This is to certify that the

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High Performance Liquid Chromatographic Analysis of Natural Fermented Yogurt

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Charles M Stine
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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF NATURAL FERMENTED YOGURT

By

Michael Lee Richmond

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ABSTRACT

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS
OF NATURAL FERMENTED YOGURT

By

Michael Lee Richmond

The popularity and consumption of yogurt has increased tremendously in the U.S. A recent study of commercial yogurts revealed wide variation in chemical content, net weight and caloric content. Improved uniformity in composition and quality would benefit yogurt processors. Because phase separation is a concern of commercial importance, experiments were designed to assess the role of secondary packaging and stabilizer blends in reducing physical damage. Stretch wrapping the stack proved effective in minimizing physical damage ($P < 0.01$).

In another study two high performance liquid chromatographic (HPLC) systems were developed to separate and quantitate various simple sugars and sugar alcohols in food matrices, fresh fruit and yogurt. Using a bonded phase system, two columns connected in tandem and a ternary mobile phase (acetonitrile/water/ethanol) fructose, glucose, sorbitol, sucrose and maltose were accurately separated in twenty minutes. Twenty-four fruits were analyzed for carbohydrate content. Fruits from the Rosaceae family generally contained sorbitol, whereas none of the other fruits examined contained sorbitol. This procedure proved to be fast, simple, and reliable for analyzing simple carbohydrates in food systems,

especially for separating glucose from sorbitol. This was accomplished by addition of a second column.

A majority of the world's population is considered lactose intolerant. Because lactose occurs naturally in many dairy foods, and is added commercially to a variety of non-dairy foods in the form of lactose, dried whey or milk, there are concerns about lactose content in many foods. An HPLC procedure was developed to separate and quantitate lactose, glucose and galactose in dairy products. The system contained a temperature elevated resin-based column ($80^{o}C$) and used water as the eleuent. The three sugar mixture was easily separated in ten minutes. Hydrolysis of lactose and accumulation of glucose and galactose was followed through ripening and long term storage of yogurt. Lactose content decreased from 7.12% to 4.19% after 14 days, while galactose content increased from 0% to 1.06% at 14 days. Glucose remained at trace levels. Autoclaved lactose containing microbiological media were also evaluated using this system. A compound having the same retention time as lactulose was observed in autoclaved media.

I wish to dedicate this book to my parents Lester and Josephine. Mom and Dad I love you both. Thank you for your support and encouragement. I could not have done it without you.

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I am also indebted to the University of Guelph for preparing and evaluating taste panel data, as well as Dr. Ramesh Chandan for helping in this endeavor. I also want to thank Sterling Thompson for performing some microbiological analyses. Others who helped and I wish to acknowledge are: Dr. J. Gill, Dr. H. R. Struck, Dr. C. J. Mackson. Numerous students also helped me with various experiments and projects to which I am deeply indebted; they include Dave Barfuss, Maxwell Sherman, John Stevenson and Dave Staley. In addition, I would like to thank the secretaries that I have worked with over the years -- Debby, Tammy, Patti and Mary. Finally, I would like to thank those who have helped me along the way and I have forgotten to mention here -- thank you all, I appreciated it.

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INTRODUCTION

In recent years, the popularity of yogurt in the U.S. has grown tremendously. While many factors are responsible for the expansion of this market, fruit addition along with multimedia advertising are considered most important. Because of this sudden growth, a definite need has arisen for broad range scientific research of yogurt.

Several important areas needing further examination are product composition and development, physical and chemical damage during distribution and storage, current literature status and various processing parameters.

Lactose intolerance reportedly affects a large portion of the world's population. Dairy products play a vital role in the diets of many individuals and because lactose is often in moderate to high concentrations in various dairy products such as yogurt, rapid and accurate measurements of lactose and other sugars are needed for industrial and academic purposes.

The separation ana analysis of simple sugars and sugar derivatives, whether added to foods commercially or occurring naturally, is a current and important nutritional topic.

This presentation addresses these concenrs and outlines them in chapter form with the intent of (1) providing a broad spectrum of practical research about yogurt, and (2) providing information on the separation and analysis of simple carbohydrates in dairy products and (simple sugars and sorbitol) in various botanical families of fruit.

CHAPTER I

INTRODUCTION AND REVIEW[1]

[1]A Review of Yogurt Technology with Emphasis on Research Since 1970. To be submitted to Dairy Sci. Absts. (England)

REVIEW OF THE LITERATURE

Yoghourt, Yoghurt, Yogurt. There seem to be as many names for this food as there are flavors. The popularity of this nutritionally healthful food has increased the world over in recent years. Although per capita consumption in the U.S. is well below that of most European countries, significant increases have been made during the past decade. The popularity of yogurt has increased for many reasons. However, fruit and other flavors, increased advertising expenditures and marketing strategies have played an important role in developing the current demand for this excellent food.

Consumption in the U.S. is currently about 1.2 kg per capita compared to 2.26 kg per capita for cultured buttermilk (Milk Ind. Found., 1981). European consumption rates for yogurt are much higher than U.S. figures (Rasic and Kurmann, 1978). Increasing consumption of yogurt in the United States by improving marketing strategies and product quality will benefit the dairy industry and other related industries (fruit, preserves, packaging) as well.

This review of yogurt will be primarily concerned with recent advances in production and research since the review by Humphreys and Plunkett (1969). This paper will primarily discuss yogurt made from bovine milk using the mixed starter culture Lactobacillus bulgaricus and Streptococcus thermophilus. Further, since previous reviews have focused on yogurt manufacture outside the Continental U.S. (Europe and Australia) this review will highlight yogurt manufacture and current research in the U.S. and abroad.

Since 1969 a number of reviews describing various aspects of yogurt have been published (Humphreys and Plunkett, 1969; Lundstedt, 1971; Mann, 1973 a,b,c; Robinson and Tamime 1975, Kosikowski, 1977; Tamime and Deeth, 1980). Rasic and Kurmann (1978) recently published a book on yogurt, and various chapters have also been devoted to this subject (Kosikowski, 1977; Chandan, 1982). An interesting article on the origin and culture of fermented milks, including yogurt was described by James (1975).

In 1969, Humphreys and Plunkett reviewed yogurt and its manufacture describing many processing parameters, and Lundstedt (1971) discussed manufacture with regard to incubation temperature, suggesting a lower incubation temperature ($30^{o}C$) and longer incubation time (12-16 h) would improve the product. Mann (1974) updated literature from around the world, discussing many concerns about yogurt in a three-part series (1973a,b,c). In 1975, Davis described developments in yogurt technology in the United Kingdom (U.K.). He reported on the history of fermented milks, the role of yogurt bacteria, and also described a process for continuous manufacture of yogurt. Robinson and Tamime's review in 1975 outlined numerous methods used world wide for the production of yogurt; methods for processing stirred, set and liquid yogurt and their respective processing parameters were described. More recently, Tamime and Deeth (1980), in an exhaustive review, covered the technology and biochemistry of yogurt. They discussed physical and chemical changes during processing, fermentation and storage.

TABLE I
Nutrient Content of Commercial Yougurts[a]

	Water g	Energy kcal	KJ (KCAL x 4.184)	Protein N x 6.38 g	Lipids					Carbohydrate g	Ash g	Minerals							Vitamins								
					Total g	Saturated Fatty Acids g	Monounsaturated Fatty Acids g	Polyunsaturated Fatty Acids g	Cholesterol mg			Calcium mg	Iron mg	Magnesium mg	Phosphorus mg	Potassium mg	Sodium mg	Zinc mg	Ascorbic Acid mg	Thiamin mg	Riboflavin mg	Niacin mg	Panthothenic Acid mg	Vitamin B_6 mg	Folacin mcg	Vitamin B_{12} mcg	Vitamin A (R.E.)	Vitamin A (I.U.)
Yogurt																											
Plain, whole milk 8 g Protein/227 g (100g)	87.90	61	257	3.47	3.25	2.10	.89	.09	13	4.66	.72	121	.05	12	95	155	46	.59	.53	.029	.142	.075	.389	.032	7	.372	30	123
Plain, lowfat 12 g protein/227 g (100g)	86.07	63	265	5.29	1.55	1.00	.43	.04	6	7.04	1.09	183	.08	17	144	234	70	.89	.80	.044	.212	.114	.591	.049	11	.562	16	66
Plain, skim milk 13 g protein/227 g (100g)	85.23	56	233	5.73	.18	.12	.05	.01	2	7.68	1.18	199	.09	19	156	255	76	.97	.87	.048	.234	.124	.641	.053	12	.613	2	7
Coffee and Vanilla, 227 g lowfat, 11 g protein/227g (100 g)	79.00	85	358	4.93	1.25	.81	.34	.04	5	13.80	1.02	171	.07	16	135	219	66	.83	.75	.042	.201	.107	.552	.045	10	.527	13	54
Fruit, low fat 9 g protein/227 g (100g)	75.30	99	415	3.98	1.15	.74	.32	.03	4	18.64	.93	138	.06	13	109	177	53	.67	.60	.034	.162	.086	.446	.037	8	.426	12	49

[a]National Dairy Council (1979)

The intent here is to review yogurt and summarize newer research related to the technology, chemistry, production, distribution and storage of yogurt.

Nutrition Information

Cultured dairy foods are an important part of the dairy industry and the consumption of yogurt is very important to the increasing growth of the cultured foods market. From a nutritional stand point increased yogurt consumption has been related to (1) greater nutritional awareness and (2) possible therapeutic benefits from continued ingestion of living bacteria, specifically L. bulgaricus (National Dairy Council, 1972; Deeth and Tamime, 1981).

In a recent review, Deeth and Tamime (1981) discussed the nutritional value of yogurt. The nutritional content of various yogurts are depicted in Table 1. Deeth and Tamime (1981) reported on many nutritional concerns including chemical composition, vitamin content and digestibility. During manufacture the biological value of the protein increases, varying amounts of lactose are hydrolyzed, but there is little change in the lipids of the yogurt, other than slight hydrolysis. In terms of possible therapeutic effects of yogurt much research is still needed.

About 10 years ago there was a controversy as to whether yogurt induced cataracts (Richter and Duke, 1970). Rats fed yogurt exclusively grew at normal rates, reproduced, and showed no signs of deficiency after one and one half years. However, formation of catacts was observed in some animals. Research indicated galactose

accumulation was responsible for this condition, a lens clouding condition of the eye (Richter and Duke, 1970; Anonymous, 1970). However, rats are deficient in the enzyme that normally breaks down galactose (galactose-1-phosphate uridyl transferase) but humans have adequate supplies of this enzyme. Bahrs (1971) reported that normal consumption of yogurt was not harmful to human health nor did it give rise to cataract formation.

Attitudes, Marketing, Sales

The attitudes about yogurt vary widely with geographic area (both Nationally and Internationally). Kroger and Fram (1975) conducted a survey to determine consumer preferences in the Northeastern U.S. Of 400 households surveyed only 40% indicated any yogurt use whatsoever. Less than half of the users surveyed knew there were bacteria in the product; 74% of 161 respondents preferred fruit yogurt to plain.

The United Dairy Industry Association (UDIA) receives more inquiries about yogurt than all other dairy products combined (Quackenbush, 1976). In 1977, a National Survey (4000 respondents) regarding attitudes toward various dairy products was conducted. Nearly one half of the people surveyed had never eaten yogurt. Females consumed yogurt more frequently and particularly women in their late teens; males 20-24 had the lowest consumption. The pacific region showed the highest usage and both high and low income groups consumed yogurt. In 1977, 109 yogurt plants (representing 75% of the U.S. yogurt production) were surveyed (Knutson, 1978). Fresh yogurt accounted for 82% of total yogurt production, while

frozen yogurt mix (soft serve) and hard frozen yogurt accounted for 5.5 and 12.5%, respectively. Yogurt was produced in over 50 flavors and 85% of the yogurt produced was low fat. Yogurt sales constituted 3.5% of total dairy sales but the profit margin was 18.8%. Fifty three percent of the fresh yogurt produced was sundae style (fruit on the bottom, stirred up) and 37% swiss style (homogenous product). The remainder was plain.

Like other yogurt markets, sales increases have paralleled fruit incorporation and this trend was also noted in Germany (Rockseisen, 1977). Plain yogurt still commands 30% of this market. In 1975 total yogurt consumption in Germany was 284.2 thousand tons. Although yogurt processors were numerous, there were less than 10 major companies selling and marketing yogurt. Rockseisen (1977) also noted that consumers did not want added preservatives in yogurt. Van Hoof (1982) reported that the yogurt market grew 148% between 1970-78 in the U.K. He reported that yogurt consumption was expected to double again over the next decade. Natural (plain) yogurt accounted for 9%, and fruit yogurt 91% of the sales in the U.K.

Duame (1979) reported that to improve yogurt consumption every processor must work to improve the quality and flavor of their product. More recently yogurt consumption appears to be leveling off in the U.S. (Anonymous, 1981). New market strategies are

currently being formulated to increase consumption. Further, post pasteurization heat treatment killing viable bacteria and the introduction of artificially acidulated yogurts may also be jeopardizing attitudes and hence growth of the yogurt market. There is agreement that there may be a market for post-pasteurization heat treated yogurt, as well as acidulated artificial yogurt (Kroger, 1976; Schock, 1977) but these products should be regarded differently.

Regulations, Quality, Composition

Over the past decade much discussion and debate has surrounded regulation of commercial yogurt. Davis (1971) provided an in-depth discussion of compositional standards and reported there was no justification for the use of emulsifiers and stabilizers. He also said that yogurt should be catagorized by fat content. Some simple tests to identify and enumerate yogurt bacteria were described noting that no legal standards were necessary but industry standards should be adopted. Davis (1971) recognized a place for post-pasteurized yogurt but noted that the product should not be called yogurt. Other topics regarding proposed standards were pursued by the author.

In 1976, Robinson discussed regulatory trends for yogurt in Europe. He felt the nutritional content should not be less than that from bovine milk with the possible exception of fat content. He addressed bacteria in yogurt from various standpoints: does the

consumer expect viable and abundant bacteria in yogurt? Do these bacteria contribute to product quality? These are hard questions to answer for a large population. Most certainly, preferences differ greatly world wide. Robinson (1976) reported that Canada did not intend to introduce compositional standards because free market competition and responsible producer attitudes would insure product quality.

Proposed definitions and standards for yogurt (United States) were published in the June 10, 1977 Federal Register (Summers, 1980). A definition of yogurt was presented and various standards described, including post-pasteurization heat treatment. If the product is heated after culturing then the parenthetical phrase "heat treated after culturing" must follow the name of the food. Since publication of these proposed standards, the Milk Industry Foundation (MIF) has outlined numerous rejections regarding formulation and manufacture. As of this writing there have been no legal standards adopted for yogurt in the U.S. Rasic and Kurmann (1978) included in appendix form the FAO/WHO draft standards (1977) for yogurt and sweetened yogurt as well as standard for flavored yogurt and products heat treated after fermentation. It becomes clear that standards vary from country to country and the many people involved in writing and using these standards are concerned about assuring a wholesome milk product.

The quality of yogurt is important from many standpoints (Kroger, 1973, 1976a, 1976b; Robinson and Tamime, 1976; Connoly, 1978). Kroger (1973) defined the study and control of quality in

yogurt as (1) expert analysis through sensory, subjective or sensory evaluation, and (2) scientific, technical or laboratory analysis, with the objective measurement of chemical, physical, microbiological, biochemical and rheological properties. While noting yogurt was a loosely defined product, he summarized essential requirements for production of high quality yogurt. Kroger (1976a) reported yogurts need to be standardized, properly defined, described and limited in its composition by regulations and by doing this a stable and important consumer-product relationship should result. Kroger (1976a) like Davis (1971) agreed there was room for a long shelf life yogurt on the market, but it should not be called yogurt. As previously stated there is much concern about this product and how it should be labeled.

Robinson and Tamime (1976) reviewed and compared international standards for yogurt. Emphasis was placed on defining yogurt and standard methods of analysis; desirable methods for compositional analyses and physical assessment were described. They reported that microbiological examinations should be made for the following reasons: (1) to insure the absence of pathogenic and spoilage organisms, and (2) to check that the desired bacteria are there and in large numbers. Robinson and Tamime (1976) concluded that although techniques necessary to assess physico-chemical and microbiological quality of yogurt are available, there is little published agreement regarding testing. In 1978, Connoly discussed

standards necessary to implement quality assurance programs, including general standards (plant facilities, buildings, grounds, restrooms, equipment, etc.) and product standards. After packaging, yogurt should be held in cold storage for 14-24 h before shipping. At this time quality assessment of the finished product should be made.

Many analyses and studies of yogurt have been made during the past 10 years showing wide variation in composition (Duitschaever et al, 1972; Kroger and Weaver, 1973; Davis and McLachlan, 1974; Richmond et al, 1979; O'Neil et al, 1979). Duitschaever et al (1972) examined 152 yogurts in Ontario, Canada and found yogurt of uniform composition was generally not available. Net weights ranged widely with mean overfill of 7.2%. pH values varied from 3.27 - 4.53 with a mean of 3.91. In another survey (Kroger and Weaver, 1973) commercial yogurts in the Eastern United States were examined. Again constant overfill was a major problem (6.87%). Wide variation of chemical components was also noted. Davis and McLachlan (1974) investigated various properties of yogurt (plain, strawberry, black currant) from the London area including sugar content, solids nonfat (SNF), fat, pH and viable bacteria. pH values ranged from 3.7 to 4.3; titratable acidity ranged from 0.64 - 1.50% lactic acid; viable bacteria counts ranged from 10^7 - 10^9 orgs/ml.

Richmond et al (1979) surveyed commercial brands of yogurt in the Michigan area. Wide variation was observed between and within brands. Mean values of all fruit-flavored yogurts (n=42) were 4.34% protein, 2.34% fat, 25.88% T.S. and 4.01 pH (Table 2).

TABLE 2 Chemical composition of various flavored yogurts.[a,b]

Product category	Protein (%)	Fat (%)	Total solids (%)	pH
Low fat yogurt (N = 28)	4.26 ± 0.35	1.56 ± 0.28	25.83 ± 2.17	4.07 ± 0.16
Full fat yogurt (N = 14)	4.51 ± 0.18	4.01 ± 1.00	26.39 ± 1.58	3.88 ± 0.13
All samples (N = 42)	4.34 ± 0.33	2.34 ± 1.29	25.88 ± 1.99	4.01 ± 0.17

[a]Mean ± standard deviation

[b]Richmond *et al* (1979)

Corresponding values for plain yogurt (n=5) were 5.68, 2.86, 16.90 and 4.23, respectively. While product overweight was again considered a problem, 10.6% of all samples surveyed weighed less than declared container net weight. This is a potentially serious problem from a legal standpoint. Results again indicated that commercial yogurt would benefit from closer compositional control. O'Neil et al (1979) surveyed commercial yogurts from Central New Jersey. They determined uniformity in composition and consistency within and among brands of plain yogurt and effect of storage time upon consistency of yogurt. Their data revealed significant variations in composition and consistency among several brands of commercial plain yogurt.

Lindsay et al (1981) used consumer preference evaluation and descriptive analysis to demonstrate influential flavor and textural properties of yogurt products. Consumer panelists were separated into three distinct age groups. Sundae-style yogurts were preferred over swiss-style yogurts by a college student population. The preference for sundae-style over swiss-style was believed related to the appearance that sundae-style yogurts contained more fruit. High tartness intensity and low sweetness intensity in sundae style yogurts caused lowered consumer preference for a general population, while lumpy consistency improved or had no effect on consumer preferences.

Processing and Manufacturing Concerns

The yogurt market differs widely from country to country in Europe and world wide; certain countries prefer set yogurts with or without

fruit, and other countries prefer stirred yogurt (Cottenie, 1978). Although many recipes are available no single recipe is best, nor will one recipe fit into all processing schemes. Bacteria supply houses (culture laboratories) supply starter cultures in liquid, freeze-dried and concentrated deep-frozen form.

Processing and manufacture of yogurt was recently reviewed (Tamime and Greig, 1979; Rasic and Kurmann, 1978). Tamime and Greig (1979) discussed many aspects of manufacture and technology. A broad range of topics were discussed including fermentation tanks and incubators, agitation of the coagulum, in-tank cooling, pumps coolers, pipes, fittings and restrictions, and packaging equipment. A flow diagram of yogurt manufacture is depicted in Figure 1. Under certain conditions homogenization and pasteurization may be interchanged provided yogurt mix is not contaminated after pasteurization. With set yogurt incubation takes place in the container, while fluid and stirred yogurt are produced in bulk vats.

Cottenie (1979) described the manufacture of set and stirred type yogurts. An interesting discussion of cooling tunnels was provided noting that too extensive cooling in the beginning may cause phase separation (whey-off). Other factors involving yogurt production also were described. Currently 80-90% of yogurt manufactured in the U.K. is of the stirred variety (Tamime and Greig, 1979). These authors described various effects of mixing stirred yogurt, noting that structural damage to the coagulum is

likely to occur during manufacture. However, different types of processing tanks and a variety of mixing paddles are availalbe to minimize this type of damage. While higher agitation speeds provide greater cooling, they also increase the shearing effect (Tamime and Greig, 1979). Tamime and Greig (1979) described advantages and characteristics of different pumping systems including design considerations. They recommended yogurt be pumped at low velocity through large diameter pipe of minimum length, restrictions and fittings.

Stocklin (1969) described the production and handling of yogurt (fruit-flavored sundae, swiss) on a commercial scale in the U.S. He reported incubation was completed when trace amounts of whey were visible on top of the coagulum, and that this criterion was a better way to determine set time than pH or titratable acidity. Aggarwal (1975) used cryoscopy for acidity measurement in commercial yogurt production. The freezing point of the mix decreased with increased acid development. He reported cryoscopy may be an alternative to titratable acidity and pH measurement during yogurt manufacture.

Lundstedt (1973) described methods for improving yogurt manufacture including (1) lowering incubation temperature ($30^{o}C$) and increasing set time (14-16 h), and (2) pumping the mix through a fine mesh in-line filter/strainer before entering the filling machine. Appel (1976) described some problems and cures in manufacture of swiss-style yogurt. He reported that with the introduction of gelatin stabilizers desired consistency (firm

product) was more easily obtained. Further, fruit supply may be a concern because plant matter such as cherry pits, peach seed pieces and other foreign contaminants interfere with proper operations. And because most processers obtain fruit from more than one supplier the problem is often more difficult to resolve. Another problem encountered is fruit incorporation; receiving fruit in drums rather than boxes enhances in-line feeding of the base to the mixing tank. Another and potentially more serious problem is the availability of sub-standard products (yogurt) at reduced cost. Appel (1976) felt that the yogurt image suffers as a result.

Lundstedt (1978) reviewed some of the problems encountered in yogurt manufacture. He suggested that single bacterial strains should not be purchased and subsequently mixed since some single strain cultures cannot be combined to produce a stable culture. Another problem may be starter milk, which should be of high quality and free of antibiotics. In case of phage attack all cultures (bulk cultures and inoculated tank milk) should be heated to 88^{o}C for 30 min, all milk discarded, and equipment cleaned with caustic soda and rinsed with a 2000 ppm hypochlorite solution. New cultures and a controlled milk supply should then be used. Kurmann (1977) described some current problems as well as new trends in the manufacture of yogurt. He reported that mechanical stirring of yogurt at temperatures above 38-40^{o}C could cause a consistency defect (sandiness). He also stated that the selection of mother cultures should be accomplished according to established technological, nutritional, dietetic and therapeutic criteria.

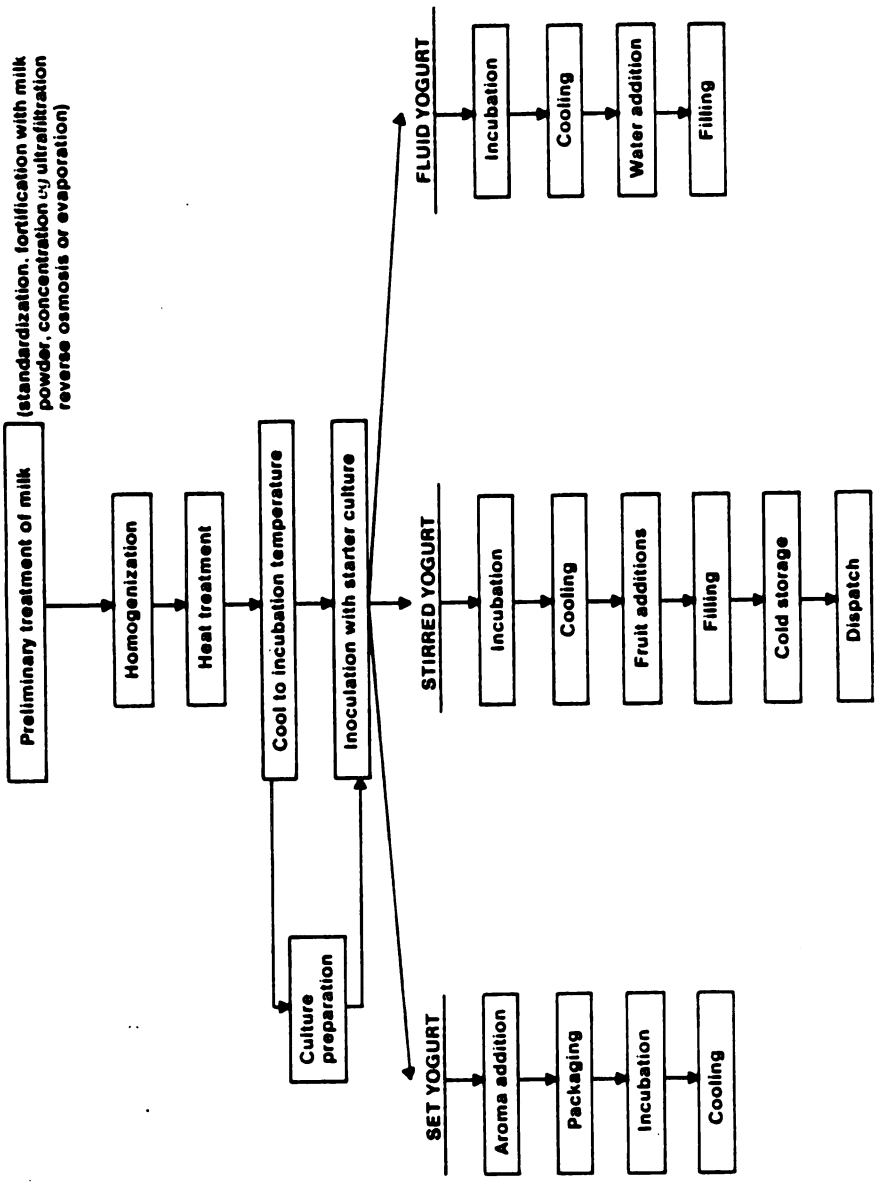

Figure 1. Flow diagram of yogurt manufacture. (Tamime and Greig, 1979)

Tamime and Robinson (1978) described the production of concentrated yogurt (labneh) traditionally consumed in the Middle East. Product concentration is achieved by putting the yogurt into a cloth bag and hanging it to allow whey seepage, or by stacking or piling bags on top of each other. Because of the popularity of this product and the desire to improve final product uniformity, labneh production was evaluated to determine factors influencing quality. Natural yogurt (16% T.S.) proved to be a good starting point for labneh production (24% T.S.). Products of solid contents less than 20%, and greater than 25%, were found to be poorer in quality. Various cultures were used to manufacture labneh.

In another article (Tamime, 1978) the production of yogurt and concentrated yogurt from hydrolyzed milk was discussed. A commercial neutral yeast lactase was used to hydrolyze the lactose. In this study 50% hydrolysis (35^{o}C) was achieved in 45 min. and 99% in 4 h. Tamime reported processing time could be reduced by up to 1 h using hydrolyzed milk. Increased starter activity was related to increased free glucose in the system. By using hydrolyzed milk to make concentrated yogurt, a sweeter product was produced. Starters played an important role in the drainage time of whey. A slime-forming culture (RR) showed the least amount of extracted whey. This culture was not desirable in concentrated yogurt production, since the product became very gummy and lost some of its traditional character.

Jepsen (1977) described the use of membrane filtration for the manufacture of yogurt. Using ultrafiltration (UF) membranes,

protein content was increased without increasing lactose content. Another advantage of UF is the ease with which it can be incorporated into a continuous system. Veinoglou (1978) discussed the production of strained yogurt from UF milk. For production purposes, best results were obtained with 8.5% protein and 9.5% fat. Sensory properties were similar to the traditional product. Nielson (1976) reported on the use of whey solids in yogurt. Dry whey (0.2 - 0.6%) increased viscosity and enhanced acid development. Advantages of using whey and dry whey in yogurt were discussed.

Macbean (1978) discussed the development of mechanized and continuous methods for the production of yogurt. Yogurt mix was heated to 92^{o}C, 45 min. and a two-stage fermentation system used. Yogurt mix was incubated to pH 5.5 (45^{o}C) in a stirred fermenter and pH controlled by inflow of unfermented mix. Mix from the first stage moved into the second stage where it was continued to ferment and allowed to coagulate in a tubular flow fermenter as the mix moved slowly downwards at controlled temperature (<45^{o}C). Difficulty in achieving tubular flow in the pilot scale second stage tubular-flow-fermenter was noted resulting in yogurt with poor texture. In another article, Macbean et al (1979) described pH-stat continuous cultivation and stability of mixed fermentation in continuous yogurt production. The general use of continuous fermentation in yogurt manufacture was discussed.

Angevine (1972) reported on processing yogurt and acidophilus yogurt. He reported long set yogurt (30-32^{o}C; 12-16 h) resulted

in better balance of yogurt bacteria producing more uniform flavor. A procedure for processing acidophilus yogurt was also described. Also discussed were nutritional incentives ascribed to acidophilus yogurt. Mann (1978) reported that acidophilus milk was rapidly becoming a product of commercial importance in the U.S., although production figures do not support this very well. Currently availability of this product as well as liquid yogurt is primarily concentrated in "health food stores" and often at high cost. Rasic and Kurmann (1978) described processing and manufacture of various cultured milks in their book.

