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ORGANIC ACIDS IN FERMENTED MILK PRODUCTS

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ORGANIC ACIDS IN FERMENTED MILK PRODUCTS

by

Maria Angela Thomaz Barroso

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In the Graduate College

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction

by Maria Angela Thomaz BARROSO

entitled Organic Acids in Fermented Milk Products

be accepted as fulfilling the dissertation requirement for the Degree

of Doctor of Philosophy.

J. W. Stull
Dissertation Director

19 Nov 79
Date

As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

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Final approval and acceptance of this dissertation is contingent on the candidate's adequate performance and defense thereof at the final oral examination.

11/78

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SIGNED: Maria Angela Thomaz Barroso

In memory of my brother-in-law

Salvador Reboucas de Souza Mello

and my grandmother Maria Jose Farias Lourenco

Dedicated to my parents

Domingos Braga Barroso and Rita Thomaz Barroso

With love and thanks for encouragement

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ABSTRACT

Various gross and detailed composition parameters in samples of commercial plain, low-fat yogurt were compared in three available indigenous brands collected and analysed during a 65-day interval. These included percent titratable acidity, pH, percent total solids, quantitative and descriptive flavor evaluation and organic acids. The latter components were extracted from the yogurt with ethanol, fractionated on Amberlite resin and then analysed as silyl derivatives by gas liquid chromatography (GLC).

Each brand appeared to have distinctly different characteristics in several of the parameters measured. In the case of percent titratable acidity, for example, the means for each brand were 1.42%, 1.63% and 1.80%. Individual sample values ranged from 1.3% to 2.0%. Comparable mean pH values for the same brands were 4.08, 3.85 and 3.98 with individual samples ranging from 3.6 to 4.2. Mean percent total solids were even more distinctly different; namely, 14.49%, 12.33%, 17.82% arranged in the same brand sequence as given for titratable acidity and pH. The range in individual samples total solids was 11.31% to 18.43%. Flavor characteristics varied widely in the samples with a tendency toward various combinations and incidences of "lacks fine flavor", "low acid" or "high acid". A general skew toward mediocre to poor was evident in the assigned numerical flavor scores distribution.

Organic acids identified were lactic, phosphoric, succinic and citric. Traces of components tentatively characterized as fumaric, aconitic and quinic acids were noted on most chromatograms. It was not possible to make conclusions regarding the significance of differences in the quantitative amounts of these acids in plain, low-fat yogurt.

CHAPTER 1

INTRODUCTION

Fermented milks and cheese have enabled populations to survive periods of famine. As staple foods in man's diet, they have provided elements vital to good health. The origin of fermented milk foods dates from many centuries before Christ. In the earliest geographical areas, they were produced in warm climates such as the Mediterranean Sea Basin. It is supposed that the first products were discovered by accident but after the principal was established related products were created and spread throughout the world. Asiatic tribes, Huns, Mongols and Goths went to Europe over successive centuries and carried with them the practice for making foods from fermented milk.

Countries in North America and Europe produce cheese and related products from cow's milk. Milk of other animals such as sheep, goats, horses, buffalo, camels and yak is used in certain other countries. In early times the kind of milk used depended on the animal ingienous to the area. Resulting fermented products from various mammals' milk differ widely in flavor, texture, and appearance.

Buttermilk, sour cream, yogurt and cheese are produced by controlling milk fermentation. Fermentation in milk generally results in the breakdown of lactose into lactic acid. Distinctive fermented milk foods are produced by additional interesting, parallel or post-fermentation reactions.

Yogurt, one of the most typical fermented milk products, is believed to have originated in Mesopotamia about 5000 BC and was produced from goat's milk. From there it spread throughout the world. The practice of souring milk to produce yogurt was refined with modern technology by discovery of the agents which cause the particular fermentation. It is characterized by the production of a smooth, viscous gel with a delicate, walnutty flavor resulting from the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria in warm milk. Yogurt is a unique food not duplicated by other fermentations or by direct acidulation processes. The two dominant yogurt bacteria are thermophilic and are able to grow well at high temperatures. Along with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* other harmless lactic acid streptococci and lactobacilli may also grow in yogurt. At the end of a typical yogurt fermentation, the microflora population comprises about one billion live *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cells per ml of product. During storage at $4^{o}C$, these bacteria tend to die with *Streptococcus thermophilus* population declining more rapidly.

Yogurt and other fermented milks are considered by many who use them as one of their principle foods to have a superior affect on health and well being.

For centuries yogurt has been eaten with fruits or honey. Flavored yogurt, therefore, is not a recent phenomenon. However, the methods used to incorporate fruit flavors into yogurt have been improved (Kosikowski, 1977, p.75).

In this work, certain composition parameters of plain, commercial yogurt material are reported. Comparisons between manufacturers' brands and relationships between analyses are shown. The characteristics considered include titratable acidity, pH, total solids, descriptive and quantitative flavor evaluation and organic acid measurements by gas liquid chromatography (GLC).

CHAPTER 2

LITERATURE REVIEW

Yogurt, a Fermented Milk Product

Yogurt is a product produced by the growth and activity of two specific microorganisms in milk. It is a fermented food and may be referred to as a cultured milk, yielding a significant amount of lactic acid but contains little or no ethyl alcohol. This product usually has characteristic viscosity and texture intermediate between other fermented beverages and soft cheese; there is no significant separation of the component liquid whey and curd fractions. The name yogurt was probably given by the Turkish people to a milk fermented by lactic acid-producing bacteria which utilized part of the lactose (Lampert, 1970, p. 212). It is probably the best known cultured milk worldwide and is spelled yogurt, yourt or yoghourt (The Encyclopedia Britanica, 1974, 10, p. 823).

Origin

Yogurt seems to have originated in the warm countries of the Middle East and probably was originally made from sheep or goat's milk. Apparently the goat was first domesticated in Mesopotamia about 5000 BC. It is logical to believe that the goat's milk was stored without cooling in gourds and in the hot climate naturally formed a curd by fermentation. People tasted it and developed a iking for it. They then started making the product routinely and as the product gained popularity, consumption increased. The starter culture used became more uniform by adding a small portion of the previous day's yogurt to warm milk that had first been boiled. Incubation temperature was controlled more uniformly by placing a blanket over the pan of inoculated milk (Kosikowski, 1977, p.68).

Development Throughout the World

Yogurt is still widely made and consumed in those countries where it originated. It is the traditional form of sour milk in Bulgaria and neighboring countries. Fermented milk foods are favored over fresh, fluid milk in certain countries with warm climates which may also be characterized by a lack of available transportation, pasteurization and refrigeration. By allowing large numbers of harmless lactic acid-producing bacteria to grow rapidly in milk, populations of undesirable bacteria which may cause spoilage, food poisoning or disease are retarded or prevented. In this way, people were able to control the growth of other milk spoilage bacteria (Porter, 1975).

Since 1900, yogurt has become widely accepted in Europe. In North America yogurt was generally accepted somewhat later but the consumption has been increasing steadily since 1940. In 1968, there were twenty five flavored yogurt combinations reported in the United States (Brown and Kosikowski, 1970).

Today yogurt is produced and consumed worldwide. It has various names according to geographic area. For example, in Armenia it is called Matzoon; in Egypt, Leben, in Bulgaria, Naja; in Italy, Gioddu; and in India, Dadhi (Lampert, 1970, p. 212). Among others this cultured milk can be made from milk of cows, sheep, goats or water buffalo. The specific milk used depends on animal availability and the culture of the people. Cows milk is used in the United States and North Central Europe; sheep and goat's milk are used in Turkey and Southeastern Europe; water buffalo's milk is used in Egypt and in India (The Encyclopedia Britanica, 1974, p. 823).

