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DEVELOPMENT AND PROPERTIES OF A DRINKABLE YOGURT SHAKE SWEETENED WITH HONEY

BY

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ABSTRACT

This study was conducted to investigate the growth and activity of various yogurt cultures when honey was used in place of sucrose in milk, then to develop and optimize product formulations for a low-fat drinkable yogurt shake sweetened with honey. Reconstituted NFDM (12% w/v) containing 5% (w/w) sucrose, fructose or honey were inoculated with *Lactobacillus acidophilus* La-7, *Lactobacillus delbrueckii* subsp. *bulgaricus* Lr-78, *Streptococcus salivarius* subsp. *thermophilus* St-134 or *Bifidobacterium* sp. Bf-13 at a 5% (v/v) level, incubated at 37°C. Plate counts were determined at 0 and 24 hr, and pH was monitored at 0, 12, and 24 hr. Activity was determined using HPLC analysis. Strawberry yogurt shakes with 5% (w/w) honey were manufactured with milk containing two fat levels (1 or 2%) and supplemented with three NFDM levels (0, 3, or 6% w/w). Sucrose sweetened yogurt shakes were used as the control. A trained sensory panel evaluated sweetness, strawberry flavor intensity, viscosity, and smoothness. Overall acceptability was determined by an untrained panel.

Results indicated that honey had no significant effect on the growth or activity of lactic acid bacteria, however it did enhance ($p<0.05$) the activity of bifidobacteria. Sweetness, strawberry flavor intensity, and smoothness of the yogurt shakes decreased ($p<0.05$) with an increase in NFDM content, viscosity increased ($p<0.05$) with an increase in NFDM. A difference in viscosity between honey and sucrose was found by the trained panel, but was not detected by the rheological study. Honey and sucrose sweetened products, with no added NFDM, were found to be the most desirable.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Some current market trends indicate that today's consumers want foods that are not only lower in fat, but also healthier for them to consume. It is stated that although fat was still the number one concern among consumers, interest in nutrition rose dramatically between May 1995 and 1996. It was also predicted that *Bifidobacterium* and *Lactobacillus* would be receiving a great deal of attention due to the growing interest in incorporating these probiotic bacteria into foods (Sloan, 1996). In addition, foods that boost the immune system, reduce the risk of disease or enhance health, are anticarcinogenic, or are antioxidants are forecasted to be some of the consumer trends for the next twenty years and beyond (Sloan, 1998).

The consumption of yogurt as well as low-fat flavored beverage milks and milk drinks increased steadily from 1970-1992 (Putnam and Allshouse, 1993). Yogurt is perceived as a healthy product, and with the addition of live probiotic cultures such as *Lactobacillus acidophilus* and/or *Bifidobacterium* sp., yogurt has an even healthier perception. Honey emanates a healthy image as well. There has been increased interest in the incorporation of honey into various foods. Honey has been applied to food products such as beverages, frozen desserts, processed meats, among others. In fact, several sport drinks on the market contain honey. Honey provides many important nutrients, such as glucose and fructose, as well as adding it's distinctive flavor when it is incorporated into a food product (National Honey Board, 1996).

The purpose of this project was to investigate the possible incorporation of these trends into a product. The product was a drinkable yogurt shake sweetened with honey and fermented with the aforementioned probiotic bacteria along with common commercial yogurt starter cultures.

The hypothesis of this research is that honey can be fermented by starter and probiotic bacteria typically used in the production of yogurt. A desirable fermented yogurt drink with optimum properties can be manufactured using honey as a sweetener rather than sucrose which is the traditional sweetener used by the dairy industry. The specific objectives of this research were (1) to investigate the growth and activity of *Streptococcus salivarus* subsp. *thermophilus*, *Lactobacilus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus,* and *Bifidobacterium* sp. when honey is used in place of sucrose in milk, (2) to develop and optimize product formulations for a drinkable low-fat strawberry-yogurt shake and determine their stability during refrigerated storage, and (3) to determine overall product acceptability.

CHAPTER 1

LITERATURE REVIEW

Section 1.1 - Background of yogurt

Although known by a variety of names around the world, the name yogurt was originally derived from the Turkish name 'jugurt' (Tamime and Deeth, 1980). Yogurt is produced by fermenting milk, which historically was a method of preserving milk. Since 1950, the technology of yogurt production as well as the understanding of the factors effecting the organoleptic properties of yogurt have been rapidly advancing. The introduction of fruits and flavors in the manufacture of yogurt have furthered the growth of its consumption. The increase in knowledge of the beneficial effects in yogurt has further increased its value nutritionally (Rasic and Kurmann, 1978).

For more than two decades, yogurt has become increasingly popular in the United States. Per capita consumption of yogurt in the U.S. has increased from 0.8 pounds per year in 1970 to 4.3 pounds per year in 1992 (Putnam and Allshouse, 1993). In addition, consumption levels of low-fat milk products, which includes low-fat yogurt, have risen between 1970 and 1987 (Senauer *et al.*, 1991).

The Code of Federal Regulations (CFR, 1997) contains standards of identity for three yogurt types: yogurt, low-fat yogurt, and nonfat yogurt. Yogurt is defined as containing no less than 3.25 percent milk-fat, no less than 8.25 percent milk solids, and no less than 0.9 percent titratable acidity expressed as lactic acid. Low-fat yogurt and non-fat yogurt have the same standards of

identity as yogurt with the exception of the fat levels which are; no more than 2 percent and no less than 0.5 percent milk-fat, and no more than 0.5 percent milk-fat, respectively. Yogurt must contain the lactic acid bacteria, *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, and the milk must be pasteurized prior to the addition of the cultures. Also, yogurt may be homogenized if desired.

In addition to the types of yogurt defined by the FDA, there are also 'styles' of yogurt. According to Tamime and Deeth (1980) there are two main styles, set and stirred yogurt, based on the way the yogurt is produced. However, many more styles exist. For example, a fluid yogurt is considered to be a stirred yogurt with a lower total solids level. Also, yogurts can be further differentiated by the incorporation of flavors resulting in three additional categories; natural or plain, fruit or flavored yogurt. Other categories of yogurt also include; pasteurized/UHT yogurt, concentrated/condensed yogurt, frozen yogurt, dried yogurt, and low or reduced calorie yogurt (Tamime and Deeth, 1980).

Yogurt starter cultures consist of two species of bacteria: *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus thermophilus*. These bacteria are classified as homofermentative (metabolize glucose via the glycolytic pathway) lactic acid bacteria. Lactic acid bacteria utilize lactose, the carbohydrate source in milk, as their principle carbon source for energy and growth by hydrolyzing it into glucose and galactose. While the resulting galactose is not utilized by most strains of lactic acid bacteria, the glucose is

metabolized through the glycolytic or Embden-Meyerhof-Parnas pathway creating lactic acid which composes 95% of the fermentation end products. In addition, small quantities of organoleptic substances (substances which impart the characteristic flavor, aroma, taste, consistency, viscosity and texture properties of yogurt) such as volatile fatty acids, ethanol, acetaldehyde, acetoin and butanone are also created (Fernandes *et al.*, 1992).

Free amino acids and peptides have been shown to increase during fermentation suggesting the presence of exopeptidases and endopeptidases in lactic acid bacteria (Amer and Lammerding, 1986; Fernandes *et al.*, 1992). Although no gross method of proteolysis is mediated by these cultures during the yogurt fermentation process, each of the two yogurt bacteria contain moderate proteolytic activity which aids in their symbiotic growth and production of flavor compounds (Figure 1) (Fernandes *et al.*, 1992).

Although *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* contain lipases, they do not effect the level of free fatty acids in yogurt significantly. Furthermore, with the use of skim or low-fat milk in the manufacturing of yogurt, lipid hydrolysis by these lipases will not contribute much to the characteristics of the product (Fernandes *et al.*, 1992).

Section 1.1.1 - Drinkable yogurt

Although there is no standard of identity for a 'drinkable', 'liquid', or 'fluid' yogurt, drinkable yogurt products have been investigated previously. Traditionally manufactured by mixing water and yogurt in equal amounts, a fluid yogurt can also be produced by using a mix with a lower level of solids such, e.g.

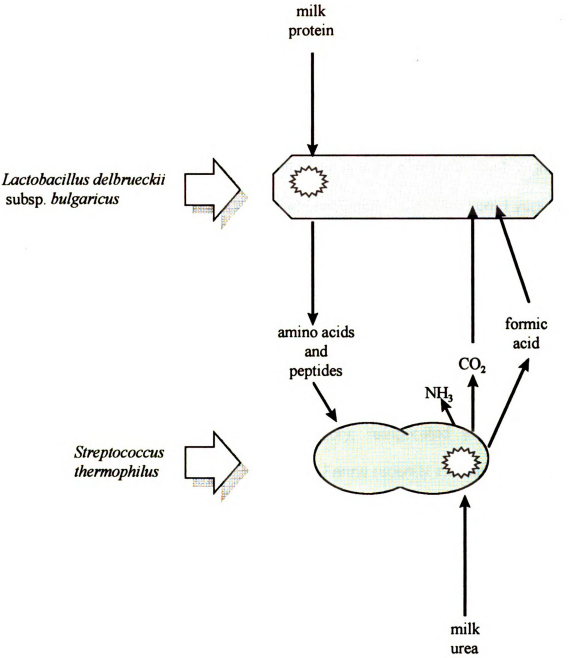


Figure 1. Schematic of symbiotic relationship between yogurt cultures (Loones, 1989)

11% (Tamime and Deeth, 1980). The market potential of liquid yogurt was discussed by Morley (1979) which included a typical yogurt drink formula and manufacturing instructions. Morley reported that a liquid yogurt had great market potential due to the large growth in the market for yogurt during the 1970's. Hong (1980) developed a drinkable yogurt. However, neither Morley's nor Hong's report included consumer testing for acceptability or intent to purchase this product. White *et al.* (1984) developed a fruit-flavored yogurt drink, which included consumer testing and concluded that fruit-flavored yogurt drinks would be accepted by a major segment of the consumer market, and should also make significant gains in acceptance as consumers become more aware of the nutritional benefits of these foods. Consumers' want of healthy foods is a major trend along with a trend toward adult-orientated instant nutrition and energy drinks (Sloan, 1996). In addition, a new category has been said to be emerging in the cultured products category; refrigerated yogurt drinks (Anonymous, 1996). For example, Sunnydale Farms recently introduced a low-fat yogurt shake and Yonique introduced a drinkable yogurt in October of 1995 (Gorski, 1997; Anonymous, 1996). This new refrigerated yogurt drink category increased by over 161 percent and produced 16.5 million dollars in sales during 52 weeks ending Sept. 8, 1996 according to Information Resources Inc. (Anonymous, 1996).

Section 1.1.2 - Nutritional aspects of yogurt associated with the consumption of live lactic acid bacteria and bifidobacteria

The term lactic acid bacteria refers to a group of gram-positive bacteria closely affiliated with one another by the similarity in their mophological, metabolic, and physiological characteristics. These bacteria are described as gram-positive, nonsporing, nonrespiring cocci or rods, in which lactic acid is the major end product produced from their fermentation of carbohydrates. Although there has been some controversy in respect to the boundaries of this group, it has been generally agreed that the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* (which now includes *Lactococcus* and *Enterococcus* according to the revised taxonomy) comprise the core (Axelsson, 1993).

First discovered in the feces of infants in 1900 by Henry Tissier and named *Bacillus bifidus communis*, bifidobacteria are not considered to be true lactic acid bacteria (Hughes and Hoover, 1991). These gram-positive curved rods produce both acetic and lactic acids as primary metabolites, in a theoretical ratio of 3:2, respectively, through an unusual glucose metabolizing system (Figure 2) (Hughes and Hoover, 1991; Scardovi and Trovatelli, 1965). In addition, small amounts of ethanol and formic acid are also produced during fermentation by bifidobacteria (DeVries and Stouthamer, 1968). It is for this reason, that although bifidobacteria exhibit beneficial health effects when used in yogurt, they have been shown to produce defects such as whey separation, sandy or slimy texture, too mild a taste, yeasty or vinegary taste or too little

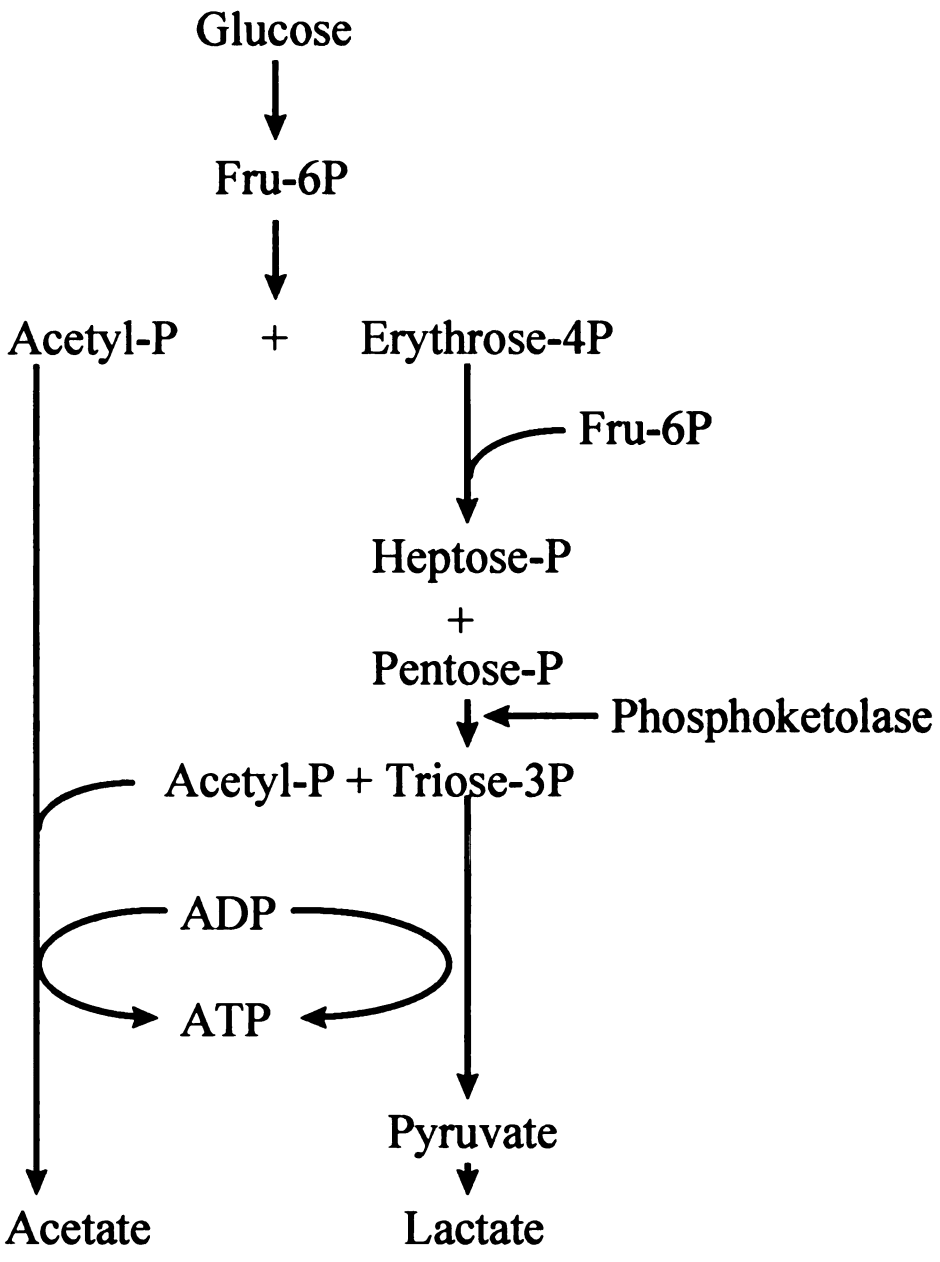


Figure 2. Bifidus pathway (Wood and Holzapfel, 1992)

aroma (Rasic and Kurmann, 1983). However, Samona *et al.* (1996) recommended using a mixed culture, containing both bifidobacteria and yogurt bacteria, in order to overcome this problem. In addition, Samona *et al.* (1996) stated that it would also decrease fermentation time (as bifidobacteria take longer to ferment than do yogurt cultures). Gilliland (1991) reported that the addition of yogurt cultures to 'bifidus' products might increase their dietetic value.

For decades, humans have consumed yogurt for its perceived prophylactic and therapeutic properties. Prophylactic properties refer to the protection against disease while therapeutic properties refer to a cure after illness (Fernandes *et al.*, 1992). Some of the beneficial health effects believed to be associated with the consumption of live lactic acid bacteria are reduction of serum cholesterol, anticarcinogenic actions, antagonism toward pathogens, and reduced lactose intolerance (Fuller, 1989; Gilliland, 1990; Fernandes *et al.*, 1992).

While most lactic acid bacteria exert antagonistic actions toward pathogens *in vitro*, *L. acidophilus* and *Bifidobacterium bifidum*, have received the most attention for the control of these pathogens *in vivo* (Gilliland, 1990). These bacteria are considered to be probiotic bacteria. The word probiotic was originally used to describe substances produced by one protozoan which stimulated another (Lilly and Stillwell, 1965). The term was later applied to animal feed supplements which had a beneficial effect on the gut flora of the host animal (Parker, 1974). Probiotic was more recently defined as 'organisms

and substances which contribute to intestinal microbial balance'. However, Fuller (1989) felt this definition to be unsatisfactory because it was too imprecise and could include antibiotics thus he redefined it as 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. Probiotics generally refer to the viable bacteria themselves or the viable bacteria contained in cultured dairy products or food supplements.

Mann (1977) reported that the daily consumption of large amounts of yogurt (2-4 liters per day) reduced serum cholesterol in human subjects after 12 days. Bazzare *et al.* (1983) observed a significant decrease in the total serum cholesterol in females after one week of yogurt supplementation (three 8-oz. cups per day), while the change in males was not significant. In contrast, neither Rossouw *et al.* (1981) nor Thompsan *et al.* (1982) found any significant differences in the serum cholesterol levels of their subjects after three weeks of consuming yogurt or other fermented dairy products (2 and 1 liter volumes respectively). Thakur and Jha (1981) reported that rabbits on a high cholesterol diet exhibited higher serum cholesterol levels than did rabbits on a similar diet supplemented with yogurt. Although these researchers attributed this to the calcium ions in the yogurt, the non-fermented milk used in the control group contained the same calcium concentration. Gilliland (1990) thought it was possible that the calcium ions were more bio-available in the fermented yogurt product than in the control milk, however, additional research would be needed to more clearly determine the effect of yogurt on hypocholesterolemia.

DeRodas *et al.* (1996) investigated the effect of *L. acidophilus* and dietary calcium on total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and total bile acids in the serum of pigs previously fed a high cholesterol diet. Supplementation of the diet with *L. acidophilus* (2.5×10^{11} total cells/feeding) or 1.4% dietary calcium decreased total cholesterol and LDL cholesterol in serum, but not HDL cholesterol. In addition, total serum concentration of bile acid was reduced by both 1.4% calcium and *L. acidophilus*. The authors concluded there was a significant correlation between total serum cholesterol and total bile acids and thus that both *L. acidophilus* and dietary calcium possess hypocholesterolemic actions, though further research is needed to simultaneously evaluate the effects of these on serum and fecal bile acids. Furthermore, although there is no relationship between the ability of *L. acidophilus* to deconjugate bile acids and to assimilate cholesterol (Walker and Gilliland, 1993), results from DeRodas *et al.* (1996) and Gilliland *et al.* (1985) indicate that both are important in the capability of *L. acidophilus* to exercise hypocholesterolemic action. Another recent study by Beena and Prasad (1997) investigated the potential hypocholesterolemic properties of bifidus and standard yogurts fortified with skim milk powder, condensed whey, or lactose-hydrolyzed condensed whey in albino rats. Total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol levels were measured. Results indicated that bifidus yogurts and yogurts fortified with whey proteins reduced total and LDL cholesterol.

Several lactic acid bacteria have been shown to possess anticarcinogenic or antimutagenic activity. Some of this activity is attributed to the substances the organism produces during growth, while it was reported that it could also be attributed to the antagonistic action of the lactic acid bacteria, especially during growth in the intestines, against those organisms that might convert procarcinogens into carcinogens (Gilliland, 1989). Fernandes *et al.* (1992) also reported that the consumption of fermented milk products could possibly prevent the initiation of cancer by causing a favorable change in intestinal microflora resulting in a reduction in the conversion of pro-carcinogens to carcinogens. Shahani *et al.* (1983) studied the influence of *L. acidophilus* on Ehrlich ascites tumor cells in rats. Twelve rats were divided equally into two groups. The control group was fed rat chow and the other was fed rat chow plus milk fermented with *L. acidophilus*. After 7 days the animals were killed and the number of tumor cells were determined. Results showed that the rats that received milk fermented with *L. acidophilus* had lower numbers of tumor cells in all three replicates. It was concluded that *L. acidophilus* produced "something" during growth that had an antagonistic effect on the growth of the tumor cells.

Goldin and Gorbach (1977 and 1984a) studied the influence of the consumption of *L. acidophilus* cells on the activity of three enzymes in the fecal material of rats. These three enzymes; β-glucuronidase, azoreductase, and nitroreductase; can convert pro-carcinogens into carcinogens in the intestinal tract. Rats were injected intraperitoneally with 1,2-dimethylhydrazine (DMH), a compound which induces tumor formation, on a weekly basis during the study.

The control group received a meat-based diet and .4 ml of milk daily, while the other group received the meat-based diet and .4 ml of milk containing 10^{10} cells of *L. acidophilus* daily. Enzyme levels in the feces were monitored during a 7 week period and results indicated significant reductions in the levels of all three enzymes in the rats that received the milk containing *L. acidophilus*.

Goldin and Grobach (1984b) showed that the consumption of *L. acidophilus* cells could also lower the activity of these three enzymes in humans. After enzyme levels were monitored for several days in the 21 test subjects, they were fed milk on a regular basis to establish the baseline for the control period. This period was followed by another period without milk and then a period with milk containing *L. acidophilus*. Consumption of milk alone had no effect on the levels of the enzymes while the consumption of the *L. acidophilus* milk caused a significant decline in the activity of the enzymes. It is still not clear, however, what mechanisms are responsible for this anti-carcinogenic activity.

As stated previously, although most lactic acid bacteria will exercise antagonistic action toward pathogens *in vitro*, *L. acidophilus* and *Bifidobacterium* have received the most attention for controlling these pathogens *in vivo* (Gilliland, 1990). These organisms have been shown to be both prophylactic and therapeutic in controlling intestinal infections when administered in milk (Mehta *et al.*, 1983; Rasic and Kurman, 1983; Colombel *et al.*, 1987). Other studies indicated that neither *L. acidophilus* nor *Bifidobacterium* had an effect on intestinal infections (Pearce and Hamilton, 1974; Pozo-Alano, *et al.*, 1978; Clements *et.al.*, 1981). However, Gilliland (1989 and 1990) suggested that

some of the studies were not documented properly and thus their reliability was in question.