While there are many patents involving cultured dairy products, Igoe (1979) described an interesting patent for direct acidified yogurt. The yogurt was prepared from milk, a thickener blend (starch and various vegetable stabilizers) and food grade acidulant. After pasteurization and acidification the mix was subjected to a shearing treatment to produce yogurt like texture. A commercial yogurt currently being introduced in the U.S. is made from goats milk. Aggarwal (1974) discussed manufacture of goats milk yogurt. Yogurt mix (goats milk, 4.3% fat) was pasteurized ($88^{o}C$, 30 min) but not homogenized. Acid development was faster in goat milk than bovine. Goat milk yogurt was whiter in color due to a lack of carotenoid pigments, and the fat globule size. Eventhough goats milk yogurt was not homogenized, there was no detectable fat or cream line due to the smaller fat globules in such milk. Aggarwal reported that acetaldehyde production masked the typical "goaty" flavor. This yogurt did not show any signs of phase separation. Duitschaever (1978) described the manufacture of yogurt

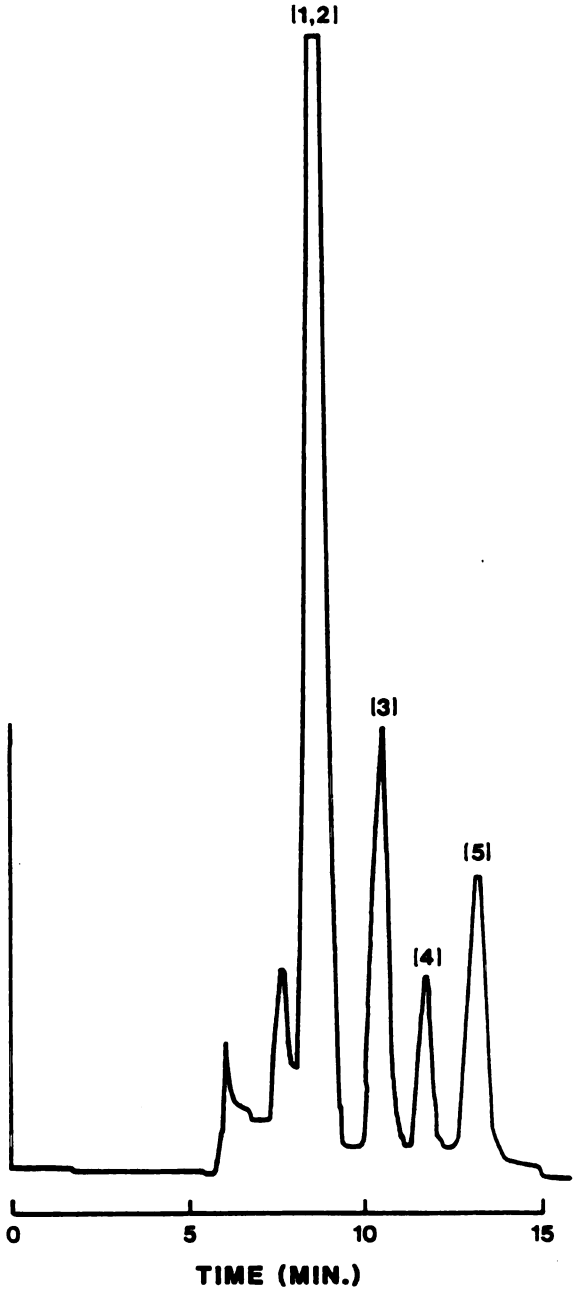

Figure 2. HPLC chromatogram of strawberry yogurt: (1) sucrose, (2) lactose, (3) glucose, (4) galactose, (5) fructose. Bio-rad HPX-87 carbohydrate column (80°C); Aminex A-25 Micro-guard Anion/OH cartridge; solvent, H_2O; flow rate, 0.6 ml/min.; Injection volume, 4µl; attenuation, 8X.

TABLE 3 Qualities of an ideal yogurt starter.[a]

1	Purity, i.e., free from contaminants.
2	Vigorous growth.
3	Production of the right consistency.
4	Production of a good flavor without off flavors.
5	Stability, i.e., its balance should be easily maintained.
6	No tendency to induce syneresis.
7	Should not develop excessive acidity on cold storage.
8	Should have a reasonable tolerance to sugar.
9	Should be resistant to penicilin and other antibiotics.
10	Its maintenance should be easy.
11	It should be phage resistant.

[a]Tramer (1973)

from goat and bovine milk. Goat milk was standardized to 2.0% fat, homogenized at 200kg/cm^2 (2800 psi), pasteurized at 80^{o}C, 15 min, cooled to 45^{o}C and inoculated with culture. Goat milk was fortified with 4% goat skim milk powder; a pH of 4.5 was attained in 170 min. The yogurt did not whey-off during storage at 4^{o}C and was well liked, especially when sugar and/or flavorings were added.

In a series of articles Pinthong et al (1980 a,b,c) described the development of a soy-based yogurt product. During development a product with acceptable acidity was produced using soy milk, 1.0% glucose and 0.1% yeast extract; glucose increased acid production and yeast extract was incorporated to stimulate L. bulgaricus (Pinthong et al, 1980a). After incubation a firm homogenous curd was produced. Phase separation was minimal. In a second article (Pinthong, 1980b) various systems (soy milk + S. thermophilus; soy milk + L. bulgaricus; soy milk + mixed culture) were used to produce a yogurt-like product. Compounds detected included acetaldehyde, acetone, methanol, ethanol, n-pentanal and n-hexanal. Sensory properties were related to levels of n-pentanal produced by S. thermophilus and n-hexanal naturally present in the soy milk.

Pinthong et al (1980c) described the effect of fermentation (using various bacteria) on the levels of oligosaccharides present. High performance liquid chromatography (HPLC) was used for carbohydrate analysis. The degree of oligosaccharide removal was small however. L. bulgaricus reduced the beany odor by removing some of the n-hexanal (Pinthong, 1980c). Richmond et al (1982) used HPLC to separate sugars in strawberry yogurt (Figure 2). Milk salts were present in the area before the sugar peaks. Using a

resin-based LC system lactose and sucrose were not adequately resolved but excellent resolution between isomers, glucose and galactose is shown in this figure.

Protein, Carbohydrate, and Fat

Baysu (1972) investigated changes in amino acid, protein and lactose content of milk during fermentation. Mean values for amino acid content in 25 yogurt samples were reported. Mean values for total protein in milk and yogurt samples were 3.38 and 3.39%, respectively. The decrease in arginine content (milk to yogurt) was significant at the 5% level. Lactose content and pH were significantly different at the 1% level, which was not surprising. Schalichev et al (1971) determined free amino acids in raw milk, heat-treated milk and yogurt. They reported accumulation of free amino acids in yogurt was dependent on heat treatment of the raw milk. The quantity of free amino acids increased with an increase in heat treatment. Free amino acids in cows milk and yogurt (24 and 48 h after production) were qualitatively and quantitatively determined by Kopac-Parkaceva et al (1975). Seventeen amino acids in raw milk and yogurt were identified but their content in yogurt varied depending on the kinds and ratios of starter used. One-day-old yogurt (1:1) showed an increase in all free amino acids, especially tyrosine, phenylalanine and leucine. When starter ratio was changed (1:3; L bulgaricus: S. thermophilus) proline was the major amino acid present. In two-day-old yogurt (1:1) valine,

phenylalanine, lysine and leucine were in greatest concentration. They reported that yogurt produced with a starter ratio of 1:1 contained larger amounts of free essential amino acids.

El-Shibiny et al (1979) discussed the effect of storage on the proteins of zabadi at refrigerator temperatures. Zabadi was periodically analyzed for total nitrogen, non-protein nitrogen, total free amino acids, soluble tyrosine and tryptophan. During storage non-protein nitrogen, soluble tyrosine and free amino acids increased but storage had no effect on the electrophoretic pattern of zabadi proteins. In a related article these same authors (El-Shibiny et al, 1979) discussed the effect of storage on various chemical properties of zabadi. During storage total solids decreased as a result of lactose hydrolysis. Fat content decreased slightly during storage. The acidity of fresh samples increased and pH decreased with increasing concentration of dry milk used. Total volatile fatty acids increased during 7 days storage. Acetaldehyde increased throughout storage as did glucose and galactose content.

Groux (1973), in a discussion of yogurt aroma, reported aromatic components found in yogurt may be from enzymatic deamination of certain free amino acids. He also reported that modified milk proteins resulting from bacterial action were important for coagulum structure. Formisano et al (1971a) discussed the proteolytic activity of L. bulgaricus and S. thermophilus in yogurt. They further discussed the role of the cooling phase on the free amino acid content of yogurt. Both organisms possessed a caseinolytic

enzyme system. Luca (1972) studied the decomposition of non-casein proteins by lactic acid bacteria (LAB) in yogurt manufacture. He reported L. bulgaricus was more proteolytic than S. thermophilus and when both organisms were inoculated together, reduced proteolysis was noted. In general, nitrogen content of the total albumin and globulin fraction decreased during the first two days and then increased. Increases in the nitogen content of the proteose-peptone fraction was also observed. Formisano et al (1974) reported the degree of proteolysis was limited during incubation and the resulting amino acid pool was characterized as having 21 amino acids and seven ninhydrin-positive substances not identified. Predominant amino acids included glutamic acid, proline, serine, x-alanine and aspartic acid. During storage they observed a decrease in neutral fat, while free fatty acid content increased. Further, fatty acids with higher carbon number ($C_{14:0}$ to $C_{18:2}$) were more numerous than those containing fewer carbon atoms (C_4-C_{12}).

Terplan et al (1973) evaluated 23 micro-organisms for their ability to produce histamine and tyramine in yogurt. Over one-half the organisms were able to form histamine; however, bacteria with decarboxylase activity were able also to reduce amines. No health concerns were apparent from the amounts of histamine and tyramine produced.

Popov and Zakhariev (1973) followed hydrolysis of lactose in Bulgarian sour milk using paper chromatography (PC). They found only a small amount of lactose was hydrolyzed; this was explained by

the weak β-galactosidase activity of the culture. Lee and Lillibridge (1976) described an ascending thin layer chromatographic (TLC) procedure for determining lactose in various foods including yogurt. Sample preparation and analysis time was quite long. Goodenough and Kleyn (1976) used TLC to follow hydrolysis of lactose during ripening of yogurt. They reported about one-third of the lactose was hydrolyzed during incubation. Mouillet et al (1977), using a GLC procedure, found 35% of original lactose was hydrolyzed during incubation, glucose was used up by the LAB and galactose accumulated during ripening. These results agree closely with Goodenough and Kleyn (1976). Recently, HPLC has been used to determine lactose and other carbohdyrates in dairy products (Waters Assoc., 1978; Euber and Brunner, 1979; Richmond et al, 1982). Both bonded phase and resin based liquid chromatographic (LC) systems have proved useful in separation and quantitation of sugars in dairy products (Figure 2). Samples are easily prepared and analysis times are generally less than 15 min. Dean (1978) used the Technicon Auto Analyzer to determine free sugars in yogurt. A nut yogurt contained 9.7% sucrose, 3.2% lactose, 1.0% fructose, 1.3% galactose and 0.9% glucose. Washuttl et al (1973) reported on the content of sugar alcohols in foods. The only sugar alcohol found in yogurt was galactitol at 893 mg/100 g.

Engel (1973) used a commerical lactase preparation (Maxilact) to modify yogurt. He reported that yogurt with 50% lactose hydrolysis would be an acceptable product that was sweeter than natural yogurt

without increasing caloric content. Gyuricsek and Thompson (1976) used a commercial yeast lactase to obtain 90% hydrolysis of yogurt milk. Yogurt culture was added and the mix incubated to pH 4.6. Reported advantages included reduced incubation time (40 min decrease). Tartness was decreased and glucose content increased due to hydrolysis. Sensory evaluations indicated that hydrolyzed lactose yogurt to be an acceptable product.

No discussion of milk sugar is complete without mentioning lactose intolerance. Because dairy products are an important part of our calcium intake, Gallagher et al (1975) studied three lactase-deficient patients and found they tolerated fermented dairy products without symptoms of this malady. In all three subjects fecal calcium paralleled increased lactose intolerance symptoms. Results indicated that calcium absorption improved when consumed in fermented dairy foods. Hurt (1972) reported that because milk and other dairy products are often regarded as staples in the diet, there is concern by some segments of the food industry regarding addition of whey and NFDM to foods because of their lactose content. Hurt recognized that lactase deficient individuals could obtain dairy food nutrition by consuming fermented products rather than fresh dairy foods. Escobar and Guillot (1974) described the use of yogurt and cheeses in the treatment of patients with lactose malabsorption. When milk was withdrawn from the diet (49 patients) yogurt administration was considered favorable in 76% of the patients. The value of these milk substitutes was emphasized for those requiring increased protein, calories and calcium in the diet.

Goodenough and Kleyn (1976) fed laboratory rats yogurt, pasteurized yogurt and simulated yogurt with sucrose or lactose for 7 d. Assays of blood galactose demonstrated that animals fed natural yogurt (containing viable bacteria) were able to absorb galactose more efficiently. Gastro-intestinal survival of culture organisms was demonstrated *in vivo* up to three hours after feeding. Hargrove and Alford (1978) observed growth rate and feed efficiency in rats fed yogurt and other fermented milks. Fermented products tested were yogurt, three types of acidophilus milk, lactic buttermilk, Bulgarian buttermilk and direct acidified milk. In six different trials yogurt gave better weight gains than other milks. Even though *L. bulgaricus* was found in the intestinal tract during feeding trials, it disappeared after 3 d when no longer fed. *S. thermophilus* was never isolated below the upper small intestine, but *L. acidophilus* was usually present and persisted when no longer in the diet.

Recently in the U.S. more emphasis is being placed on the milk solids nonfat (MSNF) fraction of milk products. Graf (1975) reported that low fat dairy products were selling surprisingly well. He discussed market implications of changing the fat content in milk and dairy products. The contribution of fat to the flavor of dairy products is well known. Because much of the yogurt consumed is low fat and because of the low lipolytic activity of yogurt bacteria, flavor contributions are considered minimal. Bills *et al* (1969) reported objective laboratory analysis and sensory evaluation has provided insight into the role of free fatty acids as flavor compounds in dairy products. They discussed the importance

of short chain fatty acids including acetic acid, and pH on flavor, when pH of the medium is below 4.5 undesirable acetic acid or vinegary flavors may be observed.

Formisano et al (1971) used gas chromatography (GC) to describe variations in free and esterified fatty acids in fermented milks and ratios between saturated and monounsaturated fatty acids. Differences in the metabolic behavior between the two yogurt bacteria were also discussed. Rasic and Vucurovic (1973) studied free fatty acid content of yogurt made from various milks. In cow's milk, saturated fatty acids generally increased when compared to initial milk except stearic acid which decreased. Oleic, linoleic and palmitoleic acid decreased. In ewe's milk yogurt relative amounts of saturated fatty acids increased, but saturated fatty acid content of goat's milk generally decreased. Oomen (1972) described the fat distribution in Dahi. Most fat was distributed in the top layer regardless of starter addition or species of milk. As the amount of starter was increased from 1% to 2.5% the amount of fat in the top layer decreased.

Stabilizers

Pette and Lolkema (1951b) reported on firmness and whey separation of yogurt. Even then there was much concern about whey separation in the bottle, the commonly used yogurt package. They reported homogenization provided a beneficial effect regarding firmness. Today yogurt is packaged much differently but whey-off or phase separation is no less a problem (Kroger, 1976; Rasic and Kurmann, 1978; Richmond et al, 1982 in press).

Powell (1969) described use of stabilizers in cultured dairy products and reported that various stabilizers and emulsifiers aid in producing and maintaining desirable characteristics of body, texture, mouthfeel and appearance. Six groups of stabilizers are generally recognized (1) plant gums (2) manufactured gums (3) seaweed derivatives (4) gelatin (5) pectins, and (6) starches. Some stabilizers hydrate in cold water such as guar, carboxymethyl-cellulose (CMC) and certain carageenans. Locust bean gum hydrates slowly in cold water but needs to be heated (85^{o}C) and then cooled for maximum viscosity. Gelatin will disperse in cold water but needs to be heated (60^{o}C) and cooled before it will attain maximum viscosity. Because of these differing characteristics it may be desirable to make various blends as is often done in industry. Powell (1969) also reported that locust bean, guar and CMC disrupt the casein complex, but their reactivity is reduced by blending with carageenan and there is a certain balance between stabilizer action on casein and developing acidity in the culture. Finally, Powell (1969) noted that when used correctly stabilizers make an important contribution to cultured dairy foods.

Volker (1970) discussed stabilization of fruit yogurt and noted that fruit yogurt has a strong tendency to synerese under conditions of transport and storage. Nielsen (1975) reviewed the factors that control the body and texture of yogurt. He felt that texture of yogurt was an important quality aspect and under typical handling should resist wheying-off. Factors responsible for controlling body

and texture of yogurt included control of (1) mix composition (2) heat treatment prior to inoculation (3) homogenization (4) starter culture and incubation conditions (5) handling ripened yogurt, and (6) stabilizer systems. Nielsen (1975) also reported that cooling, transporting and packaging were critical to protecting texture. The author further suggested that use of stabilizers is little more than patchwork. Hall (1975) described various stabilizer systems commonly used for cultured products. He reported three stabilizer systems in use were (1) gelatin (2) gelatin + plant stabilizers (3) all vegetable stabilizers. He reported swiss style yogurt, unless drinkable, required stabilization. The most widely used stabilizer was gelatin (225 or 250 bloom).

Steinitz (1977) noted that the use of stabilizers is much more complex than simply increasing or decreasing viscosity. For sundae style yogurt stabilizers may be used in the upper portion but are always used in the fruit portion; and when used in both fractions they must be compatible. Recently, non-gelatin stabilizers have increased in popularity due to increasing cost of gelatin, and dietary customs (Steinitz, 1977). Stabilizers in yogurt must function in the pH range 3.8-4.5 and allow easy blending between fruit and yogurt; pectin is commonly used to stabilize fruit perserves. Steinitz reported that swiss style yogurt is often over stabilized in the U.S. A final point was that proper stabilizers in fruit and yogurt cannot resolve such problems as poor quality ingredients, improper processing, storage, transportation and handling. Oberi et al (1978) studied major factors influencing consistency and stability of yogurt. Of the factors studied

extending ripening time (lower temperature) proved to be the most economical and effective in increasing viscosity and reducing syneresis. Decreasing lactose content to 1.5% before fermentation prevented souring during storage but a bitter defect developed. The influence of various stabilizers on the consistency of "non heat treated stirred yogurt" with and without added fruit preparation was studied (Luczynska et al, 1978). Commercial stabilizers were used according to manufacturers specifications. The most favorable consistency for yogurt using these commercial stabilizers was described in the article.

Meiklejohn (1977) indicated Australian consumers preferred highly viscous yogurt. Traditionally viscosity is increased by stabilizers or adding extra solids or both. Many countries prohibit addition of these materials, yet concentration can be achieved legally by evaporation and membrane filtration, often complicating processing and increasing costs (Meiklejohn, 1977). He reported that with careful attention throughout the entire manufacturing scheme yogurt with high viscosity and good body and texture can be produced without the addition of stabilizers or increased solids. Samuelsson and Christiansen (1978) studied stability and viscosity of fermented milk foods. The following factors aided in the manufacture of good quality fermented milk products: milk with a high protein and fat content; storage at $5^{o}C$ for 24 h before distribution; pasteurization at 85-$90^{o}C$ for 15 min; homogenization at 200-300 kg/cm^2; moderate ripening rate; minimum mechanical treatment and proper stirring.

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Using a lactodynamograph Binder (1978) studied coagulation characteristics of milk and consistency of yogurt. Eleven yogurt cultures were evaluated. There was a significant correlation between maximum amplitude and quotient of consistency; milk with an amplitude above 70 mm yielded a firm yogurt whose quotient of consistency was less than 1.0. No correlation existed between pH and consistency. Binder (1978) reported that by using a standard culture the measure of maximum amplitude with the lactodynamograph allows a judgement to be made regarding the consistency of yogurt to be expected in milk of different origin and treatment. The reaction of different cultures could then be tested using standard milk samples. Richter and Hartman (1977) used penetrometry to evaluate the body and texture of yogurt. Results indicated no relationship between body and texture ratings and penetrometer values. Penetrometer values ranged from 24.0 - 18.2. Flavored yogurts had higher values, probably due to fruit addition. Additions of 6-8% sugar also increased penetrometer values. Samples rated excellent had values of 26.2, while weak sample values were 28.9. Weak and soupy was by far the most common criticism. In summary they reported body and texture were two distinct measurements and penetrometry could not be used to rate these properties.

Andres and Hagan (1977) described a new line of stability systems for yogurt. These systems were developed with the objective of providing yogurt and yogurt products with longer shelf life for more efficient long distance distribution, as well as improving product texture, cost and appearance. These stabilizer systems were

reported to have the ability to improve stucture and texture and possibly permit use of less solids in the mix. Some advantages for plain yogurt included low level of use (0.1%), no retardation of acid development, and improved shock resistance during transportation and storage. Nash (1980) described the use of a natural yogurt stabilizer containing only milk-derived ingredients that did not contain added stabilizers or emulsifiers. This stabilizer system reportedly produces a firm-bodied smooth textured product without phase separation. Recommended use level for lowfat yogurt was 4%.

Kosikowska et al (1978) evaluated 305 strains of slime-forming yogurt bacteria for their ability to improve the consistency of stirred yogurt. Eleven strains of S. thermophilus and 12 strains of L. bulgaricus were selected and 100 combinations made for further study. Criteria for selection was highest viscosity and best sensory properties. Hamdy et al (1972) studied the stability of zabadi made from whole buffalo or whole cow milk, reconstituted skim and toned milk. They reported that gelatin, agar and calcium chloride improved the texture of zabadi from reconstituted milk. The use of rennet as a strengthening agent resulted in a rubbery texture with a sweet curd.

Heat Treatment

Heat treatment of yogurt may refer to different goals, including initial pasteurization to destroy pathogens, additional heating to

denature whey proteins for product stability, or to post-pasteurization heat treatment to extend product shelf life. This latter treatment has recently been the subject of much controversy, as noted earlier.

Gavin (1966) studied the effects of post-heat treatment on keeping quality of yogurt at room temperature. Heat treatments depended on temperature, time and acidity; the lower the pH the lower the temperature and less time needed to increase shelf life. Shalichev and Nakashev (1973) studied the influence of pH on the equilibrium of soluble and colloidal calcium in raw and heat treated milk and yogurt. In yogurt milk (S. thermophilus, L. bulgaricus), soluble calcium increased from 33 mg/100 g at pH 6.45 to 72 mg/100 g at pH 3.70. Martinez-Castro and Olano (1980) studied the isomerization of lactose during heating (120^{o}C). The maximum amount of isomeric sugars of lactose was 0.53 g lactulose and 0.08 g epilactose /100 g milk. Davies et al (1978) used electron microscopy to study the development of yogurt gels. They observed that protein micelles of milk heated to 95^{o}C for 10 min possessed superficial appendages that were not apparent on protein micelles of unheated milks. They suggested during severe heat treatment denatured whey protein associated with the surface of casein micelles forming these appendages and that sulfhydryl bonding was involved in their formation.

Rakshy (1966) reported that pasteurizing yogurt for a few minutes at 60^{o}C caused high kill rates to microorganisms present and sour foods (yogurt) could be pasteurized at lower temperatures

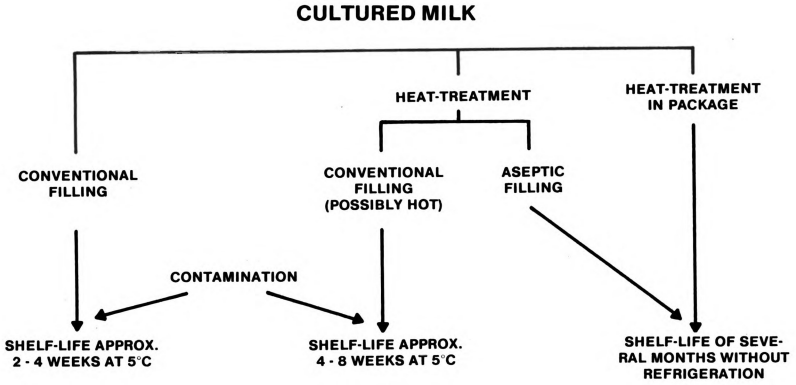

Figure 3. Dependence of shelf life of cultured milk on the technological process (Puhan, 1979).

for shorter times. Heating to 50-55°C for 30 min increased keeping quality considerably; 95 to 99% of the initial flora was destroyed under these conditions. Woods (1976) reviewed methods of higher heat, shorter time (HHST) processing and noted the terms HHST and UHT are used interchangeably. For yogurt HHST improved flavor and texture.

Puhan (1979) reviewed post-pasteurization heat treatment (PPHT) of cultured dairy foods (Figure 3). Generally, a temperature of 70°C for 30-60 seconds was sufficient to eliminate LAB and contaminating yeast and molds. In yogurt 97.5% of the LAB survived a PPHT of 65°C for 22 seconds (pH 4.55) whereas 99.9% of the LAB were eliminated at this same time/temperature combination (pH 3.82). He reported the purpose of heat treating cultured dairy products was to prolong shelf life while maintaining product quality. Speck and Geoffrion (1980) reported PPHT inactivates starter cultures as well as also inactivating β-galactosidase. Heat was more damaging to lactase at pH 4.2 than pH 4.6. No lactase activity or viable culture bacteria were detected after heating at 70°C for 2 min.

Yogurt Cultures

The lactic acid bacteria (LAB) include species of the family Lactobacillaceae. They are structurally a heterogeneous group but are characterized by their main end product--lactic acid. They are gram positive, non-sporulating rods or cocci. Their catalase

activities vary. These bacteria are widely used by the dairy industry throughout the world. Various authors have reviewed the role of LAB in cultured dairy products (yogurt) manufacture (Speck, 1979; Sharpe, 1979; Moon and Reinbold, 1974; Vedamuthu, 1974; Gordon and Shapton, 1977; Tramer, 1973). Tramer (1973) described qualities that good yogurt starters should have (Table 3). Gordon and Shapton (1977) discussed general characteristics of yogurt starters. Yogurt cultures should be obtained from reputable commercial culture houses or from research institutes. Cultures should be renewed at regular intervals (Tramer, 1973). Handling cultures requires certain skills as well as awareness of different temperature optima, differing growth rates, symbiotic or associative growth characteristics, and importance of maintaining culture balance (Tramer, 1973). Many aspects of culture maintenance were discussed by Tramer. In Figure 4, the inhibitory effect of various sugars on acid development is depicted. These results reveal a marked inhibition of cultures with increasing sugar content. Microscopic examination revealed the rods were the organisms affected. However, Tramer concluded that increased sugar concentration was not the problem but rather increased total solids (T.S.) was the critical factor. Above 22% T.S. severe inhibition occurred and further noted cultures varied in their resistance to increased T.S. The selection of a suitable starter is therefore not an easy matter. Speck (1979) reported that little work has been done on starter cultures used in yogurt manufacture. The primary purpose of cultures in yogurt manufacture are for the production of flavor compounds, lactic acid, and texture, which result from starter growth and acid production (Speck, 1979).

Various combinations of L. bulgaricus and S. thermophilus were used to define acid and flavor production and proteolytic activity in skim milk medium (Singh and Sharma, 1982). Variations in titratable acidity, volatile acidity, acetaldehyde and amounts of liberated tyrosine were noted. However, one of the combinations (Lb-RTS, St-HST) showed much higher acidities and more acetaldehyde production compared to other starters. Results agree with other authors regarding the higher proteolytic activity of L. bulgaricus.

Moon and Reinbold (1974) discussed selection of active and compatable starters for yogurt. They reported yogurt flavor depended on the acetaldehyde-lactic acid ratio. The compatibility of cultures used in yogurt manufacture was an important criterion for strain selection. These authors described a procedure (modified coagulase test) for proper strain selection of yogurt cultures. 252 possible combinations were studied (21 S. thermophilus, 12 L. bulgaricus) and 33 controls. Under test conditions, pairs that required longer times to coagulate than controls were considered inhibitory; pairs that required the same time as controls, intermediate; pairs that required shorter time than controls, stimulatory. Coagulation time differences between inhibitory and stimulatory pairs was as much as 2 h. This type of testing could be important to industry since microbial interactions can affect the overall character of yogurt.

No review about yogurt would be complete without mention of some of the important early research (Pette and Lolkema 1950 a,b,c). Pette and Lolkema (1950a) described acid production and aroma

formation in yogurt. They reported typical yogurt aroma was due to (1) lactic acid, and (2) aroma substances produced; the aroma compounds were produced by the rods which developed as acidity increased. They identified acetaldehyde in the distillate. In a second article (Pette and Lolkema, 1950b), they found yeast autolysate lengthened log growth phase and stimulated acid production in S. thermophilus. They reported S. thermophilus required certain amino acids which were not present in sufficient concentration in the original milk. In mixed culture, L. bulgaricus hydrolyzed portions of milk proteins thereby stimulating acid production by the cocci. The most important amino acid liberated was valine, and concentration of this amino acid in milk varied during the year. In the third article Pette and Lolkema (1950c) showed that mixed cultures produced more acid than single cultures. Rapid acid production was due to stimulation of the cocci by the rods. In addition to this symbiotic action, they also demonstrated an inhibitory effect of rods on the cocci due to lactic acid production.

Galesloot et al (1968) reported that S. thermophilus stimulated L. bulgaricus by producing a factor that was equal to or replaced by formic acid. However, this stimulation could only be demonstrated in moderately heated milks. These authors reported that other researchers were not able to observe the stimulation of L. bulgaricus by S. thermophilus because they used steamed or sterilized milk containing formic acid formed by the severe heat treatment. Shankar and Davies (1977) briefly reviewed associative

bacterial growth of yogurt starters. They reported some compound(s) other than an amino acid was the major stimulatory component produced by L. bulgaricus, possibly certain peptides derived from milk casein. Singh and Ranganathan (1979) discussed the caseinolytic activity of L. bulgaricus and a mutant produced using N-methyl-N-nitro-N-Nitrosoguanidine. The mutant released greater amounts of tyrosine when compared to the parent culture. Both the mutant and parent cultures degraded k-casein more readily than α_s or β-fractions. Sharpe (1979) reviewed culture symbiosis, noting that L. bulgaricus has a more active cell bound proteinase than S. thermophilus which aids the growth of S. thermophilus by releasing stimulatory peptides or amino acids from casein.

McKay et al (1971) reviewed the biochemical nature of lactose utilization by LAB noting lactic acid contributes to flavor, color, texture and keeping quality of cultured dairy products. Lactose hydrolysis by S. aureus and lactic streptococci was discussed. Lactose can by hydrolyzed and transglycosylated by the enzyme β-galactosidase. The mechanism of enzymatic synthesis of galactosyl oligosaccharides was described by Pazur (1953). He described the synthesis of four galactosyl oligosaccharides during the hydrolysis of lactose by a yeast enzyme. Asp et al (1980) also discussed the structures of various oligosaccharides produced during the hydrolysis of lactose. Rutter and Hansen (1952) described the conversion of galactose to glucose derivatives in L. bulgaricus.

