# A BACTERIOLOGICAL AND CHEMICAL STUDY OF ICE MILK

by

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# INTRODUCTION

Considerable work, in the past, has been done to determine the sanitary quality of ice cream and other products of dairy origin. Legislation has also prompted many investigations to determine the physical qualities and chemical composition of these products. Workers have carried these investigations to all phases of "ice cream" production, including homemade ice cream, seasonal differences in sanitation, the effect of different flavors, and the composition and sanitation of other types of products similar to ice cream.

As a result of these many investigations, legislation has been drawn up by state and local governments to govern the ice cream industry.

In January, 1942, the Federal Food and Drug Administration started hearings on standards for ice cream, but these hearings were discontinued after 12 weeks because of the war. These hearings were again resumed in November, 1950 with an added proposal, by the International Association of Ice Cream Manufacturers, to define and set up a regulatory standard of identity for "ice milk". This product had increased greatly in popularity with the public, since the war, and as a result was of great concern to the ice cream manufacturers. This product is a high milk solids, low fat, and low overrun product usually sold direct from the freezer and sometimes called "soft ice cream" by the consumer.

The state of Kansas, according to section 65-720 of the General

Statutes of Kansas 1949 (17), defines and regulates the sale of ice milk as follows:

> Ice milk means and includes a frozen product or semi-frozen product made in semblance of ice cream, but containing less than ten percent (10%) milk fat. Ice milk shall not be sold in packages, cans or wrappers, unless the containers are plainly labeled in legible eight point type, with the words, "Ice Milk". Ice milk shall not be sold for immediate consumption in business establishments, unless there is posted in a conspicuous place on the premises a card showing in two-inch type the following: "Ice Milk Is Sold Here", or unless such wording appears prominently on the menu with type no smaller than the largest type appearing thereon.

It was on the basis of this definition that the samples were chosen for analysis and with the government hearing in progress it seemed timely to investigate the product, "ice milk", to determine its sanitary qualities and uniformity of composition, results of such investigations being entirely absent in the literature, on this particular dairy product.

## REVIEW OF LITERATURE

Ice cream has probably received more attention from workers in the field of sanitation and quality control than any other manufactured dairy product except market milk. The tremendous growth from a by-product, to use excess milk, to a major industry itself, its occasioned association with outbreaks of traceable disease affecting large numbers of people, and, in the later years, the arrival in the ice cream field of the counter freezer have, no doubt, been responsible for these investigations.

As early as 1906 and 1907 Stiles and Pennington (27) tested 263 samples of ice cream from dealers in the city of Washington, D. C. The bacterial examination of these samples showed a total plate count ranging from a minimum of 137,500 to a maximum count of 365,000,000 bacteria per milliliter. Of the 263 samples only 19 or about 7 percent showed counts of less than 1,000,000 bacteria per milliliter.

Hammer (19) tested samples of ice cream from Des Moines, Iowa, and the Iowa State College creamery. The 10 Des Moines samples averaged 19,920,000 bacteria per milliliter with a maximum count of 39,000,000 and a minimum of 4,200,000 bacteria per milliliter of sample. The 12 samples of college ice cream averaged 19,775,000 bacteria per milliliter with a maximum of 72,000,000 and a minimum of 500,000 bacteria per milliliter.

Ayers and Johnson (6) made a comparison of 94 samples of summer made ice cream with 91 samples of winter made ice cream. Of the 94 summer made samples 19 percent were found to contain less than 1,000,000 bacteria per milliliter with 41 percent of the winter made samples falling in the range of 0 to 1,000,000 bacteria per milliliter. The acidity of 65 of the samples showed a maximum of 0.387 percent, a minimum of 0.09 percent, and an average of 0.206 percent acid calculated as lactic acid. The acidity of the samples tested, however, did not bear any relationship to the bacterial counts. The sample showing the maximum acidity of 0.387 percent had but 217,000 bacteria per milliliter in contrast

to the sample with but 0.09 percent acid and 49,000,000 bacteria per milliliter.

During a three year period from 1920 through 1923, 115 samples of ice cream from Kansas dealers were tested by Fay (12). These samples were found to range from 1,500 to 47,000,000 bacteria per milliliter with an average count of 1,895,000 bacteria per milliliter. The overall bacterial counts were lower than the results obtained by other workers in other areas. One-half of the samples showed total counts of less than 100,000 bacteria per milliliter and three-fourth of the samples had total counts of less than 300,000 bacteria per milliliter.

Fabian (10) collected over 1,110 samples of ice cream, in a nine year period prior to 1926. These samples were collected from 36 plants in 5 cities in Michigan. The ice cream was of various flavors and from plants that varied in capacity from a few gallons per week to 30,000 gallons of ice cream per day. The bacterial count of the samples ranged from a minimum of 1,000 to a maximum of 300,000,000 bacteria per milliliter, with 63 percent of the samples having a bacterial count of 50,000 or less per milliliter of sample.

Grumbine and Halliday (18) tested several retail samples of ice cream in Chicago for bacteria and the chemical constituents of the product. Tests were made for sugar (sucrose), fat, total solids, serum solids, overrun, and fat value in cents in terms of pint of product. It was found that 39 percent appeared to be the amount of total solids preferred by the customers. An overrun of

76 to 92 percent also was preferred. The sugar content was found to be a matter of individual preference. These workers gave more value to the economic significance of the constituents than to the sanitary quality of the product as indicated by the bacterial count. Included in this study were several hand packed samples of ice cream which would influence the amount of overrun obtained by these workers.

For six years prior to 1939 Dodge (8) had conducted a survey of ice creams in the state of Kansas. Samples were tested for bacterial count, percent butterfat, percent total solids, weight per gallon, source of mix, pasteurization efficiency, and sanitary condition of plant, and were judged and scored on flavor, texture, and packaging. The survey conducted in 1939 included a larger percentage of samples from counter freezers than the surveys conducted prior to 1939. It was shown that there had been a steady improvement in quality, as indicated by lower bacterial counts, each year. It was found that 76.7 percent of the counter freezer ice cream manufacturers and 72.4 percent of the wholesale manufacturers made a product with less than the state standard of 100,000 bacteria per milliliter. Approximately 20 percent more samples were within the state requirements than in the survey made in 1938.

Over 300 samples of Kansas ice cream manufactured during July, 1938 were tested by Martin, Nelson, and Caulfield (21) for total bacteria count, coliform count, phosphatase test, butterfat, and weight per gallon, and were scored on flavor, body, texture, color, and packaging. Total bacterial counts of 100,000 or less per

milliliter were obtained on 59.8 percent of all samples. These workers agree with Dodge (8) that a slightly larger percentage of the counter freezer samples were in the lower bacterial count ranges. A tendency was noted for the total bacteria count to increase as the Escherichia-Aerobacter organisms increased. Of the 313 samples tested 17 showed a positive phosphatase test, indicating the possibility of underpasteurization or the addition of some unpasteurized dairy products. Despite certain relationships between results obtained by the use of different determinations, the authors agree that there were enough instances in which the relationships did not hold to show that it is necessary to use a wide variety of tests and determinations to ascertain the true quality of a sample of ice cream.

Crowe and Downs (7) tested samples of ice cream, purchased from consumer outlets in Nebraska, for total bacterial counts, coliform counts, butterfat, total solids, and calculated overrun. The results of this work were similar to those obtained by Grumbine and Halliday (18). Among the samples were included several of hand packed ice cream. The weights of the ice cream averaged 298.0 grams per pint, with a maximum of 465.0 grams and a minimum of 212.0 grams per pint. The percent of calculated overrun averaged 80.0 percent with a maximum of 141.0 percent and a minimum of 9.8 percent. The work of Grumbine and Halliday (18) in Chicago showed a range in weight from 248.8 to 480.2 grams per pint and overrun ranging from 14.1 to 143.0 percent. On the Nebraska ice cream the total solids ranged from 37.21 percent to 40.19 percent with a

butterfat content of 12.16 percent to 16.97 percent. The state law in Nebraska at that time required a butterfat content of at least 14.0 percent for ice cream. The bacterial counts ranged from 18 samples with less than 10,000 bacteria per milliliter to 6 samples with more than 50,000 bacteria per milliliter. The coliform counts ranged from 0 to 3450 per milliliter.

A study of homemade ice cream was made by Foltz and Martin (15) during a period from September, 1938, to June, 1939. One-hundred samples of homemade ice cream were collected from 40 housewives in Manhattan, Kansas. An analysis of these samples revealed a butterfat content from 1.4 to 35.4 percent, and total solids ranging from 17.84 to 34.07 percent. The average logarithmic count of 171,000 bacteria per milliliter was no doubt affected by the ingredients used and the method of freezing employed. The samples made from pasteurized milk and cream and frozen in the refrigerator had a logarithmic average bacterial count of 17,000 per milliliter which compares vary favorably with the best factory made ice cream.

A comparison of bacterial counts on 279 samples of commercial ice cream in Kansas was made by Foltz and Martin (14) using tryptone-glucose-skimmilk agar and standard nutrient agar. These tests were made using temperatures of 32° C. and 37° C. It was found that 160 samples or 67 percent of the 279 samples were below the 100,000 bacteria per milliliter limit of the state of Kansas. The use of tryptone agar gave higher average counts of bacteria at both 32° C. and 37° C. than standard nutrient agar.