Reduced lactose intolerance is another benefit associated with the ingestion of lactic acid bacteria. Some individuals who are lactose intolerant have been shown to be able to digest yogurt with live cultures better than milk (Rubin, 1996). This relates to the lessening of problems associated with lactose intolerance such as diarrhea and gas. The yogurt starter cultures liberate the enzyme lactase during manufacture usually leaving residual levels in the finished yogurt. This residual enzyme helps to break down lactose in the gut. In addition, more of the intracellular lactase is liberated as the yogurt bacteria lyse in the gut (Vedamuthu, 1992).

In addition to the aforementioned health benefits associated with lactic acid bacteria and bifidobacteria, additional benefits reported in clinical studies associated with these organisms include: immune enhancement, vaccine adjuvant, anti-diarrhea and anti-constipation effects, and balancing of intestinal microflora (Lee and Salminen, 1995). Bifidobacteria have also been reported to produce thiamine, riboflavin, and vitamins B_6 and K. However, their impact on human nutrition is not known (Rasic and Kurmann, 1983).

In order for colonies to remain viable and colonize in the gastrointestinal tract, at least 1×10^5 colony forming units (CFU's) per gram or milliliter of the final product must be present. This is known as the 'therapeutic minimum'. Thus, 1×10^6 - 1×10^9 viable cells should be consumed per day in order for them to have any beneficial effects in humans (Lee and Salminen, 1995). In general,

populations of only 2 x 10^6 cfu/ml of each strain added to milk are targeted by the dairy industry, however, only Oregon and California have regulations on the viability of the bacteria contained in fermented milk products at the present time (Sanders, *et al.*, 1996). In addition, in order to label a yogurt product with the U.S. National Yogurt Association's "Live and Active Cultures", or "LAC" seal, it must contain at least 10^6 organisms per gram at the time of manufacture (NYA website, 1998).

In order for a microorganism to be considered an effective probiotic strain, several requirements must be satisfied. The most important of which is the ability to survive passage from the mouth, through the stomach and small intestine, to the large intestine. Hence, the bacteria must be resistant to acid and bile salts and stable under gastric conditions. In addition, the bacteria must be able to adhere to mucosal cells in the intestines, be able to grow under these conditions, produce antimicrobial substances, be antagonistic against pathogenic bacteria, and be proven safe for human consumption (Salminen *et al.*, 1993).

Section 1.2 - Honey consumption and usage

According to USDA data, the per capita consumption of honey remained relatively steady from 1970-1992 (Putnam and Allshouse, 1993). In a 1990 survey by the National Honey Board (NHB), it was reported that 72% percent of food manufacturers surveyed used honey in one or more of their products. Flavor and consumer appeal were the top two reasons why they used honey as an ingredient. The primary users of honey were cereal, bakery and health food

manufacturers. Only a small percentage of dairy and prepared foods manufacturers used honey. Lagrange *et al.* (1991) suggested that this may be due to the lack of awareness by the dairy industry as to the benefits of using honey. Honey has also been used widely in the production of beer (LaGrange, 1994), wine, and other fermented drinks dating back many centuries (Lee *et al.*, 1990). Honey is used as an ingredient in many food products around the world and is present in almost every market segment, usually as a prominently identified ingredient due to its universal appeal and marketability (LaGrange and Sanders, 1988).

Honey is a natural syrup whose properties, such as flavor, color, and composition vary depending upon the plant source from which the nectar was collected, the amount of processing, and the length of storage (Anonymous, 1979). However, the floral source seems to be the most important factor since each floral source contributes something different in terms of flavor, aroma, color, and rounding effect (NHB, 1996). Thus, these characteristics vary greatly between honey types. The color of honey ranges from very pale yellow, through ambers, to nearly black. These variations in color are due almost entirely to the plant source, however, climate may also modify the color, heat providing a darkening effect. As with the color, variations in the flavor and aroma of honey are largely determined by the floral source. An endless number of flavor and aroma variations exist eventhough there seems to be a characteristic "honey flavor". As a general rule, light-colored honey is mild in flavor and dark-colored honey has a more prominent flavor (White and Doner, 1980).

Hundreds of types of honey are produced in this country alone, and data on 452 nectar sources and 15 honeydew sources of honey were published in the *Directory of Important World Honey Sources* (Crane and Walker, 1984). Of these, only about 25 or 30 are available in large enough quantities to be considered commercially important (White and Doner, 1980). Of all the honey manufactured in the United States (consisting of over 300 types), 45% is Clover honey (NHB, 1996).

Honey is a stable, high-density, high-energy food that is produced by bees using floral nectar or sometimes honeydew. The bees change or "ripen" the thin, easily spoiled sweet liquid nectar resulting in honey. The definition of honey according to the earlier U.S. Food and Drug Act is, "the nectar and saccharine exudation of plants, gathered, modified, and stored in the comb by honey bees (*Apis mellifera* and *A. dorsata*); is levoratory; contains not more than 25% water, not more than 0.25% ash, and not more than 8% sucrose." These limits were based largely on a survey published in 1908 and allow too low of an ash content, too high a content of water and sucrose, and fails to mention honeydew. Because this definition is not totally correct, today it has only an advisory status (White and Doner, 1980).

Section 1.2.1 - Honey composition and characteristics

Honey is, on the average, composed of 38.5% fructose (levulose), 31.0% glucose (dextrose), 7.2% maltose, 4.2% trisaccharides and other carbohydrates, 1.5% sucrose, 0.5% minerals, vitamins, and enzymes, and 17.1% water (LaGrange and Sanders, 1988; NHB, 1996). Total carbohydrates in honey

equal approximately 82.4% (LaGrange and Sanders, 1988; USDA, 1962), and approximately 95% of these are fermentable (NHB, 1996). This is an important characteristic when honey is used as a carbon source for microorganisms in fermented products such as beer, wine, and bakery items. On a dry weight basis, honey is on average about 1 to1.5 times sweeter than sucrose, while liquid honey is as sweet as sucrose (NHB, 1996).

Although dextrose and levulose account for approximately 85% of the solids in honey, 22 other sugars are also present in smaller amounts. Ten disaccharides including; sucrose, maltose, isomaltose, maltulose, nigerose, turanose, kojibiose, laminaribiose, α,β-trehalose, and gentiobiose, ten trisaccharides including; melezitose, 3-α-isomaltosylglucose, maltotriose, panose, l-kestose, isomaltotriose, erlose, theanderose, centose, and isopanose, and two oligosaccharides including; isomaltotetraose and isomaltopentaose, have been identified. The majority of these compounds do not occur in the nectar but are formed either by chemical action in the honey or by the enzymes produced by the honey bee (White and Doner, 1980).

Honey typically has a pH of around 3.9. However, pH can range from 3.4 to as high as 6.1 (NHB, 1996). The organic acids present in honey, primarily gluconic, increase its microbial stability. Other organic acids that are present include; formic, acetic, butyric, lactic, oxalic, succinic, tartaric, maleic, pyruvic, pyroglutamic, α-ketoglutaric, glycollic, citric, malic, 2- or 3-phosphoglyceric, α- or β-glycerophosphate, and glucose 6-phosphate (White and Doner, 1980). These organic acids also enhance the flavor of honey by contributing to its tartness

(NHB, 1996).

In addition to water, carbohydrates, and organic acids, honey contains proteins, amino acids, vitamins, and minerals. Four out of the 8-11 proteins found in various honeys are common to all and have been reported to originate in the bee rather than in the nectar. These proteins provide lower surface tension which causes the honey to foam, form scum, and enhance the formation of fine air bubbles. Various honeys contain 11 to 21 free amino acids the most common of which are: proline, glutamic acid, alanine, phenylalanine, tyrosine, leucine, and isoleucine. These amino acids originate from the chemical or digestive break down of the proteins. The amount of amino acids is small thus, has no nutritional significance (White and Doner, 1980).

Vitamins such as thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, and ascorbic acid, as well as minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc are also present in trace amounts in honey. They are much lower than the U.S. recommended allowance, thus they have no nutritional significance (LaGrange and Sanders, 1988). However, honeydew honey is richer in minerals than are floral honeys (White and Doner, 1980).

The presence of enzymes such as invertase, diastase, and glucose oxidase, sets honey apart from other sweeteners. These may originate from the bee, pollen, nectar, or even microorganisms or yeasts in the honey. Invertase, also known as saccharase or sucrase, splits sucrose into dextrose and levulose and also has been reported to form small amounts of more complex sugars

during its action. Diastase (amylase), breaks down starch but since there is no starch in honey, its function is not yet clear. Diastase has been used in the past by European countries as a measure of quality in honey because its concentration varies among different honeys and it can be quantified. Glucose oxidase forms gluconic acid, the principle acid in honey, from the conversion of dextrose to gulconolactone. Hydrogen peroxide is also formed as a byproduct of this reaction, which provides for the heat-sensitive antibacterial property of honey (White and Doner, 1980). Catalase, acid phosphatase, protease, esterase, and β-glucosidase also have been reported to be present in honey (NHB, 1996).

Section 1.2.2 - Antimicrobial properties of honey

Because of its antimicrobial properties, honey was used in medicine for dressing wounds and inflammations during ancient times (White and Doner, 1980). Ancient Romans, Greeks, Chinese and Egyptians used honey to heal wounds and cure diseases of the gut. However, only recently has scientific evidence been provided on the therapeutic properties (and hence valid medical uses) due to the antibacterial properties of honey (Zumla and Lulat, 1989). White and Doner (1980) reported that medicinal uses of honey were largely confined to folk medicine in the 1970's. Molan (1992a) stated that honey is gaining acceptance among the medical profession for use as an antibacterial agent for the treatment of medical conditions such as surface infections created by burns and wounds. Honey has been tested for use as a topical antibacterial agent for the treatment of various types of wounds and skin ulcers, an oral agent

for the treatment of infant gastroenteritis and stomach ulcers, and for the storage of skin grafts (McCarthy, 1995). Subrahmanyam (1991) compared a conventional topical burn treatment, silver sulfadiazine, with pure, undiluted, unprocessed honey. Results showed that 91% of the infected wounds treated with honey were free from infection within seven days while only less than 7% of the silver sulfadiazine were free of infection within this time. Moreover, 87% of honey treated wounds were healed within 15 days compared to only 10% of the control group. Efem (1988) showed that various types of skin ulcers and wounds, including Fournier's gangrene, burn wounds, tropical ulcers, bed sores and diabetic ulcers, that could not be cured with conventional methods (i.e. antibiotics and medicated dressings) responded favorably to treatment with honey. It was reported that within 7 days of the first honey application, the infected wounds that had not responded to conventional treatments were free of infection.

Haffejee and Moosa (1985) discovered that the duration of diarrhea in patients with gastroenteritis was shortened when treated orally or intravenously with dilute honey. Patients in the control group (glucose solution) had a mean recovery time of 93.13 hours compared with 58.00 hours for the honey treated patients. Somal *et al.* (1994) showed that *Helicobacter pylori*, the organism responsible for upper gastrointestinal dyspepsia or stomach ulcers, was successfully inhibited by Manuka honey, a honey common to New Zealand. Honey produced from this nectar contains a phytochemical that is antibacterial to *H. pylori*. Because of its antimicrobial properties, honey also provides an

inexpensive way for skin grafts to be stored. Experimental evidence suggests that skin grafts can be stored up to 12 weeks in unprocessed, undiluted, sterile honey. This is useful for burn patients with multiple wounds which are unable to be grafted simultaneously due to infection (McCarthy, 1995).

A number of factors are responsible for the antimicrobial properties of honey. As stated previously, honey ranges in pH from 3.2-4.5 according to one source and 3.4-6.1 according to another source but generally averages at around 3.9 (White, 1975; NHB, 1996). This acidity prevents the growth of many species of bacteria, if diluted however, the acidity may be neutralized. Another factor is osmolarity. The high sugar content of honey greatly reduces the water available to microorgansims thus preventing their growth (Molan, 1992b). The mean values for water activity (a_w) or 'free' water have been reported to range from 0.562-0.62. Minimum a_w values for growth of most spoilage bacteria, most spoilage yeast, and most spoilage molds have been cited at 0.9, 0.88, and 0.80, respectively (Jay, 1992). In addition Jay (1992) reported *Clostridium botulinum* types A and B as having a 0.94 minimum a_w for growth, 0.97 for type E. It has been shown that if honey contains more than 17% moisture, it will ferment if it contains a sufficient number of yeast spores (White and Doner, 1980). A third factor which contributes to the antimicrobial properties of honey is hydrogen peroxide. Termed "inhibine" in 1937 by Dold *et al.*, the antimicrobial activity that remained after the osmotic effect had been eliminated by the dilution of honey, was later determined to be due to hydrogen peroxide formed from the activity of the enzyme glucose oxidase (White *et al.*, 1962; White and Subers, 1963).

However, White and Subers (1963) showed that some honey samples possessed antimicrobial properties in excess of what hydrogen peroxide could account for alone. In addition, antibacterial activity persisted when the hydrogen peroxide was removed from the honey (Adcock, 1962). Hence, it is believed that there are additional antimicrobial components in honey (Russell *et al.*, 1990). Molan (1992b) attributes this additional activity to antibacterial components (non-peroxide factors) from the plant source.

The existence of non-peroxide factors is indicated by several findings. These include the antibacterial activity not correlating completely with the rate of accumulation of hydrogen peroxide in honey samples, the finding of antibacterial activity in honey that is heat stable, and the existence of antibacterial activity in honey persisting after the addition of catalase to remove hydrogen peroxide (Molan, 1992a). Although the major (non-peroxide) antibacterial component(s) in honey remains to be identified, researchers have isolated some of the minor (non-peroxide) antibacterial constituents. Russell *et al.* (1990) identified some antibacterial components in manuka honey by separating an ether extract of the honey using preparative thin-layer chromatography. Further analysis using a combination of gas chromatography and mass spectroscopy led to the identification of the following components: 3,5-dimethoxy-4-hydroxybenzoate (methyl syringate), methyl 3,4,5-trimethoxybenzoate, and 3,4,5-trimethoxybenzoic acid. Another study identified 2-hydroxy-3-phenylpropionic acid as the major antibacterial component observed from an ether extraction of manuka honey analyzed using gas chromatography-mass spectrometry (Tan,

1989). Other researchers have found volatile antibacterial substances in honey. Toth *et al.* (1987) detected 41 volatile components in four Hungarian honeys using gas chromatography. However, only eight have been identified: pinene, camphene, limonene, eucalyptol, linalool, benzyl alcohol, farnesol, and eicosane. The volatile components obtained from the distillation of honey had a significant antimicrobial effect on the gram-negative pathogens tested.

Regardless of what components are responsible for the antimicrobial properties in honey, these properties vary widely among different types of honey, with some honeys having greater antibacterial activity than others (McCarthy, 1995; Molan, 1992b). However, caution should be used when considering the quantitative aspects of some studies since some of the extracted compounds are tested for their antimicrobial properties at much higher levels than they naturally occur in honey (Molan, 1992a).

The potency of the antibacterial activity in honey can be determined by two standard microbiological techniques. The first is the agar diffusion assay technique in which a small sample of honey or honey solution is dropped onto a nutrient agar plate inoculated with a microbial culture. If the honey contains antibacterial properties the culture will not grow resulting in a clear zone around the spot in which the honey was applied. The size of the zone thus determines the antibacterial potency of the honey (Molan, 1992a; McCarthy, 1995). However, there is no way to determine the effective antibacterial concentration of the honey using this method since the honey diffuses into the agar during incubation and is thus always lower than that of the solution that was applied

(Molan 1992a). The second assay used involves incorporating the honey in the nutrient agar or broth in which the culture is grown. It is then possible to find a minimum inhibitory concentration for each honey by using a series of varying concentrations. Neither method can determine whether the antibacterial activity of the honey is bactericidal or bacteriostatic. To determine if a honey is bactericidal, subsequent culturing in fresh nutrient medium is required to test if the culture survived exposure to honey (Molan, 1992a).

Several species of bacteria as well as many species of fungi are vulnerable to the antimicrobial properties of honey whether they have a bactericidal or a bacteriostatic effect on them (Table 1). Studies have indicated that *Staphylococcus aureus* is the species most susceptible to the antimicrobial effects of honey (Molan, 1992a).

There are no reports in the literature on the effects of honey on lactic starter cultures used in yogurt, specifically *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus thermophilus*, although several other species of *Streptococcus* have been shown to be inhibited by honey (Table 1). Curda and Plockova (1995) measured the impedance of growth of two types of dairy starter cultures with the addition of both unheated and sterilized honey. Honey was added to skim milk in concentrations of 0, 1, 3, 5, or 10% (w/v) and its effect on the growth of *Lactobacillus acidophilus* and a mesophilic starter culture consisting of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* was determined by measuring impedance changes. Their results indicated *Lactobacillus*

Table 1. Species found to be sensitive to the antimicrobial properties of honey[a]

Species inhibited (Bacteria)	Species inhibited (Bacteria cont'd.)	Species inhibited (Fungi)
Alcaligenes sp.	*Pseudomonas fluorescens*	*Aspergillus flavus*
Alcaligenes faecalis	*Salmonella* sp.	*Aspergillus flumigatus*
Bacillus sp.	*Salmonella cholerae-suis*	*Aspergillus niger*
Bacillus alvei	*Salmonella dublin*	*Aspergillus parasiticus*
Bacillus anthracis	*Salmonella enteritidis*	*Candida albicans*
Bacillus cereus	*Salmonella gallinarum*	*Candida pseudotropicals*
Bacillus cereus var. mycoides	*Salmonella paratyphi-A*	*Candida reukaufii*
Bacillus larvae	*Salmonella pullorum*	*Candida stellatoidea*
Bacillus megaterium	*Salmonella schottmuelleri*	*Candida tropicalis*
Bacillus pumilus	*Salmonella typhi*	*Candida utilis*
Bacillus stearothermophilus	*Salmonella typhimurium*	*Penicillium* sp.
Bacillus subtilis	*Salmonella typhosa*	*Penicillium chrysogenum*
Citrovacter freundii	*Sarcina lutea*	*Saccharomyces* sp.
Corynebacterium diptheriae	*Sarcina orangea*	
Edwardsiella tarda	*Serratia marcescens*	
Escherichia coli	*Shigella* sp.	
Haemohilus influenzae	*Shigella boydii*	
Klebsiella sp.	*Shigella dysenteriae*	
Klebsiella pneumoniae	*Shigella flexneri*	
Listeria monocytogenes	*Shigella sonnei*	
Micrococcus sp.	*Staphylococcus* sp.	
Micrococcus luteus	*Staphylococcus aureus (albus)*	
Mycobacterium tuberculosis		
Neisseria sp.	*Staphylococcus aureus*	
Pasterurella multocida	*Streptococcus* sp.	
Proteus sp.	*Streptococcus faecalis*	
Proteus mirabilis	*Streptococcus pyogenes*	
Proteus morgnii	*Streptococcus salivarius*	
Proteus vulgaris	*Streptomyces* sp.	
Pseudomonas sp.	*Vibrio cholerae*	
Pseudomonas aeruginosa	*Vibrio cholerae* biotype *Proteus*	

[a]Molan, 1992a

acidophilus was inhibited by honey at levels above 5% regardless of the heat treatment while the growth of the mesophilic starter culture was not inhibited by the sterilized honey at any concentration, but showed the maximum inhibitory effect to be in the 10% level of unheated honey. However, since these researchers used a honey common to the Central Bohemian countryside and the antimicrobial characteristics vary largely upon the floral source, it is extremely likely that the honey used in their study had markedly different antimicrobial properties than that of honeys indigenous to the U.S. Therefore, information is still needed on the effects of Clover honey (which is predominantly used in the U.S.) on lactic starter cultures.

Section 1.2.3 - Functionality of honey

In addition to its characteristic flavor, honey serves other functions in foods as well. Honey has been shown to enhance the sweetness of sugar solutions, decrease the sourness of sour solutions, decrease the bitterness of bitter solutions, modify the saltiness perception, and increase the acceptability of savory products (NHB, 1996). Honey serves a variety of other functions as well, depending upon its application. Some examples are increased humectancy in baked goods and intermediate moisture products, increased viscosity in salad dressings, volume building in frozen desserts and variety breads, preservation in dried fruits, and for clarification in apple juice and wine (synergistic effect when in combination with pectinase). In addition, honey is soluble in water, is very miscible, and is extrudable and pumpable so it can be easily handled in bulk manufacturing systems (NHB, 1996).

As previously demonstrated, honey is perceived by consumers to add value to a product. Thus, it seems likely that its addition to a product such as yogurt would increase both its nutritional and organoleptic properties. In addition, the use of honey as a total replacement of sucrose could prove to further the "natural" image of yogurt. Thus it is necessary to determine the effects of honey on yogurt cultures in order to incorporate it into a product such as this.

Section 1.3 - Analysis of organic acids in dairy products

Several techniques are used to assess the growth and activity of starter cultures before, during, and after fermentation, such as titratable acidity (TA), pH, and high performance liquid chromatography (HPLC) analysis. While titratable acidity and pH provide a rough estimate of the total acid produced, these methods do not differentiate between or subsequently quantify the amounts of different acids, while HPLC does. It is for this reason that the HPLC method of analysis is widely used when trying to determine the compounds produced by a microbial culture during fermentation and storage. For example, in order to better understand the metabolism and quality of milk products, Bevilacqua and Califano (1989) conducted a study to develop an isocratic HPLC procedure for the quantification of individual organic acids in dairy products. Nine different organic acids were quantitated for six different dairy products and recoveries greater than 85.3% were observed for all acids. Fernandez-Garcia and McGregor (1994) performed a single isocratic analysis by HPLC for the separation and quantification of ten organic acids created during the

fermentation and cold storage of yogurt. Although they were unable to detect formic and butyric acid, Fernandez-Garcia and McGregor observed varying degrees of increases and decreases for all other acids during the fermentation and cold storage of the yogurt. They reported these results to be in accordance with those in other studies. Ashoor and Monte (1983) developed an isocratic HPLC method for the determination of the growth factors of *Bifidobacterium bifidum* in human milk. Not only did they report that the HPLC method was simpler and less time consuming than the standard microbiological method used during the early 1980's, they also reported that its use revealed, for the first time, the presence of two separable growth factors in all human milk samples.

The advantages to using HPLC analysis for the determination of the organic acids produced in a dairy product can be readily seen in the examples above. However, this method alone is not adequate for the determination of a product's quality, overall acceptability and shelf-life. Other methods then need to be employed.

Section 1.4 - Sensory analysis

Sensory analysis is widely used for the determination of product attributes, quality, acceptability and shelf-life. Lin and Cunningham (1984) utilized a sensory panel to determine product acceptability of a yogurt-like product containing egg white. During the study, six panelists experienced in dairy product evaluation were asked to rank three sets of samples on a nine-point hedonic scale. Although the products did not score well for acceptability, the panel did indicate that the products would have an extended shelf-life at

refrigeration temperature. Salji *et al.* (1987) used a sensory panel as one of the determining factors of the shelf-life of plain liquid yogurt manufactured in Saudi Arabia. Similar to the previous study, this study used six trained panelists to evaluate products using a nine-point hedonic scale. Panelists indicated the product to be acceptable before its date of expiration.