Carbohydrate or lactose content of yogurt varies widely among commercial yogurts. Goodenough and Kleyn (1976a) reported lactose

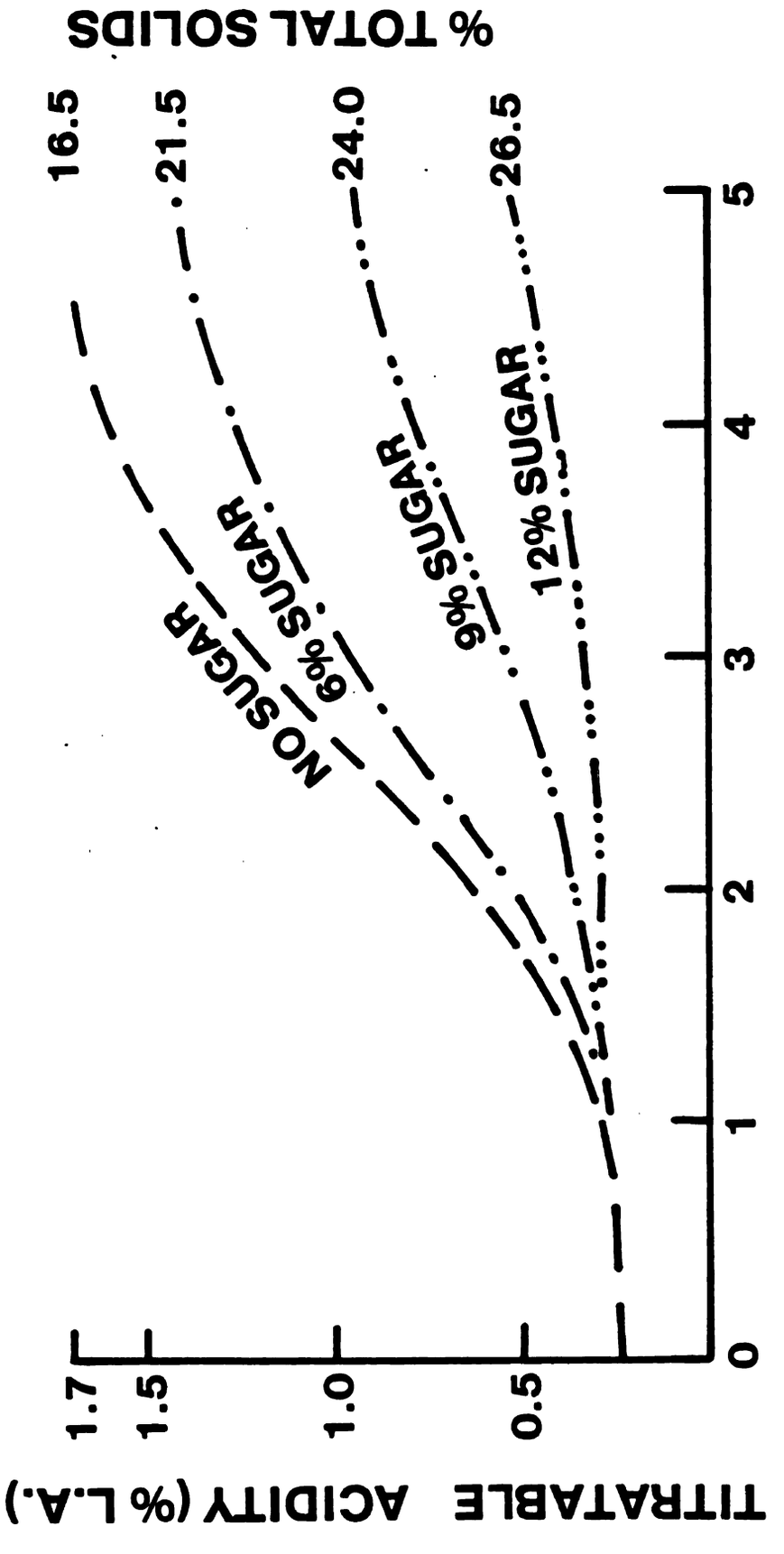

Figure 4. Inhibitory effect of various sugar additions on acid development of a yogurt culture (Tramer, 1973).

content of fresh yogurt (8.5% lactose) decreased to 5.75% after fermentation. Glucose remained at trace levels throughout incubation and galactose increased from trace to 1.2%. Lactose content in commercial samples ranged from 3.31 - 4.74% and galactose from 1.48 - 2.50%. Because of the amounts of lactose found, these authors questioned the often-made assumption that lactase deficient individuals can tolerate cultured milk products better than non fermented products. As mentioned previously, Goodenough and Kleyn (1976) reported laboratory rats fed natural yogurt containing viable cultures were able to absorb galactose more efficiently and intestinal lactase activity was greater. Culture survival was demonstrated *in vivo* up to 3 h after feeding. Kilara and Shahani (1976) discussed β-galactosidase activity of cultured dairy products including yogurt and a direct acidified yogurt product. Cultured yogurt possessed considerable enzyme activity, while the direct acidification product showed no enzymatic activity.

Because LAB are nutritionally fastidious, one must expect some change in vitamin content during incubation of yogurt. Acott and Labuza (1972) and Reddy *et al* (1976) reported on vitamin content of ripened yogurt. Reddy *et al* (1976) compared the content of cultured and acidified yogurt after ripening and storage. Folic acid and B_{12} content decreased 29 and 60% in cultured yogurt and 48 and 54% in acidified yogurt stored at $5^{o}C$ for 16 days. Biotin, niacin and pantothenic acid remained relatively stable throughout storage. Deeth and Tamime (1980) and Rasic and Kurmann (1978) provided an in-depth review of vitamin content of yogurt. Okonkwo and Kinsella

(1969) found the content of orotic acid decreased from 8.2 - 4.6 mg/100 ml during ripening of yogurt. This was attributed to action of the lactobacilli. Upon cooling, orotic acid levels remained constant and yogurt contained about one-half the concentration normally found in milk.

Culture Enumeration

Sandine et al (1976) reported on acid producing organisms and, in particular, LAB in the Compendium of Methods for the Microbiological Examination of Foods. Current methods as well as reagents and necessary media were described. Rasic and Kurmann (1978), Tamime and Deeth (1980), and Tramer (1973) also provided information regarding microbiological assessment of LAB.

In 1971 Davis et al reported on enumeration and viability of L. bulgaricus and S. thermophilus in yogurt. At that time few methods had been described for differentiating and enumerating yogurt bacteria. Using a double pour plate technique with media containing mildly reducing substances, a satisfactory method was found that differentiated yogurt bacteria by colony type under the microscope. The medium provided easy and reliable differentiation -- S. thermophilus being smooth, round or lenticular, and L. bulgaricus showing rough or irregular shaped colonies in the depth of the medium (Davis et al, 1971; Tramer, 1973). Another medium for the differential enumeration of yogurt bacteria was described (Lee et al, 1974). They reported that all S. thermophilus strains fermented lactose and sucrose, while L. bulgaricus fermented lactose but not sucrose. Commercial yogurts were successfully tested for

differential counts on Lee's agar. S. thermophilus produced yellow colonies, while L. bulgaricus appeared as white colonies (less acid production).

For enumerating and separating organisms in yogurt culture, Shankar and Davies (1977) used the inhibitory property of β-glycerolphosphate toward L. bulgaricus to selectively isolate and enumerate S. thermophilus. Johns et al (1978) used reinforced clostridial agar (RCA) to suppress the growth of S. thermophilus thereby allowing selective isolation of L. bulgaricus. Both Shankar and Davies (1977) and Johns et al (1978) used the LAB procedure (Davis et al, 1971) in confirmatory work.

Monk (1979) used microbial thermograms to investigate the growth of S. thermophilus and L. bulgaricus in single and mixed culture (milk medium). Thermograms are a continuous record of the rate of heat production. Monk (1979) reported the area enclosed by a thermogram is proportional to the heat produced and has three main components. Catabolism of lactose provided the largest amount of heat. He reported the heat effect will decrease when growth stops, when concentration of a substrate or a growth factor limits growth, or rate of catabolism decreases due to inhibition by metabolites, including changes in pH. In single culture, L. bulgaricus produced 48% more heat during a 9 h period than S. thermophilus. The maximum heat effect reached during growth is a measure of cell yield.

Rasic and Kurmann (1978) and Hup and Stadhouders (1972) described media and enumeration of yeast and molds in yogurt. Hup and Stadhouders (1972) compared media for enumeration of yeasts and

molds in dairy products. They recommended for sour dairy products oxytetracycline glucose yeast extract agar (OGY).

Culture Activity

Kondratenko et al (1978) described the inhibitory effect of antibiotics on yogurt production. Penicillin and five other antibiotics tested revealed varying results. Penicillin and streptomycin at low concentrations (.007 units/cm^3 and 0.8 mg/cm^3, respectively) inhibited yogurt microflora. When penicillin concentration was 0.2 units/cm^3 there was no growth. Chloramphenicol had the least inhibitory effect.

Rubin (1976) studied factor(s) in yogurt responsible for inhibiting the milk borne pathogen S. typhimurium. Rubin reported although there has been much work in this area, specific factors responsible for the death of this organism have not been clearly identified. Lactic acid was reported to be chiefly responsible for inhibiting this bacterium. A direct correlation was observed between intracellular dissociated lactic acid species and inhibition of growth rate of S. typhimurium, but no correlation was apparent for the intracellular undissociated moiety.

Cousin and Marth (1977) manufactured yogurt from skim milk precultured with psychrotropic bacteria. Yogurts made from milk supporting growth of normal flora and Pseudomonas sp. (No. 36) were not statistically different from control yogurt. Yogurt made from milk precultured with Pseudomonas sp. (No. 13) and Flavobacterium sp. (No. 26) were unacceptable.

Meikljohn (1978) described a seasonal inhibition of yogurt

cultures in Australia. He reported that a combination of seasonal conditions and particular manufacturing conditions used to prepare the fruited yogurt possibly lead to periodic culture inhibition. Microscopic examination revealed normal cocci but rods were nearly absent. Inhibition was overcome by reducing total solids to 22%. He suggested relatively high osmotic pressures predisposed the lactobacilli to an inhibitor that becomes apparent during a certain period of the year in heat-concentrated non-fat milk. However, this inhibitory phenomenon was transient, persisting only a few weeks.

Peake and Stanley (1978) described the specific inhibition of L. bulgaricus during commercial production of yogurt. A scheme was described to determine presence of the bacteriophage. Electronphotomicrographs revealed phage possessed hexagonal heads of 60 nm diameter and tails of 210 nm length.

Mikolajcik and Hamdan (1975a) discussed growth characteristics and metabolic by-products of L. acidophilus. Experiments using skim milk medium were conducted to determine growth rates, optimum temperature for growth, and viability upon storage. Generation times ranged from 49 min to 104 min. Storage at $5^{o}C$ resulted in little loss of viable cells. In another article these authors reported an antibiotic isolated from L. acidophilus (acidolin). After purification acidolin exhibited the following properties: (1) dialyzable with low molecular weight ca 200 (2) no nitrogen (3) highly hygroscopic (4) very acidic (5) active against a wide range of organisms, including spore formers but not LAB (6) very heat stable ($121^{o}F$, 15 min), and (7) non-toxic to tissue culture cells.

Shahani et al (1976) investigated the antibiotic production potential of various strains of L. acidophilus and L. bulgaricus. Milk was essential for production of these antibiotic substances. In another article Shahani et al (1977) detailed the procedure for isolating acidophilin. Purification fold was 247 with a 4.5% yield. The amount of acidophilin required to cause 50% inhibition of 27 bacteria was determined (17 were common pathogens). In general 30-60 µg/ml acidophilin was required for 50% inhibition, ascribing some possible therapeutic properties to acidophilus milk (Shahani et al, 1977). Speck (1975) reported literature has generally indicated that intestinal well-being is associated with high numbers of fecal lactobacilli. He reported when fecal samples are plated with lactobacillus selective agar and incubated in an atmosphere of CO_2, the microaerophilic lactobacilli, including L. acidophilus, can be enumerated. Speck (1975) also described developments in acidophilus products.

Bauer et al (1976) reported that fresh milk contained more bound than free acids. During the souring process bound, free, and total acids increased progressively through 90 min incubation and then rapidly thereafter. Free acids increased the most (208%), and bound acids the least (82%). This distribution was reported to be in correct relation to T.A. and pH values.

Steffen et al (1973) studied configuration of lactic acid formed by different lactic acid bacteria in relation to processing conditions. All S. thermophilus strains tested produced over 92% L (+) lactic acid, while L. bulgaricus strains produced D (-) lactic

acid. Puhan et al (1973) selectively isolated and enumerated lactobacilli (Rogosa agar) and streptococci (Streptoselagar) in 269 yogurt samples. Average bacteria counts were 500 million/ml with streptococci making up 60-80% depending on age and pH of the yogurt. D (-) lactic acid content increased during storage as a result of metabolic activity of the lactobacilli, whereas L(+) lactic acid remained constant throughout storage. Enumeration of S. thermophilus and L. bulgaricus on selective media revealed an increase of streptococci in the early phase of incubation (Puhan et al 1973b). The acidity of the yogurt changed in correlation to the activity of the LAB. After reaching the maximum count of LAB the content of L(+) lactic acid stabilized, whereas D(-) lactic acid increased quickly in the beginning of storage but increased more slowly throughout storage.

Lacrosse (1970, 1972) discussed development of acidity in yogurts stored at varying temperatures. He concluded that yogurt should not exceed .90% lactic acid if its flavor is to be acceptable, and under proper storage yogurt could be held at $5^{o}C$ for 15 days, or $10^{o}C$ for 5 days (Lacrosse, 1970). Fluckiger and Walser (1973) studied the effect of initial pH on susceptibility of yogurt to souring (post-acidification) during storage. Results indicated that yogurt with higher initial pH soured more than yogurt with low pH, especially in the first 14-18d. But this was not an absolute relationship. Abrahamsen (1978) used whole milk and five different yogurt cultures to examine the content of lactic acid and acetaldehyde in yogurt stored at different temperatures. During

storage the proportion of L(+) lactic acid, (as a percent of the total) decreased for all samples indicating L. bulgaricus activity in cold storage. Different starters showed varying amounts of acetaldehyde production. For some samples the content did not change through 24 d storage, while for others acetaldehyde decreased considerably throughout storage. In four of five cultures examined, acetaldehyde levels dropped during storage.

Flavor of Natural Yogurt

Pette and Lolkema (1950a,b,c; 1951a,b) published a series of articles providing some basic knowledge about yogurt. In some of the research (Pette and Lolkema, 1950c) they reported that two primary components were responsible for yogurt flavor: (1) lactic acid, and (2) aroma substance(s) produced. For best yogurt flavor an acidity of 0.85-0.90% was desirable. They also reported that the lactobacilli were responsible for lactic acid production during storage and they identified acetaldehyde as a component of yogurt flavor. In another article (Pette and Lolkema, 1951a) the optimum proportion of rods to cocci was determined for proper and typical yogurt flavor. The optimum ratio was 1:1, a practice which is now commonly accepted in commercial yogurt fermentation.

Harvey (1960) quantitated acetaldehyde and acetone production of various lactic streptococci. Cultures were grown in autoclaved skim milk and the carbonyl compounds determined using PC. Acetaldehyde content ranged from 0.5 ppm to 10 ppm in the cultures. According to threshold sensory data these concentrations should impart significant effects on flavor and aroma of milk cultures (Harvey,

TABLE 4 Acetaldehyde content and acidity of various yogurts.[a]

Yoghurt	pH	T.A.	CH_3CHO
L. bulgaricus[b]+	4.33	1.55	34 ppm
S. thermophilus			
L. bulgaricus[c]+	4.4	1.33	25 ppm
sodium formate			
S. thermophilus[d]+	4.5	1.37	25 ppm
casein hydrolysate			
Commercial samples of yoghurt[e]	4.03	2.04	20 ppm

[a]Marshall and Mabbitt (1980).
[b]Mean of 8 samples.
[c]Mean of 6 samples.
[d]Mean of 14 samples.
[e]Mean of 5 samples purchased from local supermarket.

1960). Acetone content was generally less than 1 ppm. Quantities of acetaldehyde and diacetyl produced in skim milk and MRS media by 5 species of streptococci were reported (Bottazzi and Dellaglio, 1967). In both media all strains of S. thermophilus formed more acetaldehyde and diacetyl than other homofermentative lactic streptococci. Cultures of all isolates of S. thermophilus contained mean concentrations for acetaldehyde and diacetyl of 3.0 and 1.0 ppm in skim milk and 3.5 and 3.0 in MRS medium, respectively. They reported single strain cultures produced acetaldehyde:diacetyl in an unfavorable ratio for balanced flavor.

In a recent article (Marshall and Mabbitt, 1980) yogurt of typical flavor was produced using single starters with certain prescribed additives (Table 4). Yogurt was made successfully on a laboratory scale using single starter organisms. A 2% inoculum of S. thermophilus and 0.25% casein hydrolysate or a 2% inoculum of L. bulgaricus and 30 ppm sodium formate was used. Resulting yogurts had titratable acidities and acetaldehyde contents comparable to mixed culture yogurts (Table 4). The advantages and limitations of using single starter organisms for commercial yogurt production were discussed (Marshall and Mabbitt, 1980). Yogurt made using only S. thermophilus does not exhibit over acidification during storage and sugar content can be increased more in the presence of the cocci.

Bottazzi and Vescovo (1969) reported on quantities of carbonyl compounds produced in skim milk medium from 84 strains of thermophilic lactobacilli isolated from commercial yogurts. Many strains were very active producing 15-20 ppm acetaldehyde; however,

many strains were also relatively inactive. They reported cultures producing about 8 ppm acetaldehyde gave rise to good flavored yogurt, whereas cultures producing less than 4 ppm did not have full flavor. Of all thermophilic lactobacilli studied, none produced diacetyl, and only small amounts of acetoin were formed. Cultures producing an acetaldehyde:acetone ratio of 2.8:1 had stronger yogurt flavor in milk. Acetaldehyde production of different commercial starters was determined through 7 h incubation at 45°C, as well as through four weeks cold storage at 4°C (Hamdan et al, 1971). Maximum acetaldehyde (23-27 ppm) was produced by the fifth hour of incubation. During storage some cultures reduced acetaldehyde and some did not. When single strains (of each organism) were used, acetaldehyde content was reduced considerably compared to acetaldehyde production of combined commercial cultures. Although L. bulgaricus produced more acetaldehyde than S. thermophilus, combining cultures (1:1) resulted in increased production of acetaldehyde (Figure 5).

In an interesting paper (Collins, 1972) biosynthesis of flavor compounds produced by lactic streptococci and associated organisms was reviewed. Collins reported diacetyl, acetoin and 2,3-butylene-glycol were closely related compounds representing three levels of oxidation of one four-carbon skeleton. He reported that the majority of microorganisms that synthesize diacetyl are also able to reduce it to acetoin; acetoin may then be further reduced to 2,3 -butyleneglycol. Biosynthesis of acetoin, acetyl-coenzyme A and diacetyl were discussed. Collins also discussed the importance of

these reactions and why microorganisms produce such metabolites. Influence of pH and aeration on their formation was also described.

Dumont and Adda (1978) reviewed flavor formation in dairy products. Flavor compounds resulting from carbohydrate, lipid and proteins were described. They reported that during ripening amines are formed through amino acid decarboxylation, and while their flavor significance is still questionable, biological amines are of concern because of possible health hazzards to certain susceptible individuals.

Groux (1973) studied yogurt flavor. He reported when acetaldehyde was present in small quantities diacetyl and even acetoin could partially substitute still providing typical yogurt flavor. Groux further suggested that free amino acids resulting from proteolysis could be precursors of some aromatic compounds contributing to the overall flavor of yogurt. Gorner et al (1975) studied changes of volatile substances in yogurt during ripening with the aid of GC. Acetaldehyde content of ripened yogurt ranged from 23.1 to 33.0 ppm. They reported acetone, 2-butanone and ethanol were all present in milk used for yogurt production. Gorner et al (1978) studied the volatile compounds in yogurt (21% TS) using gas liquid chromatography (GLC). Satisfactory yogurt flavor coincided with acetaldehyde contents ranging from 23-47 ppm.

There have been many different values reported for the levels of compounds needed to produce typical yogurt flavor. There is much yet to be done to adequately describe the flavor of natural yogurt, however, Groux (1976) studied components of yogurt aroma. Using

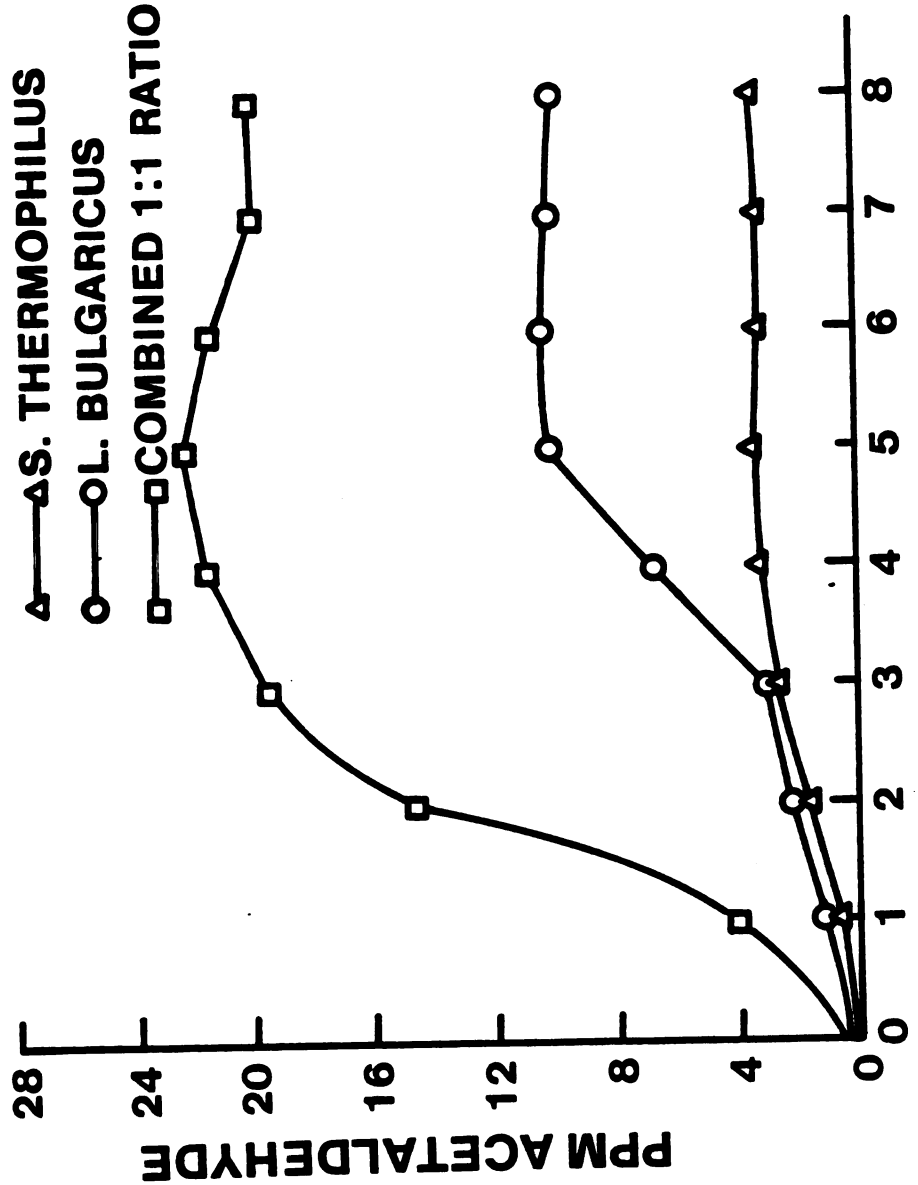

Figure 5. Acetaldehyde production by a single strain of S. thermophilus, L. bulgaricus and a 1:1 mixture of both (Hamdan et al, 1971).

freeze dried cultures cultivated in autoclaved skim milk containing 1% yeast extract (YE), volatile components (acetaldehyde, diacetyl, acetoin) and non-volatile components (free amino acids) were quantified and related to yogurt flavor. Bacterial strains used did not show any proteolytic specificity since all amino acids, except arginine and cystine appeared in free form and quantities of free amino acids were not in ratio to their concentration in milk proteins (Groux, 1976). Generally free amino acid content increased during ripening, but glycine and lysine were consumed by the culture. Ammonia nitrogen increased during ripening and proline was the most abundant amino acid produced during fermentation. In summary, Groux reported that free amino acids did not contribute much to yogurt flavor but they might be precursors of important volatile compounds. He also concluded from trained panel sensory data that diacetyl and acetoin were important contributors to typical yogurt flavor.

Viani and Horman (1976) identified numerous compounds present in yogurt aroma. They reported the mild technique used in the isolation of the aroma complex allowed them to distinguish between compounds resulting from the heat treatments used during the preparation of yogurt and eventual laboratory artifacts. Numerous aroma compounds formed during manufacture as well as possible precursors are shown in Table 5. Using GC headspace analysis, Hild (1979) quantitated acetaldehyde, ethanol, acetone and diacetyl in yogurt. He noted yogurts with distinct flavor contained at least 5-7 ppm acetaldehyde, and yeasty and spoiled products were

characterized by increased ethanol contents. Acetaldehyde content of these spoiled products was also increased. Brandao (1980) determined volatile flavor components of yogurt using a headspace GC technique. He reported acetaldehyde production of 25.5 ppm for mixed culture (1:1); ethanol and diacetyl content increased during fermentation. Brandao discussed the importance of ethanol dehydrogenase activity. During storage of yogurt (after 14 d) ethanol increased concomittantly with decreasing acetaldehyde content. Hamdan et al (1971) describing the effect of potassium sorbate on cultures found that potassium sorbate (0.05%, 0.10%) retarded growth of both yogurt bacteria as well as decreasing acid production of cultures incubated at 45^{o}C. Acetaldehyde in these cultures decreased during storage but decreased more so in samples containing potassium sorbate.

Various authors (Gorner et al, 1971; El-Sadek et al, 1972; Abrahamsen et al, 1978; Singh et al, 1980) have compared volatiles formed during manufacture of yogurt using milk from different species of mammals. Gorner et al (1971) found that acetaldehyde concentration was greatest in yogurt made from cows milk, followed by goats milk and finally sheep milk. Abrahamsen et al (1978) compared the growth of yogurt bacteria, acid development and volatiles formation in cow and goat milk yogurt. Acetaldehyde content was lower in goat milk yogurt. Cows' milk yogurt contained 17 ppm acetaldehyde at 3 h but decreased to 13 ppm after 8 h, whereas goat milk yogurt contained 5 ppm after 3 h incubation and increased to 9 ppm after 8 h incubation. Goats' milk yogurt

TABLE 5 Compounds in yogurt aroma[a].

Constituents of microbial origin		
C_2H_4O	Acetaldehyde	From the
$C_4H_6O_2$	Diacetyl	lactose-citrate
$C_4H_8O_2$	Acetoin	cycle
$C_5H_8O_2$	Acetylpropionyl	Either from
$C_5H_{10}O_2$	2-Hydroxy-3-pentanone 3-Hydroxy-2-pentanone	the threonine cycle, or thermally from fats
Constituents formed by thermal degradation of fat.		
C_3H_6O	Acetone	From Ketoacids (4)
C_4H_8O	Butanone	
C_5H_8O	3-Pentene-2-one	
$C_6H_{12}O$	2-Hexanone	
$C_7H_{14}O$	2-Heptanone	
$C_9H_{18}O$	2-Nonanone	
$C_{11}H_{22}O$	2-Undecanone	
$C_5H_8O_2$	γ -Valerolactone	From hydroxyacids (4)
$C_6H_{10}O_2$	δ -Caprolactone	
$C_8H_{14}O_2$	δ -Caprilactone	
$C_{13}H_{22}O_2$	δ -Tridecalactone	
C_5H_{12}	Pentane	
C_6H_{12}	Methylcyclopentane	

Table 5 - (Cont.)

Constituents formed by thermal degradation of fat and/or lactose.		
C_7H_6O	Benzaldehyde	
C_7H_8O	Benzyl alcohol	
$C_8H_8O_2$	Methyl benzoate	
Constituents formed by thermal degradation of lactose		
$C_5H_4O_2$	Furfural	
$C_5H_6O_2$	Furfurylalcohol	
$C_6H_6O_2$	5-Methylfurfural Furylmethylketone	
$C_6H_8O_2$	2,5-Dimethylfurane	
$C_7H_8O_2$	2-Furyl-3-propional Furylethylketone	
$C_9H_{14}O$	2-Pentylfurane	
Constituents formed by thermal degradation of proteins.		
C_2H_6S	Dimethylsulfide	From methionine
$C_2H_6O_2S$	Dimethylsulfone	
C_4H_8O	Isobutyraldehyde	From valine
C_8H_8O	Phenylacetaldehyde	From phenylalanine

[a]Viani and Horman (1973).

acidified faster and gave a final higher acidity than cows' milk. Interestingly, amounts of diacetyl, acetoin, acetone and ethanol were about the same for both milks. Singh et al (1980) studied the effect of different heat treatments and incubation temperatures on acid, flavor production and proteolytic activity by S. thermophilus and L. bulgaricus in cow and buffalo milk. L. bulgaricus and the mixed culture produced the most acetaldehyde in steam-sterilized milk, while S. thermophilus produced the greatest amount of acetaldehyde in milk heated at 65°C for 30 min. At a temperature of 42°C increased acetaldehyde, lactic acid, volatile acidity and proteolysis were noted.

El-Sadek et al (1972) quantified acetaldehyde and diacetyl production in commercial zabadi. For determination of these compounds, active cultures were grown in previously autoclaved skim milk at 30°C for 18 h. Acetaldehyde was present in all samples and ranged from 2-26 ppm with a mean value of 12.6 ppm, and diacetyl was detected in only 70% of the samples with an average value of 0.15 ppm. All of the yeasts tested formed acetaldehyde but no diacetyl. Strains of Candida pseudotropicalis produced an average of 12.6 ppm acetaldehyde, while other yeasts produced far less.

Sandine et al (1972) reported on causes and control of culture-related defects in cultured dairy products. They reported that harsh or green flavor due to excessive acetaldehyde production could be controlled by using strains high in alcohol dehydrogenase (aldehyde reductase). But for yogurt high acetaldehyde producing strains are necessary for proper flavor. Lack of flavor in yogurt

was reported to be generally due to an inactive strain of L. bulgaricus, too short incubation time, too low a temperature, or retardation of yogurt bacteria as a result of phage or antibiotic contamination. They further suggested flavor defects in fruited yogurt were difficult to detect because of the masking effect of the fruit preserves used.

Another defect in milk products is that of bitterness (Sullivan and Jago, 1970; Renz and Puhan, 1975). Sullivan and Jago (1970) described a model for formation of bitter peptides in culture dairy products. They reported bitter peptides have many similarities, including leucine C-terminal, highly hydrophobic sections, as well as increased content of glutamic acid and proline. Renz and Puhan (1975) reported bitterness in yogurt was due to the proteolytic activity of various starter cultures and resulted from bitter peptides originating from the casein fraction. Bitterness was mainly due to action of highly proteolytic strains of L. bulgaricus during storage. Yogurt made from high heat treated milk and manufactured at 38^{o}C, and having low acidities (pH 4.4) at the beginning of storage showed the strongest bitterness, whereas samples manufactured at 44^{o}C were seldom bitter.

Fruit Addition

The use of fruit or fruit preserves in natural yogurt has contributed to increased yogurt consumption in the U.S and U.K. over the past two decades. Mann (1965, 1971, 1976) described the introduction and use of fruits and fruit products in yogurt. In these articles he reported on various methods and manufacturing

conditions used to produce fruit-flavored yogurts. Craven (1975, 1976) reported that fruit-flavored yogurt in the U.S. was introduced in the early 1960's. Since then yogurt production and consumption has grown steadily. There are different styles of fruit yogurt: (1) swiss style - fruit mixed evenly throughout, (2) sundae style - fruit on the bottom, (3) Western style - fruit on the bottom and flavored syrup on top. Fruit or more commonly, fruit preserve, are issued in amounts ranging from 10-30%, with an average content of 15%. In the U.S. four flavor groups account for largest sales (1) strawberry, raspberry, blueberry (2) peach, mandarin orange, cherry, lemon (3) purple plum, boysenberry, apricot, pineapple (4) two fruit blends, seasonal fruits, coffee and spiced apple. Currently in the U.S. numerous flavor and fruit bases are being offered by commercial suppliers for addition to yogurt.

