DEVELOPMENT OF A SYNBIOTIC DRINKABLE YOGURT FOR SCHOOL-AGED CHILDREN

by

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Abstract

Fermented dairy products have long been associated with positive health benefits. Certain probiotics, such as *Lactobacillus rhamnosus*, have gained popularity as a natural way to improve health and immunity. Fermented dairy products are an easy way to incorporate and deliver probiotics to the consumer. Synbiotic systems are also being investigated as a method for promoting the growth and survival of probiotic bacteria, both during fermentation and after consumption. The purpose of this research was to develop a synbiotic yogurt drink, incorporating *L. rhamnosus* (probiotic) and inulin (prebiotic), and to investigate the effect of the synbiotic relationship on sensory, physical, chemical, and microbiological properties over time.

Yogurt drinks containing the probiotic *L. rhamnosus* HN001 (treatment P), inulin (treatment I), and a synbiotic system (treatment S) were tested using a randomized block design. Color, viscosity, brix, syneresis, and pH were measured throughout a shelf-life of 28 days. Consumer acceptability was also tested with middle-school aged children.

A shelf-life of 28 days at refrigerated temperature was found to be acceptable for the product based on chemical, physical, and microbial analysis. Based on sensory results, the synbiotic drink was found to be acceptable to the target consumers (scoring a 6.82 in “overall liking” on a 9-point hedonic scale), though the treatment with 3% inulin scored slightly higher in most categories (scoring a 7.24 in “overall liking” on a 9-point hedonic scale).

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Chapter 1 - Review of Literature

Introduction

Health and longevity were credited to the consumption of fermented milk products as early as 76 BC, when the Roman historian Plinius recommended fermented milks for treatment of gastrointestinal discomfort (Schrezenmeir and de Vrese 2001). In 1906, Eli Metchnikoff published *The Prolongation of Life,* in which he hypothesized that lactic acid bacteria in the gut improved health (Kroger and others 1992).

Consumers still associate cultured dairy products with health benefits. Yogurt, Greek yogurt, and kefir are commonly found in grocery stores across the United States. These products are perceived as healthy due to nutritional value and the presence of live active cultures. Drinkable yogurt and yogurt smoothies have emerged as an alternative way to consume yogurt. Often, these products contain additional probiotic cultures.

The term "probiotic" was used by RB Parker in 1974 to describe "organisms and substances which contribute to intestinal microbial balance." This was the first documented use of probiotics describing "good bacteria" that promote intestinal health.

Probiotics are now defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host," and when discussing probiotics in food (such as yogurt or other cultured dairy products): "live microorganisms which when *consumed* in adequate amounts *as part of a food* confer a health benefit on the host" (FAO and WHO 2001).

A prebiotic is "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid 1995). Prebiotics, in general, are dietary fibers which humans cannot digest, but certain bacteria can. This additional food source can selectively promote these bacteria.

Synbiotic, which implies synergism, is applied when probiotics and prebiotics are used in conjunction with one another. The synbiotic relationship between probiotics and prebiotics may help facilitate probiotic population of a human's digestive system. In order for a food to be considered synbiotic, it must have a prebiotic that directly benefits one or more of the probiotics present in the food (Schrezenmeir and de Vrese 2001). For example, the soluble fiber inulin has been found to have a prebiotic effect on the probiotic *Lactobacillus rhamnosus* (Boeni and Pourahmad 2012).

The goal of this study was to develop a synbiotic yogurt drink for children containing inulin and probiotic bacteria including *Lactobacillus rhamnosus*, certain strains of which have demonstrated immune-system enhancing effects in clinical studies, including studies on children in daycare and pediatric wards.

Definitions

Yogurt

Yogurt has a standard of identity which can be found in 21CFR131.200 – "Yogurt is the food produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk, or skim milk) with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophiles* … All ingredients used are safe and suitable. Yogurt … contains not less than 3.25 percent milkfat and not less than 8.25 percent milk solids not fat, and has a titratable acidity of not less than 0.9 percent, expressed as lactic acid. The food may be homogenized and shall be pasteurized or ultra-pasteurized prior to the addition of the bacterial culture. Flavoring ingredients may be added after pasteurization or ultra-pasteurization." Other optional ingredients that may be used in yogurt include nutritive carbohydrate sweeteners, flavoring ingredients, color additives, and stabilizers.

Drinkable yogurts do not meet this standard because they have lower solids than required. Therefore, they cannot be called yogurt.

21CFR131 Subpart B also contains standards of identity for acidified milk, cultured milk, low-fat yogurt, and nonfat yogurt in sections 131.111, 131.112, 131.203, and 131.206. However the product being developed in this study does not meet the standards for any of these, therefore use of any of these common names on the front label would not be legal.

Flavored Dairy Drink

Certain states have initiated their own definitions for dairy drinks. According to the New York State Statutory Authority: Agriculture and Markets Law Part 17, flavored dairy drinks are "pasteurized or ultra-pasteurized product in liquid form containing not more than two percent milk fat and not less than eight percent milk solids not fat." Water is included as an optional ingredient, along with "milk, cream, skim milk, concentrated milk, condensed skim milk or nonfat dry milk or other milk derived ingredients to increase the nonfat solids content of the food; provided, that the ratio of protein to total nonfat solids of the food, and the protein efficiency ratio of all protein present, shall not be decreased as a result of adding such ingredients." Other optional ingredients include "safe and suitable" flavors, sweeteners, colors, emulsifiers, and stabilizers.

Though cultures are not mentioned, the drinkable product does meet standards for milk fat, milk solids, and added ingredients. Similar drinks on the market, such as Danone's DanActive®

and Activia® drinks that contain probiotics, use “probiotic dairy drink” or “dairy drink” as the common name on the front label.

Therefore, it seems that the most appropriate common name for this synbiotic, drinkable product is “dairy drink,” though this is not a food standard found in 21 CFR. “Yogurt drink” may also be an appropriate term because it indicates that the primary ingredient is yogurt, but it is a drinkable product that does not meet the standards for low-fat yogurt (because water is added).

Probiotic, Prebiotic, and Synbiotic

Probiotic claims can be made on food packaging in the U.S. provided there is an acceptable amount of viable culture in the product. Companies are not allowed to make specific health claims, but they can claim that the product “contains probiotics.”

However, the EU Nutrition and Health Claims Regulation (NHCR) considers the term “probiotic” a health claim, and it is therefore not allowed on product labels (Neiburg 2013). They do not feel that there is enough evidence to support that consuming probiotics has a direct impact on the health of an individual.

In 2010, the Dietary Guidelines for Americans Committee (DGAC) appraised systematic reviews of studies over probiotics, prebiotics, and health (Brownawell and others 2012). They found insufficient evidence to make dietary recommendations for Americans concerning probiotics or prebiotics, though they did recognize that there could be potential health benefits. The DGAC also felt that since prebiotics are dietary fibers, the daily recommended intake of fiber should help incorporate some prebiotics into the diet without specific recommendations for prebiotics.

Currently, there are no regulatory definitions for "synbiotic yogurt" or "synbiotic systems" in 21 CFR. These may be defined in the future, though defining synbiotics could be a challenge because synbiotic systems can be used in chocolate, non-dairy fermented beverages such as Mageu (though this is not popular in the U.S.), as well as ice cream, meat products, and cereal products (Nyanzi and others 2010, Cruz and others 2010).

Live and Active Cultures

Live and active cultures are bacteria within a fermented product that are viable at the time of consumption. If a product states that it contains live and active cultures, that means that it should have live, functioning bacteria – capable of forming colonies on media plates or reproducing when placed in their ideal growth environment (for instance, milk at 43°C), until the end of that product's stated shelf life.

The National Yogurt Association (NYA) Seal may be used on yogurt products containing live and active cultures for a fee of $2,500/year, if the product meets the criteria set forth by the NYA. In order for the NYA Seal to be used on the label of a product, it must be fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles*. The total viable count of *L. bulgaricus* and *S. thermophiles* at the time of manufacture must be 10^8 CFU/g. The cultures must be active at the end of shelf-life.

The NYA Seal only requires there be live and active *L. bulgaricus* and *S. thermophiles*. Probiotics such as *L. rhamnosus* are not included in the bacterial counts. The NYA seal may still be desired on probiotic yogurts, though it is not an indicator of live and active probiotic bacteria.

Current Trends

According to the Specialty Food Association (2013), yogurt consumption in America is on the rise. Yogurt sales, including spoonable, drinkable, and frozen categories, were up 17 percent from 2010 to 2012. Of the $7 billion in sales in 2012, drinkable yogurt sales accounted for $422 million. Though this is down slightly from 2010, sales were expected to rebound in 2013 due to "strong performance of drinks with innovative flavors and kid-friendly packaging."

Prepared Foods Network (2012) looked into trends in yogurt and yogurt drinks. They gave a nod to Wallaby® Organic Joey Yogurt – an Australian-style yogurt for children that is high in protein, calcium, vitamin D, and contains live and active probiotic cultures. Other noted products had very few ingredients listed on the label. This "less is more" concept meets the consumer demand for clean-labeling.

Yogurt products containing probiotics or fiber fortification were expected to drive sales in 2013, due to surveys which indicated than many consumers (24 percent of survey responders) who purchased yogurt in 2012 did so to improve digestive health (Specialty Food Association 2013). The survey also indicated that women and households with children tend to consume more yogurt per month than other segments, demonstrating the potential for growth in kid-friendly products.

Some parents are jumping on the probiotic bandwagon. Probiotic cultured products for kids are emerging to meet this demand. Lifeway® (2014) now makes a probiotic kefir line called ProBugs™ with flavors like "Goo-Berry Pie," "Orange Creamy Crawler," "Sublime Slime Lime," and "Kiwi-Kale Kritter." These products come in kid-friendly squeeze pouches that feature brightly-colored animations of the bacteria. The product seems to be going for the "gross-out" factor in order to get the attention of kids and make drinking kefir a more enjoyable experience. For the parents, ProBugs™ are certified organic, and boast "seven to ten billion CFU's of 10 live and active cultures (friendly bacteria) per cup." Lifeway® claims that all of their kefir products have "twelve different live and active cultures that help promote myriad health benefits."

Stonyfield Organic™ makes a probiotic yogurt smoothie for kids. Their website says, "YoKids sMOOthies are made with real fruit and veggies for your real kid." Their "Very Berry" flavor contains 80 calories per 8 oz. serving with 10 calories from fat. It is high in potassium (170 mg/serving). However, it contains 0g of dietary fiber, and it is rather high in sugar at 13g per serving. It has only 3g of protein per 8 oz serving, which is quite low for a yogurt-based product. Ingredients (all organic) include milk and sugar, sweet potato puree, raspberry and strawberry puree, carrot juice concentrate, and tapioca starch. Live and active cultures include *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, *Bifidus*, and *L. rhamnosus.*

Recent Research

Probiotics

The idea behind probiotics is that one can alter their indigenous gut flora by consuming bacteria that will infer various health benefits. Research on the health benefits of probiotics for humans is somewhat difficult to conduct because it requires subjects to consume a prescribed amount of yogurt on a daily basis for long periods of time. Researchers must choose which probiotic, or combination of probiotics, to investigate. There are many health claims associated with probiotics, and researchers must narrow the field for certain claims. There are numerous strains of bacteria that are currently classified as probiotics. Some are included in Table 1.1.

Benefits of regularly consuming adequate amounts of probiotics may include: improved immunity and decreased susceptibility to infection, improved digestion, partial relief from lactose intolerance symptoms, relief from constipation and diarrhea, relief from conditions such as irritable bowel syndrome, and cancer prevention through the inhibition of carcinogens (Sanders 2000, Schrezenmeir and de Verse 2001).

Probiotics have also been investigated as an aid in weight loss and management. Human studies on obesity and gut microbiota have shown that individuals who suffer from obesity (and associated diseases such as type 2 diabetes, cardiovascular disease, and fatty liver disease) have a higher proportion of *Firmicutes* and a lower proportion of *Bacteriodetes* (a category which includes *Bifidobacteria*, commonly used in probiotic yogurts) than do healthy weight individuals. More research is needed to determine whether oral administration is a viable option to aid in weight loss and maintenance (Blaut and Bishoff 2010).

Lactobacillus rhamnosus

Children are a group of special interest for probiotic studies. Children who are cared for in a daycare or public school setting are more susceptible to illness than children who are cared for at home or in a small group setting (Hatakka and others 2001). This is presumably due to the higher level of exposure in a larger group setting. This results in missed work days for the parents, who have to stay home and care for their sick children; more doctor's visits, greater use of antibiotics for treating secondary illnesses; as well as greater health care costs (Hatakka and others 2001). Therefore, probiotics have been investigated as a potential means for improving immunity of children in group care settings, and subsequently preventing illness.

Studies have shown the potential of the probiotic *Lactobacillus rhamnosus* for improving immunity in children. Hatakka and others (2001) conducted a study with over 500 participants (age 1-6) in 18 daycare centers in Helsinki, Finland. In this double-blind, randomized trial, children were given either 1% milk, or 1% milk supplemented with 105/ml *L. rhamnosus* GG three times a day, five days a week, for 7 months (over flu season). The study found that *Lactobacillus rhamnosus* GG did not reduce instances of respiratory infections, though it did significantly reduce the number of sick days, doctor's visits, and antibiotic treatment for secondary infections.

In a similar study by Kumpu and others (2013), it was concluded that *Lactobacillus rhamnosus* GG significantly reduced the number of days children were sick with respiratory illness, though it did not significantly decrease the likelihood of a child becoming ill, or the severity of symptoms during the time of illness.

In another study by Hojsak and others (2010) *Lactobacillus rhamnosus* was investigated as a means of preventing nosocomial infections in children admitted to pediatric hospitals. A nosocomial infection is one that occurs after the child is admitted to the hospital for care. These infections can lengthen the hospital stay and significantly increase the cost of hospital care for these patients. Study participants were either given a fermented milk with 109 *L. rhamnosus* GG, or an identical product without the *L. rhamnosus* once daily. In this randomized, double-blind, placebo-controlled clinical trial, there were 376 participants in the *L. rhamnosus* GG group, and 366 participants in the placebo group. It was found that participants who received the placebo had a 2.89 times higher risk of contracting a nosocomial gastrointestinal infection, and a 3.17 times higher risk of developing a nosocomial respiratory tract infection.

Other strains of *L. rhamnosus* have also demonstrated potential to enhance the immune system. In a study by Sheih and others (2001), strain HN001 enhanced mononuclear cell and polymorphonuclear cell phagocytic activity (anti-microbial response), as well as "natural killer cell" (NKC) killing activity (elimination of viral-infected cells) in middle-aged and elderly study participants. This study also points to possible mechanisms for the immune-enhancing effects of *L. rhamnosus* species demonstrated in other clinical studies.

In Vivo Survival

Nearly all lactic acid bacteria can help improve the digestion of lactose in individuals. This is largely thought to be the activity of microbial β-galactosidase, the enzyme responsible for breaking down lactose (Martini and others 1991). However, these bacteria are not necessarily considered probiotics. *L. bulgaricus* and *S. thermophiles*, the required bacterial cultures for yogurt production, are not considered probiotics because they do not survive digestion and are unable to colonize the human gut.

Studies over in vivo survival of probiotics are expensive to conduct and require human subjects who are willing to participate. This, combined with little regulatory authority in the supplement industry, has led to probiotic claims for bacteria that are not known to survive digestion and flourish in the human gut.

However some studies have shown certain bacteria are able to do so when consumed in adequate amounts over a long period of time. In a study by Jacobsen and others (1999), 47 strains of *Lactobacillus* spp. including *L. acidophilus, L. rhamnosus, L. paracasei, L. reuteri*, and *L. casei* species were evaluated for their ability to survive in vitro. Viable cells were evaluated on several criteria during in vitro studies, such as adhesion (ability to attach to intestinal wall), antimicrobial activity against pathogenic bacteria, and pH and bile tolerance. These in vitro tests were designed to mimic digestion as closely as possible. Five strains were then selected from the 47 for in vivo trials.

In the in vivo trials, 12 healthy men between the ages of 18 and 37 years of age, were administered cultures for 18 days, followed by a washout period of 17 days (35 days total). Fecal samples were collected and examined at days 0, 18, 23, and 29. *L. rhamnosus* 19070-2, *L. reuteri* DSM 12246, and *L. rhamnosus* LGG were most frequently found in fecal samples. These strains performed quite well in the in vitro studies as well, indicating that adhesion, pH, and bile tolerance are important factors in the survival of bacteria to populate the intestines. Thus, these tests can be, and often are, used as methods for determining the potential of probiotic bacteria to survive digestion and populate the intestines.

Prebiotics

Prebiotics are dietary fibers that have some health benefits on their own without being paired with probiotics. Dietary fiber adds "roughage," which decreases instances of constipation, and provides a feeling of fullness without added calories. It helps the body eliminate toxins quickly, decreases instances of colon cancer, and boosts the body's natural defenses. It also helps decrease fat absorption in the small intestine, and helps lower blood cholesterol (Niness 1999).

Humans cannot digest prebiotics because they are fibers. Many microorganisms have the enzymes required to break them down. For example, humans cannot digest the fructan inulin, which is present in rye, Jerusalem artichoke tubers, and garlic. However, *Bifidobacteria* spp. can digest these fructans. In fact, when fructans are ingested regularly, *Bifidobacteria* populations increase significantly (Cseke and others 2006). Some *Bifidobacteria* spp. have been associated with improved glucose tolerance and insulin sensitivity which are factors in the development of Type II diabetes (Blaut and Bishoff 2010).

Added prebiotics have an impact on the flavor and texture of a product. Prebiotics can re-enforce molecular bonds and attractions within the food, and infer a creamy texture that makes it ideal as a fat-replacer (Cruz and others 2010). Inulin can add thickness and alter rheological properties to mimic higher fat content, as well as contribute to perceived smoothness and creaminess of low-fat and nonfat dairy-based products (Meyer and others 2011). In a study by Tarrega and Costell (2006), it was found that “adding inulin to fat-free dairy model desserts increased sweetness, thickness and creaminess.” Though flavor release seemed to vary, the inulin did improve the acceptability of fat-free samples of a starch-based dairy dessert.

Prebiotics vary in length and branching. Short-chain inulins are more soluble and have a sweeter taste than long-chain or native inulins (Tarrega and others 2010). These inulins are more quickly fermented by bacteria (such as probiotics in the gut) than long-chain inulins. On the other hand, long-chain inulins impart a thicker texture and higher water-holding capacity than short-chain inulins (Tarrega and others 2010).

In non-fermented dairy beverages, short-chain inulin is perhaps the better option. In a study by Villegas and others (2010), it was found that short-chain inulins had higher acceptability by consumers than long-chain inulin in a vanilla-flavored low-fat milk beverage. This is mainly attributed to the additional sweetness imparted by the short-chain inulin which seemed to enhance the vanilla flavor. It was found that thickness and sweetness perception were the factors that had the greatest influence on consumer acceptance.

Aryana and McGrew (2007) conducted a study over the use of prebiotics of various chain lengths in yogurt, and found that the short-chain prebiotics were fermented faster than the long-chain prebiotics, which lead to a more rapid decrease in pH, especially 24 hours after fermentation.

The use of long-chain prebiotics resulted in decreased syneresis, which results from the water holding capacity of long-chain prebiotics.

Long-chain inulins and other oligosaccharides can have a fat-like impact on the texture of dairy products. In a study by Tearrega and others (2010) short and long-chain inulins were investigated in low-fat custards for their effect on rheological and sensory properties. They found that the use of inulin blends “enhanced product flavor intensity and thickness” in the custard sensory trials.

Gussisburg and others (2009) studied inulin at various levels in low-fat yogurt, comparing it to a full-fat yogurt. Their research indicated that the gel structure was “supported” by the inulin, which improved the gel strength. However, they did not find that consistency and “creaminess” were comparable between full-fat and low-fat yogurt with 4% inulin added. Perhaps adding inulin at a higher percentage would have improved sensory perception of the low-fat yogurts. Mouthfeel is not the only barrier in fat replacement. Flavor is also important, and adding inulin at a higher level may have negatively impacted the flavor of the yogurts.

Kip and others (2006) also studied inulin in low-fat yogurt. They found that the addition of inulin significantly impacted the attributes of “thick,” “airy,” and “sticky” which were determined to positively affect creamy mouthfeel of the low-fat yogurt. Samples with 3% inulin performed best in the study, but it was recognized that 3% might not be the “optimum” usage rate for improving creamy texture (2.5 or 3.5% may have performed better had these been tested). They also found that some types of inulin performed better than others. For instance, short-chain native inulin did not perform as well as long-chain modified inulin at improving creamy texture attributes in the low-fat yogurt.

Synbiotic Systems

Inulin and probiotics have been used to make synbiotic yogurt in a laboratory setting. Boeni and Pourahmad (2012) found that the addition of inulin decreased syneresis, increased viscosity, improved flavor and texture, and improved viability of the probiotic strains *Lactobacillus acidophilus* and *Lactobacillus casei.*

Prebiotics have shown potential to improve the viability of certain probiotics over time (Hekmat and others 2009). In the previously discussed study by Aryana and McGrew (2007), it was found that long, medium, and short-chain prebiotics all increased the viable cell counts of probiotic bacteria in the study, both initially and during refrigerated storage.