Vargas *et al.* (1989) used a combination of sensory (color, consistency, and flavor), chemical (pH, TA, and tyrosine values) and microbiological (yeast and mold counts) studies to determine the shelf-life of soy-whey yogurt. The trained panel in this study was instructed to use a nine-point unstructured scale for its determination of shelf-life. Results from the sensory panel indicated that flavor was a good indicator of changes during storage. The results from the sensory panel coincided with the tyrosine values and the yeast and mold counts in regards to the product shelf-life.

Shirai *et al.* (1992) conducted trained sensory panel to evaluate the physical attributes of a yogurt-like product produced from plant foodstuffs. Panelists first participated in a preference study in order to ascertain which starter cultures were preferred. A second preference study was then conducted to determine the influence of fermentation and the addition of sugar, flavors, and calcium on the product acceptability. Results indicated that the yogurt-like product had good acceptability which increased with the addition of sugar or flavors. In addition it was reported that a suitable combination of starter cultures was also important in producing an acceptable product. Granata and Morr (1996) used a 5 member experienced panel for testing the preference of their

soy yogurt products with varying amounts of milk proteins. The panel concluded that plain and peach-flavored soy milk yogurt with 0.25% sodium caseinate or 0.1% casein hydrolyzate tested favorably for both flavor and texture compared to the milk yogurt control.

In addition to sensory analysis for the determination of various attributes and shelf-life, rheological studies can also be conducted in order to obtain information on product properties and to correlate with panel results in regards to texture and viscosity attributes.

Section 1.5 - Rheological analysis

Rheology describes the flow behavior of a product. It can be used to measure the viscosity of a product and thus can provide an instrumental method of determination that may be compared with a non-instrumental method such as a sensory panel. Similarly to sensory evaluation, rheological analysis is frequently used in the dairy industry to test attributes such as viscosity. Basak and Ramaswamy (1994) evaluated the effect of added pectin and fruit concentrate on the viscosity of stirred yogurt using a rotational viscometer. Results confirmed that the stirred yogurt displayed thixotropic behavior under all conditions. In addition, the study showed that both pectin and fruit concentrate influenced the rheology of flavored yogurt. Thus, it was concluded by the authors that the data obtained by the rheological method in this study could not only be used to describe the flow behavior of stirred yogurt, but also as a quality control tool for obtaining the desired product consistency.

Hassan *et al.* (1996) compared the rheological properties of yogurt made with encapsulated non-ropy, encapsulated ropy, and unencapusulated non-ropy lactic cultures using a rotational viscometer. Results indicated that ropy strains produced yogurts with the highest shear stress values. Encapsulated non-ropy strains produced yogurts with higher shear stress, apparent viscosity, and consistency coefficient than unencapsulated non-ropy yogurts. Keogh and O'Kennedy (1998) determined the effects of added milk fat, protein and hydrocolloids on the rheology of stirred yogurt using a controlled strain rheometer. Protein was the most effective ingredient in terms of increasing consistency followed by fat. Fat also reduced syneresis, while effects of starch and xanthan gum/LBG were insignificant in the concentrations used. Gelatin reduced syneresis.

Mitschka (1982) developed a method to calculate the average shear stress and the average shear rate of power law fluids using data from an RV model Brookfield viscometer and disc spindles. Briggs and Steffe (1997) furthered the application of this method by testing it with shear-thinning foods. Shear-thinning products are those products in which the apparent viscosity decreases with an increase in shear rate (Steffe, 1996). Briggs and Steffe (1997) proved this to be a successful method for the application of shear-thinning foods and also discovered it had a great potential for use as a quality control testing procedure for the food industry. Among its many advantages, the Mitschka method is simple in technique, and utilizes equipment (an RV model

Brookfield viscometer) that is very widely used in the industry and relatively inexpensive.

The purpose of the following study was to first determine if honey could be used as a carbohydrate source by yogurt starter cultures and probiotic bacteria in a fermented dairy product. The second part of this study was to determine if a desirable yogurt shake could then be manufactured using honey in lieu of sucrose.

CHAPTER 2

MATERIALS AND METHODS

Section 2.1 - Effect of honey on growth and activity of lactic acid bacteria and bifidobacteria

Section 2.1.1 - Culture activation

Streptococcus thermophilus (St -133) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lr-78) along with the probiotic organisms *Lactobacillus acidophilus* (La-7) and Bifidobacteria (Bf-13) were obtained from Systems-Bio-Industries (Waukesha, WI). Each starter culture was activated by adding approximately 1 ml of the initial culture to 30 ml of MRSL broth (27.5g of MRS dehydrated broth (Difco Laboratories, Detroit, MI) and 25g of lactose (Difco) in 0.5 L of distilled water) followed by incubation at 37°C for 24 hours. After incubation, 5 ml of each culture were used to inoculate 30 ml of MRSL broth. Each culture was incubated again at 37°C for 24 hr. Active cultures were refrigerated (one to two weeks after activation in order to ensure viability) until they were needed for inoculation.

Section 2.1.2 - Growth and activity determination

Figure 3 shows the experimental design for the study on the effect of honey on growth and activity of lactic acid bacteria and bifidobacteria. A 12% w/w non-fat dry milk (NFDM) (Michigan Milk Producers Association, Ovid, MI) solution was prepared and divided into four portions. Sucrose (J.T. Baker, Phillipsburg, NJ), fructose (Sigma-Aldrich, St. Louis, MO), and Grade A Clover honey (W. Stoller's Honey, Inc., Latty, OH) were added at a 5% (w/w) level to each NFDM portion.

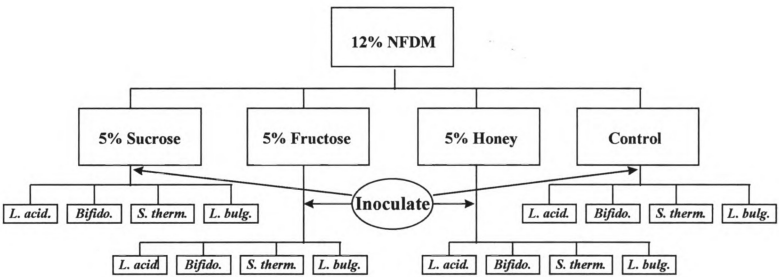


Figure 3. Experimental design for the determination of the effect of honey on growth and activity of lactic acid bacteria and bifidobacteria

The control treatment had no sweetener added. The mixtures were then further divided, pasteurized at 70°C for 30 minutes in a hot water bath and cooled to room temperature. The appropriate tubes were inoculated with the activated cultures of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *Bifidobacterium* at 5% (v/v). Inoculated tubes were incubated at 37°C for 24 hr.

Standard Plate Counts (dilutions of 10^{-4} through 10^{-6}), diluted with 0.1% bacto-peptone (Difco) were done at 0 and 24 hours using MRSL agar (27.5g of MRS dehydrated broth (Difco), 25g of lactose (Difco), and 7.5g of bacto-agar (Difco) in 0.5L distilled water) and the pour plating method for culture enumeration. Plates containing bifidobacteria were incubated anaerobically using Gas Pak® (Becton Dickinson, Co., Cockeysville, MD), all others were incubated aerobically. Plates were counted using a Quebec Colony Counter (Fisher Scientific, Pittsburg, PA). The pH was monitored at 0, 12, and 24 h.

Activity of the cultures was determined using HPLC analysis of the fermentation end products. This was accomplished by preparing standard solutions of acetic acid and lactic acid (Bio-Rad Laboratories, Hercules, CA; and Sigma-Aldrich, St. Louis, MO, respectively) in order to establish elution times and calibration curves for these acids.

Concentrations of acetic acid, 2.5, 5, 10, 20, and 40 mmol/L, and lactic acid, 2.0, 2.5, 3.0, 3.5, and 4.0, were prepared as standards and analyzed by HPLC (Shin, 1997). Acetic acid eluted from the column after approximately 9.5-9.7 min. and lactic acid between 11.2-11.4 min. Peak areas of the standards were plotted versus their known concentrations and linear regression curves

were produced for acetic acid and lactic acid. Linear regression analysis yielded standard equations: $y = 47833x-6754.2$ ($R^2 = 0.997$), and $y = 118876x + 101958$ ($R^2 = 0.988$) for acetic and lactic acid, respectively (Figures 4-5). These standard curves were used to determine acetic acid and lactic acid produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* Lr-78, *Streptococcus thermophilus* St-133, and *Lactobacillus acidophilus* La-7. However, because bifidobacteria produced less acid than lactic acid bacteria, different standard curves were prepared to determine the concentrations of acetic and lactic acids produced by bifidobacteria. These new standard curves had equations, $y = 50675x + 4523.8$ ($R^2 = 0.993$) and $y = 173649x + 7594$ ($R^2 = 0.982$) for acetic and lactic acids, respectively (Figures 6-7).

The HPLC system used consisted of an M-45 solvent delivery system (Waters Associates, Inc., Milford, MA), a 486 UV/Vis tunable absorbence detector, and a 730 data module. An Aminex HPX-87H Column (300 mm x 7.8 mm, Bio-Rad Laboratories, Richmond, CA) and guard column with disposable cartridges H^+ (Bio-Rad Laboratories) was used for the analysis. The mobile phase, a 0.009N H_2SO_4 solution, was filtered through a 0.45mm membrane filter (Millipore Corp., Bedford, MA) and vacuum degassed. The flow rate of the mobile phase was 0.6 ml/min (Shin, 1997). The detector wavelength was set at 220 nm in accordance with Shin (1997).

12% NFDM with 5% (w/w) honey, fructose, or sucrose and a control (no sweetener), fermented with either *S. thermophilus*, *L. delbrueckii* subsp.

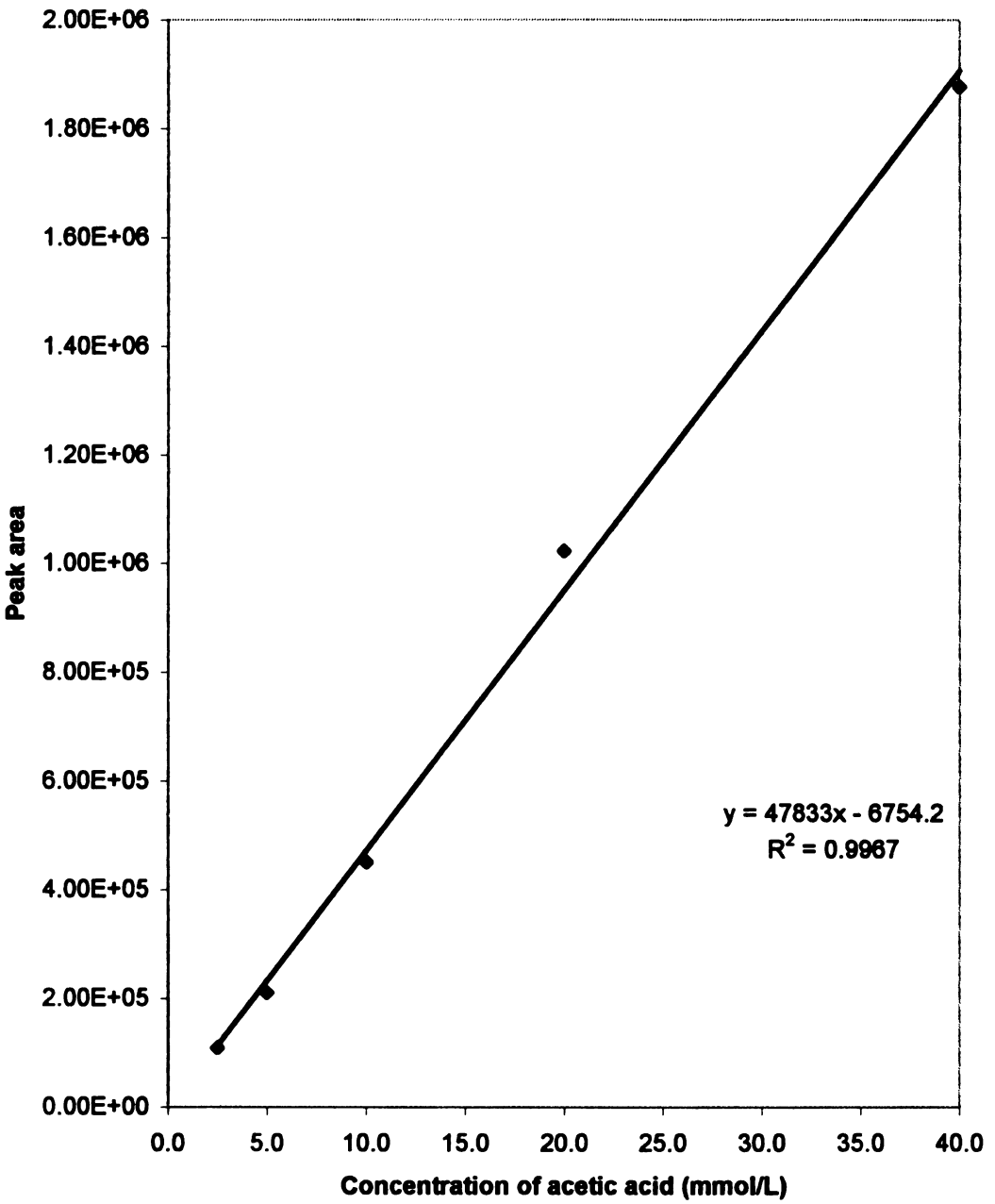


Figure 4. Acetic acid standard curve for lactic acid bacteria

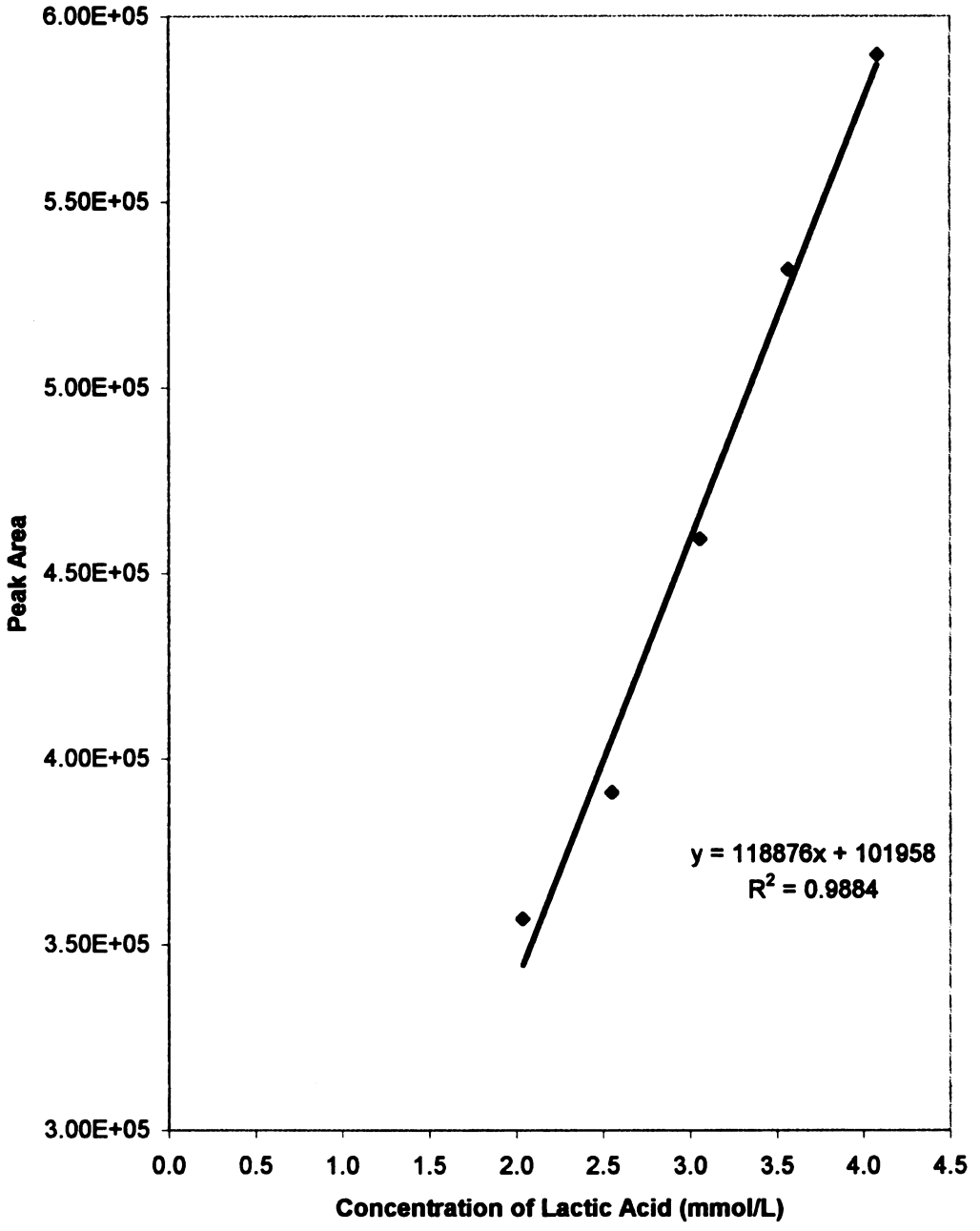


Figure 5. Lactic acid standard curve for lactic acid bacteria

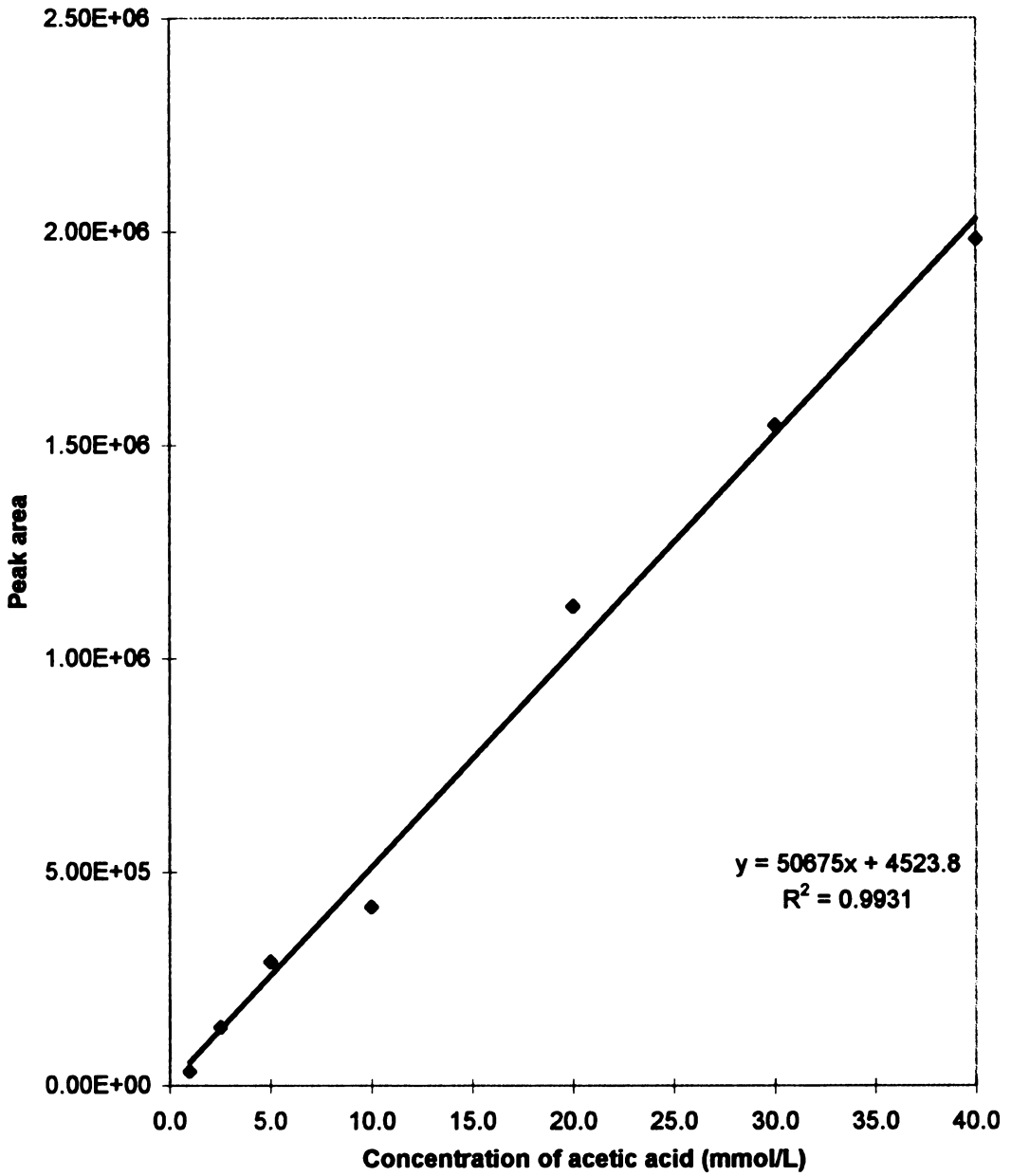


Figure 6. Acetic acid standard curve for bifidobacteria

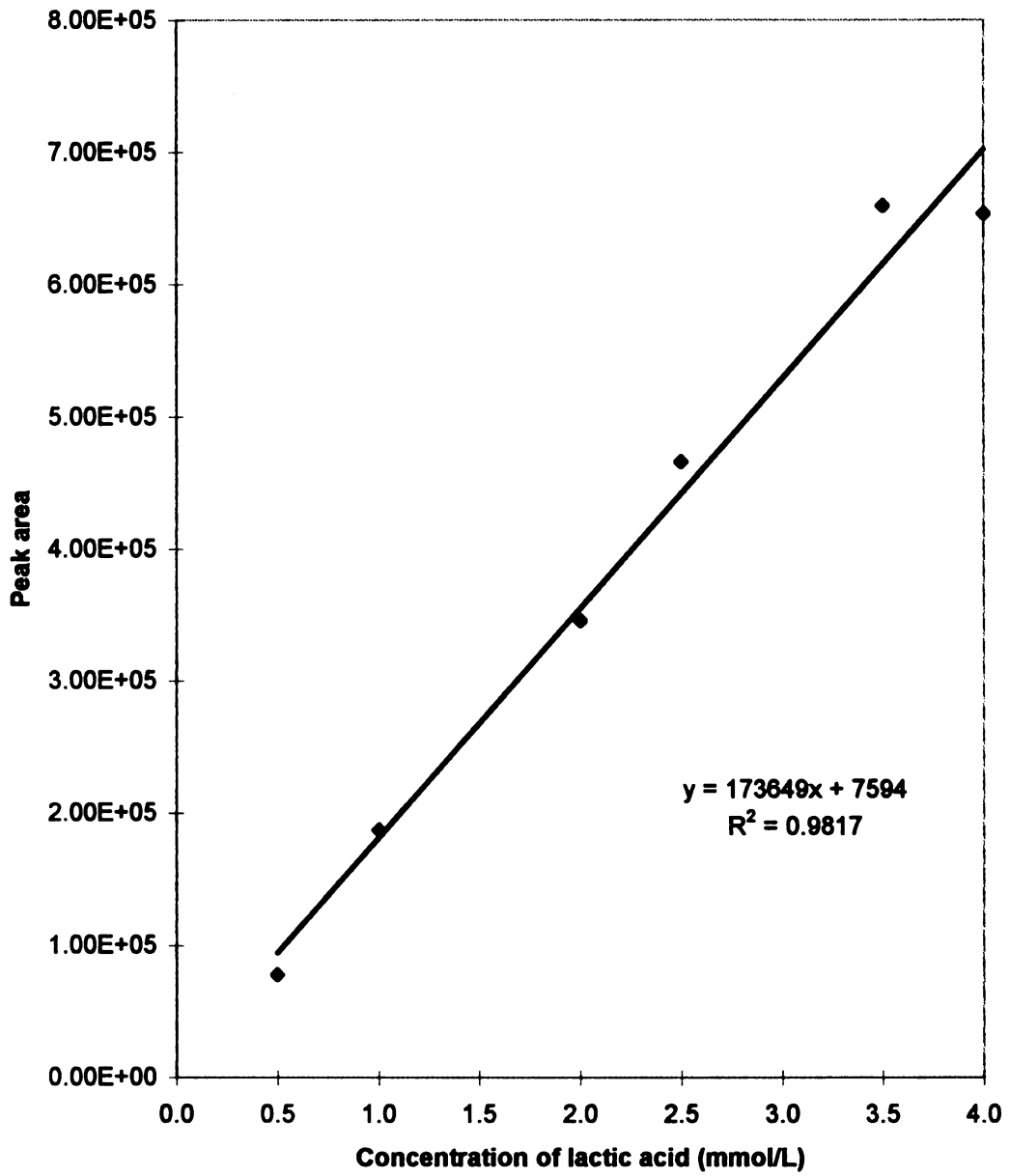


Figure 7. Lactic acid standard curve for bifidobacteria

bulgaricus, *L. acidophilus* or *Bifidobacterium*, (Figure 3) were prepared for HPLC analysis using a method similar to the procedure described by Dubey and Mistry (1996). Samples were prepared by adding 70 µl of 15.8N HNO_3 and 9.93 ml of 0.009N H_2SO_4 to 1.0 ml of each of the fermented milk samples. The mixtures were centrifuged at 1000 x g for 10 min and the supernatant was filtered through a 0.45mm membrane filter (Millipore), then a 0.22mm membrane filter (Millipore) and eluted through a reversed phase Supelclean tube (Supelco Inc., Bellefonte, PA). Samples were stored in HPLC vials at -80°C until analysis.