In various countries there is a slightly different product produced depending on the milk used, the desired texture, the desired acidity, and, in the case of flavored products, the availability and identity of fruits in each region. Major reasons for increased popularity of this fermented milk product can be attributed to the variety of available flavors and its basic nutritive value.

Current Yogurt Processing Methods

Today, commercial processes of yogurt making are highly specialized. Plants use modern equipment and have the capability of accurately determining whether or not their products are contaminated with other undesirable microorganisms which might give varying characteristics to the finished material. The yogurt can be plain or flavored. Acidity tends to be less harsh in flavored yogurt and off-flavors are less pronounced.

All the steps in the process are closely monitored in order that the end product is of uniform, high quality. In general, processing procedures have many common features but temperature and time of incubation may vary slightly. Depending on the type of end product desired, the milk used can be whole, part-skimmed, skimmed, evaporated or fortified with added non-fat dry milk solids (NFDMS). Milk intended for inoculation must be practically free from microflora since the end product's characteristics depend on the growth of two different microorganisms. Contamination of the milk by various microflora types is likely to produce an end product of undesirable characteristics. A typical procedure is to first heat the milk and then 3 to 5% of NFDMS is added to raise the total solids to 15 to 17%. This improves the body and food value of the final product. The material is homogenized at 1800-2000 pounds pressure followed by pasteurization at 195^{o}F (90.6^{o}C) for forty to sixty seconds or 185^{o}F (85^{o}C) for thirty minutes. In addition to providing nearly-sterile conditions suitable for the growth of the yogurt culture bacteria, the heat treatment denatures serum proteins thereby increasing their capacity to bind water which in turn produces a firmer, more desirable body in the finished product. The high heat treatment is also thought to partially denature milk proteins with the liberation of small amounts of certain amino acids needed for the proper growth of yogurt microorganisms (Nilsen, 1975, p. 212). After pasteurization the material is cooled to 110^{o} (43^{o}C) and inoculated. The inoculating material is a pure culture of the required microorganisms and consists of approximately equal numbers of <u>Streptococcus thermophilus</u> and <u>Lactobacillus bulgaricus</u> added at the rate of 2 to 3% by weight. Individual preparations of each culture are mixed just prior to adding the total inoculuum to the milk (Lampert, 1970).

The inoculated milk is then pumped into consumer size packages, standard twenty or forty liter milk cans, or stainless steel vats as large as 1500 gallons and incubated at 110°F (43°C). The extent of microorganism growth and activity is controlled by regulating the temperature and time of incubation. The intent is to control the fermentation in order to produce characteristic, uniform acidity, flavor and texture in the finished product. During incubation, the inoculated microorganisms convert a portion of the lactose into lactic acid and other metabolites. The exact combination of incubation time and temperature depends on the desired final acidity in the yogurt. Incubation at 105°F (41°C) requires 3 to 3½ hours for a developed acidity of about 0.75%. These conditions are common in Britain but some countries prefer higher acidity. In the U.S.A., for example, an acidity of 0.9 to 1.2% is preferred. Higher acidities appear to be preferred in countries with warmer climates. The fullest flavor development is obtained at about 1.25% acidity (Davis, 1968, p.147). At 113°F (45°C) and an incubation time of three to six hours, the pH reaches 4.4 or a titratable acidity of 0.9 to 1.2%.

A recent development in yogurt production involves incubation of yogurt mixes at lower temperatures than normal. Kosikowski (1977, p. 75) cited the studies of Angevine and Lundstedt who showed some advantages in using lower temperatures during incubation. One possible disadvantage is the increased net time required in the low-temperature incubation.

When the desired acidity is reached, the product is cooled quickly to 35°F (2°C) to stop fermentation and then stored at 40°F

($4^{o}C$) for at least eight hours before marketing. Low temperature must be maintained during storage. The normal expected shelf life for yogurt is twenty to forty days. Luisiani, Salvadori and Bianchi-Salvadori (1975) proved that yogurt should have a shelf life of approximately one month if stored continuously at low refrigeration temperatures.

The average composition of commercial, plain yogurt in the U.S.A. is 1.66% fat, 10.98% total solids, 3.46% protein, 5.15% carbohyderate, and 0.75% ash (Kosikowski, 1977, p. 72).

Flavored Yogurt

The introduction of flavored combinations increased yogurt's popularity. Added flavors are represented by mixtures of natural, synthetic or artificial materials. A common flavoring might include natural fruit puree fortified with synthetic acids and synthetic fruit flavors. Color enhancement is frequently used. The most popular flavors are strawberry, raspberry and orange. In addition to the fruit flavor, 5% sucrose is customarily added. One way to flavor yogurt is to add the flavor, color and sugar to the milk and mix by blending prior to fermentation. Larger fruits are macerated into a puree before adding. Smaller ones are used whole or crushed. The fruit, puree or syrup is layered on the bottom of the container. The inoculated milk added on top of the flavoring material, the container sealed and then incubated. Another way to flavor yogurt is to mix plain yogurt with the fruits.

The average composition of typical flavored yogurt is 1.5% fat, 3 to 5% lactose, 3 to 5% added solids, 0.3 to 0.5% stabilizer, and 17 to 21% total solids.

New Flavors in Yogurt

Considerable interest has been evidenced in the introduction of new yogurt flavors. Even though recent literature reports several approaches to the area of new flavors, it is apparent that the success rate is not high. Brown and Kosikowski (1970) made yogurt with honey flavor. They verify that, although honey is listed among the primary basic, natural foods of man, it is probably not a highly suitable flavoring material for yogurt. They used several procedures. One system was to layer the required clover honey on the bottom of the container, cover it with inoculated milk and incubate. A market test of that product indicated a relatively low acceptability of the experimental flavor as compared to other common flavored yogurts. They concluded that the flavor of the clover honey was being masked by yogurt acidity. By comparison, a buckwheat honey flavor resulted in a more acceptable product by consumer testing. In addition to being a combination of two basic, natural foods, the product is somewhat less expensive than a comparable fruit-flavored product.

One report from Switzerland (Anon, 1977a), has reported good results using exotic or unusual tropical fruits in yogurt. These fruits included kumquats, coconuts, litchi and guava. The same author (Anon, 1977b) introduced another yogurt called "Vitana yogurt" which contained the following added nine vitamins: A, B_6, D, tocopherol, thiamin, riboflavin, nicotinic acid, ascorbic acid, and pantothenic acid. It is available in a wide variety of flavors including a unique combination based on celery. It has a reported advantage over the common yogurt of providing the daily requirements of certain essential vitamins in one serving.

Characteristics of Yogurt

Yogurt must have specific characteristics related to taste, aroma, body and texture. These characteristics depend on many factors. The specific culture microorganisms and their metabolism is very important. The ratio of the two bacterial species, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*; should be 1:1 and not above 3:2 for optimum results. If this condition is observed, a desirable flavor and mild acidity is developed. Lack of typical flavor is caused by low acidities. A harsh, astringent flavor is the result of high acidity. Bitter and other foreign flavors are produced by contaminated cultures. Some defects and their causes are shown in Table 1.

Wheying-off or syneresis can be caused by the growth of some microorganisms. One of the most common is *B. subtilis* and similar types. When defects such as that occur, the product must be discarded, plant equipment thoroughly cleaned and sterilized and cultures checked for purity. Table 2 shows some methods to control the quality of yogurt.