In the U.K. (Anonymous, 1978a) television advertising was reported to be a key factor responsible for increasing sales of fruit yogurt. Innovative packaging design and the introduction of new flavors have also aided growth and popularity of yogurt. These factors certainly parallel increased sales in the U.S. market. Flavors of yogurt preferred in Europe include strawberry, raspberry, black currant, cherry, mandarin orange, apricot and pineapple (Pederson, 1971). Strawberry was reported as the most popular flavor.

Ramsey (1976) discussed the use of fruits and flavors in cultured dairy products. He reported that increased growth of swiss style yogurt in the early 1970's was related to breakthroughs in the

development of artificial extracts. While many people prefer a naturally flavored yogurt, there remains a market for artificially flavored products as well. Steinitz (1969) discussed the future of flavors in yogurt and reported flavors such as coffee and vanilla should be pure or natural products and also noted flavored yogurt will only be as good as the quality of each ingredient. In accordance with the demand for "natural" foods, processing of honey (Brown and Kosikowski, 1970; Richmond et al, 1982) and maple syrup yogurt (Duthie et al, 1977a) have been described in the literature. Brown and Kosikowski (1970) developed an all natural honey yogurt using dark buckwheat honey that had good acceptance. Duthie et al (1977a) developed a maple flavored yogurt. Concentrations of syrup ranging from 8-18% were tested. Desirable characteristics in the final product included light caramel color, soft custard body and a pleasant sweet taste with a slightly tangy maple after-taste.

Pederson (1971) described fruit bases for yogurt, including various requirements as well as ingredients used in preserve manufacture. Cravan (1975) reported that yogurt fruits have gone through many changes since their introduction. He stated that natural flavors or with other natural flavors (WONF) will increase overall costs and shorten shelf life. Moys (1979) reported that fruit supplies may be from fresh, frozen, canned, preserved or dehydrated fruit. Texture of the fruit is important and the fruit should not be over or under ripe. Moys (1979) also discussed some of the methods used to preserve fruit including freezing and canning, as well as the use of sulfur dioxide to preserve and

maintain product quality.

Bills et al (1972) studied the effect of sucrose on the production of acid and acetaldehyde by yogurt bacteria. Yogurt cultures were grown in homogenized milk containing 2% fat, 12% SNF and 0, 4, 8, 12, 16% sucrose. In general, growth and acid production were inhibited in media containing 4% or more sucrose; acetaldehyde production was decreased in media containing 8% or more sucrose. They reported from sensory data that reduction in flavor attributable to acetaldehyde in fruit flavored yogurt was likely due to the masking effect of the fruit flavor rather than decreased acetaldehyde production. In a somewhat related article, Thornhill and Cogan (1977) looked at the effect of different fruit purees on the growth of L. bulgaricus and S. thermophilus isolated from commercial yogurt cultures. In their study all fruits were first neutralized before determining any effects of the fruit on growth of the organism. Their results indicated fruit did not play a major role in growth stimulation in set type yogurt; however, in commercial use fruit is not neutralized before filling. Of further interest, no inhibitors were found in any of the fruits examined (strawberry, raspberry, black currant, orange).

Sensory Evaluation Score Cards

There are many score cards suggested for evaluating yogurt and this adds to confusion in interpreting sensory evaluations reported in the literature. Recently swiss style strawberry yogurt was included in the American Dairy Science Association (ADSA) sponsored dairy products judging contest (ADSA, 1978). For their score card

10 samples were evaluated for various defects, with no criticism being a 10. Body and texture (5 points total) and appearance (5 points) were also evaluated. Only trained individuals were expected to use this procedure for scoring, however. Duthie et al (1977b) described a score card for yogurt. They reviewed the many scoring procedures now available to evaluate yogurt. In their development of a new score card, they reported it could be used in research and development of new types of yogurt, finished product as well as shelf-life evaluation.

Storage and Packaging

The primary factor in transport and storage of yogurt is temperature control throughout the entire distribution chain; the temperature of transport and storage should be low and constant (Hamman, 1979). He reported that initial cooling temperature was quite important, because if too high, bacterial growth may continue resulting in latent acidification. Yogurt should not be transported until the temperature is 5^{o}C or below. Yogurt can be safely transported up to three days at these temperatures. Often temperatures in dairy cases are above the desired storage temperature. Hamman suggested that product shelf life may be altered considerably by breaks in this chain but the worst link is from the supermarket to the home refrigerator. Yogurt is a bacteriologically safe product with a relatively long shelf life when proper temperatures are maintained.

Luck et al (1977) studied the shelf life of yogurt and other dairy products in S. Africa. Yogurt samples were taken directly

from the manufacturer and stored at 5-7°C. Most samples kept from 4-22 days at this temperature. After storage samples became unclean or bitter and excessively sour. No coliforms were detected but yeast counts were high (60/ml) and psychrotrophs increased only after the flavor became unacceptable. Glattli et al (1974) reported yogurt stored at 5°C for 80 days contained at least one million yogurt bacteria per milliliter. When stored at 10°C live bacteria did not decrease rapidly until after day 40.

Packaging of yogurt has changed much since its introduction in glass bottles. Today yogurt is packaged in 4oz, 6oz, 8oz, 16oz, and 32oz containers made from plastic or paper and having plastic, paper or foil closures. Yogurt is often shipped over wide geographic areas involving transportation in refrigerated rail cars, semi-trucks and local delivery trucks as well as the family automobile. Lang and Lang (1971) reported that ultra high temperature (UHT) processing and aseptic packaging dramatically improves the shelf life of yogurt. They reported also the shelf life of yogurt could be extended up to 25% by injection of carbon dioxide in the head space of the container. Mann (1973c) described an interesting patent for a yogurt container that had a roughened surface designed to reduce product movement.

Over the past ten years a number of foreign processors as well as American processors have entered the U.S. yogurt market (Sahl, 1976; Anonymous, 1980a; Anonymous, 1980b). Because of the increasing popularity of yogurt in the U.S., a market survey of dairy processors was made (Anonymous, 1978). At this time only 13%

of the processors were using tamper proof packaging. 52% of yogurts were packaged in plastic and the remaining 48% in paper based materials. 91% of the processors were using 8 oz. (227g) containers but this has changed over the past few years, and now 6 oz (168g) containers nearly match 227g containers on the dairy shelf. Many of these newly introduced products have interesting package design. One manufacturer recently changed from paper to plastic containers because of a leakage problem associated with the seal (Anonymous, 1980a). This company is now using a conical plastic container with a heat sealed foil closure. Another recently introduced yogurt to the U.S. has a package called a "top front pot" that is flat on one side (Anonymous, 1980b). A one piece label is drawn over the flat side of the pot and over the top of the container providing the closure. This package is produced using thermoform-fill-seal equipment at high volumes with reduced cost per pot. Thermoplastic film is drawn from a reel, heated, preformed by dies, and then drawn into a cooled mold by compressed air producing 16 cups per stroke. Cups are then filled with fruit, followed by yogurt ($40^{o}C$), and containers are then sealed and incubated to proper pH. This design reportedly offers a 20% space savings in supermarkets. More recently thermoformed two piece spin welded yogurt containers have been introduced commercially.

Tamime and Grieg (1979) reported that yogurt packaging equipment is either a fill-seal system using preformed cups or a form-fill-seal system. They discussed ways to minimize structural damage of the coagulum, a common problem in packaging and

transportation of yogurt. Recently (Anonymous, 1980c) a new corrugated shipping container was introduced for yogurt transportation. Special ventilation holes were included to promote cool air circulation around the cups and maintain product freshness during shipment. Premature opening of lids is halted using this shipper. These shippers were also reported to palletize easily for stockers in the warehouse and supermarket. Many shipping containers are available for yogurt storage and transportation (Richmond et al 1982). These authors recently reported secondary packaging materials (shippers) could have an effect on product whey-off or phase separation. They reported minimal product damage when yogurt containers were packaged in corrugated paperboard sleeves with stretch over-wrapping.

Also of interest today is migration of various components from plastics into the contact phase. Yamashita et al (1976) described a method for determining migration of styrene monomer. Recoveries ranged from 88%-95% and the detection limit was 0.002 ppm. They reported in model systems migration of styrene monomer increased with storage temperature and time. No styrene monomer was detected in commercial fermented milks tested however.

Yogurt products

During the time that yogurt has appeared on the market the popularity of this food has increased suprisingly and is now available in different styles, flavors and sizes. During the past decade new uses for yogurt have also been developed. Today we have yogurt, frozen yogurt, liquid yogurt, yogurt dressings, and yogurt

candy to mention only a few.

Steinitz (1971) described frozen yogurt as a new product for ice cream manufacturers. Because it did not meet ingredient specifications for ice cream, but was a frozen whipped dairy food, it was decided this product was a bonafide new frozen dessert and entitled to its own Standard of Identity. In the early 1970's various states including New York and California partially defined this food (Steinitz, 1971). Chandan (1977) discussed various legal aspects for this product as well as considerations in the manufacture of frozen and soft serve yogurt. Chandan reported that frozen yogurt is easily produced using existing ice cream equipment. The mix may be made in the plant or purchased as frozen yogurt mix. Total solids range from 24-32%. A flow diagram for frozen yogurt is shown in Figure 6. Other considerations for frozen yogurt include degree of tartness, number of viable organisms, how "natural" is the product, competition with ice cream, mode of distribution, and possible novelties. (Chandan, 1977). Kosikowski (1977) described two formulations for commercial frozen yogurt. He also described processing methods for frozen yogurt mix. Jochumsen (1978) described the industrial production of frozen yogurt. He reported the mix should be homogenized (75^{o}C) and then pasteurized at 90^{o}C for 3 min. The mix should then be cooled to 43^{o}C and starter added (4-6%). Incubation takes longer than yogurt because of the high osmotic pressure of the mix. After incubation the mix is cooled to 8-10^{o}C. Yogurt is then pre-frozen and whipped in a continuous freezer using nitrogen to minimize deterioration.

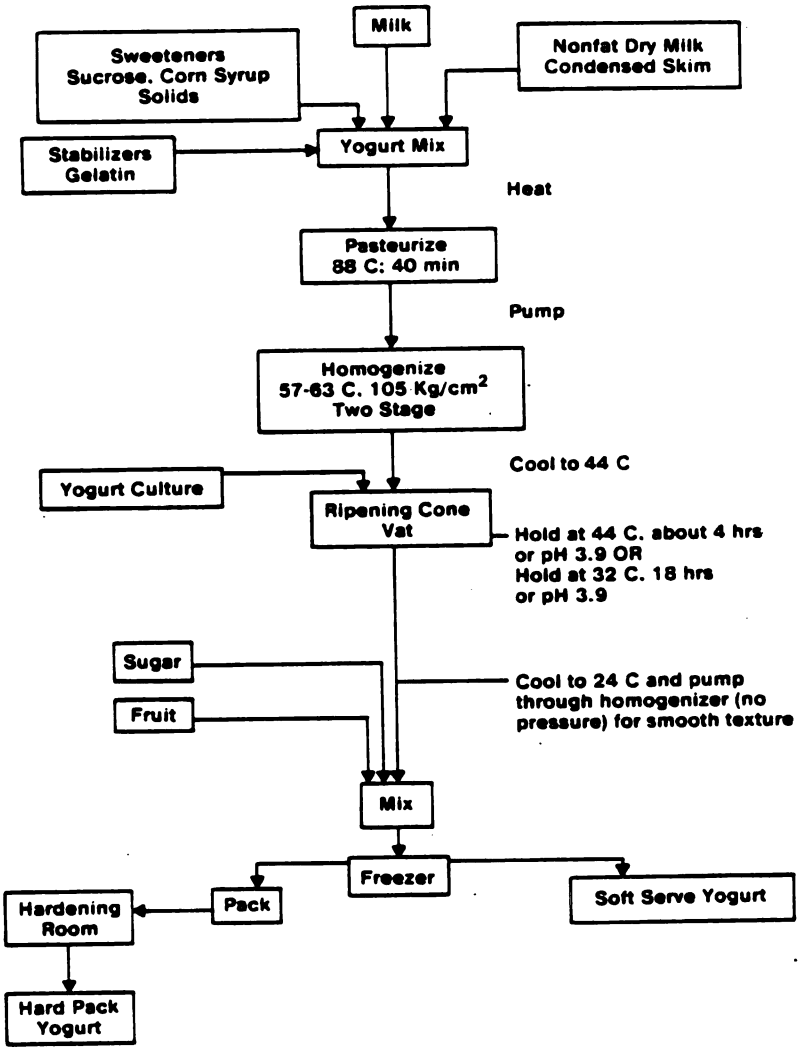

Figure 6. Flow sheet diagram for frozen yogurt manufacture (Chandan, 1977).

Drawing outlet temperature is about -6°C. He also reported packaging frozen yogurt was similar to ice cream packaging. Radaeva et al (1970) studied the effects of freezing conditions on the quality of freeze dried yogurt. Results suggested preparatory freezing temperature of -25°C was optimum, although freezing to lower temperatures better preserved yogurt qualities.

Soft serve yogurt has gained acceptance since its introduction to North America in the late 1960's because of its appeals to children, delightful taste, caloric density, good nutritional quality, and ease in merchandising, including dairy bars and convenience stores (Lavicky, 1977). He reported very few states had any standards for frozen yogurt noting possible problems in distribution. Bradley and Winder (1977) also reported a lack of standards for frozen yogurt. They discussed many interesting points, including mix compositions for soft serve frozen yogurt, fermentation slowdown due to increased sugar content, and the possibility of LAB contaminating ice cream plants. However, because the bacteria do not grow well at low temperatures and because they are easily destroyed with proper sanitation, they were not considered a problem (Bradley and Winder, 1977).

Krebs (1977) reported that yogurt sales were increasing in the U.S. and helping the frozen dessert market. He reported many dairy plants have incorporated facilities to make frozen yogurt (Krebs, 1977; Crant, 1977). In a recent survey Urbanski (1978) reported 75% of all ice cream manufacturers in the U.S. were producing some type of frozen yogurt, but only 50% of the hard frozen yogurt

manufacturers were marketing soft serve yogurt. Hard frozen strawberry and raspberry yogurt were the top sellers. Of the total frozen dessert market frozen yogurt accounted for a 6% share. Woods (1978) reported that frozen yogurt volumes would exceed 50 million gallons in 1978 and various novelties including yogurt bars, sandwiches and cones were available. Consumer preferences indicated viable culture products were preferred.

Miles and Leeder (1981) studied starter culture viability in frozen yogurt. Using two media to enumerate the yogurt bacteria individually, they reported recovery of viable bacteria in frozen yogurt of varying mix composition with respect to sucrose, glucose, corn syrup solids, MSNF and Tween 80. Initial bacterial counts were the highest and second week counts the lowest ($-29^{o}C$). L. bulgaricus was the more susceptible organism during frozen storage, especially at increased sugar levels. Lowest values after two weeks were $1.1x10^{5}$ for L. bulgaricus with 15% sucrose and $2.4x10^{7}$ for S. thermophilus.

Morely (1979) discussed the potential for liquid yogurt in the U.S. He reported that yogurt drink was a sweetened low fat cultured milk drinkable at refrigeration temperatures. He also reported many large food companies were marketing yogurt drink. However, to date yogurt drink is generally only available in health food stores or in various test markets around the U.S. Griffin (1979) described the processing of yogurt drinks, including a product that has whey protein incorporated to enhance drink consistency. He felt packaging was important, and that liquid

yogurt drinks should be easily distinguishable from fluid milk containers. Hannigan (1980) reported the key to liquid yogurts was stabilizer type and content.

Low calorie yogurts have been introduced to the U.S. market recently (Anonymous, 1980). "Lite" yogurt is being marketed with some success in the U.S. Flavored lite yogurts (227g) contain only 130 calories, about one half that of regular flavored yogurt. More recently in the U.S. yogurt sales have tended to level off and there appears to be some concern about the livelyhood of various yogurt products, including soft and hard frozen yogurt and liquid yogurts in the American market.

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CHAPTER II

YOGURT - A COMPOSITION SURVEY IN THE GREATER LANSING AREA[1]

[1]Presented at 1978 American Dairy Science Association Annual Meeting. Published in J. Food Protection. 42:424-426.

INTRODUCTION

Yogurt consumption in the United States has increased from 0.11 lb per capita in 1955 to 2.80 lbs per capita by 1977, an increase of nearly 2500%. From 1970-1976 the per capita consumption increased 170% (4). In 1976, total sales of yogurt were estimated at 510 million pounds (9) and annual sales for 1977 exceeded 600 million pounds (1). Initial projections for 1978 indicate that about 0.5% of all fluid milk produced in the U.S. will go into yogurt manufacture (2).

Previous yogurt surveys have shown wide variation in the chemical composition of commercial yogurts. Duitschaever et al. (6) surveyed 152 yogurts in Ontario, Canada, and found that yogurt of uniform composition was generally not available. It was further stated that this was evident both between and within brands surveyed. Net weights ranged widely with a mean overfill of 7.2%. The pH values varied from 3.27 - 4.53 with a mean value of 3.91. In 1973, Kroger and Weaver (7) surveyed commercial yogurts in the Central Pennsylvania area (41 fruit and 3 plain) and found constant overfill to be a major problem in this region also. Samples surveyed were inconsistent in terms of product net weights, with a mean overfill of 6.87%. Also noted in this survey were wide variations in protein, fat, total solids, pH and caloric content. In yet another survey of yogurts in the United Kingdom (5), the data revealed appreciable differences in net weight, pH, total solids, fat, protein, ash and sugar content. The survey reported in this paper was made recently in the Greater Lansing Area to assess the chemical composition of various

yogurts from commercial markets with the objective of making available meaningful data for use by the industry.

MATERIAL AND METHODS

Samples

Forty-seven samples, encompassing six major brands, were analyzed for total protein, fat, total solids, pH, net weight and caloric content. The samples were purchased in the Greater Lansing area (near MSU) and included both Sundae- and Swiss-style yogurts.

Sample Preparation

All containers were weighed to determine their gross weight. The contents were then transferred quantitatively to a Waring blender and mixed at high speed for 3 min to achieve homogeneity (6,7) for subsequent analyses.

Product Net Weight

The gross container weights (yogurt + container) were determined using a Mettler P1000 balance. After transferring and blending as mentioned earlier, the containers, including tops, were rinsed with distilled water and air-dried. The containers were then reweighed and this value was substracted from the gross weight to obtain the corresponding net weights.

Protein Content

Total protein was determined by an official AOAC Kjeldahl method. The factor 6.38 was used in conversion of reduced nitrogen values to protein (3). All chemical analyses were done in duplicate.

Fat Content

Percentage fat was determined by the Mojonnier modification of the Roese-Gottlieb extraction (8).

Total Solids

Percentage total solids was determined by the Mojonnier vacuum oven procedure (8).

pH

pH of the yogurt samples was measured using a digital pH meter equipped with glass and calomel electrodes (chemtrix Model 60A). A buffer solution of pH = 4.01 was used for scale calibration.

Caloric Content

The caloric value of each sample was determined by calculation as suggested by Kroger and Weaver (7), with a slight modification. Calories/100 g = % fat x 8.79 + [% total solids - (% fat + ash)] x 4. Mean ash contents of flavored yogurt and plain yogurt were experimentally determined to be 1.02 and 0.93%, respectively (10).

RESULTS AND DISCUSSION

The results presented herein show mean values plus or minus standard deviation for various parameters. A total of 47 individual samples were analyzed, of which 28 were fruit-flavored. Due to the lower consumption of plain yogurt, only five different commercial brands were available.

Composition of Various Brands of Low-Fat Flavored Yogurt

Examination of the data collected on composition of flavored yogurt reveals considerable variation of a single constituent within a particular brand. These variations range from wide to minimal and should therefore be of concern from an industrial quality control standpoint. There was also wide variation found in the composition between brands. With low-fat fruit flavored yogurt dominating the market (75 - 90% of total yogurt sales in the U.S.), it is noteworthy to point out that Brand II (Table 1) ranged widely in terms of its chemical composition; 3.68 - 4.42% protein, 1.29 - 1.63% fat, 21.84 - 27.07% total solids and 3.77 - 4.14 pH. In terms of the chemical composition, these data are representative of the other brands tested.

The values obtained for full-fat yogurts (not shown) showed wide variation in fat content. The mean values for percent fat of the two brands surveyed were 2.98 and 4.90% with a range of 2.39 to 5.32%. The mean pH of these samples was less than 4.00.

Composition of Flavored Yogurt

The data in Table 2 compare the mean values of all flavored yogurts, including both low and full-fat brands. These data for low-fat fruit flavored yogurt are in general accord with the published values from

Table 1. Chemical Composition of Various Brands of Low-Fat Flavored Yogurt.[a]

Product Category	Protein (%)	Fat (%)	Total Solids (%)	pH
Brand I (N = 5)	4.36 ± 0.22	1.60 ± 0.05	24.80 ± 3.13	4.14 ± 0.11
Brand II (N = 13)	3.97 ± 0.23	1.45 ± 0.10	25.26 ± 1.72	3.98 ± 0.11
Brand IV (N = 6)	4.74 ± 0.08	1.37 ± 0.05	27.25 ± 0.95	4.27 ± 0.11
Brand V (N = 4)	4.33 ± 0.04	2.20 ± 0.13	25.58 ± 1.65	3.99 ± 0.06

[a]Mean ± standard deviation.

Table 2. Chemical Composition of Various Flavored Yogurts.[a]

Product Category	Protein (%)	Fat (%)	Total Solids (%)	pH
Low fat yogurt (N = 28)	4.26 ± 0.35	1.56 ± 0.28	25.83 ± 2.17	4.07 ± 0.16
Full fat yogurt (N = 14)	4.51 ± 0.18	4.01 ± 1.00	26.39 ± 1.58	3.88 ± 0.13
All Samples (N = 42)	4.34 ± 0.33	2.34 ± 1.29	25.88 ± 1.99	4.01 ± 0.17

[a]Mean ± standard deviation.

Handbook 8-1 (10). However, Handbook 8-1 gives values for all types of yogurt except full-fat fruit flavored yogurt. Therefore, no general comparisons can be made for full-fat flavored yogurts. It would seem advisable, with increasing production and consumption of yogurt in the U.S., to make available in the future published data on full-fat flavored yogurt.

In comparing the data in Table 2, one finds that in addition to the higher fat content, protein and total solids content were also found to be greater in the full-fat brands analyzed. The pH values were comparatively different; the mean pH of full-fat products was 3.88 compared to an average of 4.07 for the low-fat yogurt.

Composition of Plain Yogurt

Both full- and low-fat plain yogurts surveyed (Table 3) were similar in protein content. Mean values for percent fat, percent total solids and pH were all found to be greater in the full-fat yogurts.

In comparing the data for low-fat flavored and low-fat plain (Table 2 vs. Table 3) the results indicated higher pH, protein and fat content in the low-fat plain yogurt while the flavored low-fat yogurts had larger values for percent total solids. The results were similar for full-fat yogurts (flavored vs. plain) in that pH, protein and fat content were all found to be greater in the plain full-fat yogurt samples analyzed. Mean total solids contents were greater in the full-fat flavored yogurts as would be expected, and mean pH values for full-fat flavored yogurts were notably less than for full-fat plain yogurt.

Table 3. Chemical Composition of Various Brands of Plain Yogurt.[a]

Product Category	Protein (%)	Fat (%)	Total Solids (%)	pH
Low fat yogurt (N = 3)	5.69 ± 0.73	1.62 ± 0.04	16.25 ± 1.01	4.22 ± 0.17
Full fat yogurt (N = 2)	5.66 ± 0.24	4.71 ± 1.54	17.88 ± 2.61	4.26 ± 0.35
All Samples (N = 5)	5.68 ± 0.58	2.86 ± 1.79	16.90 ± 1.99	4.23 ± 0.26

[a]Mean ± standard deviation.

Net Weights of Flavored Low-Fat Yogurt

Net weights are shown in Table 4. Overweight seemed to be a common denominator of low-fat flavored yogurt (actually of most yogurt examined) with a mean value for the 28 yogurts surveyed being nearly 5% overweight. Brand I had a mean net product overweight of about 2.2%. Brand II was a striking 7.5% overweight, while Brands IV and V had a net product overweights of 2.3 and 1.3 respectively. Considering all low-fat flavored yogurts surveyed, results showed a range from 1.5% under declared container net weight to as high as 12.6% over declared container net weight. In summarizing the data for full-fat yogurts (not shown), Brand IV on the average was 4.7% greater than its declared container net weight, while Brand III averaged only 0.4% overweight. In general, it appears from these data that yogurt consumers are getting more than they are paying for.

Caloric Content of Yogurt

Table 5 shows estimates of caloric content, cal/100 g, of various commercial yogurts. These values are based on the caloric equation used by Kroger and Weaver (7). The mean caloric values for flavored low-fat and full-fat yogurts were 106 and 121 cal/100 g, respectively. Mean values for plain yogurt ranged from 69 cal for low-fat plain to 92 cal/100g for full-fat plain. From these data it is apparent that low-fat plain yogurt contains 25% less calories than full-fat plain yogurt. Furthermore, it is evident that in relation to low-fat plain yogurt, full-fat and flavored yogurts account for a much increased caloric density on a per container bases. Flavoring addition alone accounted for approximately 30 cal/100 g for both the full and low-fat samples surveyed. Also of interest are the caloric ranges found in flavored yogurts. Low-fat

Table 4. Net weight of various brands of flavored low-fat commercial yogurts.[a]

Product Category	Net Weight[b] (g)
Brand I	231.9 ± 3.69
Brand II	244.0 ± 5.15
Brand IV	232.6 ± 2.63
Brand V	229.9 ± 5.83
All Samples (N = 28)	237.8 ± 7.78

[a]Mean ± standard deviation.

[b]Declared weight = 227.0 g.

Table 5. Calculated Caloric Content of Various Commercial Yogurts.[a]

Product Category	Calories/100 g
Flavored:	
Low fat (N = 28)	106 ± 9
Full fat (N = 13)	121 ± 10
Plain:	
Low fat (N = 3)	69 ± 4
Full fat (N = 2)	92 ± 20

[a]Mean ± standard deviation.

flavored yogurt ranged from 80 - 120 cal/100 g while full-fat flavored samples ranged from 102 - 135 cal/100 g. Considering the wide variation in caloric content of market yogurts along with an overfill of almost 5% for all flavored samples surveyed, the caloric content of many yogurts may be substantially greater than the value indicated on the container.

The data presented indicate that there is still much variation in yogurt composition not only between brands but within the same brand. Moreover, the results of the analyses obtained in this survey are in general agreement with those reported in the literature (5, 6, 7) in that wide variations were observed in gross composition. Apparently there has been little effort to standardize yogurt during the past 7 years despite the fact that better uniformity in composition and quality would be beneficial to both consumer and producer.

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CHAPTER III

PRODUCTION, PROCESSING AND SENSORY EVALUATION OF SWISS-STYLE HONEY YOGURT[1]

[1]To be submitted to J. Food Protection.

INTRODUCTION

The great popularity of yogurt in the U.S. has been attributed by some to the diversity of fruit, fruit preserves or other flavorings that have been introduced in the yogurt base. Currently, about 50 flavor bases are available for addition to plain (natural) yogurt. Fruit is added to yogurt in various ways: (1) Swiss - fruit and yogurt are completely mixed yielding a homogeneous product, commonly stabilized, (2) Sundae - fruit is first metered into the container and yogurt base is added prior to culturing in the container.

The "natural food" trend and increased advertising expenditures have also aided market growth of yogurt. Various researchers (Brown and Kosikowski, 1970; Duthie et al., 1977b) have incorporated flavorings such as honey and maple syrup into yogurt with varying results. Brown and Kosikowski (1970) found that darker buckwheat honey was preferred over a light colored clover honey. Duthie et al. (1977a, b) manufactured varying swiss-style maple syrup yogurts (8 - 18%), developed a score card for evaluation and then performed sensory evaluation to define the preferred syrup levels.

Because many people consider yogurt and honey as natural foods, and because lower honey levels may be used, providing desired sweetness and reduced caloric density low fat honey yogurt may prove to be an appropriate food either as yogurt or as yogurt base whereby additional fruit or fruit preserve may be incorporated. Another concern is stabilizer addition. Many experts and lay people belive yogurt need not have added stabilizers to produce a good bodied yogurt. Because of tradition as well

as increasing costs yogurt is often made in the home and commercial yogurts serve as starter culture. Home produced yogurts rarely contain added stabilizers or emulsifiers.

Using buckwheat honey, skim milk solids, whole milk, water, and yogurt bacteria isolated from commercial yogurt we manufactured and evaluated swiss-style low fat honey yogurt without stabilizer addition. Various honey levels as well as honey addition before and after heat treatment were evaluated by experienced dairy product judges as well as by a larger untrained consumer population for various sensory attributes.

MATERIALS AND METHODS

Yogurt Processing

Whole milk was obtained from the Michigan State University Holstein dairy herd. Buckwheat honey was purchased locally. Plain yogurt mix was standardized to 1.5% fat, 12.6% solids non fat with the addition of skim milk solids and water (14.1% TS). The amount of honey used ranged from 0 - 15% by weight. These additions are more fully discussed later in the paper.

Ingredients were blended together with the aid of a Lightnin^{TM} mixer and pasteurized at 88°C, 40 min. The mix was then cooled to 60°C and homogenized at 70.3 kg/cm^2 first stage and 35.2 kg/cm^2 second stage. The mix was cooled to 43°C and a previously isolated commercial culture added (no slime forming capability) to ripen the yogurt. Honey was either added before pasteurization (BP) or at the time of inoculation (AP). Yogurt was ripened at 42 - 43°C until pH 4.5 and then stored at 5° for sensory evaluation either by experienced dairy product judges or by consumer panelists. For consumer evaluation, yogurt was carefully transported by car to the University of Guelph, Ontario where expert personnel aided in experimental design for statistical analyses and scoring procedures for evaluation of 5% and 6% honry yogurt.

Culture Identification

Culture separation and identification was performed in the food microbiology laboratory at Michigan State University. Reinforced clostridial agar (RCA) was used as a selective medium for isolation of *L. bulgaricus*, since it inhibits the growth of *S. thermophilus*. M17 broth

and agar media were used for isolation of S. thermophilus by suppressing the growth of L. bulgaricus. Morphological assessment was made for size, shape, arrangement and staining properties of cells. Pour plates and streak plates were made and incubated at 37°C for 24 h for S. thermophilus and 72 h for L. bulgaricis. To identify possible slime formation, differing concentrations of sucrose in PCA were used. Various biochemical tests (Table 1) including litmus milk, salt tolerance, dye reduction (methylene blue) and acid production from various carbohydrate sources were used to aid in identification of yogurt bacteria.

Sensory Testing

Initial sensory work defining preferred honey levels and processing parameters for later consumer evaluation were performed with the aid of eight trained dairy product judges.

Statistical Methods

A balanced incomplete block design (Type I where $t = 6$, $k = 3$, $r = 10$, $b = 20$) was used to evaluate the six (t) honey yogurt samples (Cochran and Cox, 1957, p. 472, Plan 11.5). Each panelist represented one incomplete block of three (k) samples. The basic plan was replicated five times giving 50 replications (5 x r) and 100 blocks (5 x b).

The within-block sample order was balanced thus minimizing any positional effects.

An analysis of variance for each attribute was carried out to determine if any differences existed among the six samples. If a difference existed at the 95% level, the Duncan's Multiple Range test was employed to determine which samples were statistically different.

A multiple stepwise regression model was also computed to describe the relationship between overall acceptability and the four sensory attributes (color, sweetness, texture, and flavor). This analysis indicated which of the four attributes accounted for the most variability in the panelists' overall acceptability scores.