Allgeyer and others (2010) incorporated prebiotics and probiotics into a vanilla-flavored fat-free yogurt drink. The prebiotics tested included soluble corn fiber, polydextrose, and chicory inulin. The probiotics used in the study were *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* LA-5. They found that the addition of the prebiotics affected the drink's sensory properties as expected: all three increased viscosity, and the inulin increased sweet taste perception. Though the polydextrose mildly improved shelf life, the viability study concluded that there was a 2- to 3-log reduction in viable cells in yogurt drinks with or without added prebiotics, after 30 d of refrigerated storage. This indicates that none of these prebiotics are good for extending the viability of *L. acidophilus* or *B. lactis* over shelf-life.

Gonzalez and others (2011) also investigated the sensory properties of yogurt drinks containing prebiotics and synbiotics. Their experiment compared descriptive and consumer sensory tests in yogurt drinks made with whole and skim milk containing a fructooligosaccharide (prebiotic), and a fructooligosaccharide with *Lactobacillus acidophilus*. They found that the skim milk samples had a higher viscosity, and they believed it was due to the higher protein content of the drink, which created a more stable gel matrix. The descriptive panel did find differences between all samples. Perhaps most importantly, they found that the sour tastes were higher in the synbiotic samples. This was reflected in the consumer tests, in which the samples perceived as "sweeter" received higher preference scores. The more "sour" synbiotic samples had lower consumer acceptability scores, so the study concluded that "inclusion of a synbiotic had a negative impact on the acceptance by the consumers, with the skim milk beverage containing synbiotic being the least acceptable." This study utilized the prebiotic fructooligosaccharide, and the probiotic *L. acidophilus*. There is room for more studies over different probiotic/prebiotic combinations in fermented dairy beverages, such as *L. rhamnosus* (probiotic) and inulin (prebiotic).

In the study, the prebiotic and probiotic culture were added after yogurt fermentation. This may have had an impact on final solubility of the prebiotic or other sensory factors. Addition of the prebiotic at this stage would not work in an industrial setting. The prebiotic would have to be added either as part of a fruit prep or prior to yogurt fermentation in order to ensure safety of the final product. Raw ingredients can introduce microorganisms, so powders must be added to liquid to form a yogurt mix, and then pasteurized to kill potentially harmful bacteria.

Processing and Technology

Ingredients

The main ingredients used in the manufacture of yogurt are milk, additional dairy ingredients (such as cream and dried or concentrated milk products), sweeteners, and culture. Flavors, colors, and fruit preparations may be added. *Probiotic cultures* and *prebiotic fibers* are what make synbiotic yogurts different from traditional yogurts. Consequently, these are important ingredients in the manufacture of synbiotic yogurt products.

High quality milk and cream should be used for the manufacture of synbiotic yogurt (Tamime and Robinson 2007). Synbiotic yogurts may be non-fat, low fat, or whole milk products.

Dairy ingredients that may be used are defined in 21 CFR 131.20 (c) and (d) (1), include dried and concentrated milk ingredients, buttermilk, isolated dairy proteins, and lactose. Nutritive carbohydrate sweeteners (mentioned 21 CFR 131.20) may be used, as well as high intensity sweeteners if a reduced calorie product is desired. Flavors and colors are added after fermentation, so they must be ready-to-eat (RTE), and good manufacturing practices (GMPs) should be followed to ensure the yogurt is not contaminated or adulterated when flavoring agents are added.

Fruits are typically processed into fruit preparations, or "fruit preps," that are received in large totes. These are commercially sterile and should not cause contamination of the final product. However, it is important to ensure that pumps are sterile and GMPs are followed when handling cooked product such as fruit prep.

Bacterial Cultures

The lactic acid producing cultures of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* must be used in the manufacture of yogurt. Additional probiotic bacteria are added in synbiotic yogurts. Common probiotics added to synbiotic yogurts include various *Bifidobacterium* and *Lactobacillus* sp. such as *B. breve, B. bifidum, B. infantis, B. longum, B. lactis, L. acidophilus, L. casei, L. lactis,* and *L. rhamnosus* (Shin and others 2000, Boeni and Pourahmad 2012) (Table 1.1).

Table 1.1 Common Probiotics, Perceived Health Benefits, and Associated Prebiotics

Probiotic	Perceived Health Benefits	*Associated Prebiotics
Lactobacillus acidophilus	Improved immunity, relief from diarrhea, improved cholesterol levels, relief from lactose intolerance symptoms, anti-carcinogenic effects (Aryana and others 2007).	Oligofructose (from sucrose), Inulin (Kolida and others 2002)
Lactobacillus casei	Improved digestive health – namely duration and instances of diarrhea, especially in children (Pedone and others 2000).	Oligofructose (from sucrose), Inulin (Kolida and others 2002)
Lactobacillus rhamnosus	Improved immunity (Gill and others 2001), treatment/prevention of eczema in infants (Wickens and others 2008), and improved symptoms of diarrhea (Basu and others 2009).	Oligofructose (from sucrose), Inulin (Kolida and others 2002)
Bifidobacterium lactis	Enhanced cellular immunity (Gill and others 2001, Chiang and others 2000), may reduce symptoms in celiac patients (Lindfors and others 2009).	Oligosaccharides (Chiang and others 2000)
Bifidobacterium breve	Relief from constipation (esp. in children) and inflammatory bowel disease (Tabbers and others 2011, Jeon and others 2012).	Inulin, oligofructose, galacto-oligosaccharides (Kolida and others 2002)
Bifidobacterium bifidum	Improved cholesterol levels (Rasic and others 1992), improved symptoms of diarrhea (esp. in infants) (Saavedra and others 1994).	Inulin, oligofructose, galacto-oligosaccharides (Kolida and others 2002)
Bifidobacterium longum	Relief from inflammation due to ulcerative colitis (Furrie and others 2005), may have an inhibitory effect on carcinogens (Reddy and Rivenson 1993).	Inulin, oligofructose, galacto-oligosaccharides (Kolida and others 2002)

Prebiotic ingredients

Prebiotics are soluble fibers that must be isolated or transformed from their native form in order to be used in products like yogurt. Lactulose, galactooligosaccharides, soybean oligosaccharides, glucooligosacchariedes and xylooligosaccharides are all non-digestible oligosaccharides that have shown prebiotic effects in synbiotic systems.

Prebiotics have been used as functional ingredients in other products because they are water-soluble, sweet-tasting, add few calories, and aid in water-binding and gelling (Boler and

Fahey 2012). Their water binding capacity is also what makes them ideal for yogurt – they may reduce or eliminate the need for stabilizers.

In order for the system to be synbiotic, the prebiotic chosen must promote the growth of one or more probiotics in the yogurt (Table 1.1). Most *Bifidobacterium* spp. and *Lactobacillus* spp. are promoted by the use of inulin and oligofructose.

Prebiotic Manufacture

In synbiotic food production, the most commonly used prebiotics are inulin, oligofructose, and resistant starch (Cruz and others 2010). Prebiotics which are naturally occurring must be isolated from their food system. Other prebiotics must be manufactured through various processes, which are outlined in Figures 1.1, 1.2, and 1.3).

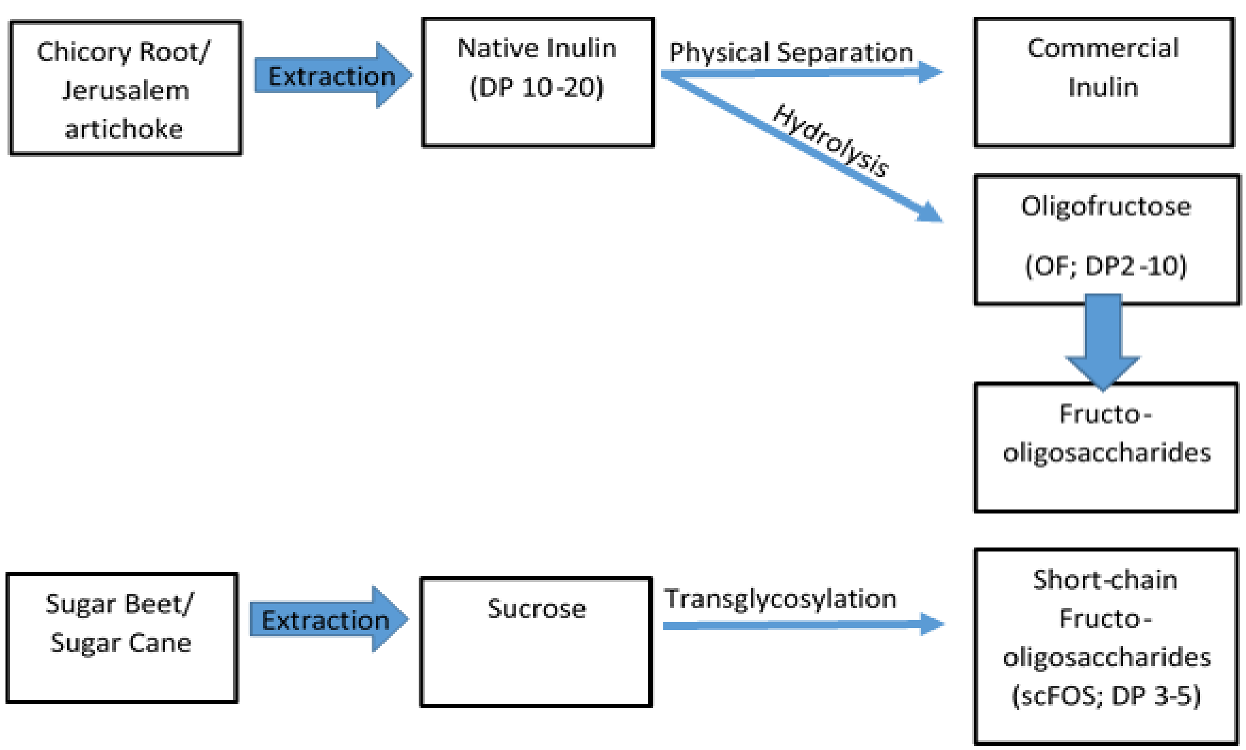

Figure 1.1 Manufacture of various prebiotics from chicory root/Jerusalem artichoke and sugar beet/sugar cane (adapted from: Boler and Fahey 2012)

According to Boler and Fahey (2012), inulin, oligofructose, and fructooligosaccharides can all be isolated from chicory root or Jerusalem artichoke through extraction, physical separation, and/or hydrolysis (Figure 1.1). Short-chain fructooligosaccharides can be created through the transglycosylation of sucrose from sugar cane or sugar beets (Figure 1.2). Xylooligosaccharides can be isolated through the hydrolysis of the xylans found in corn cobs (Figure 1.2).

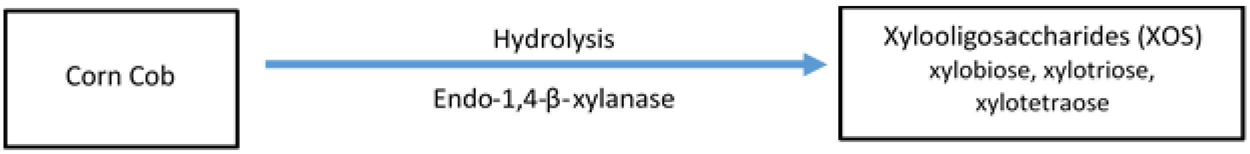

Figure 1.2 Manufacture of prebiotic xylooligosaccharides from corn cobs (adapted from: Boler and Fahey 2012)

Lactose-derived prebiotics (Figure 1.3) are less common in commercial production. However, this is a good way to utilize lactose, which is generally considered to be the least valuable component of cow's milk. The prebiotic lactulose is a disaccharide composed of two lactose molecules that is produced by alkali isomerization of lactose (Boler and Fahey 2012). Lactosucrose is a short-chain oligosaccharide composed of lactose and sucrose, which can be manufactured by transglycosylation of lactose and sucrose to form a single molecule.

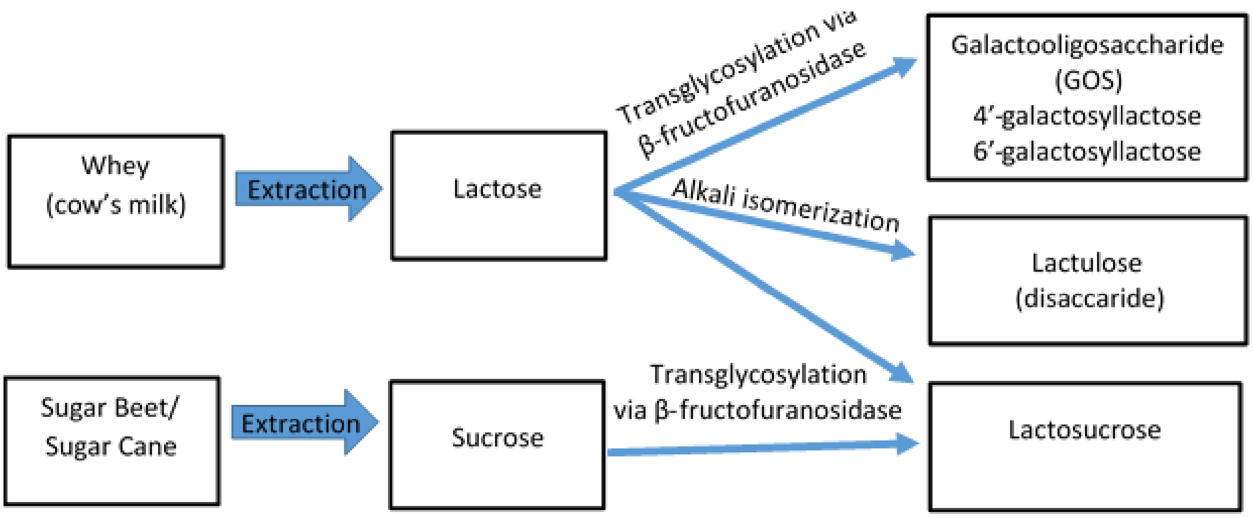

Figure 1.3 Manufacture of prebiotics from lactose and sugar (adapted from Boler and Fahey 2012)

Synbiotic Yogurt Manufacture

Synbiotic yogurt is manufactured identically to traditional yogurt. The first step in yogurt manufacturing is formulation and blending. All ingredients except colors and flavors are blended together, pasteurized at 80-85°C for 30 min or 95°C for 10 min, homogenized at 2000 to 2500 psi, and cooled to approximately 42°C (Cornell University 2014).

In batch fermented yogurt, starter culture is added to the mix and the batch is fermented in a large tank. Once the pH reaches 4.6, the yogurt is simultaneously cooled and pumped into a filler, which mixes in any flavors or fruit preparations, and distributes the yogurt into cups or tubes. The packaged yogurt is stored and distributed at refrigerated temperatures (Figure 1.4).

Equipment required for the manufacture of yogurt on a large scale includes: blending/mixing tanks for raw milk and ingredients, HTST (high temperature short time) pasteurizer, homogenizer, fermentation tanks, filling, and packaging equipment.

There are certain crucial steps in the manufacture of synbiotic yogurt. These include blending, pasteurization, and fermentation. The blending step is very important in synbiotic yogurts. Prebiotics must be properly mixed and hydrated, or they could gel and create lumps in the mix. This is not just undesirable to the consumer. Lumps of gelled ingredients can damage expensive machinery. Heat exchange plates have narrow spaces between them – clogging them could severely damage an HTST (high temperature short time) pasteurizer, and hold up production.

Air incorporation is not desired in the mixing step, because most yogurt flavor compounds can only develop under anaerobic conditions. Air incorporation it is hard to avoid at high mixing speeds. It is more beneficial to mix the yogurt for a longer period of time at a lower shear or mixing speed, prior to pasteurization.

The pasteurization of synbiotic yogurt mix is more aggressive than milk pasteurization - 80-85°C for 30 min or 90°C for 5 min versus 72°C for 15 sec. This is done to help improve the texture and body of the yogurt, explained for fully later in this chapter. Additionally, pasteurization helps solubilize the prebiotic powders that must be added to synbiotic products.

Yogurts are ideally fermented at 45°C because this speeds production without having a negative impact on flavor and texture (Radke-Mitchell and Sandine 1991). Some probiotics may require lower fermentation temperatures around 37-43°C (Tharmaraj and Shah 2003). Lower fermentation temperatures will increase fermentation time, which is undesirable in large plants because it slows production.

Synbiotic Yogurt Drink Manufacture

Once the yogurt has fermented to pH 4.6, the yogurt can be stirred, breaking the gel structure. At this time, water or fruit juice can be added to the yogurt to make it drinkable. Colors, flavors, fruits, and juices may also be added as the drink is pumped through the filler. The flow diagram (Figure 1.4) demonstrates the complete process of manufacturing a synbiotic yogurt drink.

Less water can be added at the final mixing step if the gel structure is broken thoroughly. Therefore, longer mixing times may be desired. It is important to ensure that agitation at this step is not too aggressive, as high shear can diminish probiotic cell count.

Milk solids content can be maintained in part by adding dry milk to the mix prior to fermentation. This may be desired in a beverage if protein is an important component or calories are not an issue. If a reduced calorie product is desired, adding some water to the mix or reducing/eliminating added sugar might help the developer achieve this. In the case of eliminating added sugar, high intensity sweeteners may be added. Additionally, milk protein isolates, though more costly, have a lower lactose content and may be used instead of non-fat dry milk to boost the protein content without adding sugar. Milk protein isolates are not allowed in regular yogurt, but may be used in a synbiotic yogurt drink, fermented dairy beverage, or Greek yogurt.

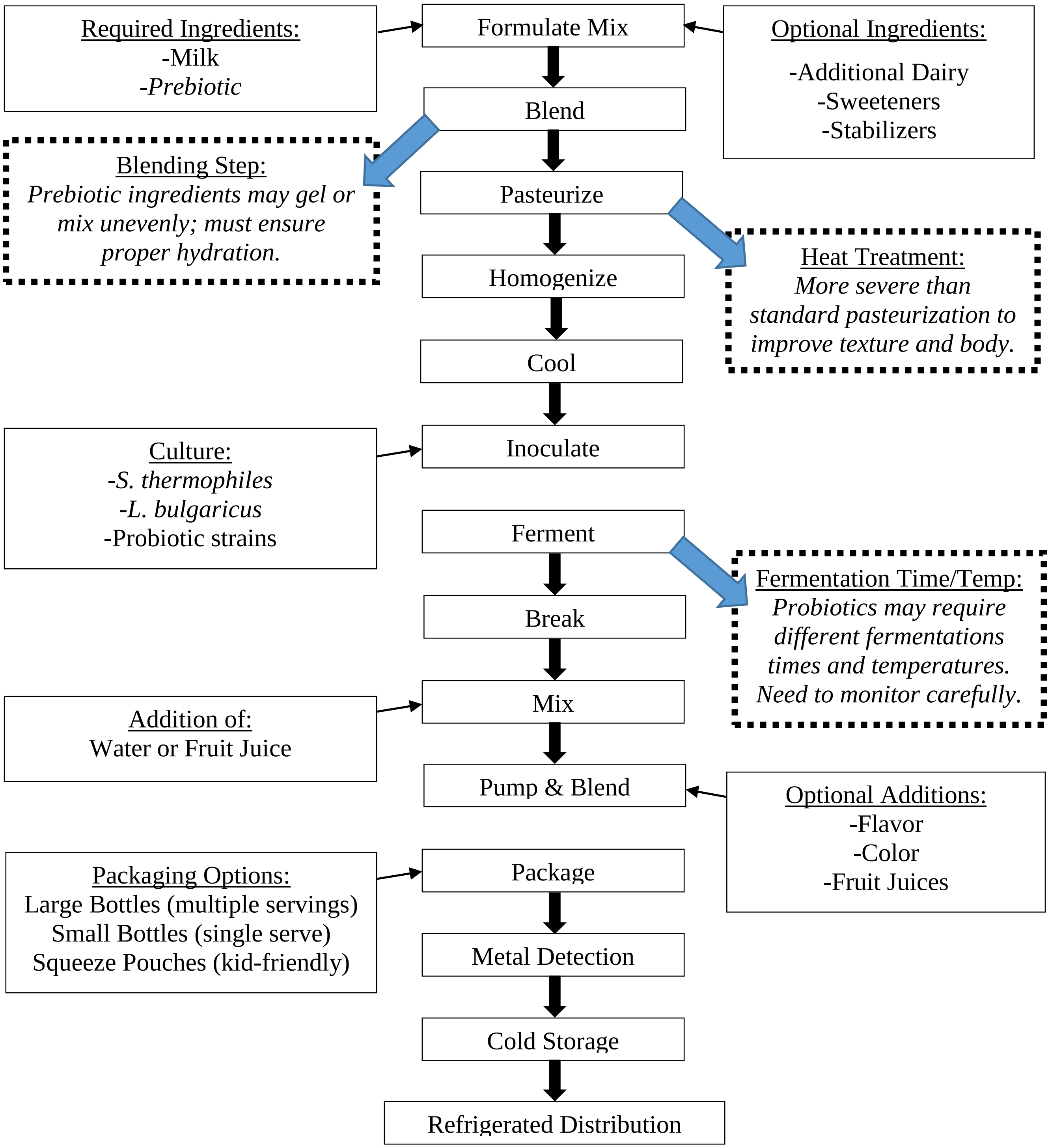

Figure 1.4 Flow Diagram for Batch Fermented Synbiotic Yogurt Drink (Adapted from: Cornell University 2014)

Quality and Shelf Life

The quality attributes desired for a drinkable yogurt or synbiotic dairy beverage include: flavor, appearance, color, syneresis, texture, body, and viscosity (Aryana and McGrew 2007). For a synbiotic yogurt drink, live and active cultures will be desired.