The consistency of yogurt is very important and is affected, in part, by the addition of milk solids. Heat treatment is also

Table 1. Yogurt: Defects and causes. --Davis, 1968, p. 148

Defects	Causes
Failure to grow	Presence of antibiotics in milk, weakened cultures, inadequate heat treatment of milk, excessive aeration.
High acidity	Too long incubation, slow cooling.
Gas formation	Growth of yeasts.
Failure to develop desired flavor	Imbalance of cultures due to faulty cultures or use of wrong temperatures.
Broken coagulum and syneresis	Rough handling, wrong acidities, abnormal milk.

Table 2. Quality control methods for yogurt.
(Davis, 1968, p.148)

Flavor	Odor and taste
Acidity	Titratable acidity on pH
Bacteriological	Microscopic examination; colony count on standard agar; tests for coli, yeasts or molds
Chemical	Fat* and solids-non-fat*

* Tests of legal significance

involved in this characteristic. Nilsen (1975) found that the best texture is one which involved a heat treatment of 195°F (91°C) for 5-30 minutes. Exposures of more than fifty or less than ten minutes reduced the firmness.

Incubation time, temperature and final pH must be controlled to produce a product with an adequate firmness. Traditional incubation, 105-110°F (41-43°C) for 2.5-3.0 hours, produces a firm coagulum at pH 4.6-4.7 (Nilsen, 1975). Excessive agitation during any step is another factor which can cause a defective body.

When permitted by law, stabilizers may be used to control body characteristics. Properly made plain yogurt does not require a stabilizer as a high viscosity, smooth gel is attained naturally. Flavored yogurt of the Swiss-style or stirred type usually need stabilizers. The most common stabilizers are gelatin, agar, or complex mixtures, consisting of guar gum, carob gum, carrageenan, tapioca and gelatin at levels of 0.4 to 0.5 percent. The stabilizer is usually added along with fruit, sucrose or glucose to the incompletely cooled yogurt and then the mixture is chilled in the container. Since stabilizers are hydrophilic colloids, they absorb water, prevent whey leakage, increase viscosity and establish proper mouthfeel. A wrong choice of type or amount may cause a gummy, slick, hard mouthfeel in the product. Yogurt with added stabilizer is less vulnerable to some factors of stress. Good yogurt should have a light, velvety, smooth texture and clean, subtle flavor. Many yogurt companies follow the suggestion of certain United Nations technical groups concerning yogurt formulations and utilize a completely natural control of product characteristics.

Nutritive Value of Yogurt

In addition to being considered nutritionally good, yogurt is frequently proposed as having some extraordinary effects on people who use it as a principal daily food. Porter (1975, p. 52) presented the following table (Table 3) comparing the composition of milk and yogurt. This data shows the considerable food value of yogurt.

Some scientists have attributed the extraordinary longevity of people in Balkan countries to yogurt consumption (Holland, 1970). Mitchnikoff, cited by Porter (1975, p. 52) attributed the good health and increased lifespan of these people to the ingestion of yogurt and beneficial activities of its microorganisms in the large intestine.

Reddy, Shahani and Banerjee (1973) have studied the effect of yogurt ingestion in rats having certain types of cancer. Retrogression of the cancer was reported but their studies are not yet complete.

The effect of yogurt on the reduction of serum cholesterol in human patients has been studied by Mann and Spoerry (1974). The fermented milk intake by the experimental groups was considerably above normal levels, but based on those experiments, they concluded that there is a factor in sour milk which influences cholesterol metabolism. Gold and Samuel (1965), reported negative results on cholesterol metabolism when the yogurt intake was drastically reduced.

Table 3. The nutrient content of milk, natural yogurt, and fruit-flavored sweetened yogurt (per 100 g).— Porter, 1975, p. 53

	Milk	Natural yogurt	Fruit flavored sweetened yogurt
Fat (g)	3.7	1.5	1.5
Protein (g)	3.2	5.0	4.3
Carbohydrate (g)	4.7	7.1	14.0
Vitamin A (retinol equivalents, µg)	30	12	12
Thiamin (µg)	45	65	55
Riboflavin (µg)	180	270	240
Energy content (Kcal)	65	62	87

CHAPTER 3

MATERIALS AND METHODS

Source of Yogurt Material

Plain, low fat yogurt representing four commercial brands was collected from retail food stores. The products were packed in 8 oz (227 g) sealed, non-transparent, plastic containers. Labels indicated the yogurt was composed of grade A pasteurized, cultured milk with other ingredients (varying with brand) including nonfat dry milk, skim milk, agar, gelatin, carrageenan or kosher gelatin. The samples were collected according to container printed pull date and kept under refrigeration at 33^{o}F (0.56^{o}C) until analyzed.

The analyses performed were: pH, titrable acidity, total solids, flavor, and organic acids. All the analyses were made at the same time the container was opened and were generally within one week of collection. Table 4 shows dates of sample collection and printed expiration of expected storage life (pull date); times from collection to storage life expiration and from collection to analyses.

Reagents

Buffer solution, pH 4 (Matheson Coleman and Bell); sodium hydroxide (Baker Analyzed Reagent); phenolphthalein (Mallinckrodt); ethyl alcohol, anhydrous 200 proof, reagent quality (U.S. Industrial Chemicals Co.); Amberlite CG-120, 100-200 mesh, strongly acidic,

Table 4. Dates of sample collection and printed expiration of expected storage life (pull date); times from collection to storage life expiration and from collection to analysis.

Sample no.	Collection date (1979)	Pull date (1979)	Time from collection to pull date (days)	Time from collection to analyses (days)
A				
1	08-28	09-24	27	1
2	08-27	10-01	35	2
3	09-04	10-06	32	2
4	08-30	10-08	39	15
5	09-12	10-13	31	2
6	09-12	10-20	38	2
7	09-17	10-22	35	3
8	09-17	10-27	40	3
9	09-25	10-29	34	2
10	09-28	11-03	36	11
11	10-01	11-10	40	8
12	10-04	11-12	39	5
13	10-16	11-24	39	8
Duration of collection period (days)	50			
B				
1	08-18	09-07	20	19
2	09-03	09-19	16	3
3	09-09	10-01	22	2
4	09-09	10-07	28	2
5	09-17	10-15	28	3
6	10-22	11-10	19	2

Table 4, Continued.

Sample no.	Collection date (1979)	Pull date (1979)	Time from collection to pull date (days)	Time from collection to analyses (days)
B				
Duration of collection period (days)	65			
C				
1	09-04	09-07	3	2
2	09-07	10-04	27	4
3	09-20	10-16	26	7
4	10-13	11-05	23	11
Duration of collection period (days)	39			
D				
1	09-07	09-27	20	4
2	09-20	10-04	14	7
Duration of collection period (days)	13			

sulfonated polystyrene type, cation exchange resin (Mallinckrodt); Amberlite CG-400, 100-200 mesh, strongly basic, quaternary ammonium polystyrene type, anion exchange resin (Mallinckrodt); formic acid, 88% reagent grade (Mallinckrodt); sodium formate, crystals, reagent grade (Matheson Coleman & Bell); hydroxylamine hydrochloride, analytical grade (Mallinckrodt); lactic acid, E, (sigma) 64.4% W/W, L isomer 49.4% (Chemical Co.); succinic acid, A grade (Calbiochem); phosphoric acid, 85.5% (J. T. Baker Chemical Co.); DL malic acid, A grade M.P. 132°C (Calbiochem); citric acid, A grade (Calbiochem); "BSTFA", N,0-bis (trimethylsilyl) trifluoroacetamide (Pierce Chemical Co.); ethanol (Matheson Coleman and Bell; Methanol (Matheson Coleman and Bell); Bacto-Brom Cresol Purple (Difco Laboratories, Inc. Detroit, Michigan); fumaric acid (Calbiochem); aconitic acid, 98% (Aldrich Chemical Co., Inc. Milwaukee, Wis.); quinic acid (Aldrich Chemical Co., Inc. Milwaukee, Wis.).