Various statistical tables for this portion of the consumer evaluation are presented in Appendix I. The sensory score card is illustrated in Appendix II.

Tasting and Scoring

Each panelist tasted three of the six honey yogurt samples. The samples were evaluated for color, sweetness, texture, flavor and overall acceptability.

A semi-structured scale (Appendix II) was used to quantify the panelists' responses for each sensory criterion. Scoring was accomplished by placing a vertical bar for each sample on a ten centimeter line which had been anchored with extreme descriptive terms for each attribute. The lines were later measured to give the intensity of the panelists' responses.

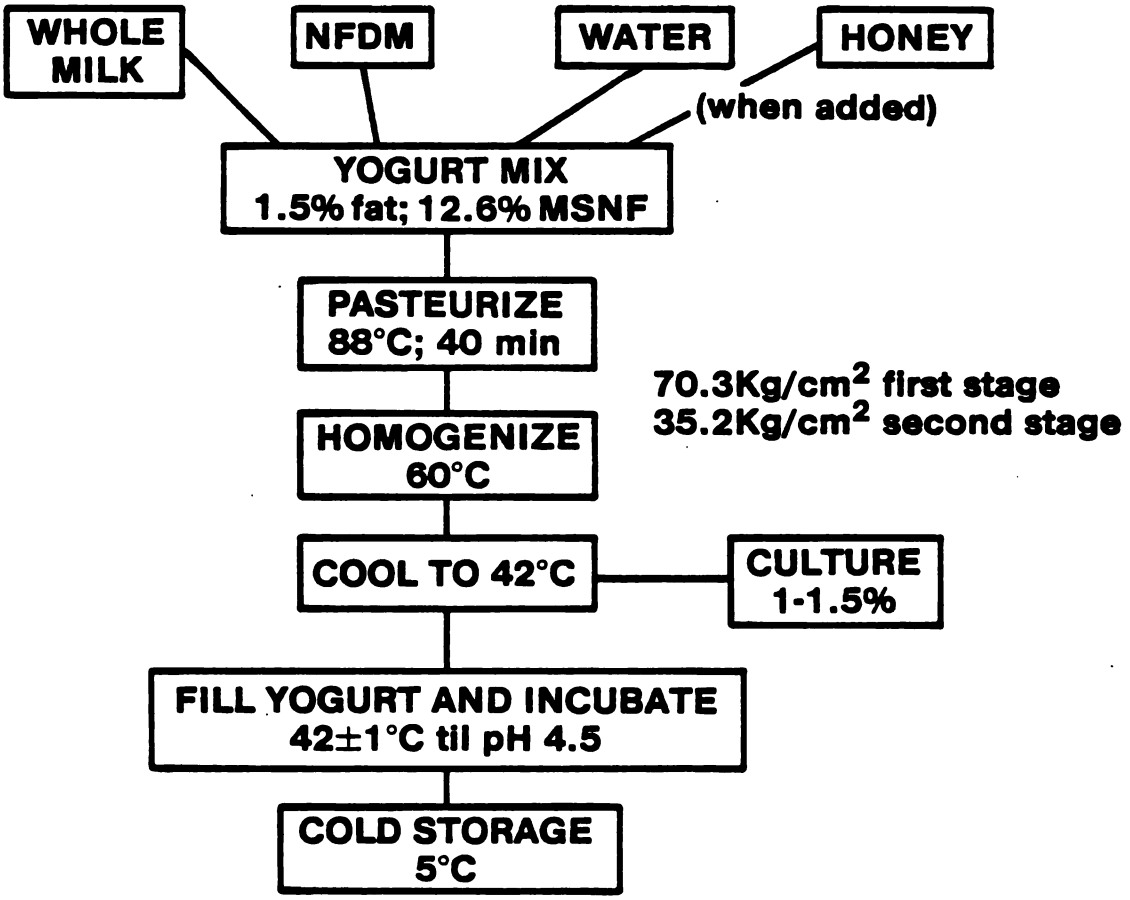

Figure 1. Yogurt processing scheme.

RESULTS AND DISCUSSION

Various biochemical (Table 1) and microbiological tests were used to confirm that the bacteria taken from commercial yogurt were actual yogurt bacteria S. thermophilus, L. bulgaricus. Tests performed indicated that the bacteria used to manufacture buckwheat honey yogurt were S. thermophilus and L. bulgaricus. No slime formation was observed for either organism on sucrose plates. Yogurt produced contained no added stabilizers or polysaccharide material which could participate in a stabilization role.

The processing scheme for production of low-fat plain and low-fat buckwheat honey yogurt is depicted in Figure 1. Low-fat plain yogurt was used through this study as a control for both set time (time required to clot yogurt mix) and as a means to discern flavor defects related to culture organisms or ingredient formulation. For all yogurts examined, pH decreased less than 0.1 pH after four days storage (5°C).

For determination of desirable buckwheat honey levels 3.6 kg lots of plain (0%), 5%, 8%, 12% and 15% by weight buckwheat honey formulations were processed as previously described. Honey was added before pasteurization. From Table 2 it is apparent that set time increased directly with concentration of honey. Buckwheat honey additions of 8% and above required 12 h or longer to set.

Trained judges reported control yogurt (no honey addition) to have good typical yogurt flavor and good body and texture. All judged (8 experienced individuals) reported 5% buckwheat honey yogurt to have good flavor and good body and texture. However, honey levels of 8% and above were found to be too sweet, as well as having poor body and texture.

Table 1. Biochemical Tests to Aid in Identification of Yogurt Culture Bacteria.

Test	*S. thermophilus*	*L. bulgaricus*
Litmus Milk	+	+
Salt tolerance		
1% NaCl	+	
2% NaCl	-	
Skim milk:		
With 0.01% MB	-	
Without MB	+	
Acid production:		
Glucose	+	+
Lactose	+	+
Sucrose	+	-
Maltose	-	-
Mannitol	-	
Rhamnose	-	-
Growth at temperature of:		
45°C	+	+
55°C	-	-
10°C	-	-
Motility and nitrite production		-

Table 2. Initial Evaluation of Buckwheat Honey Addition to Low-fat Plain Yogurt.

	Buckwheat Honey Addition (%)				
	0	5	8	12	15
Yogurt					
mix pH	6.50	6.30	6.20	6.20	6.15
final pH	4.40	4.60	4.43	4.60	-
set time	3.75h	3.75h	12 h	[a]49d	[b]-

[a]Gel formation observed at weekly intervals.

[b]No gel formation after 12 h incubation (43°C) and two months cold storage.

Because of time and energy requirements necessary for proper set of 8% honey yogurt as well as previously noted sensory panel criticisms lower levels of honey were then evaluated to define desirable levels of buckwheat honey.

In another phase of the study lower levels of added honey (5%, 6%) were incorporated into the mix before pasteurization (BP) or after pasteurization (AP) and then evaluated for flavor and body and texture by the experienced judges (Table 3). When honey was added before pasteurization set times were 3.5 h for 5% honey addition and 4.0 h for 6% honey addition, whereas when honey was added after pasteurization time required to set was doubled. Six of eight judges reported superior flavor for BP honey hogurt, while the other two judges felt that AP honey yogurt to be superior in flavor, while noting that yogurt processed this way had a coarse body and texture. Both honey levels were liked equally well by the trained panel of judges.

Scale up processing operations (3.6 kg to 13.6 kg) were made to provide for larger quantities that would be needed for consumer evaluation. Six 13.6 kg lots of yogurt (3 lots 5% honey, 3 lots 6% honey) were made and packaged in 4 oz (112 g) plastic containers with plastic lids stored for 2 d and then transported to Guelph, Ontario, Canada, for consumer evaluation.

A balanced incomplete block design was used in the evaluation of six honey yogurt samples (three lots with 5% buckwheat honey and three lots with 6% buckwheat honey). Each sample was evaluated by 50 people (100 panelists tasted three out of the six samples) for color, sweetness, texture, flavor, and overall acceptability. The samples received similar

ratings for color and texture. The 6% honey yogurt was generally rated higher than the 5% honey yogurt for sweetness, flavor, and overall acceptability of the samples (Tables in Appendix I).

Table 3. Effect of Buckwheat Honey Added to Yogurt Mix Before (BP) and After Pasteurization (AP) as Related to Set Time.

Honey Addition (%)	0	5		6.5	
Treatment	none	BP	AP	BP	AP
Initial pH	6.38	6.24	6.30	6.20	6.30
Final pH	4.25	4.32	4.42	4.38	4.60
Set time (h)	3.5	3.5	7.5	4.0	7.5

SUMMARY

Lowfat honey yogurt is a nutritious and "natural" food that has lower caloric content than typical fruited yogurts. Six percent buckwheat honey added to plain lowfat yogurt with no added stabilizers was preferred over the 5% addition. This product could be easily made in the home or on a commercial scale. Honey added before pasteurization was preferred by experienced dairy judges. When honey was added at culturing an inhibitory effect was noted and yogurt set time was doubled. Beck (1938) reported that bees may inject or spray a venom-like material that has an antifermentative and preserving effect in the honey comb.

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CHAPTER IV

PHYSICAL DAMAGE OF YOGURT - THE ROLE OF SECONDARY PACKAGING ON STABILITY OF YOGURT[1]

[1]Presented at 1981 Institute of Food Technologists Annual Meeting. To be published in J. Food Science.

INTRODUCTION

Commercial yogurt is packaged and distributed in a variety of ways (Table 1) and many factors are involved in physical damage (phase separation and broken coagula) of yogurt, including agitation during transportation and handling (Rasic and Kurmann, 1978). Other factors include over acidification, low solids content, admixture of air, temperature fluctuations and various stabilizer additions. Neilson (1975) and Rasic and Kurmann (1978) reported that some stabilizers actually caused decreased acid production and increased phase separation. Good quality yogurt can be made without the use of commercial stabilizers, although the product is then more vulnerable to stress. Whey separation (syneresis) of yogurt is a common defect and should be controlled. Desirable firmness without syneresis is essential for a quality product (Kroger, 1976). This type of product damage can be caused by the commercial distribution environment.

Vibratory motions are encountered by packaged products during shipping and distribution (Ostrem and Godshall, 1979). Vibration is common to all modes of transportation; and most products will be subjected to some vibration during shipment (MTS, 1976). There are no economically feasible means to completely eliminate the sources of vibratory motions during transportation. Therefore, it is necessary to design products and packages that will withstand vibration without a loss in product quality, while at the same time, minimizing packaging expense.

While actual evaluation of the product-package system throughout shipping is the most desirable means of testing packaged products, it is usually difficult or impossible to collect data in this manner. Therefore, laboratory test methods must be used to reduce overall evaluation time and expense. Both sour cream and yogurt in the retail shelf often show evidence of syneresis of whey, therefore, a series of trials was designed to observe the effects of vibration at the resonance frequency of yogurt products using different commercial distribution packages. Various shippers and overwrap systems were evaluated.

Because little work has been performed assessing the role of packaging in influencing product damage in yogurt (Jones, 1980), this study was made to evaluate various commercial shippers of differing structural design on the quality of plain yogurt subjected to vibrations common in the shipping and distribution environment. Other considerations included in this research were impact shock testing and the effects of stabilizer addition, shipper performance during incubation, cold storage and overwrapping.

MATERIALS AND METHODS

Yogurt Processing

Lowfat plain yogurt mix was standardized to 1.5% fat, 12.6% solids not fat (SNF). The mix was pasteurized at 88^{o}C for 40 min then cooled to 60^{o}C and homogenized at 70.3 kg/cm^{2} first stage and 35.2 kg/cm^{2} second stage. The mix was cooled to 43^{o}C and a mixed strain yogurt culture added (2% inoculation) to ripen the

product. Yogurt was packaged in 227g waxed paper containers with plastic lids, incubated at this temperature to pH 4.5 and placed in a cold room (5°C) for 2 days. Resonance search and dwell testing was then performed on primary (plastic body and waxed paper) and secondary (shipper) containers.

Experimental

Using an MTS electrohydraulic vibration table a frequency search (3-40 Hz) was made for the following shipping containers (12 cups/shipper; stacked 10 high). I. Preformed molded pulp trays individually shrink wrapped with 1 mil polyethylene (PE); II. Wax coated paperboard trays with no film overwrap; III. Corrugated fiberboard sleeves individually shrink wrapped with 1 mil PE; IV. Corrugated fiberboard sleeves stretch wrapped over entire stack.

After establishing reasonance, the stacks were vibrated at this frequency (constant input 0.5g) for 15 min (dwell time). All stacks were then stored in the cold room at 5°C. After 8 h the yogurt was initially evaluated for product damage. All yogurts were evaluated on day 2, 5 and 10 of processing.

Syneresis or whey-off was indicated qualitatively by: - no whey-off; ± very slight; + slight; ++ definite. At the termination of storage the extent of syneresis was quantitated by collecting and weighing the free surface whey using a Mettler P1210 balance.

Filled yogurt containers (waxed paper containers with plastic snap on lids) were evaluated for impact damage using an MTS model 846-240 impact shock machine. Samples were tested in duplicate.

Table 1 - Characteristics of shipping containers for different commercial yogurts.

	PACKAGING MATERIAL	SHIPPER DESIGN	PACK SIZE	PRIMARY PACKAGE
A	WAXED PAPERBOARD	CUT OUT TRAY	12-8oz/PAK	WAXED PAPER BODY and CLOSURE
B	SINGLE WALL CORRUGATED B-FLUTE	TUCKFOLD BOX	12-6oz/PAK	PLASTIC BODY FOIL CLOSURE
C	UNWAXED PAPERBOARD	CUT OUT TRAY PACKED IN MILK CASE 3 HIGH	14-6oz/PAK	WAXED PAPER BODY AND CLOSURE
D	WAXED LAMINATE INSERT	CUT OUT TRAY PACKED IN MILK CASE 3 HIGH	9-6oz/PAK	PLASTIC BODY AND CLOSURE
E	BLEACHED PAPERBOARD	SLEEVE TRAY	12-6oz/PAK	PLASTIC BODY FOIL CLOSURE
F	SINGLE WALL CORRUGATED E-FLUTE	ONE PIECE FOLDER OPENTOP	12-8oz/PAK	WAXED PAPER BODY PLASTIC CLOSURE
G	SINGLE WALL CORRUGATED B-FLUTE	SLEEVE WITH PLASTIC SHRINK OVERWRAP	12-8oz/PAK	PLASTIC BODY AND CLOSURE

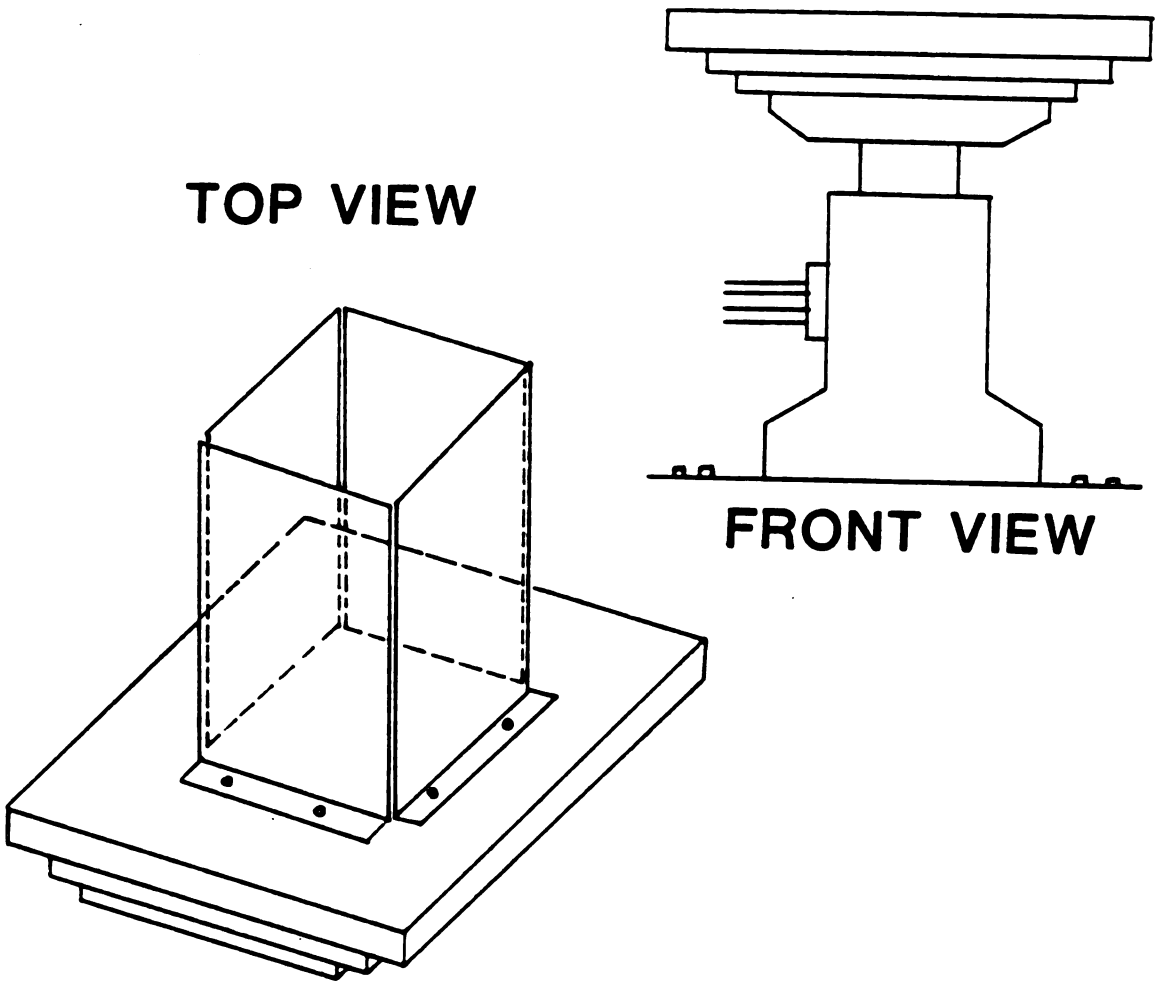

Figure 1. MTS electrohydraulic vibration table.

Vibration Table

The electrohydraulic system consists of a control console and a vibration table (Figure 1). The vibrator maintains a constant input across a broad range of frequencies and the test specimen (yogurt stack) amplifies the input at critical frequencies (Moore, 1976). Such equipment enables one to ascertain resonance, which is the frequency where the acceleration or amplitude is maximal. At this point, any adjustment of frequency will reduce the amplitude or strength of vibration. Gordon and Bains (1979) concluded that most unit loads have major resonance frequencies between 7 and 30 Hz.

This type of testing procedure employs a sine sweep and dwell over a predetermined range (3-40 Hz) to determine resonance points. The frequencies of concern are then held (dwell) for a period of time to determine the likelihood of damage. For evaluation of effects under practical commercial shipping conditions at least one stacked column of containers is tested (MTS, 1976).

In a recently published search of yogurt storage and packaging covering 1972-79, no work on vibration or shock testing was reported (Jones, 1980). Vibration during shipment of other food products has been related to product damage.

RESULTS AND DISCUSSION

Resonance vibration for primary containers, waxed paper and plastic, occurred at 22 and 24 Hz, respectively. Resonance for shippers I-IV occurred at approximately 11 Hz. After vibration, 19, 16, 38 and 16 percent of the primary containers in shippers I-IV, respectively, showed slight or definite whey-off. Ten days after processing 56, 39, 30 and 13 percent of the primary containers

exhibited whey-off. The bar graph in figure 2 illustrates the comparison between shipper types and phase separation on day two and day ten of processing.

At the end of the storage period the extent of syneresis was quantitated. Slight whey-off corresponded to 0.2% - 0.6% (w/w) whey and definite whey-off from 0.6 - 1.8%. Control samples which were not vibrated exhibited minimal or no whey-off during storage, and pH values decreased 0.1 unit during this period.

At the conclusion of the storage study, definite whey-off was visible in 10-20 percent of the samples in shippers I-III. Less than 1 percent of the samples in shipper IV (stretch wrapped) showed definite whey-off. This shipper also showed least overall damage. Twenty five and 14 percent of the samples in shippers II and III appeared to have broken (cracked) coagula, whereas in shippers I and IV this type of damage was minimal ($< 1\%$). Most damage, for all shippers, occurred in the top layers of the stacks. Stretch wrapping considerably minimized this type of damage. Statistical analysis (Gill, 1978) revealed that there was a significant difference between shipper types (I-IV) and resulting product damage (Table 2). Individual comparisons using Bonforroni Chi-square statistics showed shipper IV (stretch wrapped corrugated fiberboard sleeves) to have less damage ($p < 0.01$) than the other three shippers tested (I, II, III). The most dramatic difference was between shipper IV and II. No significant differences ($P < 0.05$) between other shipper comparisons was noted (II vs I, II vs III, III vs I). Using this type of testing, various materials and processing parameters could be evaluated for their effectiveness in minimizing whey-off or product damage during distribution.

TABLE 2. Statistical analysis of physical damage[1] occuring in yogurt vibrated in selected shippers.

SHIPPER COMPARISON	TEST STATISTIC	SIGNIFICANCE
I - IV	45.72	$P < 0.001$[1,2]
IV vs I	23.29	$P < 0.01$[3]
IV vs II	47.44	$P < 0.01$[3]
IV vs III	29.55	$P < 0.01$[3]
III vs I	6.534	*4
II vs III	3.278	*4
II vs I	0.575	*4
VII - VIII	1.690	4

1 Product damage was defined as definite whey-off and disrupted coagula.

2 Comparison of all shippers (I - IV) using Chi-square distribution ($X^2\alpha,3$).

3 Individual comparisons using Bonferroni chi-square statistics (X^2 B,α,6,1).

4 Comparisons between shippers II vs I, II vs III, and III vs I did not reveal a significant difference at $P < 0.05$.

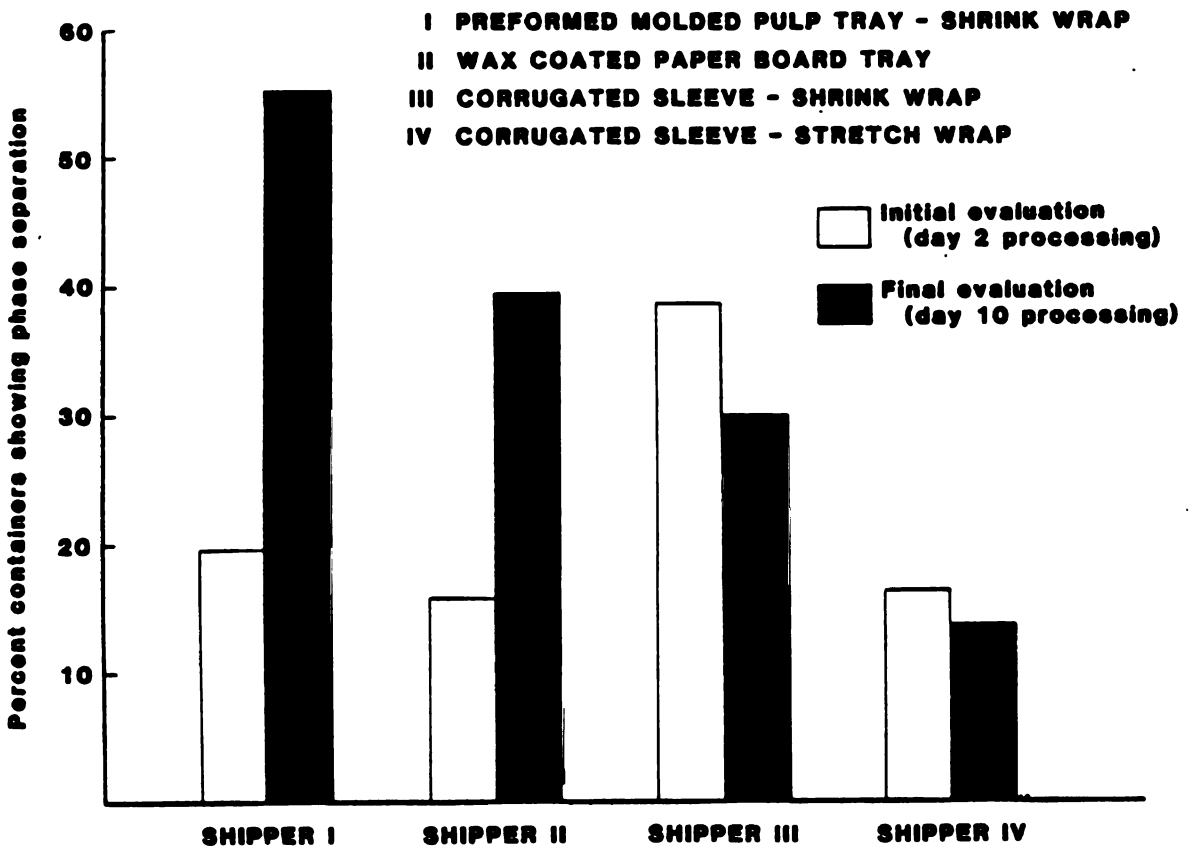

Figure 2. Comparison of shipper types and related physical damage in low fat plain yogurt following vibration.

A further study was designed to evaluate the effectiveness of a proprietary blended stabilizer on reducing physical damage. Fiberboard sleeves shrink and stretch wrapped: (V) and (VI), respectively were used as the test system. All conditions previously discussed were similar except for incorporation of the stabilizer. The commercial stabilizer (starch & gelatin) was blended according to manufactures directions (1.0% addition). No whey-off occurred in any of the samples. These data and visual observation of yogurt coagulum suggest that lower levels of stabilizer could be used providing reduced cost without increasing product damage.

Since product shock is also common in the distribution environment, waxed paper containers with plastic lids (pre-examination showed no whey-off) were examined for physical damage after shock testing. Filled yogurt containers were evaluated for shock damage using an MTS impact shock machine. Samples were dropped from height equivalents ranging from 0.3 m to 2.4 m (1 - 8 feet). All samples were carefully caught on the rebound after dropping and evaluated visually for wheying-off and disrupted coagula. No disturbances were noted in any of the yogurts dropped from equivalent heights ranging from 0.3m to 2.4m; however, all yogurts that were dropped and not caught showed disrupted and broken coagula. Control samples were not exposed to dynamic shock testing and showed none or slight whey-off after five days storage at $5^{o}C$.

In a final experiment, preformed molded pulp trays stretch wrapped (VII) and no overwrap (VIII) were evaluated for their performance through incubation and storage. Yogurt containers were filled and placed in trays; the trays plus primary containers were incubated at 40°C to pH 4.5. All trays and containers were then placed in cold storage (5°C) for later vibration testing.

Because more damage occurred in the upper shippers on the vibrated stack, dummy products (previously made yogurt) were used in the bottom 5 stacks and new product in the top 5 stacks (layers 6-10); only layers 6-10 were evaluated for product damage.

After incubation and storage many of the trays were weak and broke with minimal handling. Some of the primary containers would not sit straight in the trays. The pulp trays were especially weak after cold storage.

Evaluations were made in the same way as the previous experiments. Twelve control samples not vibrated showed none or slight whey-off during the storage period.

Preformed molded pulp trays stretch wrapped after cold storage were difficult to overwrap because of their weakened state. Statistical analyses comparing overwrap versus no overwrap did not provide strong evidence for either system evaluated (Table 2). For each system (VII, VIII) most damage was found in the top shipper vibrated. Because of weakness in the molded trays the application of a coating, laminating material, sizing agent or other additive would be suggested to increase shipper strength throughout incubation and storage including, stretch wrapping and distribution.

CONCLUSIONS

Based on statistical analysis and observation of vibration data, secondary packages (shippers) for yogurt appear to have an effect on product damage. Generally, whey-off tends to increase during storage, but not for all shippers evaluated. Three basic types of damage were apparent (1) slight or definite whey-off (2) cracked or broken coagula, and (3) completely disrupted coagula. Of the shippers tested, stretch wrapping proved most useful in minimizing product whey-off. Limited shock data revealed whey-off was not a problem when dropped from height equivalents of 2.4M or less.

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CHAPTER V

SEPARATION AND ANALYSIS OF CARBOHYDRATES IN YOGURT AND FRESH FRUITS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

A. Separation of Carbohydrates in Dairy Products by High Performance Liquid Chromatography.

B. Separation and Quantitation of Carbohydrates in Lowfat Plain Yogurt and Lactose Containing Microbiological Media.

C. Analysis of Simple Sugars and Sorbitol in Fruit by High Performance Liquid Chromatography.

Introduction

In recent years, the determination of carbohydrates by high performance liquid chromatography (HPLC) has been intensively investigated. Two recent review articles (16,21) summarized numerous HPLC applications for foodstuffs; methods of carbohydrate analysis were briefly discussed. Macrae (16) presented a discussion of HPLC hardware in which he suggested that a single technique will not suffice for analyzing carbohydrates by HPLC. Bonded phase columns resolve sucrose and lactose but not glucose and galactose, while resin based columns easily separate glucose and galactose but show only minimal separation between sucrose and lactose. Resin based columns for carbohydrate determinations by HPLC are currently receiving much attention.

The separation of carbohydrates using bonded phase columns is well documented in the literature (6, 7, 11, 20, 23) and various enzymatic techniques are also available for the determination of simple sugars (10).

Scobell et al (19) described the use of an automated liquid chromatographic (LC) system employing Aminex cation exchange resins for the separation and analysis of various simple carbohydrates. Water was used as the eluent and the sugars were detected by measurement of refractive index (RI). Elevated column temperatures

were required for adequate resolution. Using a column packed with Aminex A-5 (calcium form) resin, they separated melezitose, melibiose, glucose and galactose in 25 minutes. They reported rapid and accurate analyses of large numbers of commercial sweeteners with a minimum of operator attention.

In 1972, Hobbs and Lawrence (9) described the use of a strongly acidic cation exchange resin (lithium form) for the determination of carbohydrates. Galactose and lactose were separated in 40 minutes using this resin packed in a glass column. Solvent composition was 75% ethanol: 25% deionized water. More recently, Conrad and Palmer (3) described a wide spectrum of food applications for HPLC and also included some comparisons between bonded phase and ion exchange carbohydrate packing materials.

Pechanek et al (17) reported on the determination of mono and disaccharides in foods. They used an ion-exchange resin (lithium form); 2-propanol: H_2O (89:11) binary solvent system; tetrazolium chloride derivitization and subsequent measurement at 570nm for the determination of these sugars. They were able to separate a six sugar mixture, though not baseline, in less than one hour. Glucose and galactose were resolved adequately in 50 min.

Verhaar and Dirkx (22) used ion-exchange chromatography (Aminex A-25) for the determination of sugars, sugar alcohols and sugar acids. Compounds investigated included mannose, fructose, glucose, glucitol, mannitol and gluconic and glucaric acids; these compounds

were detected by measuring absorption between 190-200 nm. Wong-Chong and Martin (25) used ion-exhange chromatography to separate sugar cane saccharides. They were able to resolve sucrose, glucose and fructose in about 8 min., though not nearly to baseline, using water as the solvent. For adequate sample resolution by ion exchange a jacketed column was required to maintain elevated operating temperatures. Aminex A5, Q-15S and Q-150S resins were evaluated.