The quality of these attributes over time contributes to the overall shelf life because when one or more of these quality attributes becomes unacceptable, the product is no longer in its window for consumption. The quality attribute that becomes unacceptable is known as the "mode of failure."

Flavor

The flavor of a synbiotic yogurt drink should be identical to a traditional drinkable yogurt. Consumers do not want the drink to taste differently due to added prebiotics including inulin and oligosaccharides.

When considering prebiotics and their impact on flavor, it is important to note that some oligosaccharides have a sweet flavor (Aryana and McGrew 2007), which may or may not be desirable for certain applications. In sweetened yogurt, for instance, this may allow the manufacturer to use less sweetener. In plain yogurt, however, the sweetness may be perceived as an off flavor that is undesirable to the consumer.

Appearance, Color, and Syneresis

Appearance of a product is very important, especially when designing a product for children. The beverage should have a smooth appearance with no free water or syneresis. Syneresis is usually not considered the mode of failure in a drinkable yogurt because it can be easily incorporated by shaking the drink before opening. However, it is best to minimize syneresis because it is considered undesirable by consumers. In synbiotic drinkable yogurt, the prebiotic can act as a stabilizer to help minimize syneresis. This is because prebiotics are soluble fibers that gel in water. Thus, they can contribute to the water holding capacity in a yogurt system.

As the pH decreases over shelf life, lumps can occur due to protein aggregation. This may be considered as a mode of failure because consumers will want a drinkable product to have a smooth appearance. The color of the drink should correspond with the flavor; for instance, strawberry-flavored yogurt is expected to be pink. Color of the drink can be monitored in the plant if there is a colorimeter available and the desirable L, a, b values are known. If the color reading is unacceptable, the most likely cause is too much or too little coloring agent added to the batch.

Texture and Body

Mouthfeel is a very important aspect of consumer acceptability of drinkable yogurt. Children especially can be very particular when it comes to texture and consistency.

Yogurt and yogurt drinks have a thicker texture than milk due to gel formation during fermentation. As the yogurt cultures ferment lactose, they release lactic acid as a byproduct of their metabolism. The exact mechanisms of milk gelation are still disputed; however, it is known that milk casein molecules play the most significant role (Lucey 2002).

Yogurt mix is heated past the point needed to kill pathogens and competitive bacteria. At very high temperatures (>70°C), whey proteins are denatured. Some of these denatured proteins then associate with casein micelles via hydrophobic interactions and intermolecular disulfide bonds. This improves the gel-strength and has an impact on the texture of the final product. It should be noted that this high heat treatment (though it improves texture) can also increase syneresis, which is a major quality concern in yogurt products (Lucey 2002).

It is important that the proteins do not aggregate, causing a sandy texture. This can happen if the product is not acidified quickly enough. Slow acid development can happen if too little starter culture is added to initiate fermentation (Redfern and Rizk 1979). When acidification is too rapid, whey expulsion during shelf-life may increase. This is a quality concern, often caused by high incubation temperature or the addition of too much starter culture (Lucey 2002).

In the case of a synbiotic yogurt drink, the prebiotic choice cannot have negative impacts on the texture or body. However, the addition of a prebiotic often improves the texture. Inulin reportedly contributes a fat-like mouthfeel (Aryana and McGrew 2007) when added at low levels (2-2.5%) (Boeni and Pourahmad 2012). This means that it has potential to be used in low-fat or fat free synbiotic systems to contribute to the overall consumer acceptability of those products.

Viscosity is associated with texture and body of the yogurt. Once the desired thickness is known, apparent viscosity can be used as a tool to measure and monitor viscosity. If the viscosity is not on target, there may be several causes. For instance, if the viscosity is too low, there may be an issue in the fermentation process. Too much water may have been added to the batch, or an ingredient, such as nonfat dry milk, may have been omitted. It should be noted, however, that though the addition of inulin has been shown to have a positive impact on mouthfeel, it has not been shown to alter the viscosity (Niness 1999, Aryana and others 2007).

Live and Active Cultures

Live, active cultures are desired in synbiotic yogurts. An insufficient count of viable bacteria is usually the mode of failure chosen when determining shelf life of synbiotic or probiotic products. If there is no viable bacteria in the product at the time of consumption, gut population will not be achieved, and any subsequent benefits of consuming the probiotic bacteria will not occur.

Certain methods can be used to enumerate live and active cultures to determine shelf-life and ensure there are enough probiotic bacteria in the product. *Biffidobacterium* spp. can be enumerated using the pour plate technique and MRS-NNLP (nalidixic acid, neomycin sulphate, lithium chloride, and paramomycin sulphate) agar. *Lactobacillus* spp. are more difficult to enumerate. Tharmaraj and Shah (2003) investigated methods for enumerating these bacteria. They found that *L. acidophilus* can be measured with MRS-sorbitol agar and anaerobic incubation for 72 h at 37°C. *L. casei* and *L. rhamnosus* can be enumerated with MRS-vancomycine (MRS-V) agar. These can be enumerated separately by subtraction method. *L. rhamnosus* can grow at 43°C, where *L. casei* grows at lower incubation temperatures (~37°C). The 43°C plate counts can be subtracted from the 37°C plate counts – the difference will be the total viable *L. casei*.

The pH of yogurt can also be an indicator of how many live active cultures remain. This is a less accurate, but more rapid method. The viability of probiotic bacteria is greatly decreased by a pH of 4.30 or lower (Lankaputhra and others 1996). A shelf life study that correlates pH and viable bacteria may be used to validate pH measurements as a quality indicator. This is necessary because different probiotics are more sensitive to pH changes than others. While some probiotic bacteria may be able to survive at a pH of 4.30, others may die off at pH 4.50.

Synbiotic systems may improve shelf-life by extending the viability of cultures. Beoni and Pourahmad (2012) reported that *L. acidophilus* and *L. casei* decreased in synbiotic yogurt with inulin over 21 d refrigerated storage, but the decrease was not significant. Akalin and others (2004) found that the addition of fructooligosaccharides to yogurt containing the probiotic *Bifidobacterium animalis* improved viability over 28 d refrigerated storage. The viability of *Bifidobacterium longum* cells was also improved from 7 d to 21 d of refrigerated storage. Similarly, Shin and others (2000) found that the addition of prebiotics improved viability of *Bifidobacterium* in refrigerated storage. A sample with 5% fructooligosaccharide added maintained 67.3% of its viable cells versus 11.6% in the control with no added prebiotics after 4 weeks of storage. Viability of cells increased from 0.5-5% with added galactooligosaccharides, inulins, and fructooligosaccharides. All samples with added prebiotics maintained higher levels of viable probiotic bacteria.

Product Safety

Inadequate product safety is generally not considered as a mode of failure for yogurt products. This is because the milk is pasteurized prior to inoculation and fermentation. The product is refrigerated and has high numbers of favorable bacteria that should out-compete any remaining pathogens. Additionally, most pathogens cannot grow at pH ≤4.6. As long as GMPs are followed during the manufacture of yogurt, it should remain wholesome throughout shelf-life.

Study Objectives

The main objective of this research was to develop a synbiotic yogurt drink for children containing inulin and probiotic bacteria including *L. rhamnosus*. Certain strains of this probiotic have demonstrated immune-system enhancing effects in clinical studies, including studies over children in daycare and pediatric wards.

Originally the plan was to place this product in schools as an alternative choice for students to drink with meals. However, due to strict regulations and government control over school lunch items, this product does not meet the guidelines set for school lunches, and could not be offered as a milk alternative.

The USDA ruled in 2012 that "8 ounces of fluid milk must be offered with breakfast and lunch [in schools]. In addition, schools must offer at least two different milk options. However, the new standards allow only for low-fat (1 percent) or fat-free plain milk or fat-free flavored milk in the school meal programs" (National Milk Producers Federation 2012). Our product did not meet these requirements because it is a cultured dairy product, not fluid milk. The product is also strawberry-vanilla flavored and not completely fat free. Even though it is very low in sugar, it does not meet the guidelines for flavored milk.

The product could, however, be offered a la carte, or as a snack item. Therefore, the product needed to meet the requirements for "Smart Snacks in Schools," which is part of the Interim Final Rule: Nutrition Standards for All Foods Sold in School (Concannon 2012). This statute is regulated by the USDA Food and Nutrition Service Child Nutrition Division. These requirements must be met for a la carte items for sale in the cafeteria, at snack bars, or in school vending machines. This means items need to be approved in order to be sold on public school campuses, which is defined as “all areas of the property under the jurisdiction of the school that are accessible to students during the school day” (USDA 2013).

It was imperative that the product be accepted by school-age children as a product they enjoy and would choose to purchase given the chance. Therefore, a primary objective was to ensure that the product was accepted by the target consumer: children between the ages of 6 and 12 years.

A secondary objective was to ensure that the synbiotic interaction of inulin and probiotic culture did not have a negative effect on the acceptability of the product. An additional objective of the study was to determine physical and chemical parameters of the drink (pH, Brix, viscosity, syneresis, and color) over shelf-life, and determine if the synbiotic system has a significant effect on these parameters. The goal of the shelf-life study was to determine if the synbiotic yogurt maintained acceptable quality attributes throughout the desired shelf-life of 4 weeks (28 days).

Chapter 2 - Materials and Methods

Preliminary Work

Prior to any data collection, a control formula had to be determined. The product needed to meet the requirements for “Smart Snacks in Schools.” The Alliance Product Calculator (Alliance for a Healthier Generation 2014) was used to develop a formula that would satisfy these requirements. The formula that was determined to be the best for the control is shown in Table 2.1.

The original formula contained 2% milk, water, sugar, inulin, natural flavors, and a yogurt culture blend with several probiotics, including *L. rhamnosus*. Vanilla and strawberry flavored dairy drinks were informally evaluated by small panels of 5-8 Food Science students and faculty at Kansas State University, Manhattan, KS. It was determined that a mixture of the strawberry and vanilla flavors was desirable.

Different levels of flavor, sugar, and sweetness enhancer were tested, and optimal levels were chosen by the small tasting panels. The highest level of inulin that could be added without compromising texture or flavor was also determined by the small tasting panels.

The addition of nonfat dry milk (NFDM) increased the protein content of the product, which is often a welcome addition to the diet of school-aged children. NFDM was also added at such a level that the milk solids of the final product were equivalent to milk.

Sugar was reduced while maintaining sweetness by using a sweetness enhancer (Firmenich Sweetgem™, Princeton, NJ) which can be listed as “natural flavoring” on the label. This meets consumer demands for less added sugars, as well as a short, “clean” label.

The culture in the original formula was a premade blend that contained *S. thermophiles, L. bulgaricus, B. lactis, L. acidophilus, L. casei, L. lactis,* and *L. rhamnosus*. In order to more closely meet the objectives of the study, a single strain of *L. rhamnosus* was obtained to concentrate the benefits of consuming this probiotic. A *S. thermophiles* and *L. bulgaricus* blend was also obtained and mixed at a ratio of 4 to 3 with the *L. rhamnosus* culture for yogurt production.

L. rhamnosus GG would have been the ideal strain of *L. rhamnosus* in the product. However, a *L. rhamnosus* GG strain is not commercially available through DuPont/Danisco (New Century, KS), and therefore could not be obtained for the study and subsequent commercialization. Available research and data showed that the HN001 strain was the "next best thing" (Sheih and others 2001, Gill and others 2000). This strain was attainable through DuPont/Danisco.

Table 2.1 Drinkable Yogurt Baseline Formula

Ingredient	**% Total**
Milk – 1% (HyVee, West Des Moines, IA)	**81.098**
Nonfat Dry Milk (~35% protein; HyVee, West Des Moines, IA)	**1.250**
Sugar (C&H White Granulated; Yonkers, NY)	**2.500**
Prebiotic (Cargill Oliggo-Fiber Inulin; Wayzata, MN)	**3.000**
Yogurt Culture (Danisco YoMix 465; New Century, KS)	**0.004**
L. rhamnosus (Danisco HOWARU Rhamnosus; New Century, KS)	**0.003**
Water (tap)	**12.000**
Sweetness Enhancer (Firmenich Sweetgem™; New Ulm, MN)	**0.020**
Nat Vanilla Custard Flavor (Givaudan Flavors; Cincinnati, OH)	**0.055**
Nat Strawberry Flavor WONF (Givaudan Flavors; Cincinnati, OH)	**0.045**
Natural Red Coloring (elderberry and beet extracts) (Gold Coast, Commerce, CA)	**0.025**
Total	**100.000**

It was necessary to use milk with 1% milk fat and rehydrated NFDM to obtain the fat/calorie ratio where it needed to be for the "Smart Snacks in Schools" program requirements. Some milk fat was retained in the formula (1% milk was used rather than skim milk) to improve texture and help “smooth out” the flavor of the beverage. Flavor release is affected by the presence or absence of fat in a system (Reineccius 2006). Even a small amount of added fat can have an impact on the flavor of a dairy-based product. Without the fat, the flavor was perceived as “abrupt” with high intensity and fast clearing of the palate. The addition of a small amount of fat changed the perceived flavor release, so that there was a more present and prolonged flavor, followed by some lingering on the palate after swallowing.

Research Variables

Once the target market was identified and the control formula determined, variables were introduced to test the yogurt drink. It was decided that the variables should reflect consumer acceptance of the product. Based on previous studies of similar products and consumer tests, it was determined that variables which should be tested include: presence or absence of the prebiotic inulin, the probiotic culture *L. rhamnosus*, and the effects of combining both in a drink. The three treatments are shown in Table 2.2.

Table 2.2 Three Treatments of a Synbiotic Yogurt Drink

	Inulin at 3%	*L. rhamnosus*
I – Prebiotic Inulin	X	
P – Probiotic *L. rhamnosus*		X
S - Synbiotic	X	X

Since the whole premise of the product is that it is synbiotic, it was important that the prebiotic inulin and probiotic *L. rhamnosus* do not negatively impact the flavor, texture, color, or overall consumer acceptance. Though many studies have shown little difference in flavor when probiotics are added, one study on synbiotic drinkable beverages did find that samples with high levels of prebiotics and added probiotics (*B. lactis* Bb-12 and *L. acidophilus* LA-5) had an altered flavor, according to a descriptive sensory panel (Allgeyer and others 2010). Since testing was only done with a descriptive sensory panel, whether this would have a positive, negative, or neutral impact on consumer acceptance is unknown.

Variables which were measured are listed in Table 2.2. These included the color, viscosity, Brix, and pH throughout shelf-life, as well as consumer acceptability. Additionally, shelf-life was determined based on live and active bacteria present in the product, in order to ensure consumers are getting the advertised probiotic dosage throughout shelf-life. The ideal shelf-life for this product was determined to be ≥ 28d.

Statistical Design

Three treatments of yogurts were evaluated and compared. Three replications were treated as blocks in a randomized block design. Duplicate readings for pH, Brix, color, and viscosity were taken on each test day. Sensory analysis was only conducted on one occasion, not in triplicate, due to monetary constraints. Also due to monetary constraints, the third party testing (microbial, sugars, proximate analysis) was only tested on once for the necessary treatments.

Statistical analysis on the shelf-life data was conducted by the Statistical Consulting Lab at Kansas State University. SAS statistical software (SAS Institute Inc.; Cary, NC) was used for this statistical analysis. ANOVA with pairwise comparison was used to find significant differences among treatments or over time.

Sensory statistical analysis was conducted with Excel software (Microsoft Corporation; Redmond, WA) for averages and groups. two-way ANOVA was used to find significant differences between treatments.

Table 2.3 Experimental Design

Day	1	8	15	22	29
pH	X	X	X	X	X
Brix	X	X	X	X	X
Viscosity	X	X	X	X	X
Syneresis	X	X	X	X	X
Color	X	X	X	X	X
Lactic Acid Bacteria	X				X
Proximate Analysis	X				
Sugars	X				
Sensory Testing		X			

Experimental Procedures

Sample Preparation

The first step in preparing the yogurt beverage was making yogurt. The process was as follows: dry ingredients were weighed and mixed in plastic containers and labeled the day prior to the experiment. Dry ingredients included: granulated sugar (C&H, Yonkers, NY), Oligo-fiber inulin DS2 (prebiotic) (Cargill; Wayzata, MN), and nonfat dry milk (NFDM) (HyVee; West Des Moines, IA).

Dry ingredients were solubilized in milk (1% milk fat) (HyVee; West Des Moines, IA) to form the yogurt mix. Yogurt mixes were heated to 95°C on a stove top, removed from the heat, covered, and allowed to rest for 5 min. Mixes were then cooled to 42°C in a cold water bath, and transferred to sterile containers. The yogurt mix was inoculated with starter culture (Danisco YoMix 465 LYO 500 DCU) and probiotic (if applicable) (Danisco HOWARU Rhamnosus LYO 500 DCU) (Danisco USA Inc; New Century, KS) at 42°C. Inoculated yogurt mixes were placed in an incubator (Percival; Perry, IA) at 42°C for 5-6 h, until pH reached 4.6. Sample pH was monitored with an Accumet portable meter (Fisher Scientific; Waltham, MA) fitted with a 13-620-AP61 probe. The meter was calibrated with pH 4.0 buffer and pH 7.0 buffer before pH measurements were recorded.

After removal from the incubator, samples were stirred at room temperature to thoroughly disrupt gel structure. For this step, a bench-top stand mixer (KitchenAid; St. Joseph, MI) was used with a paddle attachment on speed two for 5 min. After mixing, yogurts were transferred to an ice bath and stirred by hand until the temperature reached 20°C (±2°C). Samples were then placed in a refrigerator to rest overnight (approximately 18 hours).

Drink samples were prepared on the following day. Water, strawberry flavor (Givaudan Flavors; Cincinnati, OH), vanilla flavor (Givaudan Flavors; Cincinnati, OH), sweet-type flavor (Sweetgem) (Firmenich; New Ulm, MN) and red coloring (Gold Coast; Commerce, CA) were added. The KitchenAid mixer with a paddle attachment was used (speed two, 5 min) to incorporate ingredients and further disrupt the gel structure for ideal texture.

Samples were divided into clean, 1 pint, transparent plastic containers with screw-top lids at 350 mL per treatment per week, leaving about 1 in of headspace between the yogurt and the top of the container. Each of the 350 mL samples were stored in the refrigerator at 3°C. One sample of each treatment was pulled from the refrigerator for analysis each week, and disposed of after testing.

In-Laboratory Testing – Shelf-Life Study

Syneresis

Syneresis was measured according to Koksoy and Kilic (2004) with slight modifications. The original study measured syneresis only once, on day 15. Modifications allowed for the documentation of syneresis in an undisturbed sample over time to measure changes. A sample of 100 ml was placed in a 100 ml graduated cylinder and sealed with Parafilm (Sigma-Aldrich; St. Louis, MO). The 100 ml samples were placed in a refrigerator at 3°C for the duration of the experiment. Serum separation was measured on days 8, 15, 22, and 29, and reported as ml/100 ml.

pH

The sample pH was measured with an Accumet portable meter (Fisher Scientific; Waltham, MA) fitted with a 13-620-AP61 probe. The meter was calibrated with pH 4.0 buffer and pH 7.0 buffer before pH measurements were recorded.

Brix

Brix was measured with a Fisher Scientific bench top refractometer (Fisher Scientific; Waltham, MA). Lenses were cleaned with distilled water and Kimwipes (Roswell, GA) prior to use and between each measurement.

Color

The color was measured with a MiniScan portable colorimeter (Hunter Labs; Reston, VA). The colorimeter was calibrated with white and black standardization tiles prior to use. A 150 mL sample was placed in a 250 mL beaker. The measurement was taken through the glass on the bottom of the beaker. Beakers were wiped with Kimwipes before sample was added, and again immediately prior to taking a reading.

Apparent Viscosity

Apparent viscosity was determined according to Aryana and others (2007) with slight modifications. Modifications were necessary based on equipment available. A different spindle was used, and viscosity was taken at one point in time, rather than plotting changes in viscosity. A single reading was all that was deemed necessary as an indicator of viscosity for this experiment. A Brookfield DV II+ viscometer (Brookfield Engineering Lab Inc, Stoughton, MA.) with a #62 spindle was used. A sample of 65g was placed in an insulated sample cup at 8°C. The viscometer was set to 10 r.p.m. and measurements were collected after 45 sec.

Lactic Acid Bacteria

Viable lactic acid bacteria counts were determined by a third party laboratory (Silliker Inc.; Chicago, IL). Since bacteria counts only needed to be determined in six samples, it was deemed more cost-effective to use a third party, than buying media and supplies for a limited number of samples. Cost-effectiveness was important in this research because the development of this drink was being done for an outside entity with limited funding.

For each treatment, 200g of each drink (minimum sample weight required for analysis) were collected on day 1 and day 29 of the experiment and sent to Silliker's Food Science Center in Crete, IL for analysis. Only total *Lactobacillus* and total *Streptococcus* could be measured, but it was believed that *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* could be differentiated by comparing differences in *Lactobacillus* counts between samples.

Total *Lactobacillus* species were enumerated using a "total probiotic" count method (Wehr and Frank, 2004). A sample of 11g was added to 99g MRS broth and stomached. Ten-fold dilutions were prepared with peptone buffer. One ml of diluted sample was placed in an empty, sterile petri dish. Then, 12-15 ml of tempered MRS + 0.05% cysteine-HCL agar was added, and the sample was swirled to mix. After solidifying, an overlay of tempered MRS + 0.05% cysteine-HCL agar was poured on top to create an anaerobic environment. The plates were inverted and incubated for 72 h at 37 ±2°C. Counts were reported as viable count per gram.