Equipment

pH meter, Accumet, model 220 (Fisher); The Majonnier milk tester, model D (Majonnier Bros. Co., Chicago); analytical balance (Mettler Instrument Corp.); buret, 50 ml, with Teflon stopcock (Kimax); filter paper, No. 595, 12½ cm (Schleicher and Schuell Co.); chromatographic column, 25 ml buret with Teflon stopcock (Kimble Products, Kimax); vial, screw cap vial, Pyrex (Corning), with Teflon-lined rubber; separatory funnel, 60 ml (Kimble Products, Kimax); gas chromatograph, Micro Tek (Tractor Inc., Austin, Texas), model DSS-170, equipped with a dual flame ionization detector and a glass column (0.4 cm internal diameter by 1.55 m long) fitted for on-column injection. The column packing consisted of 3% SE 52 + 1.5% SE 30, on 80-100 mesh Chromosorb W, acid washed DMCS; argon carrier gas; integrator, Infotronics model CRS-108 (Infotronics Corp., Houston, Texas), providing digital and printed readouts for retention times and peak areas; centrifuge-L-713, speed 0-3,000 rpm (Phillips-Drucker, Astoria, Oregon, U.S.A.); rotary evaporator, flash-evaporator (Buchler Instruments); TLC plate; rectangular trough chamber for TLC solvents.

Tests and Analyses on Yogurt

pH

pH was determined following the instructions provided by the instrument manufacturer. The instrument was standardized with the pH 4.0 standard buffer solution.

Titratable Acidity

To determine titratable acidity, the American Association of Official Analytical Chemists (AOAC) method (AOAC, 1975, sec. 16.023) for milk was followed. A 10.0 g sample was diluted with 40 ml of distilled water. Acidity was reported as percent lactic acid by weight with each milliliter of 0.1 N NaOH used to titrate the sample being to 0.0090 g lactic acid.

Total Solids

The Majonnier Bros. Co. (1925) method for ice cream was used to determine the total solids. This procedure is one of a series of methods prepared for determination of total solids in milk and milk products. A one gram sample was weighed into a tared, aluminum dish, one milliliter of distilled water added and the mixture spread evenly over the bottom of the dish by swirling. Total solids are expressed as percentage by weight.

Flavor Scores and Criticisms

Sensory evaluation of yogurt samples was made by an experienced panel of two judges. The American Dairy Science Association (ADSA) scoring guide for Swiss-style, flavored yogurt was used. Table 5 shows the scoring guide. The scores of each sample were recorded.

Organic Acids

Preparation of Ion Exchange Columns. Approximately 5 g cation or anion exchange resin was transferred to one of two 150 ml beakers, 50 ml distilled water added, stirred well and allowed to stand for 5 min at room temperature. After the resin had settled, the water carrying the smaller, lighter resin beads was decanted off. A second 50 ml portion of distilled water was added and the operation repeated. Finally, 100 ml distilled water was added, the mixture stirred well and allowed to stand for 30 min. The bottom of the chromatographic columns were plugged with Pyrex glass wool equivalent to approximately 0.25 ml on the column graduation, about 5 ml distilled water was poured into the column and allowed to flow through. The resin in one beaker was suspended in distilled water by stirring and then transferred to the column. The column was packed to the 18 ml level of the

Table 5. ADSA guide for scoring flavor of Swiss-style yogurt.

Flavor criticism	Intensity of defect		
	Slight	Definite	Pronounced
High acid	9	7	5
Low acid	9	8	6
Bitter	9	7	5
Cooked	9	8	6
Green	9	7	5
Lacks fine flavor	9	8	7
(Lacks flavoring)*	9	8	7
Lacks freshness	8	7	6
(Lacks sweetness)*	9	8	7
Old ingredient	7	5	3
Oxidized	6	4	1
Rancid	4	2	*
Stabilizer	8	7	6
Too high flavoring	9	8	7
(Too sweet)*	9	8	7
(Unnatural flavoring)*	8	6	4
Unclean	6	4	1

*Not applicable to plain yogurt

buret. Before application of the sample, the columns were conditioned as follows: the cationic column was washed with 50 ml 6F formic acid, followed by 100 ml distilled water; the anionic column was washed with 50 ml 2F sodium formate followed by 100 ml distilled water.

Sample Preparation and Organic Acid Extraction. The recovery of organic acids from animal tissues and plant products was studied by Kuksis and Prioreschi (1967). They worked with a variety of isolation techniques and indicated that the anion exchange method of Bush, Hurlbert and Potter (1952) appeared to be the best technique for isolating organic acids as a group.

The method used in this work was the one developed by the Associate Referee and described by Baker (1973), with some modifications. This method is based on extraction and isolation of the organic acids with the use of ion exchange chromatography.

About 20 g of well-mixed yogurt was weighed into a 150 ml graduated beaker, 20 ml anhydrous ethyl alcohol containing about 20 mg of malic acid was added and then the mixture stirred well for one minute. The entire yogurt/alcohol material was transferred to a centrifuge tube, centrifuged for 10 min at 2,600 rpm after which the supernatant was filtered.

Column Chromatography. A 10 ml aliquot of the centrifuged, filtered supernatant was added to the cationic column positioned over an anionic column. After the filtrate material was allowed to pass through both columns, they were washed with four successive, 25 ml portions of water-alcohol (1:4). The washing material was added in the same way as the filtrate allowing each portion to drain completely through the columns before adding the next one. The organic acids were eluted from the anionic column into a 100 ml volumetric flask (including a previously added crystal of hydroxylamine) with four successive, 25 ml portions of 6F formic acid.

Preparation of the Extracted Separated Acids for GLC Analysis. A 10 ml aliquot of the eluted acids was pipetted into a 50 ml beaker, 30 ml benzene was added and the beaker placed on a vigorously boiling steam water bath. The solvent was evaporated to ca 5 ml under a stream of air directed into the center of the beaker at the surface. An additional 10 ml benzene was added to the beaker and then evaporated to ca 2 ml. The beaker was quickly removed from the steam bath and the contents transferred into a screw capped vial. The benzene was evaporated to dryness on the boiling steam water bath with an air stream. The beaker was quantitatively rinsed with four, 0.5 ml portions of alcohol into the screw capped vial and the combined rinsings evaporated to dryness as before. At this point, the evaporated eluate can be stored in the closed vial under dry nitrogen in a freezer, should the assay be continued the next day.

A 0.5 ml portion of sililating reagent (BSTFA) was added to the sample vial and it was then quickly capped. The vial was placed in a 221^{o}F (105^{o}C) oven for 5 min (after ca 2 min, the vial should be taken out, shaken vigorously and quickly returned to the oven). A 2 μl sample of the silylated solution was injected into the gas chromatograph with the following operating conditions: injection port

$255^{o}C$; detector $215^{o}C$; the column programmed from 90 to $250^{o}C$ at the rate of $15^{o}C/min$. The flow rates were 25 ml/min for argon carrier gas with hydrogen and air flows adjusted for optimum detector response.