Section 2.2 - Development and optimization of formulations for a honey sweetened yogurt shake.

Section 2.2.1 - Consumer flavor preference determination

Based on a market survey by the National Honey Board, two flavor combinations were initially selected for the product formulations: vanilla-honey and strawberry-honey. A survey (Appendix A) was prepared in order to determine which flavor combination consumers preferred in a yogurt product. This survey also included questions that provided demographic information about the population being surveyed, as well as information about their yogurt consumption.

Section 2.2.2 - Development of formulations

Strawberry yogurt shake formulations supplemented with varying NFDM levels, 0, 3, or 6% (w/w), and sweetened with 10% (w/w) honey and 5% (w/w) fructose, were prepared from 1 and 2% fat milk (Figure 8 and Table 2). Sucrose

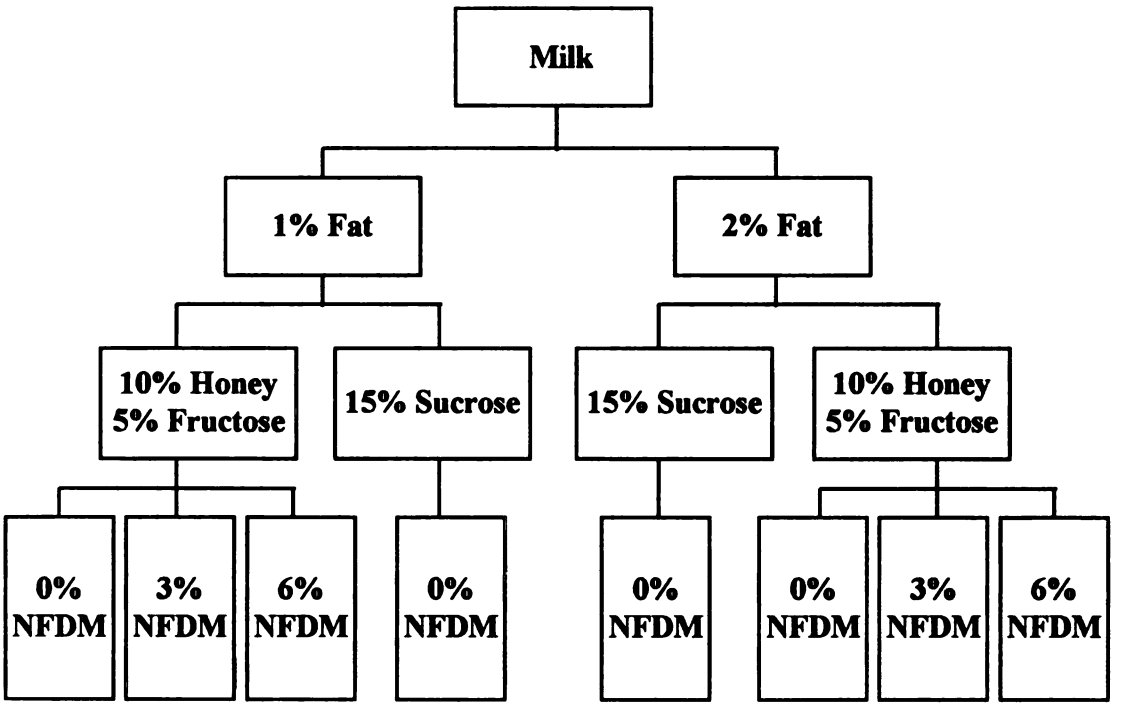


Figure 8. Experimental design of honey sweetened yogurt shake formulations for pilot plant production

Table 1. Yogurt shake formulations

	Treatments							
Ingredients (Percent)	**1% Fat Sucrose 0% NFDM**	**2% Fat Sucrose 0% NFDM**	**1% Fat Honey 0% NFDM**	**2% Fat Honey 0% NFDM**	**1% Fat Honey 3% NFDM**	**2% Fat Honey 3% NFDM**	**1% Fat Honey 6% NFDM**	**2% Fat Honey 6% NFDM**
	1 S 0	**2 S 0**	**1 H 0**	**2 H 0**	**1 H 3**	**2 H 3**	**1 H 6**	**2 H 6**
Milk	64.00	64.00	64.00	64.00	64.00	64.00	64.00	64.00
NFDM	0.00	0.00	0.00	0.00	3.00	3.00	6.00	6.00
Honey	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00
Water	6.00	6.00	6.00	6.00	3.00	3.00	0.00	0.00
Sucrose	5.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00
Honey	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00
Stabilizer	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Fructose	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00
Flavor	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Water	6.36	6.36	4.60	4.60	4.60	4.60	4.60	4.60
Sucrose	8.24	8.24	0.00	0.00	0.00	0.00	0.00	0.00
Fruit Puree	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Total:	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

was used in place of honey (dry wt. basis) and fructose as a control for both types of milk resulting in a total of eight treatments.

Milk, NFDM (Michigan Milk Producers Association, Ovid, MI), water, and 5% (w/w) grade A clover honey (North Arkansas Wholesale Co., Bentonville, AR) or sucrose (controls), were combined, pasteurized at 75° ± 2°C for 30 min, cooled to 42°C, inoculated with 0.02% (w/w) frozen commercial yogurt starter culture (Systems Bio-Industries, Waukesha, WI), containing the probiotics *Bifidobacterium* and *L. acidophilus*, and incubated for 4-6 hours until a pH of 5.0 ± 0.3 was reached. The mixes were then cooled and refrigerated overnight.

Separate mixtures consisting of strawberry flavor (Kraus and Co., Battle Creek, MI), stabilizer (NutraSweet Kelco Co., San Diego, CA), water, fructose (ADM Corn Processing, Decatur, IL) and the remainder of the honey or sucrose, were combined, pasteurized at 75° ± 4°C for 15 min, and added to the fermented milk mixtures. The fruit puree was then added to each mix and each treatment was homogenized at 500 psi single stage. Following homogenization, products were packaged in one quart plastic containers, labeled, and refrigerated at 4°C for 28 days. The products were evaluated for sensory and rheological properties, and for shelf-life at 0, 14, and 28 days of refrigerated storage. The pH was also monitored at these time intervals.

Section 2.2.3 - Trained Panel Sensory Evaluation

Sensory evaluation was conducted using a 12-member trained sensory panel consisting of faculty and graduate students at Michigan State University. Once prescreened, panelists were selected through a screening process based

on their ability to discriminate and score consistently for the characteristics being studied: Strawberry flavor intensity, sweetness, viscosity, and smoothness (Appendix A). The judges subsequently participated in one orientation and four one hour training sessions prior to data collection. The training involved sampling four samples of varying intensities for each characteristic investigated and using a structured rating scale to quantify them. Panelists were provided with feedback on their ratings.

Yogurt shakes, stored at 4°C, were poured into plastic drinking cups with random three digit codes just prior to the data collection. Sample temperature averaged 15 $\pm$ 5°C during testing. Samples were presented in two groups of four in a randomized block design, and water was provided for rinsing between samples. The panel was instructed to rate each sample on a nine-point intensity scale (where 9 indicated highest intensity and 1 indicated lowest intensity) for the four characteristics studied. A space for written comments was included at the bottom of the questionnaire (Appendix A). All testing and training sessions were conducted in a climate-controlled, sensory analysis laboratory equipped with individual testing booths. Sensory evaluation was conducted as approved by UCHRIS for use of human subjects (Appendix A).

Section 2.2.4 - Determination of shelf-life

Shelf-life of the products were determined at 0, 14, and 28 days of storage by psychotrophic counts according to the Standard Methods for the Examination of Dairy Products, (Marshall, 1992). Because psychotrophic counts remained zero through the duration of the study for the first replicate, and the

products appeared to be spoiling due to mold, mold counts (using 3M petri films for yeast and mold counts) were performed in lieu of psychotrophic counts for the remainder of the replicates in the study. However, mold counts also remained at zero for unexplained reasons. Mold counts were again performed at 14 day intervals during the storage period of the products produced for the subsequent acceptability panel. However, only one replicate was obtained.

Section 2.2.5 - Rheological analysis

An RV model Brookfield viscometer (Brookfield Eng. Labs. Inc., Stoughton, MA) with disc spindles, along with the Mitschka Method of data analysis (Mitschka, 1982), were used to determine the apparent viscosity of the treatments. The yogurt shakes were determined to be shear-thinning from preliminary testing. The Mitschka method was previously evaluated for shear-thinning foods and was proven to have superior potential in the food industry as a quality control method (Briggs and Steffe, 1997).

Temperature, separation, and time-dependency were factors taken into consideration during the development of this procedure. Insulated, pre-chilled (4°C) 600 ml glass beakers were used in order to maintain product temperature during testing and samples were shaken before being poured into the beakers to avoid separation. Dial readings were recorded for six shear rates, 1, 5, 10, 20, 50, and 100, in duplicate and averaged. The products were evaluated at 0, 14, and 28 days of refrigerated storage.

Data were analyzed by applying the Mitschka method to evaluate typical shear-thinning fluid food products as described by the power law model:

$$\sigma = K\dot{\gamma}^{n} \qquad [1]$$

where: σ = shear stress, Pa

K = consistency coefficient, Pa s^n

$\dot{\gamma}$ = shear rate, s^{-1}

n = flow behavior index, dimensionless

The readings were averaged and graphed to obtain the slope of the logarithm of shear stress versus logarithm of rotational speed, known as the flow behavior index (n) using the following equation:

$$n = \frac{d(\log_{10} \sigma_a)}{d(\log_{10} N)} \qquad [2]$$

where: σ_a = average shear stress, Pa

N = rotational speed, RPM

The average shear stress is calculated as:

$$\sigma_a = k_{a\sigma}(C * \text{dial reading}) \qquad [3]$$

where: $k_{a\sigma}$ = shear stress conversion factor, Pa

$k_{a\sigma}$ is a function of the spindle number (Table 3), C (dimensionless) is a constant dependent upon which model of Brookfield viscometer was used (C = 0.5 for the 1/2 RV model; C = 1.0 for the RV model; C = 2.0 for the HAT model; and C 8.0 for the HBT model), and the dial reading represents the percent torque of the product measured by the Brookfield viscometer. Eq. [3] was an extension of the Mitschka method developed for use only with the RV Brookfield

Table 3. Conversion factors for the method described by Mitschka (1982)

Brookfield Spindle		1	2	3	4	5	6	7
k_{∞}		0.035	0.119	0.279	0.539	1.05	2.35	8.40
n =	0.1	1.728	1.431	1.457	1.492	1.544	1.366	1.936
	0.2	0.967	0.875	0.882	0.892	0.907	0.851	1.007
	0.3	0.705	0.656	0.656	0.658	0.663	0.629	0.681
	0.4	0.576	0.535	0.530	0.529	0.528	0.503	0.515
$k_{N\gamma}$	0.5	0.499	0.458	0.449	0.445	0.442	0.421	0.413
	0.6	0.449	0.404	0.392	0.387	0.382	0.363	0.346
	0.7	0.414	0.365	0.350	0.343	0.338	0.320	0.297
	0.8	0.387	0.334	0.317	0.310	0.304	0.286	0.261
	0.9	0.367	0.310	0.291	0.283	0.276	0.260	0.232
	1.0	0.351	0.291	0.270	0.262	0.254	0.238	0.209

viscometers; hence, the introduction of C. The average shear rate is calculated as:

$$\dot{\gamma}_a = k_{N\gamma}(N) \qquad [4]$$

where: $k_{N\gamma}$ = shear rate conversion factor, min s^{-1}

Values of $k_{N\gamma}$ are a function of the flow behavior index and the spindle number (Table 3). The apparent viscosity (η), in Pa s, can then be determined by dividing Eq. [3] by Eq. [4] yielding the following expression:

$$\eta = \frac{\sigma_a}{\dot{\gamma}_a} \qquad [5]$$

Briggs and Steffe (1997) developed a simplified equation to calculate average shear rate. They established a mathematical relationship between $k_{N\gamma}$ and n (Table 3):

$$k_{N\gamma} = 0.263(n)^{-0.771} \qquad [6]$$

By using Eq. [6] to calculate the shear rate conversion factor, the need for linear interpolation in using Table 2 is eliminated, and only minor differences are found. The average shear rate is calculated as:

$$\dot{\gamma}_a = (0.263(n)^{-0.771})N \qquad [7]$$

Maintaining the temperature of the product constant during testing did not prove to be a problem due to the large sample volume being tested and the short length of time of the test. However, the beaker was pre-chilled and insulated to further maintain the storage temperature of the product. Since the samples were shaken prior to testing, there was little time-dependent behavior

present, and it was considered negligible. Separation or slip, also did not appear to present a problem for the same reasons. A middle range shear rate of 30 s^{-1} and a low shear rate of 1 s^{-1} were chosen for the statistical comparison of the data.

Section 2.2.6 - Untrained consumer panel for overall acceptability

The two most preferred honey treatments (0 and 3% NFDM) from the trained sensory evaluation and the corresponding control, prepared from 1% fat milk, were selected for the overall acceptability determination by an untrained consumer panel. The 58 untrained consumers consisted of undergraduate and graduate students, and faculty from Michigan State University. Samples stored at 4°C were poured into plastic sample cups, marked with random three digit codes, just prior to testing. The three samples were presented in a balanced order and panelists were instructed to rank each one on a nine-point hedonic scale (where 9 was like extremely and 1 was dislike extremely) (Appendix A). Water for rinsing between samples was provided. There was also a space for written comments. All testing sessions were conducted in a climate-controlled, sensory analysis laboratory equipped with individual testing booths.

Section 2.3 - Statistical analysis

Growth and activity determination experiments; including the pH determination, standard plate counts, and organic acid determination; were replicated three times in a randomized design. Statistical analysis was done using a one-way ANOVA on Sigma Stat 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using Student-Newman-Keuls test for

multiple comparisons. A $p<0.05$ was considered statistically significant.

All experiments conducted during the storage study; including the trained sensory panel evaluation, the rheological analysis, and the pH determination; were replicated three times in a randomized design. Experiments were analyzed using a two-way completely blocked ANOVA on Crunch Version 4.0 (Crunch Software Corp., Oakland, CA). Blocks were composed of repeated measures and appropriate comparisons were made using the Student-Newman-Keuls method of testing. This method of analysis was used for all data collected during the storage study with the exception of the pH data. pH data were also analyzed using a two-way completely blocked ANOVA on Crunch Version 4.0 and appropriate comparisons were made using the Student-Newman-Keuls method of testing. However, because there was a time effect (trend over time), Sigma Stat 1.0 (Jandel Corp., San Rafael, CA) was used to make comparisons over time within the same treatment. Overall acceptability panel data were analyzed using Sigma Stat 1.0. Appropriate comparisons were made using the Student-Newman-Keuls method of testing for multiple comparisons. A $p < 0.05$ was considered significant for all comparisons.

CHAPTER 3 - RESULTS AND DISCUSSION

Section 3.1 - Effect of honey on growth and activity of lactic acid bacteria and bifidobacteria

Section 3.1.1 - Standard plate counts

Tables 4, 5, 6, and 7 show the growth of *L. acidophilus* La-7, *L. delbrueckii* subsp. *bulgaricus* Lr-78, *Streptococcus salivarius* subsp. *thermophilus* St-133, and *Bifidobacterium* sp. Bf-13, respectively, in a 12% reconstituted NFDM solution containing sucrose, fructose, honey, or no added sweetener (control), at 0 and 24 hours of incubation. There were no significant differences in growth between cultures grown in the different sweeteners. This may have been due to the large amount of variation between samples. However, the SPC's did show that honey did not inhibit the growth of any of the cultures.

Ustunol (1998) reported that 3 and 5% honey decreased ($p<0.05$) the mean doubling time ($T_d = \ln 2/\mu$ (specific growth rate) and $\mu = (\ln X_2 - \ln X_1)/(t_1-t_2)$) of *Bifidobacterium* sp. Bf-1 in 12% NFDM. In addition, 3 and 5% concentrations of honey were also the most effective in enhancing the growth of *Bifidobacterium* sp. Bf-6 in 12% NFDM when compared with sucrose, fructose, and glucose at 1, 3, and 5%, and 1% honey. It was concluded that sweetener concentrations other than honey did not seem to have an effect on enhancing the growth of the bifidobacteria tested. It was also concluded that the effects of honey on bifidobacteria appeared to be strain specific because mean doubling times were lower for *Bifidobacterium* sp. Bf-1 than *Bifidobacterium* sp. Bf-6 in both 3 and 5% honey concentrations.

Table 4. Effect of sweetener type on growth of *Lactobacillus acidophilus* (La-7) in 12% NFDM

Sweetener Type	Plate Counts (CFU/ml)	
	0 Hours	24 Hours
Sucrose	$1.50 \times 10^{08a} \pm 4.58 \times 10^{07}$	$3.87 \times 10^{09a} \pm 1.42 \times 10^{09}$
Fructose	$2.00 \times 10^{08a} \pm 1.04 \times 10^{08}$	$1.80 \times 10^{09a} \pm 1.00 \times 10^{08}$
Honey	$2.43 \times 10^{08a} \pm 1.38 \times 10^{08}$	$2.73 \times 10^{09a} \pm 7.77 \times 10^{08}$
Control	$2.07 \times 10^{08a} \pm 5.51 \times 10^{07}$	$2.87 \times 10^{09a} \pm 9.07 \times 10^{08}$

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 3 for all treatments.

Table 5. Effect of sweetener type on growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lr-78) in 12% NFDM

Sweetener Type	Plate Counts (CFU/ml)	
	0 Hours	24 Hours
Sucrose	$3.83 \times 10^{07a} \pm 3.39 \times 10^{07}$	$4.90 \times 10^{08a} \pm 1.90 \times 10^{08}$
Fructose	$2.37 \times 10^{07a} \pm 1.63 \times 10^{07}$	$4.23 \times 10^{08a} \pm 2.36 \times 10^{08}$
Honey	$2.30 \times 10^{07a} \pm 2.69 \times 10^{07}$	$3.93 \times 10^{08a} \pm 1.58 \times 10^{08}$
Control	$2.43 \times 10^{07a} \pm 1.62 \times 10^{07}$	$4.60 \times 10^{08a} \pm 5.44 \times 10^{08}$

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 3 for all treatments.

Table 6. Effect of sweetener type on growth of *Streptococcus salivarius* subsp. *thermophilus* (St-133) in 12% NFDM

Sweetener Type	Plate Counts (CFU/ml)	
	0 Hours	24 Hours
Sucrose	$1.23 \times 10^{07a} \pm 8.14 \times 10^{06}$	$3.70 \times 10^{08a} \pm 2.35 \times 10^{08}$
Fructose	$1.02 \times 10^{07a} \pm 7.03 \times 10^{06}$	$2.40 \times 10^{08a} \pm 5.57 \times 10^{07}$
Honey	$1.21 \times 10^{07a} \pm 9.41 \times 10^{06}$	$2.67 \times 10^{08a} \pm 8.62 \times 10^{07}$
Control	$1.03 \times 10^{07a} \pm 6.75 \times 10^{06}$	$3.90 \times 10^{08a} \pm 1.59 \times 10^{08}$

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 3 for all treatments.

Table 7. Effect of sweetener type on growth of *Bifidobacterium* (Bf-13) in 12% NFDM

Sweetener Type	Plate Counts (CFU/ml)	
	0 Hours	24 Hours
Sucrose	$6.10 \times 10^{07a} \pm 2.62 \times 10^{07}$	$4.20 \times 10^{08a} \pm 3.98 \times 10^{08}$
Fructose	$4.70 \times 10^{07a} \pm 1.71 \times 10^{07}$	$2.90 \times 10^{08a} \pm 2.60 \times 10^{08}$
Honey	$8.30 \times 10^{07a} \pm 3.27 \times 10^{07}$	$2.30 \times 10^{08a} \pm 9.17 \times 10^{07}$
Control	$7.93 \times 10^{07a} \pm 3.17 \times 10^{07}$	$3.60 \times 10^{08a} \pm 4.40 \times 10^{08}$

[a]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 3 for all treatments.

Thus, the growth rate was found to be dependent upon the concentration of honey as well as the strain of bifidobacteria used.