Total *Streptococcus* was enumerated in a similar manner, with slight modifications. Instead of MRS + 0.05% cysteine-HCL agar, M-17 agar was used to select for *Streptococcus* bacteria. The plates were incubated at 37 ±2°C for 48 h instead of 72 h.

Chemical Analysis

Sugar profile and proximate analysis were determined by a third party laboratory (Medallion Labs; Minneapolis, MN). The product was intended for future commercialization, and development of product specifications data from a known commercial lab was deemed appropriate.

Two samples of 50g of each treatment were collected on day 1 of the experiment and sent to a Medallion Labs facility in Minneapolis, MN for analysis. Sugar profile was done by HPLC using Association of Official Analytical Communities (AOAC) method 977.20 with slight modifications. These modifications include shaking the sample with water to extract sugars, and using an amino modified HPLC column to give better peak shape and resolution.

Proximate analysis was also conducted on the yogurt drink by Medallion Labs in Minneapolis, MN. The tests determined ash using AOAC: 923.03, calories (determined by calculation), carbohydrates (determined by calculation), moisture using AOAC: 950.46, and protein using AOAC: 992.15 and American Association of Cereal Chemists (AACC) method 46-30. AOAC: 950.46 was modified slightly.

Nutritional Analysis

To generate a nutrition facts, Genesis R&D software (ESHA Research; Salem, OR) was used. The formulation was entered by ingredient weight. The data collected from proximate analysis and sugar profile was then used to correct the nutrition facts panel. These adjustments accounted for nutritional changes during processing and the fermentation process.

Sensory Analysis

Sensory analysis was conducted with the Sensory Analysis Center at K-State Olathe, Olathe, KS. Rather than bringing children to the Sensory Center to participate, a local school was selected based on criteria such as: target market (middle-upper class kids with expendable funds), willingness to participate (a $1,000 donation to the school was used for incentive), and ability to complete the questionnaire (reading and comprehension level). Oxford Middle School in the Blue Valley School District (Overland Park, KS) was selected based on these criteria.

Students who were eligible to participate were selected by a preliminary screener. The screener asked about gender, age, known allergens, lactose intolerance, and foods regularly consumed. If the child was lactose intolerant or had any known allergies, they were not allowed to participate. If the child did not eat yogurt, they were not considered eligible for the study. If the child passed the screener, parental consent was required in order for the child to participate. The screener in its entirety is available in Appendix A, Figures A.1-A.12.

Consent from parents was needed in order to meet Institutional Review Board (IRB) requirements. The IRB number for this study was #5930.1, which was a blanket IRB number that is used by the Sensory Center at K-State Olathe. It covers sensory testing involving participants who are minors, as long as the minor and a parent give consent for participation in the study, and the product is considered safe to eat.

Samples were prepared in the Kansas Value Added laboratory (Kansas State University, Manhattan, KS) according to the "Sample Preparation" procedure outlined at the beginning of this chapter. Samples were stored at ~3-4°C until day 8. On the day of the experiment, samples were removed from the refrigerator, placed in a cooler (Rubbermaid, High Point, NC), and transported cold from Manhattan, KS to Overland Park, KS.

Microbiological testing was required by the Blue Valley School District (Overland Park, KS), to prove that the product was microbiologically safe to consume. 3M™ Petrifilm™ Plates (3M; St. Paul, MN) were used for these tests. A 1:10 dilution of each treatment was plated on an *E. coli*/Coliform Count Plate, a Yeast and Mold Count Plate, and an Aerobic Count Plate. Plates were incubated and read according to manufacture instructions. A letter was then issued to K-State Olathe for their records and to share with the participating school district, stating the results of these tests and microbiological quality of the yogurt drinks. The results of the microbiological test are shown in Table 2.4.

Table 2.4 Microbiological Quality of Yogurt Drinks used for Consumer Testing

	Yeast & Mold	Total Coliforms	Total Aerobic Plate Count
B1 (S) Synbiotic	<10 CFU/g	<10 CFU/g	>1,000 CFU/g*
B2 (I) Inulin Only	<10 CFU/g	<10 CFU/g	>1,000 CFU/g*
B3 (P) Probiotic Only	<10 CFU/g	<10 CFU/g	>1,000 CFU/g*

High counts are an indicator of live active lactic acid bacteria. (n=1)

The three treatments (S, I, and P) were tasted on Day 8 after manufacturing. This allowed time for proper manufacturing and microbiological testing, but still allowed the experiment to take place on an experiment day, where known data points on syneresis, pH, Brix, color, and viscosity had been taken.

The sensory test was administered in the cafeteria/common room of Oxford Middle School, which had florescent lighting. Though florescent lighting is not generally the best for sensory testing, these conditions were ideal for this particular experiment because the product will be consumed in a cafeteria setting after commercialization. Most school cafeterias have florescent lighting.

Samples were stored on ice and served cold (~40°F; 4°C) in 1.25 fluid ounce clear labeled sample cups with lids. Sample were labeled with the following code numbers: 261 – Batch 1 Synbiotic (S), 715 – Batch 2 Inulin (I), 583 – Batch 3 Probiotic (P). Samples were administered in random order.

Compusense software (Compusense; Guelph, Ontario, Canada) was used to develop and administer the questionnaire. The random order in which samples were administered was generated by the software. Before each sample was administered, students were instructed to eat a bite of unsalted saltine cracker and take a drink of distilled water to clear their palates before tasting.

The questionnaire focused on aroma, flavor, texture, appearance, and overall liking of each sample. Liking questions were asked on a 9-point hedonic scale, with 1 being dislike extremely, 5 being neither like nor dislike, and 9 being like extremely. Questions over flavor levels were asked on a "just-about-right" scale with 3 being just-about-right, 1 being too weak, and 5 being too strong. After tasting samples, students were asked how likely they would be to purchase the yogurt drink. The students were also asked how much they would be willing to pay for their favorite yogurt drink.

Additionally, students were asked if they were able to tell a difference between the samples, and if so, to describe the differences. The questionnaire in its entirety is shown in Appendix B, Figures B.1-B.16.

Chapter 3 - Results and Discussion

Shelf-Life

Syneresis

There was a significant difference in measured syneresis values among treatments and over shelf-life. Most samples showed a significant amount of serum separation between D8 and D22. A steady increase in syneresis or serum separation is expected when yogurt samples are allowed to sit undisturbed.

There was only a significant difference among these yogurt treatments on D22, where the synbiotic sample had significantly less serum separation than the probiotic sample. These results are not consistent enough to conclude that added inulin decreased syneresis over shelf-life in this study.

Previous studies have shown mixed results as to whether or not soluble fibers such as inulin decrease syneresis in yogurt products (Aghajani and Pourahmad 2012, Boeni and Pourahmad 2012, Aryana and others 2007). Some show no significant differences in syneresis values with added prebiotics, while others show significant decreases in syneresis with added inulin, especially inulins with longer chain lengths.

Since this product will be stored in a container that is not transparent, the consumer will not be able to see the syneresis as it occurs. The product will also contain a written statement on the label that says “Keep Refrigerated. Shake before Opening,” so hopefully fluid separation will be minimized and then incorporated into the drink before the consumer opens the bottle.

A stabilizer could be added to the formula to reduce syneresis in the product. However, a clean label was desired, and the study sponsor wanted to use as few ingredients as possible.

Syneresis is a quality issue, not a safety issue, and in this instance it was decided that preventing it was not considered a priority by the study sponsor.

Table 3.1 Syneresis Values (ml/100ml) over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	$0.0^{Aa} \pm 0.0$	$1.0^{Aa} \pm 0.0$	$1.3^{ABa} \pm 0.6$	$2.0^{Ba} \pm 0.5$	$2.7^{Ba} \pm 0.6$
Inulin	$0.0^{Aa} \pm 0.0$	$0.7^{Aa} \pm 0.6$	$1.7^{ABa} \pm 1.2$	$2.2^{Bab} \pm 1.6$	$2.7^{Ba} \pm 2.1$
Probiotic	$0.0^{Aa} \pm 0.0$	$1.3^{Aa} \pm 0.6$	$3.3^{Ba} \pm 2.3$	$4.8^{Bb} \pm 2.8$	$5.3^{Ba} \pm 2.5$

A-C, means with different superscripts in a row are different among days ($p \leq 0.05$).
a-c, means with different superscripts in a column are different among treatments ($p \leq 0.05$).
(n=3)

pH

The pH was not significantly different among treatments, but it was significantly different among days (Table 3.1). This was expected, since the pH of yogurt is known to drop steadily over refrigerated storage.

Food safety is not typically a concern for yogurt products that are manufactured safely (not considering improperly pasteurized mix or mix contaminated after pasteurization). Most yogurts contain live cultures, and have a pH below 4.6. As the pH continues to drop, the likelihood of pathogenic bacteria growing decreases (Massa and Others 1997). However, the drop in pH may reduce the acceptability of the product by contributing a more sour or acid taste, which also decreases sweetness perception (Martin and others 1999).

The pH of all yogurts dipped below 4.30 between D15 and D22. This is approximately the pH that causes a significant drop in viable lactic acid bacteria (Lankaputhra and others 1996). However, bacterial counts remained high for these yogurts after 4 weeks of refrigerated storage (Table 3.1). Additionally, the cooling after fermentation will be more abrupt when the product moves to commercial production. This means that fermentation can be stopped more rapidly, and the pH will likely be closer to 4.60 on D1. With proper storage and handling, it may be able to retain a pH above 4.30 for the full duration of shelf-life.

Table 3.2 pH Values over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	$4.55^{A} \pm 0.03$	$4.40^{B} \pm 0.06$	$4.34^{BC} \pm 0.09$	$4.28^{C} \pm 0.07$	$4.27^{C} \pm 0.04$
Inulin	$4.56^{A} \pm 0.03$	$4.38^{B} \pm 0.06$	$4.33^{B} \pm 0.10$	$4.25^{C} \pm 0.05$	$4.25^{C} \pm 0.04$
Probiotic	$4.52^{A} \pm 0.02$	$4.39^{B} \pm 0.04$	$4.32^{C} \pm 0.08$	$4.24^{D} \pm 0.05$	$4.23^{D} \pm 0.02$

A-D, means with different superscripts in a row are different among days ($p \leq 0.05$).
No differences among treatments ($p \leq 0.05$). (n=3)

Brix

The Brix, or percent soluble solids (Table 3.3) was significantly different between treatments, but did not change significantly over time. Inulin is a soluble solid. Therefore, the difference between treatments was anticipated.

Soluble solids content remained stable for all three treatments throughout the shelf-life. A drop in soluble solids over time was originally anticipated. It was thought that the bacteria would use some of the inulin as fermentation continued over shelf-life. If fermentation was occurring, and inulin was being consumed by the bacteria during storage, it was not detectible by measuring the brix of the yogurt drink.

No similar studies have monitored brix values of yogurt over shelf-life, but it does not appear to be a variable that is worth monitoring in the future. However, it is important to know that the brix of this synbiotic yogurt drink should be around 12.8-12.9. This can be used as a quality check during production.

Table 3.3 Brix Values over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	12.8 ^{a} ± 0.2	12.8 ^{a} ± 0.2	12.7 ^{a} ± 0.2	12.9 ^{a} ± 0.2	12.9 ^{a} ± 0.2
Inulin	12.8 ^{a} ± 0.4	12.7 ^{a} ± 0.2	12.9 ^{a} ± 0.2	13.0 ^{a} ± 0.2	12.9 ^{a} ± 0.3
Probiotic	9.8 ^{b} ± 0.2	9.9 ^{b} ± 0.1	9.9 ^{b} ± 0.2	9.9 ^{b} ± 0.2	9.9 ^{b} ± 0.3

a-b, means with different superscripts are different among treatments($p \leq 0.05$).
No among days ($p \leq 0.05$). (n=3)

Color

L, a, and b values were measured together, but will be discussed separately for the purposes of this section. For color L values, there was no significant change over shelf-life or between treatments (S, I, or P) (Table 3.3). This means that the lightness (light versus dark) did not change over time. Color "a" values did show significant differences, both amoung treatments and over time. Color "a" values are a measure of red versus green. Positive numbers (as seen in Table 3.4) indicate red, while a negative would indicate green. Since a red food color was used, it is expected to see red color values. Color "b" values are shown in Table 3.5. Color "b" values represent tendency towards yellow (positive) or blue (negative).

Table 3.4 Color L Values over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	83.35 ± 1.22	83.50 ± 1.78	83.36 ± 1.27	83.63 ± 0.25	83.42 ± 0.89
Inulin	82.33 ± 0.50	82.85 ± 1.03	82.58 ± 1.10	83.65 ± 1.07	83.75 ± 0.68
Probiotic	83.48 ± 1.21	83.81 ± 1.45	83.93 ± 0.95	84.36 ± 1.01	85.03 ± 1.10

No differences among days or treatments ($p \leq 0.05$). (n=3)

Table 3.5 Color a Values over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	$4.84^{Aa} \pm 0.26$	$4.49^{Ba} \pm 0.25$	$4.35^{BCa} \pm 0.22$	$4.18^{Ca} \pm 0.22$	$3.95^{Da} \pm 0.25$
Inulin	$4.65^{Aab} \pm 0.11$	$4.42^{Aa} \pm 0.28$	$4.13^{Bab} \pm 0.27$	$3.99^{BCa} \pm 0.12$	$3.89^{Cab} \pm 0.19$
Probiotic	$4.53^{Ab} \pm 0.19$	$4.29^{ABa} \pm 0.18$	$4.07^{BCb} \pm 0.16$	$3.93^{Ca} \pm 0.09$	$3.79^{Db} \pm 0.22$

A-D, means with different superscripts in a row are different among days ($p \leq 0.05$).
a-b, means with different superscripts in a column are different among treatments ($p \leq 0.05$).
(n=3)

Table 3.6 Color b Values over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	$4.36^{A} \pm 0.28$	$4.48^{A} \pm 0.42$	$4.72^{B} \pm 0.31$	$4.90^{B} \pm 0.64$	$5.18^{C} \pm 0.18$
Inulin	$4.43^{A} \pm 0.44$	$4.46^{A} \pm 0.40$	$4.75^{B} \pm 0.33$	$5.12^{C} \pm 0.12$	$5.27^{D} \pm 0.41$
Probiotic	$4.61^{A} \pm 0.33$	$4.60^{A} \pm 0.33$	$4.82^{B} \pm 0.40$	$5.08^{C} \pm 0.33$	$5.28^{D} \pm 0.39$

A-D, means with different superscripts in a row are different among days ($p \leq 0.05$).
No differences among treatments ($p \leq 0.05$). (n=3)

There were significant differences between color “a” values of treatments (day 1, day 15, and day 25). This may have been due to presence or absence of the prebiotic and probiotic, though why these would change the color, and only on some days, is unknown.

Natural colorants are often not stable when exposed to light (Chapman 2011). It is possible that the significant differences in color “a” values may have been caused by uneven light exposure during storage. The refrigerator used for this study was also used for other purposes in the lab. Students open and close the refrigerator regularly. Samples at the front of the stack would receive more light than samples at the back of the stack.

Subtle differences in the formulations at the time of manufacture are also a potential cause. For instance, batches may have had slightly difference “actual” percentages of food coloring. The colorant was very concentrated and had a consistency that made it difficult to handle in “micro” quantities. This may have resulted in some error, effecting primarily the “red” color measurement (color “a” value). Therefore, the significant differences in color a values may be the result of error. More research is necessary if this is to be investigated further.

Color L values indicate that there was no significant movement from dark to light. This can be an indication of color fading. Though the color did not “fade” significantly from dark to light, it did lose red tones as indicated by significant decreases in color “a” values over time. The yogurts also became “more yellow” over time, as indicated by significant increases in color “b” values during the shelf-life study. These changes in color may be due to the acidic pH of the product. The pH continues to drop over shelf-life, and foods with such a low pH can have an effect on natural colorants. Light exposure can also have an effect on natural colorants (Chapman 2011). This may be an explanation for the fading red color over time.

For the most part, natural red colorants are stable in dairy products at pH ranges of 3.5-6.8 (Mendi and others 2000, Socaciu 2008). However, anthocyanin based colors are less stable. As the pH decreases, they should move closer to red/pink, and away from purple/blue colors (Chapman 2011). The red color values decreased over time (as pH decreased), but this may have been due to light exposure. The color did become “more yellow” which is “away from” blue on the color scale. This may have been due to the drop in pH.

Visually, very little differences were detected. The addition of more red color at the beginning of shelf-life may be desired to keep a brighter pink color throughout shelf-life. Another possible way to prevent red color loss is using light-proof packaging.

Viscosity

The viscosity of all treatments was significantly different from D1 to D8 (Table 3.6). This is likely due to processing. For all analysis days besides D1, the yogurt drink had several days to settle or reform gel networks before it was stirred. Yogurt drinks on D1 only had a brief resting period after they were very thoroughly mixed. Therefore, it is probably best that the first value is discarded in analysis of viscosity over time.

Though there were some significant differences among treatments at the level of significance used ($p \leq 0.05$), they did not show a consistent trend over time. This may be due to the method used for measuring viscosity, which may lead to a very large standard deviation.

Overall, the significant differences in viscosity over time do not indicate a negative impact on quality. Though the viscosity of one of the samples did drop slightly at the end of shelf-life, the drop in viscosity was not significant.

There could be a concern that the consistency will be too thick at the end of shelf-life. The sensory test (which was conducted on D8) showed that the consistency was acceptable to the target consumer at that point in the shelf-life. The viscosity of the synbiotic sample is significantly greater on D29 than D8. However, the product was not shaken prior to the sensory test, and it should be shaken prior to the consumer opening the individual yogurt drink container. This will thin the drink to a certain extent.

Some previous studies also showed that adding inulin to a yogurt system did not increase viscosity (Niness 1999, Aryana and others 2007). These studies did show that addition of inulin had a positive impact on the mouthfeel. However, the improved mouthfeel did not correlate with an increase in viscosity.

Table 3.7 Viscosity Values (cP) over time for Strawberry-Vanilla Yogurt Drinks

Treatment	Day 1	Day 8	Day 15	Day 22	Day 29
Synbiotic	$1903^{Aa} \pm 95$	$2243^{Ba} \pm 133$	$2272^{BCa} \pm 123$	$2259^{BCa} \pm 135$	$2312^{Ca} \pm 255$
Inulin	$1972^{Aa} \pm 281$	$2306^{Ba} \pm 360$	$2341^{BCa} \pm 302$	$2483^{Cab} \pm 306$	$2487^{Ca} \pm 411$
Probiotic	$2030^{Aa} \pm 175$	$2406^{Ba} \pm 245$	$2507^{BCa} \pm 254$	$2571^{Cb} \pm 208$	$2534^{BCa} \pm 321$

A-C, means with different superscripts in a row are different among days ($p \leq 0.05$).
a-c, means with different superscripts in a column are different among treatments ($p \leq 0.05$).
(n=3)

Lactic Acid Bacteria

Since samples were collected on D1 and D29, but were plated the next day, counts are representative of D2 and D30. Counts at the beginning of shelf-life (BSL) and at the end of shelf-life (ESL) are discussed below. Table 3.6 shows microbial counts for treatments S, I, and P at the beginning and end of shelf-life.

There were no *Streptococcus* bacteria added to the yogurt besides *S. thermophiles*. Therefore, the total *Streptococcus* is entirely made up of *S. thermophiles* bacteria. *S. thermophiles* counts at the beginning of shelf-life were approximately 10^9 CFU/g in treatments S and P, and 10^{10} CFU/g in treatment I. Viable cell counts were relatively stable throughout the desired shelf-life. Storage only appeared to reduce viable cells by about 1 log from beginning to end of shelf-life (Table 3.1).

It was reported that the BSL total *Lactobacillus* counts were 10^8 CFU/g in the S and P treatments, and < 10^7 CFU/g in the I sample. This led us to believe that there were around 10^8 CFU/g *L. rhamnosus* in the S and P samples, and ~10^{6-7} CFU/g *L. bulgaricus* in all treatments at the beginning of shelf-life. *Lactobacillus* counts were also slightly higher in S than P, which may indicate that there are slightly more *L. rhamnosus* bacteria in S.

However, ESL total *Lactobacillus* counts were greater than BSL total *Lactobacillus* counts in I, and there was a 1 log reduction in S. These results were unexpected. It is possible that there was an error in the lab oratory results. It is also possible that *L. rhamnosus* counts decreased, while *L. bulgaricus* counts increased over time.

Unfortunately, it is impossible to tell if there are viable *L. rhamnosus* bacteria by the end of shelf-life in the Synbiotic and Probiotic treatments. More specialized tests were not available through known third party labs, and in-house specialized testing was not in the research budget. However, previous studies have shown that *L. rhamnosus* can be maintained in yogurt at viable cell concentrations of 10^{5-7} CFU/g, especially in the presence of a prebiotic such as inulin (Aryana and others 2007, Hekmat and others 2009).

This product could have the LAC seal on the label. However, the process of applying for and maintaining a LAC seal from the NYA is costly (National Yogurt Association 2013). The seal is not an indicator of live and active probiotic bacteria, and the ingredient statement can still contain the declaration "Contains live cultures: *L. bulgaricus, S. thermophiles*, and *L. rhamnosus*."