The peaks were recorded and the areas of the peaks were integrated by a printing integrator. Relative area of the peaks were calculated according to the following formula:

$$\text{Relative area (R)} = \frac{\text{Area of known peak}}{\text{Area of standard peak}}$$

GLC Calibration Curves. A standard solution containing 0.10, 0.25, 0.50, 0.75, 1.0, 1.50, 2.0 mg/ml each of lactic, phosphoric, succinic, fumaric, aconitic, citric and quinic acids and 1.0 mg of malic acid in BSTFA were silylated and chromatographed as described for the purified extract. Calculation of the relative area ratios of the trimethylsilyl (TMS) derivatives of the organic acids and the malic acid (as standard) peak were made and plotted against concentration.

TLC Determination of Organic Acids. Organic acids extraction was made in the same way as for GLC analysis. The entire eluate (100 ml) containing the organic acids in 6F formic acid was evaporated under vacuum in a rotary evaporator at $45^{o}C$; distilled water and ethyl alcohol in small portions were used to help the removal of excess formic acid.

The method used to separate the organic acids by thin-layer chromatography is presented by Ganshirt, Waldi and Stahl (1965). The acids were dissolved as ammonium salts, 2% in methanol-water (1 + 1) and 10 µl of this solution were applied to the plate. The mixture of the organic acids was separated on Silica Gel G layers, with solvent composed of ethanol (96%)-water-ammonia solution 25% (100 + 12 + 16). The running time was 120 min, migration-distance of 12 cm, in plain chambers. After development, the plate was heated for 10 min at 212^{o}F (100^{o}C). Then the plate was allowed to cool at room temperature and sprayed with a solution of 0.1 g Bacto-Brom Cresol Purple in 250 ml 7.4% 0.01 N sodium hydroxide; yellow spots on a blue background were obtained.

To identify the acids in the sample, 10 µl of standard solutions of lactic, phosphoric, succinic, fumaric, malic and citric acids were applied to the same plate.

CHAPTER 4

RESULTS AND DISCUSSION

Titratable Acidity

Excluding brand D, there are three, distinctly different mean titratable acidity levels in an increasing order of 1.42%, 1.63%, and 1.80% for the brand B, A and C, respectively (Table 6). Considering the possible relationship between these values and those of mean pH, brand B has the lowest mean acidity (1.42%) and the correspondingly highest pH (4.08). In the case of the comparatively high mean acidity of brand C (1.80%), total solids probably has a bearing since this is also the highest (17.82%). A possible relationship between high acidity and low pH, however, is not borne out in this instance (1.80% and 3.98, respectively). On the other hand, brand A with the lowest mean total solids (12.33%) has the lowest mean pH (3.82) and an intermediate acidity (1.63%).

Although there was considerable difference in the number of samples upon which to base standard deviation calculations, it is apparent that the acidity values within each brand varied only slightly during the observation period. The mean titratable acidity of brand C had a standard deviation of 0.22 as compared to 0.18 and 0.08 for brands A and B, respectively.

Table 6. Individual titratable acidity values (% lactic acid) of samples from four brands of plain yogurt with comparative mean pH and percent total solids.

Sample no.	Brand A	Brand B	Brand C	Brand D
1	1.5	1.5	1.5	1.7
2	1.9	1.3	1.8	1.3
3	1.5	1.5	1.9	
4	1.6	1.4	2.0	
5	1.7	1.4		
6	1.6	1.4		
7	1.6			
8	1.7			
9	2.0			
10	1.6			
11	1.5			
12	1.7			
13	1.3			
Mean	1.63	1.42	1.80	-
Standard deviation	0.18	0.08	0.22	-
Mean pH	3.85	4.08	3.98	-
Mean total solids (%)	12.33	14.49	17.82	-

In the samples from 13 different batches of brand A collected over a 50-day period (from 8-28-79 to 10-16-79, Table 4) individual titratable acidity values ranged from 1.3 to 2.0%. In the case of brand B, acidity of the individual batches represented in the six samples collected over a 65-day period (from 8-18-79 to 10-22-79, Table 4) ranged from 1.3 to 1.5%. Titratable acidity values in the four samples of brand C ranged from 1.5 to 2.0% during the 39-day collection period. Throughout the 25 samples from all brands the acidity levels ranged from 1.3% (A13, B2, D2) to 2.0% (A9, C4).

pH

As in the case of titratable acidity, there are also three distinctly different mean pH values; namely, 3.85, 3.98 and 4.08 for brands A, C and B, respectively (Table 7). The unexplained high pH in sample A6 (4.8 and not correlated with its normal titratable acidity of 1.6%) had only a slight effect on the mean or standard deviation values. Except for this sample, the individual pH values in the other 12 samples of this brand ranged from 3.6 to 3.9 during the 50-day observation period. In brand B, the pH values ranged only from 4.0 to 4.1 in the six samples collected over a 65-day period. The pH range in brand C was from 3.9 to 4.0 in the four samples analysed during a 39-day collection interval. These variations are reflected in the standard deviations of 0.30, 0.04 and 0.05 for brands A, B and C, respectively. Among individual values in samples from the four brands, pH ranged from 3.6 in sample A8 to 4.2 in D2 (excluding A6).

Table 7. pH values of four brands of plain yogurt.

Sample no.	Brand A	B	C	D
1	3.8	4.1	4.0	3.9
2	3.7	4.1	4.0	4.2
3	3.8	4.1	4.0	
4	3.8	4.1	3.9	
5	3.8	4.0		
6	4.8	4.1		
7	3.8			
8	3.6			
9	3.7			
10	3.7			
11	3.8			
12	3.8			
13	3.9			
Mean	3.85	4.08	3.98	-
Standard deviation	0.30	0.04	0.05	-

Total Solids

The range of difference between the mean total solids values were greater than those of titratable acidity or pH (Table 8). These means were 12.33%, 14.49% and 17.82% for brands A, B and C, respectively. Individual total solids values in brand A ranged from 11.31% to 13.59% (standard deviation $\pm$ 0.64) in the 13 samples collected during the 50-day period. In brand B, the values ranged from 12.69% to 15.49 in the 65-day observation. The standard deviation of the total solids values in this brand was $\pm$ 1.13 signifying a somewhat greater lack of uniformity of this characteristic. In a 39-day interval, the range of individual total solids values in brand C was from 17.16% to 18.43% (standard deviation $\pm$ 0.52). While only two samples were available in brand D during a 13-day period they each had widely different total solids values; namely, 15.81% and 20.39%. The latter represented the highest total solids value in the samples analysed throughout all the brands. The overall range between low and high total solids levels in individual samples was 11.31% (A13) to 20.39% (D2). This large spread of 9.08% indicates widely differing concepts of quality standards or control for this plain yogurt composition parameter.

Flavor Scores and Criticisms

The mean flavor score for brand A was 8.46 ranging from 7 to 9 (Table 9). Of the 13 samples examined during a 50-day period, seven were criticised as "lacks fine flavor", six as being "high acid" with one (A13) being both "low acid" and "lacks fine flavor".

Table 8. Percent total solids of four brands of plain yogurt.

Sample no.	Brand A	B	C	D
1	12.27	13.49	17.82	15.81
2	11.63	12.69	17.16	20.39
3	12.50	15.49	18.43	
4	12.12	15.16	17.85	
5	13.59	15.03		
6	12.51	15.08		
7	13.29			
8	11.31			
9	12.53			
10	12.23			
11	12.66			
12	11.83			
13	11.78			
Mean	12.33	14.49	17.82	-
Standard deviation	0.64	1.13	0.52	-

Table 9. Flavor scores and criticisms for brand A plain yogurt.

