PROPERTIES OF DEHYDRATED
SOUR CREAM

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Rohini J. Desai

1966

ABSTRACT

A STUDY OF SOME PHYSICAL AND CHEMICAL PROPERTIES OF DEHYDRATED SOUR CREAM

by Rohini J. Desai

A study was made of the effects of foam-spray drying and freeze drying on some physical, chemical and organoleptic properties of cultured (sour) cream. Samples of sweet cream varying in fat content from 10 to 18% were individually pasteurized, homogenized and inoculated with a lyophilized mixed strain starter-culture. A portion of the ripened cream was frozen for use as a control; the remaining portion was divided into lots one of which was freeze dried and the other foam-spray dried. Samples from each dehydrated sour cream were stored at 40 F and 72 F.

Substantial losses in amounts of flavor and aroma constituents occurred on dehydration of the sour cream. Compared to the control, both foam-spray dried and freeze-dried sour cream had less volatile acids, lower titratable acidity and smaller amounts of acetoin-plus-diacetyl. In general, the retention of these volatile compounds was better in the freeze dried than in the foam-spray dried sour cream. Diacetyl on the other hand, increased following

drying of the sour cream by both methods. The free fat values were consistently higher in the freeze-dried cream, conferring on the product a distinct yellowness of appearance while the foam-spray dried counterpart was a light cream in color. Though the ease of dispersion of both powders decreased with increasing fat content, the results could not be correlated to the corresponding amounts of free fat. Organoleptic evaluations also demonstrated the superiority of the freeze-dried sour cream when compared to the foam-spray dried product.

A STUDY OF SOME PHYSICAL AND CHEMICAL PROPERTIES OF DEHYDRATED SOUR CREAM

By

Rohini J. Desai

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1966

Dedicated

to

Mr. and Mrs. Jayantilal B. Desai

ACKNOWLEDGMENTS

The author is greatly indebted to her major professor, Dr. C. M. Stine, for his guidance and encouragement throughout this study, his gift of understanding and for his efforts in the preparation of the manuscript.

Grateful thanks are extended to Dr. H. A. Lillevik and Dr. J. R. Brunner, who served on the examining committee and reviewed the manuscript, to Dr. G. M. Trout for his help in the preparation of the manuscript and to Dr. B. S. Schweigert for providing financial assistance.

Grateful appreciation is also extended to Mr. Lee Blakely, Mr. Don Wallace, Mr. Jaswant Singh and Mr. Jim Kirk for their help in various ways.

Last but not the least, the author thanks her parents, Mr. and Mrs. Jayantilal B. Desai, for their sacrifices and constant inspiration for a higher education, and to whom this thesis is respectfully dedicated.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Cultured (sour) cream is a ripened cream with a pleasant acid flavor, distinctive aroma, smooth texture and moderately heavy body. This fine food is made by inoculating sweet pasteurized cream with a culture of acid and flavor producing organisms and allowing the fermentation to proceed until the desirable qualities of the product are developed.

Until fairly recently, the market for commercial cultured cream was somewhat restricted to the metropolitan areas of New York and other major cities. Today however it is a food commonly enjoyed throughout the United States. Per capita consumption of fresh sour cream in the United States averaged 0.7 pounds in 1965.

In addition to direct consumption as a food, cultured cream finds increasing acceptance on salads, as a dressing for vegetables, in fillings for cakes and as a replacement for buttermilk or sweet cream in many exotic recipes.

All foods are subject to deterioration sooner or later, depending on the particular food and conditions of storage. Cultured cream keeps well for 2 weeks at ordinary refrigerator temperatures of 40 F. Though storage for 4

THE

weeks or longer is possible under such conditions, bitterness resulting from the growth of psychrophilic organisms eventually sets in, often accompanied by undesirable yeast and mold growth on the surface. Such defects render any product unacceptable for consumption. Hence, in order to prolong shelf life, improved and economical methods of preservation are needed. Storage at sub-zero temperatures was early recognized to be quite effective in inhibiting microbial spoilage for extended periods but such conditions also proved to be detrimental to the body and texture of thawed sour cream.

The advent of many new and improved drying methods and their widespread application to the food industry has been an ultimate boon to the American homemaker in a multitude of ways. Easily prepared food products that can be stored at room temperature for many months are being utilized in increasing numbers by today's modern housewife. However, many fresh foods remain unexploited and continue to be consumed in the fresh state. Cultured cream might well be utilized in numerous convenience foods if dehydrated sour cream of superior quality could be developed.

Hence, the intent underlying this undertaking was to make a comparative study of the flavor properties and chemical and physical properties of fresh, cultured cream and of dehydrated sour cream prepared by spray and freeze drying.

REVIEW OF LITERATURE

Body, Texture and Flavor of Cultured Cream

From the marketing standpoint, cultured cream should have a smooth, rather heavy body with the moisture homogeneously incorporated. For consumer acceptability most markets require a product which yields a plummet reading of 7.5 or 8 as determined by the Hilker-Guthrie method. In the majority of states and cities the same quality regulations are applied to cultured cream as to sweet cream. In general, the product must contain at least 18% fat and be made from inspected creams (Guthrie, 1952).

An acid gel, accompanied by a delicate flavor resulting from the growth and activity of lactic acid streptococci and flavor producing leuconostoc bacteria, characterizes cultured cream. Thus, excellent cultured cream possesses a mild, subtle, aromatic acid flavor reminiscent of the flavor of a 93 score or AA grade ripened cream butter (Kosikowski, 1966).

Various factors influence and contribute to the overall excellence of cultured cream. Certainly, the starter culture employed is one of foremost importance in the production of a good body and the desired flavor characteristics (Guthrie, 1963).

Cream separated from milk at 42 F had a higher viscosity as determined by a Borden flow meter than corresponding cream separated at 90 F, irrespective of whether pasteurization was before or after separation (Roberts, Blanton and Marley, 1953). However, cream separated from cold pasteurized milk was found satisfactory by Glazier et al. (1954) from the standpoint of bacterial contamination, in spite of the lower viscosity as compared to cold separated cream from raw milk.

Guthrie (1952) found that "different makes of homogenizers are not important factors in the manufacture of cultured cream." His results indicated, however, that the final product made from cream which was homogenized twice in either the first or the second stage with a total of 5000 psi was superior in body to that obtained if two-staged homogenization were employed at a gauge pressure of 5000 psi. Double homogenization at 2500 psi has been considered optimum for obtaining desired body and smoothness in sour cream (Guthrie, 1952).

The temperature of the cream at the time of homogenization also affects the final body of the sour cream. The body was best with cream homogenized at 165 F and poorest when homogenized at 120 F, according to Guthrie (1952). Aule and Storgards (1958) also reported that viscosity and stability of the cream on standing increased directly with increasing homogenization temperatures. The adverse effects

produced by lower homogenization temperatures could not be overcome by increasing the homogenization pressure to higher than 300 kg/cm^2. Homogenization of cream after, instead of before, pasteurization, as is sometimes practiced, does not affect the viscosity when all other conditions remain the same. However, homogenization followed by pasteurization is often preferred to avoid an increase in the coliform and the standard plate counts due to recontamination (Savage and Brown, 1953).

Hening and Dahlberg (1943) observed that cream required a longer holding time than milk at 160 F to achieve phosphatase inactivation and the equivalent of 99.9% destruction of coliform organisms. On the basis of their study the optimum time-temperature relationships for pasteurization of cream ranged from 145 F/30 mins to 170 F/3 sec. Guthrie (1952) also noted that extending heat treatment at 165 F beyond 30 minutes resulted in a noticeably weaker body in the sour cream. Processing sweet cream at 165 F/30 min has been demonstrated by Savage et al. (1953) to have the least effect on changes in viscosity due to homogenization. Further studies conducted by Guthrie (1963) to determine the optimum time/temperature relationship for pasteurization of raw sweet cream confirmed his earlier choice of 165 F/30 min. The body of the final sour cream was weak or weak and grainy when pasteurization temperatures of 145 F/30 min and

180 F/30 min respectively were used. Heating cream to excessively high temperatures precipitates the "casein" and results in a thicker body, according to Guthrie (1952).

The relationship of fat content of the cream to body characteristics of cultured cream was investigated by Guthrie (1952). He found that 18% fat was optimal, with progressive decreases in quality as the fat content was raised or lowered from that value. The addition of milk-solids-not-fat (MSNF) to "normal" cream did not cause an improvement in physical characteristics of cultured cream. However, Guthrie noted (1963) that MSNF improved the body of low solids cream.

Although the main contributions to the body of sour cream are made by the fat and casein, the addition of stabilizers has also been found to be important in order to maintain uniform viscosity and plasticity from batch to batch, day to day and from season to season. The use of various stabilizers has been investigated (Guthrie, 1963), yet rennet at levels of 0.5 ml/10 gals of cream has been the stabilizing agent most commonly recommended. According to Guthrie (1952), agitation of the warm cultured cream expresses some moisture, creating graininess of texture. This can be avoided by stirring the ripened cream only after partial cooling.

Savinovsky (1948) stored sour cream for 6 months at -10, -15 and -25 C with very little decrease in acidity at the end of that period. An increase in acidity was observed when cultured cream was stored at 3 C. Freezing has a weakening effect on the body of the cream (Guthrie, 1952). Thawing of creams stored at -10 and -15 C yielded a thin liquid in which lumps of protein and fat granules were suspended. Creams stored at -25 C appeared normal (Savinovsky, 1948). Addition of stabilizers to cream before frozen storage considerably improved physical stability during storage and subsequent thawing, according to Bell (1947).

Starter Culture

The first starters used in manufacturing cultured dairy products were natural cultures obtained by allowing milk to sour. The isolation of Streptococcus lactis in 1873 stimulated interest in the identification of various other strains and their ultimate cultivation and laboratory propagation ensued. By the turn of the 19th century, commercial cultures for use in creameries were available all over Europe and America.

During this period the lactic streptococci were considered synonymous with starter cultures and it was not until 1919 that this rather restricted outlook was broadened to accommodate a set of "associated" organisms, now known as

the citric acid fermenters. These organisms were simultaneously isolated by Bailey and Hammer in the United States and Boekhout and Otto de Vries in Europe (Hales). Further work by various other investigators led to a greater enumeration of newer strains and today we have at least 12 distinct types of starter cultures for milk fermentations, which have marked differences in morphology and substrate utilization.