Table 3.8 Reported Lactic Acid Bacteria (LAB) counts for Strawberry-Vanilla Yogurt Drinks

	LAB	BSL[1] CFU/g	ESL[2] CFU/g
Synbiotic	Total *Lactobacillus*	1.2×10^{8}	7.7×10^{7}
	Total *Streptococcus*	1.6×10^{9}	1.0×10^{9}
Inulin	Total *Lactobacillus*	$< 1.0 \times 10^{7}$	7.3×10^{8}
	Total *Streptococcus*	1.8×10^{10}	1.2×10^{9}
Probiotic	Total *Lactobacillus*	1.1×10^{8}	1.0×10^{8}
	Total *Streptococcus*	1.8×10^{9}	8.9×10^{8}

[1]BSL (Beginning of Shelf-Life), [2]ESL (End of Shelf-Life), (n=1)

Sugars and Proximate Analysis

Sugars and proximate analysis were only analyzed once. After fermentation, the nutrition composition should remain fairly stable throughout shelf-life. Results from Sugars by HPLC and proximate analysis are included in Table 3.9.

Sucrose levels were expected to be around 2.5%, because sucrose is usually not broken down during fermentation. It was a little lower than expected at 2.23%. This could be testing error, or the cultures in the yogurt may be able to break down some sucrose.

The lactose content was 3.50%. This is slightly low for yogurt, but the lactose content of the milk may have been somewhat low to begin with. We did not test the yogurt mix prior to fermentation. That would have been useful information to find out just how much the composition of the mix changed during fermentation. Total sugars is made up of the lactose and sucrose. Total sugar content was 5.73% (11.35g/200ml serving).

Inulin is a soluble dietary fiber. However, because it is an oligosaccharide (short-chain carbohydrate), it is not possible to determine the fiber content using crude fiber. In proximate analysis, sugars, lactic acid, and inulin all fall under total carbohydrates. The fiber content will likely remain close to 6g/200ml. This makes the net carbohydrates 15g, not 21g. The 6g of fiber is not included when calculating the calorie content. This explains the discrepancy between the proximate analysis results, and the results generated by the nutrition facts software.

Table 3.9 Reported Sugars and Proximate Analysis for a Synbiotic Strawberry-Vanilla Yogurt Drink

Test	Method	Result	Total per 200ml Serving
Galactose	AOAC: 977.20	Less than 0.1%	0g
Fructose	AOAC: 977.20	Less than 0.1%	0g
Glucose	AOAC: 977.20	Less than 0.1%	0g
Sucrose	AOAC: 977.20	2.23%	4.42g
Maltose	AOAC: 977.20	Less than 0.1%	0g
Lactose	AOAC: 977.20	3.50%	6.93g
Total Sugars	AOAC: 977.20	5.73%	11.35g
Protein	AOAC: 992.15	3.42%	6.77g
Ash	AOAC: 923.03	0.73%	1.45g
Moisture	AOAC: 950.46	84.46%	167g
Total Fat	Gravimetric	0.71%	1.41g
Carbohydrates	Calculation	10.70%	21g
Calories	Calculation	63/100g	125 cal
Calories from Fat	Calculation	6/100g	12 cal

(n=1)

The primary carbohydrates are sugar and fiber. If there are 11g of sugar, and 6g of fiber, then there should be 17g of carbohydrates. As mentioned earlier, lactic acid, the byproduct of fermentation of lactose, is included under the carbohydrates in proximate analysis. Lactic acid is metabolized does contribute a similar number of calories as other carbohydrates. Therefore, there must be around 4g of lactic acid in the yogurt, contributing to the "other carbohydrates" not from sugar or fiber.

Moisture, fat, ash, and protein did not change significantly during fermentation. No adjustments were needed on the nutrition facts panel.

Nutrition Facts

The basic ingredients were entered into Genesis R&D to generate a nutrition facts panel. The nutrient composition of yogurt can be altered during the fermentation process, hence the need for proximate analysis and sugar profile. After taking into account any changes in composition, the following nutrition facts panel (Figure 3.1) was developed.

Nutrition Facts

Serving Size 7 floz (200mL)
Servings Per Container 1

Amount Per Serving	
Calories 100	Calories from Fat 15
	% Daily Value*
Total Fat 1.5g	**2%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 75mg	**3%**
Potassium 300mg	**9%**
Total Carbohydrate 21g	**7%**
Dietary Fiber 6g	**24%**
Sugars 11g	
Protein 7g	

Vitamin A 8% • Vitamin C 0%
Calcium 25% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500 mg	3,500 mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

INGREDIENTS: Cultured Skim Milk, Water, Inulin (soluble fiber), Cane Sugar, Cream, Nonfat Dry Milk, Natural Flavors, Natural Red Color (beet juice extract).

Contains live cultures: L. bulgaricus, S. thermophilus, and L. rhamnosus.

Contains Milk

Figure 3.1 Nutrition Facts and Ingredient List for Strawberry-Vanilla Synbiotic Yogurt Drink

The nutritional information from this nutrition facts was entered into the “smart snack” calculator (Alliance for a Healthier Generation, 2015). It was found to meet the requirements necessary to be a “dairy snack” available for sale in schools.

The total fat in a 7 oz serving is 1.5g, and the saturated fat is 1.0g. The saturated fat content had to be very low in order for the product to be accepted. The sodium content also had to be low, but that was not a challenge for this particular product, as there is no added salt. Carbohydrate content could be relatively high, but the sugar content needed to be very low. The final sugar content of this product was 15g/serving.

This was a particular challenge for our study. Children tend to enjoy sweet-tasting snacks and beverages. Because milk already contains lactose, added sugars had to be kept at a minimum. Some lactose is converted to lactic acid during the fermentation process, but the remaining lactose does not contribute a perceptible amount of sweetness to yogurt.

Artificial sweeteners could have been added to increase the sweetness without affecting the calorie or sugar content. However, artificial sweeteners are not considered “clean label” because they are not natural ingredients, and therefore they did not meet the requirements for this project. Natural high intensity sweeteners were also considered, but due to the off-flavors associated with such sweeteners, a sweetness enhancer was chosen as the best option for this project.

Sweetness enhancers allow for the use of less sugar by increasing the sweet taste perception of the sugar. They are thought to do this by enhancing the ability of sucrose to bind to sweet taste receptors on the tongue (DuBois and Prakash 2012). So less sugar can be added, but it will still taste sweet. Many sweetness enhancers can be listed as “natural flavor” or “natural and artificial flavor" on the label. Therefore, a natural sweetness enhancer, which could be included as a "natural flavor" on the label, was chosen as the best option for sugar reduction in the yogurt drink.

Sensory Analysis

Liking and Just About Right (JAR) Scales

There were significant differences among "overall liking" scores of the yogurts. The I treatment scored significantly higher in overall liking and texture liking (Table 3.9). There were no significant differences among treatments in the aroma and flavor categories. There were no significant differences between the S and P treatments.

Table 3.10 "Liking" Mean Scores for Strawberry-Vanilla Yogurt Drinks.

Treatment	Overall Liking	Aroma Liking	Texture Liking	Flavor Liking
Synbiotic	$6.82^{b} \pm 1.66$	$7.13^{a} \pm 1.47$	$6.47^{b} \pm 1.67$	$6.75^{a} \pm 1.78$
Inulin	$7.24^{a} \pm 1.53$	$7.36^{a} \pm 1.24$	$7.01^{a} \pm 1.73$	$7.05^{a} \pm 1.81$
Probiotic	$6.72^{b} \pm 1.54$	$7.18^{a} \pm 1.50$	$6.53^{b} \pm 1.57$	$6.61^{a} \pm 1.86$

a-d – means in columns with different superscripts are different ($p \leq 0.05$). (n=112)
Hedonic Scale: 1=dislike extremely, 5=neither like nor dislike, 9=like extremely.

Previous studies have shown that the addition of inulin improved some sensory attributes of low-fat and fat-free dairy products, such as perceived smoothness, creaminess, thickness, sweetness, and flavor intensity (Meyer and others 2011, Tarrega and Costell 2006, Tearrega and others 2010, Kip and others 2006). Since I scored better in most categories than S and P, this is consistent with previous studies. However, this conclusion would be better supported if a sample with no inulin or probiotic added had been tested in addition to the three treatments (S, I, and P).

One of the objectives of this research was to determine if the synbiotic interaction of inulin and the probiotic culture *L. rhamnosus* HN001 would have a negative effect on the consumer acceptability of the product. Gonzalez and others (2011) found that the sour tastes were higher in synbiotic samples when using a fructooligosaccharide with *Lactobacillus acidophilus* as a synbiotic system. This was reflected in their consumer tests, where the more “sour” synbiotic treatments had lower consumer acceptability scores. The study concluded that “inclusion of a synbiotic had a negative impact on the acceptance by the consumers, with the skim milk beverage containing synbiotic being the least acceptable.”

Based on the sensory data collected, it does not appear that the synbiotic interaction decreased liking scores. There were no significant differences in tartness scores between samples (Table 3.10). It is more likely that the addition of *L. rhamnosus* HN001 was the main contributor to a reduction in scores for treatments P and S.

Previous studies have shown that the addition of probiotics can have an impact on flavor (Bakirci and Kavaz 2008, Hekmat and Reid 2006, Oliveria and Jurkiewicz 2009, Tamime and others 1995). There were no significant differences in the “liking” scores of the synbiotic and probiotic samples, but the sample without the probiotic (treatment I) scored significantly better in flavor and texture liking. Based on significant differences in the data, it appears that adding *L. rhamnosus* HN001 an effect on the flavor and texture of the drink.

Though treatment I was the only treatment that scored above a 7 in all categories, the treatment S was close to 7 in most categories. This was determined acceptable to proceed with commercialization. Some flavor defects will be eliminated in the commercialization process. For instance, some off flavors can develop in the milk when it is heated on the stove in a stock pot, which tends to have “hot spots,’ rather than pasteurized with a commercial heat-exchanger, which can heat the fluid mix more evenly (Azzara and Campbell 1992). High temperature short time (HTST) pasteurization is the method primarily used in the industry, both for its efficiency and higher quality of the final product.

There were no significant differences in the JAR (just-about-right) scale attributes (Table 3.10). The JAR scale indicates that respondants (in general) thought the tartness of samples was good. JAR scale mean for all samples was very near the JAR mark (S=3.06, I=3.04, P=3.14). The JAR scale means for sweetness on all samples was < 3.0 (S=2.67, I=2.73, P=2.70), which indicates that the majority of students would prefer a drink with a sweeter taste.

The mean JAR scale score for strawberry flavor was somewhat low for all samples (S=2.56, I=2.66, P=2.74). Strawberry flavor of treatment P was closest to the JAR mark. It also had the highest rating for tartness, so it is possible that the additional tartness helped to enhance the strawberry flavor of the drink. It appears that more strawberry flavor may be necessary to make the drink a success with the target consumer.

Table 3.11 "JAR" Mean Scores for Strawberry-Vanilla Yogurt Drinks

Treatment	Strawberry Flavor	Sweetness	Tartness
Synbiotic	2.56 ± 0.79	2.67 ± 0.82	3.06 ± 0.81
Inulin	2.66 ± 0.81	2.73 ± 0.73	3.03 ± 0.75
Probiotic	2.74 ± 0.80	2.70 ± 0.78	3.14 ± 0.77

No differences between treatments ($p \leq 0.05$). (n=112)

Likes and Dislikes

After tasting each sample, students were asked what they liked and disliked. This was an open-ended question, so students were allowed to say anything. Frequent responses were categorized, and are shown in Figures 3.3 and 3.4.

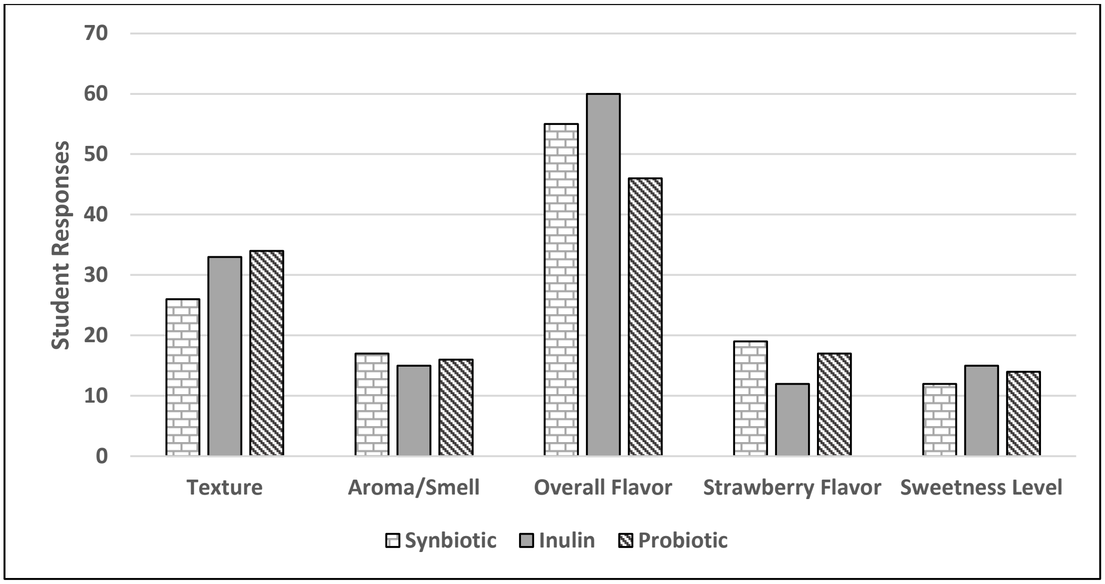

Figure 3.2 “Likes” by category for 3 Strawberry-Vanilla Yogurt Drinks

The most frequent responses in the “likes” category were texture, aroma/smell, overall flavor or taste, strawberry flavor, and sweetness level. A handful of students also indicated that they liked aftertaste, tartness, and appearance of certain treatments, but there were not enough responses to create categories for these (responses in these categories were $n < 5$ for all treatments).

Over half the respondants indicated that they liked the overall flavor of treatments I and S. Many students (nearly a third of respondents) also commented that they enjoyed the texture of the yogurt drinks. More students indicated they liked the texture of treatment P, but the texture of the treatment I had significantly better liking scores on the hedonic scale. The students may have been trying to find an attribute they liked, even though they did not particularly enjoy treatment P.

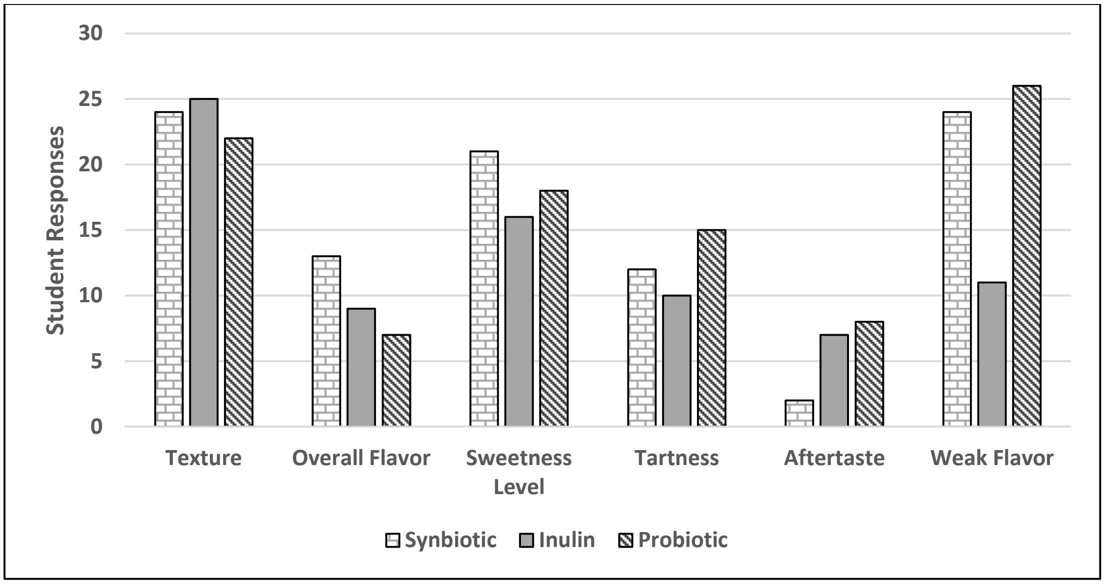

Figure 3.3 "Dislikes" by category for 3 Strawberry-Vanilla Yogurt Drinks

The most frequent responses in the "dislikes" category were texture, overall flavor or taste, sweetness level, tartness, aftertaste, and weak flavor. Some students also indicated that they disliked the strawberry flavor, aroma, and appearance, but there were not enough responses to create categories (responses in these categories were n < 5 for all treatments).

There are different extremes in the like and dislike categories. Some students indicated that the samples were too tart, while others said they were not tart enough. Some students thought samples needed to be sweeter, while others said they were too sweet. The JAR scale indicates that respondents (in general) thought the tartness of samples was near the mark, but that most drinks could have a sweeter taste.

Sweetness can be difficult to adjust in a product that must be low in sugar and carbohydrates. The addition of the sweetness enhancer did help with this issue, but the "weak flavor" and "watery" character cited by many respondents may be due to the lack of sugar in the product. Unfortunately, this cannot be helped by the addition of more sugar, because the limit has already been reached if the product is going to be accepted by schools as a "smart snack."

This data is limited in what can be interpreted from it. Respondents were allowed to write anything, as long as they wrote something. Some students mentioned two or three attributes they enjoyed, some responded that they liked everything about a sample and others responded that there was nothing they liked. Additionally, not all comments fell into one of the selected “common categories.”

The data is useful to determine if there is anything striking about any of the samples. For instance, many students indicated that they disliked the amount of flavor. They stated that samples needed more strawberry flavor or that the taste was weak. This was most commonly sited for the S and P treatments. The JAR scale did not show any significant differences in strawberry flavor or sweetness, but still many students commented that they wanted the drinks to have a stronger flavor.

This is something that may be improved upon, perhaps by adding more strawberry flavoring to the drink, removing some water, or by even changing the yogurt culture to include bacteria which produce stronger “yogurt-type” flavors. It is unclear why the samples that had the added probiotic (which may have added some flavors) were more commonly sighted for having too little flavor. Possibly, some of the flavor-producing *Lactobacillus blugaricus* were over-crowded by the probiotic, so they produced fewer flavorful byproducts during fermentation.

Purchase Intent and Pricing

After tasting each sample, students were asked whether they would purchase the drink. The question was asked on a 5-point scale, with 1 being definitely would not purchase, and 5 being definitely would purchase.

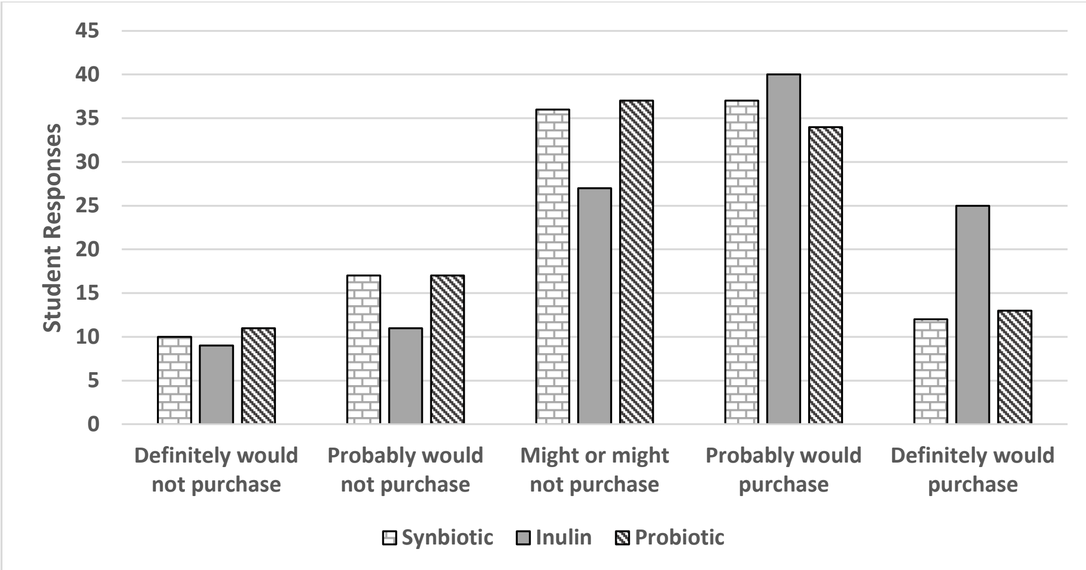

Figure 3.4 Willingness to buy Strawberry-Vanilla Yogurt Drinks

As expected from the overall liking scores, more students indicated that they “probably would” or “definitely would” purchase treatment I (no probiotic added). Treatments S and P have similar distribution on the chart. The graph as a whole is skewed to the right, so if it was available as an a la carte item, more students indicated that they might purchase or would purchase the drink (all treatments) than would not.

One of the major barriers for this drink is that it will not be part of school lunch. It will have to be an a la carte item that students choose to purchase outside of what is already provided by the school. These results show that a good percentage of students would be willing to purchase the drink if it was available to them as an ala carte item. However, Figure 3.6 shows the amount students would be willing to pay for this product, and most of them would not go over $1.50, and many of them would prefer to pay less.