The Aminex HPX-87 carbohydrate column is an 8% crosslinked, tightly sized styrene divinyl benzene copolymer functionalized to give a strong acid cation exchange resin (14). The resins are converted to their desired ionic form by washing with dilute HCl, rinsing with deionized water and washing with the basic salt of the desired cation. Column packing and regeneration were also discussed in a bulletin issued by Bio-Rad (13). Bio-Rad also recently published a useful bulletin regarding the care and use of resin based columns (1).

In this paper we describe a procedure for determining simple carbohydrates (lactose, glucose, galactose) in dairy foods using an Aminex HPX-87 cation exchange (calcium form) carbohydrate column maintained at 80^{o}C. Reverse-osmosis, ion-exchanged water was the only solvent, and a refractometer was used to detect eluting sugars. There are many compounds found in dairy products (fats, proteins, acids, salts, etc.) that can interfere and/or reduce analytical column life (salts and other compounds). Therefore, a

Bio-Rad Microguard™ guard column was incorporated in the system to improve resolution and increase column life.

Materials and Methods

Standard Carbohydrate Solutions

Two standard carbohydrate solutions were prepared from analytical grade reagents, while single carbohydrate solutions were used to establish elution times and order. One solution contained 1.00% (w/v) of each of the following compounds: lactose, glucose and galactose; the second solution contained 4.00% (w/v) lactose plus 1.00% (w/v) glucose and 1.00% (w/v) galactose. Prior to injection all solutions were filtered through a 0.45μm Metricel membrane filter (Gelman Filtration Products, Ann Arbor, MI).

Preparation of Carbohydrate Extracts from Foods

Dairy products were purchased at local markets and prior to sampling, were blended in a laboratory blender. Samples of various dairy products (strawbérry yogurt, plain yogurt, buttermilk, milk, dried acid and sweet wheys) were accurately weighed (10.0g) into glass centrifuge tubes and absolute ethanol was added to make the final concentration of ethanol 80% (v/v). The slurries were mixed well and allowed to stand for 20 min. to insure precipitation of the proteins. Ethanol (80%) was then added to give a total volume of

50.0 ml. The samples were then centrifuged at 5000 rpm for 5 min., the supernatant decanted and the residue washed with ca 25 ml of 80% ethanol (9). The extract plus washings were reduced to dryness using a rotary vacuum evaporator. Finally, the sample extracts were made to 25.0 ml with water and filtered through Whatman No. 42 paper. Lipids and colored materials were removed with a Sep-Pak C_{18} cartridge (Waters Assoc.) using a previously described procedure (18).

HPLC Equipment

The system used consisted of a Waters Associates M-45 solvent delivery system, a U6K septumless injector and a Model RI-401 differential refractometer with a Linear Instruments Model 232 chart recorder. The column was a Bio-Rad Aminex Carbohydrate HPX-87 column (300mm X 7.8 mm) held at 80 C using a 30 cm Alltech Associates water jacket (cat #9502) and a Precision Scientific 66600 circulating waterbath and 62538 thermoregulator. A Bio-Rad Laboratories Aminex A-25 (40mm X 4.6 mm) Microguard Anion/OH cartridge (cat#125-0130) was used as a guard column to remove unwanted anions, especially lactate and to prolong analytical column life. The eluent was water purified by reverse osmosis, followed by ion exchange and vacuum degassing. The purified water was stored at 50 C to minimize resorption of oxygen. A Hamilton 10 µl syringe was used to inject 1-8µl sample volumes.

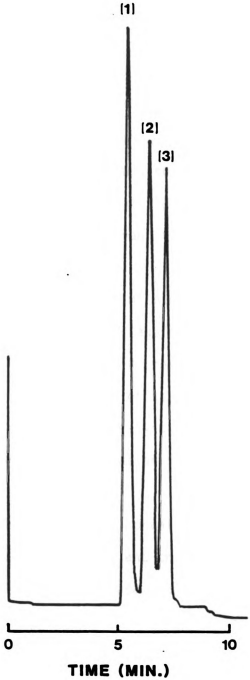

Figure 1. HPLC chromatogram of standard carbohydrate solution: (1) lactose, (2) glucose, (3) galactose. Bio-Rad HPX-87 carbohydrate column ($80^{o}C$); Aminex A-25 Micro-guard Anion/OH cartridge; solvent, H_2O; flow rate, 1.0 ml/min; injection volume, 4µl; attenuation, 8X.

Results and Discussion

This system at a flow rate of 1.0 ml/min reproducibly separated lactose, glucose and galactose with near base line resolution in only 8 min. (Figure 1). By increasing lactose concentration to 4% in this three sugar mixture there was no loss of resolution between sugars. This is important since dairy products usually contain much higher concentrations of lactose than other carbohydrates occurring naturally in milk.

By increasing flow rate to 1.8 ml/min. the three carbohydrate mixture was separated in four min., again without sacrifice of near base line resolution for lactose. Decreasing the flow rate to 1.2 ml/min resulted in good resolution of standard sugars in six minutes. Finally, by further decreasing the flow rate to 0.6 ml/min, we achieved baseline separation of the three sugars in 12 min.

When a four component solution containing sucrose, lactose, glucose and galactose was injected and the flow rate set at 1.0 ml/min, the elution order was sucrose, lactose, glucose and galactose (Figure 2). This figure also shows the lack of resolution between the two disaccharides using this resin based system. However, by slowing the flow rate to 0.6 ml/min lactose is differentiated (figure 3); further reduction of flow rate (0.3 ml/min) showed that resolution between sucrose and lactose was

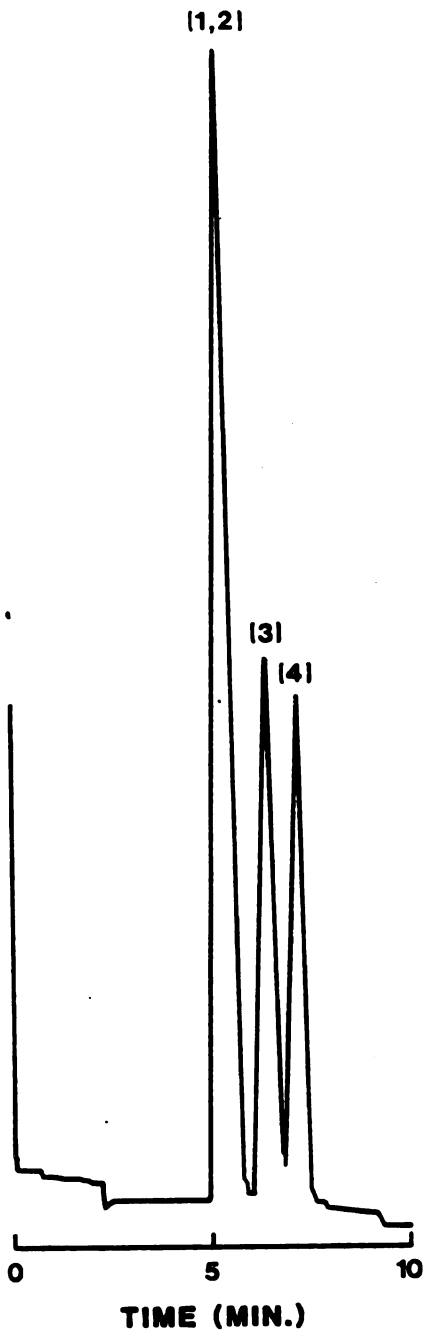

Figure 2. HPLC chromatogram of standard carbohydrate solution: (1) sucrose, (2) lactose, (3) glucose, (4) galactose. Bio-Rad HPX-87 carbohydrate column (80°C); Aminex A-25 Micro-guard Anion/OH cartridge; solvent, H_2O; flow rate, 1.0 ml/min; injection volume, 2.5 µl; attenuation, 8X.

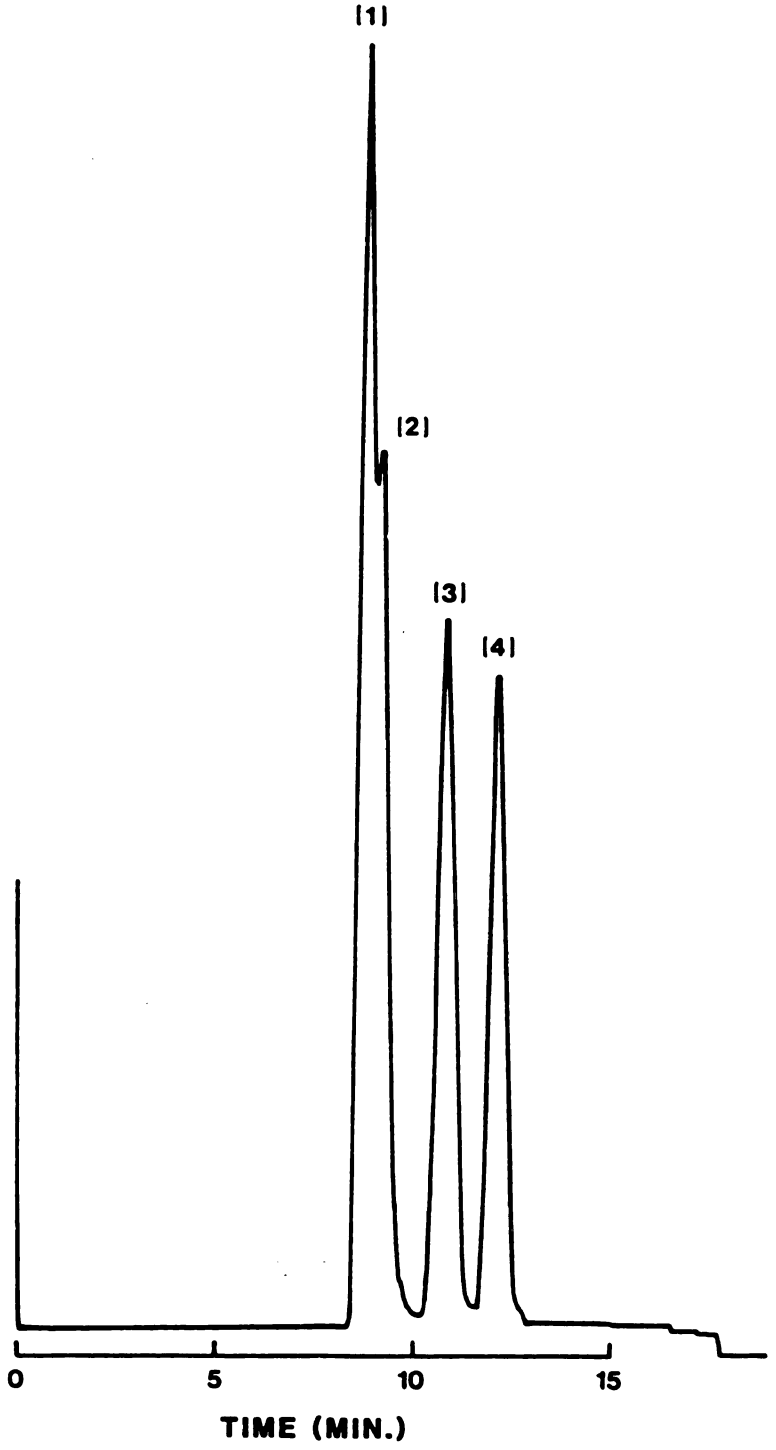

Figure 3. HPLC chromatogram of standard carbohydrate solution: (1) sucrose, (2) lactose, (3) glucose, (4) galactose. Bio-Rad HPX-87 carbohydrate column (80°C); Aminex A-25 Micro-guard Anion/OH cartridge; solvent, H_2O; flow rate, 0.6 ml/min; injection volume, 2.5 µl; attenuation, 8X.

nearly quantifiable, while total time remained under 20 min.

Although Bio-Rad (14) recommends the Aminex HPX-87 carbohydrate column be operated at 85 C, little improvement was noted by increasing column temperature from 80 C to 85 C. Operating at 80 C shortened start-up time and may also relieve stress on the system when operated over long periods of time. However, decreasing column temperature to 65 C resulted in poor resolution.

During this study changing detector attenuation from 8X to 2X did not impair peak resolution. Peaks were adequately resolved at 2X when greater detector sensitivity was required.

Various sugars were present in the extract from strawberry yogurt (Figure 4). The only change in operating conditions for this product was a reduction of flow rate to 0.6 ml/min. Sugars present included sucrose, lactose, glucose, galactose and fructose; retention times of standard sugars correlated well with actual sample peaks and total analysis time was less than 20 minutes. In addition to sugars present, three early eluting peaks were present as well as a small late eluting peak. This late peak (not shown) had nearly the same retention time as ethanol which is present in yogurt but could also be present due to the extraction procedure (23).

The early eluting peaks are likely contaminating anion peaks resulting from salts present in the milk system (8). The first two early peaks had retention times that were similar to calcium chloride and calcium phosphate, respectively. The third early eluting peak in the strawberry yogurt extract was not present in

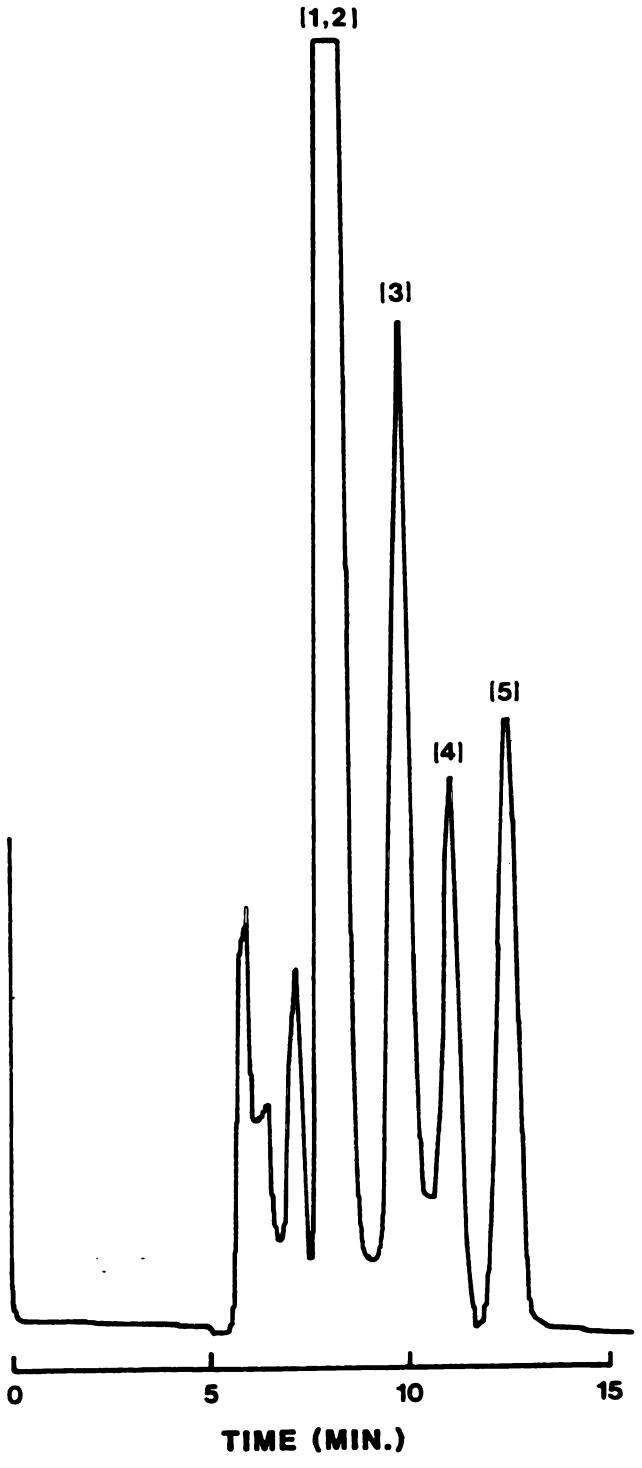

Figure 4. HPLC chromatogram of strawberry yogurt: (1) sucrose, (2) lactose, (3) glucose, (4) galactose, (5) fructose. Bio-Rad HPX-87 carbohydrate column (80°C); solvent, H_2O; flow rate, 0.6 ml/min; injection volume, 4 µl; attenuation, 8X.

Figure 5. HPLC chromatogram of strawberry yogurt: (1) sucrose, (2) lactose, (3) glucose, (4) galactose, (5) fructose. Bio-Rad HPX-87 carbohydrate column (80°C); Aminex A-25 Micro-guard Anion/OH cartridge; solvent, H_2O; flow rate, 0.6 ml/min; injection volume, 4 µl; attenuation, 8X.

plain yogurt. The retention time for this peak was similar to that of citric acid. Since this peak was present only in the fruited yogurt, it is possible that this compound originates in the flavoring material rather than the yogurt base. Further, since larger molecular weight species elute earlier than the sugars, oligosaccharides produced by the transglycosylation action of β-galactosidase may be present in this area.

Initial chromatograms (no guard column) for both plain and strawberry yogurt extracts were difficult to interpret because of poor resolution and interference in both the early and sugar eluting peak areas. As discussed by Macrae (16), while column and detector technology has improved greatly in recent years, there is still a need for improving sample preparation and extraction procedures when analyzing foods. Standards can easily be separated but problems often arise when undesired compounds in extracts interfere.

Lactic acid, for example, appeared to co-elute with glucose and galactose in our system. To eliminate this interference, an attempt was made to precipitate the lactate ion as an insoluble calcium salt before centrifugation. This method was reportedly used successfully for citric acid by Davis and Hartford (5), but in both ethanol and acetonitrile solutions, the calcium lactate was soluble.

Various Amberlite (Mallinckrodt) ion exchange resins were prepared using a modification of the procedure of Wong-Chong and Martin (25); however, excessive carbohydrate loss made them

unacceptable. Sample chromatograms were greatly improved with the addition of a Bio-Rad Microguard™ Anion/OH guard column in this system (2). This guard system proved to be a convenient and effective means of removing milk salts as well as cleaning up the interferences in the late (sugar) eluting peak areas (Figure 5). This latter interference could possibly have been due to lactic acid since (a) injections of reagent grade lactic acid showed peaks in these problem areas and (b) by using the Micro-guard system interference was considerably reduced. Some carbohydrate material is adsorbed by the guard system, however.

Figure 6 shows the separation of lactose from a cultured buttermilk; lactose was also resolved from milk, and dry and fluid wheys (not shown).

Cummings (4) separated a variety of carbohydrates using different ion-exchange resins and found that several monosaccharides co-eluted when using the Aminex HPX-87 column. Co-eluting sugars included galactose, mannose, sorbose, xylose, rhamnose; and fucose, fructose, and arabinose. There were no co-elution problems with our samples, however, since interfering sugars were not present. By using the Aminex HPX-85 column Cummings (4) reported many of these sugars were better resolved.

Davis and Hartford (5) used the Aminex HPX-87 column for the analysis of isomerized syrups. Sample clean up before injection included the use of Waters C_{18} Sep-Paks (24). A modification of

this procedure (18) proved useful in removing coloring materials present in strawberry yogurt. The nonpolar hydrophobic C_{18} packing is normally used with polar solvents and is recommended for preparing samples for carbohydrate analysis. For removing interfering acids Davis and Hartford (5) suggested using ion-exchange prior to HPLC injection; and for removing citric acid, they suggested precipitation with calcium carbonate before deionization. The Bio-Rad Microguard cartridge proved effective in removing unwanted acids from yogurt and cultured butter milk samples. Other HPLC applications for determination of proteins and organic acids in yogurt are described in the literature (12, 15).

Certain advantages and disadvantages are apparent for both bonded phase (18) and resin based systems for the determination of carbohydrates by LC. The resin system uses only high purity water, which is easily prepared or already present in the lab, and affords a more readily available and less expensive solvent source. Moreover, the ease of handling the disposal of water is convenient, since this solvent is not hazardous like many LC solvents.

Another major difference between these column types is operating temperature. Bonded phase columns are commonly operated at ambient temperatures, while resin systems often require elevated operating temperatures. To maintain these temperatures heating blocks or circulating water baths are required.

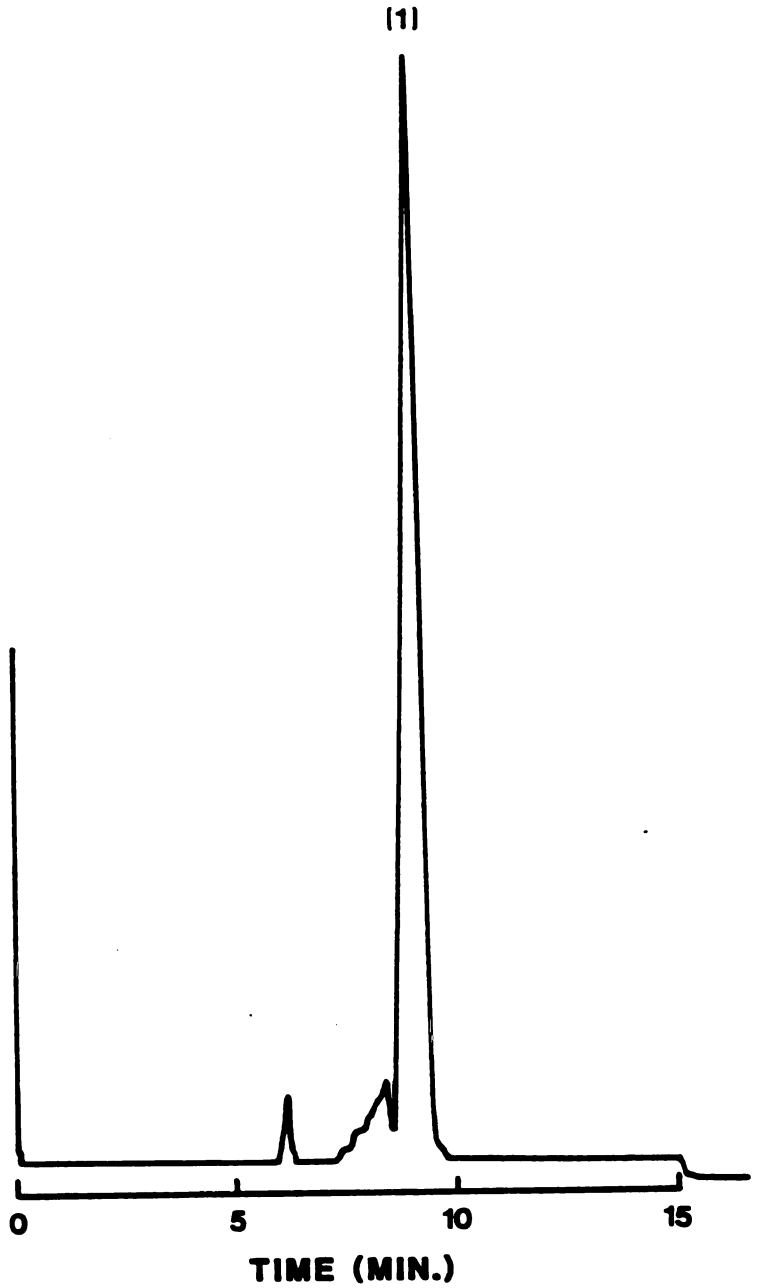

Figure 6. HPLC chromatogram of cultured buttermilk: (1) lactose. Bio-Rad HPX-87 carbohydrate column (80°C); Aminex A-25 Micro-guard Anion/OH cartridge; solvent, H_2O; flow rate, 0.6 ml/min; injection volume, 2.5 µl; attenuation, 8X.

In addition to the applications described herein, HPLC offers a simple solution to monitoring the hydrolysis of lactose to glucose and galactose, which could be useful in commercial enzyme reactors designed for hydrolysis of cheese whey.

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Introduction

Milk and other dairy products are considered staples in the diet of many people. Dairy products are an especially important nutrient source for infants, the young, and the elderly. Primary nutrients include calcium, riboflavin, protein and energy. However, because many people are not able to adequately digest lactose the content of this sugar in food systems can be of importance. Beside lactose, the concentration of other sugars including galactose is currently of interest.

There are a variety of techniques available for determining simple carbohydrates in dairy products (Richmond et al, 1982). Over the past decade many chromatographic procedures have been described for determining lactose and other sugars in dairy foods. More recently, high performance liquid chromatography (HPLC) has been used for analysis of carbohydrates in foodstuff (dairy products). Both bonded phase (Euber and Brunner, 1979) and resin based systems (Richmond et al, 1982) are widely used.

The hydrolysis of lactose in cultured dairy products has been followed using a variety of techniques. Popov and Zakhariev (1973) followed hydrolysis of lactose in Bulgarican sour milk using paper chromatography (PC). They reported only a small amount of lactose was hydrolyzed; this was explained by the weak β-galactosidase activity of the culture. Lee and Lillibridge (1976) described an ascending thin layer chromatographic (TLC) procedure for determining lactose in foods, including yogurt. Sample preparation and analysis time were quite long however.

Goodenough and Kleyn (1976) used TLC to follow the hydrolysis of lactose during ripening of yogurt. They reported about one-third of the lactose was hydrolyzed during incubation; glucose remained at trace levels, and galactose accumulated during fermentation. Mouillet et al (1977) used gas-liquid chromatography (GLC) and found 33% of the original lactose was hydrolyzed during incubation of yogurt. Euber and Brunner (1979) and Richmond et al (1982) found HPLC to be a useful, fast and reliable technique for determining carbohydrate content of yogurt. Samples are easily prepared and analysis time is less than 15 min. Brandao (1980) described an HPLC procedure (bonded phase column) for determining carbohydrate content of yogurt. The procedure was quite time consuming, including an enzymatic step to convert glucose to gluconic acid so as not to interfere in the detection of galactose. In a previous paper (Richmond et al, 1982) a resin based HPLC procedure for determining lactose, glucose and galactose in dairy products was described. This simple procedure was used to quantitate the lactose, glucose and galactose during initial heat treatment, incubation, and storage of lowfat plain yogurt. The effect of autoclaving on lactose content of various microbiological media was also studied using HPLC.

MATERIALS AND METHODS

Yogurt Processing

Whole milk was obtained from the Michigan State University Holstein dairy herd and standardized to 1.5% fat and 12.6% MSNF by addition of non-fat dry milk (NFDM) and water. The lowfat plain yogurt mix was pasteurized at 88^{o}C for 40 min., cooled to 60^{o}C, and homogenized at 70.3 kg/cm^{2} first stage and 35.2 kg/cm^{2}

second stage. The mix was then cooled to 43°C and a mixed strain yogurt culture (1:1) added (Chris Hansen, Milwaukee, WI, 2% inoculation) to ripen the product. Yogurt was packaged in 227 g waxed paper containers with plastic lids, incubated at 43°C to pH 4.5 and stored at 5°C. Samples were evaluated at hourly intervals durng ripening and then weekly or longer intervals thereafter.

Standard Carbohydrate Solutions

Two sets of carbohydrate standards were made for use in preparing standard curves for lactose, glucose, and galactose from analytical grade reagents: (1) six lactose solutions of concentration 0.20 - 2.00% (w/v), and (2) six solutions containing both glucose and galactose with each component ranging from 0.10 - 1.00% (w/v) [total carbohydrate concentration ranging from 0.20 - 2.00, w/v]. Prior to injection, all solutions were filtered through a 0.45 µm Metricel membrane filter (Gelman Filtration Products, Ann Arbor, MI). For standard curve preparation as well as sample extracts injection volumes were 2 µl for lactose and 4 µl for glucose and galactose analyses.

Preparation of Carbohydrate Extracts from Yogurt

At predetermined intervals two containers of yogurt were randomly selected. The contents of the two cartons were mixed, allowed to equilibrate for 10 min. at room temperature and pH measured with a combination electrode accurate to 0.1 pH units. Ten gram samples were accurately weighed into large conical centrifuge tubes and absolute ethanol added to bring the final ethanol concentration to 80% (v/v). The slurries were mixed and allowed to stand 20 min. at room temperature to precipitate proteins. Ethanol

(80% v/v) was then added to give a total volume of 50.0 ml. The samples were centrifuged at 5000 rpm for five min., the supernatant decanted and the precipate washed with ca 25 ml additional 80% (v/v) ethanol. The combined extract and washings were then concentrated (absence of alchohol odor) using a rotary vacuum evaporator (25-27^{o}C). The sample extracts were made up to 25.0 ml with water and filtered through Whatman No. 42 paper. The samples were filtered through a 0.45 µm Metricel membrane, placed in vials, sealed and frozen (-10^{o}C) for subsequent high performance liquid chromatographic (HPLC) analysis.

Before injection, samples were thawed at room temperature and mixed. Occasionally sugar and salt crystallization occurred and sample extracts were shaken until the crystals went into solution. To maintain accuracy, injection volumes were identical to those used for standard sample injections 2 µl for lactose, 4 µl for glucose and galactose (Euber and Brunner, 1979).

HPLC Equipment

The HPLC apparatus was constructed from a Waters Assoc. (Milford, MA) M-45 solvent delivery system, a Waters U6K septumless injector, a Waters Model RI-401 differential refractometer and a Linear Instruments Model 232 chart recorder. The HPLC column was a Bio-Rad Aminex HPX-87 carbohdyrate column (300 mm x 7.8 mm) maintained at 80^{o}C using a 30 cm Alltech Assoc. water jacket (cat. # 9502), a Precision Scientific 66600 circulating waterbath and a 62538 thermoregulator. A Bio-Rad Aminex A-25 (40 mm x 4.6 mm) Microguard Anion/OH cartridge (cat #125-0130) was used as a guard column to remove unwanted anions, and to increase column life. The

eluent (reverse osmosis ion-exchanged deaminated water) was vacuum degassed at room temperature and held at 50°C to minimize reabsorption of gases during HPLC analysis.

Separation and Quantitation

All injections were made with a Hamilton 10 µl syringe (Hamilton Co., Reno, NV). A flow rate of 0.6 ml resulted in elution times of 9.0, 11.0 and 12.5 min. for lactose, glucose, and galactose, respectively. Elution times increased slightly toward the end of the experiment due to extended guard and analytical column use without regeneration or replacement.

For standard curve preparation and anaysis of carbohydrate extracts injection volumes were 2 µl for lactose and 4 µl for glucose and galactose. A best fit standard curve (peak height vs. sugar concentration) for each of the three sugars was prepared using linear regression (Table 1). During early stages of yogurt ripening lactose concentrations were relatively high and 2 µl injection volumes resulted in off-scale peaks for lactose. To correct for this the samples were diluted with distilled water (1:1). Duplicate injections were made for all yogurt samples.

Recovery

The efficiency of the extraction procedure was evaluated by determining the recovery of lactose and galactose from a standard solution and a yogurt-sugar preparation (Table 2). Ten grams of a standard solution (2.00% lactose and 1.00% galactose, w/v) and 10.0 grams of yogurt were accurately weighed, extracted, and extract sugar concentration measured. One gram of the standard sugar solution was mixed with 10.0 g yogurt and also extracted. Duplicate

Table 1 Statistical data: regression equations and correlation coefficients for lactose, glucose and galactose standard curves.

Sugar	Regression Equation[a]	Correlation Coefficient: (r)
Lactose[b]	y=0.1976 x + 0.5150	0.999
Galactose[c]	y=0.2204x + 0.4012	0.999
Glucose[c]	y=0.2407x + 0.2092	0.998

[a] y= µg sugar/injection; x= peak height (mm). All values are averages from duplicate injections.

[a] Injection volume (2 µl)

[b] Injection volume (4 µl)

Table 2. Recovery of lactose[a] and galactose[b] from a spiked sample of lowfat plain yogurt[c].