Milk is the substrate most widely used for the growth of dairy cultures, but it is not the natural habitat for all of them. Some appear to be of plant origin (Speck, 1964). However, the complement of nutrients in milk is essentially adequate for the lactic streptococci though the required nitrogen does not necessarily exist in forms that are readily available. Milk as it is secreted contains very little non protein nitrogen; hence, the nitrogen requirements of the cultures have to be met by the hydrolysis of proteins (Speck, 1964). Not all bacteria are endowed with equal proteolytic activity and this limits the ability of cultures to obtain their nitrogen in amounts or forms necessary for maximum growth. In addition, analyses of individual milk samples from various cows, as conducted by Anderson et al. (1955), indicated a variation in the peptide content, providing evidence that the activity of starters could be correlated to the amount of the peptide fraction in milk. Thus, the quality of the original raw milk is important (Greene and Jezeski, 1955) and the nature of its subsequent treatment

THES

has been shown to affect its suitability as a starter medium. Certain manufacturing processes, especially heating, were found by Greene and Jezeski (1957) to alter the substrate, rendering it stimulatory in some cases and inhibitory in others. Foster (1952) reported an improved growth of 6-8 species of homofermentative lactobacilli in autoclaved milk, presumably due to the resulting partial hydrolysis of casein, while grossly overheating the milk had a deleterious effect on the same organisms. Gilliland and Olson (1963) observed that acid production by lactic cultures incubated for 10-12 hours was more rapid in whole milk and buttermilk than in skim milk. The use of fresh skim milk or reconstituted non-fat dry milk (NFDM) is, however, widespread as a substrate for culture propagation. Horral and Elliker (1950) reported that reconstituted milk promoted more constant activity in starters than did selected whole milk. On the other hand, different lots of NFDM varied in their ability to provide a satisfactory medium. In general, however, commercial high heat powders supported starter activity more favorably than did low heat powders, with the exception that those powders with an excessively severe heat history exerted a deleterious effect.

Increasing the MSNF content of reconstituted NFDM was found to stimulate acid production by strains of organisms belonging to the lactobacilli and the streptococcus genera (Yano *et al.*, 1960), possibly because of the buffering

action of MSNF and a higher concentration of growth factor(s) in the milk.

Many strains of streptococci, leuconostoc and lactobacilli are known to require pantothenic acid, nicotinic acid and biotin for maximum growth; riboflavin is necessary for the growth of some strains and is stimulatory to others (Nambudripad et al., 1957; Anderson and Elliker, 1953). Folic acid and pyridoxine requirements have been shown to be variable. The presence of various amino acids such as proline, valine, leucine, isoleucine, histidine and methionine in the growth medium was also demonstrated by Anderson and Elliker (1953) as being essential for growth of various strains of S. cremoris and S. lactis. Cystine, tryptophan, aspartic acid and serine were shown to be dispensable. Similarly, all strains of Leuconostoc citrovorum studied required arginine, histidine, isoleucine, leucine, lysine and valine, while threonine, asparagine, aspartic acid, glycine and cystine were not essential by any of them (Prouty, 1961).

Production of Diacetyl and Acetoin by Aroma Bacteria

In manufacturing cultured or fermented dairy products, starter cultures are added to produce lactic acid or to produce a desired aroma in the cultured food product. Beginning in the late 19th century, there was much confusion

as to whether the desired aroma imparted to butter by starters was the result of a single organism or a mixture of organisms (Collins, 1962). Later interest was centered around certain low acid producing organisms which produced good butter aroma only when grown in association with S. lactis. Further research by various workers led to the establishment of their identity by several different names. Hammer (1920) called them Streptococcus citrovorus and Streptococcus paracitrovorus, Krishnaswamy and Babel (1951) suggested S. lactis var. aromaticus, while Knudsen and Sorensen (1929) named the organisms Betacoccus cremoris. A year later the terms Leuconostoc citrovorum and Leuconostoc paracitrovorum, were coined by Hucker and Pederson (1930). The terms currently used (Breed, Murray and Smith, 1957) are Leuconostoc citrovorum and Leuconostoc dextranicum.

For many years, little attention was given to the fact that some single strain cultures had been found able to produce good butter aroma in the absence of S. lactis or S. cremoris. Shown to be variants of the lactic streptococci, many strains of organisms have been reported in the past 35 years which are characterized by their ability to ferment citrate actively with the production of carbon dioxide, volatile acids and C_4 compounds such as diacetyl, acetoin and 2,3-butylene glycol. Matuszewski (1936) was the first to isolate and identify the organisms as Streptococcus diacetilactis. At about the same time, van Beynum and Pette

THES

(1936) described two citrate utilizing organisms capable of producing lactic acid and diacetyl in milk and suggested for them the name Streptococcus citrophilus. Swartling (1951) isolated 35 strains of acetoin-producing lactic streptococci from raw milk starter cultures and dairy products identical to the strains accounted for earlier and concluded that the name S. diacetilactis, rather than the other names proposed, should be retained. Czulak (1953) characterized S. diacetilactis strains isolated from Australian Cheddar cheese starters.

Present day starter cultures employ either L. citrovorum or S. diacetilactis or both for the production of aroma compounds desirable in certain fermented milk foods. The leuconostocs grow best in association with any of a variety of strains of S. lactis or S. cremoris, which produce lactic acid from lactose. The presence of either S. lactis or S. cremoris is beneficial to sufficiently reduce the pH of the medium and thereby initiate leuconostoc activity.

Flavor has long been recognized as a major factor in the quality and acceptability of foods. The value of selected cultures of bacteria for the development of a desirable flavor and aroma in many dairy products has been thoroughly established. Rapid development in analytical techniques and instrumentation over the past two decades has enabled the elucidation of the complex flavor chemistry of

many foods. Some families of flavor compounds have been studied more thoroughly than others. In dairy products, the aliphatic carbonyls are one of the most important of the various groups of flavor compounds encountered and they are important as contributors to the flavor spectrum of most dairy products (Day, 1965).

Diacetyl is one of the more important of these but other compounds such as the volatile acids are also significant. Generally, no single carbonyl compound can be implicated as the sole source of a typical flavor; rather the flavor appears to result from a composite of many compounds (Day, 1965). Wong and Patton (1962) indicated the presence of formaldehyde, acetaldehyde, methyl sulfide, acetone, butanone, pentanone-2 and hexanone-2 in milk and cream. Most carbonyls produced as a result of lipid oxidation are objectionable; however, a recent paper by Begemann and Koster (1964) has identified cis-4-heptenal as an important component of the "cream-like" flavor.

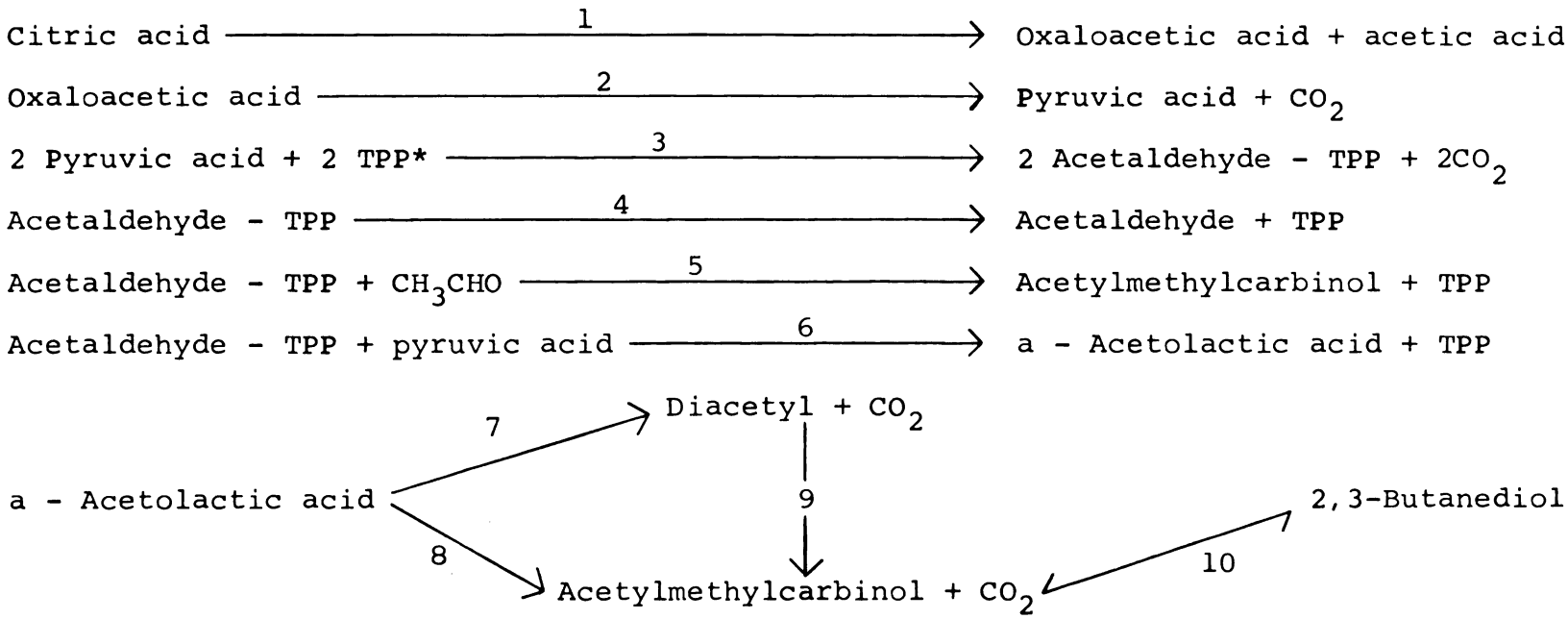
The importance of acetoin and diacetyl was first emphasized by Michaelian et al. (1933) who found that butter cultures with a desirable flavor and aroma contained relatively large amounts of these compounds while those lacking in flavor were quite low in acetoin and diacetyl. Other investigations have confirmed this observation (Hoecker and Hammer, 1941; Dolazalek, 1952; Calbert and Price, 1949).