This price point may not be realistic if any profit is going to be made from these drinks. Similar products on the market (in grocery stores) are rarely priced below $1.25 per bottle.

Figure 3.5 Purchase Price for Strawberry-Vanilla Yogurt Drink (preferred sample)

Differences

Some students claimed they noticed no differences between yogurt drinks, or that they did notice a difference, but could not pin-point what that difference was. Differences in flavor, sweetness or tartness (usually mentioned together), texture, and aroma were noted by some respondents. These responses were grouped into the most common, and those are displayed in Figure 3.8. Some students cited differences in more than one category. These are included in the category “multiple differences.”

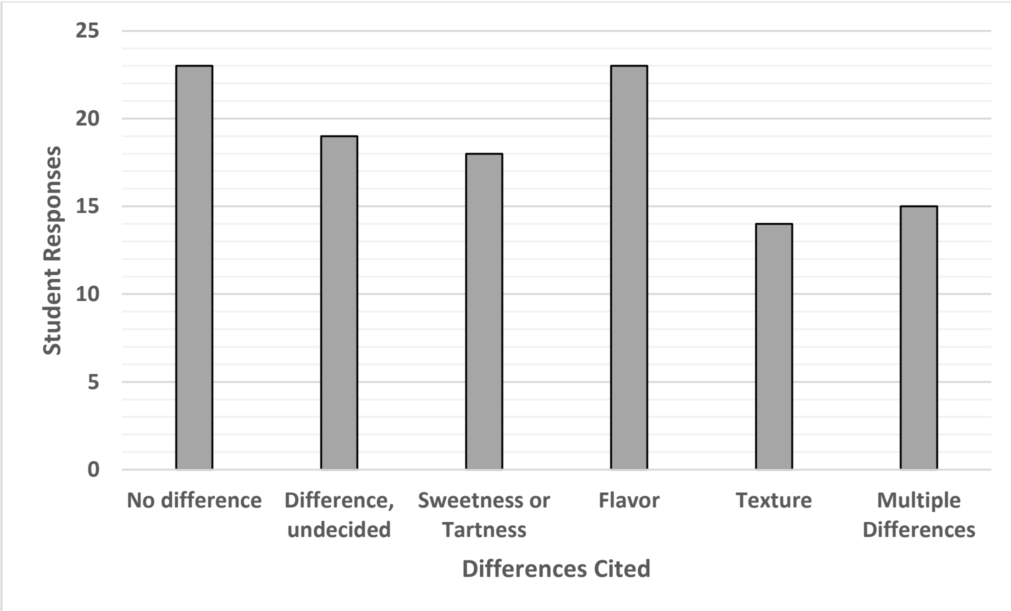

Figure 3.6 "Differences" by category for 3 Strawberry-Vanilla Yogurt Drinks

Differences between samples were noted by respondents. However, the question was asked in such a manner that students rarely indicated what differences they noticed with specific samples. Therefore, this data cannot be used to determine if there were consistent noticeable differences between specific samples. However, it does tell us that over 75 percent of students surveyed claim that there was some kind of noticeable difference among treatments .

Consumer Behavior

Some demographic information was collected and is shown in Figure 3.8. It shows that over half of students surveyed do purchase a la carte items at least once per week. It also shows that over half of the students do not consume yogurt more than once every 1-2 weeks, and about two thirds of students consume milk every day.

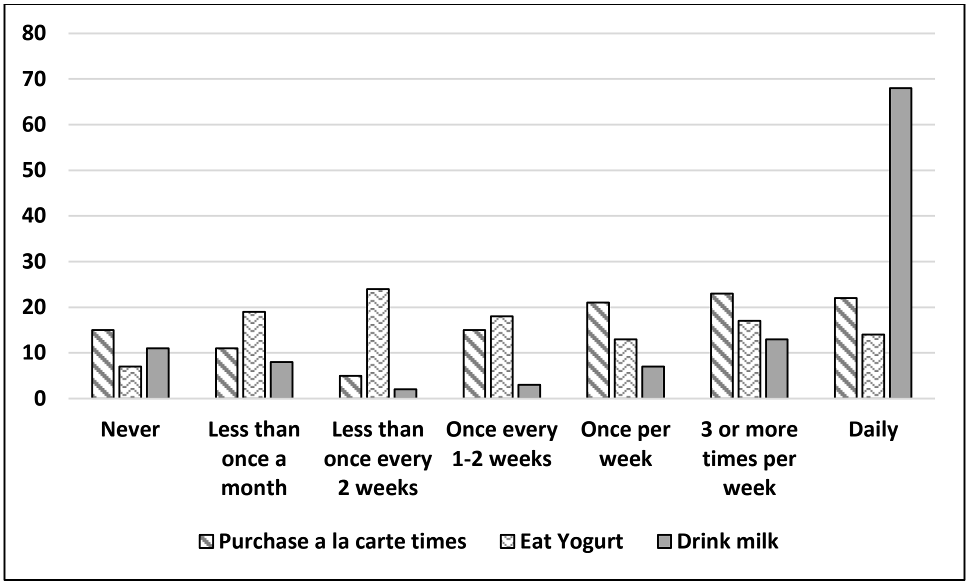

Figure 3.7 Purchasing Behavior, Yogurt and Milk Consumption of Survey Participants

Due to the low yogurt consumption and relatively low frequency of a la carte purchases, it is unlikely that the drink will have the desired effect of improving immunity in children and reducing sick days. If this product was offered as an alternative to milk (as was originally hoped), some students would likely choose to drink it every day. If that were to occur, a reduced number of sick days may be seen. This is not currently possible, due to strict regulatory control over what can and cannot be served at school lunch. Regulations clearly state that children can only be offered fluid milk, and that it must be low-fat, or non-fat if sugar and flavor is added.

Chapter 4 - Conclusions

There were few significant differences between the physical and chemical parameters of the three yogurt treatments over shelf-life. Based on sensory results, the synbiotic drink was found to be acceptable to the target consumer, though the sample with inulin only (I) scored slightly higher in most categories, including "overall liking." A shelf-life of 28 days was found to be acceptable for the product, based on chemical, physical, and microbial analysis.

Most students indicated that they did detect a difference between samples. Whether these differences are truly due to the synbiotic nature of the product is unknown. Because there were no significant differences between treatments S and P, it appears more likely that the difference comes from the addition of the probiotic *L. rhamnosus*.

In order to determine if this particular synbiotic relationship produces off-flavors, a descriptive panel will likely be required. A control yogurt with no prebiotic (inulin) or probiotic (*L. rhamnosus*) added should be included in such a study. Different strains of *L. rhamnosus* (including *L. rhamnosus* GG) should also be included, because it is possible that different strains of this probiotic bacteria produce different, greater, or fewer byproducts in the presence or absence of inulin or other prebiotics.

An adequate amount of data demonstrates the potential of *L. rhamnosus* strains for immunity enhancement, both in children and adults, though children have been most frequently studied (Hatakka and others 2001, Sheih and others 2001, Hojsak and others 2010, Kumpu and others 2013). Yogurt and yogurt drinks are likely an acceptable and cost-effective delivery medium for this probiotic, especially if inulin or another acceptable prebiotic is added to facilitate probiotic growth.

If school lunch regulations were changed to include dairy drinks that are nutritionally equivalent to milk, drink options that have health benefits beyond their nutritional value may be offered. Further research should be conducted in schools to see if a drink like this could reduce overall sick days, if introduced as a milk option. If studies in public schools are not possible, home studies, studies in private schools, or additional studies at private daycare facilities may be conducted. Positive results could be used as evidence to support regulatory changes for public schools. The economic impact of fewer secondary infections (doctors' visits and antibiotic treatments) and sick days (parents staying home/missing work to take care of sick children) could be astounding.

Future Work

The sensory test indicated that some additional work should be done on this product before moving to scaled-up processing. Goals of additional work would be to increase the sweetness level and strawberry flavor, if possible (without compromising nutrition or clean labeling). These adjustment should be made after the product can be made using an HTST pasteurizer/homogenizer, and an industrial yogurt manufacturing setting, to see what kind of impact the process changes have on the flavor. Adjustments to the formula will be relatively small. It is unlikely these adjustments will have any negative impact on consumer acceptance.

Additionally, it was discovered that the "natural red color" obtained from Gold Coast Flavors had added preservatives (sodium benzoate and potassium sorbate). Unfortunately, this was discovered after all analytical and sensory testing had been completed. It was assumed that their "natural" claim indicated a preservative-free product. However, a spec sheet could not be obtained for a significant period of time, after which it was too late to replace it with an alternative color. A new coloring with no preservatives will be obtained prior to commercialization.

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Appendix A - Sensory Screener

Figures A.1 and A.2 were shown at the beginning of the sensory screener. Figure A.1 was the initial screen parents saw after they had successfully logged into the system. Figure A.2 shows the screen on which the parents were asked about their child's gender. This was for demographic information only; students were not eliminated or accepted to the test based on gender.

Figure A.3 shows the screener page that asked for the child's age (demographic information). Figure A.4 shows the allergen question. If the child had any known allergens, they were immediately disqualified (Figure A.12).

Children were disqualifed if they had lactose intolerance (Figure A.5), or if the parent did not select yogurt as a "food their child eats" (Figure A.6). If disqualifed, parents were taken directly to the disqualification page (Figure A.12).

Figure A.7 shows the question about frequency of yogurt consumption. If "never" was selected, the child was disqualified. Otherwise, children were allowed to participate. Figure A.8 shows a question about purchasing food at school. This was for demographic information only.

Figure A.9 shows the screen on which parents were asked to provide their child's first and last name, if they qualified for the study. The parents were then asked if their child would want to participate (Figure A.10), and if so, to give them consent to participate (Figure A.11).

If, at any point, the child was disqualified from participating in the study, a screen (Figure A.12) would be displayed which informed them that their child was not qualified to participate in this particular study.

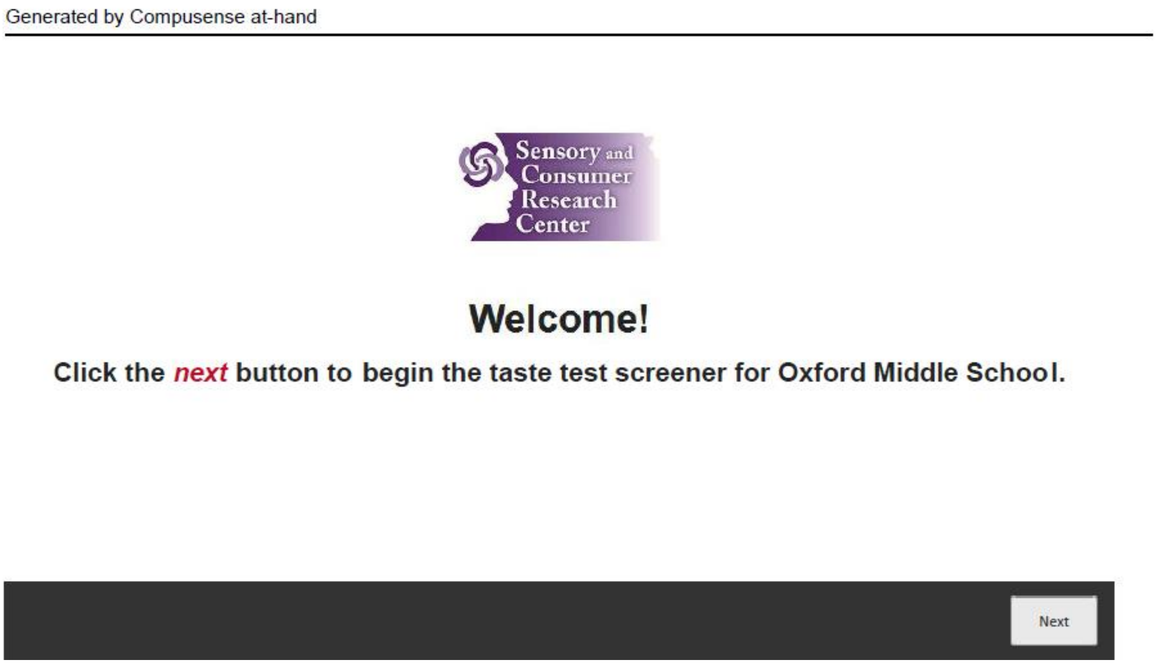


Figure A.1 Compusense Screener – Welcome

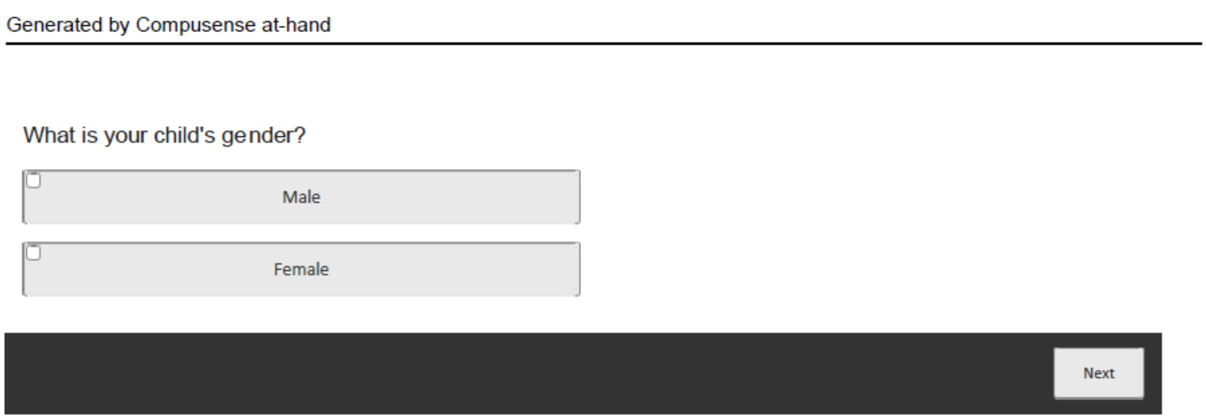
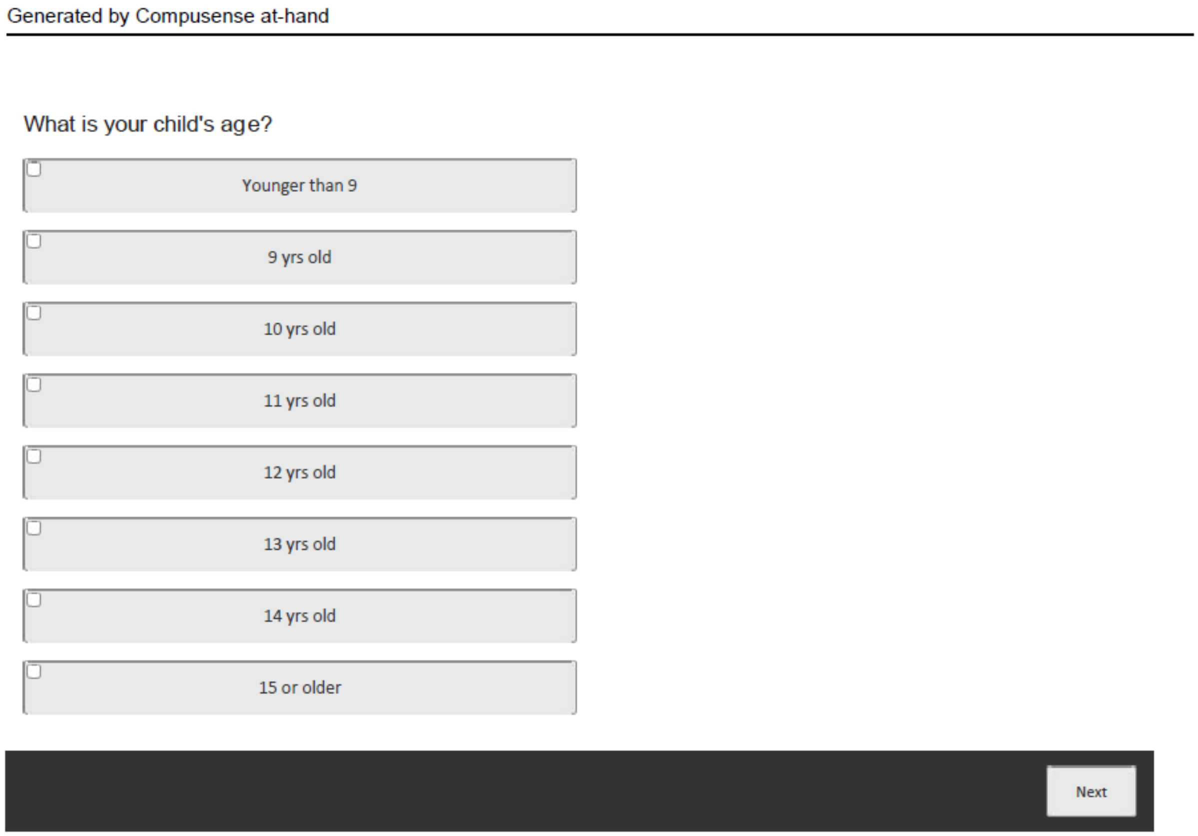


Figure A.2 Compusense Screener - Gender

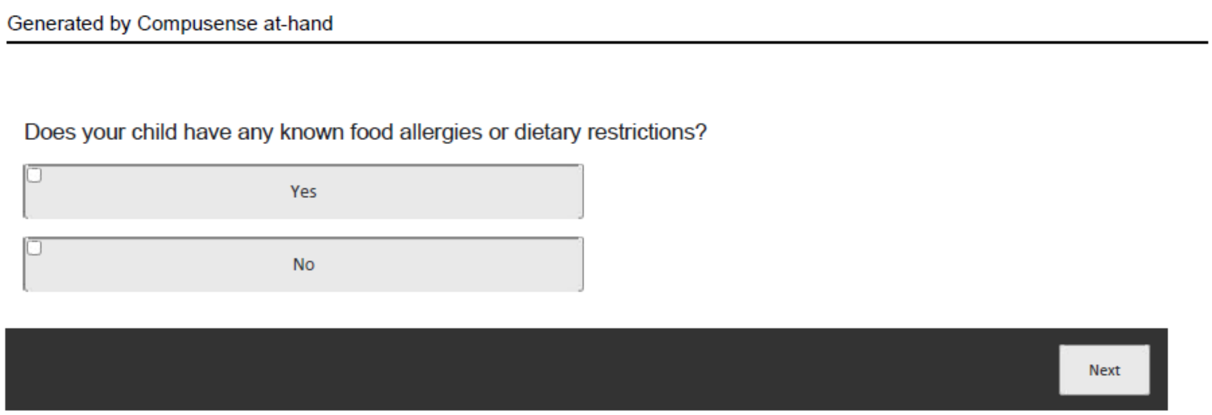

Generated by Compusense at-hand

What is your child's age?

- Younger than 9
- 9 yrs old
- 10 yrs old
- 11 yrs old
- 12 yrs old
- 13 yrs old
- 14 yrs old
- 15 or older

Next

Figure A.3 Compusense Screener - Age


Generated by Compusense at-hand

Does your child have any known food allergies or dietary restrictions?

- Yes
- No

Next

Figure A.4 Compusense Screener - Food Allergies

Generated by Compusense at-hand

Is your child lactose intolerant?

- [ ] Yes
- [ ] No

Next

Figure A.5 Compusense Screener - Lactose Intolerance

Generated by Compusense at-hand

Which of the following foods does your child eat? (Check all that apply)

- [ ] Bread ???
- [ ] Pasta ???
- [ ] Yogurt ???
- [ ] Rice ???
- [ ] Fruit ???
- [ ] Milk ???
- [ ] None of the above

Next

Figure A.6 Compusense Screener – Foods

You indicated that your child eats yogurt. Approximately how often does he/she consume yogurt or yogurt drinks?

- Daily
- 3 or more times per week
- 1-2 times per week
- Once every 2 weeks
- Less than once every 2 weeks
- Less than once a month
- Never

Next

Figure A.7 Compusense Screener - Frequency of Yogurt Consumption

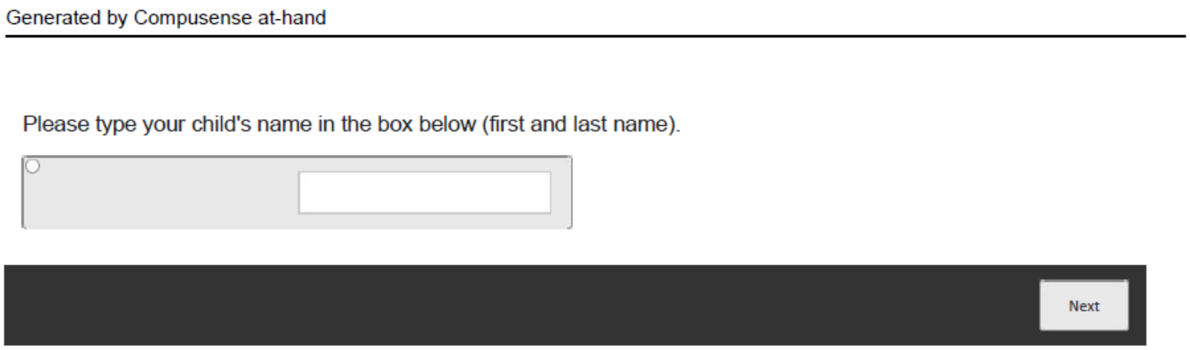
Generated by Compusense at-hand

How often does your child purchase lunch or a la carte items at school?

