the optical rotation of gelatin solution decreased as the temperature was raised, reaching a minimum value at 86°–95° F., after which no change occurred. The necessity for accurate thermal control of gelatin solutions is emphasized by Reiger (14), who showed that if a gelatin solution is gelatinized at a low temperature and is brought to a higher temperature, the rotation decreases. The writer is not aware of any reported experimental work on the influence of the thermal treatment of a gelatin sol on its optical rotation when the higher temperature preceded the lower.

The general plan was to determine the optical rotation of one per cent gelatin-water solutions when using various thermal treatments. Gelatin-water solutions were prepared by soaking the gelatin in distilled water for one-half hour at room temperature, then heating for 30 minutes at 145° F. This method of preparation was standard procedure, not only for the optical rotation studies, but also for the entire study. The Schmidt and Haensch, triple-field saccharimeter and 100-mm. jacketed polarizing tubes were used for optical rotation determinations. Condensation of moisture on the cover glasses of the polariscope tubes was prevented by attaching short tubes containing calcium chloride, as recommended by Browne (1).

1. *The change in optical rotation with time at various temperatures.* Gelatin solutions having a pH of 4.4 were held at the following temperatures: 34.5°, 50°, 68°, 86°, 104°, and 140° F. Optical rotation was determined at various intervals of time until equilibrium was practically reached. The time-interval used depended upon the rate of change in optical rotation.

The results are given in table 1 and shown graphically in figure 1. Opti-

cal rotation decreased as the temperature was raised, which is in agreement with the results reported by Trunkel (16) and by Reiger (14). The results show also that at and above 104° F. the optical rotation readings remained unchanged, and at temperatures below 104° F. the readings changed upon aging, and the greatest change occurred during the first two hours' time. After 4 hours the increase in optical rotation for the solution which was held

TABLE 1

The change in optical rotation with time at various temperatures

Time	Specific rotation					
	34.5° F.	50° F.	68° F.	86° F.	104° F.	140° F.
0 minutes	−172	−146	−133	−124	−121	−116
3 "	191	164	.....	.....	.....	.....
6 "	204	177	.....	.....	.....	.....
9 "	209	184	.....	.....	.....	.....
12 "	220	189	.....	.....	.....	.....
15 "	227	198	146	124	121	.....
18 "	230	202	.....	.....	.....	.....
21 "	234	207	.....	.....	.....	.....
24 "	238	210	.....	.....	.....	.....
27 "	241	214	.....	.....	.....	.....
30 "	242	217	155	124	121	.....
36 "	246	221	.....	.....	.....	.....
42 "	250	224	.....	.....	.....	.....
48 "	254	228	.....	.....	.....	.....
54 "	256	230	.....	.....	.....	.....
1 hour	260	232	164	126	121	115
2 "	267	245	175	128	.....	.....
3 "	275	253	182	128	.....	.....
4 "	278	257	188	129	.....	.....
5 "	279	258	191	129	.....	.....
6 "	280	262	193	129	.....	.....
7 "	282	264	194	.....	.....	.....
8 "	283	266	.....	.....	.....	.....
9 "	284	.....	.....	.....	.....	.....
10 "	284	268	201	.....	.....	.....
11 "	285	.....	.....	.....	.....	.....
12 "	286	270	206	.....	.....	.....
1 day	291	275	218	129	121	115
2 "	295	281	229	133	121	.....
3 "	303	284	236	.....	.....	.....
4 "	.....	287	239	135	.....	.....
5 "	304	290	242	135	.....	.....
6 "	.....	.....	245	.....	.....	.....
1 week	308	290	246	134	.....	.....
2 "	310	294	250	.....	.....	.....

at 68° F. was approximately one-half of that attained by the solution held at 34.5° F. At each temperature below 104° F., the change from the sol to the gel modification, as indicated by optical rotation, proceeded to a characteristic equilibrium. It should be pointed out that the final values are only approximate since specific rotation near the optical equilibrium becomes an infinitesimal value. Specific rotation increased more rapidly and the final value was higher at the lower temperatures.

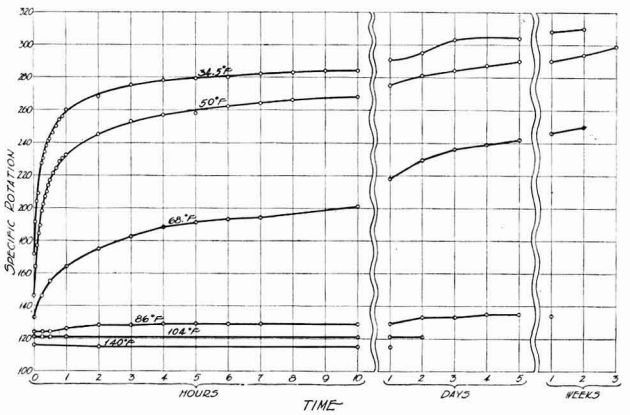

FIG. 1. Change in optical rotation with time at various temperatures.

2. *The effect of initially aging for 4 or 6 hours at various temperatures on the optical rotation after 24 and 48 hours.* Gelatin solutions having a pH of 4.4 were held for 4 hours at the following temperatures: 50°, 68°, 86°, 104°, 122°, and 254° F., and one solution was held at 68° F. for 6 hours. At the close of the initial aging period the solutions were cooled rapidly to 34.5° F. and held at this temperature for a total aging period of 48 hours. Optical rotation was determined after a total aging period of 24 and 48 hours. A portion of the gelatin solution was aged only at 34.5° F. for 48 hours, and

TABLE 2

The effect of various initial aging temperatures when using a short initial aging period on the optical rotation of a one per cent gelatin solution

Initial aging treatment °F.	Optical rotation after 24 hrs.		Optical rotation after 48 hrs.	
	34.5° F. (a) D	Per cent increase or decrease over control	34.5° F. (a) D	Per cent increase or decrease over control
Control*	−283		−296	
50° for 4 hrs.	290	+ 2.4	294	− 0.6
Control	277		281	
68° for 4 hrs.	284	+ 2.5	287	+ 2.1
Control	287		295	
86° for 4 hrs.	279	− 2.7	285	− 3.3
Control	279		291	
104° for 4 hrs.	279	0.0	282	− 3.0
122° for 4 hrs.	280	+ 0.3	287	− 1.3
254° for 4 hrs.	204	−26.8	208	−28.5
Control	286		292	
68° for 6 hrs.	293	+ 2.4	295	+ 1.0

* The control samples were aged only at one temperature (34.5° F.). The high initial aging temperature period was always followed by aging at 34.5° F. until the total aging time was equal to that of the control.

served as the control. As it was impossible to use portions of the same gelatin solution for all the various initial aging temperatures used, solutions were made up at different times. In order to eliminate variations in optical rotation measurements which might occur in identical solutions made up at

TABLE 3

The change in optical rotation at 34.5° F. with time after optical equilibrium was obtained at the various initial aging temperatures

Aging time at 34.5° F.	Optical rotation after			
	Initially aged at 50° F.	Initially aged at 68° F.	Initially aged at 86° F.	Control*
	34.5° F. (a) D	34.5° F. (a) D	34.5° F. (a) D	34.5° F. (a) D
0 minutes	−295	−252	−180	−172
3 "	.....	.....	199	191
6 "	.....	259	213	204
9 "	.....	259	221	209
12 "	.....	259	233	220
15 "	.....	266	235	227
18 "	.....	.....	240	230
21 "	.....	.....	242	234
24 "	.....	.....	243	238
27 "	.....	.....	251	241
30 "	297	276	251	242
45 "	.....	279	262	252
1 hour	297	284	266	260
2 "	298	290	276	267
3 "	.....	.....	282	275
4 "	.....	.....	284	278
5 "	.....	.....	.....	279
6 "	299	292	285	280
1 day	301	300	295	291
2 "	.....	304	.....	295
3 "	306	305	299	303
4 "	.....	309	.....	.....
5 "	.....	.....	301	304
6 "	.....	308	.....	.....
7 "	.....	311	301	308
8 "	307	.....	.....	.....
9 "	.....	308	300	.....
10 "	.....	312	.....	.....
11 "	.....	315	300	.....
12 "	308	.....	300	.....
2 weeks	308	.....	.....	310
3 "	311	.....	.....	.....
4 "	314	.....	.....	.....

* Control was a freshly prepared gelatin solution which received no initial aging temperature treatment.

different times, a control was used each time a solution was prepared. The results were then reported as increases or decreases in specific rotation over the control. However, optical rotation variations in the control solutions were not great.

Data obtained in this study are given in table 2. The solutions aged at

the various high initial aging temperatures, with one exception, did not differ significantly in optical rotation from a solution aged at only 34.5° F. after an aging period of 24 hours or 48 hours. The initial aging temperature of 254° F. decreased the optical rotation, no doubt, because of a partial hydrolysis of the gelatin.

3. *The change in optical rotation at 34.5° F. with time after obtaining optical equilibrium at the various high initial aging temperatures.* The gelatin solutions aged at 50°, 68°, and 86° F. in the first experiment were used for this study. After optical equilibrium had been reached at the various temperatures, the gels were cooled to 34.5° F. and held at this temperature until equilibrium was again attained. An unaged gelatin solution cooled to 34.5° F. was used for the control.

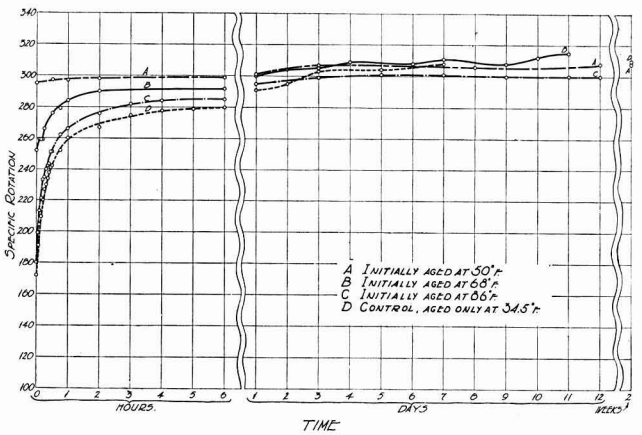

FIG. 2. Change in optical rotation at 34.5° F. with time after optical equilibrium was attained at the various initial aging temperatures.