Sample no.	Scores	Criticisms
1	9	Lacks fine flavor
2	9	Lacks fine flavor
3	8	High acid
4	9	High acid
5	8	Lacks fine flavor
6	8	Lacks fine flavor
7	9	Lacks fine flavor
8	8	High acid
9	9	High acid
10	9	High acid
11	9	High acid
12	8	Lacks fine flavor
13	7	Low acid, lacks fine flavor
Mean	8.46	
Standard deviation	0.66	

In the case of brand B, the mean flavor score was 7.83. The range was the same as for brand A; namely, 7 to 9 (Table 10). Of the six samples collected over a 65-day period, four were criticised as "lacks fine flavor" and two as "low acid".

While only four samples of brand C were available during a 39-day period, the mean flavor score of 7.25 covered a range of 6 to 8 (Table 11). This brand had two samples that were characteristically "green", one as being typical "lacks fine flavor" and one that had been subjected to an apparent unusually high heat treatment with a resultant pronounced "cooked" flavor.

Of the two samples represented in the available brand D products, one was criticised as "lacks fine flavor" and one as being both "low acid" and "lacks freshness" (Table 12).

Considering all brands, the most commonly occurring flavor criticism was "lacks fine flavor" with 13 samples being so identified. Next most common flavor criticism was six samples with a "high acid" characteristic. "Low acid" was descriptive of the flavor of four samples. Overall, two samples were considered "green", one as being "cooked" and one typical of "lacks freshness".

Even though a relatively few samples could be compared in the "high acid"/"low acid" categories, it is of interest to consider the titratable acidity and pH values for these. The mean acidity and pH values for the six "high acid" samples were 1.65% and 3.73 respectively while the similar means for the four "low acid" samples were 1.38% and 4.08, respectively. This would indicate that combinations

Table 10. Flavor scores and criticisms for brand B plain yogurt.

Sample no.	Scores	Criticisms
1	9	Lacks fine flavor
2	9	Lacks fine flavor
3	7	Low acid
4	7	Low acid
5	8	Lacks fine flavor
6	7	Lacks fine flavor
Mean	7.83	
Standard deviation	0.98	

Table 11. Flavor scores and criticisms for brand C plain yogurt.

Sample no.	Scores	Criticisms
1	8	Green
2	8	Green
3	7	Lacks fine flavor
4	6	Cooked
Mean	7.25	
Standard deviation	0.96	

Table 12. Flavor scores and criticisms for brand D plain yogurt.

Sample no.	Scores	Criticisms
1	9	Lacks fine flavor
2	6	Low acid; lacks freshness

of the flavor criticisms and titratable acidity/pH values were fairly descriptive under the conditions of this series of observations.

A summary of the number of samples falling into each flavor score category from the range 6 to 9 is:

Flavor score		Number of samples
6		2
7		5
8		8
9		10
	Total	25

Together with the previously discussed criticism array, this distribution can be interpreted as not only showing considerable variation in flavor but also a general skew toward the mediocre to poor part of the scale.

Organic Acids

Relative Retention Times for the Silyl Derivatives of Organic Acids in Plain Yogurt Detected by GLC

Relative retention times of the silyl derivatives of organic acids extracted from plain yogurt and then subjected to GLC analysis were calculated according to the following formula:

$$\text{R.R.T.} = \frac{t_R \text{ of unknown compound}}{t_R \text{ of malic acid}}$$

Where:

R.R.T. = relative retention time

t_R = time for appearance of GLC peak following injection

Similar calculations were made using derivatives of pure compounds of lactic, phosphoric, succinic, fumaric, aconitic, citric and quinic acids.

On the basis of comparisons between the relative retention times of pure standards and the chromatograms of purified extracts from plain yogurt, identifications of lactic, phosphoric, succinic, fumaric, aconitic, citric and quinic were made (Figures 1 and 2, Table 13).

Mabesa, Baldwin and Garner (1979) had previously identified lactic, succinic, malic and citric acids in peas and carrots using this technique with fumaric acid being one of the materials in his standard mixture.

Philip and Nelson (1973), working with ion-exchange chromatography followed by GLC, analyzed grape juice for malic and tartaric acids. As a part of the study, they made silyl derivatives of several organic acids, including fumaric, malic, aconitic, citric and quinic and reported the sequence of their appearance on the chromatogram.

Baker (1973) showed the TMS derivatives of organic acids in grape, orange and apple juice. Malic, transaconitic and citric acids were found in those three juices. Tartaric acid was found in grape juice and quinic acid in apple juice.

Comparison of the relative retention time values in Table 13 indicates that the silyl derivatives of pure organic acid standards and those of plain yogurt extracts are close enough to be considered identical.

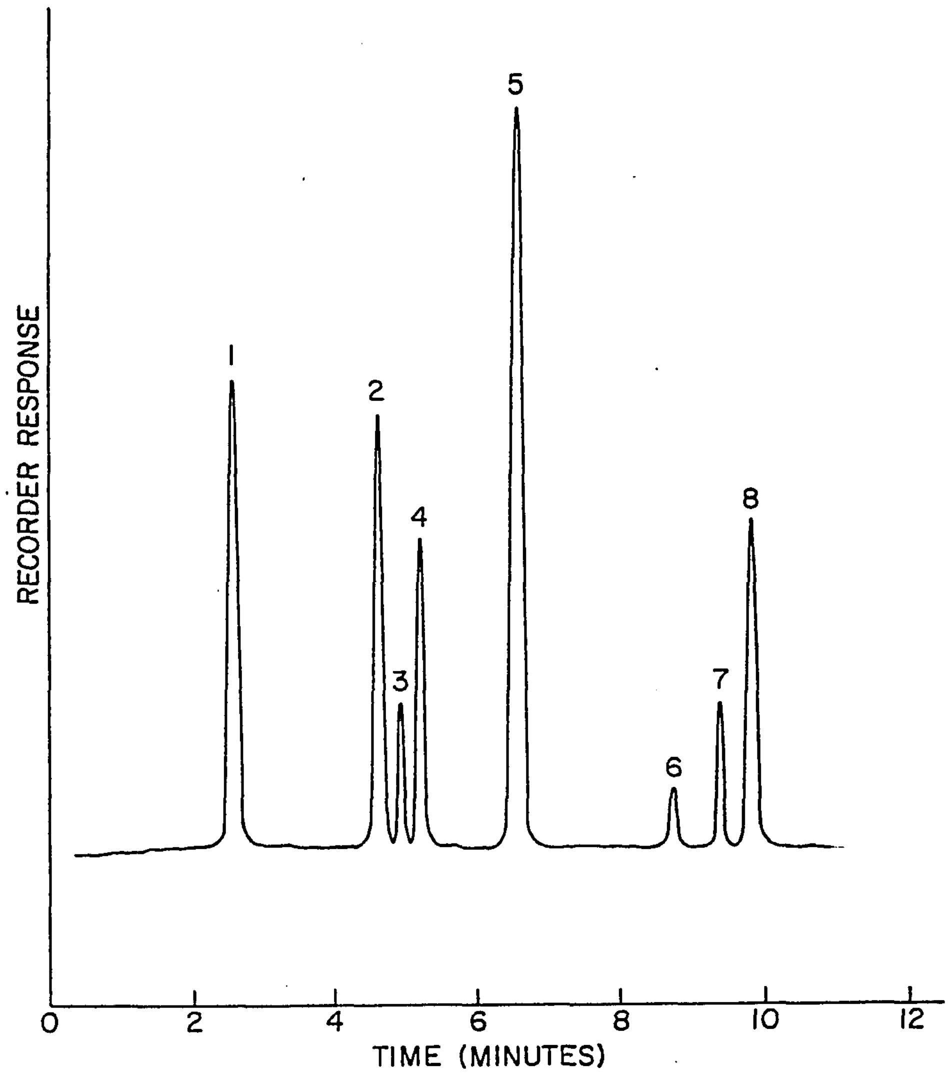

Figure 1. GLC chromatogram of a standard mixture of organic acids as TMS derivatives.