Section 3.1.2 - Acetic acid and lactic acid production by lactic acid bacteria and bifidobacteria as determined by HPLC

Activity of the cultures grown in various sweeteners was determined by HPLC analysis. Figures 9-13 show production of acetic and lactic acid production by *L. delbrueckii* subsp. *bulgaricus* Lr-78, *S. salivarius* subsp. *thermophilus* St-133, *L. acidophilus* La-7, and *Bifidobacterium* sp. Bf-13 in 12% NFDM containing either fructose, sucrose, honey, or no added sweetener (control), after 0, 12, and 24 hours of incubation. As expected, the lactic acid bacteria (*L. delbrueckii* subsp. *bulgaricus*, *S. salivarius* subsp. *thermophilus*, and *L. acidophilus*) did not produce any acetic acid, and there were no significant differences ($p<0.05$) in the concentrations of lactic acid produced by these cultures regardless of the sweetener present or the incubation time. When bifidobacteria was grown in the presence of honey or other sweeteners, lactic acid levels were not detectable by HPLC at 0 and 12 hours of incubation, however, after 24 hours of incubation concentrations of lactic acid in 12% NFDM were higher ($p<0.05$) in honey than those in the sucrose, fructose, or control treatments. Acetic acid production by bifidobacteria was detected at 12 hours and concentrations increased at 24 hours. However, these concentrations were not significantly different between sweetener types at either time. These results not only indicate that honey does not inhibit yogurt starter cultures but also that honey may enhance the activity of bifidobacteria. Although it is not clear which

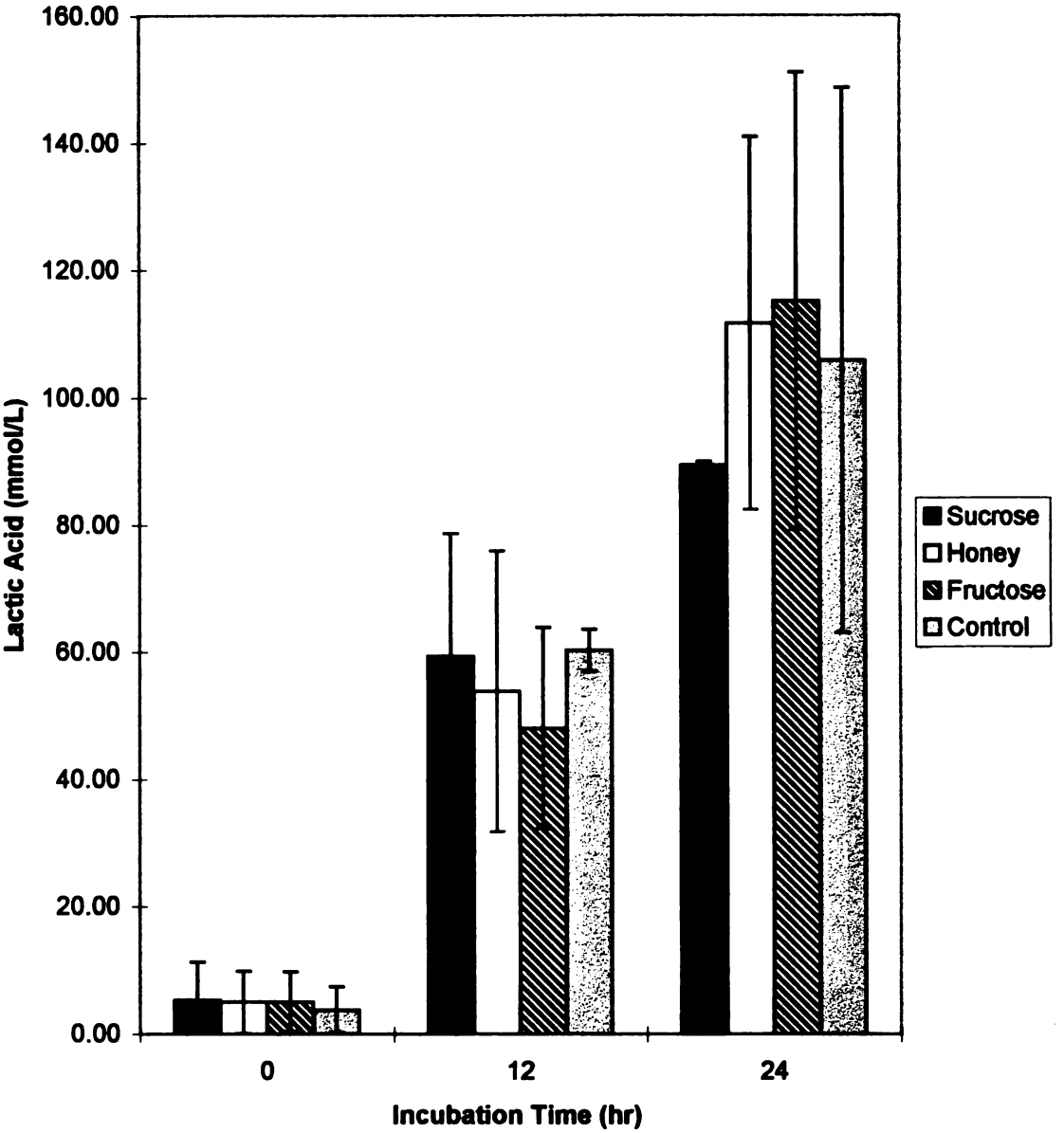


Figure 9. Effect of sweetener type on lactic acid production by *Lactobacillus delbrueckii* subsp. *bulgaricus* over 24 hours of incubation in 12% NFDM

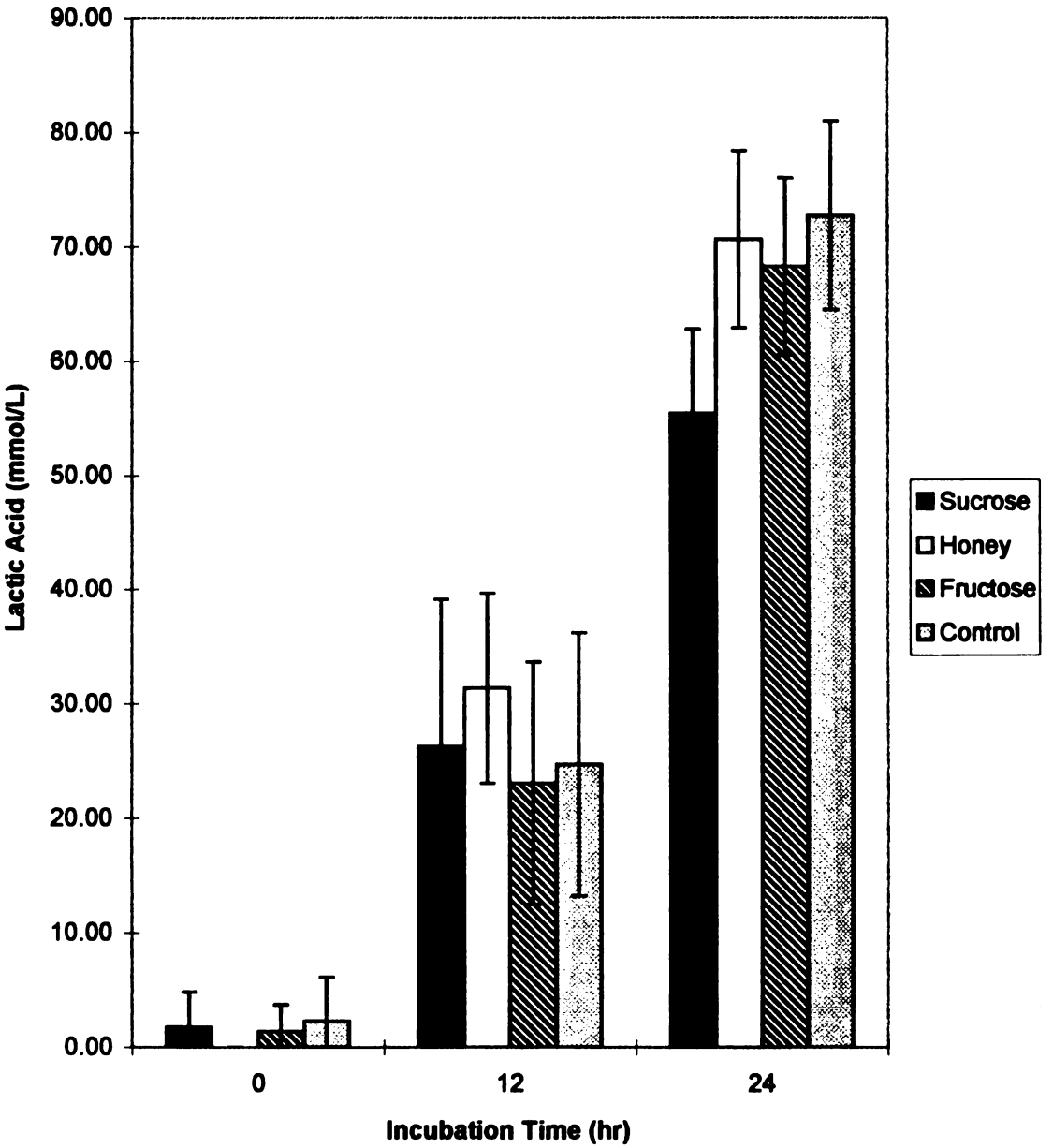


Figure 10. Effect of sweetener type on lactic acid production by *Streptococcus salivarius* subsp. *thermophilus* over 24 hours of incubation in 12% NFDM

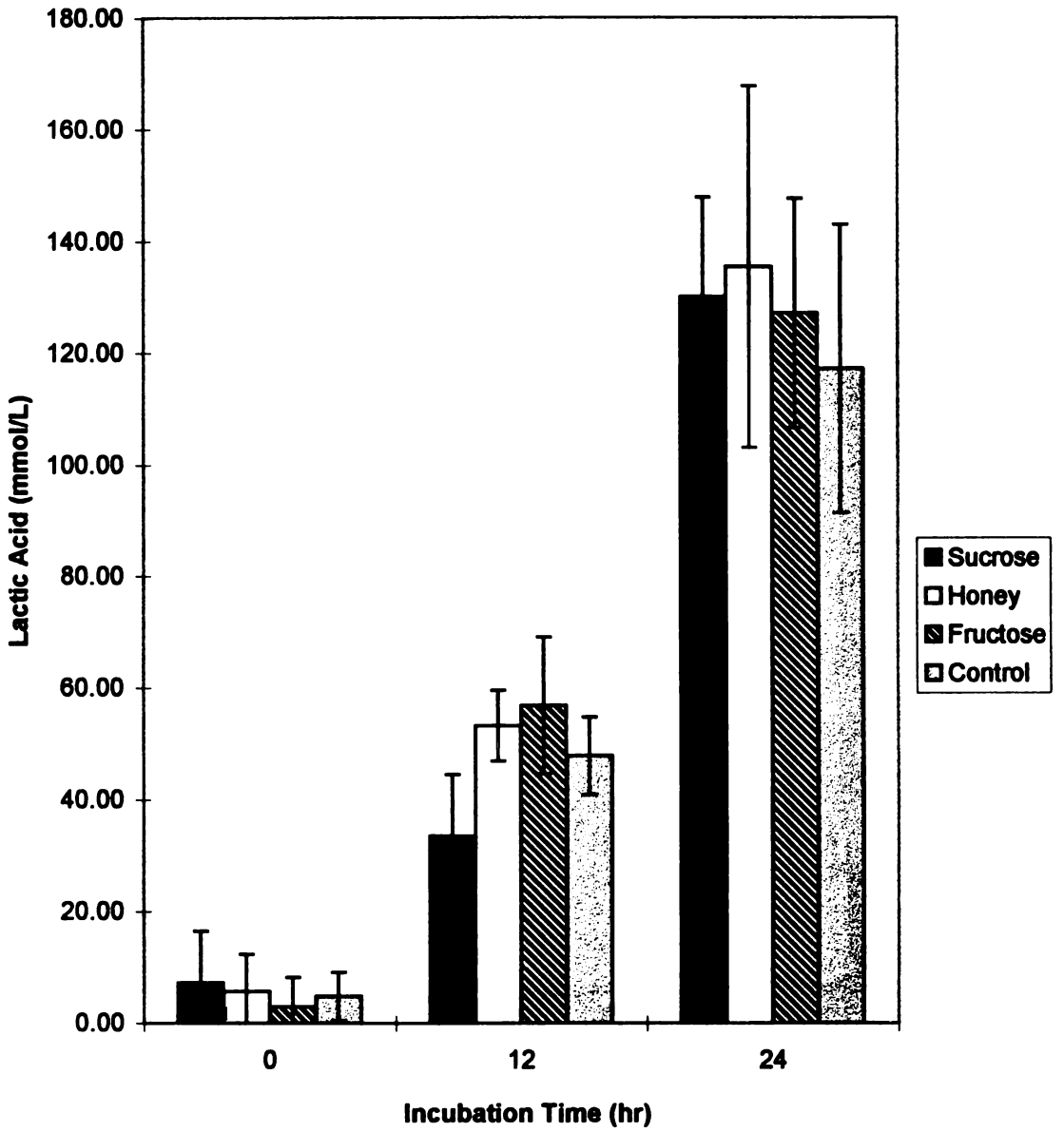
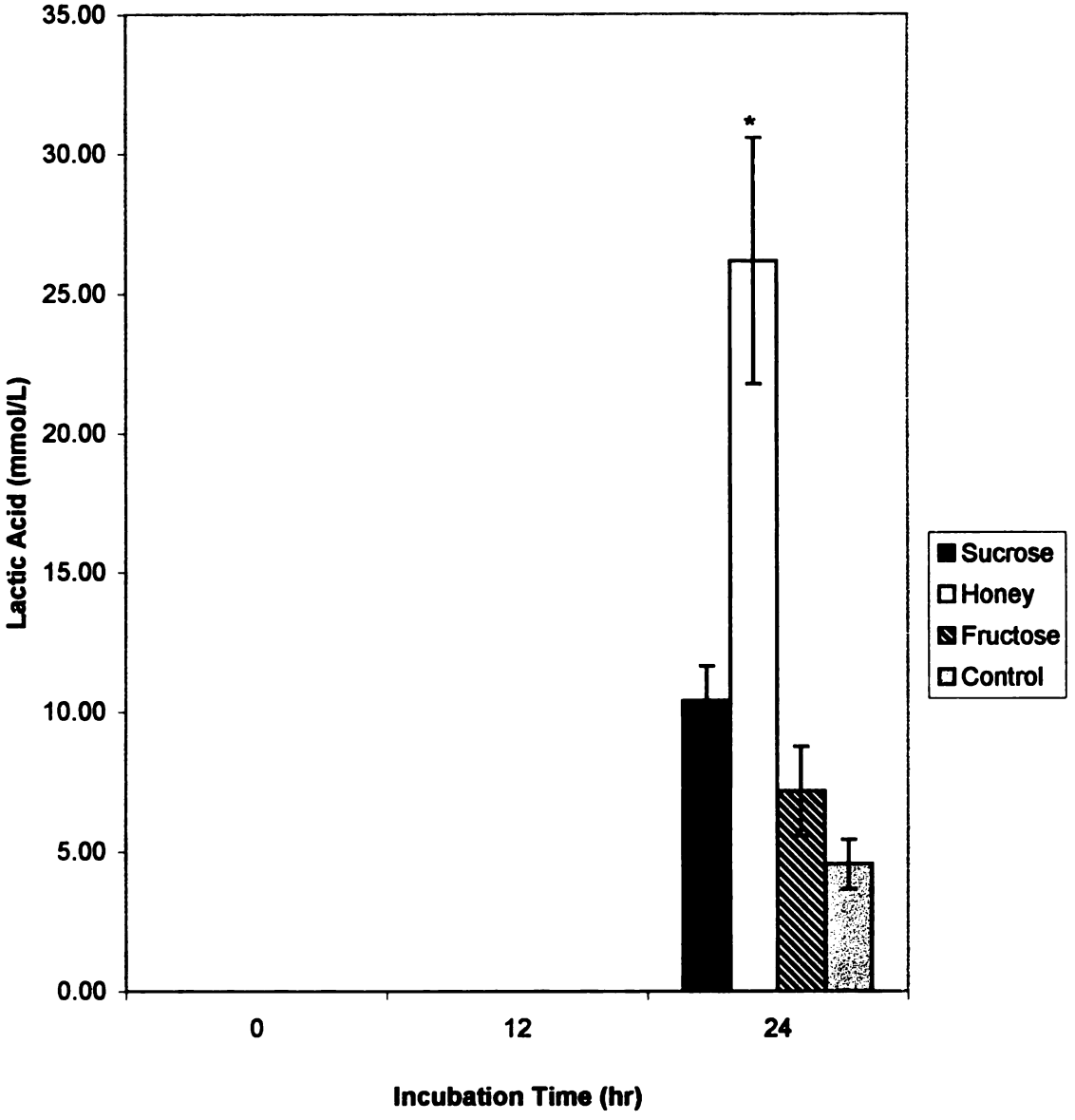


Figure 11. Effect of sweetener type on lactic acid production by *Lactobacillus acidophilus* over 24 hours of incubation in 12% NFDM



*Indicates significantly different ($p<0.05$).

Figure 12. Effect of sweetener type on lactic acid production by *Bifidobacterium* BF-13 over 24 hours of incubation in 12% NFDM

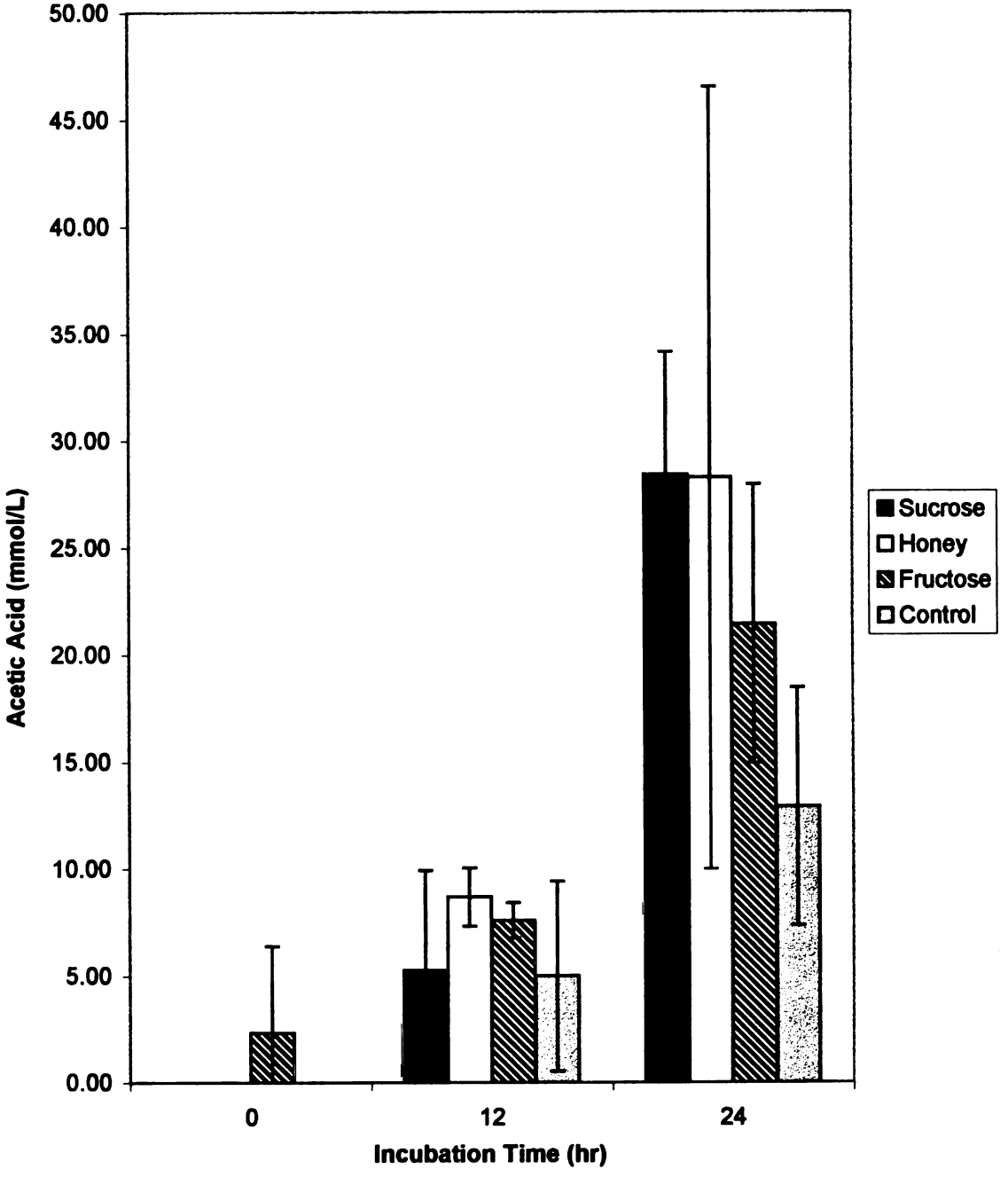


Figure 13. Acetic acid production by *Bifidobacterium* BF-13 over 24 hours of incubation in 12% NFDM

component(s) in honey increased the activity of Bf-13 the presence of complex sugars in honey may have some influence. Further research is needed to investigate this.

Although honey is known to have antimicrobial properties, the following hypothesis may explain as to why its presence did not have an inhibitory effect of the starter bacteria. As stated in the literature review, honey owes its antimicrobial properties to its high osmolarity, its low pH, the production of hydrogen peroxide from its glucose oxidase enzyme, and its small amounts of non-peroxide components (Molan, 1992a). When the honey is diluted (negating the osmotic effect and buffering the acidity) only the hydrogen peroxide and non-peroxide antibacterial constituents remain. Furthermore, the glucose oxidase enzyme that is activated by diluting the honey can be destroyed by heating, leaving only the non-peroxide factors to inhibit culture growth (Molan, 1992b). Therefore, because the honey was diluted (5% honey in 12%NFDM) and had been pasteurized in this experiment, only the non-peroxide constituents could influence antimicrobial activity, however since they were less concentrated, this did not occur.

Section 3.1.3 - pH determination

The pH of the NFDM was not affected by sweetener type when fermented by *L. delbrueckii* subsp. *bulgaricus* (Lr-78) or *S. salivarius* subsp. *thermophilus* (St-133) over a 24 hour period (Tables 8 and 9). There were no significant differences in pH between any of the sweeteners (sucrose, fructose or honey) or the control at any of the time points (0, 12, and 24 hours) when NFDM was

Table 8. Effect of sweetener type of pH of milk fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus*

Incubation Time (hr)	Sweetener Type			
	Sucrose	Fructose	Honey	Control
0	5.88 ± 0.09[a]	5.84 ± 0.12[a]	5.90 ± 0.10[a]	5.93 ± 0.08[a]
12	4.36 ± 0.26[a]	4.44 ± 0.23[a]	4.42 ± 0.29[a]	4.35 ± 0.12[a]
24	3.93 ± 0.06[a]	3.94 ± 0.07[a]	3.90 ± 0.06[a]	3.88 ± 0.04[a]

[a]Means with different superscripts are significantly different ($p \leq 0.05$). Comparisons are made only within the same row. Means ± standard deviations; n = 3 for all treatments.

Table 9. Effect of sweetener type on pH of milk fermented with *Strepococcus salivarius* subsp. *thermophilus*

Incubation Time (hr)	Sweetener Type			
	Sucrose	Fructose	Honey	Control
0	6.13 ± 0.06[a]	6.14 ± 0.08[a]	6.05 ± 0.03[a]	6.13 ± 0.09[a]
12	4.17 ± 0.13[a]	4.17 ± 0.14[a]	4.15 ± 0.14[a]	4.15 ± 0.13[a]
24	3.92 ± 0.14[a]	3.91 ± 0.13[a]	3.88 ± 0.12[a]	3.91 ± 0.14[a]

[a]Means with different superscripts are significantly different ($p \leq 0.05$). Comparisons are made only within the same row. Means ± standard deviations; n = 3 for all treatments.

fermented with these cultures. When NFDM was cultured with *L. acidophilus* (La-7) a significantly lower ($p<0.05$) pH was detected after 12 hours of incubation when grown in the presence of fructose and honey. However, after 24 hours of incubation, pH of all treatments were similar (Table 10). The increased acidity in the fructose and honey treatments could be attributed to their being or consisting of monosaccharides, specifically, unbound fructose. When glucose goes through the Embden-Myerhof pathway (EMP), it is immediately converted into fructose-6-P. When fructose and honey are used as carbon sources for these organisms, this first step is eliminated. In addition, when disaccharides penetrate the cell, they are found as either free sugars or sugar phosphates. In the case of free sugars, the disaccharides are split to monosaccharides by specific hydrolases before entering the EMP. While in the case of sugar phosphates, before going into the EMP, the disaccharide phosphates are split into one part free monosaccharides and one part monosaccharide phosphates by specific phosphohydrolases (Salminen *et al.*, 1993). In either case, the disaccharides have to be split before entering the EMP, while fructose and honey (which contains 38.5% fructose) do not have to undergo the first step in the EMP (the conversion of glucose to fructose-6-P). This may explain why *L. acidophilus* was able to utilize these sugars faster, producing more acid at 12 hours, then slowing down and producing more equal amounts of acid compared to the other organisms at 24 hours. It is unclear, however, as to why this did not occur with other lactic acid bacteria in this study (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*). One possibility is that the

Table 10. Effect of sweetener type on pH of milk fermented with *Lactobacillus acidophilus*

Incubation Time (hr)	Sweetener Type			
	Sucrose	Fructose	Honey	Control
0	5.81 ± 0.09[a]	5.83 ± 0.09[a]	5.82 ± 0.06[a]	5.86 ± 0.07[a]
12	4.91 ± 0.35[a]	4.30 ± 0.12[b]	4.25 ± 0.14[b]	4.90 ± 0.23[a]
24	3.92 ± 0.06[a]	3.82 ± 0.03[a]	3.79 ± 0.03[a]	3.94 ± 0.12[a]

[a-b]Means with different superscripts are significantly different ($p \leq 0.05$). Comparisons are made only within the same row. Means ± standard deviations; n = 3 for all treatments.

Table 11. Effect of sweetener type on pH of milk fermented with *Bifidobacterium* sp.

Incubation Time (hr)	Sweetener Type			
	Sucrose	Fructose	Honey	Control
0	6.03 ± 0.08[a]	6.00 ± 0.04[a]	6.03 ± 0.07[a]	6.09 ± 0.07[a]
12	5.53 ± 0.07[a]	5.48 ± 0.06[a]	5.02 ± 0.17[b]	5.47 ± 0.13[a]
24	5.10 ± 0.22[a]	5.10 ± 0.14[a]	4.55 ± 0.09[b]	5.06 ± 0.05[a]

[a-b]Means with different superscripts are significantly different ($p \leq 0.05$). Comparisons are made only within the same row. Means ± standard deviations; n = 3 for all treatments.

presence of fructose had an enhancing effect the amount of acid created by *L. acidophilus*. This is supported by activity data (HPLC), shown in Figures 9-13, at 12 hours (although not statistically different, a trend was apparent), however, because growth data (SPC's) were not collected at the 12 hour period, it cannot be determined whether or not it also supported this trend. Further studies would need to be conducted before any definite conclusions could be made.

The decrease in pH of the NFDM containing *Bifidobacterium* sp. (Bf-13) was found to be enhanced by the presence of honey (Table 11). At both 12 and 24 hours of incubation, the pH of the honey treatment was found to be lower ($p<0.05$) than that of all other treatments. Since no significant difference was observed in the fructose treatment compared to the sucrose and control treatments, it stands to reason that the fructose in honey did not contribute to the faster decrease in pH, unless a synergistic effect occurred. This also shows that whether the sugar was a mono- or disaccharide was of no consequence.

Ustunol (1998) showed that glucose (the second highest sugar component in honey) did not have a significant ($p<0.05$) effect on the growth or activity of NFDM containing *Bifidobacterium* Bf-1 or Bf-6 when compared with sucrose, fructose, honey, and no added sweetener (control). Shin (1997) reported the growth of bifidobacteria to be enhanced by certain oligosaccharides. The oligosaccharides present in honey, of which two have been identified thus far - isomaltotraose and isomaltopentaose, could explain this effect on the pH and activity. There are many influencing factors regarding the enhancing effect of oligosaccharides on bifidobacteria. Shin (1997) found

that the type of oligosaccharide (fructooligosaccharide (FOS) vs. galactooligosaccharide (GOS)), the concentration used (0.5, 1.0, 3.0, or 5.0%) as well as the strain of bifidobacteria (Bf-1 vs. Bf-6) had a significant effect on the growth and activity of bifidobacteria. Dubey and Mistry (1996) reported that the growth of bifidobacteria in infant formulas was not stimulated by FOS at 0.5%, while Shin (1997) found FOS stimulated the growth of bifidobacteria at levels of 1, 3, and 5%. The degree of polymerization of the FOS has also been shown to be important. Short chain fructooligosaccharides with degrees of ploymerization between 3 and 5, were found to exhibit maximum activity (Gibson and Roberfoid, 1995).

Section 3.2 - Development and properties of a drinkable yogurt shake sweetened with honey

Section 3.2.1 - Consumer flavor preference determination

The flavor preference survey indicated that 66.2%, of the 74 people surveyed, preferred a strawberry-honey combination. Thus, strawberry was the flavor used for the yogurt shake formulations. Interviewees suggested the possibility of a peach-honey combination and an orange-honey combination. However, these flavors were not investigated in this study.

Section 3.2.2 - pH determination

Although the pH of all treatments decreased over the 28 day storage period, the decrease was only significant ($p<0.05$) at 28 days, with the exception of the 6% NFDM treatments (Table 12). This was expected since the lactic cultures were viable during refrigerated storage. Studies have shown

Table 12. The pH of strawberry yogurt shakes over 28 days of refrigerated storage

Treatments (Formulations)			pH		
Fat	SW	NFDM	Day 0	Day 14	Day 28
1	S	0	4.22 ± 0.02[a]	4.20 ± 0.03[a]	4.15 ± 0.01[b]
2	S	0	4.16 ± 0.02[a]	4.14 ± 0.02[a]	4.11 ± 0.02[b]
1	H	0	4.08 ± 0.01[a]	4.02 ± 0.02[a]	3.97 ± 0.02[c]
2	H	0	3.97 ± 0.01[a]	3.96 ± 0.02[a]	3.92 ± 0.02[b]
1	H	3	4.08 ± 0.02[a]	4.06 ± 0.02[a]	4.03 ± 0.01[a]
2	H	3	4.12 ± 0.01[a]	4.06 ± 0.01[ab]	4.00 ± 0.02[b]
1	H	6	4.25 ± 0.01[a]	4.19 ± 0.01[b]	4.15 ± 0.02[c]
2	H	6	4.31 ± 0.02[a]	4.27 ± 0.02[b]	4.17 ± 0.01[c]

[a-c]Means with different superscripts are significantly different ($p < 0.05$). Comparisons are made only within the same row. Means ± standard deviations; n = 3 for all treatments.

bifidobacteria to be viable during refrigerated storage. Shin (1997) studied the viability of bifidobacteria and lactic acid bacteria in two brands of commercial yogurt. In the first brand of yogurt, up until two weeks after the product's date of expiration, viability of bifidobacteria was reported to have remained above 10^6 cfu/g. Lactic acid bacteria counts, for the same brand of yogurt, remained above 10^7 cfu/g until the date of expiration. Bifidobacteria counts in the second brand of yogurt also remained above 10^6 cfu/g until 2 weeks after product expiration while lactic acid bacteria counts remained above 10^6 cfu/g through the duration of the study (3 weeks post-expiration). In addition, while the pH of the first brand of yogurt declined only slightly over the duration of the study, the pH of the second brand decreased significantly.

Section 3.2.3 - Trained sensory panel evaluation

Trained sensory panel data is summarized in tables 13, 14 and 15. At 0, 14, and 28 days, sweetness of the strawberry yogurt shakes decreased ($p<0.05$) with an increase in NFDM content. It is likely that the additional milk solids masked the panelists' perception of sweetness. In addition, with the exception of the two treatments with 3% NFDM, all treatments scored lower in sweetness after 28 days of storage than after 0 days of storage. The decrease in the perception of sweetness could be associated with the pH data mentioned previously. As the pH decreased, the yogurt became slightly more acidic which may have masked the sweetness. Several panelists commented on the increasing acidity, and increasing sour and tart flavors in the yogurt shakes as the storage study progressed (Appendix B). The panel rated the sucrose

Table 13. Evaluation of strawberry yogurt shakes by a trained sensory panel at day 0

Treatments (formulations) Fat/SW/NFDM			Sweetness	Strawberry Flavor Intensity	Viscosity	Smoothness
1	S	0	6.20 ± 0.20[a]	5.50 ± 0.82[a]	2.77 ± 1.06[a]	8.53 ± 0.15[a]
2	S	0	6.53 ± 0.42[b]	6.03 ± 0.25[a]	2.27 ± 0.29[a]	8.60 ± 0.27[a]
1	H	0	5.33 ± 0.57[c]	5.63 ± 0.61[a]	3.27 ± 0.81[ab]	8.53 ± 0.21[a]
2	H	0	4.87 ± 0.96[cd]	6.03 ± 0.68[a]	3.63 ± 0.49[b]	8.57 ± 0.31[a]
1	H	3	4.33 ± 0.76[cd]	4.90 ± 0.52[a]	5.33 ± 0.67[c]	8.40 ± 0.20[a]
2	H	3	4.20 ± 0.20[d]	4.57 ± 0.70[a]	5.00 ± 0.30[c]	8.27 ± 0.50[a]
1	H	6	3.43 ± 0.65[e]	3.63 ± 0.58[b]	7.87 ± 0.51[d]	7.73 ± 0.51[b]
2	H	6	3.53 ± 0.25[e]	3.33 ± 0.42[b]	7.47 ± 0.29[d]	7.80 ± 0.40[b]

[a-e]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 36 for all treatments (3 replicates x 12 judges).

Table 14. Evaluation of strawberry yogurt shakes by a trained sensory panel at day 14

Treatments (formulations) Fat/SW/NFDM			Sweetness	Strawberry Flavor Intensity	Viscosity	Smoothness
1	S	0	5.53 ± 0.60^{a}	4.97 ± 0.23^{a}	2.63 ± 0.85^{a}	8.63 ± 0.12^{a}
2	S	0	6.23 ± 0.59^{b}	4.60 ± 0.76^{a}	2.43 ± 0.67^{a}	8.77 ± 0.06^{a}
1	H	0	4.90 ± 0.40^{c}	4.87 ± 0.51^{a}	3.00 ± 0.96^{ab}	8.53 ± 0.25^{a}
2	H	0	4.70 ± 0.82^{cd}	5.13 ± 0.12^{a}	3.80 ± 0.10^{b}	8.50 ± 0.52^{a}
1	H	3	4.87 ± 0.50^{cd}	4.80 ± 0.10^{a}	5.07 ± 1.04^{c}	8.37 ± 0.21^{a}
2	H	3	4.33 ± 0.46^{d}	4.20 ± 0.66^{a}	5.37 ± 0.32^{c}	8.17 ± 0.23^{a}
1	H	6	3.37 ± 0.40^{e}	3.27 ± 0.06^{b}	8.00 ± 0.76^{d}	7.47 ± 0.38^{b}
2	H	6	3.53 ± 0.21^{e}	3.60 ± 0.44^{b}	7.80 ± 0.53^{d}	7.63 ± 0.32^{b}

$^{a-e}$Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 36 for all treatments (3 replicates x 12 judges).

Table 15. Evaluation of strawberry yogurt shakes by a trained sensory panel at day 28

Treatments (formulations) Fat/SW/NFDM			Sweetness	Strawberry Flavor Intensity	Viscosity	Smoothness
1	S	0	5.43 ± 0.40^{a}	4.80 ± 0.61^{a}	2.67 ± 0.61^{a}	8.70 ± 0.10^{a}
2	S	0	6.27 ± 0.64^{b}	4.90 ± 0.56^{a}	2.63 ± 0.47^{a}	8.60 ± 0.27^{a}
1	H	0	5.00 ± 0.27^{c}	5.37 ± 0.35^{a}	3.23 ± 0.50^{ab}	8.50 ± 0.17^{a}
2	H	0	4.90 ± 0.36^{cd}	5.03 ± 0.68^{a}	3.27 ± 0.15^{b}	8.57 ± 0.15^{a}
1	H	3	4.63 ± 0.35^{cd}	4.70 ± 0.36^{a}	4.70 ± 0.46^{c}	8.20 ± 0.20^{a}
2	H	3	4.40 ± 0.36^{d}	4.77 ± 0.50^{a}	5.07 ± 0.40^{c}	8.27 ± 0.06^{a}
1	H	6	3.80 ± 0.76^{e}	3.53 ± 0.47^{b}	7.57 ± 0.47^{d}	7.47 ± 0.29^{b}
2	H	6	3.73 ± 0.55^{e}	3.60 ± 0.20^{b}	7.60 ± 0.36^{d}	7.47 ± 0.15^{b}

$^{a-e}$Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 36 for all treatments (3 replicates x 12 judges).

treatments as being sweeter than the honey treatments with no added milk solids which was not expected since, the sweetness of sucrose is equivalent to that of honey on a dry weight basis (NHB, 1996) and this adjustment was made in the product formulations. Strawberry flavor intensity also decreased with an increase in NFDM. However, this decrease was only significant ($p<0.05$) in the samples containing 6% NFDM. Similarly to the sweetness, the increase in milk solids probably masked the perception of strawberry flavor intensity as well. With the exception of two samples (2% milk with 3% NFDM and 2% milk with 6% NFDM) all samples scored lower for strawberry flavor intensity after 28 days of storage compared to those at 0 days. This trend also supports the thinking that the increasing acid in the product caused a decrease in the perception of flavor as well as sweetness. Smoothness decreased ($p<0.05$) with an increase in NFDM concentration in the strawberry yogurt shakes and remained relatively stable over the 28 day storage period. Panelists may have perceived the increase in NFDM as an increase in "grittiness" as indicated by some of their comments (Appendix B). Viscosity of the strawberry yogurt shakes increased ($p<0.05$) with an increase in NFDM concentration. This was expected as an increase in total solids leads to a thicker product. In addition, the panel scored the honey treatments with no added NFDM to be more viscous than the sucrose controls. This may explain why the panel rated these same honey samples as being less sweet than the sucrose control as the added viscosity may have decreased the perception of sweetness. Similar findings were reported by Kokini *et al.* (1982) who found that the perceived sweetness intensity decreased

for both sucrose and fructose solutions containing tomato solids, as tomato solids were increased. The panel did not appear to perceive a difference between the two fat levels (treatments made with 1 and 2% fat milk) in the products. Panelists indicated the treatments with 3% NFDM to be the most desirable of those tested.

Section 3.2.4 - Apparent viscosity determination

Apparent viscosity versus shear rate curves can be seen in Figures 14-16. Consistency coefficients and flow behavior of the yogurt shakes during 28 days of refrigerated storage can be seen in Tables 16-18. Apparent viscosity increased with an increase in NFDM content at a shear rate of 30 (1/s). However, this was only significant ($p<0.05$) in the treatments with 6% NFDM (Table 19). Since there appeared to be a significant difference in viscosity between treatments at the lower shear rates in these figures, apparent viscosity was also determined at 1 (1/s). Results were the same as with a shear rate of 30 (1/s), with significant differences ($p<0.05$) in only the treatments with 6% NFDM. The rheological method was less sensitive for viscosity determination than the trained panel since the viscometer was unable to detect the differences in viscosity perceived by the trained panel judges. Because the use of the RV Brookfield viscometer with the Mitschka method of analysis had been previously determined by Briggs and Steffe (1997) to be a good procedure for evaluating the apparent viscosity of shear-thinning foods, its lack of sensitivity for the strawberry yogurt shakes may suggest that slip could have been a factor. Slip could have occurred if a thin layer of fluid, which had a viscosity lower than that

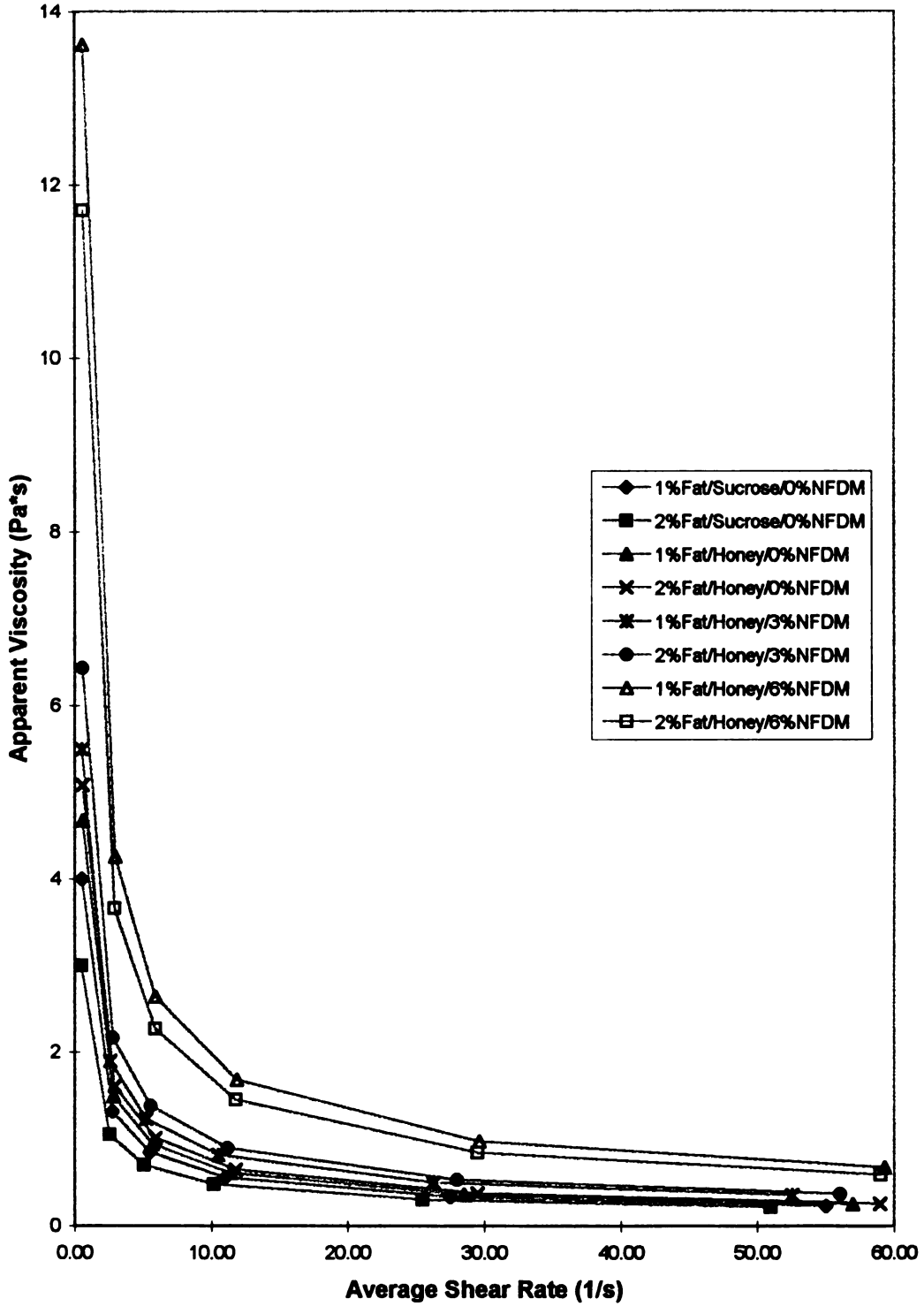


Figure 14. Apparent viscosity of yogurt shakes at day 0

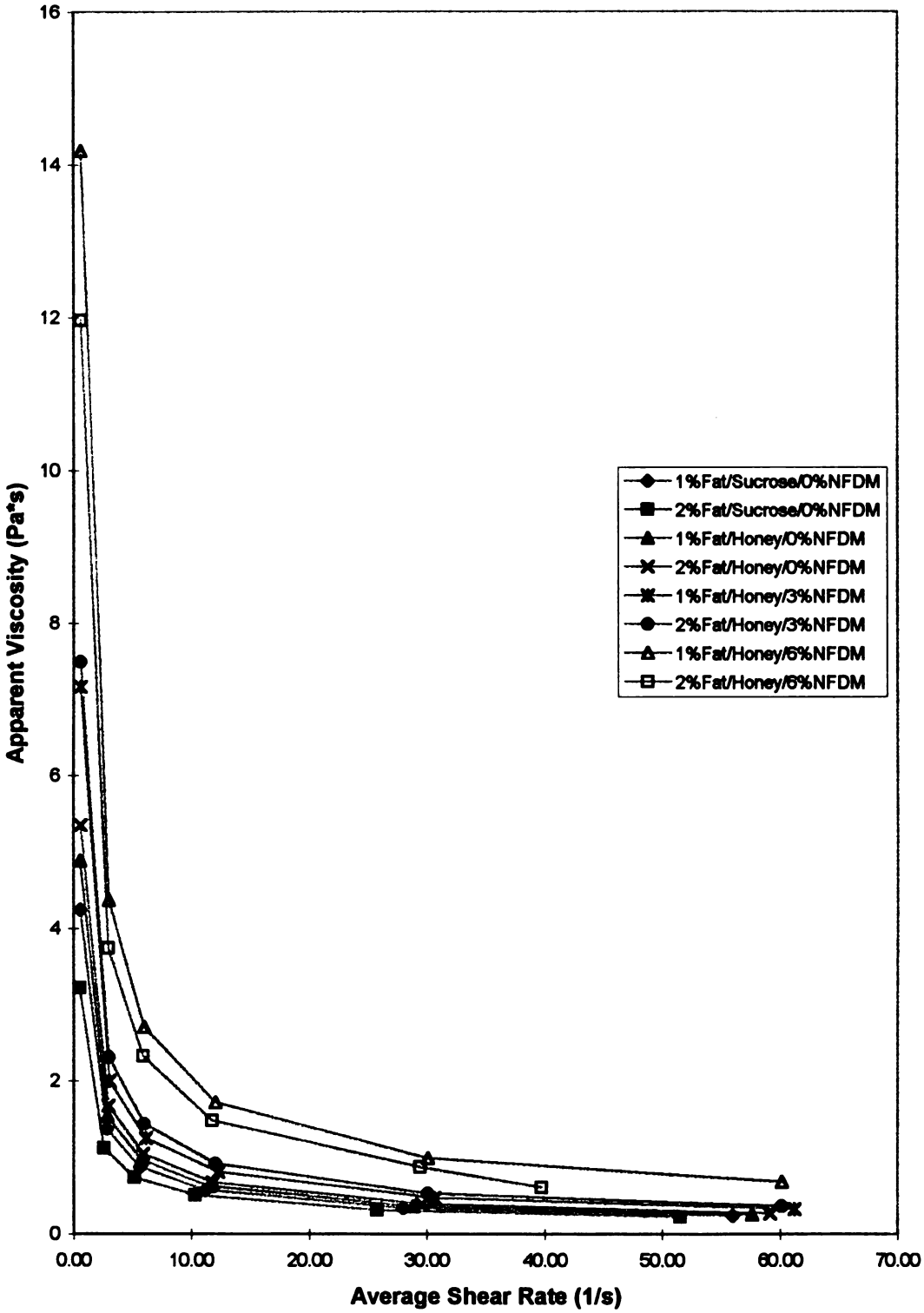


Figure 15. Apparent viscosity of yogurt shakes at day 14

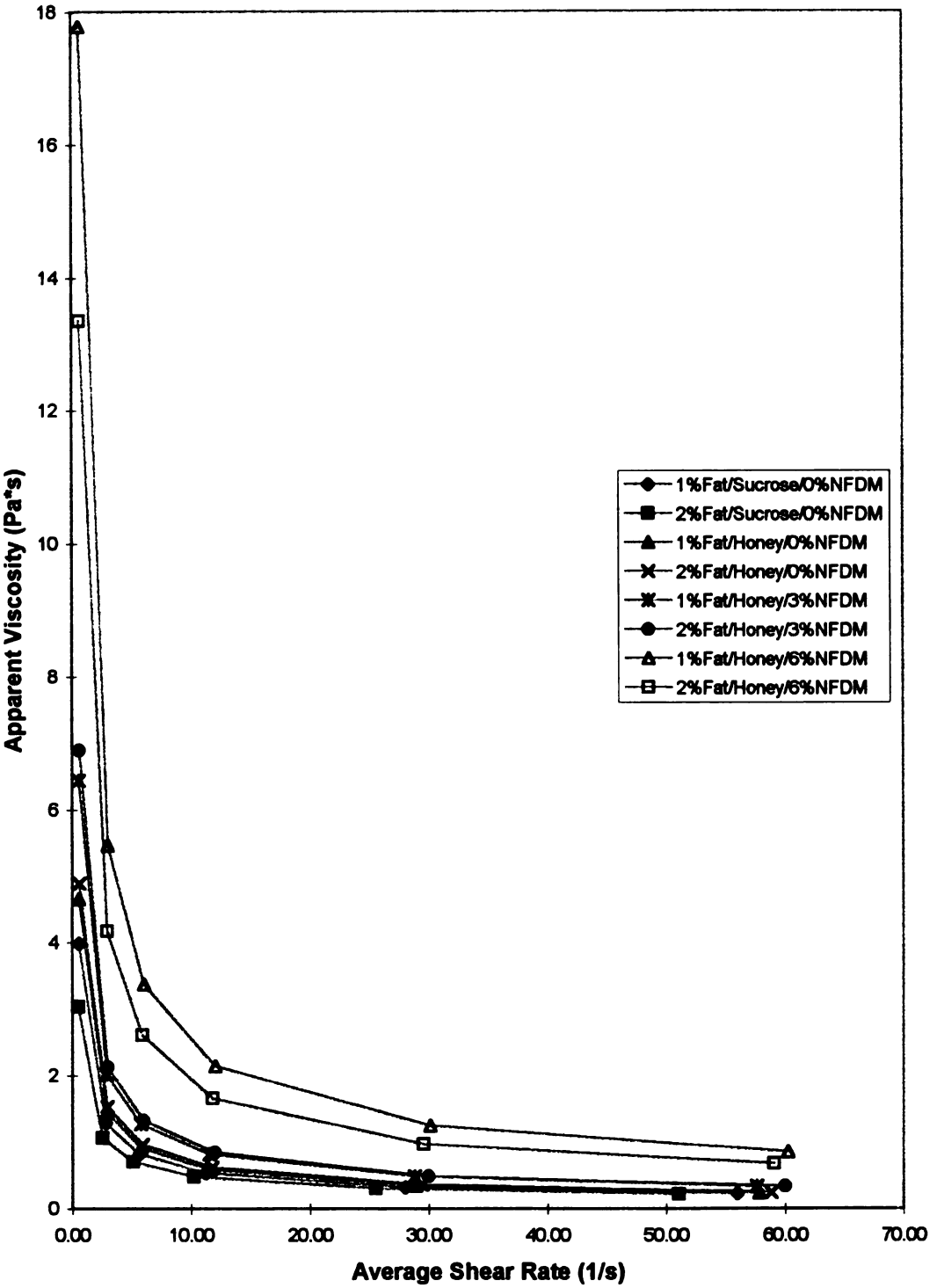


Figure 16. Apparent viscosity of yogurt shakes at day 28

Table 16. Consistency coefficient and flow behavior of strawberry yogurt shakes at day 0

Treatment Fat/SW/NFDM	K (Pa s^n)	n (-)	R^2
1 S 0	2.5885	0.3848	0.9922
2 S 0	1.9008	0.4277	0.9927
1 H 0	3.0900	0.3691	0.9910
2 H 0	3.3934	0.3515	0.9916
1 H 3	3.6326	0.4194	0.9891
2 H 3	4.3226	0.3829	0.9928
1 H 6	9.0459	0.3491	0.9877
2 H 6	7.7528	0.3512	0.9873

Table 17. Consistency coefficient and flow behavior of strawberry yogurt shakes at day 14

Treatment Fat/SW/NFDM	K (Pa s^n)	n (-)	R^2
1 S 0	2.7471	0.3760	0.9903
2 S 0	2.0448	0.4210	0.9919
1 H 0	3.2248	0.3636	0.9892
2 H 0	3.5584	0.3501	0.9898
1 H 3	4.5825	0.3341	0.9550
2 H 3	4.9965	0.3428	0.9877
1 H 6	9.4564	0.3424	0.9873
2 H 6	7.8963	0.3530	0.9865

Table 18. Consistency coefficient and flow behavior of strawberry yogurt shakes at day 28

Treatment Fat/SW/NFDM	K (Pa s^n)	n (-)	R^2
1 S 0	2.5953	0.3756	0.9899
2 S 0	1.9168	0.4256	0.9919
1 H 0	3.0643	0.3618	0.9880
2 H 0	3.2382	0.3527	0.9894
1 H 3	4.2387	0.3657	0.9881
2 H 3	4.5807	0.3440	0.9870
1 H 6	11.811	0.3418	0.9861
2 H 6	8.8077	0.3512	0.9871

Table 19. Apparent viscosity (at an average shear rate of 30 (s^{-1})) of strawberry yogurt shakes

Treatments (formulations) Fat/SW/NFDM			Day 0 η(Pa*s)	Day 14 η(Pa*s)	Day 28 η(Pa*s)
1	S	0	0.315 ± 0.07[a]	0.326 ± 0.07[a]	0.306 ± 0.05[a]
2	S	0	0.269 ± 0.06[a]	0.284 ± 0.08[a]	0.272 ± 0.08[a]
1	H	0	0.348 ± 0.13[a]	0.358 ± 0.13[a]	0.339 ± 0.12[a]
2	H	0	0.370 ± 0.07[a]	0.386 ± 0.08[a]	0.355 ± 0.06[a]
1	H	3	0.467 ± 0.17[a]	0.474 ± 0.14[a]	0.474 ± 0.12[a]
2	H	3	0.511 ± 0.10[a]	0.530 ± 0.08[a]	0.488 ± 0.06[a]
1	H	6	0.974 ± 0.30[b]	1.000 ± 0.24[b]	1.250 ± 0.51[b]
2	H	6	0.840 ± 0.26[b]	0.863 ± 0.26[b]	0.965 ± 0.10[b]

[a-b]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same column. Means ± standard deviations; n = 3 for all treatments.

of the yogurt shake, formed around the spindle disc (Steffe, 1996). This may be probable since the yogurt shakes seemed to separate (synerese) readily, especially in the thinner shakes. This phenomenon would make apparent viscosity determination difficult because the free whey (or syneresis) would cause approximately the same amount of friction in each sample.

Variability may have been another factor that contributed to the lack of sensitivity in the Mitschka method. In comparing the raw data from all three replicates, the third replicate appears to vary greatly from the other two. Factors contributing to this variation could have been the day to day variability between the manufacturing of each replicate, the different brands of milk used for manufacture, and human error.

The power law equation, which this study was based on, has been the most frequently used mathematical model for analysis of yogurt with the Herschel-Bulkley model occasionally incorporated to fit the non-Newtonian behavior of yogurt (Velez-Ruiz and Canovas, 1997). Other researchers have incorporated more complex models to analyze the rheological properties of yogurt. Ramaswamy and Basak (1991 and 1992) expressed the rheological behavior of commercial stirred yogurt in a three-cyclic shearing sequence in which upward curves usually followed the Herschel-Bulkley model and downward curves followed a linear relationship. Although many authors referred to the thixotropic property of yogurt, no model was used to describe it (Labropoulos, *et al.* 1981; Mottar, *et al.* 1989; Steventon, *et al.* 1990; Ramaswamy and Basak 1991 and 1992; and Basak and Ramaswamy, 1994).

Since the yogurt shakes did not exhibit many of the properties found by these authors to be common to yogurt, it can be established that the methods of analysis they used to analyze yogurt would not be applicable to the yogurt shakes in this study. While the yogurt shakes were non-Newtonian, they did not exhibit any thixotropic tendencies. They also separated much faster than yogurt and appeared to have the problem of slip commonly associated with food suspensions such as vegetable and fruit purees (Steffe, 1996). In addition, when the yogurt shakes were shaken, many small air bubbles appeared throughout the product. This also may have affected the analysis.

Although the Mitschka method did not possess the sensitivity that was needed for analysis of the yogurt shakes, it is very likely that other methods would. Other methods of analysis initially considered appropriate for this study included the parallel plate, cone and plate, and concentric cylinder viscometers (Steffe, 1996). These methods were not chosen, however, because they were not as readily available or used by the food industry as are the Brookfield viscometers in addition to their being more expensive. Because separation or slip seemed to be the reason for the insensitivity of this method in testing the yogurt shakes, it appears that the mixer viscometer would be the method of choice for this product.

Section 3.2.5 - Untrained consumer acceptability panel

Untrained panelists rated the sucrose control and the honey treatment with no added NFDM as being equally preferred at a score of 7 (like moderately). The honey with 3% NFDM added, although still liked, scored a 6

(like slightly), which was lower ($p<0.05$) than the other two treatments (Table 20). Panelists indicated the optimum viscosity to be between the 0% and 3% NFDM level, with the 0% being too thin and the 3% being too thick. In addition, out of the 58 panelists, 36 indicated they would purchase at least one of the products if they were available commercially, 8 said "maybe", "I would try it", or "would peak my interest", while 13 said they would not purchase any of the products tested. Additional questions that were asked during the panel provided no conclusive data and therefore were not included.

Section 3.2.6 - Mold count determination

Mold counts increased rapidly between 14 and 28 days of storage, though visual mold started to appear around the 28 day mark (Table 21). Thus shelf-life was estimated to be between 14 and 21 days though further studies need to be done. Shelf life of these products is expected to be longer when the product is manufactured in a more sanitary environment. In addition, product shelf-life might be further extended by removing oxygen or gas flushing the headspace.

Table 20. Overall acceptability of strawberry yogurt shakes by an untrained consumer panel

Treatments (Formulations) Fat/SW/NFDM	Median	25%	75%
1 S 0	7.00[a]	6.00	8.00
1 H 0	7.00[a]	6.00	8.00
1 H 3	6.00[b]	4.00	7.00

[a-b]Medians with different superscripts are significantly different ($p<0.05$). Comparisons are made within the same column. Medians and interquartile ranges; n = 58 judges for all treatments.

Table 21. Mold counts of strawberry yogurt shakes over 28 days of refrigerated storage

Treatments (Formulations)	Mold Counts (CFU/ml)		
Fat/SW/NFDM	Day 0	Day 14	Day 28
1 S 0	5	2	~147
1 H 0	3	1	~133
1 H 3	5	4	4
Strawberry Puree	0	0	0

n=1 for all treatments.

CHAPTER 4 - CONCLUSIONS

(1) The use of honey as a sweetener did not inhibit the growth or activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* Lr-78, *Streptococcus thermophilus* St-133, *Lactobacillus acidophilus* La-7, or *Bifidobacterium* sp. Bf-13 in 12% NFDM.

(2) Honey appeared to enhance the activity of Bf-13 as determined by pH determination and HPLC analysis.

(3) Sweetness, strawberry flavor intensity, and smoothness all decreased ($p<0.05$) with an increase in NFDM level, while viscosity increased ($p<0.05$) with an increase in NFDM level. A difference in viscosity between the sweeteners (honey vs. sucrose) was found by the trained panel but not detected by the Brookfield viscometer. The panelists however, did not perceive any difference between milk fat levels. The pH of the products declined and mold counts increased over the 28 day refrigerated storage period. The product shelf-life was estimated to be between 14 and 21 days. However, shelf-life could be further lengthened by removal of oxygen in the headspace.

(4) Honey and sucrose sweetened products, with no added NFDM, were most preferred by the untrained consumer panel and the majority of the panelists indicated they would purchase at least one of the products tested if they were available commercially.

CHAPTER 5 - FUTURE RESEARCH

During this study, it was observed that the presence of honey enhanced the activity of bifidobacteria. Additional studies need to be conducted, however, before any definite conclusions can be made. As reported by Shin (1997), the concentration of the sweetener as well as the strain of bifidobacteria are also factors that effect the growth and activity of bifidobacteria in 12% NFDM. In addition, the component(s) in honey that is/are responsible for the enhancement of bifidobacteria are unknown. Additional sweeteners, sweetener levels, and different strains of bifidobacteria should be studied in order to better understand the interaction between bifidobacteria and various sweeteners. Further studies are needed to determine the responsible component(s).

In terms of the strawberry yogurt shake, further optimization of the NFDM and sweetener levels to produce the most desirable yogurt shake is needed. Panelists commented that the products were a little too sweet and indicated the most desirable thickness would probably occur in a product between with 0 and 3% NFDM. Further optimization of ingredients may also be needed to make this product more feasible in terms of cost.

APPENDIX A

QUESTIONNAIRES FOR SENSORY EVALUATION TESTS

MICHIGAN STATE
UNIVERSITY

November 15, 1996

TO: Zeynep Ustunol
136 G.M. Trout Food Science Bldg

RE: IRB#: 96-718
TITLE: MANUFACTURING OF DRINKABLE YOGURT SHAKE CONTAINING HONEY
REVISION REQUESTED: N/A
CATEGORY: 1-G
APPROVAL DATE: 11/14/96

The University Committee on Research Involving Human Subjects'(UCRIHS) review of this project is complete. I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the UCRIHS approved this project and any revisions listed above.

RENEWAL: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Investigators planning to continue a project beyond one year must use the green renewal form (enclosed with the original approval letter or when a project is renewed) to seek updated certification. There is a maximum of four such expedited renewals possible. Investigators wishing to continue a project beyond that time need to submit it again for complete review.

REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB # and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.

PROBLEMS/ CHANGES: Should either of the following arise during the course of the work, investigators must notify UCRIHS promptly: (1) problems (unexpected side effects, complaints, etc.) involving human subjects or (2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of any future help, please do not hesitate to contact us at (517)355-2180 or FAX (517)432-1171.

Sincerely,

David E. Wright, Ph.D.
UCRIHS Chair

DEW:bed

cc: Heather Vachon
Fred Salas

OFFICE OF
RESEARCH AND GRADUATE STUDIES

University Committee on Research Involving Human Subjects (UCRIHS)
Michigan State University
202 Administration Building
East Lansing, Michigan
48824-1046

517/355-2180
FAX: 517/432-1171

Table A.2. Flavor combination survey

Product: Drinkable Yogurt Shake

Gender: M F

Are you:
__Less than 25 years of age
__Between the ages of 25-35
__Between the ages of 36-55
__Over 55 years of age

Do you consume yogurt and if so, how often?

__More than once a week
__About once a week
__Two to three times a month
__About once a month
__Less than once a month
__Never

Considering all factors (i.e. price) being equal, indicate which yogurt shake flavor combination you think you would prefer by circling your choice:

Strawberry-honey Vanilla-honey

Comments:

If you would be interested in being involved in the actual taste testing of this product, please fill out the following section:

Name:_____ Phone number:_____

Thank you for taking the time to fill out this survey.

Table A.3. Trained panel prescreening questionnaire

Name _____ Phone (Day)_____
(Evening)_____

Gender M or F

Age __ 18-25, __ 26-35, __ 36-55, __ > 55

Time

1. Do you plan to be on campus during the summer? _____

2. Are there any weekdays that you will not be available on a regular basis?

3. What part of the day are you normally available?
   ___ Morning (8-11)
   ___ Early Afternoon (11-2)
   ___ Afternoon (2-5)

Health

1. Do you have any food allergies? (e.g. lactose intolerance)

2. Do you take any medications which affect your senses?

3. Are you currently on a restricted diet? If yes, please explain.

4. What foods can you not eat?

5. What foods do you not like to eat?

Yogurt Consumption

1. Do you consume yogurt?_____

   If yes, how often? ___ More than once a week
   ___ More than once a month
   ___ Less than once a month

Thank you for your time!

Table A.4. Consent form for taste panel members

Department of Food Science and Human Nutrition
Michigan State University

Drinkable low-fat strawberry honey yogurt shake prepared from pasteurized low-fat milk, non-fat dry milk, grade A honey, sucrose, fructose, seedless strawberry puree, starter cultures, strawberry flavor, water, and color.

I _____ have read the above list of ingredients and find none that I am allergic to. I have also been informed on the nature of the research (including experimental materials and procedures) which will be used during the tasting session. I understand that the taste panel will take approximately 10-15 minutes. I agree to serve on the taste panel which will be conducted on _____, 1997. I understand that I am free to withdraw my consent and to discontinue participation in the panel at any time without penalty.

_____
Signature

_____
Date

Table A.5. Panel screening test

Product: Strawberry Drinkable Yogurt Shake

Name_____ Date_____

Characteristic: **Strawberry Flavor Intensity**

Instructions: Rank these samples for strawberry flavor intensity. The sample with the most intense strawberry flavor is ranked first, the second most intense is ranked second, and the least intense is ranked third. Taste the samples in the order indicated below rinsing with water between each sample.

Place the code number on the appropriate lines.

194 975 782

Ranking: Most intense 1. _____
2. _____
Least intense 3. _____

Comments:

Table A.6. Refrigerated storage test

Product: Strawberry Drinkable Yogurt Shake

Name_____ Date_____

Characteristic: **Strawberry Flavor Intensity**

Instructions: Evaluate the strawberry flavor intensity of the sample of strawberry yogurt shake indicated below. Taste the sample and rinse with water between tasting for each characteristic. Place an X next to the value which best describes the strawberry flavor intensity of the sample.

637

____ 1 Not Very Strawberry
____ 2
____ 3
____ 4
____ 5 Moderately Strawberry
____ 6
____ 7
____ 8
____ 9 Very Strawberry

Comments:

Table A.7. Trained panel questionnaire

Product: Drinkable Yogurt Shake

Name: _____ Date: _____

PLEASE ANSWER QUESTION #1 DURING THE SAMPLING OF THE PRODUCT THEN ANSWER THE REMAINDER OF THE QUESTIONS WHEN YOU ARE FINISHED.

1. As you sample and rate the characteristics of the products today, please evaluate the appropriateness of each characteristic (Strawberry Flavor Intensity, Sweetness, Smoothness, and Viscosity) for each sample in your own words:

Sample Number:
399

212

747

585

151

974

628

436

2. If these product characteristics were optimized the level you find most appropriate, would you want to buy this product if it were available? Why or why not?

3. Would knowing this product contains the probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium* sp. increase your intent to purchase this product? Why or why not?

4. Do you find honey to be a good replacer of sucrose for health reasons, taste reasons, or any other reasons? Why or why not?

5. Are there any comments you would like to make in general about this product at any time during this storage study?

Thank you for your faithful participation through the duration of this study.

Table A.8. Untrained panel acceptability test

Product: Strawberry Drinkable Yogurt Shake Sweetened with Honey

Name: _____ Date: _____

Please evaluate the three strawberry yogurt shake samples by tasting each sample in the following order and rinsing between each one. Indicate how much you like or dislike each sample by checking the appropriate phrase.

931	716	143
__like extremely	__like extremely	__like extremely
__like very much	__like very much	__like very much
__like moderately	__like moderately	__like moderately
__like slightly	__like slightly	__like slightly
__neither like nor dislike	__neither like nor dislike	__neither like nor dislike
__dislike slightly	__dislike slightly	__dislike slightly
__dislike moderately	__dislike moderately	__dislike moderately
__dislike very much	__dislike very much	__dislike very much
__dislike extremely	__dislike extremely	__dislike extremely

Do you consume yogurt and if so, how often?

__Less than once a month
__About once a month
__Two or three times a month
__About once a week
__More than once a week

How do you feel about the following characteristics of these products:

Sweetness?
Viscosity?
Flavor?
Texture/Mouthfeel?

If any of these products were available for purchase would you want to buy this product? Why or why not?

Would knowing this product contains the probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium sp.* increase your intent to purchase this product? Why or why not?

Do you find honey to be a good replacer of sucrose for health reasons, taste reasons, or any other reasons? Why or why not?

Comments:

Thank you for your time!

APPENDIX B

TRAINED PANEL CODES AND COMMENTS

Table B.1. Trained panel treatment codes

Treatment Fat/SW/NFDM	Rep #1 Code Day 0	Rep #1 Code Day 14	Rep #1 Code Day 28
1%/S/0%	128	352	847
2%/S/0%	637	378	254
1%/H/0%	873	131	455
2%/H/0%	764	495	744
1%/H/3%	285	769	397
2%/H/3%	516	913	661
1%/H/6%	949	586	246
2%/H/6%	491	622	113

Treatment Fat/SW/NFDM	Rep #2 Code Day 0	Rep #2 Code Day 14	Rep #2 Code Day 28
1%/S/0%	185	838	778
2%/S/0%	722	522	257
1%/H/0%	937	989	435
2%/H/0%	313	564	122
1%/H/3%	885	291	644
2%/H/3%	117	656	893
1%/H/6%	394	879	566
2%/H/6%	931	448	863

Treatment Fat/SW/NFDM	Rep #3 Code Day 0	Rep #3 Code Day 14	Rep #3 Code Day 28
1%/S/0%	578	452	399
2%/S/0%	763	299	212
1%/H/0%	256	777	747
2%/H/0%	449	188	585
1%/H/3%	622	563	151
2%/H/3%	824	336	974
1%/H/6%	945	381	628
2%/H/6%	611	919	436

Table B.2. Trained panel comments rep.1 day 0

#491 Leaves a bad taste.
#128 Tasted a tiny bit grainy, but not lumpy. Taste like more berry flavor than from training.
#637 A tiny bit grainy, but not lumpy.
#285 Bad after taste.

#949 Didn't care for. Too thick.
#285 Didn't care for.
#489 Didn't care for. Too thick.
#873 Didn't care for. Again - I taste a weird (yuck) after taste.
I would like #128 the best of all four if it was a little thicker.
#764 Kind of grainy. Hard to tell (sweetness and strawberry flavor intesity) has burnt (yuck) aftertaste!

#949 Gritty feel - slight. Very bitter. The bitter taste was overwhelming.
#516 No grittyness at all.
#491 The sample was not "lumpy" but it was very "gritty" so I did not mark it smooth. It kind of tasted slightly acidic and I could taste the non-fat dry milk. Non-fat dry milk and acidity stand out and mask the strawberry.
128 seemed very slightly gritty. The oversweetness made this a little hard (strawberry flavor intensity) but I think what I am tasting is a lot of strawberry also.
#873 Seemed slightly gritty again.
#285 Acidic and non-fat dry milk taste again. Didn't seem gritty. Acidic.

#637 Good stuff.

#491 Noticeable honey flavor.
#516 Strong honey taste.

#949 Seemed to be able to taste the honey in this product.

#949 I can taste honey!

#128 Tastes horrible!
#491 Too thick!

#491 Not very good - but I think you already know this.

#873 This sample was the wateriest in terms of thickness/lack of lumps.

Table B.3. Trained panel comments rep.1 day 14

#495 Slightly grainy. Initial taste is full of strawberry flavor, but turns sour.
#622 Difficult to determine strawberry taste, because of the bad aftertaste. Bitter aftertaste.

#378 Very good.
#622 Off (burnt) after taste.
#131 Off (burnt) after taste.
#586 Yuck.

#622 Gritty.
#352 Gritty.
#586 I tasted honey big time.

#352 My favorite of the four today!

#131 Honey flavor.

#622 Some bitter/chemical aftertaste.
#586 Can taste honey!

#586 A little sour and bitter.

#378 Awesome!! Very good.

#913 Felt a few lumps.
#622 This was bland tasting.
#352 Has a few slight lumps.
#586 This was the thickest sample. I did not give it a 9 as that means " you could use a spoon" to drink it.

Table B.4. Trained panel comments rep. 1 day 28

#397 Yummy.
#246 Aftertaste.

#113 Strong honey flavor.
#246 Can taste some honey.

#113 Gritty.
#455 I taste honey again. Acid like.

#254 The sweetness is overbearing. Masks strawberry flavor.
#246 Very bland tasting.
#113 Slightly sour.
#847 Slightly grainy. Awful.
#744 Bad aftertaste.
#661 Slightly sour.

#254 Gross!

#661 Somewhat acid.

#661 Taste a little sour.
#113 Very honey taste - too intense.
#254 Too sweet but the intense wash out pretty easy.
#246 Too sweet.

#254 It has some kind of artificial/chemical flavor I cannot identify.
#744 Artificial/chemical taste again!

#254 Sweetness had an aftertaste. Too sweet to tell - sweetner over powered.

#847 Too runny - needs more texture.
#254 A lot of bubbles, made the texture strange.

Table B.5. Trained panel comments rep. 2 day 0

#117 Best of group.

#394 Gritty.
#937 Slight grit.
#117 Acidic. Slightly gritty.
#931 Gritty. Sour acid like.

#722 The sweetness is overbearing that it makes it difficult to taste strawberry flavor.
#937 Has a salty aftertaste.

#117 Strong honey flavor.

#931 This is gross!

#394 This was the thickest sample but drinkable. If it had been thicker where I needed to tap bottom of cup, I then would have rated as "9".
#185 Slight aftertaste.

#117 Taste less sweet and more bitter.
#394 Flavor very acidic and taste like old yogurt and bitter.
#885 Too much milky flavor

#937 Too sweet.

Table B.6. Trained panel comments rep. 2 day 14

#448 Bad smell.

#522 This is very disgusting.

#448 Very slight grit.
#291 Very slight grit.
#879 Acid and taste like honey. Slight grit. Can't taste (strawberry flavor intensity) over acid.
#522 Very slight grit. Hard to distinguish strawberry-acidic.
#989 Very sour.

#291 Tart.
#989 Slightly grainy.
#879 Sour.

#989 Aftertaste.

#656 Smooth but had a "souring" taste - like it may be going spoiled.
#989 This was the sweetest sample. I marked it "8" as it didn't pose an aftertaste.
#879 This was thickest sample but not so thick that you'd need to use a spoon, therefore I called it 8 instead of 9. Nine is for spoon-thickness. Starting to curdle and taste spoiled.
#448 Starting to have a "spoiled" taste and slight curding.

#291 A little sourness in the aftertaste.
#879 A little aftertaste of honey and bitter.
#448 Too honey.

Table B.7. Trained panel comments rep. 2 day 28

#893 Bad taste.

#644 Gritty.
#435 Has a slight sour aftertaste.
#863 Slight grit. Bitter.
#778 Very slight grit. Has sour aftertaste. Tasted a little like it was going bad (mold taste). Couldn't really get a good taste (sweetness). I was tasting mold or something.
#122 Very slight grit.
#122 Does have sour aftertaste.
#566 Gritty.

#644 Large lumps.
#893 Slightly sour.

#863 Tangy/sour.
#566 Can taste some honey also somewhat "tangy"/sour.

#644 Honey taste!
#566 Some artificial taste (strawberry flavor intensity)!
#863 Honey taste!
#893 Honey taste!

#893 Very tart.
#644 Slightly granular texture.
#435 Somewhat granular texture.
#863 Granular texture.

#893 Tastes a little too sweet.
#863 Too much honey flavor, too intense.
#644 Very chalky aftertaste.
#566 Too much milk solids and honey flavor. Chalky.

#644 This is one of the better tasting ones.
#257 Terrible!
#435 Tastes terrible!

Table B.8. Trained panel comments rep. 3 day 0

#256 Honey flavor.

#611 Can taste honey!
#622 Some honey taste.
#945 Need a spoon!!!

#256 Gritty.
#611 Gritty.
#824 Gritty - slight.
#622 Slight grit.
#945 Gritty.
#449 Slight grit.

#578 Less acid.
#824 Somewhat more acid than 578 and 622.
#622 Somewhat more acid.

#611 Yuck.
#622 Don't like it that thick.
#449 Aftertaste.

#611 Strange aftertaste.

#945 This was the thickest sample.
#763 In my opinion, the thinnest mixture but not water - very slight thickness. This was the sweetest of the set of 4 samples. I tended to get a sweet flavor with slight strawberry taste.
#622 This sample tended to be the thickest of the sets but not where one needs to used spoon to drink solution.

#449 A little sour.
#611 Too chalky and too thick to drink it.
#622 A little sour.
#945 A little chalky

Table B.9. Trained panel comments rep. 3 day 14

#563 Tangy.
#336 Can taste honey.
#188 Tastes tangy.

#919 Bitter.
#381 Not really lumpy but definitely texture to mouth.
#777 Sour aftertaste.

#563 Honey flavor!
#336 Honey flavor.
#452 Some unidentified - artificial flavor! (strawberry flavor intensity)
#381 Some artificial flavor! (strawberry flavor intensity)

#563 Sour aftertaste.
#336 Sour.
#299 Shake is starting to separate.
#381 Bland tasting.

#452 I like very much this sample just a little less sweet.
#381 Little flavor, I don't like it. I don't care for this sample.
#188 Too much honey flavor. Good flavor, just too sweet.

#919 This was the thickest sample of this set, but not as thick as one in prior set. Bland.
#777 A bit grainy tasting.
#563 Had very few slight chunks/bits.
#452 Tastes a little sour.

#381 Sick!
#777 Very good.

Table B.10. Trained panel comments rep. 3 day 28

#436 Tastes terrible. Too thick.
#747 Good taste.

#585 Sour.
#151 Bitter/sour. Very very slight grit.
#628 Bitter/sour. Gritty.
#974 Bitter.
#436 Sour.

#974 Can taste slight strawberry taste with sourness of product.
#436 Slightly granular.
#628 Granularly texture.
#151 On tart/tangy side. Slight granular texture.

#628 Need a spoon.

Figure B.11. Trained sensory panel end questionnaire responses

399 - By far the worst one. Terrible flavor and sweetness. Viscosity is alright but the taste ruins it. Not lumpy at all. Smoothness is good.

Not sweet but not tangy. A little bit too thin. Can't taste the strawberry flavor.

Better than 212.

Slightly too sweet. Texture good. Thickness could be very slightly thinner. Maybe a little more flavor.

More milky flavor than strawberry, a little too sweet.

I like the amount of strawberry flavor, maybe a little too sweet, and I like the consistency.

This sample is a bit sweet for my taste. The thickness and smoothness was good. The strawberry flavor could be just a little bit thicker.

Too sweet for my likes.

More strawberry - it's too sweet.

The sweetness and strawberry flavor are very close to my ideal. It can be a little thicker.

Good flavor, appropriately sweet, nice color, appropriately thick for a shake, very smooth, appropriate thickness.

I think the strawberry is good but it may be masked by the sweetener which is overbearing. Good smoothness but not enough viscosity.

212 - Tastes terrible. Thickness is good, but maybe a little better thicker. Strawberry flavor is mediocre, but flavor is bad.

Too thin (just a little). Good sweetness and strawberry flavor levels.

Very nice.

Texture good. Good thickness. Good strawberry. Good sweetness.

Nice strawberry flavor, a little too sweet, appropriate viscosity for a drinkable yogurt.

Too thin and watery, nice sweet taste, maybe a little more strawberry.

The sweetness kills the strawberry flavor. Needs to be just a little bit thicker.

Too sweet to have much taste.

Good - very close to ideal but still a little sweet.

It is too runny and a little too sweet for my taste. Good strawberry flavor.

Good color, good flavor, appropriately sweet and thick. Very smooth - good product.

Very good strawberry, sweetness and smoothness but lacks viscosity.

747 - Has good strawberry flavor. Very good sweetness. Not too sweet. Good thickness and viscosity. Maybe a little on the thick side.

Good thickness for a drink. Very smooth and creamy (not watery). Strawberry not as strong - seems too sweet.

Needs more strawberry and sweet.

Good thickness - but maybe could be slightly thinner. OK flavor - maybe slightly more. Needs to be slightly sweeter - has sour aftertaste. OK texture.

Not very strawberry, but overall I liked it. Good in sweetness and viscosity. Different mouthfeeling - almost like a film-forming.

Good - nice viscosity - I like the amount of strawberry flavor and sweetness.

The sour taste killed the strawberry intensity and sweetness. The thickness and smoothness were good!

Slight sour/spoilage taste - has most strawberry taste.

Very good product! It's perfect.

The sweetness and the honey flavor cover the strawberry flavor. Thickness is what you expect in yogurt drink.

Good flavor appropriate sweetness, good color, very smooth product, overall excellent product.

Needs more sweetener and viscosity. Strawberry is good as is smoothness. Has an aftertaste.

585 - Terrible flavor. Not sweet enough. Good thickness and viscosity.

Good thickness for a drink. Good level of strawberry flavor (not too strong). Slightly sweet.

Perhaps needs a little more sweet but otherwise good.

Good thickness. Good texture. Needs to be sweeter. Good strawberry.

Very similar to 747 but with some honey flavor.

Just a bit too watery, very sweet and maybe a little more strawberry - or the sweetness covers the strawberry.

This is my favorite! There is a good mixture of sweetness and strawberry intensity. The drink was smooth and not thick.

Aftertaste of spoilage.

A little sweet and too smooth.

It will be nice if it was just a little less sweet.

Good flavor, good sweetness, good color, very smooth. Overall excellent product.

Needs a bit more sweetness and strawberry. Also more viscosity. Smoothness is good. Has and aftertaste.

151 - Overall pretty good. A little too thick and viscous. Needs more sweetness and strawberry flavor.

Adequate strawberry flavor. Sweet (not too sweet - no other after taste). Slightly thick for a drink.

Same comments as 974.

Needs to be sweeter. Needs to be slightly thinner. I think OK strawberry, OK texture.

More milk and honey flavor than strawberry, appropriate sweetness and viscosity.

Too thick, needs more strawberry, I do not care for the flavor of the sweetness too much.

Difficult to taste strawberry flavor since the powdered milky taste is overbearing. A little too thick for me, but smooth tasting.

Slight aftertaste.

Too sweet but close to 747 (my ideal) a little thick also - for a drink.

Too sweet in flavor and strawberry flavor is too low. A little too thick.

Good flavor, appropriately sweet, very smooth, little bit more thick than 747, good color. Overall a very good product.

Very good viscosity. A little too much strawberry, very good smoothness. Needs to be sweeter.

974 - Terrible flavor. Not sweet enough. Too thick. Good viscosity.

Very smooth and creamy (not lumpy at all). Adequate thickness - (not too thick or thin). Moderate strawberry - (not too strong). Doesn't taste sweet slightly tangy.

Needs more flavor and sweetness.

Needs to be sweeter. Good texture. Maybe slightly thinner. Could need more strawberry flavor but hard to tell because it is bitter.

Not much strawberry flavor. It's in the units of viscosity for a drinkable yogurt.

Yuck, not enough strawberry, weird taste and too thick and chalky.

Too thick for me. Needs a greater strawberry flavor with a bit more sweetener. There is a slight sour aftertaste.

Tasting sour/spoilage with a slight strawberry taste.

Too thick and too sweet!

Too thick and too sweet!

Good flavor, appropriately sweet and very smooth. Overall a very good product.

Excellent product in all areas but maybe a bit too much strawberry.

628 - Sweetness and strawberry flavor are somewhat lacking. Way too thick and a little viscous.

Too thick. Not sweet enough or strawberry.

Real nasty (similar to 436 but worse).

Much too thick. Needs more flavor. Definitely needs to be sweeter. Texture needs to be less gritty.

Too thick, not enough strawberry flavor or sweetness.

Way, way too thick, has an aftertaste and seems bland, not full of sweetness or strawberry.

Nasty! Way too thick, no flavor, no sweetness! Would never buy this one!

Too thick and granular tasting for a drinkable yogurt shake.

Too thick!! Not enough strawberry.

Too thick, not enough sweetness and strawberry flavor. It needs a spoon due to thickness.

Way too thick for a drink otherwise very smooth product. Not very good overall.

Needs more strawberry and sweetness. Way too much viscosity. Smoothness is good. Overall poor.

436 - Way too thick. Terrible flavor. Sweetness is too little. Very viscous.

Too thick. Doesn't taste like strawberry or sweet enough.

Nasty, too thick and non-sweet.

Too thick - needs to be pourable. Needs to be sweeter. Not sure about strawberry flavor - the sour flavor masks that. Texture OK - could be smoother though.

Too thick for a drinkable yogurt, honey masks strawberry flavor.

Terrible, not sweet enough, no flavor and way too thick.

Way too thick. Strange taste, sort of tastes like powdered milk. I really don't like the taste of lumps either even if there is just a few.

Tart, thick and slightly granular, slightly sour tasting (spoilage).

Similar to 628 - this one was too sweet for the thickness.

Too thick, not enough sweetness and strawberry flavor. It needs a spoon due to thickness.

Way too thick for a drink - otherwise smooth texture. Not very good overall.

Too viscous which hurts ability to evaluate. Smoothness is good. Too sweet but strawberry is good.

Question #2 - Yes, but it is not something I would drink all the time. It would be good every once in a while, but too much for everyday.

No. I only eat yogurt occasionally (strawberry isn't my favorite).

Yes. It is a nice snack. If the price is comparable to yogurt (~ $ 0.50 for 6-8 oz.)

Yes. If you want yogurt but the tub and spoon are inconvenient. It tastes the same. Also would fill you up and taste better than the instant breakfast.

Yes, if price is reasonable.

Most likely, if it is low in calories and no fat, and offers some nutritional value.

Yes, I enjoyed the shakes when they weren't too thick, too sweet or too lumpy. However, I might wait a while, since I have had my fill on yogurt shakes.

It would depend upon shelf-life of product, whether it was too sweet or bland (no purchase) - would have to have a good strawberry flavor for purchase by me.

Yes - because I like yogurt but think that it is too thick sometimes. Also - a drink yogurt is more convenient and can be consumed "on the go" - takes less time.

Yes, it will be a nice snack or quick breakfast.

Yes, overall excellent product(s).

No, because calories are probably too high. However, based just on taste - I would buy the product.

Question #3 - No. I wouldn't know the benefits or drawbacks without learning more.

Yes and no. I know they are beneficial, but again, I only eat yogurt occasionally.

Probably not. I would buy it if I liked it and the price was good. If the price is high, I would just buy yogurt.

Not really - I don't think I could really notice any type of probiotic effect (from just one product eaten occasionally).

Yes, for health reasons.

Not a lot, but it would help - *L. acidophilus* is good for you.

It would not make a difference, since I know what could be in other dairy type products.

Yogurts already has a culture, so it would not affect my decision on whether to purchase a product or not.

No - I don't know what they are good for.

No, it will not make any difference what sort of culture is used.

Yes - fermented foods with viable cultures are good for you.

Yes, because of the potential health benefits.

Question #4 - Yes, but not in this product. Gives a distinct flavor, but not one that is better than sucrose.

I like the taste of honey and use it for baking, etc., but don't believe the nutritional value differs much from other sweeteners.

Doesn't matter to me although I've raised bees in the past.

I generally like the flavor of honey on cereal or tea - it brings out the flavor more than sugar without making it too sweet.

Yes, but it depends on how much honey has been used.

I don't care too much either way. Both are sugars - flavor is more important.

Yes, it doesn't make the shakes too sweet.

Yes - honey is a natural ingredient and not a chemical. I do feel that chemicals can cause forms of diseases/illnesses.

Yes - I really liked the honey taste because it was different and didn't leave a bad aftertaste.

No, or maybe in a small proportion. The taste of honey is too intense.

No particular preference.

If honey was used in all samples then yes for taste reasons, but I would be concerned about honeys replacement of sucrose if the product was to be used by children (microbiology of honey).

Question #5 - Toward the end of the study some of the samples were noticeably tangy or acidic which greatly altered the taste. Early in the study the taste - quality was much higher.

Strawberry and sweetness seem inseparable to me. Thickness and smoothness were not critical parameters for me although the thicker ones were also less sweet and strawberry. If the thick ones were still as sweet and strawberry, I would like them as much as the thin ones.

The thick samples were objectionable! I don't like powdered milk and I could taste it and it added grit to the texture and gave it a weird bitter taste especially at the end.

All the very thick yogurts were REALLY bad. In addition, most of the samples were too sweet.

No.

The really thick ones are SICK! and GROSS! But the ideal one was very good and I would definitely buy it.

During storage samples got to be too thick and have a little aftertaste.

I feel the quality of the products maintained very well during the duration of the study - I did not detect any quality deterioration from sensory aspects.

Has potential.

APPENDIX C

RAW RHEOLOGY DATA

Figure 33. Raw rheology data

Day 0	REP #1			REP #2			REP #3			AVG.		
sample	K(Pa s^n)	n(-)	R2	K(Pa s^n)	n(-)	R2	K(Pa s^n)	n(-)	R2	K(Pa s^n)	n(-)	R2
1S0	1.9684	0.4119	0.9944	2.3649	0.381	0.9924	3.4322	0.3616	0.9897	2.5885	0.38483	0.99217
2S0	2.0254	0.4632	0.9941	1.3071	0.45	0.9934	2.3699	0.37	0.9907	1.9008	0.42773	0.99273
1H0	2.0245	0.3963	0.9917	2.3404	0.3842	0.9919	4.9051	0.3268	0.9893	3.09	0.3691	0.99097
2H0	2.8975	0.3621	0.9925	2.8895	0.3626	0.9904	4.3931	0.3298	0.9919	3.39337	0.3515	0.9916
1H3	2.1875	0.4212	0.9816	2.4128	0.5098	0.9993	6.2975	0.3272	0.9864	3.6326	0.4194	0.9891
2H3	3.651	0.3669	0.9894	3.0537	0.4639	0.9996	6.2632	0.3179	0.9894	4.32263	0.3829	0.9928
1H6	5.8854	0.3667	0.9854	8.6813	0.3524	0.9905	12.571	0.3281	0.9873	9.0459	0.34907	0.98773
2H6	6.6803	0.364	0.9878	5.4392	0.362	0.9849	11.139	0.3277	0.9891	7.75283	0.35123	0.98727
Day 14 sample												
1S0	2.1915	0.3975	0.9934	2.5019	0.3711	0.9894	3.548	0.3594	0.988	2.74713	0.376	0.99027
2S0	2.2176	0.4624	0.9947	1.3623	0.4316	0.9909	2.5546	0.3689	0.9901	2.04483	0.42097	0.9919
1H0	2.1746	0.3862	0.9908	2.411	0.3803	0.9904	5.0888	0.3242	0.9864	3.2248	0.36357	0.9892
2H0	3.1341	0.3592	0.9917	2.8837	0.3637	0.9895	4.6573	0.3273	0.9881	3.55837	0.35007	0.98977
1H3	3.3335	0.3338	0.89	4.2326	0.3412	0.9889	6.1814	0.3272	0.986	4.5825	0.33407	0.95497
2H3	4.0866	0.37	0.987	4.6206	0.3364	0.9875	6.2823	0.3219	0.9887	4.9965	0.34277	0.98773
1H6	7.0663	0.3622	0.9859	8.7169	0.339	0.9888	12.586	0.3261	0.9872	9.4564	0.34243	0.9873
2H6	7.6202	0.3733	0.9875	5.1217	0.3603	0.9841	10.947	0.3253	0.9879	7.8963	0.35297	0.9865
Day 28 sample												
1S0	2.0754	0.3919	0.9932	2.4601	0.3766	0.9893	3.2503	0.3584	0.9871	2.59527	0.37563	0.98987
2S0	2.2025	0.464	0.9934	1.2968	0.4366	0.993	2.2511	0.3763	0.9892	1.9168	0.42563	0.99187
1H0	2.1207	0.3847	0.9908	2.3404	0.3779	0.9883	4.7318	0.3229	0.9849	3.0643	0.36183	0.988
2H0	2.906	0.3613	0.992	2.6942	0.369	0.9895	4.1145	0.3279	0.9866	3.23823	0.35273	0.98937
1H3	2.5972	0.4245	0.9907	4.2488	0.3434	0.9883	5.8701	0.3291	0.9853	4.2387	0.36567	0.9881
2H3	4.089	0.3678	0.987	4.0532	0.3456	0.9876	5.6	0.3185	0.9864	4.58073	0.34397	0.987
1H6	7.2308	0.3615	0.9856	17.031	0.3405	0.9865	11.17	0.3234	0.9862	11.8106	0.3418	0.9861
2H6	7.5207	0.36	0.9877	8.6975	0.3723	0.9848	10.205	0.3212	0.9889	8.80773	0.35117	0.98713

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