- Daily
- 3 or more times per week
- 1-2 times per week
- Once every 2 weeks
- Less than once every 2 weeks
- Less than once a month
- Never

Next

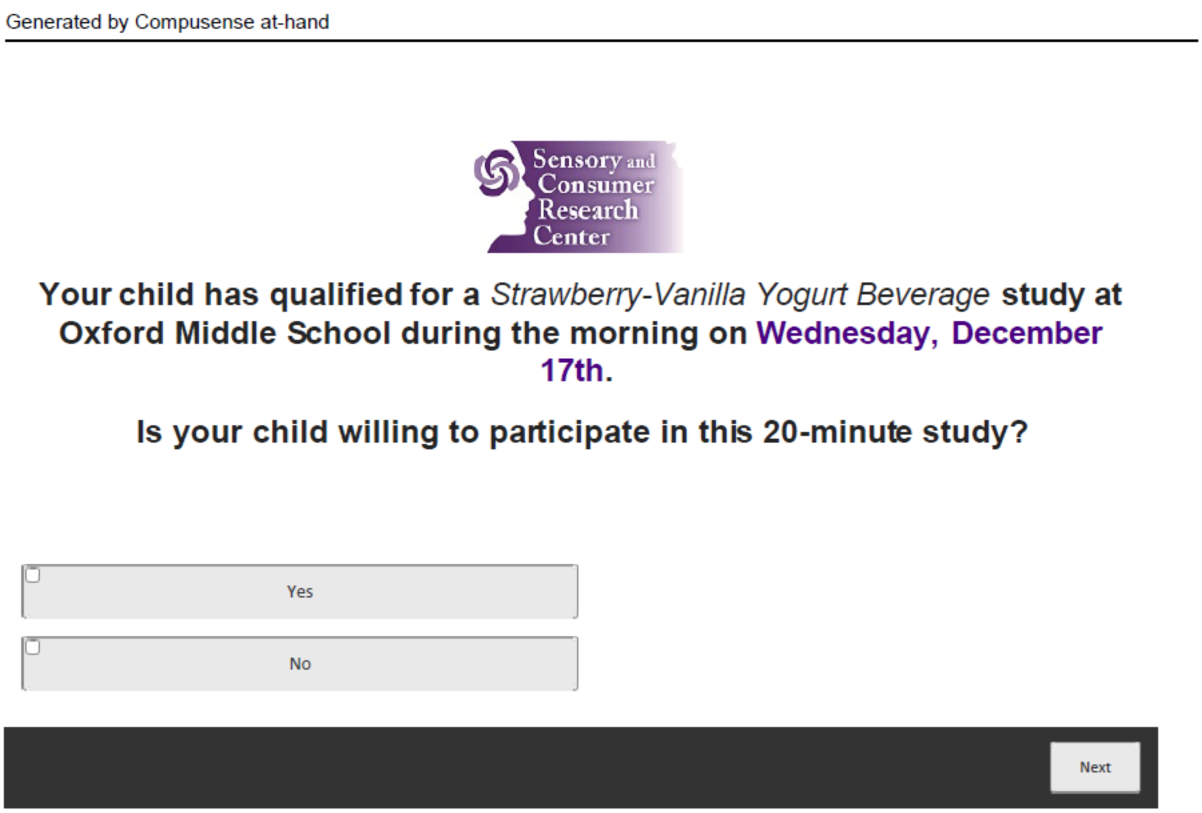
Figure A.8 Compusense Screener - Purchase of Lunch or A la Carte Items


Generated by Compusense at-hand

Please type your child's name in the box below (first and last name).

Next

Figure A.9 Compusense Screener - Name of Child


Generated by Compusense at-hand

Sensory and Consumer Research Center

Your child has qualified for a *Strawberry-Vanilla Yogurt Beverage* **study at Oxford Middle School during the morning on Wednesday, December 17th.**

Is your child willing to participate in this 20-minute study?

Yes

No

Next

Figure A.10 Compusense Screener – Child Participation

Generated by Compusense at-hand

1. I will allow my child to participate as a panelist in research conducted by K-State Sensory and Consumer Research Center.

2. I understand that the purpose of this project is to participate in a tasting of strawberry-vanilla flavored yogurt beverage.

3. My child has no food allergies and is not lactose intolerant.

By typing my name in the space below, I am providing my electronic signature and acknowledging that I understand the above statements.

Thank you for your consent. After entering your name, the survey is completed.

Please click NEXT to save your responses.

You may see an error message after this screen- Please ignore the error message, as your results have been submitted.

Next

Figure A.11 Compusense Screener – Parental Consent

Generated by Compusense at-hand

Sensory and Consumer Research Center

Thank you for completing this survey.

Unfortunately, your child did not qualify for the taste test.

Finished

Figure A.12 Compusense Screener – Not Qualified

Appendix B - Sensory Questionnaire

Figures B.1 and B.2 were shown at the beginning of the sensory test. Figure B.1 was the initial screen the students saw after they had successfully logged into the system. Figure B.2 shows the screen on which the students were asked to give consent to the terms of the sensory test prior to tasting anything.

Figure B.3 is an image of the slide shown prior to tasting each of the samples, which gave instructions for students to clear their palates with water and unsalted saltine cracker before tasting. Figure B.4 gives instructions to smell the drink, in order assess the aroma of the drinks in the questions which followed (Figure B.5). Figures B.4 and B.5 were shown for all samples in order following Figure B.3.

Figure B.6 gave students the instruction to taste the drink, in order to answer questions over the flavor and texture of the yogurt. Figures B.7 and B.8 show the questions over flavor and texture which the students were asked. Questions over "liking" were asked on a 1-9 hedonic scale. Questions over flavor levels were asked on a "just-about-right" scale with 3 being "just-about-right", 1 being "too weak", and 5 being "too strong" These screens were shown in order, after the aroma questions, for each sample.

Figures B.9 and B.10 were shown for all drinks. Figure B.9 shows the questions asked after the "just-about-right" scales for all samples. The questions were designed to force students to comment on what they liked and did not like about each sample. The following screen (Figures B.10) was used to determine how likely students would be to purchase the yogurt drink if it was available as an a la carte item.

Figure B.11 shows a question which was asked after all of the samples were tasted. The students were asked how much they would pay for the sample they liked the best. Figure B.12

shows the difference question. The question was open-ended so students could express what they thought was different about samples without introducing bias.

Figures B.13-B.15 show demographic questions which were asked after all samples were tastes. The purpose of asking these questions was to see if there were any relationships between students who liked or did not like the samples, and what they considered to be their normal consumer behavior. The last screen (Figure B.16) indicated that they were finished with the test, and thanked them for participating in the study.

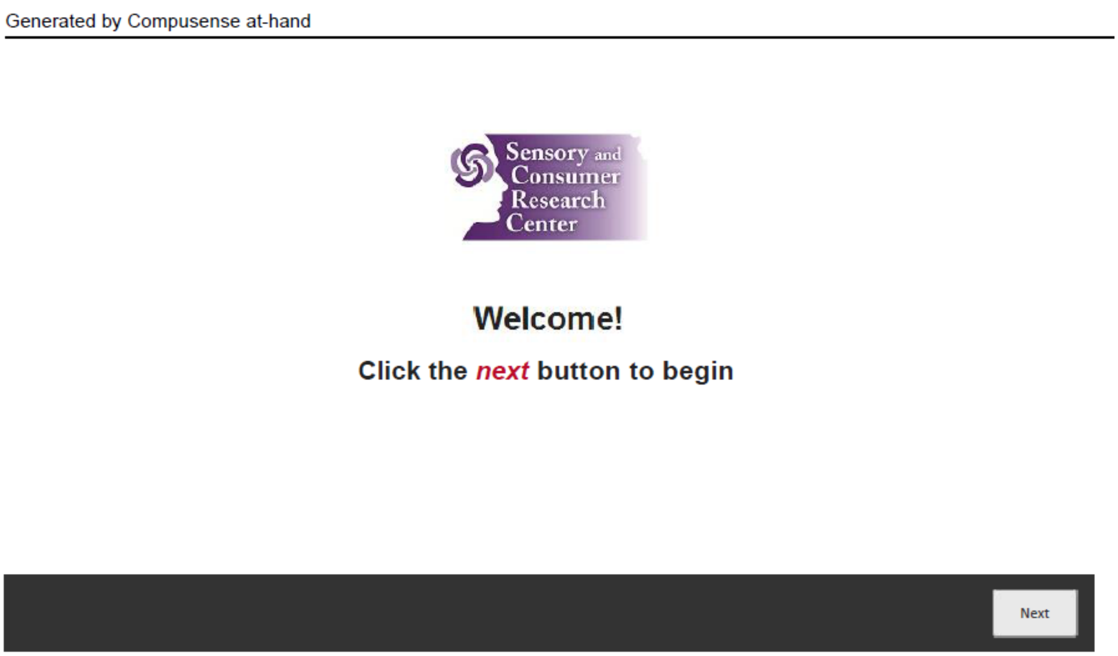
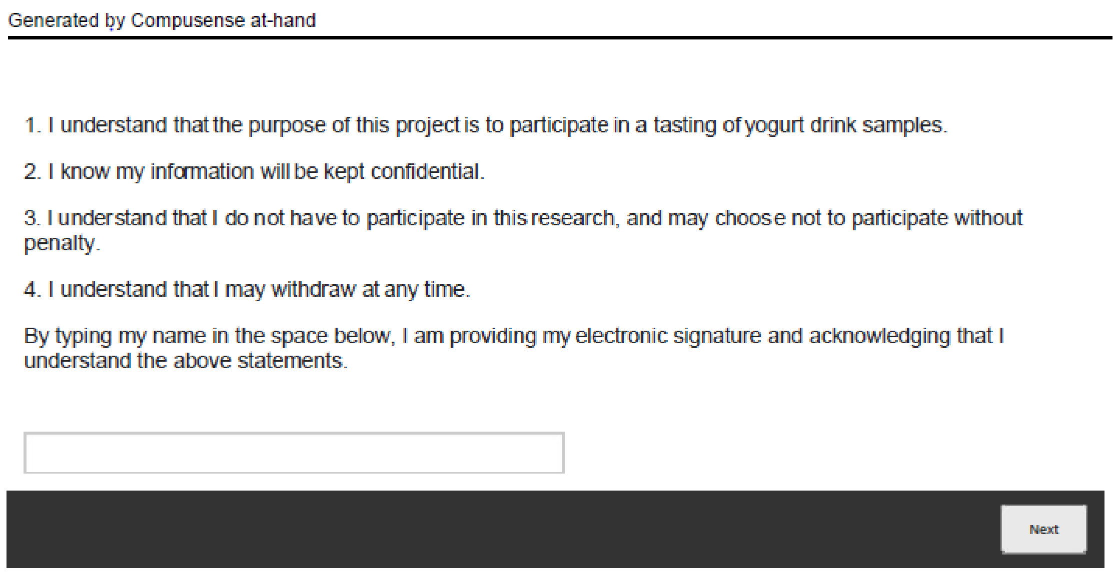


Figure B.1 Compusense Sensory Test - Welcome



Generated by Compusense at-hand

1. I understand that the purpose of this project is to participate in a tasting of yogurt drink samples.

2. I know my information will be kept confidential.

3. I understand that I do not have to participate in this research, and may choose not to participate without penalty.

4. I understand that I may withdraw at any time.

By typing my name in the space below, I am providing my electronic signature and acknowledging that I understand the above statements.

Next

Figure B.2 Compusense Sensory Test - Consent

Figure B.3 Compusense Sensory Test - Palate Cleanse

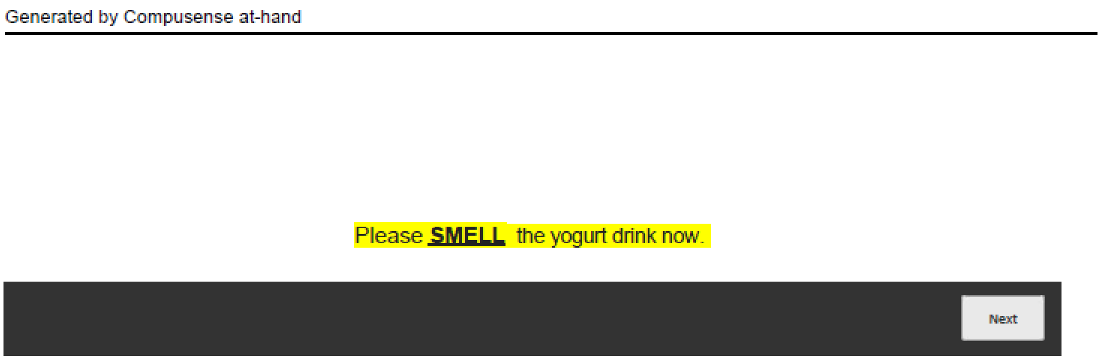


Figure B.4 Compusense Sensory Test - Aroma 1

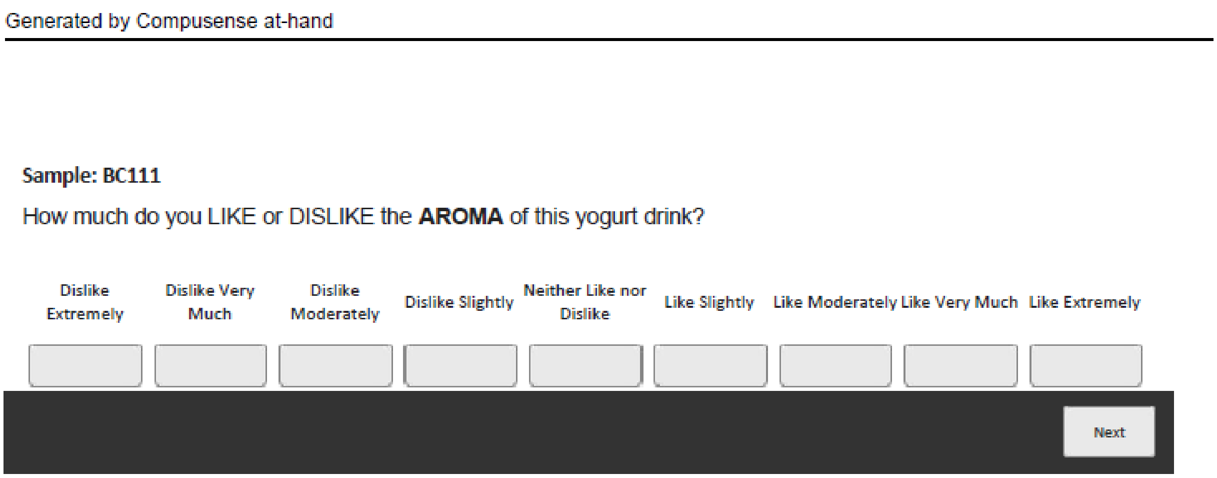


Figure B.5 Compusense Sensory Test - Aroma 2 (Q1 for each drink)

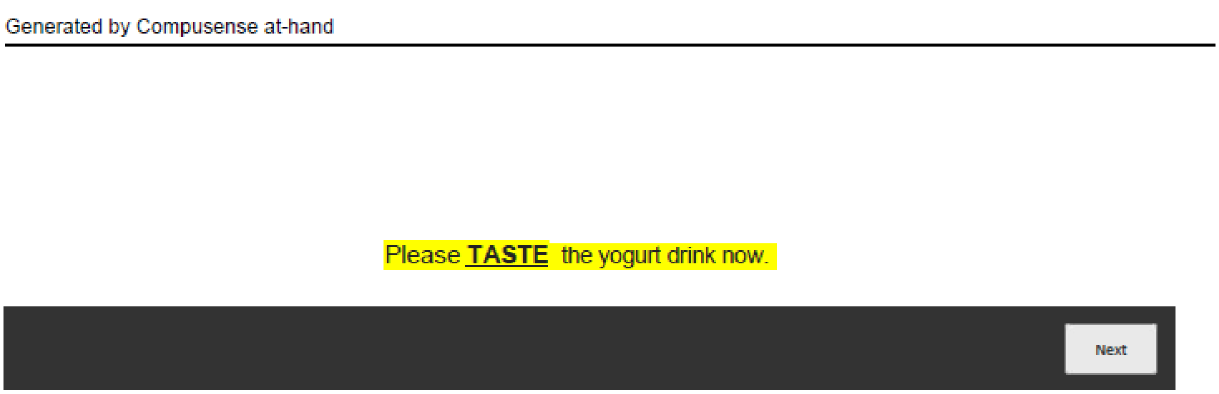
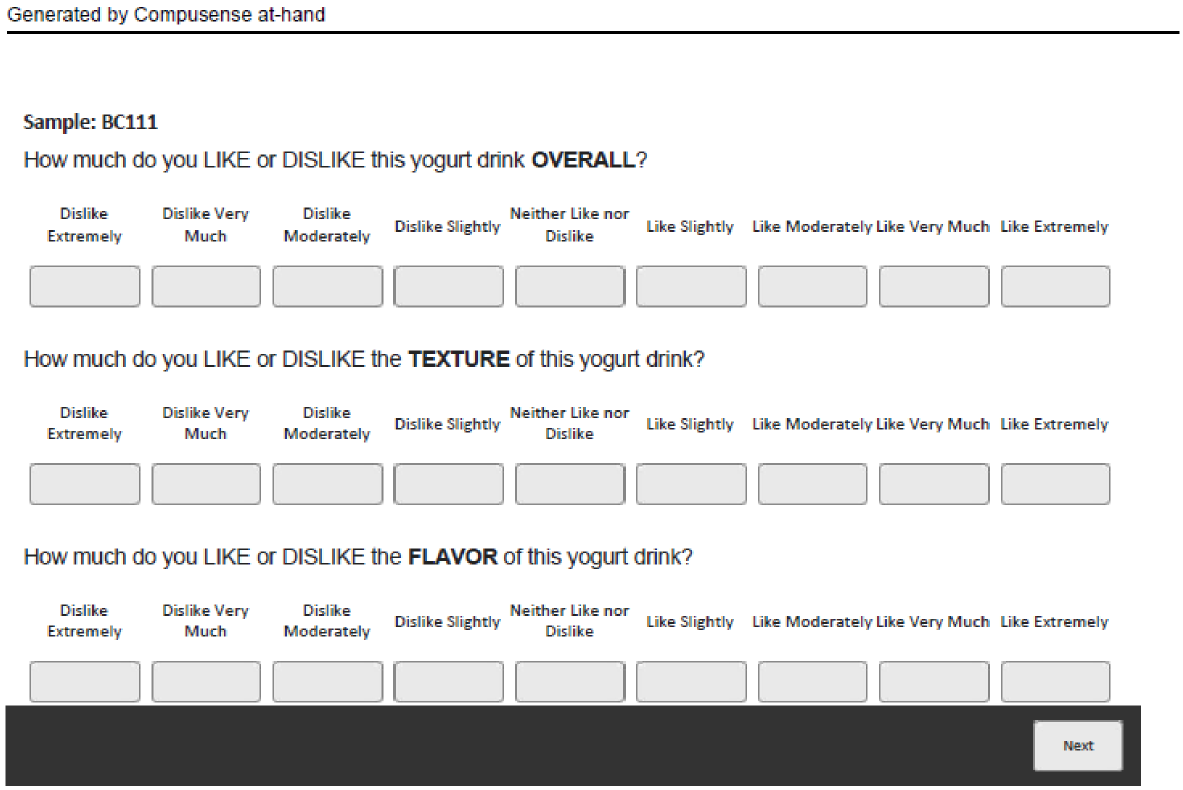


Figure B.6 Compusense Sensory Test - Taste 1



Generated by Compusense at-hand

Sample: BC111

How much do you LIKE or DISLIKE this yogurt drink **OVERALL**?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you LIKE or DISLIKE the **TEXTURE** of this yogurt drink?

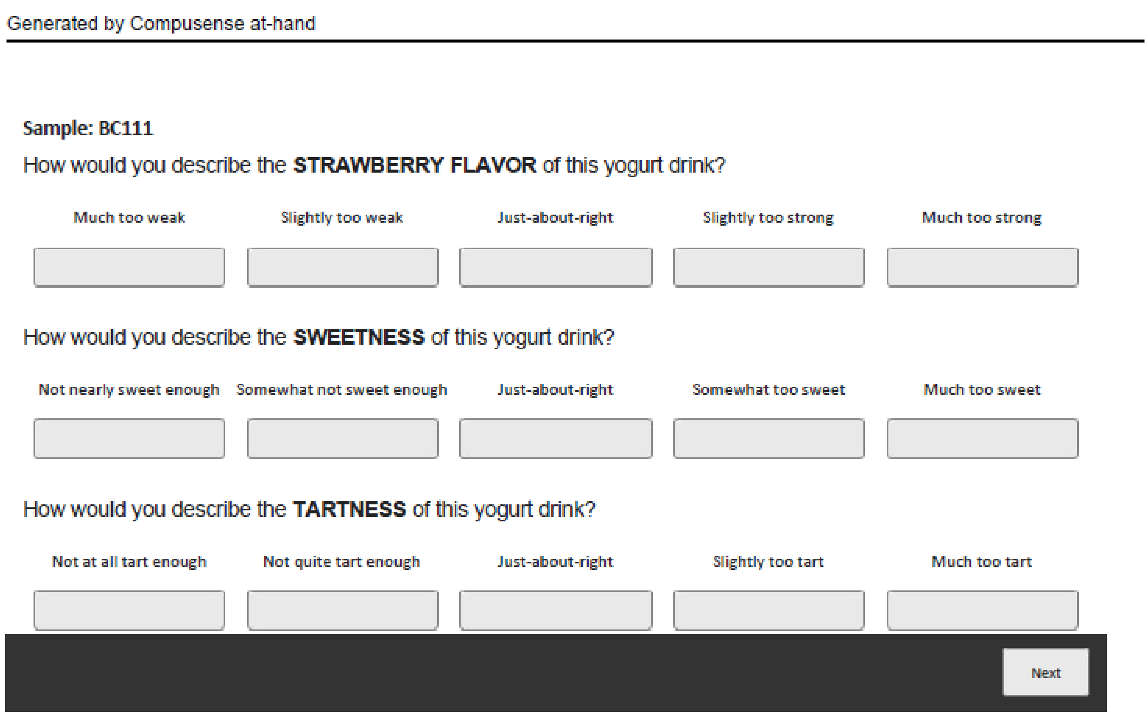
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How much do you LIKE or DISLIKE the **FLAVOR** of this yogurt drink?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Next

Figure B.7 Compusense Sensory Test - Taste 2 (Q2-4 for each drink)



Generated by Compusense at-hand

Sample: BC111

How would you describe the **STRAWBERRY FLAVOR** of this yogurt drink?