Tryptone agar gave higher bacterial counts at an incubation temperature of 32° C. than at an incubation temperature of 37° C. or than standard agar at either 32° C. or 37° C.

Fifty-five samples of ice cream in Maine were tested by Tobey (30) for butterfat and bacterial content. Twelve of the samples were found to be below the 10 percent butterfat standard adopted by Maine in 1943. Thirty-four of the 55 samples were found to be below 50,000 bacteria per milliliter. This figure was used arbitrarily by the author because the state of Maine did not have any statutory limitations in regard to the bacterial content of ice cream.

According to Abele (1) the control of safety and sanitary quality of the products of frozen desserts manufacturers is a procedure in the interest of public health and is of benefit to the entire industry. Regulations have existed on a local and state basis for some time and in 1929 "Sanitary Regulations for Ice Cream" were prepared by a joint committee of the International Association of Ice Cream Manufacturers and the International Association of Dairy and Milk Inspectors. A frozen desserts ordinance was released by the Public Health Service in 1935 but few, if any cities adopted this ordinance. This ordinance was known as the "Proposed Ice Cream Ordinance" and included definitions of such products as ice cream, frozen custard, sherbet, ices, and imitation frozen desserts. This ordinance was studied in 1937, 1938, and 1939 and in late 1939 an almost completely changed ordinance was issued by the United States Public Health Service

Sanitation Advisory Board (16). This revised ordinance provided for the grading of the plant and not the product.

The "Ice Cream Review" (2) in 1936 supervised a poll of various workers in the dairy industry, to find the opinion as to the need of standards for the manufacture of frozen products other than ice cream, sherbets, and ices. Thirty-three of the voters voted "yes" to the question while only eight were opposed to such a provision.

According to Fistere (13), "American Butter and Cheese Review" (5), and "Ice Cream Review" (4) the forthcoming standards on ice cream will include also definitions and standards for products other than ice cream. The Federal Food and Drug Administration started hearings on, "ice cream", standards January, 1942, and continued for 12 weeks but the hearing, after compiling a record of 6,000 pages of testimony, was discontinued because of the war. On November 13, 1950 the hearing was resumed with testimony directed at (a) text of the original proposal, (b) proposed "Findings of Fact", prepared by the Food and Drug Administration, based upon the first hearing record, and (c) a proposed regulation on definitions and standards prepared by the Food and Drug Administration. The International Association of Ice Cream Manufacturers at this latest hearing are proposing a definition and standard of identity to be established for "ice milk", by saying that consumers do not readily distinguish between "ice milk" and "ice cream", and that "ice milk" is easily passed off for "ice cream".

State-wide standards for ice milk are discussed for

California by Turnbow (31) and for Oregon by "Ice Cream Review" (3). The California standard allows a maximum of 4 percent butterfat and not more than 150,000 bacteria per gram of sample. The Oregon standards allow a maximum of 12 percent but not less than 3.2 percent butterfat. A maximum bacterial count was not given but a labeling and advertising clause prohibiting the use of words such as "ice cream", "cream", or "creamy" in the sale or distribution of ice milk. The weight of ice milk in Oregon can not be less than 4.5 pounds per gallon of finished product.

According to Fabian et al. (11), and Palmer et al. (24) the counter freezer first appeared in great numbers about 1929 and has increased in numbers until today there are a great many in operation in the United States. This increase in manufacturing units within a given area greatly increases the inspection load. The gross quality and intelligence of the workers, access to plenty of hot water and/or steam, adequate refrigeration facilities, and good location and condition of the manufacturing unit are conditions not always present in counter freezer operations and add to the load on the already inadequate inspection and enforcement agencies. It is apparent that whether it is a counter freezer or an ice cream plant it should be required to meet the same requirements, regardless of the size of the unit.

The first direct-from-the freezer products according to Swenson (28) were high butterfat ice creams. Retailers soon learned that a low butterfat mix produced a better soft ice cream and that the customers preferred it over a high-fat, freezer-fresh

product. As this product grew in popularity the term "soft ice cream" became established in the minds of the public regardless of the butterfat content. These products which today are sold under such trade names as Dairy Treet, Zesto, Dairy Queen, Sweeden Freeze, and others are mostly of the low fat content variety of product terms "ice milk" by authorities in the dairy industry. Swenson also reports that soft ice cream sales, nationally, have a volume equal to 15 percent of all the hard ice cream reported as being manufactured. This volume of sales has had a very pronounced effect on the ice cream industry, especially in the field of bulk ice cream.

Since the end of World War II and the return of "joy-riding" to the American public, drive-in stores selling a low fat soft ice cream product have increased in numbers until today there is hardly a town of any size that does not have at least one of these units in operation. This product is very popular with the public and as a result the number of these units increases every year.

With the standards hearing reopening November 13, 1950, with a definite objective of defining and setting up standards for such a product, the question arises as to just how good are these products both from the standpoint of sanitation and of nutrition. It was with these thoughts in mind that the following investigation was carried out during the fall of 1950 on several samples of soft ice cream or ice milk purchased from retail outlets in various cities in Kansas.

# EXPERIMENTAL PROCEDURES

## Collection of Samples

Fifty-six samples of ice milk were collected for this experiment during September, October, and November, 1950. These samples were purchased from retail outlets in 18 Kansas towns by Professor W. H. Martin and John P. Mellott of the department of Dairy Husbandry and Professor V. D. Foltz and Richard W. Ripper of the department of Bacteriology at Kansas State College, Manhattan, Kansas.

Samples were collected in the following Kansas towns: Abilene, Arkansas City, Augusta, El Dorado, Emporia, Herington, Hutchinson, Junction City, Kansas City, Lawrence, Manhattan, McPherson, Newton, Salina, Topeka, Wellington, Wichita, and Winfield. These samples were sold under the trade names of Dairy Custard, Dairy Delight, Dairy Freeze, Dairy Queen, Dari-Ann, Frigid Queen, Frosty Creme, Frozen Delight, Jersey Cow, Keen Kreme, Melo-Freeze, Newton Dairy Bar, and Zesto.

The samples were purchased in pint or quart containers at the retail price and immediately marked with an identification number. After marking, the samples were placed in an insulated ice cream packer containing dry ice and maintained in a frozen condition until samples were taken for the various tests. Identification cards, numbered to correspond with the sample reference number, were kept, containing data concerning the date, reference number,

name of product, flavor of product, price, name of store, address of store, collector, and any remarks that seemed pertinent concerning the collection of the sample. As soon as practical the samples were returned to Manhattan and immediately placed in the ice cream hardening room, at the College Creamery, at a temperature of about -17.8° C. (0° F.).

## Sampling

The samples were removed from the hardening room and while tempering enough to take a representative sample the gross weights, in grams, were obtained by weighing on a laboratory balance scale. After tempering, the lid was removed from the container and the top one inch of the product was removed with a sterile wooden tongue depressor and discarded. A representative sample was taken for bacterial analysis and placed in a sterile 2 ounce, wide mouth, sample jar. In order to avoid the possibility of any container contamination care was taken not to include any material from closer than one inch from the edge of the container. This bacteriological sample was immediately stored in a deep freezer at -17.8° C. (0° F. ) until the bacterial analysis was made. Samples were also taken at this time for the analytical tests. These samples were stored in a refrigerator at a temperature of 4.4 to 10.0° C. (40 - 50° F.) to prevent excess bacterial growth and subsequent souring. The remainder of the pint or quart sample was returned to the hardening room for storage in

case future reference or other samples were needed. At a later period all containers were emptied, washed, dried, and weighed to obtain the net weights.

## Bacteriological Analysis

All bacterial counting procedures used were in accordance with Standard Methods (26) unless otherwise noted. A total viable bacterial count, a presumptive count for the coli-aerogenes (coliform) group using violet-red-bile agar, and a total microscopic count was made on each sample. The bacterial counts were made as soon after taking the sample as was practicable with all counts being made within one week of the date of purchase. According to Hammer (20) this storage at -17.8° C. (0° F.) should cause little if any change in bacterial counts.

The two-ounce sample jars were removed from the deep freezer and tempered in a water bath at 37° C. (98.6° F.) until the contents were thoroughly melted and then removed within 10 minutes.

Dilutions were made by using volumetric measurements as outlined in section 13.18 of Standard Methods (26). Eleven milliliters of the melted sample was pipetted into a sterile 99 milliliter water blank to give a 1-10 dilution. One milliliter of the 1-10 dilution was transferred to a second sterile 99 milliliter water blank giving a dilution of 1-1,000. From these dilution blanks and the original melted sample, by using sterile 1.1 milliliter dairy pipettes, dilutions of 1-100, 1-1,000, and 1-10,000

were made for the total plate count and dilutions of 1-1, 1-10, and 1-100 were made for the coliform count. These diluted test portions were pipetted into sterile 100 x 15 millimeter petri dishes marked with the sample number and respective dilutions. Ten to twelve milliliters of liquified agar, cooled to 42-44° C. (107.6 - 111.2° F.) was poured into the petri dishes and thoroughly mixed with the sample by rotating slowly.