Peaks:
1. Lactic
2. Phosphoric
3. Succinic
4. Fumaric
5. Malic
6. Aconitic
7. Citric
8. Quinic

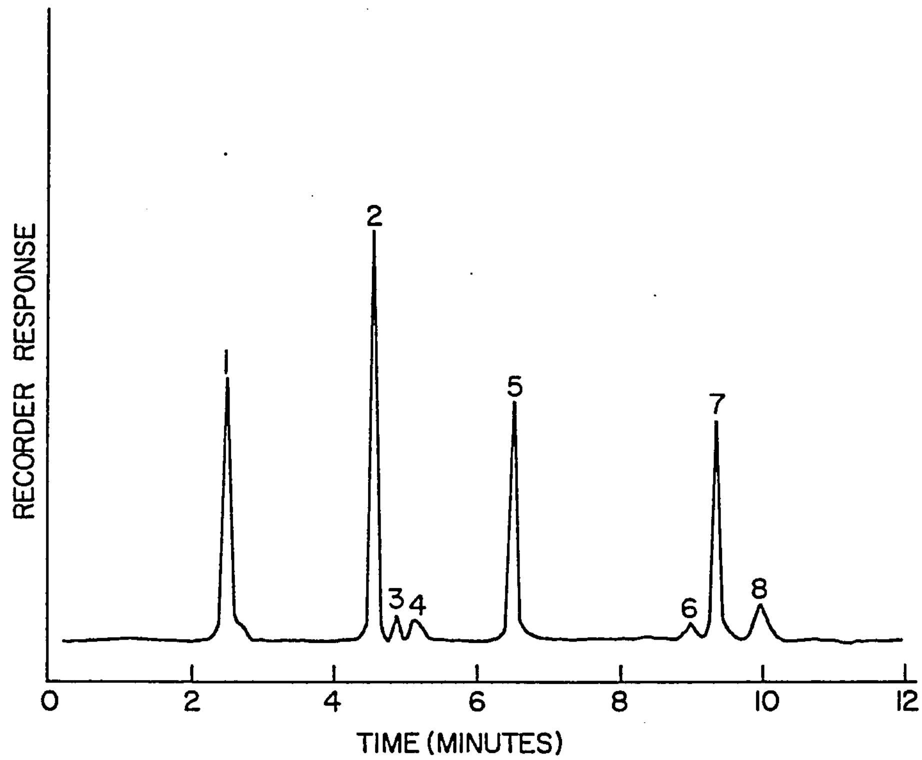

Figure 2. GLC chromatogram of organic acids isolated from plain yogurt.

Peaks: 1. Lactic
2. Phosphoric
3. Succinic
4. Fumaric
5. Malic
6. Aconitic
7. Citric
8. Quinic

Table 13. Relative retention times of pure organic acid standards and organic acids extracted from plain yogurt.

Pure compound	Organic acid						
	Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
Standard mixture	0.44	0.73	0.77	0.81	1.31	1.40	1.46
Plain yogurt extract	0.46	0.74	0.78	0.81	1.33	1.41	1.49

TLC Identification of Organic Acids

TLC was used as a further attempt to identify the organic acids extracted from the plain yogurt material. Ganshirt et al. (1965) cited Braun and Greenen who used the same solvent mixture as employed in this study to extract and dissolve the acids. Using the same silica gel G layered plates and solvent mixture for separation, three extracted materials were identified as having Rf-values identical to pure standards of lactic, malic and citric acids. Under the conditions of this TLC separation, phosphoric acid did not migrate on the silica gel G plate as it appeared in both the sample and standard at the initial point of application. Succinic and fumaric did not appear on the TLC plates after development in the case of both pure standards and yogurt extract.

GLC Recovery Characteristics of Silyl Derivatives of Organic Acids

An aliquot of a solution of organic acids in water:alcohol (20:60) was taken to give 0.5 mg each of lactic, phosphoric, succinic, fumaric, malic, aconitic, citric and quinic acid. After drying on a water bath, it was silylated with BSTFA and chromatographed. An aliquot of another solution to give 5.0 mg of the same organic acids excluding malic acid was taken. This later solution was chromatographed through the ionic resin columns and subjected to the analytical procedure as described for the yogurt samples in this work. At the end of the extract evaporation, 0.5 mg of malic acid was added to the vial. Peak areas from the printing integrator analyses of the chromatograms were used to obtain the mg of each organic acid in the samples. Recovery percent was calculated comparing these two results (Table 14).

Under the conditions of this procedure, the percent recovery of lactic, phosphoric and quinic acids can be considered reasonably realistic (106%, 113% and 115%, respectively). Two acids (succinic and fumaric) portrayed unusually high recovery percents (158% and 199%, respectively). Additionally, two compounds (aconitic and citric) gave unrealistically low recovery rates (67% and 74% respectively). As seen in later analyses, since fumaric and quinic acids occur in plain yogurt in such low concentrations their percentage recovery considerations are of minimum significance. On the other hand, succinic acid probably occurs in intermediate amounts. The recovery factor in this case (158%) is unexplained and introduces a degree of uncertainty in the interpretation of certain subsequent analyses for this acid.

Percentage Calculation of Organic Acids Determined in Plain Yogurt by GLC

The relative peak areas (R) were obtained for each identified organic acid as described in Material and Methods. These areas were then applied to the calibration curves for the specific organic acids and the values in mg were determined. From these values, the percentage of each organic acid was determined.

Replicated Analyses of Organic Acids in a Single Plain Yogurt Sample

Four aliquots from a well-mixed container of brand A were taken to make four replicated analyses. Percentages of organic acids in the

Table 14. Percentage recovery of organic acids determined by direct GLC injection as compared to GLC following column chromatography.

	Organic acid						
	Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
Direct GLC injection (mg)	0.70	0.67	0.67	1.41	0.54	1.17	0.66
GLC after resin fractionation (mg)	0.74	0.76	1.06	2.81	0.36	0.87	0.76
Recovery (%)	106	113	158	199	67	74	115

samples were determined in the same way as described in the experimental methods section of this work.

It is of interest to compare certain values in Table 15 with ones reported as being characteristic or average for a milk product of the composition represented in this yogurt sample. The first of these is in connection with the characteristics of titratable acidity. When products such as yogurt (or other fluid milk products) are titrated directly with 0.1 N NaOH using phenolphthalein as an end point indicator the resultant titratable acidity value (T.A.) is expressed as percent lactic acid. However, it is actually comprised of two different components. One is lactic acid, per se (L.A.). This is also referred to as real or developed acidity. The other is referred to as apparent acidity (A.A.) and is due to the fact NaOH reacts with certain non-fat milk constituents. These include protein, salts (including phosphates and citrates) and dissolved CO_2. In addition, the color change of the indicator phenolphthalein occurs at about pH 8.3 or slightly on the alkaline side of the pH scale. This relationship can be summarized arithmetically as T.A. = L.A. + A.A. In a fresh, unfermented product T.A. = A.A. The normal T.A. of a fresh, unfermented fluid milk product can be estimated by multiplying the percent milk solids non-fat (M.S.N.F.) by the empirical factor 0.018 (Frandsen and Nelson, 1950,p.107). The low fat, plain yogurt sample analysed in this replicated observation was standardized to contain 2% fat. The remaining 10.72% M.S.N.F. should account for 0.19% A.A. (0.018 x 10.72% = 0.19%). The L.A. portion of the T.A. becomes 1.96% (2.15% - 0.19% = 1.96%). This compares with the mean GLC-determined

Table 15. Percentages of organic acids in a replicated analysis of plain yogurt.