Much too weak	Slightly too weak	Just-about-right	Slightly too strong	Much too strong

How would you describe the **SWEETNESS** of this yogurt drink?

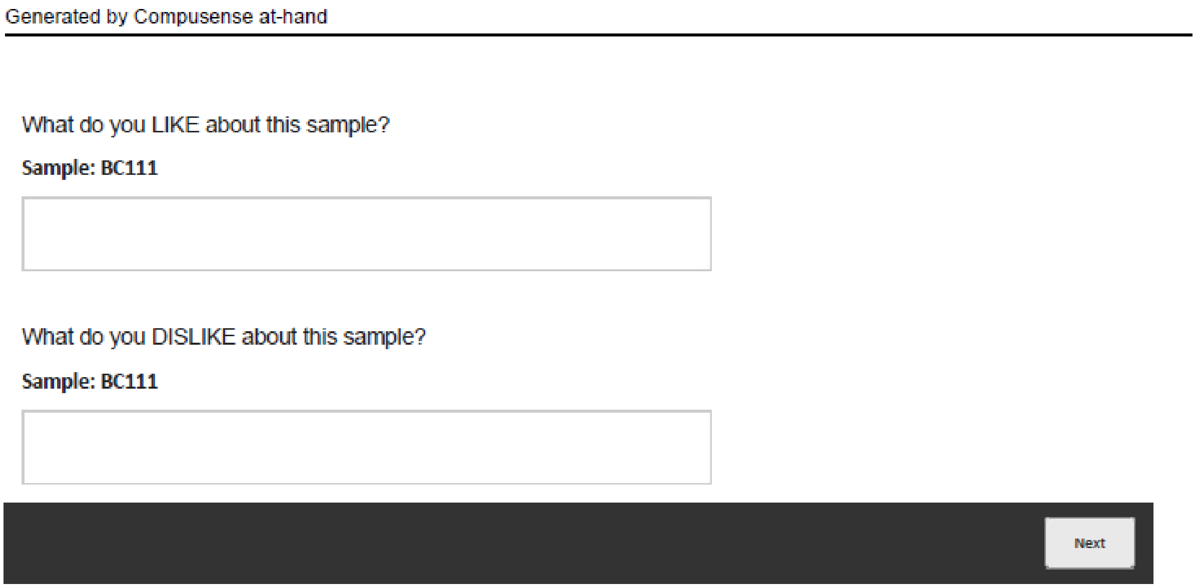
Not nearly sweet enough	Somewhat not sweet enough	Just-about-right	Somewhat too sweet	Much too sweet

How would you describe the **TARTNESS** of this yogurt drink?

Not at all tart enough	Not quite tart enough	Just-about-right	Slightly too tart	Much too tart

Next

Figure B.8 Compusense Sensory Test - Taste 3 (Q5-7 for each drink)



Generated by Compusense at-hand

What do you LIKE about this sample?

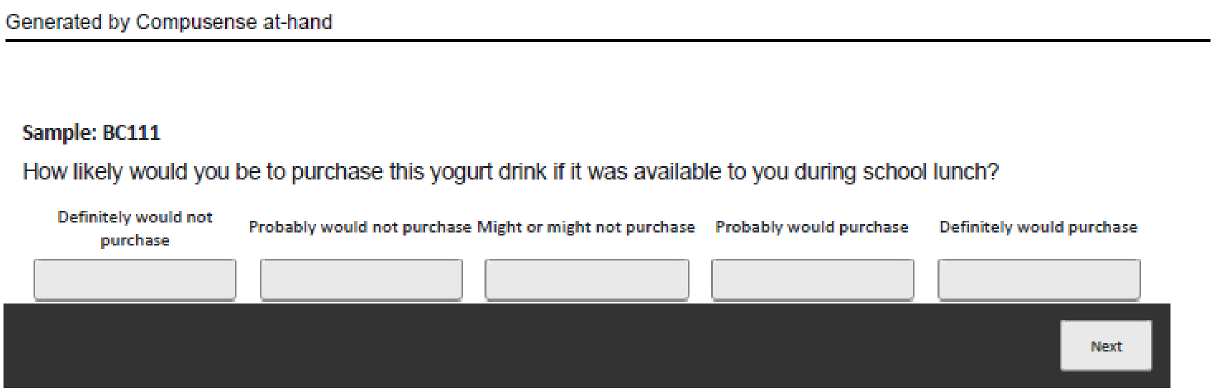
Sample: BC111

What do you DISLIKE about this sample?

Sample: BC111

Next

Figure B.9 Compusense Sensory Test - Taste Like and Dislike (Q8-9 for each drink)



Generated by Compusense at-hand

Sample: BC111

How likely would you be to purchase this yogurt drink if it was available to you during school lunch?

Definitely would not purchase	Probably would not purchase	Might or might not purchase	Probably would purchase	Definitely would purchase

Next

Figure B.10 Compusense Sensory Test - Purchase Intent 1 (Q10 for each drink)

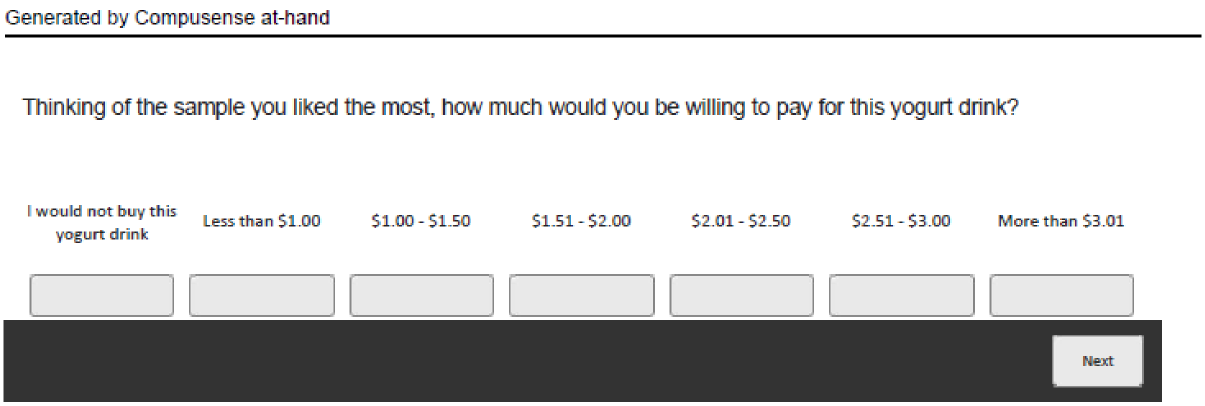


Figure B.11 Compusense Sensory Test - Purchase Intent 2

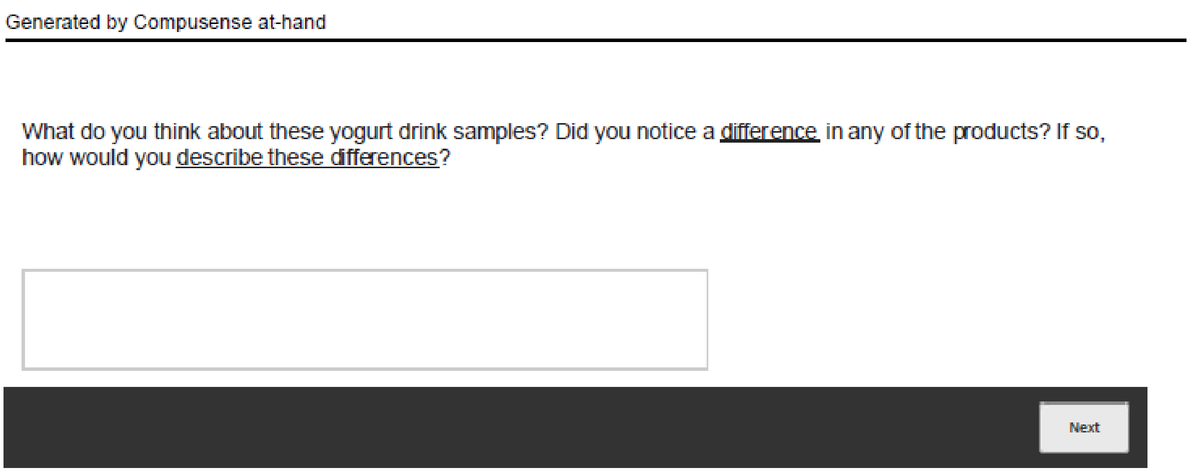
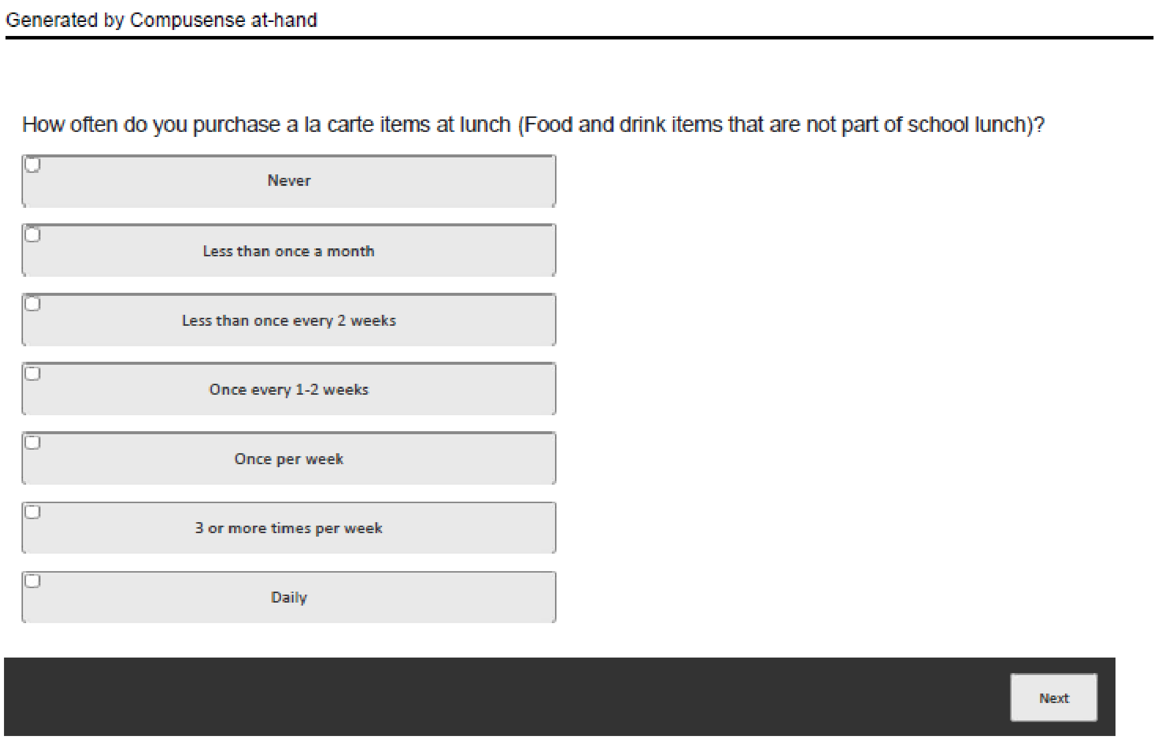


Figure B.12 Compusense Sensory Test - Differences

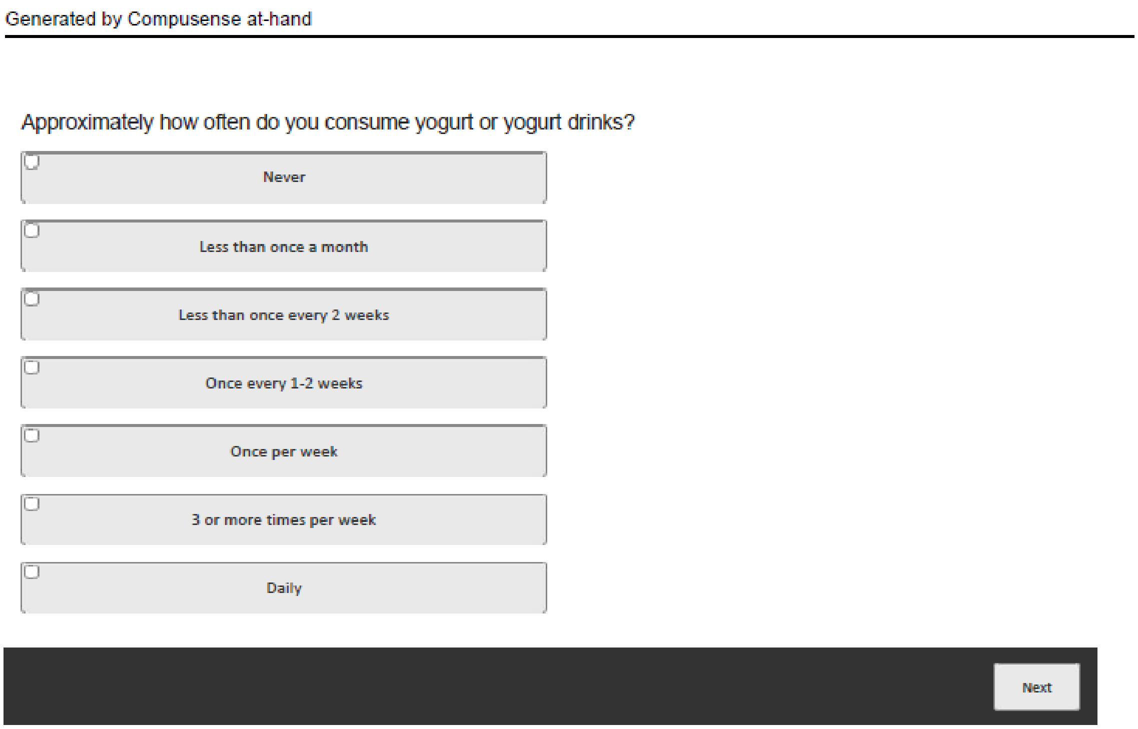

Generated by Compusense at-hand

How often do you purchase a la carte items at lunch (Food and drink items that are not part of school lunch)?

- Never
- Less than once a month
- Less than once every 2 weeks
- Once every 1-2 weeks
- Once per week
- 3 or more times per week
- Daily

Next

Figure B.13 Compusense Sensory Test - Consumer Behavior A la Carte


Generated by Compusense at-hand

Approximately how often do you consume yogurt or yogurt drinks?

- Never
- Less than once a month
- Less than once every 2 weeks
- Once every 1-2 weeks
- Once per week
- 3 or more times per week
- Daily

Next

Figure B.14 Compusense Sensory Test - Consumer Behavior Yogurt Consumption

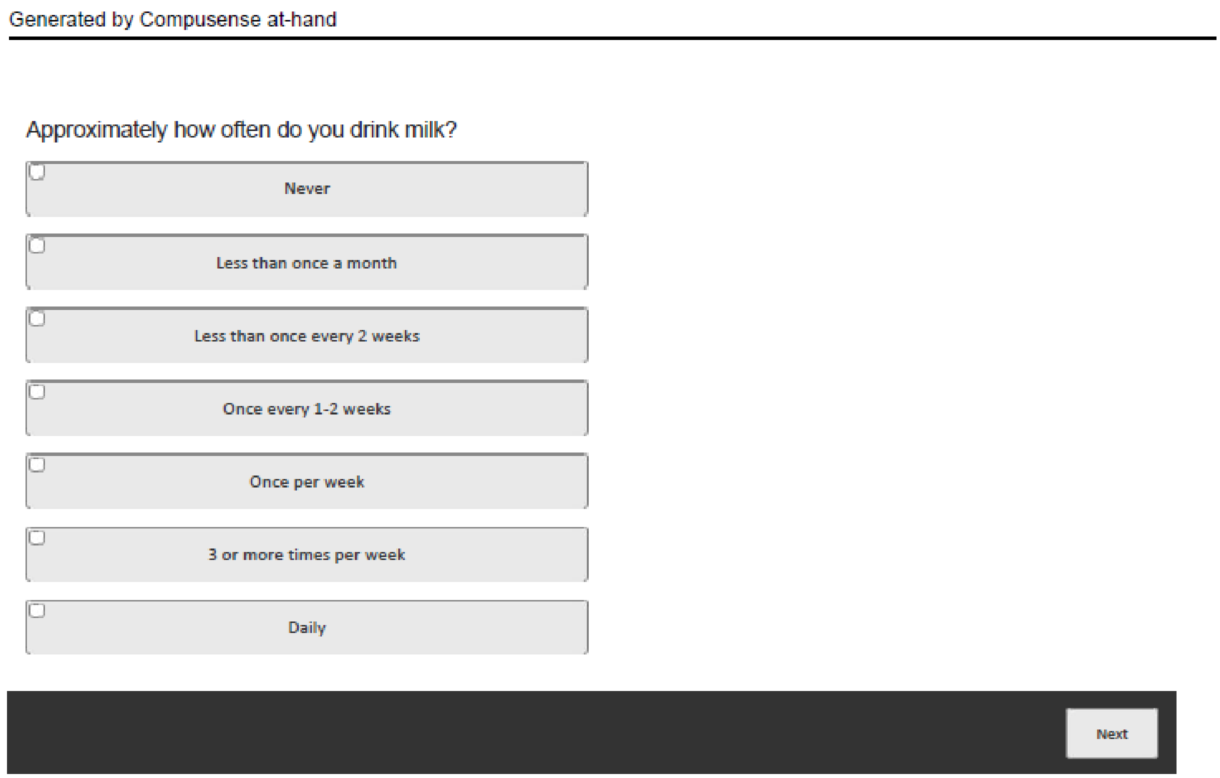


Figure B.15 Compusense Sensory Test - Consumer Behavior Milk Consumption

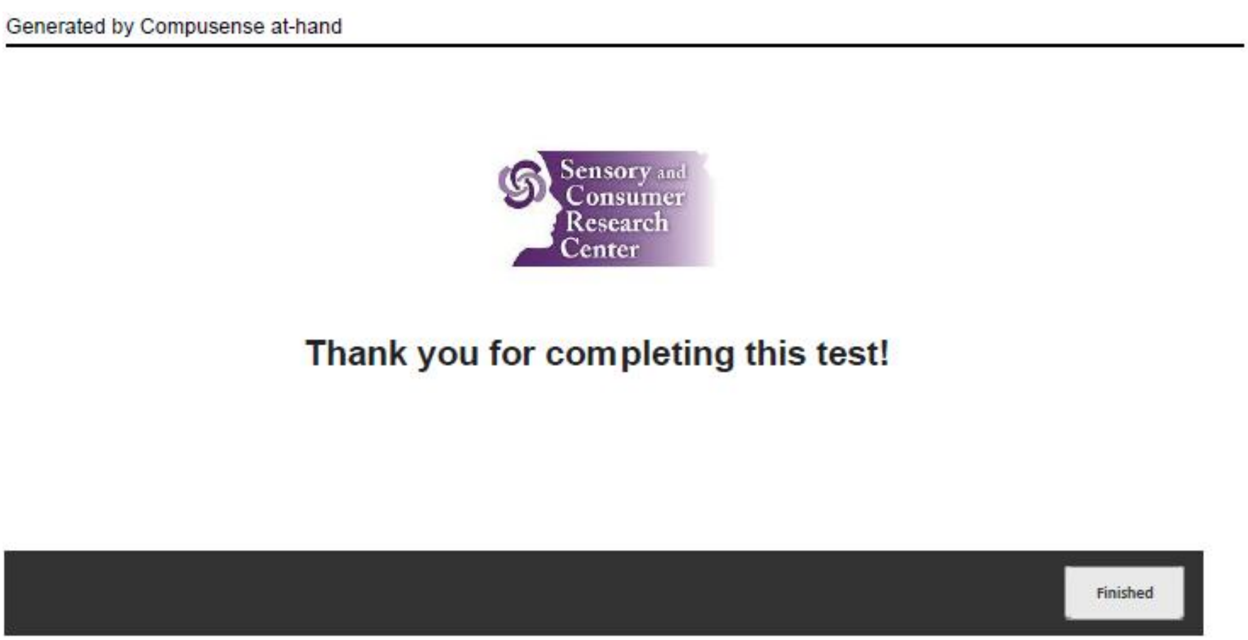


Figure B.16 Compusense Sensory Test - Thank You