The results are given in table 3 and figure 2, and show that the long high initial aging temperature treatment at 50°, 68°, and 86° F. had no significant effect upon the final specific rotation values. It should be noted that all of the gelatin sols and gels reached approximately the same specific rotation value after being held for one day at 34.5° F. The curves in figure 2 show that the solution initially aged at 86° F. almost paralleled the control, while the solution initially aged at 50° F. showed no marked increase in specific rotation. The solution initially aged at 68° F. showed an increase in specific rotation, but to a lesser extent than the solution which was initially aged at 86° F.

4. *The change in optical rotation with time when initially aging for 4 hours at 68° F. with and without agitation.* A gelatin solution having a pH of 6.2 was used. After pasteurization the gelatin solution was divided into three parts. Solution 1 was aged only at 37° F. for 48 hours; solution 2 was

TABLE 4

The change in optical rotation with time when initially aging at 68° F. with and without agitation

Total aging time	Solution 1 Aged at 37° F. only	Solution 2 Initially aged at 68° F. for 4 hrs. with no agitation		Solution 3 Initially aged at 68° F. for 4 hrs. with agitation	
	37° F. (a) D	68° F. (a) D	37° F. (a) D	68° F. (a) D	37° F. (a) D
0 minutes	−164	−134	.....	−134	.....
15 "	222	150	.....	.....	.....
30 "	235	159	.....	.....	.....
45 "	242	164	.....	.....	.....
1 hour	246	168	.....	168	.....
2 "	251	175	.....	176	.....
3 "	255	183	.....	177	.....
4 "	257	187	−202	181	−202
4 " 15 min.	.....	.....	228	.....	235
4 " 30 "	.....	.....	241	.....	243
4 " 45 "	.....	.....	247	.....	249
5 "	.....	.....	250	.....	252
6 "	261	.....	257	.....	256
8 "	262	.....	262	.....	261
12 "	265	.....	267	.....	265
24 "	268	.....	271	.....	269
48 "	269	.....	275	.....	276

aged for 4 hours at 68° F. with no agitation; and solution 3 was aged for 4 hours at 68° F. with agitation. At the end of the 4 hours the two solutions held at 68° F. were cooled to 37° F. immediately and were then held at the low temperature until the total aging period was equal to 48 hours. Agitation at 68° F. was accomplished by a motor-driven paddle consisting of a

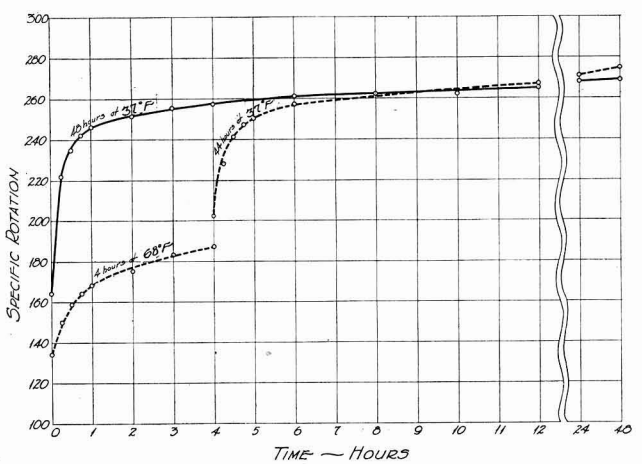

FIG. 3. Change in optical rotation with time when initially aging for 4 hours at 68° F., and when aging at only 37° F.

0.25-inch-mesh wire screen, driven at the rate of 116 r.p.m. The device was arranged so that the incorporation of air could be prevented and also so that the samples could be agitated while in the constant-temperature bath.

Determinations of optical rotation were made at intervals throughout the aging period. The results are given in table 4 and figure 3, and show that the solution which was initially aged at the high temperature caught up with the control at approximately the eighth hour, but did not surpass it in optical rotation. Mild agitation during the high temperature initial aging period had no significant effect on optical rotation.

II. *Light Scattering (Tyndall Phenomenon)*

The optical property of light scattering appeared most likely to give data on the relative size of particles (indicating the degree of dispersion) and

TABLE 5

The change in relative light intensity with time when initially aging a one per cent gelatin solution for 4 hours at 68° F., and when aging at only 38° F.

Aging time	Relative light intensity	
	Aged only at 38° F.	Aged first 4 hours at 68° F., remaining period at 38° F.
0	30.1	20.2
15 minutes	35.0	22.8
30 "	37.6	24.3
45 "	39.4	25.0
1 hour	42.0	26.2
2 "	44.6	27.7
3 "	46.2	29.0
4 "	46.5	30.0
5 "	46.8	48.0
6 "	48.4	53.8
9 "	49.3	58.5
1 day	54.7	62.6
2 "	56.4	64.6
4 "	59.0	70.3
7 "	59.9	72.5

their connection with gel structure. A review of the theory of the Tyndall effect is too lengthy for discussion in this paper. However, a review on this subject has been published by Wells (17), which will furnish a basis for the interpretation of the data to be presented.

A photo-electric Tyndall meter assembled in the dairy laboratory was used in this study and its description has been published elsewhere (10). A one per cent gelatin solution having a pH of 6.2 was held for 4 hours at 68° F., then cooled to 38° F., and held at that temperature until the total aging time was equal to that of the control (aged only at 38° F.). Changes in Tyndall intensity were observed at intervals throughout the aging period.

It will be noted from table 5 and figure 4 that the high initial aging temperature treatment greatly affected the light-scattering ability of the gelatin

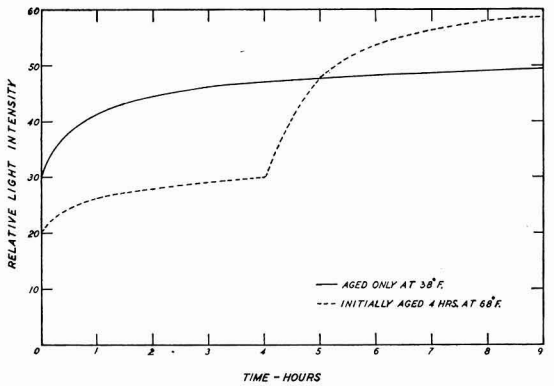

FIG. 4. Change in relative light intensity with time when initially aging at 68° F., and when aging at only 38° F.

sol and gel. During the first 4 hours of aging time the Tyndall intensity was much lower at 68° F. than at 38° F. However, the gelatin solution aged at the high initial temperature surpassed the control in Tyndall intensity soon after it was cooled to 38° F. It required approximately one-half hour to cool from 68° to 38° F. Thus, the observed increase in Tyndall intensity indicated a change in the gel structure or the size of gelatin molecules or aggregate of molecules.

III. *Electrical Conductance Measurements*

It seemed logical to expect that any change in gel structure as a result of

TABLE 6

The velocity of the hydrogen and the chromate ion in a 3 per cent gelatin gel

Time (minutes)	Distance traveled in millimeters	
	Aged 5 hours at 68° F. plus 19 hours at 38° F.	Aged only at 38° F. for 24 hours
	Hydrogen ion	
0	0.0	0.0
5	17.7	18.0
10	37.0	36.2
15	56.2	54.7
20	76.2	75.0
25	91.2	91.5
	Chromate ion	
0	0.0	0.0
5	11.0	11.7
10	21.0	21.5
15	30.0	31.2
20	39.0	40.0
25	47.0	48.2
30	54.7	56.5

the high initial aging temperature might be detected by observing the velocity of various ions through the gel. Therefore, the velocities of the hydrogen ion and chromate ion were determined by the use of an apparatus which was similar to the one described by Lodge (5) for measuring the velocity of the hydrogen ion. A gelatinized 3 per cent gelatin solution having a pH of 6.2 was used. The aging treatment of 5 hours at 68° F. plus 19 hours at 38° F. was compared with the control, held at 38° F. for 24 hours. All determinations were made after a total aging time of 24 hours. The rate at which a color was either discharged or produced indicated the velocity of the ion under observation.

Results are given in table 6, and show that the high initial aging temperature of 68° F. had no measurable effect on the velocity of the hydrogen ion. This is in close agreement with the findings of Lodge (5), who concluded that the velocity of the hydrogen ion suffers almost no retardation from the high viscosity of the gelatin solution.

The high initial aging temperature of 68° F. decreased slightly, but consistently, the velocity of the much larger chromate ion in the gelatin gel. This indicates that the high initially aged gelatin solution, upon cooling, has a more closely knit structure than a gel produced by cooling rapidly to 38° F. In all probability the hydrogen ion was small enough so that it was not retarded by the gel structure, while the chromate ion was too large to pass unmolested. This interpretation is given on the assumption that no significant chemical reaction or colloidal adsorption occurred between the hydrogen or chromate ion and the gelatin. It should be pointed out that the results for the hydrogen ion are not directly comparable to those for the chromate ion because the voltage and length of observation tubes used were not the same in the two experiments.

IV. *Effect of a High Initial Aging Temperature on the Gold Number of Gelatin*

Elliott and Sheppard (3) and Menz (7) found that the gold number decreases (protective action increases) with increases in concentration of gelatin. This was explained by the observation that the amicrons increase in size as the concentration of gelatin decreases. It has also been shown that aging increases the gold number (protective action decreases), probably because the amicrons form larger aggregates of less protective power. Moore, Combs, and Dahle, (8) concluded that there was no direct relation between the gold number of gelatin and gel strength or other common tests used by the ice cream manufacturer in selecting gelatin.

A 0.5 gelatin solution having a pH of 5.25 was used to ascertain whether the gold number would be affected by the high initial aging temperature of 68° F. After the 24-hour total aging period the solutions were diluted to 0.01 per cent, and the following results were obtained:

Temperature treatment	*Gold number*
68° F. for 5 hours plus 19 hours at 38° F.	0.0140
38° F. for 24 hours	0.0160

The high initial aging temperature of 68° F. slightly decreased the gold number of the gelatin. However, the difference seemed too slight to indicate significant changes in relative size of amicron aggregates.

V. *The Effect of Agitation While Initially Aging at 68° F.*

A 1 per cent gelatin solution having a pH of 4.4 was prepared in the usual manner. After heating, the solution was divided into three portions, which received the following treatments: 1) aged only at 38° F. for 24 hours, without agitation; 2) aged 6 hours at 68° F. plus 18 hours at 38° F., without agitation; and 3) aged 6 hours at 68° F. with agitation, plus 18 hours at 38° F. without agitation. Agitation was accomplished by the device which is described in the study of the effect of agitation on optical rotation. At the end of the 24-hour aging period the gelatin samples were tempered to 68° F. and basic viscosity determined with the MacMichael viscometer, using a No. 34 wire. The following results were obtained:

Gelatin Solution No.	*Basic Viscosity* °M.
1	134.7
2	304.5
3	382.0

These results again illustrate the effect on viscosity of initially aging a gelatin solution at 68° F., and also show an additional increase in basic viscosity due to agitating the gelatin solution while aging at 68° F.

A theory for explaining the high initial aging temperature phenomenon. Cooling the gelatin solution rapidly from 145° F. to a low temperature of 40° F. or less results in the formation of a large amount of the gel modification in a relatively short period of time. It is readily conceivable that this rapid formation of the gel modification may interfere with the building of the skeleton gel structure by retarding the development of gel filament; which in turn would result in many unconnected side branch filaments. At the higher initial aging temperature the gel modification is formed more slowly; thus the viscosity is not sufficient to interfere to any great extent with the process of gel structure building. However, when the temperature is later lowered, the increased viscosity cannot interfere with the gel structure building because the skeleton structure has already been laid down at the higher initial aging temperature. According to this theory the optimum temperature for the formation of the more closely knit structure should be that temperature at which the viscosity does not greatly interfere with further structure building and at which there is present sufficient material

for structure building, which is the gel modification. This optimum temperature was found to be approximately 68° F., as reported in an earlier publication (9).

DISCUSSION

The chief purpose for the following discussion is to note how the experimental facts substantiate the proposed theory for explaining the high initial aging temperature phenomenon. Gel structure of gelatin will be considered as being filamentous in nature (14) and composed of the gel modification, because it best serves to explain the experimental results.

The consistency or viscosity of both gelatin-ice cream mix and gelatin-water solution is due chiefly to a structure or gel formation. According to Ostwald and Stuart (13) two kinds of structural viscosity are present in a gelatin sol, mechanically labile and mechanically stable. These two types of structural viscosity, no doubt, have much in common with "apparent viscosity" and "basic viscosity," commonly used in dairy literature. The relationship between particle size and viscosity in ordinary suspensions cannot be expected to hold for gelatin since the particles are filamentous and knit together to form a gel. If the proposed theory—that the high initial aging temperature produces a more closely knit structure, consisting of many more inter-connected filaments—is correct, then it is readily conceivable how such a structure would have increased structural viscosity and gel strength. Both were increased by the high initial aging temperature (9, and this study).

The effects of the high initial aging temperature on the optical rotation did not parallel the effects on structural viscosity and gel strength. Optical rotation was not increased, while viscosity and gel strength have been shown (9) to be increased as much as 100 per cent by the use of a high initial aging temperature. The lack of correlation between the effects of the high initial aging temperature on viscosity and gel strength, and optical rotation, is further emphasized by the results from the study in which the gelatin solution was agitated while aging at the high temperature. Agitation had no effect on optical rotation, but increased basic viscosity approximately 25 per cent. Kraemer and Fanselow (4) concluded that the optical activity of a gelatin is a function of such colloid features as degree and state of dispersion, in addition to the intramolecular and stereo-chemical relations. Unfortunately we do not know the amount that each factor contributes to the mutarotation of a gelatin solution. The reported (9) temperature of 99.86° F., which is the approximate upper limit where a high initial aging temperature produces an effect, coincides quite closely with the sol-gel transition temperature of 100.46° F. as reported by Davis and Oaks (2). Therefore, optical rotation was interpreted in this study to indicate the amount of the gel modification present, which is capable of forming a gel structure. The fact that the gelatin solutions were found to be equal in optical rotation, whether they

were initially aged at a high temperature or not, indicated that they contained equal quantities of the gel modification. This conclusion is based upon two assumptions; namely, that changes in optical rotation are due chiefly to changes in molecular structure, and that the altered molecular structure is the gel modification. If the proposed theory, namely that of a more closely knit structure, is correct, then optical rotation was not a function of this changed structure, and the changed structure was not brought about by an increased amount of the gel modification.

Like basic viscosity and gel strength, the light-scattering ability of the gelatin solution was increased by the high initial aging temperature. An increase in light-scattering ability does not always indicate an increase in size of particles. Mecklenburg (6) working with sulfur sols below 10^{-4} cm. in diameter, found the Tyndall beam to become more intense the larger the particles. On the other hand, Tolman (15) showed that in smokes 5×10^{-6} cm. in diameter, and for suspensions 10^{-4} cm. in diameter and larger, the Tyndall beam became more intense, at a given concentration, the greater the subdivision. No attempt was made in this study to determine the size of particles in the gelatin solution, because lyophilic systems contain particles whose exact size is not readily measured. However, from the small amount of information found in the literature, it may be assumed that the diameter of the gelatin particle would be below 10^{-4} cm. Obviously, from what has been said, it is difficult to interpret the light-scattering studies. However, the increased Tyndall effect, as a result of the high initial aging temperature, is readily conceivable to be due to an increase in the surface area of the micelle as a result of the more closely knit structure. It should be recalled that the optical rotation data indicated that the amount of gel modificaion (structure building material) is the same whether a high initial aging temperature was used or not. Therefore, with a fixed amount of the structure building material, the closer the structure is knit the greater becomes the filament surface area.

The slight, but consistent, decrease in rate of migration of the chromate ion through the high initially aged gelatin solution points to a more closely knit structure. Likewise, the very slight increase in protective action (decrease in gold number) indicates a decrease in size of amicron aggregates, which would seem more adapted to producing a closely knit structure than larger aggregates of amicrons. The gold number determination must be made with a very dilute gelatin solution. However, if a more concentrated gelatin solution could be used, the effect on the protective action might be much greater. Agitation of the gelatin solution while at the high initial aging temperature increased the gel structure. Thus, agitation within limits, might tend to make a more closely knit structure, because more frequent contacts of gelatin micelle may occur.

SUMMARY

1. Optical rotation of a 1 per cent gelatin solution was determined at various intervals of time until equilibrium was practically reached, when the following temperatures were used: 34.5°, 50°, 68°, 86°, 104° and 140° F. The optical rotation decreased as the temperature was raised. Aging at and above 104° F. did not change the optical rotation, while holding at temperatures below 104° F. increased optical rotation. The greatest change occurred during the first two hours of aging. The time required for reaching optical equilibrium increased as the temperature was lowered.

2. Initially aging a 1 per cent gelatin solution for 4 hours at a temperature range of 50° to 122° F., followed by aging at a low temperature, did not affect, after 24 and 48 hours of aging, the optical rotation when compared with aging at the low temperature (34.5° F.) only. The initial aging temperature of 254° F. decreased the optical rotation, perhaps because of a partial hydrolysis of the gelatin.

3. One per cent gelatin solutions were held at 50°, 68°, and 86° F. until optical equilibrium was attained, then cooled to 34.5° and held at this temperature until optical equilibrium was again attained. The long high initial aging temperatures had no significant effect upon the final optical rotation values when compared with aging at 34.5° F. only. All of the gelatin solutions reached optical equilibrium at 34.5° F. after approximately 2 days.

4. The optical rotation of a gelatin solution initially aged at 68° F. for four hours caught up with the control (aged only at 38° F.) at approximately the eighth hour of total aging time, but did not surpass the control upon further aging.

5. Mild agitation of the gelatin solution while at the higher initial aging temperature (68° F.) had no significant effect on optical rotation.

6. The light-scattering ability of a 1 per cent gelatin solution was lower at 68° F. than at 38° F.

7. Initially aging a 1 per cent gelatin solution for 4 hours at 68° F., followed by aging at a low temperature, increased the light-scattering ability when compared with aging at the low temperature (38° F.) only.

8. The gelatin solution initially aged at a high temperature surpassed the control (aged at 38° F. only) in Tyndall intensity immediately after cooling from 68° F. to 38° F.

9. The high initial aging temperature of 68° F. had no significant effect on the velocity of the hydrogen ion through the gelatin gel when compared with an aging temperature of 38° F. only.

10. The high initial aging temperature of 68° F. slightly decreased the velocity of chromate ions through the gelatin gel, when compared with an aging temperature of 38° F. only.

11. The high initial aging temperature of 68° F. slightly increased the protective action of gelatin, when compared with an aging temperature of 38° F. only.

12. Agitation of the gelatin solution while at the high initial aging temperature of 68° F. increased the basic viscosity when compared with an unagitated solution.

CONCLUSION

This study indicates that the high initial aging temperature produced a more closely knit gel structure, which has many more inter-connected filaments than a structure produced by aging at the low temperature only. The increased number of gel filaments are more effective in mechanically obstructing the formation of large ice crystals. This appears to be the most plausible explanation of the increased efficiency (smoother texture) of gelatin in ice cream when initially aged at 68° F.

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THE FUMIGATION OF DAIRY PRODUCTS WITH METHYL BROMIDE

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With the rapidly increasing use of methyl bromide fumigation for insect control in various food industries, the question arises from time to time as to the possible presence and effect of residual bromides in the fumigated foods. Some experimental work (1, 3, 6) tended to indicate that while the residues were negligible in many foods, they might be higher in others, particularly those in finely divided form or containing much oil or fat, such as milled grains, cheese or nutmeats. Within the past few years, however, extensive commercial fumigations of some of these products have been conducted successfully and recent publications (4, 5) have presented additional information about the bromide residues. These residues are generally lower following commercial fumigations than in laboratory experiments, because of the smaller ratio of fumigant to product. After suitable periods of airing, the residues consist entirely of inorganic bromides which are harmless in the amounts ordinarily present.

Laboratory experiments, as well as commercial fumigations involving millions of pounds of dairy products, have been carried out to demonstrate the suitability of methyl bromide for fumigating these products. They may be exposed satisfactorily in vaults, coolers, curing rooms, warehouses, or factories which are tight enough to confine the gas. Pests such as the cheese mite (*Tyroglyphus siro*), cheese skipper (*Piophila casei*), weevils and roaches, which may often be found under the usual manufacturing and aging or storing conditions, are controlled most readily with methyl bromide because of its toxicity to all forms of insect life including the eggs, and because of its effectiveness at low or high temperatures. Although a larger dosage is required at lower temperatures, it is noteworthy that this fumigant is effective under such conditions; many common fumigants become ineffective when the temperature drops. Methyl bromide also has the advantages of relative insolubility in water, ease of application, and rapid venting. The exceptional penetrating property of the gas makes it unnecessary to move or lift stock or to open boxes in order that the pests will be reached. Careful inspections of fumigated dairy products have revealed no off-tastes or odors, so that the only question to be discussed in this paper concerns the magnitude of the bromide retention under various fumigating conditions.

FUMIGATION OF CHEESE

Dudley and coworkers (1) reported bromide residues in American cheese corresponding to about 0.008 per cent Br after laboratory fumigation and

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0.0034 per cent after commercial fumigation. Laug (3) obtained much higher residues by treating grated Italian cheese with extreme dosages, and he reported that most of the bromide was non-volatile. A method for distinguishing between inorganic and organic bromide was developed by Shrader, Beshgetoor and Stenger (5), who also gave the results of a laboratory fumigation of American cheese. They concluded that methyl bromide does not penetrate more than a quarter of an inch inside the surface of the cheese. Using an unwrapped half-pound cube of cheese, the outside quarter-inch layer after aeration contained 0.0080 per cent Br, corresponding to 0.0043 per cent on the entire sample. This cheese had been fumigated for 12 hours at 76–77° F., with a methyl bromide concentration of two pounds per 1000 cubic feet.

In table 1 are shown the retentions of bromide by whole Longhorn cheeses

TABLE 1

Fumigation of whole Longhorn cheeses
Five lbs. CH_3Br per 1000 cu. ft. for 24 hours at 50° F.

Hours aired at 75° F.	Per cent bromide remaining in outer 0.25 inch					
	Cheese A			Cheese B		
	Total	Inorganic	Organic	Total	Inorganic	Organic
0.5	0.0077	0.0015	0.0062	0.0101	0.0023	0.0078
4	0.0061	0.0021	0.0040	0.0084	0.0030	0.0054
24	0.0042	0.0022	0.0020	0.0047	0.0038	0.0009
48	0.0025	0.0025	0.0000	0.0043	0.0039	0.0004
96	0.0024	0.0024	0.0000	0.0039	0.0038	0.0001
168	0.0026	0.0025	0.0001	0.0038	0.0036	0.0002

fumigated under conditions which might be encountered in practice, except that the dosage was higher than is ordinarily required. (The normal dosage is from one to two pounds per 1000 cubic feet, depending on the temperature.) The cheeses, weighing about 12 pounds each and having their original cloth and wax coatings, were exposed for 24 hours at 50° F. in a 500-cubic foot chamber containing 2.5 pounds of methyl bromide. After various periods of standing in air at about 75° F., the cloth was removed from one-inch sections of each cheese and samples were taken from the outer quarter-inch and from interior sections. Analyses for total and inorganic bromides, with organic bromide by difference, were made by the methods previously described (5). Since within experimental error no more bromide was ever found in the interior portions than in the unfumigated controls, only the results obtained on outside samples are shown in the table. Cheese B appears to have taken up more bromide than Cheese A, apparently because it had a thinner coating of wax. The data concerning Cheese B are plotted in figure 1.

The results show that all of the absorbed methyl bromide has been lost,

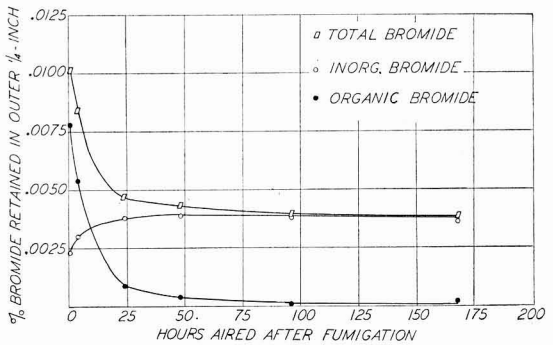

FIG. 1. The retention of bromides in cheese exposed for 24 hours at 50° F. to methyl bromide fumigation at the rate of 2.5 lbs. per 500 cubic feet.

either by volatilization or by conversion into inorganic bromide, within two or three days after removal from the fumigation chamber. In another set of experiments, the magnitude of the residual bromide content after a week of airing was studied as a function of dosage. Whole Longhorn cheeses were fumigated with different concentrations of methyl bromide for 24 hours at 50° F., and later analyzed for total bromide in the outer quarter-inch (after removal of the cloth). A quarter-inch layer from the top of each cheese was also tested; this was not covered with cloth, but had a wax coating which was included in the samples. The data, which are listed in table 2, show a general tendency toward higher retention with higher dosage, but the effect appears to be modified somewhat by the condition of the coating, since considerable variations occur between samples from different positions. Interior samples again were found to be free from bromide.

It should be mentioned that while methyl bromide does not penetrate solid cheese, it enters any hole or crevice which is open to the air. Therefore infestations on either external or open internal surfaces can be con-

TABLE 2

Fumigation of whole Longhorn cheeses with various dosages
Fumigation for 24 hours at 50° F., airing for 168 hours

Cheese	Methyl bromide (lbs. per 1000 cu. ft.)	Temperature during airing (° F.)	Per cent total bromide found	
			Outer 0.25 inch	Top 0.25 inch
A	5	75	0.0026	0.0029
B	5	75	0.0038	0.0032
C	3	30–50	0.0035	0.0030
D	3	30–50	0.0029	0.0028
E	2	30–50	0.0025	0.0017
F	2	30–50	0.0021	0.0025
G	1	30–50	0.0018	0.0019
H	1	30–50	0.0020	0.0015
I	Unfumigated control		0.0000	0.0002

trolled. With porous cheese, the retention of bromide in interior portions is practically the same as that on the outside. For example, during a commercial fumigation a sample quarter of a rather porous cheddar was exposed, unwaxed, to 1.8 pounds of methyl bromide per 1000 cubic feet for 8 hours at 60–70° F., then it was immediately waxed and shipped to the laboratory for analysis. The total bromide, which was entirely inorganic at the time of analysis, amounted to 0.0012 per cent on the outer quarter-inch (average from top, curved side and cut side after removal of the wax) and to 0.0011 per cent at a region about three inches inside the cheese. Another more solid cheese (American cheddar), treated in a comparable way, showed bromide on the outer portions, but no more in the center than was found in a duplicate unfumigated portion.

In another commercial fumigation, unwrapped one-pound bricks of limburger cheese were fumigated in a curing room at 70° F. A dosage of 1.5 pounds of methyl bromide per 1000 cubic feet was employed and about 5000 pounds of cheese were present in the room. After fumigation the room was aired for three hours, then two bricks of the cheese were wrapped in waxed paper and shipped to the laboratory where they were analyzed six days later. Cheese 1, the less ripe of the two, contained 0.0021 per cent bromide in the outer quarter-inch and 0.0006 per cent in the interior. The corresponding figures for Cheese 2 were 0.0038 per cent and 0.0012 per cent. Controls on similar unfumigated bricks showed contents of the same magnitude as those of the centers of the fumigated samples, but there were individual variations which may be associated with the salt content. Common salt ordinarily contains up to 0.2 per cent bromide.

Methyl bromide does not penetrate moisture-proof wrapping material such as cellophane with a rubber-like lining. Numerous fumigations of storage rooms and warehouses containing half-pound and two-pound packages of cheese so wrapped have been carried out, and no more bromide has been found in the cheeses than in unfumigated controls. The controls on Brick, Swiss and American process cheese contained no bromide, while a prepared cheese spread contained 0.0005 per cent probably introduced with salt or some other ingredient of the mixture. When a half-pound tinfoil-wrapped package of this cheese spread was fumigated for 9 hours at 70° F. with 3 pounds of methyl bromide per 1000 cubic feet, the bromide content rose only to 0.0009 per cent.

From the above it is evident that the amount of bromide retained by fumigated cheese is dependent primarily upon the area exposed and upon the kind of coating or wrapping. In direct fumigations of large cheeses or in fumigations of storage rooms, etc., containing large or small packages of cheese in moisture-proof wrappers, the retentions are for all practical purposes negligible.

FUMIGATION OF DRIED SKIM MILK

Samples of skim milk powders which had been dried by different processes were fumigated for a 12-hour period at 70° F. and 30 per cent relative humidity with 2 pounds of methyl bromide per 1000 cubic feet. The milk powders were fumigated in 5-pound paper bags, in a 500-cubic foot chamber at atmospheric pressure. After fumigation the bags were removed to a large room in which they were allowed to stand in normal air for definite time intervals. Before each sampling the contents of each bag were well mixed. The amounts of bromide retained after various periods of standing are shown in table 3. The unfumigated controls contain much more bromide than is added by the fumigation. All of the retained bromide is inorganic after four days of airing. These observations are consistent with those of Laug (3) who fumigated skim milk powder with much higher dosages of methyl bromide and found relatively little bromide retained after sufficient airing.

TABLE 3

Fumigation of skim milk powders
Two lbs. CH_3Br per 1000 cu. ft. for 12 hours at 70° F. and 30% R.H.

Hours aired	Per cent total bromide	
	Spray-dried product	Roll-dried product
0.5	0.0029	0.0059
4	0.0029	0.0050
24	0.0028	0.0054
51	0.0027	0.0054
96	0.0024	0.0048
Unfumigated control	0.0024	0.0044

Portions of the same fumigated samples were later refumigated in the same manner with the same concentration of methyl bromide, but for 16 hours at 68° F. and 70–80 per cent relative humidity. After four days of airing the percentages of bromide were 0.0026 in the spray-dried milk and 0.0058 in the roll-dried material. These data indicate that no difficulty with high bromide residues is to be expected in fumigations of skim milk powders.

FUMIGATION OF BUTTER

There is no point in fumigating butter itself, but since it might be stored in creameries or warehouses undergoing general fumigation, an experimental fumigation has been carried out. A cube of butter 2.5 inches on a side was exposed for 12 hours to one pound of methyl bromide per 1000 cubic feet, at 72–77° F. Immediately after fumigation the outer quarter-inch was cut off and the outer and inner portions were aired separately, being analyzed from time to time for total and inorganic bromide. The findings are reported in table 4. They show that methyl bromide does not penetrate

more than a quarter of an inch into the butter, and that most of it escapes by volatilization, but a small part is converted to inorganic bromide.

No analyses have yet been made of packaged butter fumigated under commercial conditions. Some packaged oleomargarine which had been present during fumigation of a cold storage room was found to contain very little bromide, the highest results amounting to 0.0007 per cent Br in the outer quarter-inch and 0.0003 per cent Br in interior portions. It may be concluded that fumigation of rooms containing butter or oleo will produce no objectionable bromide residues in these products.

In no case does methyl bromide remain as such in any of these dairy products for more than two or three days after removal from the fumigation chamber. The bromide residues are entirely inorganic. Flinn (2) has shown that no injurious effects were produced in adults who consumed 30 to

TABLE 4

Fumigation of butter

One lb. CH_3Br per 1000 cu. ft. for 12 hours at 72–77° F.

Hours aired	Per cent bromide remaining			
	In outer 0.25 inch		In inner portions	
	Total	Inorganic	Total	Inorganic
0.5	0.0058	0.0003	0.0001	.....
4	0.0056	0.0004	0.0001	0.0002
24	0.0027	0.0004	0.0002	.....
48	0.0016	0.0005	.....	.....
96	0.0010	0.0011	.....	.....
168	0.0009	0.0010	.....	.....
Unfumigated controls	0.0001	0.0001	.....	.....

45 grains (2 to 3 grams) of sodium bromide daily over a period of four months. Assuming that an adult might eat a kilogram of fumigated foods each day (a liberal allowance) and that the bromide residues in these foods might average 0.01 per cent (which is more than was found in any of the above experiments), the possible daily bromide intake would amount to only 0.10 gram, or 0.13 gram as sodium bromide. It is evident that an ample margin of safety exists.

SUMMARY

The use of methyl bromide for fumigating dairy products has been discussed and its advantages mentioned. The residual bromide contents of dried skim milk, butter and several kinds of cheese have been determined after fumigation under various laboratory and commercial conditions. After a reasonable period has been allowed for escape of the fumigant, the residues are entirely inorganic in nature and are present in insignificant amounts.

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(6) STENGER, V. A., SHRADER, S. A., AND BESHGETOOR, A. W. Analytical Methods for Methyl Bromide. Indus. and Engin. Chem., Analyt. Ed., **11**: 121. 1939.

COMMITTEE REPORT ON SILAGE METHODS, EVALUATIONS, ETC., FOR THE AMERICAN DAIRY SCIENCE ASSOCIATION, PRESENTED AT THE EAST LANSING MEETING IN JUNE, 1942

1. Crop Ensiled, if mixtures of species, specify approximate percentage of each.
   a. Stage of maturity.
      1. Weather conditions at time of storage.
   b. Moisture content at time of ensiling.
   c. Carotene or other vitamin data.
   d. Soil type and level of fertility (fertilizer data, etc.).
2. Method of Harvesting and Rate of Filling (days to complete job).
   a. Length of cut.
   b. How compacted—tramping, addition of water, etc.
   c. Method of sealing.
3. Preservatives Used.
   a. List amounts of preservatives used on a ton of green weight.
   b. Method of adding preservative.
   c. Wilting (method used in determining extent of wilting).
   d. Cultures—kind, quantity, and method of introduction.
4. Sampling Set Up to Measure Silage Losses.
   a. Weigh all material blown into the silo and weigh all the material removed; classify it as to edible and spoiled portion.
   b. If it is impossible to weigh material out of the silo, place 5–10 burlap or cotton bags that have been moistened and wrung as dry as possible holding approximately 10 kilos of representative chopped material in each 8 to 10 ft. section of the silage. Place these samples in the silo at the same level about in the middle of each section. Weigh each sample. At the same time take a like sample for immediate feed analysis. Carotene analysis, moisture and pH should be run at once and Kjeldahl nitrogen determination should be run on undried samples. Upon removal of bags from the silo, not earlier than 30 days after filling, they should again be weighed and prepared immediately for analysis of same factors as determined in fresh sample.
5. Silage Classification.
   a. Very good. (At silo.) Clean, acid odor and taste, no butyric acid, mold sliminess or proteolysis. (In laboratory.) pH of 3.5–4.2 and less than 10% ammonia nitrogen of total nitrogen.
   b. Good. (At silo.) Acid odor and taste, not more than trace of butyric acid. (In laboratory.) pH of 4.2–4.5 and ammonia nitrogen of 10–15 per cent.

c. Fair. (At silo.) Butyric acid, slight proteolysis or some mold. (In laboratory.) pH 4.5–4.8, ammonia nitrogen, 15–20 per cent.
d. Poor. (At silo.) High butyric acid, high proteolysis, sliminess or mold. (In laboratory.) pH above 4.8 and ammonia nitrogen above 20 per cent.
e. Color of silage—green, brown, dark brown, black.
f. Determination of other acids such as lactic, acetic, etc., that may be volatilized in drying or by steam distillation, might aid in classifying the silage.

6. Palatability of Silage.
   a. Good.
      Readily eaten.
   b. Fair.
      Medium intake—slow rate of consumption.
   c. Poor.
      Very low intake—considerable refusal.

   A factor of eating habits enters into this classification and it should not cloud the issue of palatability.

7. Silos.
   a. Type—tower, pit, trench, snow fence, etc.
      1. Material used in silo construction.
   b. Condition.
   c. Dimensions.
   d. Drains.

8. Costs.
   a. Crop cost—to compare with corn costs in terms of man-hours, machine-hours, etc.
      1. Plowing, seed bed preparation, seed cultivation, and fertilizer.
      2. Harvesting.
      3. Preservative costs.

   These studies should be made in cooperation with the Departments of Agricultural Engineering and Agricultural Economics, and if possible, should be based on actual farm conditions rather than on experimental procedures.

9. Experimental Feeding and Digestion Trials.
   a. Animals.
      Age, weight, sex, etc.
   b. Preliminary feeding period of 2–4 weeks or longer depending on the feeding history of the animals.
   c. Feed a control silage.
   d. Rations should be balanced on T.D.N. and protein basis or on pro-

tein and net energy basis. With certain silages the calcium-phosphorus relationship should be considered.

e. Production, live weight, appearance, or any physiological changes.

10. Data should be obtained on the weights of grass silage of various kinds at different levels in the silo. The committee suggests that all stations working on this problem could pool their data for publication. This committee could work as a clearing house in the assembling and compilation of the data.

J. G. ARCHIBALD, Massachusetts
G. BOHSTEDT, Wisconsin
J. C. KNOTT, Washington
C. F. MONROE, Ohio
T. E. WOODWARD, Washington, D. C.
C. B. BENDER, New Jersey, *Chairman*
Committee on Silage Methods, Evaluations, Etc.

American Dairy Science Association Announcements

NOTE FROM THE ASSOCIATION PRESIDENT

Milk and dairy products are now being recognized by governments, as well as nutritionists, as munitions that can help win the struggle for freedom. In bringing this about, the members of the American Dairy Science Association, whatever their particular duties may be, have a vitally important role in doing their utmost to insure that the largest possible quantities of clean milk are produced and that processing of that product shall be the most efficient possible. Past research has enabled us to meet the challenge well, but our efforts must not be slackened for there are still numberless problems awaiting solution. Dairy teaching whether in the class room or to farm audiences has accomplished much, yet still there is a great deal to be done. Dairy farmers of today are much more efficient and produce a much better quality of product, but our milk production could be raised at least 25 per cent by a more universal adoption of the most approved methods. The processing of the many types of dairy products has evolved from the simple home making of butter to large industrial manufacture of many diverse products. Yet there are many problems in this field remaining unsolved. While sanitarians have made the milk supply of the United States safe for public consumption, there still remains a need for more effort to insure a healthful product. In other words while the achievements have been legion, the present emergency imposes a great rsponsibility upon all those who are connected with the dairy industry.

We, of the American Dairy Science Association, in our forthcoming meeting next June at Columbia, Missouri, have much to consider in the various impacts of war on the dairy industry for that will be the general theme. Chairman Judkins and his committee are formulating the program and every member is urged to submit suggestions as to how the meetings may be made more effective. Because the time is short, please send in titles and abstracts promptly.

Signed,
H. P. Davis, *President*

THIRTY-EIGHTH ANNUAL MEETING, UNIVERSITY OF MISSOURI, COLUMBIA, MISSOURI. JUNE, 1943

CALL FOR TITLES

Titles of papers to be presented should be in the hands of the program committee not later than April 1, 1943. Program Chairmen are as follows: General Chairman, H. F. Judkins, 230 Park Ave., New York, N. Y.

Extension Section Chairman, E. C. SCHEIDENHELM, Michigan State College, East Lansing, Mich.

Manufacturing Section Chairman, P. F. SHARP, 317 Ramona Ave., Piedmont, Cal.

Production Section Chairman, K. L. TURK, Univ. of Maryland, College Park, Maryland.

Titles should be sent to the section chairman concerned.

VOLUME XXVI FEBRUARY, 1943 NUMBER 2

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ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
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MILK AND MILK PRODUCTS

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JOURNALS

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New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEW

72. **Advances in Enzymology.** Volume I and Volume II, published by Interscience Publishers, Inc., 215 Fourth Avenue, New York, N. Y., both edited by F. F. NORD of Fordham University and C. H. WERKMAN of Iowa State College. Volume I has X + 433 pages, $5.50, and Volume II, VIII + 374 pages, $5.50; both volumes include author, and subject index, and references.

Excellent features of the two volumes on Enzymology are the arrangements of the chapters, which in nearly every case includes an introduction whereby the reader is given background for what may be otherwise unfamiliar material, a text discussion of the subject, in which the material is conveniently presented under sub-headings for the orientation of the reader, a summary or conclusion by the author on pertinent points, and finally, a complete reference list. Advances in Enzymology is a compilation of reviews on the subject of enzyme behavior and chemistry. The two volumes contain 10 and 12 chapters, respectively, written by recognized authorities in their respective fields. The sections in Volume I include: An excellent review by H. M. Bull on Protein Structure dealing principally with fiber and globular proteins. It includes arrangement, molecular weight and stability of protein structures. The section, Specificity of Proteinases, by M. Bergmann and J. J. Fruton presents clearly a discussion of factors known to determine or affect specificity of this group of enzymes, including role of molecular weight and nature of linkages of the proteins, and activation and synthesis of enzymes. J. B. Summer discusses the Chemical Nature of Catalase including occurrence, role, stability, inactivation, determination, theories of mechanism and products. D. E. Green in a section, Enzyme and Trace Substances, analyzes the significance of the components of enzyme systems in terms of the prosthetic group, and presents a very enlightening discussion of differentiation and similarity of vitamin, hormone and enzyme relationships. In view of the identification of some of the vitamins with the prosthetic groups of enzymes, the discussion on the relation between nutritional requirement and enzyme function is interesting. Other chapters in this volume of perhaps lesser interest to research in the dairy industry are: Physical Chemical Viewpoint of the Problem of Virus Activity (in German); Metabolic Generation and Utilization of Phosphate Bond Energy; Photosynthesis, Facts and Interpretations; Bacterial Photosynthesis; Enzymatic Processes in the Living Plant (in German), and Digestion in the Lower Vertebrae (in German). The Volume II contains the following: In a chapter on Bacterial Viruses (Bacteriophages) Max Delbrücke reviews

distribution, life cycle, assay, inactivation, etc. D. D. Van Slyke reviews Kinetics of Hydrolytic Enzymes and Their Bearing on Measuring Enzyme Activity. In complement with the discussion in Volume I, M. Bergmann discusses A Classification of Proteolytic Enzymes, based upon the splitting of peptide bonds. Included in this section are the activation of the enzymes, and coupled reactions. M. J. Johnson and J. Berger review Enzymatic Properties of Peptidases. This review classifies peptidases in the manner of poly and di peptidase groups, and separately reviews other peptidase systems of animal, plant, bacterial, etc., origin. Respiration of Aspergillis and Related Systems is discussed by H. Tamiya (in German). One of the most concise but complete reviews on Vitamin K, Its Chemistry and Physiology is presented by H. Dam, and followed by a chapter on the Adrenal Cortex Hormones, by J. J. Pfiffner. Other chapters cover Diamine Oxydase (in German); Chemistry of Tea Fermentation; Heterotrophic Assimilation of Carbon Dioxide; Cellulose Decomposition by Microorganisms; and Reciprocal Integration of Carbohydrate and Fat Catabolism.

Enzymology as a whole has been a subject seemingly apart from the most of research activities in the dairy industry. Yet the importance of enzymes in the ripening of cheese, the manufacture of by-products, activity of starters, or vitamin chemistry is recognized. These two volumes in Enzymology will be of sufficiently broad interest to be of value for research libraries in the dairy industry. K.G.W.

BACTERIOLOGY

73. Thermoduric Bacteria and Their Control. C. C. Prouty, State Col. Wash., Pullman, Washington. Milk Dealer, *32*, No. 1: 76–77. Oct., 1942.

Following a discussion of the types, thermal resistance, laboratory diagnosis, and the control of thermoduric bacteria, the author states: "In brief, the control of thermoduric types involves the use of equipment in a good state of repair and the practice of adequate methods of sanitation designed to remove, destroy, and prevent the growth of such bacteria." C.J.B.

BREEDING

74. A New Type of Recessive Achondroplasia in Cattle. P. W. Gregory, S. W. Mead, and W. M. Regan, Div. Anim. Husb., Univ. Cal., Davis. Jour. Hered., *33*, No. 9: 317. 1942.

A hereditary recessive defect, usually lethal and resembling but not identical with previously reported types of "bull dog calves," appeared in the Jersey herd at the California Station in the course of inbreeding experiments. The defect is described and four pictures are included. The defect

is quite variable in its expression. It seems to be inherited as a monofactorial autosomal recessive. One heifer lived to 14 months of age and even then did not succumb directly to the defect but was slaughtered. J.L.L.

CHEESE

75. Contributions to the Study of Rancidity in Canadian Cheddar Cheese. I. Butyric Acid Clostridia in Rancid Cheese. C. H. CASTELL, Ontario Agr. College, Guelph, Ontario. Sci. Agr., *23*, No. 2: 131. 1942.

The two chief factors suspected of causing cheese to become rancid are the butyric-acid-forming bacteria and the enzyme lipase. In this study an attempt was made to isolate and enumerate the butyric acid anaerobes in rancid and normal cheese. Typical forms were not found by direct microscopic examination but they were frequently observed in low dilutions of rancid cheese made in sterile water and incubated at room temperature. The addition of minute amounts of ammonium hydroxide hastened sporulation.

Dilutions of both rancid and non-rancid samples were transferred to three types of enriched media, incubated for 5 days at 37° C., pasteurized at 85° C. and reincubated for at least 7 days. Iodophilic clostridia were thus shown to be more prevalent in the rancid than in the non-rancid samples. However, the numbers found were not considered large enough to be a significant factor in the production of rancid flavours. O.R.I.

CHEMISTRY

76. Refractometric Determination of Casein in Skim Milk. JOHN G. BRERETON AND PAUL F. SHARP, Dept. Dairy Indus., Cornell Univ., Ithaca. Jour. Indus. Engin. Chem., Analyt. Ed., *14*, No. 11: 872. Nov., 1942.

From methods for the determination of casein in skim milk which have been suggested, the refractometric method was selected because of the general satisfaction of refractometric methods, because of the high degree of accuracy which can be obtained on medium-sized samples, and because neither accurately standardized solutions nor accurate adjustment to a single specific temperature is necessary. The casein in 25 gr. of skim milk is precipitated with dilute acetic acid at 40 to 42° C. The precipitate is washed and redissolved using a technique developed to give clear filtrates and reproducible refraction of the solution obtained on dissolving the precipitate. The casein is redissolved in 0.1 N sodium hydroxide and the refraction is compared with a 0.1 N sodium hydroxide solution containing no casein. The refractive index of the solution is increased by .00181 when 1 gr. of purified casein is dissolved in 100 ml. of 0.1 N sodium hydroxide. The average deviation between results obtained with the refractometer procedure and the

A.O.A.C. method was 0.04% of casein and the maximum error was 0.06% when applied to 21 samples of skim milk of a wide range of composition. B.H.W.

77. **Preparation of Samples for Microbiological Determination of Riboflavin.** F. N. STRONG AND L. E. CARPENTER, Col. Agr., Univ. Wis., Madison. Jour. Indus. Eng. Chem., Analyt. Ed., *14,* No. 11: 909. Nov., 1942.

Results are given of a comparative study of modified procedures proposed for the riboflavin assay, of a study of the effect of pure fatty acids, glycerides and other organic materials on the test organism. A procedure for the preliminary treatment of samples has been worked out. Interfering substances are removed and reliable values for riboflavin are obtained on such materials as cereals, dry whey and mixed diets which previously were difficult to assay by the microbiological method. B.H.W.

DISEASE

78. **A Discussion of Mastitis.** H. L. DAVIS, W. H. KIMMER, AND J. A. ANDERSON. Dairy Indus., *7,* No. 8: 206. Aug., 1942.

The authors review the general field of mastitis including the definition, detection, cure and prevention of the disease. D.V.J.

79. **An Epidemic of Typhoid Fever Due to Infected Cheese.** MAXWELL BOWMAN, Dept. Health and Pub. Welfare, Winnipeg, Manitoba, Canada. Canad. Pub. Health Jour., *33,* No. 11: 541. 1942.

During the winter of 1939–40 three outbreaks of typhoid fever occurred in sections of Manitoba. Epidemiological evidence is presented indicating that the three outbreaks were due to infected cheese. O.R.I.

HERD MANAGEMENT

80. **The Effect of Number of Daily Milkings upon Persistency of Milk Production.** I. LUDWIN, Washington, D. C. Jour. Anim. Sci., *1,* No. 4: 300. 1942.

The effect of frequency of milking upon persistency of milk production was studied by selecting 364 Holstein-Friesian A. R. cows meeting the following requirements: (a) Every record was at least 305 days in length, (b) The cows must have been milked the same number of times daily for at least the first nine months after calving except for the first 45 days, (c) The cows must have had at least two records both made in the same herd (d) under (c) must have been made under a different number of daily milkings, (e) records must be capable of fitting a composite comparison.

The results showed that the persistency measured by actual rate of decline (slope) is not affected by frequency of milking. If a proportional measure of persistency is used then persistency is increased by more frequent milking. The decline in milk yield from the third to the ninth month is best considered in a straight line. C.F.H.

ICE CREAM

81. Frozen Fruits Available. D. G. SORBER, Agr. Res. Admin., U. S. Dept. Agr., Albany, Calif. Ice Cream Field, *40,* No. 4: 32. Oct., 1942.

The ice cream trade is utilizing more and more frozen sliced, crushed, or pureed fruit in small sized containers. The advantages claimed for the latter products are uniformity of pack, exclusion of air, preservation of fresh fruit flavor and greater convenience.

Corn syrup, both enzyme-converted and regular are satisfactory replacements for sucrose in frozen-pack fruits, but corn sugar is not satisfactory except possibly in 10% replacement or less.

Results of experiments reported by the author show that satisfactory strawberry puree was prepared by using from 30% to 50% added sugar, ½% to 1% medium-set citrus pectin and up to ¾% citric acid for the less tart fruits if desired. 40% added sugar gave the best results.

The author briefly discusses the characteristics of about twenty varieties of fruits that can be used as sources of flavor in ice cream, stress being placed upon the mineral and vitamin contents of the fruits under consideration. W.C.C.

82. How to Stretch Chocolate Coating. J. H. ERB, Ohio State Univ., Columbus, Ohio. Ice Cream Field, *40,* No. 4: 22. Oct., 1942.

It is pointed out that under the present shortage of chocolate the advisability of (1) altering chocolate formulas and (2) maintaining a constant fluidity of the coating to avoid waste.

Chocolate coating consists of a blend of chocolate liquor, sugar, cocoa butter or some other fat, and about 0.3% to 0.4% lecithin. Ice cream coating usually contains from 55% to 65% fat, and full milk chocolate coatings must contain at least 12% whole milk solids, it is stated. Typical formulas for 100-pound quantities of two types of ice cream coatings are given, and two formulas are included for converting confectioner's coating to ice cream type coating.

Milk chocolate type coating requires less chocolate than the dark type and is therefore recommended. Directions are given for standardizing this type of coating by adding the required ingredients to the dark coating. Instructions are also given for compounding a coating from cocoa.

Until the war coconut oil with a melting point of 76° F. was the most

commonly used fat in the preparation of chocolate ice cream coatings. Proper blends of other fats and oils are now available to replace coconut oil for this purpose.

Changes in viscosity most often result from (1) absorption of moisture at the dipping tank and (2) temperature fluctuations of the coating.

W.C.C.

83. Economy Through Serum Solid Changes. S. T. COULTER, Univ. Minn., St. Paul, Minn. Ice Cream Field, *40,* No. 4: 18. Oct., 1942.

Increasing the serum solids content of ice cream offers a means of partially replacing the decreases in solids resulting from sugar restrictions, the author states.

Mention is made of the method suggested by Sommer for ascertaining the maximum serum solids content of ice cream not only makes the development of sandiness more likely, but also produces a typical "condensed milk" or "serum solids" flavor, both of which are limiting factors in the use of serum solids, it is claimed.

The author briefly describes the more common serum solids concentrates that can be used in ice cream, such as condensed skim milk, condensed whole milk, superheated condensed milk, sweetened condensed milk, dried milk and dried buttermilk. Mention is also made of the process of making ice cream mixes in the vacuum pan. W.C.C.

84. The Use of Fruits under War Conditions. W. C. COLE, Univ. Calif., Davis, Calif. Ice Cream Field, *40,* No. 4: 21. Oct., 1942.

The emergency restrictions on sugar have not limited the amount of frozen fruit packed, although in certain instances it has necessitated a change in the proportion of sugar to fruit used. Certain processors have doubled their output of frozen pack fruit as compared with last year, it is stated.

Results of experiments by the author are reported which show that both dates and raisin syrup furnished pleasing flavors in ice cream and also supplied considerable of the desired sweetness. Thus it is claimed that mixes containing only 10% added sugar when properly flavored with either dates or raisin syrup were sufficiently sweet whereas mixes with 15% sugar so flavored were considered too sweet. It is suggested that under these conditions low sugar mixes can be used to advantage.

Lack of shipping facilities have imposed certain restrictions on the fruits available and the tin shortage has made necessary the use of substitute containers. Fruit is likely to be more extensively used because of limitations in our supply of vanilla and chocolate or cocoa.

The tartness desired in ices and sherbets, other than that supplied by the fruit used as a source of flavor, can be satisfactorily supplied by lactic acid

as well as by citric or tartaric acids which are more commonly used but are now difficult to obtain. W.C.C.

85. Hold Down Deliveries. R. T. SMITH, Scranton, Pa. Ice Cream Field, *40,* No. 4: 34. Oct., 1942.

It is stated that the industry as a whole has reduced mileage 25% or more, and some average as high as 50% of the total mileage saved. Instances are cited where three-day instead of six-day deliveries have been successfully put in operation. In other instances competitors are working together in the use of transport trucks and some companies are using common carriers for their shipments, the author states.

Although pooling of delivery or combination deliveries with competitors has not been generally accepted the author recommends the practice along with capacity loads as one means of saving rubber, gasoline and equipment. He concludes, "the life line of this industry is its ability to distribute its products within minimum of tire mileage." W.C.C.

86. How to Make Better Marshmellow Cream. TULIO CORDERO AND CHARLES SHAKELFORD. Food Indus., *14,* No. 10: 58–59. Oct., 1942.

Simple tests and controls make possible the manufacture of marshmellow cream with good volume and keeping properties. Shrinkage and separation in storage are prevented by proper mixing temperatures and formulas balanced to give proper pH.

Good marshmellow cream should be brilliantly white, capable of being whipped to twice its volume on addition of water in the right proportions, have a good vanilla flavor and stay stable for at least nine months after manufacture.

It may deteriorate by losing air and shrinking, separating sugar syrup and decomposing due to fermentation. The basic formula for marshmellow is given and tables on the effect of temperature and pH on its stability and tests for its stability are listed. J.C.M.

MILK

87. Why Has Milk Consumption Not Increased. R. W. STUMBO, Sec., Wis. Dairymen's Assoc., Fort Atkinson, Wis. Milk Dealer, *32,* No. 1: 78–81. Oct., 1942.

Statistics are cited to prove that for some reason or other the consuming public have not increased their demand for fluid milk. The author believes that there is no doubt but what this is partly due to the failure of producers and distributors to promote consumption and sales by furnishing products that sell themselves; that it is mostly a production, processing, and marketing problem. The following important steps in developing a market are

discussed: (1) Marketing only high-quality products; (2) Dependable service; (3) Good business ethics; (4) Good advertising and salesmanship; (5) Cooperation with health departments. C.J.B.

88. Factors Affecting the Accuracy of the Phosphatase Test. FRANKLIN W. BARBER, Dept. Agr. Bact., Univ. Wis., Madison. Milk Dealer, *32,* No. 1: 31, 66–68. Oct., 1942.

Inaccuracies in the phosphatase test due to methods, added substances, bacteria in cream, and bacteria in milk are discussed. The author points out that the paper is not intended, in any way, to question the reliability of the phosphatase test, but to emphasize the importance of good technique and proper interpretation of positive results. C.J.B.

89. Filtering Milk on the Farm. K. G. WECKEL, Dept. Dairy Indus., Univ. Wis. Milk Dealer, *32,* No. 1: 28–29, 46–48. Oct., 1942.

The mechanical, biological, and physical factors involved in filtering milk are discussed. The mechanical factors are: (a) Design and construction of strainer; (b) Type and application of filter disk; (c) Use of strainer and disk. The biological factors are: (a) Temperature of the milk; (b) Variability in milk composition; (c) Abnormal milk, or procedures. The physical factors are discussed under: (a) Volume of milk per disk; (b) Amount and kind of extraneous material; (c) Ultimate effect of straining on milk.

The author stresses that the justification for filtering milk is two-fold: (1) to remove inadvertently acquired foreign material from the milk and (2) to provide at the point of production a sediment test of the milk. In no sense whatever should the function of filtration be considered primarily for "cleaning up the milk." C.J.B.

90. Recent Developments of the Resazurin Test. JOHN G. DAVIS. Food Mfr., *17,* No. 11: 308–311, 324. Nov., 1942.

Developments in the resazurin test have been so fast in this country during the last year that few people can be familiar with the advances that have been made. This article is an attempt to summarize the present position and deals principally with the synthesis, purification, properties and standardization of the dye, its behavior in milk, the preparation of the solution, the use of the comparator and the various modifications of the test.

This article is very detailed. It lists methods for purifying the dye, namely precipitation and also its properties of absorption and adsorption. Tables are given for testing samples of resazurin and their compositional analysis. J.C.M.

91. The Cellular Content of Milk. S. B. THOMAS AND K. MORGAN. Dairy Indus., *7,* No. 6: 148. June, 1942.

A high catalase index was found to have a close relationship to the leuco-

cyte count. In 83% of the raw milk analyzed, samples containing over 500,000 leucocytes per ml. showed abnormally high catalase activity. A few samples contained large numbers of catalase producing micrococci, sarcinae and proteolytic types. Therefore, the authors suggest that the test not be used on milk having less than a one-hour methylene blue reduction time. They point out, however, that morning's milk analyzed within 4–8 hours after milking should not contain sufficient numbers of the catalase-producing bacteria to invalidate the test. High catalase activity was found in mixed herd milks having high leucocyte counts.