	Organic acids				
Aliquot no.	Lactic	Phosphoric	Succinic	Fumaric	Citric
1	1.07	0.59	0.35	-	0.16
2	2.21	0.32	0.41	0.12	0.21
3	2.21	0.35	0.56	0.07	0.33
4	1.31	0.37	0.27	0.03	0.32
Mean	1.70	0.41	0.40	0.07	0.26
Standard deviation	0.60	0.12	0.12	0.04	0.08

Titratable acidity (%)	2.15
pH	3.65
Total solids (%)	12.72

value of 1.70%. It is possible that at least a portion of the difference between the calculated value (1.96%) and determined amount (1.70%) can be attributed to succinic acid a probable metabolite of microflora growth.

The second comparison of interest is phosphoric acid. The value, in this case, is calculated on the assumption that all the phosphorus (both inorganic and organic), at the low pH involved, is extracted and determined as H_3PO_4 (silyl derivative) in this method. Jenness and Patton (1959, p. 161) report that normal fluid milk (8.5% M.S.N.F.) contains 95 mg P/100 ml. Transformed to the equivalent of 10.72% M.S.N.F. this gives 119.8 mg P/100 ml ($95 \times \frac{10.72}{8.5} = 119.8$). Since H_3PO_4 has 32% P this is equivalent to 0.38% H_3PO_4. The GLC determined value is 0.41%.

Finally, Jenness and Patton (1959, p. 161) report the average citrate content of milk (expressed as citric acid) with 8.5% M.S.N.F. is 0.175%. In the case of the analysed sample with 10.72% M.S.N.F., the comparable calculated value is 0.22% ($0.175\% \times \frac{10.72}{8.5} = 0.22\%$). The GLC-determined value is 0.26%.

These three comparisons (lactic, phosphoric and citric acids) between calculated and determined values would indicate that the means from the replicated analyses are reasonably representative of expected values.

Organic Acids in Plain Yogurt
Samples from Three Brands

Comparison of the results from the replicated analyses of a single plain yogurt (Table 15) with the mean values obtained from three brands (Table 16, 17, and 18) is difficult. The summary is:

		Mean percentages of organic acids				
Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
Analyses Brand A						
0.36	0.33	0.29	-	0.09	0.32	0.04
Analyses Brand B						
0.57	0.35	0.17	-	0.16	0.32	0.06
Analyses Brand C						
0.43	0.36	0.14	-	-	0.35	0.06
Four replicates of Brand A						
1.70	0.41	0.40	0.07	-	0.26	-

The major question concerns the lactic acid values. As previously discussed, the mean lactic acid concentration determined in the GLC replicated analyses agrees closely with a calculated amount based on percent titratable acidity considerations. The low mean lactic acid values for each of the three brands can only be explained on the basis of unexpected irregularities in the integration of peak areas of the chromatograms concerned.

On the other hand, the mean values for phosphoric (0.33%, 0.35% and 0.36%) and citric acids (0.32%, 0.32% and 0.35%) are not only very similar for each brand but also reasonably comparable to the replicated analyses means (0.41% and 0.26%, respectively).

Table 16. Percentage of organic acids in brand A of plain yogurt.

Sample no.	Organic acid						
	Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
1	0.84	0.42	0.17	0.05	0.09	0.50	0.06
2	0.23	0.32	0.24	-	0.09	0.43	0.04
3	0.73	0.25	0.15	0.01	0.11	0.28	0.04
4	0.10	0.41	0.09	0.11	0.08	0.38	0.04
5	0.55	0.45	0.60	-	0.11	0.50	0.05
6	0.10	0.25	0.42	-	0.10	0.29	0.05
7	0.18	0.34	0.53	-	0.09	0.24	0.03
8	0.65	0.36	-	-	0.08	0.24	0.04
9	0.38	0.43	0.62	-	0.07	0.27	0.03
10	0.24	0.26	0.15	-	-	0.27	0.03
11	0.23	0.32	0.13	-	0.08	0.33	0.04
12	0.27	0.31	0.14	0.15	-	0.19	0.03
13	0.15	0.23	-	-	-	0.24	0.04
Mean	0.36	0.33	0.29	-	0.09	0.32	0.04
Standard deviation	0.25	0.07	0.21	-	0.01	0.10	0.01

Table 17. Percentage of organic acids in brand B of plain yogurt.

Sample no.	Organic acid						
	Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
1	0.65	0.37	0.20	0.11	0.18	0.38	0.05
2	1.18	0.37	0.21	0.07	-	0.44	0.04
3	0.43	0.34	0.17	0.01	0.15	0.33	0.09
4	0.67	0.38	0.15	-	0.15	0.32	0.08
5	0.27	0.35	0.17	-	0.14	0.25	0.07
6	0.23	0.28	0.11	-	-	0.25	0.05
Mean	0.57	0.35	0.17	-	0.16	0.33	0.06
Standard deviation	0.35	0.04	0.04	-	0.02	0.07	0.02

Table 18. Percentage of organic acids in brand C plain yogurt.

Sample no.	Organic acid						
	Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
1	0.61	0.10	0.06	-	-	0.29	0.04
2	0.54	0.63	0.21	0.08	0.15	0.55	0.07
3	0.31	0.36	0.18	-	-	0.22	0.05
4	0.26	0.34	0.11	-	0.15	0.32	0.07
Mean	0.43	0.36	0.14	-	-	0.35	0.06
Standard deviation	0.17	0.22	0.07	-	-	0.14	0.02

Mean succinic acid concentration tends to be high in both the replicated analyses of brand A and in the 12-sample value of this product (0.29% and 0.40%, respectively) as compared to the similar amounts for brands B and C (0.17% and 0.14%, respectively).

It is not possible to compose the relatively low to trace amounts of fumaric, aconitic and quinic acids between brands or in the replicated results.

Since there are only two samples involved in brand D, comparison of their organic acid values with other analyses is not possible (Table 19).

Table 19. Percentage of organic acids in brand D of plain yogurt.

Sample no.	Organic acid						
	Lactic	Phosphoric	Succinic	Fumaric	Aconitic	Citric	Quinic
1	0.26	0.52	0.19	-	0.16	0.56	0.10
2	0.63	0.13	0.14	-	0.12	0.20	0.03

CONCLUSIONS

Samples of three available commercial plain, low-fat yogurt were collected and analysed during a 65-day interval. Various gross and detailed composition parameters were characterized by:

1. Mean percent titratable acidity values for each brand were 1.42%, 1.63% and 1.80%. Individual samples values ranged from 1.3% to 2.0%.

2. For the same three brands, mean pH values were 4.08, 3.85 and 3.98 with individual samples ranging from pH 3.6 to pH 4.1.

3. Mean percent total solids values arranged in the same brand sequence were 14.49%, 12.33% and 17.82%. The range in individual samples was 11.31% to 18.43%.

4. The most common flavor criticisms encountered were various combinations and incidences of "lacks fine flavor", "low acid" or "high acid". A general skew toward mediocre to poor was evident in the assigned numerical flavor score distribution.

5. Gas liquid chromatography (GLC) analysis of silyl derivatives identified lactic, phosphoric, succinic and citric acids. It was not possible to detect significances in quantitative amounts of these acids between brands or individual samples.

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