The source of these C_4 compounds remained a highly speculative and controversial issue for many years. The available literature on the subject is replete with contradicting reports stemming from individual experimentation. In the early stages of flavor research, workers believed that diacetyl and acetoin were metabolites resulting from the fermentation of lactose (Virtanen et al., 1941; Coppens, 1954). Others viewed citrate as the source (DeMan, 1956; Pette, 1949; Bang, 1945; Glenn and Prouty, 1955; Federov and Kruglova, 1955), and some felt that both compounds are involved (Mizuno and Jezeski, 1959; van Beynum and Pette, 1939; Andersen, 1959; Taufel and Krusen, 1952). Storgards (1941) on the other hand stated that neither glucose nor citrate, alone or in combination, supported production of acetoin and believed the presence of barium or calcium salts were essential to initiate the reaction. He further propounded the involvement of pyruvic acid in the synthesizing mechanism and much evidence is now available (Bang, 1943; van Beynum and Pette, 1939; Mizuno, 1956; Harvey and Collins, 1961; Juni, 1952a; Taufel and Behnke, 1960) which confirms his early observation. The pyruvate is derived from citrate by reversal of the condensing enzyme and decarboxylation of the oxaloacetate formed (Andersen, 1959). Various studies have succeeded in isolating and characterizing the citritase enzyme implicated in catalyzing the cleavage of citric acid

into oxaloacetate and acetic acid (Taufel and Behnke, 1960; Harvey and Collins, 1963; Sandine et al., 1961; Seitz et al., 1963).

van Beynum and Pette (1939) and Federov and Kruglova (1955) discussed possibilities for the pathway between pyruvate and acetoin, postulating acetaldehyde as a likely intermediate. Acetaldehyde in turn is thought to polymerize to acetoin directly (van Beynum and Pette, 1939) or condense with pyruvic acid to form alpha acetolactate (Andersen, 1959). DeMan (1956) detected alpha acetolactic acid in the formation of acetoin by L. citrovorum while Juni (1952a) demonstrated a similar phenomenon in organisms of the genus Aerobacter. Thus L. citrovorum appears to form acetoin from pyruvate by the pathway most generally used by acetoin-producing bacteria, namely, the formation of active acetate from pyruvate and reaction of active acetate with pyruvate to give alpha acetolactate which is subsequently decarboxylated to acetoin. These observations are in agreement with those of Andersen (1959) and Taufel and Behnke (1960). The same scheme is valid for S. diacetilactis, according to Seitz et al. (1963). They isolated the various enzymes involved and presented the following schematic for the mechanism of acetoin and diacetyl synthesis by bacteria:

Pathways for conversion of citric acid to diacetyl, acetylmethylcarbinol and 2,3-butanediol by S. diacetilactis



1. Citritase
2. Oxaloacetate decarboxylase
3. Pyruvate decarboxylase
4. Non-enzymatic
5. AMC synthetase
6. a - Acetolactate synthetase
7. a - Acetolactate oxidase
8. a - Acetolactate decarboxylase
9. Diacetyl reductase
10. 2,3-Butanediol dehydrogenase

*TPP represents thiamine pyrophosphate.

Breakdown and Interconversion of Diacetyl, Acetoin and 2,3-Butanediol

As evident from the foregoing schematic, diacetyl, acetoin and 2,3-butanediol are related through an oxidation-reduction mechanism. The amount of oxidized or reduced substances in the medium determines the corresponding proportion of these compounds. Obviously, the presence of oxygen or highly oxidized substances will favor the formation of diacetyl; on the other hand, a strongly reducing potential would promote the predominance of acetoin or butanediol, both of which are flavorless and odorless compounds (Marshall, 1961). This interrelationship is of great significance to industry due to the established importance of diacetyl in many dairy products and the ease of its destructive conversion into acetoin and butanediol, with an accompanying loss of flavor.

The most potent diacetyl-producing organisms are paradoxically, the ones most active in its subsequent destruction. Thus, of the lactic streptococci, S. diacetilactis exhibits the strongest reducing potential favoring the formation of butanediol (Sandine, 1964). This ability is attributed to the presence of certain enzyme systems with which the bacteria are endowed and which are activated under favorable conditions.

Various investigations have provided an insight into these mechanisms, shedding light on new theories to replace the old. Strecker and Harary (1954) reported the isolation and purification of two enzyme systems, one catalyzing the reversible oxidation by DPN^+ of butanediol to acetoin and the other catalyzing an essentially irreversible reduction by DPNH of diacetyl to acetoin. They named the enzymes 2,3-butylene glycol dehydrogenase and diacetyl reductase respectively. This observation refutes the hitherto accepted concept of acetoin being the immediate precursor of diacetyl.

A slightly different mode of diacetyl breakdown was suggested by Green et al. (1947) in a study of a diphosphothiamine-dependent enzyme which catalyzed the conversion of two molecules of diacetyl into two molecules of acetic acid and one molecule of acetoin. They called this enzyme diacetyl mutase. Strecker and Harary (1954) indicated that the diacetyl reductase was possibly a component of the diacetyl mutase reported since acetoin was not oxidized in the presence of the reductase.

Recent studies by Juni and Heym (1956) revealed yet another pathway for the reduction of diacetyl and acetoin to butanediol, proceeding through the intermediate compounds diacetylmethylcarbinol and acetylbutanediol, which is dependent on the presence of diphosphothiamine and DPN^+. These mechanisms would serve to explain the increase in butanediol content which parallels the decrease in diacetyl

and acetoin contents and causes a deterioration in the flavor of cultured dairy products.

Culture Preservation

Interest in the preservation of starter cultures has intensified during the past decade. The ideal method of preservation would be to take the organisms at the peak of their metabolic activity, hold them for days or months in a state of arrested development and have them resume their work immediately on restoration to a favorable environment (Foster, 1962). Unfortunately, this ideal has never been realized since it is virtually impossible to keep a living organism in a completely inactive state. Hence, alternative methods have had to be resorted to, based on one of two principles involving either the reduction of the metabolic rate of the organisms or the separation of the cells from their metabolic waste products. The choice of a preservative method depends largely on the ultimate purpose for which the culture is to be used and maybe any one of the following:

(a) Refrigeration at low temperatures between transfers, as often employed by many dairy plants and research laboratories,

(b) Freezing, where extended storage is required,

(c) Freeze drying or lyophilizing, involving initial freezing of the cultures, subsequent drying by sublimation and final storage at low temperatures,

THES

(d) Spray drying of the culture. Although not commercially used, spray drying has been investigated as a possible method of economically producing dehydrated starter cultures (Foster, 1962).

Liquid Cultures

Normally, ripened cultures can usually be held at 4-8 C for several days without a serious change in activity. Storage at higher temperatures, however, resulted in an accelerated loss of activity. According to Swartling and Lindgren (1960) the activity of cultures refrigerated immediately after inoculation was better retained than that of cultures permitted to ripen before storage.

The effect of the addition of various compounds on prolonging storage activity has been investigated by many workers. Heinemann (1958) was one of the first to show that glycerol has a protective effect on starter bacteria. The cultures under study remained active as long as two months at 35 F and six months at 5 F and -20 F. Under similar conditions, the activity of cultures without glycerol was appreciably decreased. Olson (1959) added various insoluble buffers to starter cultures and found that $CaCO_3$ gave the best protection of those investigated. The findings of Lindgren and Swartling (1960), however, did not indicate storage of cultures in "chalk milk" as a reliable method of preservation. Certain concentrations of glycerol, salt and

sugar also aided in preserving the cultures, a combination of 20% glycerol, 3% salt and 30% sugar being the most effective, with or without added $CaCO_3$, according to Olson (1959).

Frozen Cultures

Freezing can be used to preserve many types of microorganisms. Although the process kills some of the cells, as many as 75-90% of the viable bacteria have been recovered on thawing of the frozen cells. A further probe into the matter has revealed that the infliction of greatest injury to the bacteria occurs during the early part of storage and injury increases further with time (Moss and Speck, 1962). A rise in death rate is thus continuous resulting in a decrease of activity with length of storage (Rudnik and Glenn, 1960). Though Foster (1962) could demonstrate no effect on the rate of freezing and thawing on survival, Moss and Speck (1962) have shown that some cultures survive best when frozen rapidly. The converse has been demonstrated for many other cultures. Addition of glycerol confers protection from damage (Heinemann, 1958) while use of fresh liquid skim or 2% dried skim milk was found definitely superior to other media (Moss and Speck, 1962; Simmons and Graham, 1959; Foster, 1962). Greatest destruction of cells was found by Moss and Speck (1962) to occur when the cells were frozen in distilled water. The acidity and physiological age of cultures prior to freezing also influences their

TH

survival and overall activity. Swartling and Lindgren (1960) observed that concentrated suspensions of younger cultures (15-18 hours old) were definitely more active on thawing than cells from older cultures. They also recorded an even better performance when inoculated milk, frozen without prior incubation, was thawed and ripened.

Lindgren and Swartling (1960) considered deep freezing a very satisfactory method of preserving the activity of a freshly inoculated culture for as long as one year. The successful use of frozen cultures for direct inoculation in the commercial manufacture of fermented products (Simmons and Graham, 1959; Rudnik and Glenn, 1960) has served to confirm this observation. Simmons and Graham (1959) regularly made good buttermilk with frozen culture stored as long as three months; the activity of the thawed culture compared favorably with that of fresh starters transferred daily. Similarly, Rudnik and Glenn (1960) employed frozen culture up to 5 months old to inoculate milk directly for cottage cheese manufacture. All 39 lots of cheese so made were salable.

These and similar investigations have so far been encouraging enough to advocate freezing as a means of preserving organisms for extended periods of time.

THES

Dried Cultures

Lactic cultures can be dried by lyophilization or by spray drying. The former is the less destructive of the two processes and is readily adapted to the preservation of small amounts of culture (Foster, 1962).

Freeze drying. This process, which has enjoyed widespread use in the food industry for dehydrating foods, has likewise been successfully employed in the preservation of stock cultures. Freeze-dried cultures can be used for months or years as the seed material for developing vigorous starters. Such powdered cultures stored by Maxa and Teply (1960) at refrigeration temperatures retained their activity at almost the initial levels throughout the two-year experimental period. The ability of organisms to endure the drying process varies with the species, according to Foster (1962). Several other factors, including age of the culture and nature of the suspending medium play influential roles on activity of the culture. Watts (1955) for example, lyophilized a milk culture at various stages in the growth cycle up to 19 hours. Samples dried at 9 and 12 hours of age, which represented the late logarithmic and early maximum stationary phases of growth respectively, showed the highest survival values, namely 76 and 84%. On rehydration, their acid-producing ability approached that of the undried culture. No changes were observed by Morichi et al. (1961) in the physiological characteristics of the freeze-dried

cultures. Death rates could be minimized by maintaining the acidity of the cultures between pH 6-9. Hence, the beneficial effect of diluting cultures with skim milk on the survival rate can actually be attributed to the consequent increase in pH. Once dried, lactic cultures must be stored at low temperatures and be protected from moisture and light.

Spray drying. Several investigators have considered the possibility of spray drying large quantities of culture since it offers considerable economic advantage by way of lower processing costs over other methods of drying. Mamaeva (1955) spray dried a mixture of lactobacilli and yeasts used for koumiss culture, but recovered only a fraction of 1% of the cells in a viable condition. Nonetheless, these dried cultures after reconstitution with water exhibited a high rate of acid production and retained their activity for six months, depending on the storage conditions. Attempts to spray dry ordinary milk cultures of lactic acid bacteria were not very successful initially and early efforts by Richardson (1960) were abandoned because the product, in addition to being less active than the lyophilized culture, was difficult to rehydrate. S. lactis in 5% reconstituted skim milk dried to a 3.5% moisture level yielded 50-60% viable cells immediately after drying, as reported by Lattuada and Foster (1963). Residual moisture, within the 2.4 to 4.4% range, did not affect stability during storage, and low storage temperatures prolonged shelf life of the

THES

dried culture. Extensive studies conducted by Sapp and Hedrick (1960) show that with favorable conditions, appreciable activity can be maintained in spray-dried cultures. Outlet air temperatures of 135-165 F favored greater survival while neutralization of the acidity of the cultures before drying decreased rather than increased the activity of the dry product. Cultures dried at 12, 16 and 24 hours of age showed practically the same activity but those dried at 8 hours were less active.

Foster (1962) reported consistent differences between the survival values of S. lactis and S. cremoris, the former being more resistant both to spray drying and to storage in the dry state. Use of 5% NFDM as the suspension medium was recommended over others such as phosphate milk or dextrin-ascorbic acid-thiourea diluent. Under the best of conditions, dry cultures could be stored at least four months without a loss of greater than 15%, even though spray dried cultures have been shown by others to die rapidly if stored at temperatures above freezing. Cultures stored at 40 F by Sapp and Hedrick (1960) were active after one week but found unsatisfactory after three weeks. Retention of initial activity was considerably extended at -15 F.

EXPERIMENTAL PROCEDURES

Sources of Cultures

Commercial freeze-dried sour cream cultures were obtained from the Michigan State University (MSU) Dairy Plant and from a culture supply house. These cultures were propagated in skim milk which had been heated in flowing steam for one hour. The cultures were incubated at 72 F and were transferred daily during the course of the research.

Preparation of Fresh Sour Cream

For the research reported herein, creams of three different fat contents were prepared: 10, 14 and 18%. Each lot of cream was standardized at the MSU Dairy Plant and was processed in 10 gal stainless steel cans. The cream was pasteurized at 165 F for 15 min under constant agitation, homogenized twice at 2000 psi single stage using a Manton Gaulin three plunger homogenizer and immediately cooled to 72 F. The cream was inoculated with 1% starter culture and incubated until at least 0.70% titratable acidity, calculated as lactic acid, was attained.

Approximately four gallons of the sour cream thus obtained was then layered (½ inch thick) in enamel trays,

covered with aluminum foil and quick frozen in a -10 F moving air hardening room. Half of this frozen sour cream was broken up into small pieces and dried for 40 hr in a Stokes freeze drier chamber evacuated to 100 microns of mercury, as measured on a McLoed gauge. The temperature of the platens was gradually raised from 28 C to 42 C within the first 24 hrs.

The remaining half of the ripened sour cream was atomized into a Rogers cocurrent inverted tear drop drier using two Spraying Systems SX high pressure nozzles with number 17 spinners and number 70 cores. The dryer was operated at an exit air temperature of 165 F. Nitrogen was injected into the feed at a rate of 2.0 ft^3/gal cream in a mixing cylinder located between the high pressure pump and the atomizing nozzle.

Method of Storage

The foam-spray dried and freeze dried sour creams were stored in cryovac plastic bags at 40 F. Small amounts of these powders which were to be used for organoleptic evaluations were bottled and stored at 40 F and 72 F.

Preparation of Samples for Analyses

Control

The frozen sour cream stored at -10 F was used as the control. Each day as per requirement, portions of the frozen cream were thawed at room temperature and homogenized once in a stainless steel hand homogenizer.

Reconstituted Foam-spray Dried and Freeze-dried Sour Cream

The powders were reconstituted to the total solids content of the corresponding control by blending with distilled water. This mixture was stirred, allowed to stand at ambient temperature for 15 min and was then homogenized in the hand homogenizer.

Analytical Methods

Moisture

The moisture content of all foam-spray dried and freeze-dried sour cream samples was determined by a standard vacuum oven technique employing a Mojonnier milk tester. A sample approximately 0.3 g in weight, accurately weighed directly into a Mojonnier moisture dish, was spread evenly over the entire bottom of the dish by adding 2 ml hot distilled water (ca. 100 C). The dish was kept in direct contact upon the outside hot plate having a temperature of 180 C and heated until the first traces of brown began to

THES

appear. The sample was then transferred to a 100 C vacuum oven and kept for 10 min under 27 inches of vacuum and thereafter placed in a cooling dessicator for 5 min with the water circulating pump operating continuously. At the end of this period, the dish was weighed rapidly and the moisture content calculated and expressed to the nearest one-tenth of one per cent.

The total solids of the fresh unfrozen sour cream was similarly determined using approximately 1 g samples, accurately weighed.

pH

The pH measurements on the control and reconstituted samples were made with a Beckman Zeromatic pH meter using a calomel half cell and a glass electrode standardized to read accurately in the range of pH 4.0 to 5.0. The results were expressed to the nearest one-tenth of a pH unit.

Titratable Acidity

Nine gram aliquots of the control and the reconstituted samples were titrated with 0.1 N NaOH to the phenolphthalein endpoint. The acidity was reported as per cent lactic acid.

Volatile Acidity

A rapid direct-distillation method of Kosikowski and Dahlberg (1946) was adapted to quantitate the volatile acid content of the control and the reconstituted samples of sour

cream. To a 10 g sample of cream was added 50 ml 10% H_2SO_4 (at 50 C) and 35 g $MgSO_4 \cdot 7H_2O$. The mixture was stirred, refluxed for 5 minutes to drive off the CO_2 and cooled by standing at ambient temperature for 30 min. Distillation was then begun and continued for 60-70 min until the boiling mixture reached a temperature of 116 C. The distillate so collected contained the water soluble volatile acids; the condenser was rinsed with 25 ml neutral alcohol to recover the water insoluble volatile acids. Each fraction was then titrated with 0.1 N NaOH to the phenolphthalein endpoint and the sum of the titers reported as ml of volatile acid per 100 g sample.

Diacetyl Determination

The diacetyl content of the control and the reconstituted sour cream samples was determined by the method of Prill and Hammer (1938) employing a 25 g aliquot weighed into a 500 ml, two necked distillation flask. The flask was connected to the distillation apparatus and a slow stream of CO_2 was passed over the sample and through the apparatus for 5 min. Steam was then admitted under reflux to displace any remaining air and the CO_2 from within the system. When bubbles of gas ceased to appear in the collection trap, distillation was permitted to proceed at a slow rate for 25-30 min collecting 5.0 to 5.2 ml distillate in 1 ml hydroxylamine acetate solution. The absorbance of diacetyl (as ammono

ferrous dimethylglyoxime) was measured at 530 mμ in a model 14 Coleman spectrophotometer. This value was then converted to mg diacetyl by referring to a standard curve.

Diacetyl and Acetoin Determination

For the diacetyl-plus-acetoin determination, 15 ml 40% $FeCl_3$ solution was added to 25 g of the control or reconstituted samples being analyzed and the mixture refluxed for 10 min before distillation was commenced. The development and measurement of the colored ammono ferrous dimethylglyoxime complex were accomplished as previously described.

Total Fat

The total fat of the spray-dried and freeze-dried powders was extracted by slightly modifying the standard Roese-Gottlieb procedure to include addition of 3 ml NH_4OH instead of the suggested 1.5 ml, since the acidity of the sour cream necessitates the use of additional alkali. The results were expressed as per cent fat on a dry basis.

Free Fat

The freeze dried and foam-spray dried powders were analyzed for free fat by the method of Thomas, Holgren, Jokay and Bloch (1957) and the findings reported as mg free fat/g total fat.

Dispersibility

The method outlined by Stone et al. (1954) was modified to determine the dispersibility of the freeze-dried and foam-spray dried cultured cream. A 10 g sample of the powder was blended with 90 ml distilled water at 25 C for 30 sec in a high speed blender and immediately filtered under vacuum using a medium porosity sintered glass funnel. The resulting filtrate was transferred to a 100 ml volumetric flask and filled to the mark with distilled water. The solids content of a 10 ml aliquot of this filtrate was determined by the vacuum oven technique employing a Mojonnier milk tester and the dispersibility reported as g of powder dispersed/100 g sample.

Organoleptic Evaluation

The flavor of the reconstituted freeze-dried and foam-spray dried sour creams, stored at 40 F and 72 F for 8 weeks, was judged by a panel of 3-4 members at the end of 0, 4 and 8 week intervals. The frozen sour cream served as the control. The hedonic preference scale with a range of 0 to 9 was used in evaluating the samples and the average value for each sample was reported.

RESULTS

Some Physical and Chemical Characteristics of Dehydrated Sour Cream

The data collected on selected physical and chemical characteristics of dehydrated sour cream are presented in Table 1.

The moisture content of the foam-spray dried sour cream ranged from 1.8 to 2.9% and of the freeze-dried samples varied from 2.2 to 2.6%. In four of the six pairs of samples analyzed, the moisture content of the freeze-dried sour cream exceeded those of the corresponding foam-spray dried sour cream. The total solids of the control increased from 17.9 to 25.8% with increasing fat content of the cream.

The pH of the dehydrated sour cream, in both trials, was higher than the control. Results obtained for the freeze-dried samples, ranging in value from 3.9 to 4.5, were consistently lower than those of the corresponding foam-spray dried cream, which varied from 4.1 to 4.7.

The titratable acidity of the dehydrated sour cream, with the exception of the sample foam-spray dried from 14.6% fat cream, was lower than the control. The losses resulting

THE

Table 1. Some physical and chemical characteristics of dehydrated sour cream

Fat Content of Cream (%)	Analytical Determinations of Control			Foam Spray Dried			Freeze Dried		
	Total Solids (%)	pH	Titratable Acidity (%)	Moisture (%)	pH	Titratable Acidity (%)	Moisture (%)	pH	Titratable Acidity (%)
Trial I									
10.0	19.1	3.9	0.78	2.2	4.1	0.74	2.6	3.9	0.77
14.6	22.3	3.9	0.78	1.8	4.1	0.79	2.3	4.0	0.76
18.0	25.8	4.3	0.83	2.0	4.6	0.78	2.3	4.4	0.81
Trial II									
10.I	17.9	4.3	0.78	2.9	4.5	0.68	2.2	4.5	0.77
13.2	20.7	4.4	0.77	2.5	4.7	0.68	2.5	4.5	0.75
17.5	24.8	4.2	0.77	2.5	4.5	0.70	2.6	4.3	0.76

THES

from foam-spray drying the cream are relatively greater than those incurred due to freeze drying.

Effect of Drying on the Volatile Acidity of Sour Cream

The data in Table 2 illustrate the effect of drying on the volatile acids content of sour cream. Although there is an obvious decrease in volatile acidity on dehydration, the quantity retained by the freeze-dried powders is consistently higher than the corresponding foam-spray dried samples. An exceptional 100% retention is observed in the freeze-dried 14.6% fat sour cream analyzed in Trial I.

Effect of Drying on the Diacetyl Content of Sour Cream

The changes in the diacetyl content of sour cream as a consequence of drying are presented in Table 3. The overall trend indicates an increase in diacetyl in the resulting powders although, a decrease in the case of four samples is also recorded. In Trial II, the diacetyl content of the freeze-dried product is substantially greater than of the corresponding foam-spray dried counterparts. This observation, however, is not duplicated in Trial I.

Table 2. Effect of drying on the volatile acidity of sour cream

Fat Content of Cream (%)	Volatile Acidity (ml of 0.1N NaOH/100 gm. sample)		
	Control	Foam Spray Dried*	Freeze Dried*
Trial I			
10.0	25.0	13.4	16.0
14.6	20.0	9.8	20.5
18.0	23.8	17.3	21.5
Trial II			
10.1	17.0	9.0	12.0
13.2	16.0	12.0	13.0
17.5	18.0	9.5	14.0

*Reconstituted.

Table 3. Effect of drying on the diacetyl content of sour cream

Fat Content of Cream (%)	Diacetyl (mgs/kg)		
	Control	Foam Spray Dried*	Freeze Dried*
Trial I			
10.0	0.89	1.35	0.84
14.6	0.68	0.74	0.72
18.0	0.52	0.48	0.58
Trial II			
10.1	0.49	0.55	0.89
13.2	0.52	0.41	0.81
17.5	0.56	0.44	0.77

*Reconstituted.

THES

Effect of Drying on the Diacetyl-plus-acetoin Content of Sour Cream

Table 4 contains data which enumerate the effect of drying on the diacetyl-plus-acetoin content of sour cream. The results are significant in the drastic decreases caused by foam-spray drying, from 122-185 ppm diacetyl-plus-acetoin in the control to 4-8 ppm in the resulting powders. Losses incurred by the freeze-dried samples are also substantial, i.e., a decrease from 122-185 ppm in the controls to amounts ranging from 29-66 ppm in the powders. However, retention of diacetyl-plus-acetoin is substantially higher in freeze-dried powders than in foam-spray dried powders.

Effect of the Method of Drying on the Free Fat of Dehydrated Sour Cream

As evident from inspection of the data in Table 5, the free fat content of freeze-dried sour cream is considerably higher than of the corresponding foam-spray dried powders, by amounts varying from 138 mg/g total fat to as high as 231 mg/g total fat. An increase in free fat values of both foam-spray dried and freeze-dried powders with increasing total fat content of the original cream is noticed in Trial II. However, this trend is not evident in the powders studied in Trial I and may be related to processing conditions not studied.

THES

Table 4. Effect of drying on the Diacetyl-plus-acetoin content of sour cream

Fat Content of Cream (%)	Diacetyl-plus-acetoin (mgs/kg)		
	Control	Foam Spray Dried*	Freeze Dried*
Trial I			
10.0	122.4	6.3	52.0
14.6	122.5	5.6	39.5
18.0	138.9	4.6	29.2
Trial II			
10.1	153.5	7.0	57.3
13.2	185.0	7.6	65.3
17.5	151.3	6.4	53.0

*Reconstituted.

Table 5. Effect of the method of drying on the free fat of dehydrated sour cream

Fat Content of Cream (%)	Fat Values Of: Foam Spray Dried — Total Fat (%)	Foam Spray Dried — Free Fat (mg/g) Total Fat	Freeze Dried — Total Fat (%)	Freeze Dried — Free Fat (mg/g) Total Fat	Increased Differences in Free Fat Values of Freeze Dried Over Spray Dried Sour Cream (mg/g) Total Fat
Trial I					
10.0	53.7	735.0	55.5	881.8	146.8
14.6	64.3	712.8	64.8	872.7	159.9
18.0	70.1	726.6	70.1	864.6	138.0
Trial II					
10.1	55.6	668.0	56.3	889.0	221.0
13.2	61.6	685.0	61.7	916.0	231.0
17.5	68.8	688.0	68.7	918.0	230.0

THE

Effect of the Method of Drying on the Dispersibility of Dehydrated Sour Cream

The dispersibility of dehydrated sour cream decreased with increasing total fat content of the cream, as indicated by the data enumerated in Table 6. Foam-spray dried powders, possibly due to their lower free fat value, were more dispersible than the corresponding freeze-dried samples in four analyses out of six. However, no definite correlation can be established since many other factors, not taken into consideration in this research project, are found to influence the dispersibility of dehydrated samples.

Effect of Storage at 40 F and 72 F on the Flavor Scores of Foam-spray Dried and Freeze-dried Sour Cream

Within a period of 8 weeks duration, there was no appreciable difference in the effect of storage at 40 F or 72 F on the flavor scores of foam-spray dried and freeze-dried sour cream. Evidence in support of this observation is presented in Table 7. The freeze-dried powders of varying fat contents, scored much higher ratings ranging from 5.6 to 7.5, than the corresponding foam-spray dried samples (2.0 to 4.6). The superiority of the freeze-dried powders over their foam-spray dried counterparts was established at the very outset and a continued preference sustained by all the judges during the entire study.

Table 6. Effect of the method of drying on the dispersibility of dehydrated sour cream

Fat Content of Cream (%)	Dispersibility (g/10 g. powder) of	
	Foam Spray Dried	Freeze Dried
Trial I		
10.0	2.56	2.19
14.6	1.59	1.82
18.0	1.64	1.61
Trial II		
10.1	2.40	2.52
13.2	2.19	1.97
17.5	1.87	1.61

THE

Table 7. The effect of storage at 40 F and 72 F on the flavor scores of foam-spray dried and freeze-dried sour cream

Period of Storage (In Weeks)	Control	Hedonic Scores For Spray Dried* Stored at 40 F	Spray Dried* Stored at 72 F	Freeze Dried* Stored at 40 F	Freeze Dried* Stored at 72 F
10.1% fat cream					
0	7.3	3.3		6.3	
4	7.0	3.6	4.6	6.3	6.3
8	7.0	3.5	4.0	6.0	6.3
13.2% fat cream					
0	4.6	3.3		5.6	
4	6.5	3.6	3.6	7.2	7.3
9	7.3	...	2.0	...	5.6
17.5% fat cream					
0	8.2	3.7		6.7	
4	8.3	2.3	3.2	7.5	7.5
8	7.0	2.0	2.0	6.0	4.0

*Reconstituted.

DISCUSSION

Six batches of sour cream, varying in total fat content, were prepared during the entire course of study. Cultures employed to inoculate the sweet creams listed under Trial I and Trial II were from two separate sources. The characteristics of a culture depend a great deal on the particular strain(s) of bacteria present. Differences in the activity of the culture, stemming from variations in the mode and conditions of propagation are subsequently reflected in the resulting cultured food product. The obvious differences in physical characteristics and chemical composition of the controls of Trial I and Trial II (Tables 1-6) could partly be accounted for on this premise. The aroma producing leuconostocs are activated only after the pH of the medium is sufficiently lowered. Hence the production of adequate amounts of acidity is an important aspect of sour cream manufacture and contributes substantially to the overall flavor and desirable body characteristics of the resulting product. Values ranging from 0.6 to 0.8% titratable acidity, expressed as lactic acid, are generally achieved; the optimum level varies with such factors as the total solids content of the cream, cultures employed and manufacturing procedures used, as well as the ultimate flavor

desired. In this study, the samples of cream were cultured to an average acidity of 0.78%. As evident from Table 1, the pH values and the corresponding titratable acidity could not be correlated. Two batches of sour cream prepared from 13.2% and 17.5% fat creams with a titratable acidity of 0.77% recorded a pH of 4.4 and 4.2 respectively, while two other samples of identical pH values registered a considerable difference in their respective titratable acidities.

Frozen sour cream was employed as the control. Its body, on thawing, was considerably destabilized with much fat clumping and protein flocculation. Such destabilization defined by Favstova and Vlodavets (1955) as "a reduction of fat dispersion due to fusing of fat particles," is a consequence of the destructive action of freezing on the fat emulsion of a product. Due to the expansion of water on freezing and a possible denaturation of the membrane protein, the fat globule membrane is ruptured, resulting in the liberation and fusion of globular fat causing a subsequent loss of viscosity (Knoop and Wortmann, 1959). Rapid freezing minimizes this effect if the rate of contraction of the fat is the same at which the water expands. Slow freezing inflicts greater damage on the structure of the cream. Hence, destabilization is largely dependent on the rate and magnitude of the temperature change (Lagoni and Peters, 1961) and is favored by low temperatures of storage and a high fat content of the product. The thawed cream therefore, had to

be homogenized using a hand homogenizer, to disperse the fat and ensure homogeneity in composition of the samples analyzed.

There was a distinct difference in color of the dehydrated sour cream obtained by the drying methods employed in this study, the freeze-dried powder being lemon yellow in contrast to the light cream appearance of the corresponding foam-spray dried sample. The state of the fat in the particles and the particle size itself may account for the difference in color. The amount of fat liberated as free fat and the state of its dispersion either on the surface or throughout the entire mass of the powder, is largely dependent on the method of dehydration employed. As evident from Table 5, there is a substantial increase in the free fat content of the freeze-dried over the foam-spray dried powder. Berlin et al. (1964) observed by fluorescence microscopy, that the free fat in foam dried whole milk powders exists on the surface of the powder particles in the form of small fat globules, whereas the same is present on the surface of the spray-dried powders as lakes or films. Microscopic examinations by Nickerson et al. (1952) showed that the fat globules of freeze-dried whole milk were dispersed throughout the mass of the particles. Particle size also is governed by the method of dehydration. Since the size and shape of the frozen particles do not alter during freeze drying, the resulting powder particles are irregularly

shaped and porous in nature. Spray-dried particles, on the other hand, tend to be uniform in size and were relatively smaller as obtained in this study. Thus, a high free fat content and larger particle size may be responsible for the deepening of color in the freeze-dried over the spray-dried samples.

The ease of dispersion of a dehydrated product is greatly influenced by its lipid content. The dispersibility of dried milks was observed by Ashworth (1955) to decrease with increasing total fat in the samples. As evident from the data in Table 6, a similar decrease in dispersibility occurs in both foam-spray dried and freeze-dried sour cream with increasing total fat content. However, a similar correlation between free fat values and their effect on dispersibility could not be established in this study since the results of Trial I totally contradicted those of Trial II, indicating that other unknown factors are involved. In Trial II the dispersibility of the samples decreased with increasing amounts of free fat, whereas, with the samples reported in Trial I, dispersibility decreased with decreasing amounts of free fat. Investigations by Tamsma _et al._ (1958) indicated a positive relationship between the free fat content of foam-spray dried whole milk and its dispersibility, which was unaffected by amounts of free fat up to 40% but decreased as the levels increased from 40 to 95%.

Data reported by Reinke and Brunner (1959) failed to reveal any such interdependence. Certain variations in their processing procedures yielded greater amounts of free fat in the resulting whole milk powder but did not diminish the ease of dispersion of the product. Spraying of the feed through large orifice nozzles at low pressures favored free fat formation but also enhanced dispersibility while homogenization of the condensed milk prior to atomization decreased both. Reducing the total fat content on the other hand, yielded smaller amounts of free fat and increased the dispersibility.

The volatile acidity of sour cream was diminished on dehydration; the losses incurred on foam-spray drying were greater than those due to freeze drying. According to Bradley (1964), foam-spray drying of natural cheese slurries permitted a greater retention of flavor volatiles than could be achieved by conventional spray drying. This was attributed in part to the increased particle size of the powder, as a consequence of gassing the feed, which entrapped greater concentrations of the volatiles. In addition, sparging with an inert gas increases the porosity of the droplets thereby accelerating the evaporation of moisture. This, in turn, promotes rapid cooling of the particles during the drying period. As observed by Bradley (1964), the retention of flavor volatiles is enhanced by lower powder temperatures. On this premise, one might also account for the better

retention of the flavorful compounds in the freeze-dried samples over the foam-spray dried counterpart (Table 2). The freeze-dried powders obtained in this study were relatively more porous and larger in size than the corresponding foam-spray dried sour cream. Subjection to high temperatures, another cause for losses induced on spray drying, is totally absent in freeze drying; hence, losses in the volatile acids content is considerably minimized and restricted to a decrease in amounts of those water soluble, low molecular weight compounds which are most easily removed during sublimation of the ice.

This explanation can further be applied to account for the substantial losses in acetoin-plus-diacetyl, encountered in both powders. The loss incurred by the foam-spray dried samples is remarkably high. Both acetoin and diacetyl are low molecular weight compounds (88.1 and 86.1 respectively), very soluble in water and greatly prone to volatilization on drying. The boiling points of diacetyl and acetoin are 85 C and 144 C respectively. The acetoin content which always greatly exceeds the amount of diacetyl present, is diminished either as a direct loss or via oxidation to diacetyl due to air incorporation in the drying chamber.

On the other hand, contrary to expectations, the diacetyl content of sour cream registered an increase on

dehydration (Table 3) in eight out of twelve analyses. The additional amounts of diacetyl recorded for the two powders over initial quantities of the control, very possibly result due to conversion of precursor compounds such as acetoin to diacetyl induced by incorporation of air during stirring of the ripened cream prior to drying or occurring as a result of oxidative changes during drying itself. Shaking the cultures during ripening is conducive to increased yields of diacetyl (Prill and Hammer, 1939) while slow churning of the cream in preference to holding it for the same time without churning resulted in a butter with improved flavor, presumably due to the aeration involved (Peterson, 1943). Consequently, losses of original diacetyl incurred by the powders may be obscured by the concurrent oxidation of acetoin during processing, resulting therefore in an apparent overall gain in diacetyl content of the powder.

The analytical results discussed heretofore, are significant. However no study directed towards product development in the food industry is complete without accompanying flavor scores since palatability of the commodity in question largely influences its success on the consumer market. The dehydrated sour creams employed in Trial II of this study were also organoleptically evaluated using the conventional hedonic scale (Table 7). The flavor scores for the 10% fat cream are, in general, in good agreement. However, some discrepancies occur in the two succeeding samples;

for example, the ratings of the control containing 13.2% fat increased from 4.6 to 6.5 in 4 weeks. This does not represent an amelioration of flavor on storage. The hedonic scale is purely comparative in function and the figurative range of preference varies with each evaluation conducted at different times. Hence an implicit correlation cannot be established. On a relative basis it is evident from the data in Table 7 that the freeze-dried sour cream is distinctly superior to its foam-spray dried counterpart, this preference being sustained by the judges during the entire period. The original flavor and aroma of the cultured sour cream was considerably retained in the freeze-dried product for as long as 8 weeks. The foam-spray dried samples on the other hand were repeatedly described as being chalky, astringent and stale at the very outset; the stale flavors intensified greatly during 8 weeks storage at both 40 F and 72 F. These stale flavors are associated with the temperature dependent deteriorative changes occurring in the milk fat phase (Tarassuk and Jack, 1946; Tamsma et al., 1963). Studies conducted by Nawar et al. (1963) indicate the possibility that the stale flavor sensation, observed in dried whole milk, exists in one or both of two chemical forms identified as being an aldehyde similar to heptaldehyde and an unsaturated dicarbonyl or hydroxy carbonyl compound. The amounts of these components of the stale fraction vary with the time and temperature of storage. The stale flavor

development is most rapid in the initial months of storage and is greatly accelerated by higher temperatures. Oxygen promotes staling, hence deaeration and inert gas packing of the powders results in beneficial effects of extending the storage life. Another important factor involving subjection to high heat treatment prior to drying greatly enhances the quality of the dehydrated product by serving to minimize staling (Christensen et al., 1951).

SUMMARY AND CONCLUSIONS

1. Cultured (sour) cream ranging in fat content from 10 to 18% was dehydrated by foam-spray drying and freeze drying.
2. The moisture content of the foam-spray dried sour cream ranged from 1.8 to 2.9% and was generally lower than the corresponding freeze-dried samples, the values for which varied from 2.2 to 2.6%.
3. Compared to the control, both foam-spray dried and freeze-dried sour cream contained less volatile acids, lower titratable acidity and lower levels of acetoin-plus-diacetyl. In general, losses of volatile constituents were lower in the freeze-dried than in the foam-spray dried sour cream.
4. An increase was recorded in the quantity of diacetyl in the dehydrated samples over the controls, possibly due to an incomplete volatilization of the additional diacetyl formed on oxidation of some of the acetoin during drying.
5. Free fat values of the freeze-dried samples were consistently higher than the amounts extracted from the corresponding foam-spray dried creams.

6. The dispersibility of both the dehydrated sour creams decreased with increasing fat content of the original cream. However, no correlation could be established from the results obtained in this study, between the free fat value and its influence on the ease of dispersion of the powders.
7. The flavor and aroma of the freeze-dried sour cream was far superior to the foam-spray dried equivalent. After storage at 40 F and 72 F for 8 weeks the freeze-dried sour cream was rated acceptable to good. The foam-spray dried samples were repeatedly described as being chalky, astringent and stale. The stale flavors became more pronounced during storage.

LITERATURE CITED

Andersen, V. B. 1959. Formation of Diacetyl and Acetylmethylcarbinol by Certain Starter Organisms. Milchwissenschaft, 15:429.

Andersen, V. B. 1959. Production of Diacetyl and Acetoin in Cultures of Aroma Producing Bacteria. Maelkeritidende, 72:985. (Dairy Sci. Abstr., 22:247. 1960.)

Anderson, A. W., and Elliker, P. R. 1953. The Nutritional Requirements of Lactic Streptococci Isolated from Starter Cultures. I. Growth in a Synthetic Medium. J. Dairy Sci., 36:161.

Anderson, A. W., Parker, R. B., and Elliker, P. R. 1955. The Nutritional Requirements of Lactic Streptococci Isolated from Starter Cultures. III. Variation in Growth Promoting Properties of Fresh Whole Milk. J. Dairy Sci., 38:1083.

Ashworth, U. S. 1955. The Influence of Processing Procedures on the Dispersing Properties of Dry Milks. Dry Milk Prod. Symp. QM Food Cont. Inst., Univ. Chicago, Sept., 1954, pp. 131. (Dairy Sci. Abstr., 18:254. 1956.)

Aule, O., and Storgards, T. 1958. Homogenization of Cream. Svenska Mejeritidn., 50:483. (Dairy Sci. Abstr., 21:14. 1959.)

Bang, F. 1943. Aerobic Metabolism of Glucose by Aroma Bacterium Streptococcus citrovorus. Svenska Mejeritidn., 35:366. (Dairy Sci. Abstr., 11:88. 1949-50.)

Begemann, P. H., and Koster, J. C. 1964. 4-Cis-Heptenal: A Cream-flavored Component of Butter. Nature, 202:552.

Bell, R. W. 1947. Effect of Stabilizers on Frozen Cream. J. Milk Food Tech., 10:149. (Dairy Sci. Abstr., 9:260. 1947-48.)

Berlin, E., Howard, N. M., and Pallansch, M. J. 1964. Specific Surface Areas of Milk Powders Produced by Different Drying Methods. J. Dairy Sci., 47:132.

Bradley, R. L. 1964. Spray Drying of Natural Cheese. Ph.D. Thesis, Michigan State University.

Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. The Williams and Wilkins Company, Baltimore.

van Beynum, J., and Pette, J. W. 1936. Versl. Landbouwk. Ond., 42:360. (Sandine, W. E., Elliker, P. R., and Hays, H. 1962. Cultural Studies on Streptococcus diacetilactis and Other Members of the Lactic Streptococci Group. Canad. J. Microbiol., 8:161.)

van Beynum, J., and Pette, J. W. 1939. The Decomposition of Citric Acid by Betacoccus cremoris. J. Dairy Res., 10:250.

Calbert, H. E., and Price, W. V. 1949. A Study of Diacetyl in Cheese. I. Diacetyl Content and Flavor of Cheddar Cheese. J. Dairy Sci., 32:515.

Christensen, L. J., Decker, C. W., and Ashworth, U. S. 1951. The Keeping Quality of Whole Milk Powder. I. The Effect of Preheat Temperature of the Milk on the Development of Rancid, Oxidized and Stale Flavors with Different Storage Conditions. J. Dairy Sci., 34:404.

Collins, E. B. 1962. Culture Identity and Selection. J. Dairy Sci., 45:1263.

Coppens, R. 1954. Quelques Observations Sur la Biochimie des Ferments d'Aroma. Neth. Milk Dairy J., 8:14. (Andersen, V. B. 1959. Formation of Diacetyl and Acetylmethylcarbinol by Certain Starter Organisms. Milchwissenschaft, 14:429.)

Czulak, J. 1953. Streptococcus diacetilactis in Starters for Cheddar Cheese. Aust. J. Appl. Sci., 3:462. (Sandine, W. E., Elliker, P. R., and Hays, H. 1962. Cultural Studies on Streptococcus diacetilactis and Other Members of the Lactic Streptococci group. Canad. J. Microbiol., 8:161.

Day, E. A. 1965. Some Properties of Carbonyl Compounds Encountered in the Flavor Isolates of Dairy Products - A Review. Food Tech., 19:1585.

Dolazalek, J. 1952. The Possibility of Diacetyl Formation in Cream, Butter and Cheese. Sborn. csl. Akad. Zemed., 25:429. (Dairy Sci. Abstr., 16:63. 1954.)

TH

Evenhuis, N., Lerk, J., and Brouwer, H. 1951. Formation of Diacetyl in Starters. Neth. Milk Dairy J., 5:110.

Faustova, V., and Vlodavets, I. 1955. Destabilization of Cream at Different Temperatures. Mol. Prom., 16:38. (Dairy Sci. Abstr., 18:167. 1956.

Federov, M. V., and Kruglova, L. A. 1955. Formation of Acetylmethylcarbinol and Diacetyl in Cultures of Lactic Acid Bacteria. Dokl. Akad. Nank., 103:161. (Dairy Sci. Abstr., 17:1035. 1955.)

Foster, E. M. 1952. The Effect of Heat on Milk as a Culture Medium for Lactic Acid Bacteria. J. Dairy Sci., 35:988.

Foster, E. M. 1962. Culture Preservation. J. Dairy Sci., 45:1290.

Gilliland, S. E., and Olson, H. C. 1963. Rates of Acid Production by Lactic Cultures in Various Fractions of Milk. J. Dairy Sci., 46:609.

Glazier, L. R., Dowd, L. R., and Manke, A. H. 1954. Cold Milk Separation of Pasteurized Milk - Its Effect on Cream. Milk Dealer, 43:65. (Dairy Sci. Abstr., 16:970. 1954.)

Glenn, W. E., and Prouty, C. C. 1955. Bacterial Studies of Cultured Buttermilk. III. Effect of Additions of Citric Acid, Sodium Citrate and Lactic Acid on the Progressive Changes in the Numbers of Leuconostoc citrovorum and Streptococcus cremoris as Associated with Acetylmethylcarbinol plus Diacetyl and pH Levels. Appl. Microbiol., 3:317.

Green, D. E., Stumpf, P. K., and Zarundaya, K. J. 1947. Diacetyl Mutase. J. Biol. Chem., 167:811.

Greene, V. W., and Jezeski, J. J. 1955. The Activity of Lactic Acid Starters in Reconstituted Nonfat Dry Milk Solids and Fluid Milks. J. Dairy Sci., 38:587.

Greene, V. W., and Jezeski, J. J. 1957. Studies on Starter Metabolism. I. The Relationship between Starter Activity and the Predrying History of Reconstituted Nonfat Dry Milk. J. Dairy Sci., 40:1046.

Guthrie, E. S. 1952. A Study of the Body of Cultured Cream. Bull. Cornell agric. Exp. Sta., 880.

Guthrie, E. S. 1963. Further Studies of the Body of Cultured Cream. Bull. Cornell agric. Exp. Sta., 986.

Hales, M. W. Cultures and Starters. 3rd ed. Chr. Hansen's Laboratory Inc., Milwaukee, Wisconsin.

Hammer, B. W. 1920. Volatile Acid Production of Streptococcus lacticus and the Organisms Associated with it in Starters. Iowa agric. Exp. Sta. Res. Bull., 63.

Harvey, R. J., and Collins, E. B. 1961. Role of Citritase in Acetoin Formation by Streptococcus diacetelactis and Leuconostoc citrovorum. J. Bact., 82:954.

Harvey, R. J., and Collins, E. B. 1963. The citritase of Streptococcus diacetilactis. Substrate, Products, and Equilibrium. J. Biol. Chem., 238:2648.

Heinemann, B. 1958. Preserving the Activity of Lactic Cultures. J. Dairy Sci., 41:705.

Hening, J. C., and Dahlberg, A. C. 1943. Effect of Pasteurization Times and Temperatures on Certain Properties and Constitutents of Creams. Tech. Bull. New York agric. Exp. Sta., 269.

Hoecker, W. H., and Hammer, B. W. 1941. Flavor Development in Salted Butter by Pure Cultures of Bacteria. Preliminary Results. Res. Bull. Iowa agric. Exp. Sta., 290: 320.

Horrall, B. E., and Elliker, P. R. 1950. The Use of Reconstituted Nonfat Dry Milk for Propagating Mother and Batch Starter Cultures. J. Dairy Sci., 33:250.

Hucker, G. J., and Pederson, C. S. 1930. Studies on the Coccaceae. XVII. The Genus Leuconostoc. New York State agric. Exp. Sta. Tech. Bull., 156.

Juni, E. 1952a. Mechanism of Formation of Acetoin by Bacteria. J. Biol. Chem., 195:715.

Juni, E., and Heym, G. A. 1956. A Cyclic Pathway for the Bacterial Dissimilation of 2,3-Butanediol, Acetylmethylcarbinol and Diacetyl. I. General Aspects of the 2,3-Butanediol Cycle. J. Bact., 71:425.

Knoop, E., and Wortmann, A. 1959. Microscopic Changes in the Structure of Frozen Cream. Milchwissenschaft, 14: 106. (Dairy Sci. Abstr., 21:270. 1959.)

Knudsen, S., and Sørensen, A. 1929. Bidragtil Syrevaek-kernes Bacteriologi. p. 64. Kgl. Veterinaer-og Landbohøjskole Aarsskrift. (Collins, E. B. 1962. Culture Identity and Selection. J. Dairy Sci., 45:1263.)

Kosikowski, F. V., and Dahlberg, A. C. 1946. A Rapid Direct-Distillation Method for Determining the Volatile Fatty Acids of Cheese. J. Dairy Sci., 29:861.

Kosikowski, F. V. 1966. Cheese and Fermented Milk Foods.

Krampitz, L. O. 1948. Synthesis of alpha-Acetolactic Acid. Arch. Biochem., 17:81.

Krenn, J., and Valik, D. 1949. Investigation of the Type and Mode of Formation of Aroma Ingredients of Butter. (English Summary.) 12th Intr. Dairy Congr., 2:516.

Krishnaswamy, M. A., and Babel, F. J. 1951. Diacetyl Production by Cultures of Lactic Acid Producing Streptococci. J. Dairy Sci., 34:274.

Lagoni, H., and Peters, K. H. 1961. Destabilization of Milk and Cream by Freezing - a Problem of Heat Conductivity. Milchwissenschaft, 16:197. (Dairy Sci. Abstr., 23:561.)

Lattuada, C. P., and Foster, E. M. 1963. Survival of Streptococcus lactis after Spray Drying. Bact. Proc., p. 16.

Lindgren, B., and Swartling, P. 1960. Maintenance of Active Lactic Cultures. Medd. Svenska Mejeriern. Riksforen, 61: (Dairy Sci. Abstr., 23:379. 1961.)

Mamaeva, P. 1955. Dry Koumiss Culture. Konevodstvo, 25:46. (Dairy Sci. Abstr., 18:639. 1956.)

Man, J. C. De. 1956. The Formation of Diacetyl in Cultures of Betacoccus cremoris. Neth. Milk Dairy J., 10:38. (Dairy Sci. Abstr., 18:589. 1956.)

Marshall, R. D. 1961. Effect of Lactose Fortification on the Body and Flavor of Cultured Buttermilk. M.S. Thesis, Michigan State University.

Marth, E. H. 1962. Certain Aspects of Starter Culture Metabolism. J. Dairy Sci., 45:1271.

Matuszewski. T., Pijanowski, E., and Supinska, T. 1936. Streptococcus diacetilactis and its Use in Buttermaking. (Eng. Summ.) Roczriki Nauk Rolniczyeh i Lesnych, 36:1. (Biol. Abstr., 11:1787. 1937.)

Maxa, V., and Teply, M. 1960. The Activity of Dried Dairy Cultures and Their Use Under Manufacturing Conditions. Prumysl Potravin, 11:589. (Dairy Sci. Abstr., 23:230. 1961.)

Mizuno, W. G. 1956. A Study of the Formation of Acetoin by Mixed Lactic Streptococci and Leuconostocs. Diss. Abstr., 16:2280. (Dairy Sci. Abstr., 19:490. 1956.)

Mizuno, W. G., and Jezeski, J. J. 1959. Studies on Starter Metabolism. IV. Effect of Various Substrates on the Formation of Acetoin by a Mixed Strain Starter Culture. J. Dairy Sci., 42:251.

Mojonnier, T., and Troy, H. C. 1922. Technical Control of Dairy Products. Mojonnier Bros. Co., Chicago.

Morichi, T., Yano, N., Irie, R., and Kembo, H. 1961. Studies on the Lyophilization of Lactic Organisms. II. Physiological Characteristics and Acid Production Activities of Lyophilized Cultures. J. agri. chem. Soc. Japan, 35:150. (Dairy Sci. Abstr., 23:380. 1961.)

Moss, C. W., and Speck, M. L. 1962. Injury and Death of Streptococcus lactis Due to Freezing and Storage. J. Dairy Sci., 45:654.

Nambudripad, V. K. N., Gonsalus, A., Laxminarayana, H., and Iya, K. K. 1957. Studies on the Nutrition Requirements of Lactic Acid Bacteria. II. Vitamin Requirements. Indian J. Dairy Sci., 10:153. (Dairy Sci. Abstr., 20: 593. 1958.)

Nawar, W. W., Lombard, S. H., Dall, H. E. T., Ganguly, A. S., and Whitney, R. McL. 1963. Fractionation of the Stale-Flavor Components of Dried Whole Milk. J. Dairy Sci., 46:671.

Nickerson, T. A., Coulter, S. T., and Jenness, R. 1952. Some Properties of Freeze Dried Milk. J. Dairy Sci., 35:77.

Olson, H. C. 1959. Preservation of Lactic Cultures. Abstr. J. Dairy Sci., 42:388.

Pette, J. W. 1949. Some Aspects of the Butter Aroma Problem. Intr. Dairy Congr., 2:572.

Prill, E. A., and Hammer, B. W. 1939. Changes in the Diacetyl and the Acetoin Contents During the Manufacture of Butter. J. Dairy Sci., 22:79.

Prill, E. A., and Hammer, B. W. 1938. A Colorimetric Method for the Microdetermination of Diacetyl. Iowa agric. Exp. Sta., J548:385.

Prouty, C. C. 1961. Amino Acid Requirements of Leuconostoc citrovorum and Leuconostoc dextranicum Isolated from Dairy Starters and Cultured Milk Products. J. Dairy Sci., 44:2100.

Reinke, E. F., and Brunner, J. R. 1959. Effects of Variations in Processing Methods on the Free Fat and Dispersibility of Whole Milk Powder. J. Dairy Sci., 42: 911.

Richardson, G. H. 1960. Studies Involving the Preservation of a Lactic Culture. Ph.D. Thesis, University of Wisconsin. (Foster, E. M. 1962. Culture Preservation. J. Dairy Sci., 45:1290.)

Roberts, W. M., Blanton, L. F., and Marley, G. P. 1953. Cold Milk Separation and the Effects of Pasteurization, Separating Procedure and Product Used for Standardization on Properties of Cream. Milk Pl. Mon., 42:19. (Dairy Sci. Abstr., 15:1000. 1953.)

Rudnik, A. W. Jr., and Glenn, W. E. 1960. The Use of Frozen Culture as a Direct Inoculum for Making Cottage Cheese. Abstr. J. Dairy Sci., 43:845.

Sandine, W. E., Seitz, E. W., Elliker, P. R., and Day, E. A. 1961. Studies on the Mechanism of Diacetyl Synthesis by Streptococcus diacetilactis. J. Dairy Sci., 44:1158.

Sandine, W. E., Elliker, P. R., and Hays, H. 1962. Cultural Studies on Streptococcus diacetilactis and Other Members of the Lactic Streptococci Group. Canad. J. Microbiol., 8:161.

Sapp, C. W., and Hedrick, T. I. 1960. Factors Affecting the Activity of Spray Dried Cheese Culture. Quart. Bull. Mich. agric. Exp. Sta., 43:96.

Sasaki, R., Tsugo, T., Imamura, T., and Murai, M. 1953. Studies on the Production of Diacetyl by Lactic Acid Bacteria. Intr. Dairy Congr., 4:611. (Dairy Sci. Abstr., 16:489. 1954.)

Savage, K. G., and Brown, R. W. 1953. The Viscosity of Cream. Canad. Dairy Ice Cr. J., 32:46, 54 & 56. (Dairy Sci. Abstr., 16:190. 1954.)

Savinovsky, N. 1948. Prolonged Storage of Sour Cream at Various Temperatures. Mol Prom., 9:15. (Dairy Sci. Abstr., 11:119. 1949-50.)

Seitz, E. W., Sandine, W. E., Elliker, P. R., and Day, E. A. 1963. Studies on the Diacetyl Biosynthesis by Streptococcus diacetilactis. Canad. J. Microbiol., 9:431.

Sherman, J. M. 1955. Streptococcus lactis and the Streptococci of the So Called Lactic Group. J. Dairy Sci., 38:1184.

Simmons, J. C., and Graham, D. M. 1959. Maintenance of Active Lactic Cultures by Freezing as an Alternative to Daily Transfer. J. Dairy Sci., 42:363. (Dairy Sci. Abstr., 21:263. 1959.)

Slade, H. D., and Werkman, C. H. 1941. Anaerobic Dissimilation of Citric Acid by Cell Suspensions of Streptococcus paracitrovorus. J. Bact., 41:675.

Speck, M. L. 1964. Starter Culture Growth and Action in Milk. J. Dairy Sci., 45:1281.

Stahley, G. L., Michaelian, M. B., Werkman, C. H., and Hammer, B. W. 1935. The Disappearance of Acetylmethylcarbinol and Diacetyl in Butter Cultures. J. Dairy Sci., 18:473.

Stone, W. F., Conley, T. F., and McIntire, J. M. 1954. The Influence of Lipids on Self Dispersion and on Ease of Dispersion of Milk Powder. Food Tech., 8:367.

Storgards, T. 1940. The Formation of Aroma Substances of Butter. Meijeritiet Aikakausk, 2:22. (Dairy Sci. Abstr., 9:204. 1947-48.)

Storgards. T. 1941. Development of Aroma in Starters. Milchw. Zbl., 70:157. (Dairy Sci. Abstr., 3:197. 1941-42.)

Strecker, H. J., and Harary, I. 1954. Bacterial Butylene Glycol Dehydrogenase and Diacetyl Reductase. J. Biol. Chem., 211:263.

Swartling, P. F. 1951. Biochemical and Serological Properties of Some Citric Acid Fermenting Streptococci from Milk and Dairy Products. J. Dairy Res., 18:256.

Swartling, P., and Lindgren, B. 1960. Must Starters be Subcultured Every Day? Svenska Mejeritidn., 52:631. (Dairy Sci. Abstr., 23:230. 1961.)

Tamsma, A., Edmondson, L. F., and Vetter, H. E. 1958. Free Fat in Foam Dried Whole Milk. J. Dairy Sci., 41:710.

Tamsma, A., Berlin, E., and Pallansch, M. J. 1963. Contribution of the Fat Phase to the Development of Stale Flavor in Whole Milk Powder. J. Dairy Sci., 46:614.

Tarassuk, N. P., and Jack, E. L. 1946. Biochemical Changes in Whole Milk and Ice Cream Mix Powders During Storage. J. Dairy Sci., 29:486. Abstr.

Taufel, K., and Krusen, F. 1952. The Breakdown of Citric Acid by Lactic Acid Bacteria. Biochem. Z., 322:371. (Dairy Sci. Abstr., 15:397. 1953.)

Taufel, K., and Behnke, U. 1960. Formation of Acetoin and Diacetyl by Starter Cultures. (Eng. Summ.) Nahrung, 4:197.

Thomas, R. H., Holgren, C. J., Jokay, L., and Bloch, I. 1957. A Rapid Method for Determining Free Fat in Dry Milks. J. Dairy Sci., 40:605.

Virtanen, A. I., Kontio, P., and Storgards, T. 1941. The Development of Aroma in the Ripening of Cream. Biochem. Z., 307:215. (Dairy Sci. Abstr., 3:117. 1941-42.)

Watts, J. C. 1955. A Study of the Factors Influencing the Activity of a Lyophilized Lactic Starter. Ph.D. Thesis, University of Wisconsin. (Foster, E. M. 1962. Culture Preservation. J. Dairy Sci., 45:1290.)

Wong, N. P., and Patton, S. 1962. Identification of Some Volatile Compounds Related to the Flavor of Milk and Cream. J. Dairy Sci., 45:724.

Yano, N., and Ozawa, Y. 1956. Studies on Milk Culture. I. Effects of Citric Acid and Various Factors on the Aroma Production of Milk Culture. J. agric. chem. Soc. Japan, 30:15. (Dairy Sci. Abstr., 20:676. 1958.)

Yano, N., Morichi, T., Irie, R., and Kembo, H. 1960. Influence of Solids Not Fat on the Growth of Lactic Acid Bacteria. Jap. J. Zootech Sci., 31:204. (Dairy Sci. Abstr., 23:226. 1961.)