The catalase test conducted in conjunction with the resazurin-rennet and solids-not-fat tests was found to be a satisfactory means of detecting the presence of abnormal milk being received at cheese factories. D.V.J.

92. The Resazurin Test in Factory Practice. G. F. V. MORGAN, Dairy Indus., *7*, No. 5 : 117. May, 1942.

Investigations were made on a large number of raw milk samples using a Lovibond dual-purpose tintometer and resazurin color disc. The results indicated that during periods of moderate temperature the resazurin test is a satisfactory method for determining the hygienic quality of milk. The test can detect abnormalities in milk due to infected udders, colostrum or late lactation milk.

In discussing the discrepancies in certain data where the Breed smear was compared with the resazurin test the author points to the possibility that gram-negative coliform organisms which are active producers of reductase and questionably stained and counted in the Breed technique may be responsible for this lack of agreement.

The resazurin-rennet test may give very unreliable results and therefore is not recommended as a quality test on raw milk. D.V.J.

MISCELLANEOUS

93. Automatic Control of Air Conditioning Plant. FRANK H. SLADE. Food Mfr., *17*, No. 11 : 317–321. Nov., 1942.

Air conditioning plants in food manufacture are needed to reduce production costs and wastes. Wastes can be reduced by closer control of humidities and temperatures. Proper selection of automatic control equipment should be made. To summarize why automatic control should be a part of an air conditioning plant, they provide the required conditions of temperature, etc., they are a safety measure and produce economical results.

No set system of control can be laid down. There are three kinds of control: electrical, pneumatic, and self contained. Each one is explained. Also it gives explanations of thermostats of different types of makes. The same procedure is used with humidistats and control valves. A diagram of a control system is shown. J.C.M.

94. Chemical Sterilization. D. V. JOSEPHSON, Pa. State Col., State Col. Pa. Ice Cream Field, *40,* No. 4: 42. Oct., 1942.

The United States Public Health Service Milk Ordinance and Code recognizes sterilization by chlorine, hot water or steam, provided each of the methods is conducted in accordance with Code specifications. Nearly half of the states are operating under this Code, whereas the rest have specified one or a combination of these treatments. Although no state prohibits the use of chlorine sterilizing agents, the author states that where heat is specifically required the use of chlorine in addition is unnecessary duplication and added expense.

The ineffectiveness of live steam for sterilizing open surfaces is mentioned as well as the deteriorating effect steam has upon rubber. Spraying a strong chlorine solution on the surfaces of large storage tanks, tank trucks and large surface coolers is recommended by the author as being very satisfactory.

Three general types of chlorine sterilizing agents are recommended: (1) sodium hypochlorite, (2) calcium hypochlorite, and (3) chloramines. A brief description is given of the characteristics of these products so far as stability and rate of reaction are concerned.

The alkalinity of the chlorine solution influences the rapidity with which it destroys bacterial cells and it is stated that a compound which produces a highly alkaline solution (pH 10–11) will be very slow acting whereas if the solution is too low in alkalinity or is slightly acid the activity is accelerated; it becomes corrosive to metals and decomposes rapidly in contact with organic matter. It is pointed out that the chlorine concentration of a rinse solution is not alone a reliable index of its potential germicidal activity. The author warns against the use of so-called "combined cleaners and sterilizers" and stresses the necessity of thorough cleaning before attempting to sterilize with chlorine. For general purposes 100 p.p.m. of chlorine is recommended and for spraying 200 to 250 p.p.m. is considered desirable.

W.C.C.

95. More Results With an Acid Detergent but in a Standard Can Washer. F. M. SCALES, Sheffield Farms Co., New York City. Milk Dealer, *32,* No. 2: 30–31, 64–72. Nov., 1942.

Data are presented showing the results of 2 alkaline detergents as compared with an acid detergent in a standard Rice & Adams straight-away can washer designed for the use of alkaline detergents. The results as summed up by the author are as follows: The results indicate that the use of the acid detergent in a standard straight-away can washer gave more nearly sterile cans than either of the alkaline powders tested. The acid detergent was much more economical than the alkaline powders. C.J.B.

96. Conservation of Power Through Efficient Operation of Refrigerating Equipment. L. C. THOMSEN, Univ. Wis., Madison, Wis. Milk Dealer, *32*, No. 2: 33–35, 54–58. Nov., 1942.

A discussion is given of some of the possible conditions which may serve as a basis for economizing on refrigeration. Data are presented to show how to determine the rated capacity and the effect of suction and discharge pressures. C.J.B.

97. How the Government Buys Food. H. C. ALBIN. Food Indus., *14*, No. 10: 64–68. Oct., 1942.

The Agriculture Marketing Administration moves daily from 700 to 1000 carloads of products, primarily processed foods, to seaports for shipping. Pointers on how to get some of this business and how to meet the requirements are given. They do most of their buying according to specific announcements. The purchasing procedure is set up for immediate action. Five suggestions for making offers of sale are: (1) Read announcements carefully; (2) get offers in on time; (3) watch packaging specifications; (4) know your price ceilings and do not overbid them; (5) allow adequate time for delivery of the commodity. Each one is explained in detail.

There are two main reasons for using the offer and acceptance purchasing method: (1) To provide greater efficiency in large scale purchasing and (2) to create the broadest and most equitable market possible for government purchases. Purchases are made at announced prices. Most of the canned goods have been purchased on the offer acceptance basis but in the future many purchases probably will be made according to the terms of a special program recently announced by the Department of Agriculture. J.C.M.

98. Cooling Storage Rooms by Brine Spray Method. Part II. G. O. WEDDELL. Food Indus., *14*, No. 10: 56–58. Oct., 1942.

A method is introduced for food storage where rapid pre-cooling, followed by holding at a steady temperature, is desired, and this has improved results in fruit storage. This article forecasts combination with quick freezing of fruits and vegetables.

Recent and unusual installations of brine spray storage are described. The humidity factor is discussed and 9 advantages of these systems are listed. J.C.M.

99. The Selection of Refrigerating Equipment. ANONYMOUS. Food Mfr., *17*, No. 10: 276–279. Oct., 1942.

Information of a general nature is given concerning the selection of types of refrigerating plant components and considerations which determine their selection.

A refrigerating plant consists of three main components; compressor, condenser and evaporator is the most important. A general description of a refrigerating plant is given, concerning mainly the types of coolers and solutions used. Grid, wet, dry, and gilled or wire wound tube types of coolers are discussed in great detail.

Selection of compressors is governed by the refrigerant to be used. Compressors are made with high and medium speed.

The condensers are mainly of three types: air cooled, water cooled non-evaporative, and water cooled evaporative. Each one is explained in detail.

J.C.M.

100. Daylight in Factories. ANONYMOUS. Food Mfr., *17,* No. 11: 316. Nov., 1942.

The government has changed its ideas about roof lights in factories. A committee has been formed and it urges the use of daylight as far as possible, not only to save power but to save the health of the workers. They are considering methods of restoring daylight conditions preferably with shutter installations, which can be closed during blackouts. An expert's opinions on the subject of factory lighting and supplies can be obtained in a booklet recently issued by writing to the office of Food Manufacture. J.C.M.

101. War Gases and Foodstuffs. ANONYMOUS. Book Review from book by W. R. Woolridge. Food Mfr., *17,* No. 10: 290. Oct., 1942.

This book answers such questions as "Where and under what conditions can we store our food to protect it against contamination with gas" and many others. The author does not overload his book with repetitive matter. He refers the reader to less specialized literature. The principles of decontamination of food are discussed. A chapter on the detection of gas is included. The author does not waste words or bury the facts. "It should be available everywhere," is the reviewer's general comment on the book.

J.C.M.

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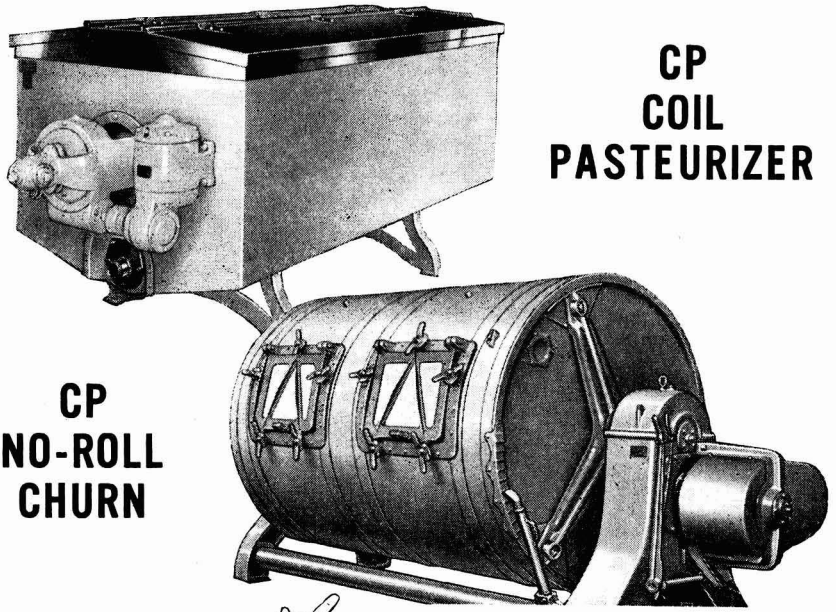

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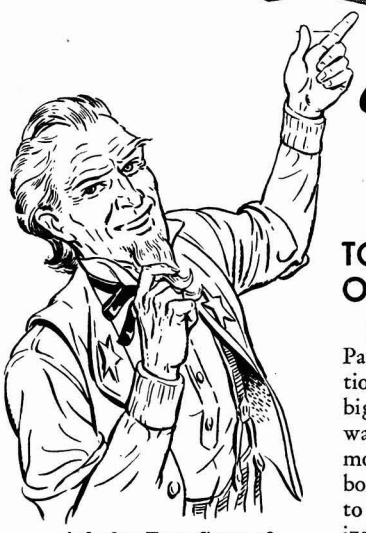

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