Lactose	Concentration(mg/ml)	Amount of Sample(ml)	Total (mg)
Authenic lactose	20.014	1.0	20.014
Processed Yogurt	22.688	10.0	226.885
Processed yogurt +authentic lactose	23.264	11.0	255.907
Galactose			
Authenic	8.172	1.0	8.172
Processed yogurt	6.484	10.0	64.839
Processed Yogurt +authentic galactose	6.865	11.0	75.515

[a]Lactose recovery 103.6%
[b]Galactose recovery 103.4%
[c]Average of duplicate injections

injections were made for each sample (unprocessed standard solution, processed standard solution, yogurt and yogurt plus added sugar). Injection volumes of 2 µl and 4 µl were used for measurement of lactose and galactose, respectively. Percent recovery was calculated from the equation

$$\% \text{ recovery} = \frac{\text{amount of sugar in 11.0 g yogurt-sugar preparation}}{\text{amount of sugar in 10.0 g yogurt + amount in 1.0 g sugar solution.}}$$

Results and Discussion

In a previous paper (Richmond et al, 1982) we described an HPLC procedure for reproducibly separating lactose, glucose and galactose using a resin based system. In this paper this procedure has been applied to (1) quantitate the carbohydrate content of lowfat plain yogurt during ripening and storage; and (2) quantitate lactose content of various lactose containing microbiological media before and after typical media preparation (121^{o}C, 15 min.), as well as separate and tentatively identify lactulose, apparently formed during autoclaving of litmus milk.

Regression equations and correlation coefficients for lactose, glucose and galactose standard curves are described in Table 1. To evaluate the efficiency of the extraction procedure a recovery experiment was performed by spiking known amounts of lactose and galactose into actual carbohydrate extracts from yogurt (Table 2). Lactose recovery was 103.6%, while galactose recovery was 103.4%.

From HPLC chromatograms (Figure 1) and Table 3 it is apparent that lactose content decreased during initial heat treatment ripening and storage of lowfat yogurt. Progressive chromatograms (Figure 1 a-d) as well as Table 3 illustrate decreasing lactose

content and increasing galactose content of yogurt over time. Glucose remained absent throughout incubation and storage. Only after 50 d were trace amounts of glucose present (Figure 1d).

Decrease of lactose content as a result of initial pasteurization and heat treatment was 7.3%. Lactose content decreased 26.7% during ripening and continued to decline throughout storage ($5^{o}C$). Lactose content decreased from 7.12% (yogurt mix-not heat treated) to 3.70% after 50 d, corresponding to a 48% decrease (Table 3). However, after 21 d (typical shelf life) lactose content decreased to 3.99%. pH of the yogurt decreased rapidly during ripening but continued to decrease during the storage period (pH 4.55-pH 4.27) possibly suggesting bacterial activity. L. bulgaricus has often been implicated in post-acidification (souring) of yogurt.

In another experiment, relative changes in lactose content of various microbiological media was examined before and after autoclaving ($121^{o}C$, 15 min.). From these data (Table 4) it is apparent that lactose content decreased 6%, 8% and 12% for litmus milk, lactose broth and brilliant green bile broth, respectively. While these decreases in lactose content may be of concern to the microbiologist, a new peak was evident in samples after autoclaving (Figure 2b). Because lactose is known to isomerize in heated milks (Martinez-Castro and Olano, 1980) we obtained reagent grade lactulose for possible identification of the unknown peak in chromatogram 2b. From Figure 3 (standard lactose/lactulose solution) confirmatory work suggests that some of the lactose undergoes isomerization at autoclaving temperatures (121^{o}, 15 min.) resulting in formation of a new peak (Figure 2b) that has the

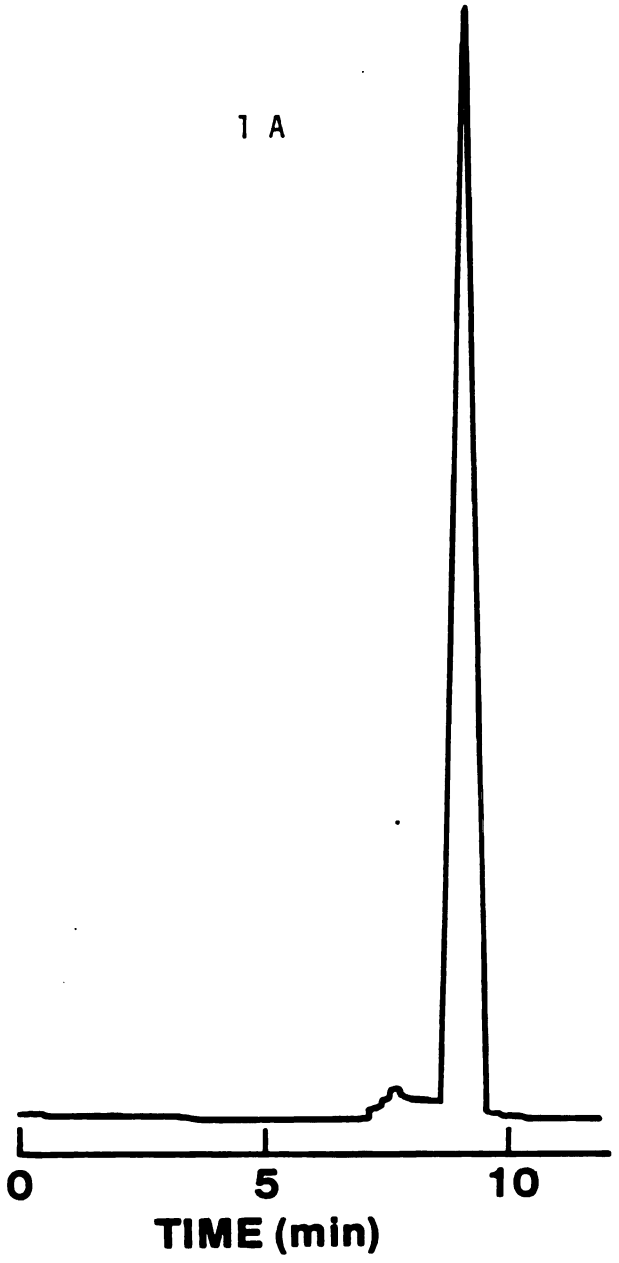
Figure 1 Progressive HPLC chromatograms of carbohydrate extracts from yogurt mix (1.5% fat, 12.6% SNF) and yogurt. Conditions: Aminex HPX-87 carbohydrate column maintained at 80°C; Bio-Rad Aminex Anion/OH Microguard™ cartridge. Refractive index detector; attenuation, 8X; flow rate, 0.6 ml/min; solvent, reverse osmosis ion-exchanged water (ROIE).

A. Carbohydrate extract from unheated lowfat plain yogur mix before heat treatment; large peak lactose. 2 µl injection volume; 50/50 dilution with ROIE water.

B. Carbohydrate extract from heated lowfat plain yogurt mix (88°C, 40 min.); large peak lactose. 2 µl injection volume; 50/50 dilution with ROIE water.

C. Carbohydrate extract from low fat plain yogurt after ripening to pH 4.6. Large peak lactose, next peak galactose. 2 µl injection volume; 50/50 dilution with ROIE water.

D. Carbohydrate extract from low fat plain yogurt after ripening and storage. Large peak lactose, next peak glucose and last peak galactose. 2 µl injection volume; no dilution.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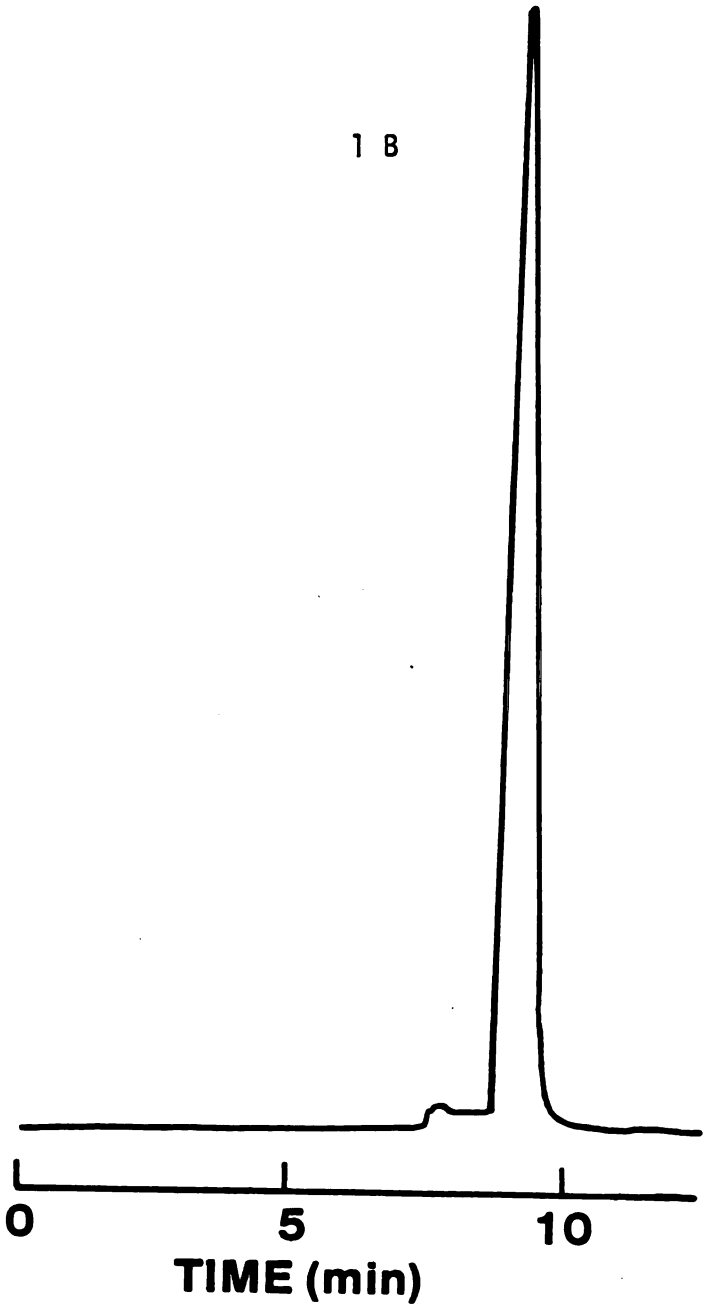
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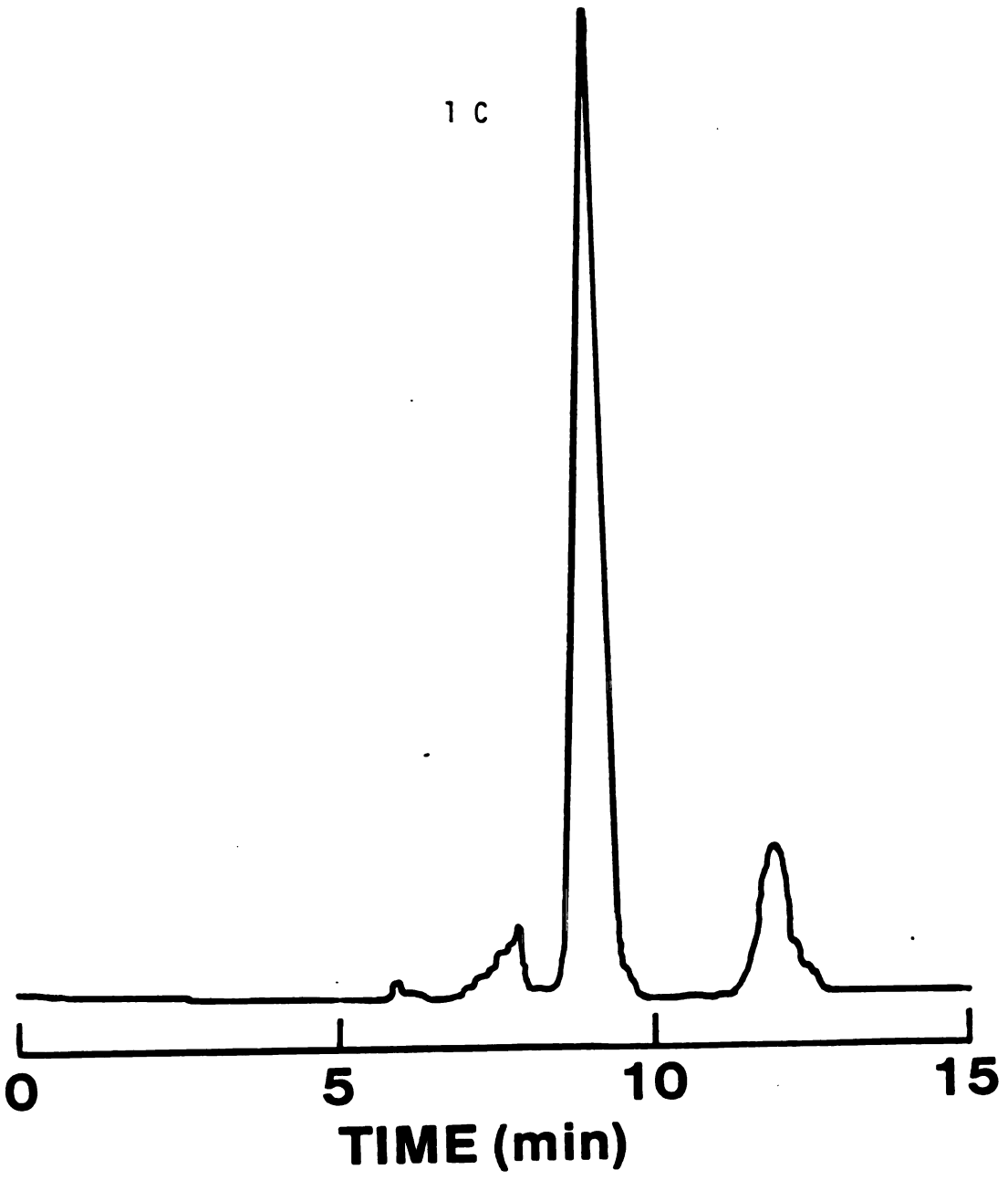
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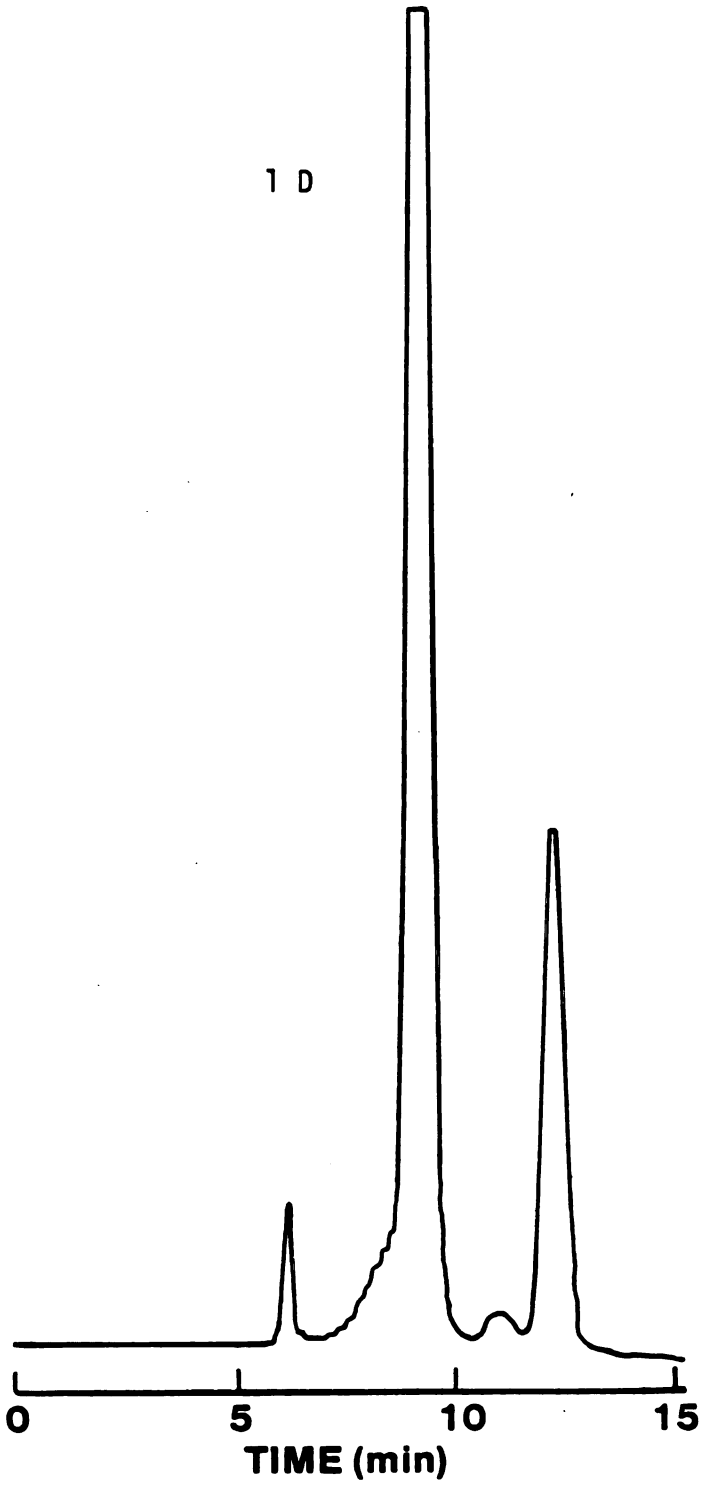
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Table 3 pH, lactose content and galactose content of low fat plain yogurt mix and yogurt during ripening and storage[a].

Time	pH	Lactose (%)	Galactose (%)
0h (mix-not heated)	-	7.12	-[b]
0h (mix-after heat treatment)	-	6.60	-[b]
1h	6.30	6.41	-
2h	6.00	6.19	0.23
3h	5.30	5.80	0.42
4h	4.80	5.44	0.59
5h	4.55	4.84	0.88
1d	4.53	4.34	0.86
7d	4.50	4.24	0.97
14d	4.40	4.19	1.06
21d	4.35	3.99	1.09
40d	4.28	3.68	1.10
50d	4.27	3.70	1.04

[a]average of duplicate injections

[b]not detected

same retention time as standard lactulose (Figure 3). When some lactulose was added to the carbohydrate extract of the autoclaved litmus milk the apparent lactulose peak increased in size without any noticable shoulder, which could suggest the presense of yet another component.

Discussion

Carbohydrate content varies widely among commercial yogurts. This may be due in part to mix composition, heat treatment, incubation temperature and time, starter culture activity, as well as storage temperature. A common industrial practice is fortification of yogurt mix with skim milk solids thereby considerably increasing initial lactose content. Goodenough and Kleyn (1976) reported lactose content of fresh yogurt was 8.5%. Our research indicated an original lactose content of 7.1%, while a recent paper (Alm, 1982) reported initial lactose content of yogurt to be 4.8%. This latter yogurt was apparently not fortified with additional solids. Because of these wide ranges for lactose content of fresh yogurt, corresponding values for ripened yogurt will also vary widely. Although Alm (1982) suggests administration of yogurt should be considered for lactose intollerant individuals, some caution should be observed since lactose content after fermentation may range from 5.75% (Goodenough and Kleyn, 1976) to 2.3% (Alm, 1982). In general, between 30-40% of original lactose is hydrolyzed during heat treatment and ripening (Goodenough and Kleyn, 1976a; Mouillet et al, 1977; our research). Original lactose content is quite important since a large portion of the worlds population is considered lactose intolerant. However, these individuals are generally able to consume lactose in dairy foods in lesser

quantities than persons that are not lactose intolerant.

Numerous procedures have been described for determining carbohydrate content of dairy products, including gas chromatography (GC), thin layer chromatography (TLC), HPLC, Technicon^{TM} Auto-Analysis, as well as enzymatic procedures. But for routine fast analyses HPLC has proven itself. Samples are easily prepared and analysis times are often less than 15 min. for samples containing various sugars. As described in a previous paper (Richmond et al, 1982) resin based systems separate glucose and galactose but not sucrose and lactose. On the other hand, bonded phase systems separate sucrose from lactose but are not able to resolve glucose from galactose. Therefore, depending on carbohydrates of interest one or both systems may be necessary for proper sample evaluation.

Concerning severe heat treatments of milk systems, Martinez-Castro and Olano (1980) described the influence of thermal processing on carbohydrate composition of milk. Using GLC these authors reported that under conditions of industrial sterilization (120^{o}C, 15-20 min.) 2-3% lactulose and 0.3-0.4% epilactose were formed. In heated milks (120^{o}C) maximum amounts of isomers of lactose were 0.53 g lactulose/100 g and 0.08 g epilactose/100 g milk. Mendez and Olano (1979) reviewed the significance of lactulose in infant feeding and medicine, reporting that lactulose in infant feeding formula encourages the development of Bifidobacterium bifidum in intestinal flora, mimicing the flora found in breast fed infants. These authors also described various

medical applications for lactulose (treatment of portal systemic encephalopathy and chronic constipation) and concluded that little work has been published on the isolation and characterization of lactulose. HPLC provides a nondestructive technique to separate lactulose from lactose so that further analyses can be made.

Table 4 HPLC analysis of lactose content (%) of various lactose containing microbiological media[a].

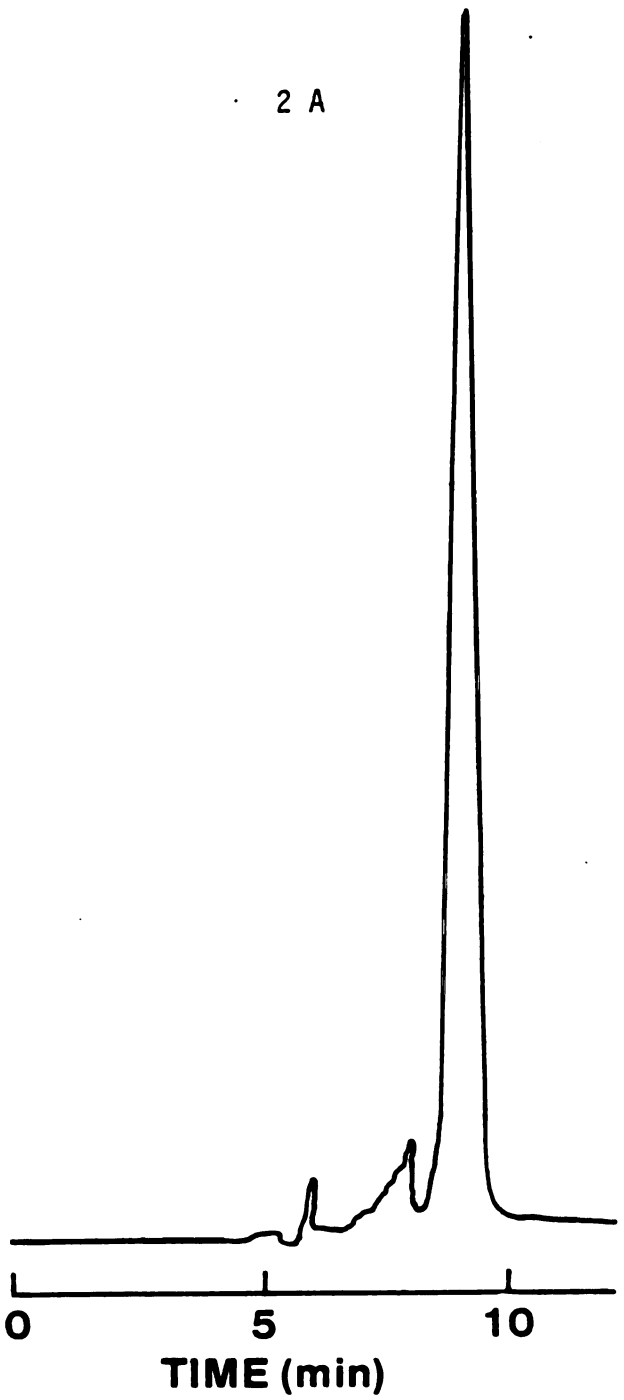
Microbiological Medium	Before Autoclave	After Autoclave ($12^{o}C$, 15 min.)
Litmus milk	3.78	3.55
Lactose broth	0.76	0.70
Brilliant green Bile broth (BGBB)	1.53	1.34

[a]Average of duplicate injections.

Figure 2 - HPLC chromatograms of carbohydrate extract from litmus milk medium. Conditions: Aminex HPX-87 carbohydrate column maintained at 80°C ; Bio-Rad Anion/OH microguard™ cartridge. Refractive index detector; attenuation, 8X; flow rate, 0.6 ml/min; solvent, reverse osmosis ion-exchanged water.

A. Carbohydrate extract from non-autoclaved litmus milk, 2 µl injection.

B. Carbohydrate extract from autoclaved litmus milk (121°C, 15 min.),2 µl injection.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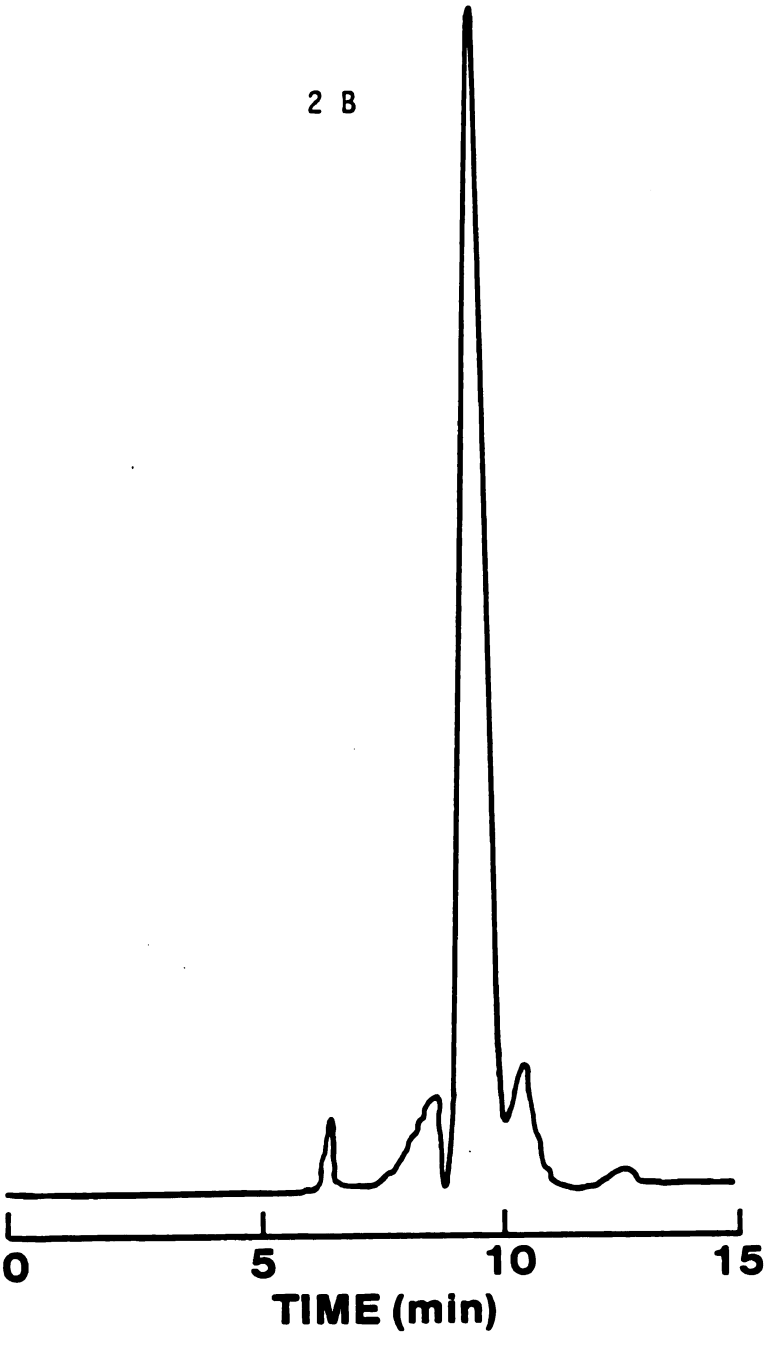
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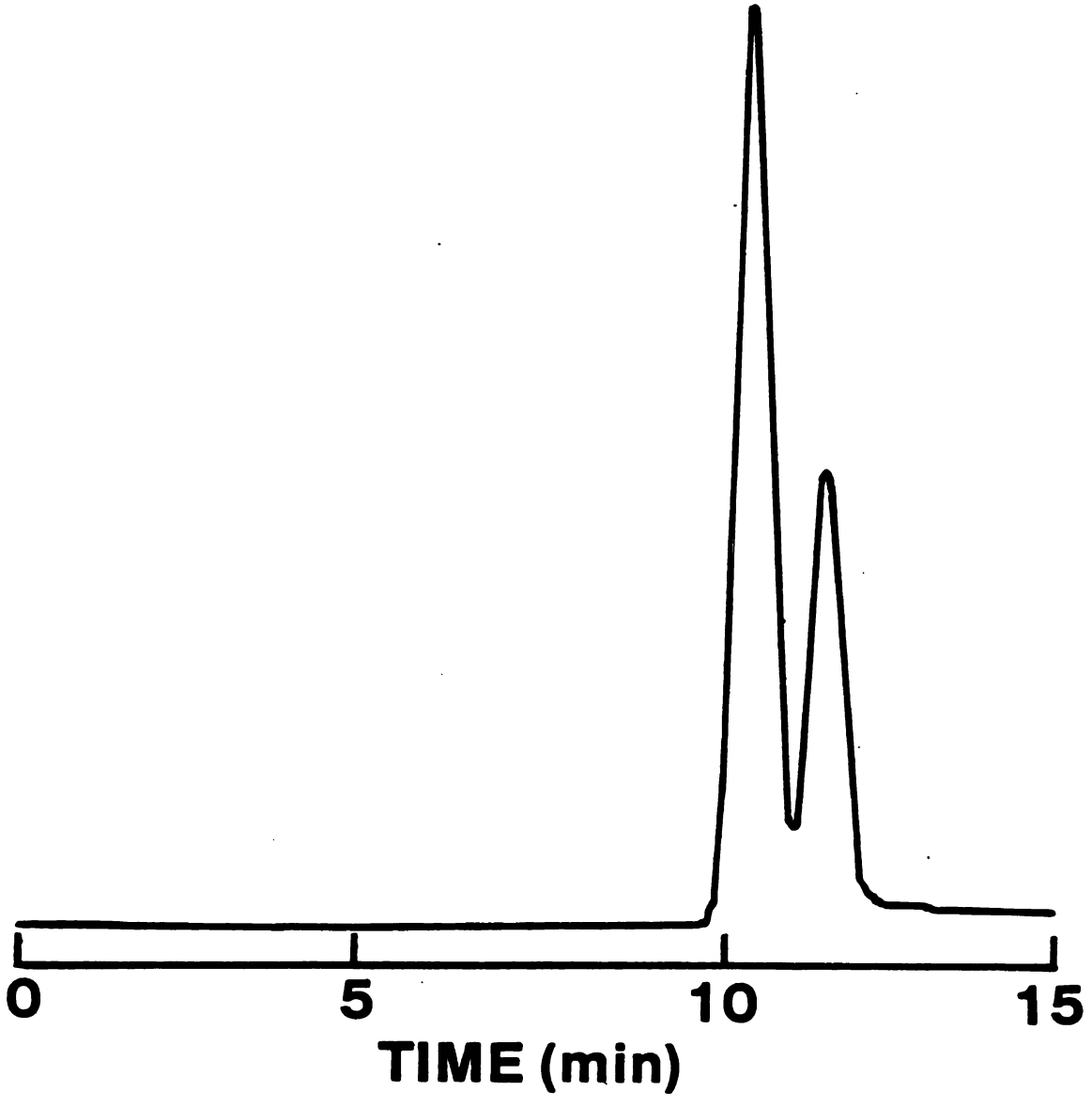

Figure 3. HPLC chromatogram of standard lactose (0.50% wt/vol) and lactulose (0.25% wt/vol). Large peak lactose, next peak lactulose. 4 µl injection; flow rate, 0.4 ml/min; HPX-87 carbohydrate column (80°C) and Bio-Rad Anion/OH Micro-guard Column. Refractive index; attenuation, 8X.