Tryptone-glucose-beef extract skim-milk agar was used for the total plate counts. This agar consisted of:

| | |
|---|---|
| Agar | 15 grams |
| Beef extract (Difco) | 3 grams |
| Bacto-Tryptone (Difco) | 5 grams |
| Glucose | 1 gram |
| Distilled water | 1,000 milliliters |

The ingredients were dissolved by heating and after adjusting the reaction to a pH 7.0, bottled in 100 milliliters portions in 6-ounce prescription bottles. This medium was then sterilized by autoclaving at 15 pounds steam pressure, 121° C. (250° F.), for 20 minutes. One percent sterile skim milk was added aseptically to the cooled media just before pouring the plates. Enough media was made at one time to check all of the samples, to insure uniform composition and results.

Bacto violet-red-bile agar (Difco dehydrated) was used for the presumptive coliform count. This media was made by suspending 41.5 grams of medium in 1,000 milliliters of cold, distilled water and dissolved by heating to boiling. This media was bottled in 100 milliliter portions in 6-ounce prescription bottles and sterilized by autoclaving at 15 pounds steam pressure, 121° C. (250° F.), for 15 minutes.

The bottles of agar were capped tightly to prevent evaporation and checked before use for evidence of contamination or faulty sterilization. The agar was melted before using, in a steamer, and then cooled to 42-44° C. (107.6 - 111.2° F.). A thin layer of violet-red-bile agar was poured on the surface of the violet-red-bile agar plates after the original agar had solidified. This was done to eliminate the occurrence of surface colonies causing all colonies of coliform bacteria to be typical dark-red subsurface colonies.

After solidifying, the plates were inverted and incubated at 37° C. (98.6° F.). Counts were made on the coliform plates after incubating 20-24 hours. Typical dark-red colonies at least 0.5 millimeter in diameter were counted, using a Quebec Colony Counter and a hand talley. Dilutions were chosen to count where the number of colonies ranged between 30 and 300. If less than 30 colonies were on the lowest dilution a count was made and reported, the same as with higher dilutions, by multiplying the count times the dilution and reporting the result as the coliform count per milliliter. After 45-51 hours incubation the tryptone-glucose-beef extract skim-milk agar plates were removed from the incubator and counts made on plates showing from 30 to 300 colonies. The count multiplied by the dilution was reported as the total plate count per milliliter of sample.

A direct microscopic count was made using the method described by Standard Methods (26). Slight deviations, however, were made

in certain techniques for convenience and to obtain better results on the product being examined.

The sample was melted and diluted 1-1 in sterile distilled water because difficulty was encountered by the high percentage of solids-not-fat of the product when the slide was stained. New 1 x 3 inch microscope slides were cleaned and 0.01 milliliter of the diluted sample, delivered by a 0.01 milliliter calibrated capillary pipette, was spread over an area of 1 square centimeter as determined by the use of a special guide plate under the slide. The slide was marked for identification and dried on the top of a microscope lamp at approximately 40-45° C. (104-113° F.) for five minutes. The slide was fixed and defatted simultaneously by immersing in a mixture of 40 percent tetrachlorethane, 54 percent of 95 percent ethyl alcohol, and 6 percent glacial acetic acid for two minutes. The slide was then air dried overnight before staining.

The slides were stained by using a differential stain as given by Elliker (9). This stain is the Barber modification of the Newman-Lampert stain and consists of the solvent used for the defatting-fixing process with 0.08 gram basic fuchsin and 1.1 grams methylene blue added per 100 cubic centimeters of solvent. By this procedure the bacteria and leucocytes stain "blue" and the background "pink". The slides were stained for 1 minute and allowed to air dry then washed gently in water to remove the excess stain.

A binocular microscope with a field diameter of 0.160 millimeter with 10.0x oculars and a 1.8 millimeter oil immersion objective was used to make the counts. Fifty microscopic fields

were counted on each sample, a record of the number of fields and the bacterial cell count being kept by the use of two hand tallies. The average count was obtained by dividing the total number of individual cells counted by the number of fields counted. The individual microscopic count per milliliter was obtained by multiplying the average count by the microscopic factor of 500,000. This value was multiplied by 2 to compensate for the 1-1 dilution of the original material. The microscopic factor was obtained by using the formula:

$$M.F. = \frac{10,000}{3.1416 \times r^2}$$

This formula combines conversion of the field area into square centimeters, determination of the number of fields per square centimeter of sample area (0.01 milliliter on an area of 1 square centimeter) and the volume of sample, in milliliters, per field.

After the examinations were made the immersion oil was removed, by dipping the slide into xylol and then allowing the slide to dry in a flat position, before storing the slide for future reference.

## Butterfat Determinations

Butterfat determinations were made by the use of a Model "A" Mojonnier Milk Tester following the method presented by Mojonnier and Troy (22), and by using the Minnesota modification of the Babcock milk fat test as outlined by Standard Methods (26).

The Mojonnier method of fat determination is a modification of the Roese-Gottlieb ether extraction process. This process is a gravimetric quantitative determination of fat by extraction with ether. All weighings were made to the fourth decimal place on a chainomatic laboratory analytical balance. The weighing containers used were cleaned and dried then placed in a vacuum oven to evaporate any moisture. The containers were then cooled in a desiccator before using.

Approximately 5 grams of melted sample was weighed and put into a fat extraction flask. To this sample was added 5 milliliters of distilled water and mixed thoroughly to dilute the sample to approximately 10 milliliters. One and one-half milliliters of commercial, chemically pure ammonia containing about 29.40 percent ammonia gas ($NH_3$) was added and mixed thoroughly to dissolve the casein not in true solution and to neutralize the acidity of the product. This permits the solvent which is later added to more readily dissolve the fat. Ten milliliters of 95 percent, 190° proof, ethyl alcohol was added and the flask shaken for 30 seconds to mix. The alcohol enables the solvent to come in contact with the fat globules, both the aqueous portion and the ether to be added later being mutually soluble in alcohol. Twenty-five milliliters of the best commercial quality ethyl ether was added to the sample and the flask shaken for 20 seconds to dissolve the fat and hold it in solution. Ethyl ether also dissolves small amounts of milk sugar and other solids not fat. Twenty-five milliliters of the best commercial quality petroleum ether with a

boiling point of not over 49-60° C. (120-140° F. ) was added to the sample to throw out, from the ethyl ether-fat solution, the last traces of water and any solids-not-fat that may be dissolved in the solution.

The flask was centrifuged 30 turns in the centrifuge contained in the testing machine. The ether layer was decanted into a previously weighed fat evaporating dish. A second extraction was made following the same procedure as was used for the first extraction except that the water and ammonia were omitted and only 5 milliliters instead of 10 milliliters of alcohol were added. The second extraction was centrifuged 30 turns and the ether layer decanted into the same evaporating dish as the first extraction. The ether was evaporated on a hot plate, after each extraction, at 135° C. (275° F.) and after the second evaporation the dish was placed in a vacuum oven at 135° C. (275° F.) under 22 inches of mercury vacuum for 5 minutes. The dish was cooled for 7 minutes in a cooling desiccator and then weighed. The percentage fat was calculated by the following formula:

$$\text{Percent fat} = \frac{\text{weight of fat extracted}}{\text{weight of sample before extraction}} \times 100$$

The Minnesota modification of the Babcock milk fat test was run on each sample using twenty-percent calibrated, 9-gram, 6-inch, ice cream test bottles and number 735 Minnesota Babcock Reagent manufactured exclusively by the Kimble Glass Company of Toledo, Ohio.

The test was performed by weighing 9 grams of the sample into a 20 percent ice cream test bottle using a cream-weighing scales. Fifteen milliliters of Minnesota reagent was added and the bottle shaken thoroughly and then incubated in a boiling water bath for 12-15 minutes with the test bottle held at least 2.5 inches above the bottom of the water bath. The test bottle was shaken after about 2.5 minutes and then again about 1 minute later, care being taken not to allow the isopropyl alcohol, in the reagent, to boil off through the neck of the bottle. The test bottles were centrifuged for 0.5 minute at the proper speed for the machine used and then water was added at a temperature of 55-65° C. (130-150° F.) to float the milk fat up into the neck of the test bottle. The test bottles were then centrifuged again for 0.5 minute. The test bottles were then incubated in a 55-60° C. (130-140° F.) water bath for 4-5 minutes. A few drops of glymol (white mineral oil with oil-soluble artificial color added) were allowed to flow down the neck of the test bottle onto the surface of the fat column. The tests were read immediately by measuring from the bottom of the lower meniscus to the sharp line of demarcation between the glymol and the fat, by applying divider points to the smooth side of the bottle neck while the bottle was held in a vertical position. The measurement was then transferred to the graduated side of the bottle neck and the percentage of fat read directly. Averages of duplicate samples were reported.

## Total Solids and Solids-not-fat Determinations

The total solids determination was made following the procedure outlined by Mojonnier and Troy (22) using the Model "A" Mojonnier testing machine. Clean solids dishes were heated in a vacuum oven at 100° C. (212° F.) for 10 minutes under at least 20 inches of mercury vacuum. The dishes were transferred to a cooling desiccator for 5 minutes before weighing. The empty dishes were weighed with a lid and then weighed after adding about 1 gram of sample. About 1 milliliter of solid-free distilled water was added to the sample in the dish as a diluent. The dish was placed on a hot plate at 180° C. (355° F.), to dry the material in a porous layer, until the first trace of brown began to appear. The dish was transferred to the vacuum oven and heated for 10 minutes at 100° C. (212° F.) under a vacuum of at least 20 inches mercury, then to the cooling desiccator for 5 minutes, after which it was weighed. Care must be taken to maintain proper temperatures and time to prevent the breaking down of lactose-monohydrate to lactose and giving erratic results. The final weighing represents the total solids and the percent total solids was determined by use of the formula:

$$\text{Percent total solids} = \frac{\text{final weight after treatment}}{\text{weight of sample before treatment}} \times 100$$

The percent solids-not-fat was determined by subtracting the percentage of fat from the percentage of total solids.

## Sediment Tests

A 100 gram sample was placed in a 400 milliliter beaker and diluted with 100 milliliters of distilled water at a temperature of not less than 82° C. (180° F.). The sample was thoroughly mixed and passed through a standard lintine sediment disk (1.25 inches diameter), using a hand sediment tester. After drying, the disks were scored and classified according to the standards of the American Public Health Association as outlined in Standard Methods (26).

## Acidity Titration

The acidity titration was run on the filtrate from the sediment test. Eighteen milliliters of the filtrate were titrated with N/10 sodium hydroxide (NaOH) from a burrette using 4-6 drops of 1 percent alcoholic solution of phenolphthalein as an indicator. The sodium hydroxide solution was added slowly, with constant stirring, until the first definite and relatively permanent shade of pink was observed. The percent acid, expressed as lactic acid, was determined by use of the formula:

$$\text{Percent acid as lactic acid} = \frac{\text{milliliters N/10 sodium hydroxide} \times 0.009}{\text{weight of sample}} \times 100$$

The weight of the sample used was 9 grams because the 18 milliliters of filtrate, used in the sediment test, were diluted

one-half. The value 0.009 represents the weight of lactic acid, in grams, which will react with 1 milliliter of N/10 sodium hydroxide.

## Phosphatase Test

The Scharer or New York method of determining the pasteurization efficiency or for contamination by unpasteurized products, was used on each sample. This test is based upon the presence of enzyme phosphatase which is always found in raw milk and which is destroyed almost completely or inactivated by efficient pasteurization. The enzyme phosphatase even in small quantities is easily detected by a chemical test. The principle of the test is that when di-sodium phenyl phosphate (buffer substrate solution) is added to phosphatase and incubated, free phenol is liberated. Free phenol plus a suitable colorimetric reagent will give a blue color, the intensity of which depends upon the amount of free phenol present, which, in turn, is dependent upon the amount of phosphatase present in the original sample.

A Phax Kit, Model C, portable kit and incubator manufactured by Applied Research Institute, New York, was used for the test. Reagents were prepared fresh for each test by dissolving 1 Indo-Phax tablet in 5 milliliters of 95 percent ethyl alcohol to make the B. Q. C. (2,6 dibromoquinonechloromide) or colorimetric reagent and by dissolving 1 Phos-Phax tablet in 50 milliliters of distilled water to make the buffered substrate solution. Special

graduated test tubes with rubber stoppers, included in the kit, were used for the test.

The graduated test tubes were filled with the buffered substrate solution until the top of the meniscus reached the 5 milliliter graduation. Using a clean medicine dropper the sample was added until the top of the meniscus reached the 5.5 milliliter graduation. A clean rubber stopper was inserted into the tube and the tube shaken thoroughly. The tubes were incubated in the special incubator, containing water at 36-44° C. (97-111° F.), for 10 minutes. After incubation, 6 drops of the B. Q. C. solution were added to each tube and the tubes shaken thoroughly. The tubes were allowed to stand for 15 minutes before 2 milliliters of neutral N-butyl alcohol were added. Indophenol was extracted by carefully inverting the tubes ten times allowing the alcohol and bubbles to separate after each 180° arc. Rapid inversion will result in an emulsion making an accurate reading impossible. The blue color in the alcoholic layer was compared with prepared color standards included in the kit. Controls were run on a boiled sample, to check for free phenol in the sample, and a boiled sample with 2 percent raw milk added, to check the reagents. Results were recorded according to the unit number on the color standard and reported as N. Y. C. (New York City) units.

## Overrun Calculation

The amount of overrun was calculated according to a procedure set up by Martin.[1] It was assumed that all the mix before freezing weighed 4177 grams (9.2 pounds per gallon). This assumption is justified because, as shown in the results, the composition of the mix did not vary a great deal from the composition recommended for such a product, which would have a weight of 9.2 pounds per gallon. The amount of overrun was calculated from the formula:

$$\text{Percent overrun} = \frac{522 - \text{net weight of pint sample}}{\text{net weight of pint sample}} \times 100$$

The value 522 is derived by multiplying 9.2, the weight of a gallon of mix in pounds, by 453 grams in a pound, and dividing by 8 pints per gallon. Therefore, it was assumed that there are 522 grams of mix per pint of unfrozen product. In cases where a quart sample was purchased the net weight per quart/2 was assumed to be the net weight per pint, the percent overrun remaining constant.

## Calculated Fuel or Caloric Value

Although the composition varies greatly among products such as these examined, it is known that the source of mix for nearly

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[1] Martin, W. H. Professor of Dairy Husbandry, Agricultural Experiment Station, Kansas State College, Unpublished data.

all the samples examined came from sources where the approximate composition is known. With this thought in mind it is believed to be within experimental error to approximate the caloric or fuel value of each sample, by the following procedures.

The fuel value of each sample was determined using a modification of a method outlined by Turnbow, Tracy, and Raffetto (32) for ice cream. It was assumed that the solids-not-fat (total solids minus fat) of the mix contained 14 percent added sugar (sucrose) and 0.3 percent added stabilizer (figured as gelatin protein). The percentage of fat, total solids, and the net weight of the finished product was determined by direct analysis. It may be safely assumed that the milk solids (solids-not-fat minus sugar and stabilizer) contain 54 percent milk sugar (lactose) and 39 percent protein. Seven percent of the milk solids is unavailable for fuel. From this information an approximate determination of the fuel value can be determined by using the following values for energy normally derived, by the body, from carbohydrates, fats, and proteins.

Carbohydrates . . . 4 calories per gram
Proteins . . . . . 4 calories per gram
Fats . . . . . 9 calories per gram

% total solids - % fat = % solids-not-fat
14% added sugar + 0.3% stabilizer = 14.3% total solids not milk
% solids-not-fat - 14.3% = % milk solids
54% lactose + 39% protein = 93% of milk solids available
% milk solids x .93 = % milk solids usable
% usable milk solids + 14.3% = % total solids usable for fuel

$$\frac{\%\ \text{solids usable}}{100} \times 4 = \text{calories per gram of solids}$$

$$\frac{\%\ \text{fat}}{100} \times 9 = \text{calories per gram of fat}$$

calories per gram solids + calories per gram fat = total calories per gram of product
calories per gram x net weight per pint = calories per pint

The results were recorded as the number of calculated calories for the pint of sample tested.

By collecting and analyzing 56 samples of ice milk for bacterial content, percent butterfat, percent solids, acidity, pasteurization efficiency, and calculated overrun and fuel value, a fairly complete picture of the product tested should be obtained from the results gathered.

## RESULTS

The results of the bacterial analysis on 56 samples as shown by the total plate count, presumptive coliform count, and the direct microscopic count, are shown in Tables 1, 2, and 3, respectively.

Table 1. Total plate count on 56 samples.

| Count | Number of samples | Percent of samples |
|---|---|---|
| 1,000 or less | 4 | 7.14 |
| 1,001 - 5,000 | 15 | 26.79 |
| 5,001 - 10,000 | 14 | 25.00 |
| 10,001 - 50,000 | 15 | 26.79 |
| 50,001 - 100,000 | 3 | 5.35 |
| over 100,000 | 5 | 8.93 |
| Total | 56 | 100.00 |

The minimum total plate count on the 56 samples shown in Table 1 was 300 with a maximum of 307,000 colonies per milliliter of sample. The logarithmic average was 9,300 as compared to an arithmetic average of 29,100.

The presumptive coliform counts on the 56 samples shown in Table 2 ranged from a minimum of 0 to a maximum of 7,500 colonies of typical coliform-type bacteria on violet-red-bile agar. An arithmetic average of 234 colonies per milliliter of sample was determined as a result of a large number of 0 counts and a high maximum count. However, by assuming that a sample having 0 colonies in 1 milliliter of sample would give 1 colony in 10 milliliters of sample, a logarithmic average of 1.84 colonies per milliof sample was determined for the 56 samples, by substituting the logarithm of 0.1 for all counts of 0.

Table 2. Presumptive coliform count on 56 samples.

| Count | Number of samples | Percent of samples |
|---|---|---|
| 0 | 22 | 39.29 |
| 1 - 5 | 16 | 28.57 |
| 6 - 10 | 4 | 7.14 |
| over 10 | 14 | 25.00 |
| Total | 56 | 100.00 |

Table 3. Direct microscopic count on 56 samples.

| Count | Number of samples | Percent of samples |
|---|---|---|
| 10,000 - 50,000 | 8 | 14.28 |
| 50,001 - 100,000 | 14 | 25.00 |
| 100,001 - 250,000 | 23 | 41.07 |
| 250,001 - 500,000 | 9 | 16.07 |
| 500,001 - 1,000,000 | 1 | 1.79 |
| over 1,000,000 | 1 | 1.79 |
| Total | 56 | 100.00 |

Table 3 shows the results of a direct microscopic count on 56 samples with a maximum count of 1,020,000 and a minimum count of 15,000 bacterial cells per milliliter of sample. These results

gave a logarithmic average of 124,000 as compared to an arithmetic average of 173,000.

The results of a chemical analysis for butterfat on 54 samples are given in Tables 4 and 5. Sample numbers 11 and 28 were omitted because the results on these samples were not representative of the product studied.

Table 4. Minnesota butterfat determination on 54 samples.

| Percent butterfat | Number of samples | Percent of samples |
|---|---|---|
| 3.5 - 4.0 | 2 | 3.70 |
| 4.1 - 4.5 | 1 | 1.85 |
| 4.6 - 5.0 | 26 | 48.15 |
| 5.1 - 5.5 | 17 | 31.48 |
| 5.6 - 6.0 | 8 | 14.82 |
| Total | 54 | 100.00 |

Table 5. Mojonnier butterfat determination on 54 samples.

| Percent butterfat | Number of samples | Percent of samples |
|---|---|---|
| 4.0 - 4.5 | 1 | 1.85 |
| 4.6 - 5.0 | 2 | 3.70 |
| 5.1 - 5.5 | 7 | 12.96 |
| 5.6 - 6.0 | 35 | 64.82 |
| 6.1 - 6.5 | 9 | 16.67 |
| Total | 54 | 100.00 |

The maximum percentage of butterfat as determined by the Minnesota method, shown in Table 4, was found to be 5.8 percent with a minimum of 3.8 percent. The overall average for the 54 samples was 5.1 percent butterfat. Sample numbers 11 and 28, not recorded in Table 4, showed 0.3 and 9.1 percent butterfat, respectively.

The maximum percentage of butterfat as determined by the Mojonnier method, shown in Table 5, was found to be 6.39 percent with a minimum of 4.34 percent. The overall average was 5.77 percent butterfat. Samples number 11 and 28, not recorded in Table 5, showed 0.54 and 10.60 percent butterfat, respectively, using the Mojonnier method.

A statistical analysis using, "Students t-test on pairs of observations," showed that the percent fat from the Mojonnier method was higher on the average than the percentage fat from the Minnesota method. Statistically, on the samples examined, the true average amount by which the Mojonnier determination exceeds the Minnesota value is almost certain (odds 999 to 1) to exceed 0.56. With a little more risk of error it could be concluded that the difference, on the results obtained in this study, between the two methods is at least 0.60 (odds approximately 99 to 1).[1]

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[1] Statistical analysis by Statistics Department of Kansas State College.

Table 6. Mojonnier total solids determination on 54 samples.

| Percent total solids | Number of samples | Percent of samples |
|---|---|---|
| 29- 30 | 4 | 7.40 |
| 31 - 32 | 10 | 18.50 |
| 33 - 34 | 38 | 70.40 |
| 35 - 36 | 2 | 3.70 |
| Total | 54 | 100.00 |

The results on a determination of total solids, using the Mojonnier method, on 54 samples are shown in Table 6. Sample numbers 11 and 28 were omitted because the results, due to variation in composition, on these samples were not representative of the product studied.

The maximum percentage of total solids, shown in Table 6, was found to be 35.54 percent with a minimum of 29.21 percent. The average for the 54 samples was 33.85 percent total solids.

The results showing the percent acidity, as determined by titration with N/10 sodium hydroxide (NaOH), are shown in Table 7 on 56 samples.

Table 7. Acidity titration on 56 samples.

| Percent acid | Number of samples | Percent of samples |
|---|---|---|
| .00 - .05 | 2 | 3.57 |
| .06 - .10 | 4 | 7.14 |
| .11 - .15 | 19 | 33.93 |
| .16 - .20 | 22 | 39.29 |
| .21 - .25 | 5 | 8.93 |
| .26 - .30 | 4 | 7.14 |
| Total | 56 | 100.00 |

The maximum acidity was found to be .29 percent acid, calculated as lactic acid, with a minimum of .05 percent. The overall average for the 56 samples was .16 percent.

The results of the sediment scores on 56 samples are shown in Table 8. These results are recorded as milligrams of sediment, according to the American Public Health Association, as outlined in the ninth edition of Standard Methods (26).

Table 8. A.P.H.A. sediment score on 56 samples.

| Milligrams of sediment | Number of samples | Percent of samples |
|---|---|---|
| 0 | 3 | 5.36 |
| 0 - .19 | 43 | 76.78 |
| .20 | 7 | 12.50 |
| over .20 | 3 | 5.36 |
| Total | 56 | 100.00 |

The sediment scores, as shown in Table 8, ranged from 0 milligrams to more than .2, but less than .5 milligrams, according to A.P.H.A. standards.

Table 9 shows the results on the phosphatase test on 56 samples. The results are expressed as N.Y.C. units based on standards included in the kit used for the test.

Table 9. Phosphatase score on 56 samples.

| N.Y.C. units | Number of samples | Percent of samples |
|---|---|---|
| less than 2 | 56 | 100.00 |

All 56 samples shown in Table 9 gave a reading of less than 2 N.Y.C. units, indicating proper pasteurization, on the mix from which the finished product was made.

The net weights of 54 samples were determined and the results are given in Table 10.

Table 10. Net weight of 54 samples.*

| Grams per pint : | Number of samples : | Percent of samples |
|---|---|---|
| 276 - 300 | 1 | 1.85 |
| 301 - 325 | 1 | 1.85 |
| 326 - 350 | 4 | 7.40 |
| 351 - 375 | 15 | 27.78 |
| 376 - 400 | 16 | 29.64 |
| 401 - 425 | 12 | 22.22 |
| 426 - 450 | 3 | 5.56 |
| 451 - 475 | 2 | 3.70 |
| Total | 54 | 100.00 |

*Samples numbered 17, 30, and 35 were quart samples and were counted as the net weight of the quart/2 to give a total of 54 samples figured in Table 10.

The maximum net weight of the 54 samples, shown in Table 10, was 463 grams with a minimum weight of 293 grams and an overall average of 387 grams for one pint of finished product. Samples number 11 and 28 were omitted from the data presented in Table 10 as not being representative of the samples tested.

The results of the percentage of overrun calculations are given in Table 11 on 54 samples. The percentage of calculated overrun, as shown in Table 11, ranged from a maximum of 78 percent to a minimum of 13 percent with an overall average of 36 percent. Samples numbered 11 and 28 were not considered as

representative and were not figured in the calculations in Table 11.

Table 11. Calculated overrun on 54 samples.

| Percent overrun | Number of samples | Percent of samples |
|---|---|---|
| 11 - 20 | 3 | 5.56 |
| 21 - 30 | 14 | 25.93 |
| 31- 40 | 22 | 40.74 |
| 41 - 50 | 11 | 20.37 |
| 51 - 60 | 2 | 3.70 |
| 61 - 70 | 1 | 1.85 |
| 71 - 80 | 1 | 1.85 |
| Total | 54 | 100.00 |

The results of the calculated caloric fuel value on 54 samples are shown in Table 12.

Table 12. Calculated fuel value on 54 samples.

| Calories per pint | Number of samples | Percent of samples |
|---|---|---|
| 401 - 450 | 1 | 1.85 |
| 451 - 500 | 1 | 1.85 |
| 501 - 550 | 6 | 11.12 |
| 551 - 600 | 17 | 31.48 |
| 601 - 650 | 17 | 31.48 |
| 651 - 700 | 11 | 20.37 |
| 701 - 750 | 1 | 1.85 |
| Total | 54 | 100.00 |

The caloric fuel values on the 54 samples shown in Table 12 ranged from a maximum of 738 calories per pint to a minimum of 440 calories per pint, with an overall average of 609 calories per pint for the 54 samples. Samples number 11 and 28 were not considered representative and were omitted from Table 12.

The overall averages of Tables 1 through 12 are placed in Table 13 to show an approximate average composition of 54 of the 56 samples studied. Samples number 11 and 28 are omitted as non-representative.

Table 13. Average results of 54 samples.

| Test | Results |
|---|---|
| Total plate count | 9,300 per milliliter |
| Coliform count | 1.84 per milliliter |
| Microscopic count | 124,000 per milliliter |
| Fat (Minnesota) | 5.1 percent |
| Fat (Mojonnier) | 5.77 percent |
| Total solids (Mojonnier) | 33.85 percent |
| Acidity (titrated as lactic acid) | .16 percent |
| Sediment score (A.P.H.A. standard) | less than .2 milligrams |
| Phosphatase (N.Y.C. units) | less than 2 units |
| Net weight | 387 grams |
| Calculated overrun | 36 percent |
| Calculated caloric fuel value | 609 calories per pint |

# DISCUSSION OF RESULTS

In discussing the results, of the analyses of the 56 samples, it is necessary to draw from numerous sources of material and information for standards, and in cases where there are no legal standards, recommended standards were used. The sources of these standards are cited and where standards are not drawn up specifically for ice milk, ice cream standards are used, based upon Federal and Kansas legislation.

According to item 24-p of the Frozen Desserts Ordinance and Code (16), the average bacterial plate count shall not exceed 50,000 per gram on pasteurized mix or frozen desserts. Upon analysis of the 56 samples, given in Table 1, 85.72 percent of the samples were found to have 50,000 or less colonies as shown by the total plate count. Of the 56 samples tested, 91.07 percent showed counts of 100,000 or less which is the standard recommended by many states, according to Bulletin Number 121 of the National Research Council (23). The Kansas standard is 100,000 colonies per milliliter of sample, with the city of Kansas City, Kansas having its own standard of 50,000. Of the seven Kansas City, Kansas, samples examined, six or 85.71 percent met the requirement of 50,000 or less. The other sample showed a count of 139,000 exceeding the maximum of both the state and local requirements.

According to Section 1.91 of Standard Methods (26) there are no official standards for the number of coliform-type bacteria

in frozen dessert products. A few states, according to the National Research Council Bulletin 121 (23), require a count of 0 coliform-type colonies, but according to Shadwick (25) a count of 10 or less coliform colonies is an acceptable standard. Of the 56 samples examined, shown in Table 2, 39.29 percent met the standard of 0 coliform and 75.00 percent of the samples met the requirement of 10 or less coliforms. According to Shadwick (25) there is no correlation between the coliform and the total plate counts.

Standard Methods (26) recommends a direct microscopic count of 1.67 cells or less per microscopic field of 0.160 millimeter diameter. Using a microscopic factor of 500,000, for this diameter, a maximum count of 835,000 bacterial cells would be obtained. Of the 56 samples examined in Table 3, 98.21 percent showed a microscopic count of less than 835,000. There is much disagreement whether there is any significant relationship between the counts obtained by the agar plate method and the direct microscopic method. According to Hammer (20) and Tanner (29) ratios of from 1/1 to 44/1 have been reported. The ratios between the logarithmic averages of the agar plate method and the direct microscopic method on the 56 samples given in Tables 1 and 3, respectively, was 13/1. This high ratio is to be expected due to the relatively low total plate counts, in which case the low count probably included types of organisms not growing on the medium used for the plate count but observed by the microscopic method. The use of the direct microscopic count on a product of this type is questioned by this author as being accurate, due to

the large percentage of milk solids, in a product of this type. Even by diluting the sample to one-half and with extreme care using a differential stain technique, extreme difficulty was encountered in making accurate counts due to the overstaining of the milk solids.

In all cases, the averages used in the bacterial analyses were logarithmic averages as recommended in Section 1.08 of Standard Methods (26). Arithmetic averages were compiled only for comparison to show the amount of variation between the two methods of averaging bacterial counts.

According to Section 12.85 of Standard Methods (26) the Mojonnier or Roese-Gottlieb method of butterfat determination is recognized as the official test on frozen dairy products. In this study the results of the Mojonnier test and the Minnesota modification of the Babcock test were compared on the same samples. The results of the Mojonnier test were used as the basic results for the samples tested. According to Section 65-720 of the General Statutes of Kansas 1949 (17) "ice milk" must contain less than ten percent milk fat. Of the 56 samples tested, 55 samples or 98.21 percent fell within the prescribed range of 0 to 10 percent milk fat. One sample, picked up by mistake, gave results of 10.60 percent butterfat and would be classified as "ice cream" by the Kansas law. In Tables 4 and 5 this sample (number 28), and one other sample (number 11) having an extremely low fat test of 0.54 percent, were omitted as not being representative. By comparing the two methods of butterfat determination on

all 56 samples the Mojonnier method reading was .08 to 1.5 percent higher, in all cases, than the reading obtained by the Minnesota method. According to Turnbow, Tracy, and Raffetto (32) the alkaline methods, such as the Minnesota method, should check within ± 0.3 of the percentage of the standard. By statistical analysis it was found that the difference between the two tests in this examination was +0.6. The large difference obtained is no doubt a result of laboratory error due to the extreme delicateness of the test and the non-experienced operator preforming the test.

There are no standards in Kansas concerning the total solids in "ice milk" but according to Section 65-707 of the General Statutes of Kansas 1949 (17), "ice cream" shall contain not less than 20 percent milk solids and not less than 33 percent total solids. Of the 56 samples tested, 41 samples or 73.21 percent exceeded the 33 percent total solids minimum. The average of all 56 samples was 33.91 percent and, with the omission of the two non-representative samples (numbers 11 and 28), the average for the 54 samples presented in Table 6 was 33.85 percent. The only sample tested that failed to meet the 20 percent milk solids requirement was sample number 59 with 19.56 percent milk solids.

The results of the titratable acidities, shown in Table 7, will vary somewhat with the solid content. Under normal conditions the acidity of the mix should be from .16 to .20 percent, the acidity of normal mixed milk being .14 to .16 percent. Providing no neutralization has taken place, any acidity above .16 to .20 percent would indicate acidity as a result of microbial

activity. Acidity below the .14 to .16 range would almost definitely indicate neutralization. It was found that of the 56 samples, given in Table 7, 7 samples or 12.50 percent were above the .20 percent limit indicating possible acid formation due to bacterial activity. Of the 56 samples examined, 32.11 percent showed acidity below .14 percent indicating possible neutralization of the mix.

Although there are no standards for sediment, it is desirable to have a product entirely free of extraneous material. However, considering the manufacture of a product entirely free of sediment as almost an impossibility, the standard chosen was anything less than .20 milligram sediment according to the A.P.H.A. score, outlined by Standard Methods (26). Of the 56 samples examined, and presented in Table 8, 46 or 82.14 percent meet the requirements of less than .20 milligram sediment.

The entire lot of the 56 samples, shown in Table 9, gave a phosphatase score of less than 2 N.Y.C. units. This is the score obtained by heating milk to a temperature of 62° C. (143° F.) for 30 minutes and shows complete and efficient pasteurization.

Based upon the assumption that one gallon of "ice milk" mix weighs 4177 grams (9.2 pounds per gallon), one pint of unfrozen mix would weight 522 grams. If an overrun of 33 percent is obtained, a pint of frozen "ice milk" should weight 392 grams. It was found that 46 samples or 80.70 percent of the 54 samples, shown in Table 10, fell within a range of 350 - 425 grams, with

an average of 373 grams per pint of finished product. The great variations in the weights are no doubt due to differences in the amount of overrun obtained by the use of different types of freezers. There are no regulations in Kansas concerning weight or overrun except the amount of butterfat and milk solids in the mix, which have a slight influence upon the weight of the mix.

The calculated overrun is dependent upon the net weight and the assumption that one pint of mix weighs 522 grams. It was found that of the 54 samples examined, as given in Table 11, 47 samples or 87.04 percent of the samples ranged from 20 to 50 percent overrun with an average of 36 percent overrun for the 54 samples examined. The optimum overrun for ice milk is 30 to 35 percent as compared to overruns of 70 to 100 percent for most ice cream. The variation in overrun is most likely due to the type of freezer and the formula of the mix used.

The calculated fuel values, based upon the net weight and a standard mix formula, of the samples given in Table 12 show an average caloric value for the 54 samples tested of 609 calories per pint. The caloric value of "ice cream" with a mix composition of 12 percent fat, 10 percent milk solids not fat, 15 percent sucrose, 0.3 percent stabilizer, and 70 percent overrun is 627 calories per pint. The "ice milk" mix formula, upon which the calculations in Table 12 are based, is 6 percent fat, 13 percent milk solids not fat, 14 percent sucrose, 0.3 percent stabilizer, and with 35 percent overrun would give a fuel value of 617 calories

per pint. The differences in overrun and composition make the fuel values of the two products almost equal.

The overall average composition on 54 of the 56 samples tested gave results that are well within either the legal or "ideal" standards for a product of this type. There were a few variations within individual samples but most of the samples were within the limits of the standards.

## CONCLUSION

From the analytical data obtained on 56 samples of a frozen dairy product known as "ice milk" gathered from retail outlets in 18 Kansas towns 55 of the samples would qualify, according to Kansas law, as "ice milk". One of the 56 samples, picked up by mistake, would qualify as "ice cream", due to a butterfat content of over 10 percent.

The average sanitary quality of "ice milk" in Kansas, according to the information gathered from the 55 samples examined, is equal to or better than specified by state and local legislation for ice milk, ice cream, and similar products. The data indicate that quality of materials, efficient pasteurization, careful and adequate refrigeration after pasteurization, and care in sanitizing the equipment used, were being observed in the case of most of the samples examined.

Although there was some variation between individual samples, the overall averages of the various tests indicate that "ice milk"

is being made from quality raw materials, is being handled properly before reaching the consumer, and is almost equal to ice cream in food value. Hence, there would seem to be no logical reason why it should not be given the same consideration in the dairy industry as ice cream itself.

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# APPENDIX

Table 14. Results of all tests on individual samples.

| Sample: | Name | Town | Price per pint | Date of collection | Total plate count | Coliform count | Microscopic count | Percent fat: Minnesota | Percent fat: Mojonnier | Percent total solids | Percent acid | Sediment score: milligrams | Phosphatase score | Net weight: grams per pint | Calculated percent overrun | Calories per pint |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Dairy Delight | Hutchinson | 30¢ | 9-19-50 | 44,000 | 0 | 586,000 | 5.8 | 5.98 | 33.43 | .16 | less than 0.2 | less than 2 | 400 | 31 | 640 |
| 2 | Dairy Queen | Hutchinson | 30 | 9-19-50 | 18,500 | 175 | 403,000 | 5.7 | 5.95 | 34.32 | .16 | 0.0 | less than 2 | 410 | 27 | 656 |
| 3 | Dairy Queen | McPherson | 30 | 9-19-50 | 6,800 | 1 | 173,000 | 5.6 | 5.88 | 33.78 | .18 | less than 0.2 | less than 2 | 375 | 39 | 600 |
| 4 | Dairy Queen | Salina | 30 | 9-19-50 | 2,600 | 1 | 288,000 | 5.7 | 5.92 | 33.88 | .16 | 0.0 | less than 2 | 400 | 31 | 640 |
| 5 | Dairy Queen | Abilene | 30 | 9-19-50 | 5,800 | 2 | 269,000 | 5.7 | 5.78 | 34.53 | .18 | less than 0.2 | less than 2 | 436 | 20 | 698 |
| 6 | Dairy Queen | Junction City | 30 | 9-19-50 | 4,400 | 0 | 158,000 | 5.8 | 5.93 | 33.99 | .18 | less than 0.2 | less than 2 | 396 | 32 | 634 |
| 7 | Dairy Queen No. 1 | Topeka | 30 | 9-25-50 | 6,500 | 48 | 104,000 | 4.6 | 5.76 | 33.52 | .15 | 0.2 | less than 2 | 426 | 23 | 682 |
| 8 | Dairy Queen No. 2 | Topeka | 30 | 9-25-50 | 14,700 | 5 | 71,000 | 4.6 | 6.07 | 34.39 | .14 | less than 0.2 | less than 2 | 428 | 22 | 685 |
| 9 | Dairy Freeze | Topeka | 30 | 9-25-50 | 1,600 | 0 | 94,000 | 4.8 | 5.82 | 35.06 | .29 | less than 0.2 | less than 2 | 394 | 33 | 670 |
| 10 | Dairy Queen No. 3 | Topeka | 30 | 9-25-50 | 1,900 | 0 | 48,000 | 4.4 | 4.95 | 29.29 | .12 | 0.0 | less than 2 | 403 | 30 | 564 |
| 11 | Frozen Delight | Ringling Bros. Circus | 25 | 9-15-50 | 42,000 | 1 | 261,000 | 0.3* | 0.54** | 32.76*** | .14 | less than 0.2 | less than 2 | # | ## | ### |
| 12 | Dairy Queen | Hutchinson | 30 | 9-30-50 | 5,200 | 0 | 138,000 | 5.3 | 5.86 | 34.37 | .12 | less than 0.2 | less than 2 | 405 | 29 | 648 |
| 13 | Dairy Queen | Wellington | 30 | 9-30-50 | 9,700 | 0 | 92,000 | 5.0 | 5.76 | 33.99 | .13 | 0.2 | less than 2 | 401 | 30 | 642 |
| 14 | Frigid Queen | Wellington | 30 | 9-30-50 | 28,000 | 0 | 75,000 | 5.3 | 5.90 | 34.25 | .20 | 0.2 | less than 2 | 372 | 40 | 595 |
| 15 | Dairy Queen No. 4 | Wichita | 30 | 10- 1-50 | 4,500 | 3 | 13,000 | 5.0 | 5.69 | 34.30 | .12 | less than 0.2 | less than 2 | 409 | 28 | 654 |
| 16 | Dairy Queen No. 2 | Wichita | 30 | 10-1-50 | 14,000 | 10 | 56,000 | 5.0 | 5.71 | 33.75 | .11 | 0.2 | less than 2 | 408 | 28 | 653 |
| 17 | Dairy Queen No. 7 | Wichita | 30 | 10- 1-50 | 2,200 | 0 | 188,000 | 4.9 | 5.69 | 33.59 | .20 | less than 0.2 | less than 2 | 382 | 37 | 611 |
| 18 | Keen Kreme | Wichita | 30 | 10- 1-50 | 53,000 | 1 | 200,000 | 5.1 | 5.89 | 34.11 | .15 | more than 0.2 | less than 2 | 311 | 68 | 498 |
| 19 | Dairy Queen | Wichita (Planeview) | 30 | 10- 1-50 | 5,600 | 5 | 166,000 | 4.9 | 5.91 | 33.00 | .13 | more than 0.2 | less than 2 | 364 | 43 | 546 |
| 20 | Dairy Queen No. 1 | Wichita | 30 | 10- 1-50 | 3,000 | 0 | 130,000 | 5.0 | 5.85 | 33.47 | .17 | 0.2 | less than 2 | 374 | 40 | 598 |
| 21 | Dairy Queen No. 6 | Wichita | 30 | 10- 1-50 | 27,300 | 1 | 100,000 | 5.3 | 6.03 | 33.15 | .16 | less than 0.2 | less than 2 | 357 | 46 | 571 |
| 22 | Zesto | Wichita | 30 | 10- 1-50 | 21,000 | 0 | 45,000 | 5.0 | 5.61 | 32.63 | .13 | less than 0.2 | less than 2 | 463 | 13 | 695 |
| 23 | Dairy Freeze | Wichita | 30 | 10- 1-50 | 15,500 | 2100 | 15,000 | 5.3 | 6.12 | 30.86 | .12 | less than 0.2 | less than 2 | 293 | 78 | 440 |
| 24 | Dairy Queen No. 3 | Wichita | 30 | 10- 1-50 | 6,400 | 1 | 34,000 | 5.1 | 6.11 | 34.57 | .20 | less than 0.2 | less than 2 | 352 | 48 | 563 |
| 25 | Dairy Queen No. 5 | Wichita | 30 | 10- 1-50 | 3,000 | 1 | 20,000 | 4.8 | 5.67 | 33.83 | .11 | less than 0.2 | less than 2 | 386 | 35 | 618 |
| 26 | Zesto | Wichita | 30 | 10- 1-50 | 41,000 | 64 | 50,000 | 5.1 | 5.45 | 33.41 | .15 | less than 0.2 | less than 2 | 461 | 13 | 738 |
| 27 | Dairy Queen | Newton | 30 | 10- 1-50 | 2,500 | 0 | 213,000 | 4.8 | 5.93 | 34.28 | .13 | less than 0.2 | less than 2 | 373 | 40 | 597 |
| 28 | Newton Dairy Bar | Newton | 24 | 10- 1-50 | 8,000 | 6 | 1,020,000 | 9.1* | 10.60** | 38.60*** | .25 | more than 0.2 | less than 2 | 259#### | ## | ### |
| 29 | Frigid Queen | Herington | 30 | 10- 1-50 | 144,000 | 26 | 333,000 | 5.4 | 6.12 | 33.81 | .27 | less than 0.2 | less than 2 | 389 | 34 | 622 |
| 30 | Dairy Queen No. 2 | Kansas City | 30 | 10- 1-50 | 9,000 | 46 | 156,000 | 5.1 | 6.15 | 33.80 | .09 | less than 0.2 | less than 2 | 382 | 37 | 611 |
| 31 | Dari-Ann | Kansas City | 30 | 10- 1-50 | 5,900 | 7 | 419,000 | 4.9 | 5.82 | 34.49 | .19 | less than 0.2 | less than 2 | 350 | 49 | 560 |
| 32 | Dairy Queen No. 3 | Kansas City | 30 | 10- 1-50 | 4,100 | 3 | 160,000 | 5.7 | 5.93 | 35.54 | .07 | 0.2 | less than 2 | 353 | 48 | 600 |
| 33 | Dairy Queen No. 4 | Kansas City | 30 | 10- 1-50 | 5,100 | 1 | 96,000 | 5.5 | 6.04 | 33.39 | .06 | 0.2 | less than 2 | 371 | 41 | 594 |
| 34 | Dairy Queen No. 5 | Kansas City | 30 | 10- 1-50 | 3,100 | 2 | 240,000 | 5.5 | 6.14 | 31.46 | .05 | less than 0.2 | less than 2 | 386 | 35 | 579 |
| 35 | Dairy Queen No. 1 | Kansas City | 30 | 10- 1-50 | 139,000 | 400 | 460,000 | 5.8 | 5.97 | 33.01 | .05 | less than 0.2 | less than 2 | 366 | 43 | 586 |
| 36 | Zesto | Kansas City | 30 | 10- 1-50 | 6,500 | 0 | 107,000 | 5.4 | 5.99 | 34.09 | .17 | less than 0.2 | less than 2 | 416 | 25 | 666 |
| 37 | Dairy Queen | Lawrence | 30 | 10- 1-50 | 307,000 | 2490 | 302,000 | 5.3 | 5.88 | 33.84 | .15 | less than 0.2 | less than 2 | 369 | 41 | 590 |
| 52 | Dairy Queen | Arkansas City | 30 | 10- 7-50 | 78,000 | 0 | 187,000 | 5.0 | 5.66 | 32.36 | .14 | less than 0.2 | less than 2 | 409 | 28 | 614 |
| 53 | Frosty Creme | Arkansas City | 30 | 10- 7-50 | 8,100 | 0 | 101,000 | 5.0 | 5.49 | 33.67 | .23 | less than 0.2 | less than 2 | 400 | 31 | 640 |
| 54 | Jersey Cow | Winfield | 20 | 10- 7-50 | 10,400 | 0 | 92,000 | 4.9 | 6.28 | 32.43 | .17 | less than 0.2 | less than 2 | 387 | 35 | 619 |
| 55 | Dairy Queen | Winfield | 30 | 10- 7-50 | 5,000 | 0 | 87,000 | 5.0 | 5.68 | 33.81 | .13 | less than 0.2 | less than 2 | 425 | 23 | 680 |

Table 14. (concl.)

| Sample: | Name | Town | Price per pint | Date of collection | Total plate count | Coliform count | Microscopic count | Percent fat: Minnesota | Percent fat Mojonnier | Percent total solids | Percent acid | Sediment score: milligrams | Phosphatase score | Net weight: grams per pint | Calculated percent overrun | Calories per pint |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56 | Dairy Queen | ElDorado | 30 | 10- 8-50 | 5,200 | 2 | 89,000 | 5.0 | 5.44 | 33.69 | .17 | less than 0.2 | less than 2 | 360 | 45 | 576 |
| 57 | Dairy Queen | Emporia | 30 | 10- 8-50 | 12,900 | 22 | 333,000 | 5.1 | 5.45 | 33.27 | .20 | less than 0.2 | less than 2 | 386 | 35 | 618 |
| 58 | Melo-Freeze | Emporia | 25 | 10- 8-50 | 3,900 | 0 | 50,000 | 5.0 | 6.25 | 33.31 | .20 | less than 0.2 | less than 2 | 390 | 34 | 624 |
| 59 | Dairy Custard | Emporia | 30 | 10- 8-50 | 209,000 | 77 | 60,000 | 5.0 | 6.39 | 29.21 | .29 | less than 0.2 | less than 2 | 366 | 43 | 549 |
| 60 | Dairy Queen | Manhattan | 30 | 10- 9-50 | 3,700 | 10 | 125,000 | 5.2 | 5.85 | 31.78 | .19 | less than 0.2 | less than 2 | 348 | 50 | 522 |
| 61 | Melo-Freeze | Emporia | 25 | 11- 9-50 | 300 | 0 | 75,000 | 5.1 | 5.63 | 33.24 | .19 | less than 0.2 | less than 2 | 374 | 40 | 598 |
| 62 | Dairy Queen | Emporia | 30 | 11- 9-50 | 134,000 | 38 | 192,000 | 5.0 | 5.77 | 33.51 | .18 | less than 0.2 | less than 2 | 394 | 32 | 630 |
| 63 | Dairy Queen | ElDorado | 30 | 11- 9-50 | 600 | 36 | 71,000 | 5.0 | 5.62 | 33.19 | .19 | less than 0.2 | less than 2 | 375 | 39 | 600 |
| 64 | Jersey Cow | Winfield | 20 | 11- 9-50 | 13,600 | 0 | 116,000 | 5.0 | 5.34 | 31.75 | .16 | less than 0.2 | less than 2 | 338 | 54 | 507 |
| 65 | Dairy Queen | Winfield | 30 | 11- 9-50 | 1,000 | 1 | 88,000 | 4.8 | 5.24 | 32.90 | .10 | less than 0.2 | less than 2 | 419 | 25 | 629 |
| 66 | Frosty Creme | Arkansas City | 30 | 11-12-50 | 12,400 | 0 | 108,000 | 4.0 | 4.96 | 32.23 | .20 | less than 0.2 | less than 2 | 405 | 29 | 608 |
| 67 | Frigid Queen | Augusta | 25 | 11-13-50 | 4,500 | 0 | 140,000 | 3.8 | 4.34 | 29.39 | .22 | less than 0.2 | less than 2 | 379 | 38 | 531 |
| 68 | Frigid Queen | Herington | 30 | 11-13-50 | 97,000 | 7500 | 172,000 | 4.7 | 5.74 | 32.49 | .28 | less than 0.2 | less than 2 | 341 | 53 | 546 |
| 69 | Dairy Queen | Junction City | 30 | 11-13-50 | 900 | 0 | 200,000 | 5.1 | 5.69 | 33.50 | .12 | less than 0.2 | less than 2 | 408 | 28 | 653 |
| 70 | Dairy Queen | Manhattan | 30 | 11-13-50 | 10,100 | 37 | 115,000 | 4.9 | 5.55 | 32.02 | .20 | less than 0.2 | less than 2 | 397 | 32 | 596 |

* Not shown in Tables 4 and 13.
** Not shown in Tables 5 and 13.
*** Not shown in Tables 6 and 13.
# Not calculated or shown in Tables 10 and 13.
## Not calculated or shown in Tables 11 and 13.
### Not calculated or shown in Tables 12 and 13.
#### Not shown in Tables 10 or 13.

# A BACTERIOLOGICAL AND CHEMICAL STUDY OF ICE MILK

by

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AN ABSTRACT OF A THESIS

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As a result of many investigations, legislation has been drawn up by state and local governments to govern the ice cream industry. In recent years a product known as "ice milk", defined by the 1949 General Statutes of Kansas as a product similar to ice cream but containing less than 10 percent butterfat, has become popular with the public and has greatly concerned the ice cream manufacturers. In November, 1950 the Federal Food and Drug Administration started hearings to define and set up standards for such a product. With these government hearings in progress, it seemed timely to investigate this product to determine its sanitary qualities and uniformity of composition, results of such investigations being entirely absent in the literature, on this particular dairy product.

For this study 56 samples of a frozen dairy product known as "ice milk" were gathered from retail outlets in 18 Kansas towns. These samples were analyzed, following the procedures given in Standard Methods for the Examination of Dairy Products, ninth edition, for total bacterial count, coliform count, direct microscopic count, butterfat, total solids, acidity, sediment, pasteurization efficiency, net weight, calculated overrun, and calculated fuel or caloric value. All samples were kept under adequate refrigeration, before analysis, to prevent any bacterial or chemical changes.

Using tryptone-glucose-beef extract agar with one percent sterile skim-milk added, total plate counts with a maximum of 307,000 and a minimum of 300 were obtained on 56 samples

examined. The logarithmic average was 9,300 for all 56 samples, as compared to an arithmetic average of 29,100. A presumptive coliform count was made, using Bacto violet-red-bile agar, giving a minimum count of 0 in 22 samples and a maximum of 7,500 with a logarithmic average, for the 56 samples tested, of 1.84 per milliliter. The arithmetic average for the coliform counts, on 56 samples, was 234. Using a differential-type stain with the direct microscopic method a maximum count of 1,020,000; a minimum count of 15,000; an arithmetical average of 173,000; and a logarithmic average of 124,000 bacterial cells per milliliter were obtained.

Butterfat determinations, using two methods, were made on all 56 samples but because of extreme variation, only 54 of the samples were considered significant. A minimum of 3.8 percent, a maximum of 5.8 percent, and an average of 5.1 percent butterfat were obtained upon analysis of 54 samples, using the Minnesota method, however, a minimum of 4.34 percent, a maximum of 6.39 percent, and an average of 5.77 percent butterfat were obtained when the 54 samples were analyzed using the Mojonnier method. A statistical analysis showed that, on the samples examined, the true average amount by which the Mojonnier determination exceeds the Minnesota value is almost certain (odds 999 to 1) to exceed 0.56.

The results of a total solids determination, using the Mojonnier method, gave a maximum of 35.54 percent, a minimum of 29.21 percent, and an average of 33.85 percent total solids for the 54 samples tested.

Acidity, titrated with N/10 sodium hydroxide (NaOH) and

calculated as lactic acid, gave a maximum of 0.29 percent, a minimum of 0.05 percent and an average of 0.16 percent, for all 56 samples tested.

Sediment scores, according to A. P. H. A. standards, ranged from 0 milligram to more than 0.2 milligram, but less than 0.5 milligram, with 82.14 percent of the 56 samples giving a reading of less than 0.2 milligram sediment.

All 56 of the samples gave a reading of less than 2 N. Y. C. units, on the phosphatase test, indicating proper pasteurization of the mix from which the finished product was made.

The net weights were determined on 54 of the 56 samples and found to range from a maximum of 463 to a minimum of 293 grams per pint. The average weight of the 54 samples examined was 387 grams per pint.

Overrun and fuel values were calculated on 54 of the 56 samples. The calculations were made on the basis of the net weight obtained on the sample, the butterfat and total solids determinations, and the formula of mix generally used for such a product. The percent overrun on the 54 samples tested ranged from a minimum of 13 to a maximum of 78, with an average of 36 percent. Based upon a mix that contained 14 percent added sugar and 0.3 percent added stabilizer, the maximum caloric value was found to be 738 calories per pint as compared to a minimum of 440 calories per pint. The average for the 54 samples tested was 609 calories per pint.

Although there was some variation between individual samples

the overall averages of the various tests indicate that "ice milk" is being made from quality raw materials, is being handled properly before reaching the consumer, and is almost equal to ice cream in food value. Hence there would seem to be no logical reason why it should not be given the same consideration in the dairy industry as ice cream itself.