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Introduction

Recently, Lee (1978) reviewed the many methods available for determining carbohydrates in foods. Carbohydrate analysis may be separated into the following categories: physical, chemical, colorimetric and enzymatic. Of the different techniques available, enzymatic and chromatographic (a physical method) procecedures are most commonly used. The various chromatographic procedures include paper, thin layer (TLC) gas-liquid (GLC), ion exchange (IE) and more recently high performance liquid chromatography (HPLC). Automated enzyme assays are also being used to determine carbohydrate content.

Huntington (1978) described the use of an enzymatic analyzer for determining glucose, sucrose and lactose. Immobilized enzymes are used for the sugar assays and they have only a 2 week life span. Also, for each sugar assayed a specific enzyme kit must be used. More recently, Prager and Miskiewicz (1979) reported a GLC procedure for separating and quantifying sucrose, lactose, maltose and glucose in commercial confectionary products. They quantified the trimethylsilyl (TMS) derivatives of the sugars; chromatographic separations, recoveries and reproducibility were all very good.

Significant advances in carbohydrate analysis by HPLC have led to many different procedures that are considered fast, simple, accurate and reproducible. Further, samples need not be altered and they may be collected for additional analyses, if desired. Linden and Lawhead (1975) reported that the analysis of saccharides by HPLC equals the precision and accuracy of GLC. They also describe a

number of applications and problems that are likely to be encountered when doing carbohydrate analyses by HPLC. In 1976, Conrad and Palmer used HPLC to rapidly analyze carbohydrate mixtures in various food and beverage matrices; they also discussed briefly the separation of certain sugar alcohols. Moreover, these authors list numerous HPLC advantages and GC disadvantages. Wong-Chong and Martin (1979a) described a rapid method for determining fructose, glucose, sucrose and raffinose in sugar cane juice by adsorption chromatography. They were able to resolve these carbohydrates in less than 27 minutes using HPLC. In another article (Wong-Chong and Martin, 1979b) these same authors used ion exchange (IE) chromatography for the separation of sugar cane saccharides. They were able to resolve sucrose, glucose and fructose in less than 8 minutes using water as the only solvent. To attain adequate resolution of samples by IE, the column must be jacketed to maintain the elevated operating temperatures which are required. Wong-Chong and Martin (1979b) evaluated Aminex A5, Q15S and Q150S ion exchange resins for their ability to effectively and reproducibily separate saccharides in sugar cane juice.

Recently, Dunmire and Otto (1979) determined the carbohydrate contents of various food products via HPLC. Their method is reported to be fast, simple, specific and reliable over a wide concentration range. They were able to resolve fructose, glucose, sucrose, maltose, lactose, melibiose, raffinose and stachyose in less than 45 minutes. Using this procedure they examined cereals, protein products, processed fruits, chocolate products, baby foods and health bars. The authors also describe a "minicolumn" sample clean-up procedure to increase column life. Woidich, et al. (1978)

described two different procedures for determining simple sugars and sorbitol (D-glucitol) in food. They used a modified silica gel column (Lichrosorb-NH_2) for the determination of fructose and glucose in the presence of various disaccharides. And by using a strongly basic cation exchanger (Bondapak-AX-Corasil), they were able to separate fructose, glucose and sorbitol.

As with carbohydrate analyses, many different procedures are described in the literature for the determination of sorbitol and other sugar alcohols. In 1974, Lara and Yabiku described a TLC method for the identification of sorbitol. Boehringer Mannehim GmbH Biochemica (1979), in their new applications manual, detail the enzymatic determination of D-sorbitol in foodstuffs. Finally, Makinen and Soderling (1980) discussed the quantitative analysis of various sugar alcohols in wild berries and commercial fruits. They made polyacetyl ester derivatives of the sugar alcohols and then used GC to determine polyol concentrations.

Frattali (1980) recently reviewed the regulatory and nutritional aspects of fructose and sugar alcohols in foods. A major point of concern in this article was directed to the nutritional needs of the diabetic. By providing qualitative and quantitative values for simple carbohydrates (including sorbitol) in food, the diabetic, in consultation with a professional, would be able to select from a much broader range of products. Sorbitol occurs naturally in many fruits and is frequently found in fruits of the family Rosaceae. Some fruits in this family include apples, pears and plums. In the apple, sorbitol apparently plays a major role in the translocation of carbohydrates to the developing fruit and during low temperature

storage it is believed that fructose is reduced to sorbitol (Bollard, 1970).

Because of concerns for labeling dietetic and other foods containing sorbitol in the presence of glucose and other saccharides, and because of ripening and storage changes involving sorbitol and other simple sugars--a multiple component HPLC assay was developed in this laboratory (Brandao, et al., 1980). In order to show application of this technique, fresh fruit from various families were assayed for their simple sugars and sorbitol content. Elution order is fructose, glucose, sorbitol, sucrose and maltose. Total analysis time is only 18 minutes for the 5 saccharide mixture.

Experimental

Apparatus

The HPLC system was assembled from a Waters Model M-6000 pump; a Waters model RI-401 differential refractometer detector; and a Whatman Partisil PXS 10/25 PAC column (4.6 mm x 25 cm) connected in tandem to a Waters prepacked μBondapak/carbohydrate^{R} column (4.2 mm x 30 cm). Samples were loaded onto the column with a Waters U6K septumless injector containing a 2 ml sample loading loop. Generally, volumes injected ranged from 2-10 μl, and a Precision Sampling Corporation syringe (Baton Rouge, La.) was used. When injection volume ranged from 10-50 ul, a 100 μl syringe was used. These large injection volumes were sometimes necessary to adequately resolve trace sugars present in the sample. Detector attenuation was maintained at 8X, and a Linear Instruments Model 232 recorder (Costa-Mesa, Ca.) was used to monitor detector response. Temperature was relatively constant at 23^{o}C ± 1^{o}C.

Mobile phase and operating conditions

Isocratic separations were made with the mobile phase, acetonitrile/water/ethanol (80/15/5 v/v/v) Non-spectro grade acetonitrile (Burdick and Jackson, Muskegon, MI.), reverse osmosis, ion-exchange, deaminated water (Culligan system, MSU) and absolute pure ethyl alcohol, reagent quality (U.S. Industrial Chemicals Co., Tuscola, Il.). The mixed ternary mobile phase was degassed under vacuum on a stirring plate for 5 minutes. Flow rate was adjusted to deliver 1.8ml/min.

Carbohydrate standards

Fructose, glucose, sorbitol, sucrose and maltose (all analytical reagent grade) were dried in vacuo at 65^{o}C for 24 hours before making up the standard solution mixture. These standards were carried through the HPLC system both individually and as a mixture for accurate determination of retention times. Peak height measurements were used to quantify the free sugars and sorbitol in the fruit, and linear regression equations were established for each compound. All quantitative determinations were made in duplicate (two aliquots from the same fruit macerate).

Sample preparation

One to two kg of fruit samples were obtained from a local farmers' market . From several ripe, sound fruits, slices of edible tissue weighing a total of 20 to 40g were excised and placed in a Waring blender. The fruits were covered with sufficient 100% ethanol to make the final concentration of ethanol 80%. Fruit and ethanol were then blended at high speed for 2-3 minutes (depending on tissue softness). The resulting slurry was refluxed under stirring for 2 hours with a condenser. The extract was then

filtered through Whatman No. 54 paper; the residue and flat bottom flask were washed with additional 80% ethanol (approximately 200ml). The extract plus washings were then reduced to a volume less than 25ml using a rotary vacuum evaporator. Samples were concentrated until the ethanol odor was completely gone. Finally, the fruit concentrate was made to 25 ml with distilled water and filtered through Whatman No. 42 paper.

All of the fruit sample concentrates were deeply pigmented and would thus severly reduce analytical column life if they were to be injected directly into the system. Therefore, Sep-Pak C_{18} cartridges (Waters Assoc., Inc.) were used to retain these varied and colorful pigments. Resultant solutions were water clear with all the coloring material being retained on these small columns.

The Sep-Pak was easily placed at the end of a 10 ml graduated syringe. The C_{18} cartridge was first pre wet with 2 ml of acetonitrile and then flushed with 5 ml of distilled water. After this, the cartridge was flushed with 2-3 volumes of air before the sample was placed into the syringe. The first 2 ml of sample were discarded, while the second 2 ml of sample were collected for HPLC analysis. Before the injection however, the samples were filtered through a 0.45 µm Metricel membrane (Gelmann Fitration Prdts., Ann Arbor, MI) to further ensure removal of any particulate impurities that might be present.

Results and Discussion

Recovery experiments were conducted by spiking known quantities of standards into an apple sample and then assaying the sample before and after the addition. Further, the prepared standard solution mixture (discussed previously) was also assayed in the same way. Sample recovery (%) in the apple was as follows: fructose,

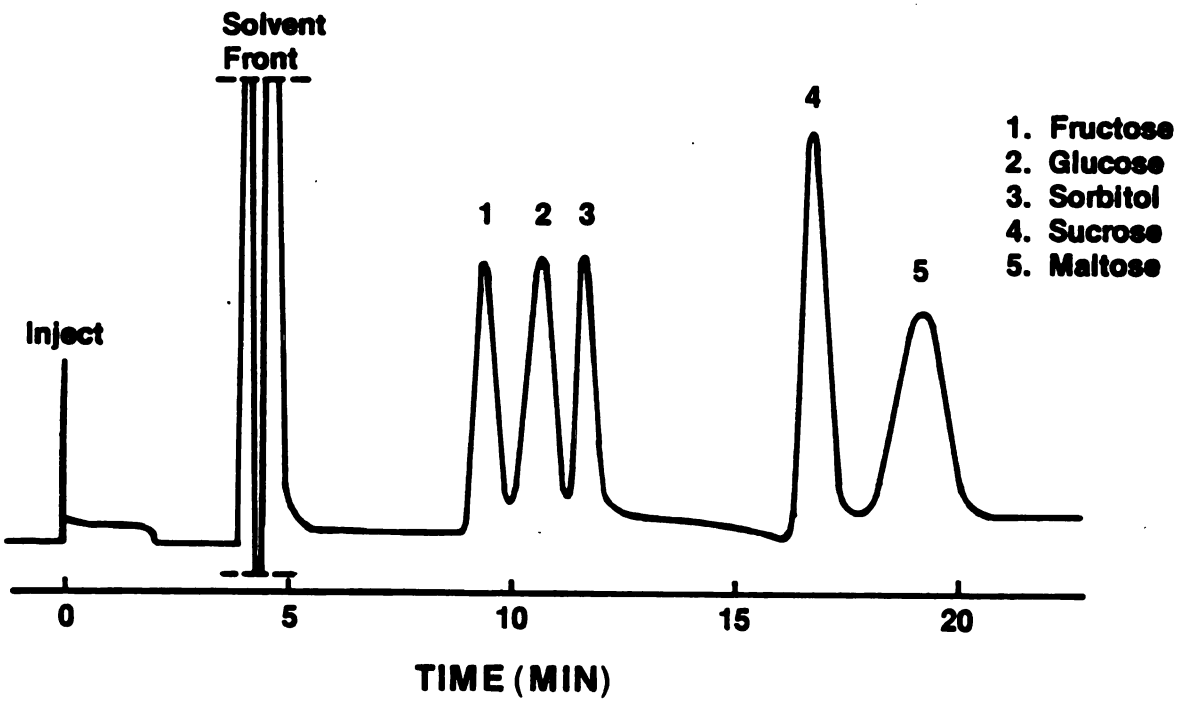

Figure 1. HPLC chromatogram of standard carbohydrate mixture. Dual column arrangement; mobile phase, acetonitrile/water/ethanol (80/15/5; v/v/v); flow rate 1.8 ml/min; injection volume, 10 µl; attenuation, 8X.

Table 1. Linear regression equations and correlation coefficients (r) for carbohydrate standards.

Sugar	Regression equations*	Correlation coefficients
Fructose	Y=1.609x + 0.787	r=0.9995
Glucose	Y=2.165x + 1.939	r=0.9999
Sorbitol	Y=1.541x + 1.378	r=0.9999
Sucrose	Y=2.448x - 2.357	r=0.9998
Maltose	Y=5.325x + 4.413	r=0.9990

*Y=µg sugar standard
x=peak height (mm)

98.3; glucose, 101.3; sorbitol, 98.0; sucrose, 101.2; maltose, 102.1. These values were also very similar to recoveries in the standard solution mixture. The identification of the sugars and sorbitol was based on HPLC retention times (Figure 1). Regression equations and correlation coefficients (r) are shown in Table 1. These equations and coefficients were true in the concentration range 30-130 µg for fructose, glucose and sorbitol; and 30-180 µg for sucrose and maltose.

In order to be consistent with other literature, our data are presented as percent fresh weight of edible tissue (% fresh weight). In general, the data in Tables 2 and 3 compare favorably with values reported in the literature (Whiting, 1970; Lee, et al, 1970). Sugar analyses of fruits from a number of different families are depicted in Table 2. None of these fruits contained sorbitol. Even when large volumes were injected no sorbitol peak was present. An actual chromatogram for the orange is shown in Figure 2. On the other hand, when examining fruits of the Rosaceae family (Table 3), sorbitol was often, but not always, present. Sorbitol was not detected in the red plum, blackberry or peach. A chromatogram of the purple plum is shown in Figure 3. When using large injection volumes, maltose was observed in only a few fruits (Tables 2 and 3). In addition, we have also presented sugar profiles of some novel fruits that are not often reported in the literature.

By using two carbohydrate columns connected in tandem and a ternary mobile phase of acetonitrile, water and ethanol sorbitol can be adequately and reproducibly separated in one simple procedure from its parent sugar glucose, in systems containing fructose, sucrose and maltose.

Table 2. HPLC analysis of simple sugars in some common fruits.

Fruit	Family	Sugar content (% fresh weight)			
		Fructose	Glucose	Sucrose	Maltose
Avocado	Lauraceae	---*	0.15	---	---
Banana	Musaceae	2.41	2.58	14.0	---
Blueberry	Vaccinium	3.21	2.99	0.25	---
Cherry tomato	Solanaceae	1.94	0.87	0.09	---
Grape	Ampelidaceae	7.33	8.05	4.65	0.05
Honey Dew melon	Cucurbitaceae	2.66	1.91	12.09	0.20
Lime	Rutaceae	0.32	0.33	0.03	---
Mango	Anacardiaceae	3.18	0.49	9.86	---
Orange	Rutaceae	3.02	2.93	7.02	0.32
Papaya	Caricaceae	2.34	2.48	4.43	---
Pineapple	Bromeliaceae	2.32	1.65	9.50	---
Strawberry	Fragaria	2.59	2.41	1.64	0.10
Watermelon	Cucurbitaceae	2.98	1.32	7.39	0.49

*not detected

Table 3. HPLC analysis of simple sugars and sorbitol in fruits of the Rosaceae family.

Fruit	Sugar and Sorbitol Content (% fresh weight)				
	Fructose	Glucose	Sorbitol	Sucrose	Maltose
Apple (Golden delicious)	7.87	1.65	0.26	1.11	---*
Apple (Red delicious)	7.96	3.46	0.24	0.51	---
Pear (cv. Bartlett)	9.03	0.90	1.66	1.24	---
Pomegrante	6.05	4.71	0.30	0.70	---
Red plum	0.83	1.26	---	4.24	0.05
Prune plum	3.29	3.08	2.65	4.41	0.11
Yellow plum	1.04	2.05	0.26	1.58	---
Sour cherry (cv. Montmorency)	3.74	4.06	1.04	---	---
Sweet cherry	4.92	4.77	2.10	0.13	---
Blackberry	1.55	1.18	---	0.14	---
Peach	0.45	0.32	---	3.13	---

*not detected

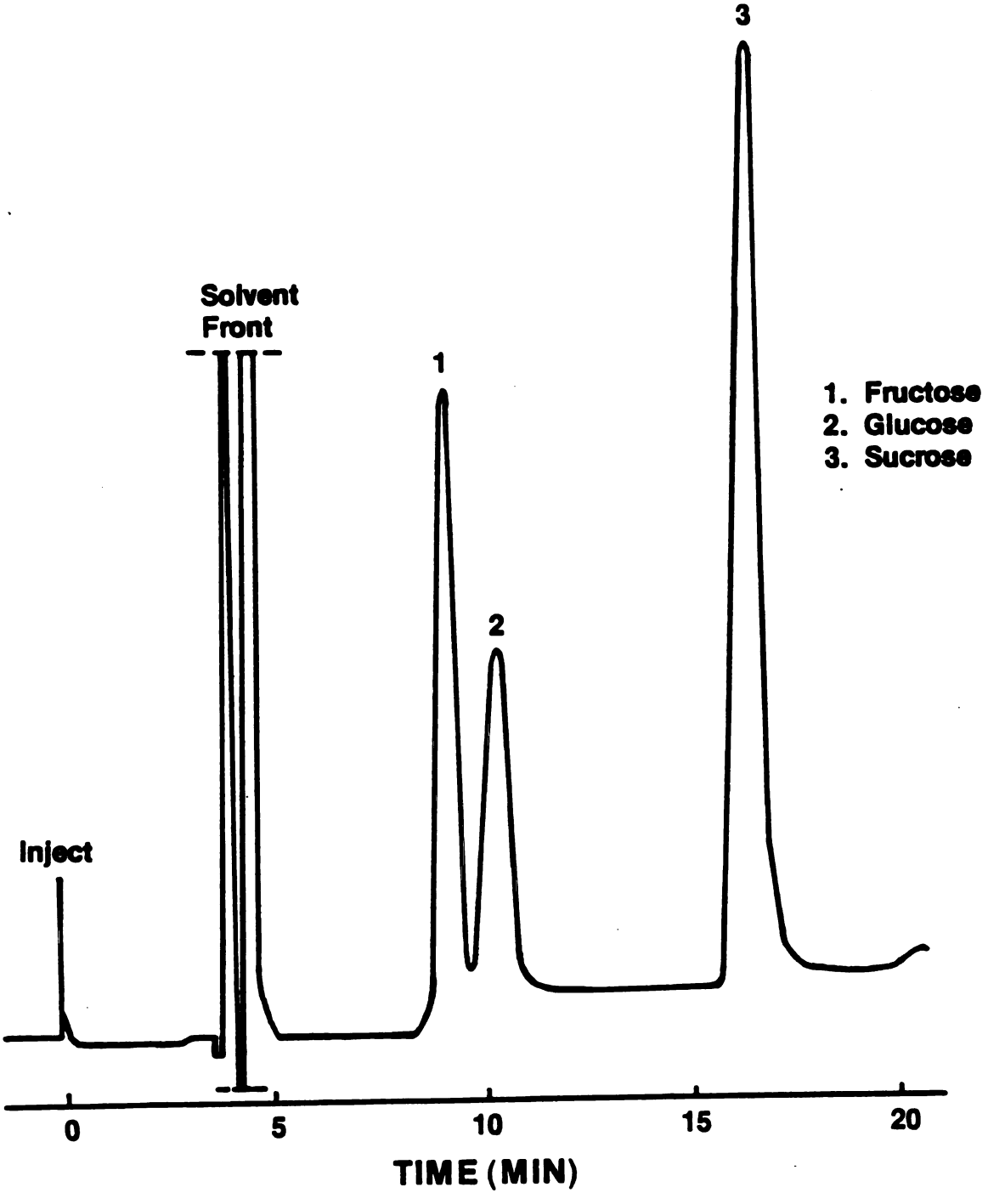

Figure 2. HPLC chromatogram of carbohydrates in the orange. Dual column arrangement; mobile phase, acetonitrile/water/ethanol (80/15/5; v/v/v); flow rate, 1.8 ml/min; injection volume, 5 µl; attenuation, 8X.

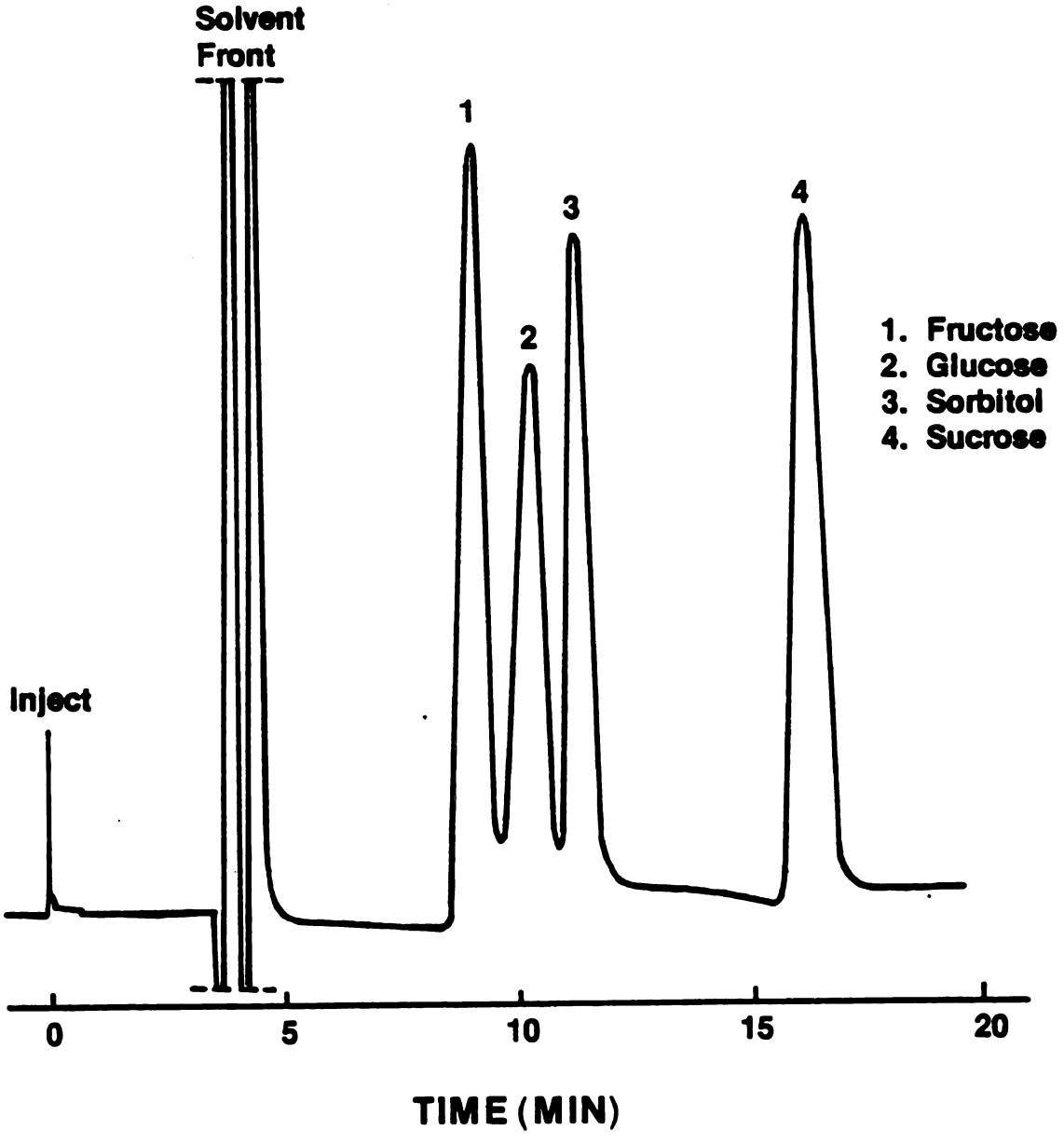

Figure 3. HPLC chromatogram of carbohydrates and sorbitol in the purple plum. Dual column arrangement; mobile phase, acetonitrile/water/ethanol (80/15/5; v/v/v); flow rate, 1.8 ml/min; injection volume, 5 µl; attenuation, 8X.

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CHAPTER VI

SUMMARY

SUMMARY

1. Yogurt consumption in the United States and abroad has increased tremendously over the past decade due to (1) fruit and flavor additions, (2) increased marketing and advertising expenditures, and (3) growing interest in good nutrition and health.

2. Composition of commercial yogurt varies widely both between and within brands. Industry would benefit by improving quality control; yogurts are often excessively overfilled and sometimes considerably underfilled yielding an illegal product.

3. Yogurt is not necessarily a low calorie food. Plain or natural yogurt is commonly low in calories but fruited yogurts may contain two times as many calories as plain. Lowfat yogurt does not mean low calorie yogurt.

4. Many people are now making yogurt in the home because of increasing retail costs and because they enjoy making it. Buckwheat honey combined with lowfat plain yogurt is a "natural" food, and because of honey's sweetness provides a low calorie flavored yogurt when compared to traditional fruited yogurts containing from 15-18% fruit preserves.

5. Phase separation or whey-off is a concern in transportation and distribution of yogurt. Secondary packaging (stretch wrapping corrugated sleeves) was found to be statistically significant in reducing phase separation of yogurt using simulated testing methods.

6. Impact damage may or may not be a problem in distribution. When yogurt containers or trays are caught before impact, physical damage is often not a concern.

7. High performance liquid chromatography (HPLC) has proved to be a valuable technique for separation and analysis of carbohydrates and simple sugars. Analysis times are generally less than 15 minutes for multi-component samples. Further, samples may be collected after separation for further testing.

8. With new column and hardware technology, difficult separations are made easy. Special HPLC carbohydrate columns (bonded phase and resin based) are made for resolving once difficult separations, including separation of glucose from galactose.

9. The introduction of protective guard columns for HPLC have also improved many qualitative and quantitative aspects of carbohydrate analysis.

10. Rapid HPLC separation and quantitation of lactose, glucose and galactose is an important technique for use by industry and academia. New immobilized enzyme technology has brought about commercial reactors for hydrolysis of lactose from cheese whey. HPLC is a valuable tool for monitoring hydrolysis rates in these enzyme reactors. Because many people are lactose intolerant HPLC offers an easy technique to determine lactose content in many foods, and also allows a means to follow hydrolysis of lactose and accumulation of galactose during ripening and storage of yogurt.

11. Bonded phase HPLC using two microparticulate columns connected in tandem is a useful and fast way to separate and quantitate various multiple component samples containing lactose, sucrose, glucose, fructose and sorbitol in a very short time. Separation of sorbitol in the presence of glucose is important since many food systems contain numerous sugars and sorbitol together.

12. While much has been learned about the physical and chemical properties of yogurt since 1950, more research is needed, especially work on practical problems of industrial concern in order to keep improving market sales.

APPENDIX I

Table 1. Analysis of Variance Table for Color.

Source Variation	DF	MS	F
Repetitions	49	12.25	10.56*
Treatments (Adj. Totals)	250	3.34	2.87
Blocks in Reps. (Adj.)	50	10.33 E_b	
Error	195	0.95 E_e	
Total	299		

*Significantly different at the 95% level.

NOTE: The F-ratio for differences among treatments was based on the adjusted treatment total sums of squares with r (t-1) d.f. as the numerator and the Effective Error Variance (E.E.V.) with (tr-t-b+1) d.f. in the denominator. The F-ratio for Repetitions was estimated by MS Reps ÷ E.E.V. with 49 and 195 d.f.

The Effective Error Variance was estimated by the following equation:

$$E.E.V. = E_e[1 + (t-k)\mu]$$

where $$\mu = \frac{r(E_b-E_e)}{rt(k-1)E_b + k(b-r-t+1)E_e}$$

In this case E.E.V. = 1.16 with 195 d.f.

APPENDIX I

Table 2. Analysis of Variance Table for Sweetness.

Source of Variation	DF	MS	F
Repetitions	49	9.64	3.63*
Treatments (Adj. Totals)	250	13.92	5.25*
Blocks in Reps. (Adj.)	50	8.92 E_b	
Error	195	2.25 E_e	
Total	299		

*Significantly different at the 95% level.
Effective Error Variance = 2.65

Table 3. Analysis of Variance Table for Texture.

Source of Variation	DF	MS	F
Repetitions	49	9.91	4.12*
Treatments (Adj. Totals)	250	0.45	0.19
Blocks in Reps. (Adj.)	50	11.09 E_b	
Error	195	2.01 E_e	
Total	299		

*Significantly different at the 95% level.
Effective Error Variance = 2.41.

APPENDIX I

Table 4. Analysis of Variance Table for Flavor.

Source of Variation	DF	MS	F
Repetitions	49	7.73	2.01*
Treatments (Adj. Totals)	250	24.86	6.47*
Blocks in Reps. (Adj.)	50	9.48 E_b	
Error	195	3.35 E_e	
Total	299		

*Significantly different at the 95% level.
Effective Error Variance = 3.85.

Table 5. Analysis of Variance Table for Overall Acceptability.

Source of Variation	DF	MS	F
Repetitions	49	9.22	2.50*
Treatments (Adj. Totals)	250	21.02	5.71*
Blocks in Reps. (Adj.)	50	9.82 E_b	
Error	195	3.18 E_e	
Total	299		

*Significantly different at the 95% level.
Effective Error Variance = 3.68.

APPENDIX I

Table 6. Sample Means for Each Attribute.

Attribute	A1	A2 (5% Honey)	A3	B1	B2 (6% Honey)	B3	S.D.*
Color	5.7^{a}	5.5^{a}	6.2^{b}	5.5^{a}	5.5^{a}	5.7^{a}	0.15
Sweetness	3.7^{a}	3.9^{a}	4.1^{ab}	5.0^{c}	4.8^{c}	4.8^{bc}	0.23
Texture	6.0^{a}	6.0^{a}	6.2^{a}	6.2^{a}	6.0^{a}	6.1^{a}	0.22
Flavor	4.0^{a}	4.5^{ab}	5.0^{bc}	5.4^{cd}	6.0^{d}	5.4^{cd}	0.28
Overall Acceptability	4.3^{a}	4.7^{ab}	5.3^{bc}	5.8^{c}	6.0^{c}	5.5^{c}	0.27

Sample means for each attribute with the same superscript are not statistically different at the 95% level (Duncan's Multiple Range test).

*Standard deviation of the attributes ($\sqrt{\text{E.E.V.} \div r}$) used in the Duncan's Multiple Range Test.

APPENDIX II

HONEY YOGURT

NAME: _____

Please taste the yogurt samples in the order presented. Evaluate each characteristic by placing a vertical line across the scale at the point that best describes that property in the sample.

Clearly label each vertical line with the sample number at the time of evaluation.

Very Poor Color ⊢——————————————————⊣ Excellent Color

Not at all Sweet ⊢——————————————————⊣ Extremely Sweet

Very Poor Texture ⊢——————————————————⊣ Excellent Texture

Very Poor Flavor ⊢——————————————————⊣ Excellent Flavor

Overall Acceptability (evaluate all 3 samples):

Dislike Extremely ⊢——————————————————⊣ Like Extremely

COMMENTS: