ABSTRACT

Title of Document:	AN INVESTIGATION INTO THE EFFICACY OF A PH-SENSITIVE MATERIAL FOR MILK SPOILAGE DETECTION
	Tom Blackmon, Jonathan Chang, Amy Cheng, Tiffany Jen, Hannah Kravis, Raishay Lin, Michael Lu, Erin Ong, Tanya Pakzad, Nima Sarfaraz, Yvonne Shiau, Jacklyn Wong
Directed By:	Dr. Nam Sun Wang, Associate Professor, Department of Chemical Engineering

Ambiguous expiration dates on milk cartons can mislead consumers into prematurely disposing unspoiled milk and potentially drinking spoiled milk. These misconceptions can lead to wastage that harms the environment, or potential discomfort and illness. The incorporation of pH-sensitive indicators into plastic milk cartons has the potential to replace stamped expiration dates as the traditional method of milk spoilage indication. We studied the correlation between bacteria count and milk pH to establish pH measurement as an effective indicator of milk quality. We then developed a method for incorporating bromothymol blue, a pH-sensitive color-changing dye, into a hydrogel made of polyacrylamide. This hydrogel can be added to existing packaging for milk or other products with detectable pH changes. Additionally, we conducted a consumer survey and analyzed current food packaging trends in the market. Our research indicates that a spoilage-indicating milk carton could have strong market potential as food industries increasingly adopt intelligent packaging designs.

AN INVESTIGATION INTO THE EFFICACY OF A PH-SENSITIVE MATERIAL FOR MILK SPOILAGE DETECTION

By

Team Milk

Tom Blackmon, Jonathan Chang, Amy Cheng, Tiffany Jen, Hannah Kravis, Raishay Lin, Michael Lu, Erin Ong, Tanya Pakzad, Nima Sarfaraz, Yvonne Shiau, Jacklyn Wong

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Advisory Committee:

Dr. Nam Sun Wang, Chair
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1. Introduction

1.1 How does milk spoil, and why is it a problem?

Milk spoils when microorganisms packaged with the milk, such as bacteria, yeasts, and molds produce by-products that cause further contamination (Ledenbach, 2009). The most common types of bacteria that cause milk spoilage grow at the low temperatures at which milk is typically stored (3–7◦C) and use large molecules of proteins and lipids for growth (Ledenbach, 2009).

Industry standards define the pH of fresh bovine milk as 6.7 (O'Connor, 1995). Bacterial growth in stored milk increases as time passes. As bacteria grow and increase, the by-products of those bacteria increase as well. Many of the bacteria found in milk, including those found in in aseptically packaged pasteurized milk, create lactic acid as a by-product. Levels of lactic acid and levels of acidity share a direct relationship: as the former increases, the latter increases as well, which causes a drop in pH. Thus, increased bacteria causes milk to "sour" as its acidity increases such that the pH level falls below 6.4. The standard for "spoiled" milk can be subjective, as milk can change to other edible forms such as buttermilk or yogurt. However, a general consensus exists among consumers and manufacturers that particular sensory characteristics, such as an acidic aftertaste, chalky mouth-feel, sourness, or pungent odor, indicate milk spoilage (Bandler & Barnard, 1984).

Consumers currently determine milk spoilage by checking a preset expiration date on the surface of milk cartons by suppliers. There are no federal regulations that require manufacturers to provide expiration dates for their products; thus, food dating is a voluntary and often inaccurate process. Discrepancies between estimated and actual

spoilage dates can differ by as much as a week when milk is kept at optimal conditions and by an even greater time span when kept in suboptimal conditions. In particular, inaccuracies arise from variable processing, shipping, and storage conditions of the milk (U.S. Department of Agriculture, 2007).

Expiration dates can be misleading because they generally come in one of three forms: “best before,” “use by,” “sell by.” Due to the multiple possible meanings of expiration dates, misconceptions among consumers are common. For example, 61 percent of surveyed consumers perceived that "sell by" dates represent the last day on which the product should be consumed rather than sold. In fact, products may be consumed for seven days or longer after that date (Tsiros & Heilman, 2005).

Whether the expiration date errs on the side of being too conservative or too generous, serious consequences may occur. Expiration dates that are listed too conservatively often lead to wastage due to premature disposal. Retailers and consumers discard milk that has reached the printed expiration date, but has not yet actually spoiled (United States Department of Agriculture, 2011). In 1995, retailers lost more than 96 billion pounds of edible food with 18.1% of that being fluid milk. This approximates to 17.4 billion pounds of milk wasted by retailers because consumers would have assumed it was spoiled (Kantor et al. 1997).

Conversely, milk may spoil before the printed expiration, which can lead to food poisoning if consumed. In the U.S., more than 76 million cases of foodborne illness occur each year, resulting in 32,500 hospitalizations and 5,000 deaths (U.S. Food and Drug Administration, 2008). The discomfort and illness usually associated with the consumption of spoiled milk rarely results in hospitalization, resulting in the issue being

underreported and understudied. However, it is still a problem that many consumers have had personal experience with and would value preventing through new detection methods. This study aims to not only provide an overview of the causes and effects of milk spoilage, but also to evaluate milk bottle designs that can accurately identify the freshness of milk.

1.2 The problem with current milk spoilage detection methods

Currently, the most common method of indicating milk spoilage is through an expiration date stamp. Typically, manufacturers determine the dates of their dairy products. In some areas of the United States, manufacturers must follow local regulations regarding expiration dates. Most notably, until 2010, milk sold in New York City carried two expiration dates: one date represented the manufacturers' estimation of expiration, which is about 15 days after pasteurization; and a second date that followed stricter New York City health provisions, which dictated that milk could only be legally sold up to 96 hours, or 4 days, after 6:00 AM on the day after pasteurization (Hager, 2010; Fleischer, 2010). In Montana, the Board of Livestock forbids milk from being sold more than 12 days after pasteurization (Johnson, 2012). These varying laws further show the inconsistencies and confusion that stems from current methods of displaying milk spoilage.

Milk that is past the stamped expiration date is not necessarily unfit for consumption. There is usually a grace period spanning from several days to more than a week between the expiration date and the time that the milk actually spoils (Johnson, 2012). In addition, if milk is consumed from a bottle that was opened after the expiration date, the milk may still be fine (Johnson, 2012). Factors such as the type of milk, brand,

storage conditions and usage cause variation in the exact time until milk becomes actually spoiled. Temperature can be just as an important factor as time. A 2009 revision of the Pasteurized Milk Ordinance released by the FDA in 2009 requires that vehicles used for milk transport must be equipped to keep milk at a temperature of 45°F or below (U.S. Food and Drug Administration, 2009). However, milk may still be subjected to volatile temperatures during transfer points such as delivery from a truck to a retailer's storage area (He et al. 2010).

Aside from looking at the expiration date, the simple "sniff test" is a common informal method of milk spoilage detection. Many people check the quality of their milk by smelling or tasting their milk, but this experience can be unpleasant, misleading, and inaccurate (Moore, 2009).

We have chosen to use pH as the primary method of detecting and indicating milk spoilage. Using a modified combination of current methods, we should be able to integrate a pH indicator into a plastic material that would be able to accurately detect and indicate milk spoilage.

1.3 Why more milk spoilage detection research is necessary

Plagued by lack of regulation and consistent unreliability resulting in wastage, expiration dates are not an effective method of indicating milk spoilage. Furthermore, other food industries are demonstrating a trend towards more environmentally friendly and health-conscious packaging. Increasing interest in intelligent packaging indicates that the time is ripe for more advanced milk packaging such as packaging that can detect and indicate spoilage. While a handful of packaging companies have floated ideas for an updated milk packaging design, none of the proposed alternatives have yet succeeded on

the market.

Recently, many researchers have been searching for other ways to determine milk spoilage. Proposed theoretical methods include: gas sensor array technology, which Haugen et al. (2006) showed monitors microbial growth and signals dangerous bacterial growth; an electronic nose system, which Magan, Pavlou, and Chrysanthakis (2008) used to find the relationship between bacterial concentration and the sensitivity of detection of spoilage in milk; and short length near infrared (SW-NIR) spectroscopy, which was hypothesized by Al-Qadiri et al. (2008) and has already been established as a method for detecting spoilage in animal products such as chicken meat. A 2003 study by Sim et al. investigated disposable and low-cost taste sensors as a method for testing milk quality. Though results proved successful, taste sensors as a concept are unwieldy and difficult to integrate into a mass-marketable product.

1.4 Outline of study

In all the time since, no alternative milk spoilage detection and indication method has successfully emerged on the market. The proposed method of incorporating pH-sensitive dye into plastic packaging holds potential as a simple and cost-effective option. This study discusses the methodology for creating a pH-sensitive plastic that detects and indicates milk spoilage, and also analyzes the market potential of a product containing such a plastic.

Research Questions

This paper explores the following research questions:

1. To what degree of accuracy can an integrated pH sensor effectively indicate the spoilage of milk?
2. Would a milk spoilage detection product for individual consumers be successful in the US market?

Overview of Methodology

Laboratory methodology in this study occurred in two parts: first, creation of the plastic prototype; and second, testing the efficacy of the plastic's pH-sensitivity as pertinent to milk spoilage. Creation of the plastic required generating a polyacrylamide-based hydrogel, infusing it with bromothymol blue dye, and then allowing it to dry to become thin plastic sheet. The prototype was then subject to efficacy testing focused on two factors: whether the plastic would display a color change when in contact with a pH change and whether the plastic could specifically detect the pH change associated with milk spoilage. Tests also assessed consumer safety factors such as leaching of the dye from the plastic into any contacted liquids.

A second methodology, a survey approved by the Institutional Review Board (IRB), was created to test market potential. Researchers distributed copies of the seven-question survey at grocery stores in the Washington, D.C. metropolitan area, and collected 276 (collected 295, but had 19 incomplete surveys) completed responses. These responses were then analyzed for correlations. In particular, survey responses analysis focused on consumer values (about health, food wastage, and cost) and preferences regarding a milk spoilage indicator. The surveys also collected demographic information such as age. Additional market research was collected and used to create a hypothetical

business plan which would garner the most success in the US.

1.5 General Study Hypotheses

Hypotheses

We hypothesize:

1. An integrated pH sensor will accurately detect the spoilage of milk in a given sample.
2. A milk spoilage detection product for individual consumers would be successful in the US market.

2. Literature Review

2.1 Science Literature Review

2.1.1 Milk and Bacteria

Our research into the problems with the current milk packaging techniques encouraged us to develop a product that would accurately detect milk spoilage and convey the information to the consumer. Before determining the appropriate method of milk spoilage detection, we first analyzed the chemical nature of spoilage in milk to make an educated decision on the most proper method.

Milk spoils due to the activity of microorganisms, including aerobic psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria (Ledenbach, 2009). In particular, psychrotrophic bacteria are a major determinant of milk shelf life because they produce by-products which contaminate milk. Of the psychrotropic bacteria present in raw milk, most are pseudomonads and related aerobic, Gram-negative, rod-shaped bacteria. Pseudonomads

are especially popular due to their abilities to grow at the low temperatures at which milk is typically stored (3–7°C), and to use large molecules of proteins and lipids for growth (Ledenbach, 2009).

As bacteria grow and increase, the by-products of those bacteria increase as well. A large proportion of bacteria in milk, including *Bacillus circulans*, the dominant spoilage microbe in aseptically packaged pasteurized milk, create lactic acid as a by-product. Ostlie, Helland, and Narvhus (2003) conducted a study to analyze the amount of metabolic products produced by five specific probiotic strains in ultra-high temperature (UHT) treated milk. They discovered that the pH of the UHT milk decreased from 6.7 initially to 3.9 - 4.4 after 24 hours of incubation. This decrease in pH was due to the lactic acid produced as a byproduct of the bacteria. High bacterial levels in milk correlate to high levels of lactic acid. The presence of lactic acid lowers pH.

Industry standards define the pH of fresh bovine milk as 6.7 (O'Connor, 1995). Over time, bacterial growth in stored milk increases. With increased levels of bacteria come increased levels of acidic bacterial by-product. Thus, increased bacteria causes milk to "sour" as its acidity increases such that the pH level falls below 6.4. The standard for "spoiled" milk can be subjective.

To determine the composition of spoiled milk, Fromm and Boor (2004) conducted a research study in order to discover the external and internal conditions of pasteurized fluid milk (2% HTST milk) during its shelf life. They discovered that free fatty acid levels, which contribute to the sour taste of spoiled milk, had increased during shelf life while casein levels dropped (Fromm & Boor, 2004). Lactic acid buildup contributes the most to the rise of fatty acid levels in milk.

Ideally, any milk spoilage detector should be effective for a wide a range of milks types. Deeth, Khusniati, Datta, and Wallace (2002) confirmed that all bacteria isolated from both skim and whole milk during spoilage were strains of *Pseudomonas* species and grew at similar rates. However, their enzymatic analysis showed that the bacteria displayed different metabolic behaviors in the different types of milk. The different spoilage patterns of skim and whole milk are not due to bacterial growth rates, but rather the acidity that arises from by-products of lipid and protein breakdown. Another study that correlated *Pseudomonas aeruginosa* counts with lowest percentage of fat and protein content in milk supports the conclusion that *Pseudomonas aeruginosa* is associated with milk spoilage (Yagoub et al. 2008). Other species of *Pseudomonas* have also been linked to spoilage patterns (Deeth et. Al. 2002).

2.1.2 Detecting Spoiled Milk

After establishing an understanding of the spoiled milk and its properties, we then had to identify a system for detecting milk spoilage. In order to best appeal to our projected demographic, this proposed system must be accurate, consumer-friendly, and cost-efficient.

Many possible methods of detecting spoilage had already been considered. Our research focused on seven different methodologies currently being researched that have potential applications for use in intelligent packaging, specifically in the milk packaging industry. A detailed analysis of each of the methods is completed in the sections below.

2.1.2.1 Utilizing pH Indicators as a Measure of Spoilage

Bacteria growth varies from one species of bacteria to another. While one may prosper under certain conditions, others may die out. These conditions are interactive and

include nutrient availability, moisture, oxygen levels and the level of other gases, the presence of inhibitors, temperature, and pH.

The pH of normal, unspoiled milk is around 6.7, a level where many bacterias thrive. In addition, the Lactic Acid Bacteria (LAB) that produce lactic acid can grow at lower pH values (pH levels of 4.0-5.0) (Klaenhammer 1988). While these organisms are intentionally used to "ferment" milk to make other dairy products, like yogurt and cheese, and inhibits the growth of many spoilage or harmful bacteria, they can also cause undesirable spoilage in certain products. Coliforms are another common bacteria that indicate the presence of pathogens. They can cause rapid spoilage in milk, since they ferment lactose with the production of acid and gas, and can degrade milk proteins. *Escherichia coli* is a well-known example of a coliform (United States Department of Agriculture, 2012).

Other properties of milk also promote bacteria growth, such as the high availability of moisture and dissolved oxygen (which supports both aerobic and facultatively anaerobic microorganisms). Temperature is frequently controlled to limit bacteria growth. Extreme heat is lethal to many organisms, such as coliforms, which explains the process of milk pasteurization (63°C for 30 minutes). In pasteurized milk, the sources of bacteria are those that survive pasteurization (thermoduric) and post-pasteurization contamination (insufficient sanitizing and environmental). Psychrotrophs play an important role in the dairy industry and are principally the cause of spoilage, since they can grow in temperatures of refrigeration, at or below 7 degrees Celsius (Lopez, 2009).

When milk spoils, the acidity increases, and is thus a factor that can be quantified to measure milk quality. Acidity in dairy products can be expressed in two ways: (1) Titratable acidity, which shows total acidity but not acid strength, and (2) hydrogen ion concentration or pH, which indicates acid strength. The natural acidity of milk is 0.16-0.18% and samples with higher figures indicate developed acidity (Training Programme, 2013). At normal levels of pH, the main protein in milk, casein, remains evenly dispersed. At lower levels of pH (about pH of 4.6), through the addition of acid from fermentation, the protein can no longer remain in solution, so it coagulates.

Studies confirm the link between pH changes in milk to spoilage. Fromm and Boor (2004) researched the attributes of pasteurized fluid milk (2% HTST milk) during its shelf life. Milk samples were randomly collected from three fluid milk processing plants in New York State. Thirteen panelists evaluated 2% HTST processed fluid milk products using a quantitative descriptive analysis methodology. They tasted and scored the perceived intensity of aroma, taste, and aftertaste of milk samples varying in degree of freshness using a numeric scale ranging from 0 to 15.

The free fatty acid (FFA) content of the samples significantly increased throughout shelf life. Between the initial day and day 7, the FFA content was not significantly different. However there were significant increases at day 14 and day 17. FFA levels increased because of milk fat lipolysis and contribute to rancid off-flavors. The higher the FFA, the more likely sensory panelists were able to detect lipolyzed or rancid off-flavors in 2% fat milk. Casein levels also decreased about 2% after day 17 of refrigerated storage from all the milk samples. There was a relatively rapid decrease over

time of casein levels. This is associated with off-flavors in fluid milk, particularly bitterness.

This study concludes that each milk-processing plant has different microflora species and needs to have plant-specific strategies to identify and reduce or eliminate sources of contamination. However, these species, while different all cause milk to decrease in pH. It proves that increases Free Fatty Acid and drop in Casein levels correlates with a decrease in pH. This suggests pH can be used as a measurement not only of milk spoilage, but also milk drinkability, since panelists determined FFA and Casein levels affect rancidity and off flavors in fluid milk. This specific study, however, does not establish a lactic acid level and corresponding pH at which milk is still drinkable. As shown before, pH readers are readily available, even though the current versions are likely inconvenient and cumbersome for individual consumers.

Ostlie, Helland, and Narvhus (2003) conducted a second study to analyze the amount of metabolic products produced by five specific probiotic strains in ultra-high temperature (UHT) treated milk. PH was measured during fermentation with a Radiometer pH meter with a combined glass electrode and temperature probe. Volatile compounds were analyzed by headspace gas chromatography and organic acids were analyzed by high pressure liquid chromatography. Quantitative analysis of carbon dioxide production was determined by an infrared CO_2 gas analyzer.

Preliminary studies showed the growth varied considerably according to the concentration of the added supplements. After 6-16 hours of incubation, all strains attained viable cell numbers (above 8.7-9.18 log cfu ml^{-1}). Depending on the probiotic

strain used, the pH of the ultra-high temperature (UHT) milk decreased from 6.7 initially to 3.9-4.4 after 24 hours of incubation.

One of the disadvantages found in this study was that the various probiotic strains in this study had very different metabolic profiles in fortified milk, which will affect the sensory quality of products using these different organisms. Nevertheless, the results were successful. The increase in strain growth (cell numbers) led to increases in amounts of lactic acid produced, which led to a drop in pH. Whereas, the methodology before correlated pH with the amount of acid and protein, this study correlates pH directly with the amount of bacteria strains. Both confirm the quantifiable nature of pH and conclude that it can be used to measure spoilage in milk. However, there must be further studies to address the shift in metabolism of probiotic bacteria in response to environmental changes and the effect of different milk treatment regimes on the metabolism of probiotic bacteria in milk.

In addition, the range after incubation that defines a limit where milk matches the definition of "spoiled" does not match the colloquial definition of spoilage. Just as with the study before, at those pH levels, milk is far below any reasonable drinking quality. For the future development of pH as an indicator of milk quality, a more accurate pH range must be established to define the point at which milk is no longer drinkable. This can be accomplished by combining Fromm and Boor's methodology involving a group of panelists to determine the level of consumable spoilage with Ostlie, Helland, and Narvhus' study of pH values and acidic byproducts.

There are currently a few devices that can be used to determine the acidity levels of milk. These are typically used by manufacturers as a means of quality control, rather

than end consumers. The Orion 3-Star pH Benchtop Meter and Orion ROSS Sure-Flow pH electrode quickly and accurately measure pH. The HI84429 Titratable Acids mini Titrator and pH Meter also serves the same purpose. A prototype, the Milkmaid smart jug, is a new product that detects when milk is starting to turn by using a pH sensor in the base. It informs its owner by changing the color of its LED lights. This product is not yet sold and the price has not yet been determined, but one of the main downfalls is the fact that consumers need to pour their milk into a separate container, rather than just using the plastic container it is sold in. So, while the method of using pH to detect spoilage is applicable to commercial products, there is no proof that this particular design will be commercially successful.

2.1.2.2 Electrical methods for the detection of bacteria

Some traditional methods of detection rely upon bacterial enumeration, where spoilage is detected when the multiplication of bacteria in a color solution causes the solution to turn colorless. The methylene blue reduction test is such an example; however, known flaws include time-consumption, redundant procedures, and an inability to discriminate between bacterial types. Lee et al. (2009) sought to improve upon the methylene blue reduction method while maintaining its advantages by using it in supplementary measures with an amperometric sensor. An amperometric sensor composed of a circuit with a potentiostat and a pair of electrodes measures current change. Amperometric sensors are miniaturizable and inexpensive, and in past research have been used in a variety of media to detect microbial changes in bacteria such as *E. coli*.

Two concentrations of *Escherichia coli* and *Enterobacter aerogenes*, types of coliform that indicates the sanitary condition of the substance, were inoculated with milk. A third sample contained milk and methylene blue. Methylene blue is blue until the metabolic activity of bacteria causes it to lose color. Resultantly, when the bacterial metabolism of the *E. coli* caused the reduction of methylene blue in the three samples, it also resulted in a current change. Any current change more than 0.05 μA was detected by the amperometric sensor and recorded. Detection time (DT) was tracked, and provided an estimate of the approximate number of microorganisms in the sample. Results were shown in graphs produced with a calculated R^2 of 0.9192, corroborating high accuracy. They showed an inverse linear relationship between the logarithm of the bacterial concentration against the frequency DT; the increase of microbial organisms was exponentially related to the time from the inoculation to the initial small change in current.

Advantages to this method include a detection time 0.5-2 hours shorter than those obtained by the methylene blue reduction method and a very broad detection range of 10^2 - 10^4 CFU/mL. Furthermore, where the methylene blue reduction method required constant supervision and sampling every 30 minute interval, the amperometric sensor was able to record the data independently. The procedure was relatively simple and inexpensive; accuracy was also a non-issue.

However, this method cannot discriminate between viable and non-viable cells. Furthermore, type of bacteria detection was lacking. The amperometric sensor could only detect *E. coli* and *Ent. aerogenes* coliforms; when other bacteria such as *B. subtilis, Lactobacillus* sp., *Saccharomyces* sp., and *Staph. aureus* were tested upon, they

produced only a negligible current change.

2.1.2.3 Wireless detection and monitoring of milk spoilage

Application of remote-query (wireless, passive) technology to detect milk spoilage is an emerging research field of experimentation. The remote-query magnetoelastic sensor platform is a free standing, ribbon-like magnetoelastic thick-film coupled with a chemical or biochemical sensing layer such as an enzyme that vibrates at a characteristic resonance frequency. A pickup coil is then used to remotely detect the magnetic field generated by the mechanical oscillations. Magnetoelastic sensors have already been developed and trialed in a number of different types of analyses; past research has used it for the analysis of glucose concentration, blood clotting, and detection of *Escherichia coli* as well as *Salmonella typhimurium*. However, it has not directly been applied to the detection of milk spoilage before.

Huang et al. (2008) used a remote-query magnetoelastic sensing platform to test for the bacterial count of *Staphylococcus aureus* ssp. *anaerobius (S. aureus*) in milk. *S. aureus* is a bacterium that resides in milk and multiples as milk spoilages; infection with *S. aureus* can result in such human diseases as toxic shock syndrome, endocarditis, and septicemia. After cutting magnetoelastic sensors from Metglas alloy ribbon, culture mediums of *S. aureus* were prepared and then used the sensor to measure sensor resonance characteristic. Because the sensor responds to mass loading due to bacterial adhesion and changes in solution viscosity, they increased the viscosity of one of the culture mediums—one a nutrient broth and the other a Trytic Soy Broth—through different trials. Ultimately, the sensor showed a higher sensitivity in the milk than in the

culture medium because of the higher viscosity of milk. Results concluded that the sensor platform is feasible for use in remote detection of spoiled milk samples.

Bacteria detection is, overall, an effective method. Typically, material costs are low. The method of using magnetoelastic sensors, for example, are able to facilitate the use of sensors on a disposable basis at $300/km, while the combination of an amperometric sensor with methylene blue also involved low costs. However, deficiencies in this method still occur regularly. Many times, bacteria detection becomes complex, as with the ATP bioluminescence method and the PCR method, and speed often becomes an issue. The methylene blue reduction method was very ponderous as bacteria detection requires constant supervision.

Two methods presented in this paper attempted to improve upon these inefficiencies. Not only does Lee et al.'s experimentation with the amperometric sensor provide a broad detection range (10^2 - 10^4 CFU/mL), much broader than the PCR-Elisa method, it also showed a detection range that was 1000-fold greater than the SPQC-TAL technique. Furthermore, electrochemical techniques are easier to use and have lower costs. It solved the problem of speed and, perhaps most important of all, provides a user-friendly experiment procedure for all to use.

2.1.2.4 Utilizing Gas-Sensor Arrays as a Measure of Spoilage

Haugen, Knut, Langsrud, and Bredholt (2006) conducted experiments using gas-sensor array technology in an effort to utilize this technology to predict the shelf-life of milk. In the experiment, a commercial solid state based gas-sensor array system was used to monitor the growth of disinfectant-resistant bacteria in milk which are known to cause

spoilage, namely *Serratia marcescens, Serratia proteamacufans,* and *Pseudomonas putida*.

Gas chromatography and mass spectrometry were used to identify the quality and amount of major volatile microbial metabolites, while statistical tests were used for analysis of data and correlation with microbiological data. Gas-sensor arrays patterned and fingerprinted complicated odors, while secondary volatile metabolites may be correlated with microorganisms producing them. This allows the identification of organisms responsible for spoilage and determining quality and shelf life based on objective criteria. Twenty-six Gram-negative bacteria were picked and identified using cellular fatty acid analysis and three isolates (*Cedcea* sp., *Serratia* sp., and *Pseudomonas* sp.) that grow well in milk and cause coagulation and spoilage were chosen.

Aside from the CO_2 signal, which stayed constant at baseline level, the sensor signals first decreased during the first 6-7 hours, due to the fact that background volatile compounds from the medium were extracted dynamically from the cultures during each measurement. The production of volatile secondary bacterial metabolites started to exceed the background volatiles and increased significantly around 7-8 hours (excluding the pure *Pseudomonas* culture). This increase in signal coincided with the onset of the exponential growth phase. At 18-20 hours, a peak in CO2 production corresponded to a plateau in *S. marcescens* N9, *S. proteamaculans* O6A and the mixed cultures. The sensor signals from the pure cultures correlated significantly with the cell counts.

Gas-sensor readings and the major volatile compounds identified by GC/MS were highly correlated (except for acetate). These results show that most of the contributions to the sensor response signals are ascribed to these compounds and the gas-sensors are

detecting the major volatile metabolites produced by the bacteria during growth. They investigated the possibility of developing a system for early detection and monitoring growth of undesirable bacteria. The high correlation between the sensor readings and the cell counts of the pure cultures suggests that gas-sensor measurements can predict bacterial cell numbers in pure cultures. Therefore, a design of specific sensors can be adapted to follow the development of specific bacteria (e.g. spoilage organisms in specific food products).

Secondary compounds identified by GC/MS were fermentation products produced by *Serratia*, *Enterobacter*, and *Erwinia*. Both *Enterobacteriaceae and Ps. putida* produce spoilage off-odours in pasteurized milk, but the *Ps. putida* culture contributed a far lower production of volatiles and CO2 than the *Serratia* strains at 25 degrees Celsius. At incubation at less than 10 degrees Celsius, *Ps. putida* would have been the major spoilage organisms in milk. Therefore, sensors must be selected and developed to give specific signals typical of target organisms in specific food products under specified conditions of temperature, pH, and water activity.

From this study, there are some constraints when using gas-sensor measurements of single strain temporal sensor response patterns to detect bacterial strains in complex cultures. This holds true particularly with *Ps. putida*, which produces secondary volatile metabolites, only contributed less than 0.1% of the temporal response pattern, even though it obtained a significant concentration in the mixed culture. For the applied experimental conditions, the gas-sensors were not sensitive enough to detect *Ps. putida* in the mixed culture, since the vapor phase was masked by the volatile metabolites generated by *S. marcescens* N9. The sensors also have a certain extent of cross-

sensitivity, so it is important to select sensors with high sensitivity for detection of strain specific metabolites, especially when the spoilage bacteria constitute a lesser percentage of the total flora.

Gas-sensors could be designed to detect the characteristic metabolites of specific bacteria in specific food products and can offer accurate rapid, accurate determinations of shelf life. To obtain correct detection of target strains of bacteria, it is necessary for sufficient strain specific volatile compounds to occur. Therefore, a drawback for gas-sensor technology is that it has the greatest potential for strains where the strain specific metabolites represent the major volatile compounds in the vapor phase. When bacteria with a low production of volatile compounds is present, it is necessary to develop relevant sensors with enough sensitivity in order to detect their characteristic metabolites.

2.1.2.5 Infrared Spectroscopy as Spoilage Indicator

Spectroscopy is a nondestructive technique, where spectral features provide biochemical information regarding the molecular composition of and interactions between different cells and tissues. It had originally and widely been used in food industry already in order to detect spoilage in chicken meat and rainbow trout fillets and quality management of beef and meat products. However, it has not been experimented upon milk until an attempt by Al-Qadiri, M. Lin, Al-Holy et al. in 2008. They evaluated visible and short wavelength near-infrared diffuse reflectance spectroscopy (600 to 11000 nm) as a technique to detect milk spoilage in pasteurized skim milk. They wanted to see the feasibility of applying visible and SW-NIR spectroscopy to monitor spoilage of pasteurized skim milk that can be used in industrial or retail settings.

In doing so, Al-Qadiri et al. first counted the total aerobic plate count and pH measurements, and then spectrally examined the milk samples at 22°C to control for spectral changes that could result from temperature differences during spectral collection. The mean pH measurement for control milk samples was 6.66, and they found no obvious decrease for milk samples stored at 6°C even after 30 hours of storage. In experiment samples, the visible and SW-NIR diffuse spectroscopy detected the formation of metabolic by-products proteolysis, and lipolysis caused by bacterial cell growth (led to a reduction in pH). Though this method was effective, it was also costly. Further work will be need in order to investigate specific spoilage microorganisms and to precisely determine which biochemical changes correlate with specific SW-NIR spectral features.

Nicolaou et al. (2012) attempted to take infrared spectroscopy a step further with the new technology of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). MALDI-TOF-MS has already been used in protein and peptide identification and quantification; however, Nicolaou wanted to see if it was useful for microbial spoilage assessment too, because usually techniques of identification and quantification of spoilage bacteria in pasteurized milk is time-consuming. Their methodology included incubating samples of milk and raw pork meat samples at 15°C and at room temperature, and then analyzed them with MALDI-TOF-MS at a regular interval rate of 4 minutes.

MALDI-TOF-MS was advantageous in many aspects, particularly when pertained to its sensitivity, accuracy, and speed. Spectrum can be generated within minutes following sample preparation. Its most comparable technology is Fourier transform

infrared (FT-IR) spectroscopy is similar; however, MS allows identification of important proteins and FT-IR spectroscopy doesn't. Furthermore, MALDI-TOF-MS makes use of low running costs and minimal sample preparation, which contributes to the rapid speed of data collection. Typical sample times only indicate 4 minutes per sample, which is considerably faster than classical microbiological plating approaches. Typical sample times take up an average of 2 days. Drawbacks, however, include the limited use of infrared spectroscopy in the field. The technology is still perceived as a tool that is capable of assessing protein qualitative qualities rather than microbial bacterial count. Additional use will be needed and propagated in order to change this perception.

2.1.2.6 Utilizing Protein or Fat Count as a Measure of Spoilage

Several studies analyze variation in lipid and protein levels in correlation with bacteria that are present in milk and known to be involved in the spoilage of pasteurized milk. Yagoub, Bellow, and El Zubeir (2008) concluded that the lowest percentages of lipid and protein in milk occur when *Pseudomonas* levels are at their highest. High *Pseudomonas* levels cause high levels of proteolytic activity in all food systems, which means they breakdown lipids and proteins, which in turn cause low lipid and protein levels. The by-products of these reactions increase milk acidity and directly correlate to milk spoilage. Additionally, Yagoub, Bellow, and El Zubeir concluded that the bacteria *Pseudomonas aeruginosa* is "associated with milk spoilage as indicated by the change of milk constituents."

There are many common industry practices that incorporate this method into testing milk properties. Protein levels are typically determined by the standard Kjeldahl method or the more favorable Dumas method. The Kjeldahl method measures nitrogen

levels by using a fitting titration technique. There are three steps involved: digestion, neutralization, and titration. The protein content is calculated from the nitrogen concentration in the milk. While the standard method, it does not directly measure protein content, and thus needs a conversion factor (which varies among different proteins) to convert measured nitrogen concentration to a protein concentration, which can lead to inaccuracies. Another disadvantage is the amount of time (1-2 hours) required to perform this test. The Dumas method is a similar, but enhanced version of Kjeldahl method. It is an automated instrumental technique that combusts a sample of known mass in a high temperature chamber, with oxygen present. Byproducts CO_2 and H_2O are filtered out, leaving only N_2 or nitrogen content to be read by a thermal conductivity detector. This test is much quicker and can be done in less than 4 minutes. Like the Kjeldahl method, the Dumas method also needs to convert nitrogen content to protein content, as it does not measure the true protein. There are also high initial costs and it can be difficult to obtain a representative sample. Other ways of protein counts are very time consuming and require intensive sample preparation before analysis.

Lipid or fat content is primarily determined by Gerber method in Europe (or the very similar Babcock method in the United States). The Gerber method requires adding dairy product into a butyrometer and adding concentrated sulfuric acid and amyl alcohol to dissolve the non-fat milk solids. The mixture is centrifuged for a set time at 1100 rpm and placed in a water bath to standardize the samples before reading the fat content off the calibrated scale of the butyrometer. It is simple, fast, low cost, and suitable for high volumes of sample. However, disadvantages include not being able to automatically

determine levels and the risk of handling highly concentrated sulfuric acid. In addition, reading the butyrometer takes acquired skill, so it cannot be used by normal consumers.

Sorhaug and Stepaniak (1998) show that "psychotropic *Bacillus* spp. secretes heat-resistant extracellular proteinases, lipases, and phospholipases that are of comparable heat resistance to those of pseudomonads." These proteins are produced by the bacteria that are able to survive the pasteurization process. Quality of dairy products are affected by heat-resistance enzymes that are secreted by psychrotrophs in raw milk before heat treatment, or produced by psychrotrophs growing during the cold refrigeration of dairy products. In fact, 25 percent of all shelf life problems associated with conventionally pasteurized milk/cream products in the US may be linked to thermoduric psychrotrophs. Sorhaug and Stepaniak also measured the psychrotroph count at the time of spoilage in CFU/ml for different dairy types and determined the average psychrotroph counts at the time of spoilage for pasteurized milk to be between 6 CFU/ml and 7.5 CFU/ml. They however did not record the protein count at this level. For this method to be used as a spoilage detector, more testing is needed to determine the equivalent protein count at the psychrotroph count of 6-7.5 CFU/ml and the amount correlation between protein count and spoilage.

The feasibility of doing a protein or fat count is low for everyday consumers, especially when considering the required know-how, needed time, and financial costs. So while levels of post-pasteurization contamination correlates extremely well with shelf life and is arguably the most accurate variable in equations used in predictive microbiology to calculate expected shelf life of pasteurized milks for mass manufacturers, it is impractical to incorporate this technology into commercially sold products. The study by Yagoub et

al. also determined increased acidity comes from the by-products of lipid and protein breakdown. It is simpler to read acidity levels through pH or some other means than it is to conduct and calculate protein or fat counts for individual consumers.

2.1.2.7 Smell and Taste Sensors in Detection of Milk Spoilage

The electronic nose unit uses 14 conducting polymer gas sensors to detect and differentiate bacteria species and bacteria concentrations through their levels of gas by-products in spoiled and unspoiled milk samples. Magan, Pavlou, and Chrysanthakis (2008) tested between six of the most common bacteria found in spoiled milk and used discriminant function analysis to differentiate between milk samples. The electronic nose was able to differentiate among all milk samples and was able to detect concentrations as low as 10^3 CFUs ml^{-1}, however reference standards among the milk industry are in concentrations of 10^2 CFUs ml^{-1} and more work is required to increase detection strength. While the electronic nose shows potential for detection of milk spoilage, further advancements need to be made. In particular, detection strength needs to be improved and bacteria species detection needs to be modified to further quantify bacteria concentration. These improvements would aid in early detection.

Another physical detection system is the disposable multi-channel taste sensor composed of many types of lipids to transmit data to a computer for analysis. The sensor array utilizes non-conventional electrodes on a single strip to decrease the size of the sensor. The primary results of this system show strong distinctions of the quality of milk over time. Results confirmed that “changes in milk quality due to bacterial activities are quite subtle and it occurs well before any physical changes can be noticed”. Further research is proposed to widen the application of this system. While this strip is quick and

simpler to use than chromatography, it can only be used once before disposal making this unconventional for milk packaging institutions.

2.1.3 Creating the Milk Spoilage Detector

Based on the existing research detailed in the above section, we determined that the best solution was using pH as the primary method of detecting and indicating milk spoilage. However, the exact methodologies detailed in the above section need to be altered with commerciality in mind and other extraneous factors, such as environmental-friendliness, should be taken into account as well.

An environmentally friendly commodity would be an added advantage, as we seek to consider all angles at which we can target potential consumers. Chen (2001) warns that a company should market its product through either an entirely green or entirely functional angle; attempts to reconcile both angles are cost-ineffective. When designing our prototypes we attempted to find a balance between maximizing the environmentally-friendly features or the efficiency of the product. Using different combinations of methodologies, we began experimenting with various designs in an attempt integrate a pH indicator into a plastic material that would be able to accurately detect and indicate milk spoilage.

2.2 Business Literature Review

2.2.1 Consumer Interest

Previous research indicates that the potential market for our product consists primarily of health conscious shoppers with high levels of income. Our product must meet that market's criteria to be successful. Lopez and Lopez (2009) conducted a market study and found that those with higher incomes prefer more expensive specialty milks,

especially organic milk, lactose-free milk, or lowfat milk. Conversely, families with younger children tend to buy milk with higher fat content and prefer conventional, manufacturing brand milks.

Furthermore, Tsiros and Heilman's study (2005) found that willingness to pay for specialty milk products is lower among younger consumers and consumers with lower incomes. A study conducted by Dimitri and Venezia (2007) indicates that consumers with younger than 54 years old with an income of $70,000 or more and a college degree are more likely to spend extra on organic and specialty milks. This established research indicates a potential target market in consumers at high-end grocery stores.
Research shows considerable market demand for improved milk spoilage detection technology. Sen and Block (2009) examined the role of ownership in customers' willingness to consume products past their freshness date. They found that consumers attribute more value to their food when they own the product and thus are more willing to consume it past the expiration date. Tsiros and Heilman (2005) found that the willingness to pay for a product diminishes in correlation to the decreasing lifespan of a product. Therefore, discounting prices to give consumers an incentive to buy milk closer to its expiration date may be an effective way to decrease wastage of milk. Williams (2005) was conducted to see how consumers interpreted health claims and the effects they had on purchasing behavior. He found that consumers generally attribute value to health claims and are more likely to purchase products with them. Therefore, the health claim that consumption of spoiled milk increases consumer exposure to dangerous bacteria is an important marketing tool for our product.

2.2.2 Packaging as a tool for communication

Packaging has emerged as a key marketing tool that was largely overlooked until recently. Agariya et. al (2012) stresses that an ideal package design should: (1) attract the buyer; (2) communicate a message to the buyer; (3) create a desire for the package; and (4) sell the product. Color is a particularly important method of communicating messages, as lighter or whiter colors denote cleanliness and purity while more vivid colors such as red convey vivacity and force of will.

In surveying a sample population in Uttar Pradesh, one of the most populous states in India, Ali and Kapoor (2008) found that consumers were far more likely to buy packaging that focused on safety and quality of the product. Kuvykaite et al. (2009) argued instead that verbal elements (product information, producer, country-of-origin, and brand) are more important to consumers making a purchasing decision than visual ones (graphics, color, size, form, and material). Underwood (2003) reminds us that the traditional role of the package is still to "protect, contain, and deliver the product to the retail shelf. Thus, packages must above all be functional and either enhances product use or convenience. Unlike advertising, packaging is a tool for communication that is tangible to the consumers, and therefore effective.

According to Goel-lal (2012), nearly 1/3 of milk drinkers find 1-gallon milk packaging to be inconvenient, with adults aged 65+ significantly more likely to feel this way. 1/4 of all milk drinkers say they find milk packaging boring. Because milk is seen as a commodity, milk manufacturers do not find it cost effective to invest in making the packaging more attractive. 45 percent of consumers favor “green” or recyclable milk cartons to one that cannot be recycled.

Green packaging is on the rise. Consumers are increasingly aware of the recent

environmental deterioration and companies are capitalizing on their corresponding willingness to buy "green" by churning out environmentally friendly products and packaging (Min and Galle, 1997). Forty-five percent of consumers favor “green” or recyclable milk cartons to one that can't be recycled (Goel-lal, 2012). Even as early as 1993, 67 percent of American consumers responded that they would willingly swap a product that didn't have environmentally safe packaging for a product that did have environmentally safe packaging. Unsurprisingly, studies have shown that 68 percent of marketers had taken note of this reaction and had already begun to make environmental related changes to their packaging (Ottman, 1993).

2.2.3 Innovation in the dairy industry

Many sectors have already endeavored to produce innovative packaging that enhances either the product's appearance or performance. Tobacco and cigarette-makers have traditionally been at the forefront of packaging innovation, and often use color and shape to attract consumers. Kool's Smooth Fusions, for example, introduced an entirely new cigarette package design that splits in the middle into two halves; this novel packaging design became a key tool in brand differentiation (Lewis and Wackowski, 2006).

The dairy industry is late to the modern innovative packaging wave. Some previous attempts have been made to improve the quality of the dairy product. As recently as 2007, Conte et al. (2007) discovered an active packaging system using lemon extract that could be used to successfully prolong the shelf life of mozzarella cheese. However, the milk carton industry remains stagnant.

Tetra Pak is a leading food processing and packaging solutions company in the world that emphasizes innovation and environmental friendliness in their products. In the general food industry, they have introduced the Tetra Recart, which is the first retortable carton package designed for shelf-stable products traditionally filled in cans, glass jars, or pouches. The Tetra Recart guarantees freshness for up to 24 months. Though this product is currently only available for use with shelf-stable products only to shelf-stable products at the moment, they have announced further plans to begin development on a similar type of carton for milk which would guarantee freshness for up to 6 months (Tetra Pak International S.A., 2013).

Evergreen Packaging, Inc. is another company that develops packages according to a customer's specific needs, specializing in dairy, juice, and liquid packaging. Unlike Tetra Pak, Evergreen Packaging caters in large amounts to the milk industry. Evergreen Packaging focused their milk carton design on light and temperature changes. Because dairy products are sensitive to these alterations and milk reacts negatively to increases in temperature and light exposure, specifically, Evergreen Packaging claims a fiber-based packaging designed to keep light and oxygen out while keeping vitamins and taste in, which helps to lock in freshness. Specifically, they have patented their barrier technology, which offers a superior oxygen and moisture-barrier board. They heavily stress environmental friendliness in their advertisements, and market their cartons as "paperboard packaging," in which over 70% of the package is made from paper and derived from trees, a renewable resource (Evergreen Packaging, 2013)

The TempTime Corporation, an international manufacturer specializing in time-temperature sensitive indicators for food products, is headquartered in Morris Plains,

New Jersey. Their product, Fresh-Check, is attached to the outside packaging of temperature-sensitive food products and pharmaceuticals. The World Health Organization became one of the first users of time temperature indicators when they used it to ensure the effectiveness of their vaccines in Africa. Since then, this technology has transformed the administration of vaccines and has been recently applied to the food industry (Sahin, Dallery, and Vaillant, 2007). Approximately the size of a quarter, this indicator changes color when the food product on which it is attached is exposed to temperature and is no longer fit for consumption. A study conducted by the Department of Chemistry, National Veterinary, and Food Research Institute in Finland determined that a correlation exists between the color change of the indicator and the sensory and microbiological quality of the food product tested (Fortin and Goodwin, 2008). Typically priced between $.025 and $.035 per package, the Fresh Check indicators are a more accurate representation of the quality of the food than the use-by-date, which cannot account for abuses that may have occurred during the cold chain process (Temptime Corporation, 2013).

Smart Lid Systems, based in Sydney, Australia, also utilizes temperature sensitive indicators. Their product, the Smart Lid, is a color changing disposable plastic lid made from thin HIPS (High Impact Polystyrene) that has increased its recognition in the field by winning several awards already in the industry. The Smart Lid is a plastic lid that will become lighter when it is on a beverage that is too hot; when the beverage cools down to the appropriate temperature, the Smart Lid returns to its original color. Currently the plastic lid changes from maroon to bright red when introduced to heat. Another lid that will change color from pale green to white when exposed to heat is currently in

development. The Smart Lid is BPA (BisphenolA) free and the color changing additives abide by the requirements of the FDA and EU. While the superlative brewing temperature for coffee is around 190 degrees Fahrenheit, human skin will usually burn above temperature of 120 degrees Fahrenheit: therefore, the Smart Lid allows for an alternative solution to testing the temperature of hot beverages with one's mouth. Smart Lid Systems has distributors in countries such as Denmark, Sweden, England and Ireland (Smart Lid Systems, 2013).

The meat industry has also been progressing in the field of intelligent packaging. TechPak has developed an indicator called The Fresh Stripe that is able to detect the freshness of meat. The Fresh Stripe is a multi-layer polymer indicator, where the polymer layer decomposes when in contact with the bacterial enzymes that degrade meat. As the meat quality deteriorates, the indicator's appearance changes. Though the Fresh Stripe is inexpensive to manufacture and is unaffected by freezing, refrigeration, or heat, its integration into packaging is still in progress as the indicator must be both visible and in contact with the meat. This technology can further be applied to other food products such as milk, soft cheese, and tofu whose enzymes are broken down by bacteria, such as other polymeric foods. However, The Fresh Stripe is difficult to incorporate into milk cartons because the stripe can only be able to be visible and in contact with the milk in clear milk packaging. The Fresh Stripe's patent is issued in some countries and pending in other countries (yet2.com Inc, 2013).

Another novel technology that can detect spoilage emerged from Cox Technologies. FDA scientists, Dr. Dwight Miller and Dr. Jon Wilkes, have created the FreshTag, a visual indicator of the spoilage level of seafood. The FreshTag detects the

production of gases that give seafood a “fishy odor,” signaling that the seafood has spoiled. The buildup of volatile amines causes this odor in spoiled seafood. This reaction of the volatile amines with the chemicals in the indicator then causes the dye in the indicator to change color, and becomes a visible sign to show the consumer that the seafood has spoiled. This new technology is needed because it can be hard for people to know whether seafood has been properly handled and is fit for consumption.

Although private labels beat sales of branded milk in 2011, branded milk processors accounted for over 71 percent of total new product count. The new milk products are focused on health contributions, with many innovations coming from rice/nut/grain/seed-based drinks (Goel-lal, 2012).

In addition to new milk products, producers are attempting to make milk more functional by adding attributes like heart health and post-workout recovery in hopes of bringing milk sales up to equal those of other beverages that make such functional claims, such as yogurt drinks and bottled smoothies. More functional, high-tech packaging conforms well to the trend among producers to market milk as more than a basic commodity. (Goel-lal, 2012).

2.2.4. Challenges in the market

Milk processors currently face challenges to the cost stability of milk as a household staple. First, consumers show increasing concern about the presence of growth hormones in non-organic milk (Thornley, 2001). Second, the milk market has struggled to shed its status as a basic “commodity”--a staple that consumers drink mainly for its nutritional profile. Competitor products such as yogurt drinks and fruit-based smoothies are often perceived as more exciting than milk and command higher price points and

generate greater brand loyalty. To increase consumer interest in milk, the milk industry needs to offer value-added products with functional benefits. According to Goel-lal's compilation of Mintel survey findings (2012), 35 percent of all milk drinkers show interest in milk that improves heart health and one-third are likely to buy "probiotic" milk that is good for digestive health. To grow in the future, the milk market must expand beyond milk's commodity status to serve a wider variety of consumer needs.

2.2.5. Trends in the Market

Organic milk, while still only accounting for a small percentage of total volume sales (3.9%) of the total cow's milk, has experienced a great amount of growth (8%) during 2010-2011. A large portion of this increase stems from parents' concerns about the growth hormone rBST in non-organic milk and the growing awareness of the benefits of consuming organic and natural foods and beverages. These parents do not mind spending twice as much to buy organic milk for their children (Thornley, 2001).

The aging population also provides concerns for the industry, since they exhibit lower-than-average interest in drinking milk. This group is significantly more likely to find 1-gallon milk packaging inconvenient compared to other groups (Goel-lal, 2012). This is likely due to the packaging design, which holds too much milk for them to drink before the expiration. Those who consume the most milk are Hispanics and Black households. The number of households with children has also been decreasing, which is detrimental to the market growth. Households with more people were more likely to purchase more milk (Goel-lal, 2012).

2.2.6. Contribution to the Research Field

Upon surveying the literature, it is evident that there is a void of information in

the field of milk spoilage definitions and detection products. The research produced from our preliminary survey, product experimentation, and market strategy should serve to fill this gap. There is a clear consumer interest in milk spoilage detection, and to date, no entity has filled this market niche.

3. Methodology

3.1 Lab Methodology

3.1.1 Bacterial/pH Correlation

This test sought to establish a correlation between the amount of bacteria (*Pseudomonas aeruginosa*) present in a milk sample and the pH level of the milk sample. To meet this objective, bacteria count on LB agar was conducted simultaneously with milk sample pH measurements.

3.1.1.1 Preparing the LB Broth

LB broth in which the bacterial cells were to be cultured was prepared. 10 g of Bactrotrypton, 5 g of yeast, 10 g of NaCl, and 0.025 g of Irgasan were weighed out and dissolved in 800 mL deionized water in a 1 L bottle. Irgasan is a chemical product that selects for *Pseudomonas aeruginosa*, the primary bacteria involved in causing illness in spoiled milk (Dinsmore). The pH of the solution was adjusted to 7.5 using NaOH. Next, the volume of the solution was adjusted with deionized water to obtain a final volume of 1 L. The 1 L bottle and its contents were then sterilized in an autoclave.

3.1.1.2 Preparing LB Agar

The LB broth for the bacterial cell culture was prepared by dissolving 10 g of Bactrotrypton, 5 g of yeast, 10 g of NaCl, 15 g of agar, and 0.025 g of Irgasan in 1 L of

deionized water. The solution was then autoclaved for sterilization.

3.1.1.3 Preparing Samples

The experiment was set up to find results for two comparisons: the relationship between bacteria content and pH, and the accuracy of this relationship in different temperatures. The "Off the Shelf" samples mimicked the milk a typical consumer would purchase and consume, while the "Sterilized" samples served as a control to compare the pH measurements from the "Off the Shelf" samples. In effect, the comparison served to establish that the bacteria are indeed causing the pH changes. The three different temperatures used for this experiment mirrored sample environments to which milk of typical consumers are exposed (refer to Table 1). Comparing the pH measurements from the three temperatures could establish that temperature differences do not affect the correlation between pH level and bacteria count in the milk sample. Fifteen samples of skim milk were obtained for the following categories:

Table 1: Samples of Milk for Bacteria/pH Correlation

Type of Sample	Heated (37C)	Room Temperature (25C)	Refrigerated (4C)
Off the Shelf	OTS1	OTS2	OTS3
Sterilized	ST1	ST2	ST3

For each temperature, 25 ml of both sample types (Off the Shelf and Sterilized) were streaked onto an LB agar plate. Five plates were prepared for each sample type for a total of 60 plates.

3.1.1.4 Testing pH

The EL-20, Education Line pH probe was properly calibrated using standard reference buffers using a 2-point calibration. A reference buffer of pH 4 was used as

point 1, and a buffer of pH 10 was used for point 2. The probe was then tested for accuracy on a buffer of pH 7 to confirm the calibration. Every two hours, the pH of each milk sample was measured and recorded using the calibrated electrode.

3.1.1.5 Bacterial count testing

The viable plate count method was used to determine the concentration of bacteria in each milk sample. The viable plate count method uses increasing dilutions of the sample to be counted in a solution that will not harm the microbe, yet does not support its growth. The diluted samples are then spread over a solid medium that will support the growth of the microbe. Since a large range of concentrations of the bacteria are used, we can more accurately assess the count of the bacteria ("Viable plate counts", 2012). Each sample was diluted after the initial incubation period. The serial dilution scheme was as follows:

$$\text{Dilution} = a/(a+b)$$

where a=amount milk transferred, and b= amount in dilution tube. The final dilutions used were in a range of 10^{-2} to 10^{-6} ug/ml.

Using a P1000 pipette, 100 ul of each dilution sample was pipetted into an appropriately labeled Trypticase Soy Agar (TSA) plate and spread the sample with a sterilized glass rod for even distribution. The plates were then incubated overnight at 37C for optimum bacterial growth. Bacterial colonies were then counted the next day by hand, using the dot technique and a number counter. The number of bacteria per sample was calculated using the formula (Wise, 2006):

CFU/mL = (number of colonies growing on a plate) x (dilution factor)/(milliliters plated)

3.1.1.6 Correlating Bacterial Count and pH

In order to confirm a relationship between bacterial count and pH and to establish the relationship, a linear regression analysis was performed. Using time as the independent variable and bacterial count and pH levels as the dependent variables, a graphical representation of the data was created. The change over time, or slope of the curves, was then compared first by visual inspection. The range of pH levels correlated to a time at which the literature value of bacterial count industry standards was counted was then compared.

3.1.2 Heat Treatment

The primary intention of heating and melting plastic was to disrupt the bonds between linear polymer chains found in plastic. Once the plastic is in a melted state, the incorporation of the pH indicator bromothymol blue would proceed. Bromothymol blue was used as the indicator for all experiments due to its sensitivity range (pH 6.0-7.6), changing from yellow to blue. A dark green color indicates fresh milk and a lightening to a yellow color indicate spoiled milk. This indicator was incorporated with the expectation that once the melted plastic solidified, new bonds would form between the plastic polymers and the pH indicator, creating a pH-sensitive product. A series of heat treatment variations were conducted in order to determine the appropriate conditions necessary to melt plastic. The goal was to find the protocol that created the more homogenous mixture of dye and plastic.

3.1.2.1 Open Flame Variation

This test involved melting plastic using direct flame from a Bunsen burner. The plastic from a standard milk carton was used since it has already been optimized for milk packaging.

A 0.5 L standard plastic milk carton was cut into several 2-inch square pieces. After the ignition of the Bunsen burner, each plastic square section was held over the flame with tongs until it became soft and malleable. The malleable plastic pieces were then placed inside a heat-proof container. 0.002 grams of bromothymol blue indicator was added into the partially melted plastic and mixed with a glass stirring rod. After the dye had been thoroughly mixed into the plastic, the product was left to cool and solidify. To test whether the pH indicator kept its pH sensing abilities, drops of acidic and basic buffers were added onto the newly molded plastic. Ten trials were conducted and for each trial, the presence of a color change was recorded.

3.1.2.2 Hot Plate Variation

Similar to the previous procedures, a 0.5 liter standard plastic milk carton was cut into several 2 square inch pieces. Enough pieces of the plastic were placed in a 25mL beaker to cover the bottom of the beaker. The beaker was placed onto a pre-heated hot plate and heated until the plastic deformed and became soft and malleable. To prevent burning of the plastic, which can be indicated by a brown or black color change, decrease the temperature. Bromothymol blue (0.002g) was added and stirred into the melted plastic using a glass stirring rod. Once the dye had been homogeneously mixed in, the product was left to cool and harden. Once the plastic solidified, the ability of the indicator-plastic complex to detect pH changes was tested with acidic and basic buffers.

Any color change was recorded. As previously done during the open flame tests, a total of 10 trials were conducted.

In addition, the experiment was conducted again using an aqueous solution of bromothymol blue in place of the solid bromothymol blue. Ten trials were again conducted and Observations were recorded and compared.

3.1.2.3 Precision Plastic Melter Variation

We were interested in determining the efficiency and efficacy of using an electric melting chamber advertised to specifically melt plastic. For this experiment, a Lee Precision Production Pot IV Plastic Melter (LPP; 900 degrees F max temperature; 500 Watt power) was purchased (refer to Figure 1). Once again, a standard 0.5 L standard plastic milk carton was cut into several 2-inch square pieces. Ten plastic pieces were placed in the pre-heated LPP and then heated until the plastic changed into a molten consistency. The plastic was removed from the LPP with a glass stirring rod. 0.002g bromothymol blue was added and mixed into the melted plastic with a glass stirring rod. Afterwards, the mixture was left to cool and solidify. Drops of acidic and basic buffers were placed onto the dye-infused plastic. Ten total trials were conducted and observations were recorded.

The entire procedure was also repeated using high density polyethylene (HDPE) and low density polyethylene (LDPE) beads instead of the milk carton plastic samples. Currently, HDPE is used in the milk packing industry to create milk cartons and LDPE is the low density version of the material (Speece & MacLachlan, 1992). Testing with these two additional materials will give a better understanding of

which plastic is most optimal for prototyping the intended milk spoilage detector. Observations for all experiments were recorded and compared.

Figure 1: Lee Precision Production Pot IV Plastic Melter

3.1.2.4 Oven Test Variation

A ceramic oven in the Advanced Manufacturing Laboratory within the Mechanical Engineering Department at the University of Maryland, College Park and ran by Dr. David Bigio, was used as another heat treatment variation. The oven theoretically provided a more controlled environment due to its adjustable temperature settings. This variation was used to determine if melting plastic under high temperature and low oxygen conditions could achieve the desired plastic consistency.

In order to determine the optimum temperature needed in the oven, the following procedure was used. First, the oven was set to its lowest setting of 500 degrees Celsius. A layer of HDPE beads was placed onto an aluminum liner and the liner was placed into the oven. The temperature of the oven was increased by 50 degrees after every 10 minutes

that the plastic beads did not melt. In this case, the temperature at which the plastic finally melted was 800 degrees and that became the ideal temperature to work at.

Melting a layer of HDPE beads in an 800 degree oven takes approximately 10 minutes. In order to prevent combustion of the plastic beads, pressurized argon gas was pumped into the oven through a rubber tube via an opening on the side of the oven. Once the plastic has melted, the liner was removed using metal tongs. Solid bromothymol blue indicator was immediately stirred into the melted plastic using a glass rod. The plastic-dye mixture was allowed to cool and re-solidify. Acidic and basic buffer solutions were placed onto the surface of the cooled plastic. Observations of any color changes were recorded. The experiment was repeated a total of 10 trials. Similar to previous test variations, aqueous bromothymol blue was also used in place of the solid version to see which was most effective. LDPE beads were also experimented with.

3.1.3 Effectiveness of Microfiltration Membranes

In order to test the properties of microfiltration membranes used in water treatment systems, a system of filtering dye-infused water was set up. Afterwards, the filtered water's optical density readings were measured.

A cuvette was filled with deionized water as a blank control reference for the spectrophotometer. A 50 ml vacuum beaker was fitted with a Buchner funnel, and the 0.45 μm pore size membrane was placed inside the funnel. A vacuum was attached by tubing to the beaker. 10 ml of deionized water was infused with 0.002g of bromothymol blue dye and measured in the spectrophotometer as another reference. The dye-infused water was poured over the membrane in the funnel, and allowed to filter through vacuum

filtration into the beaker. The liquid that passed through the funnel was then collected, and loaded into a cuvette for an optical density reading.

3.1.4 Nafion Testing

Nafion is a synthetic polymer belonging to a class called ionomers. It has the property of allowing for hydrogen ion transport, thereby rendering it as a proton exchange membrane (PEM). Because of its property of allowing the transport of hydrogen ions while being impermeable, we proposed that Nafion would be a suitable material to separate bromothymol blue and the milk while still allowing for bromothymol blue to react to any changes in pH (Mauritz & Moore, 2004). The separation would prevent the leaking of the dye into the milk itself and rendering the milk inconsumable before it has spoiled. In order for Nafion be finalized as the separating component, Nafion must meet two criterias: 1) Nafion must be able to separate bromothymol blue from the milk with absolutely no leakage of the fluids from either direction, and 2) bromthymol blue must be able to accurately detect the pH level of the milk at all times via proton transport through the Nafion. Failure of either one or both tests will deem Nafion as an unsuitable material to be used in our application.

3.1.4.1 Leakage Test

Leakage was tested in order to assess Nafion as a possible barrier between milk and bromothymol blue. Since bromothymol blue is a non-FDA approved pH indicator, it is imperative that the bromothymol blue does not make direct contact with the milk. Direct contact with the milk renders the bromothymol blue as a food contaminant and causes the milk to be no longer fit for human consumption. For this experiment, a two-

chambered well in which chambers are connected by a small hole was used (refer to Figure 2). The two chamber setup allows for a thin material to be placed between the chambers in order to test the permeability of the material between two different liquids. Rubber O-rings sealed the chambers against the desired material, and four screws at each corner of the device secured the two chambers against the material. Different liquids could then be injected into each chamber via a small hole at the top of each chamber.

To begin, a 9 square inch piece of Nafion, measuring 3 inch by 3 inch, was cut to serve as the separating membrane between the wells. The dimensions of the cut Nafion fit so that the Nafion would cover the entirety of the O-rings while still allowing the 4 screws to secure the apparatus. The Nafion was inserted between the chambers so that it completely covered the hole separating the chambers. The four screws were secured tightly against the chamber walls to hold the Nafion in place (refer to Figure 2). Bromothymol blue was dissolved in 100mL water with two drops of alcohol added to make an orange-colored solution. A syringe was used to slowly and carefully inject 3 mL aqueous bromothymol blue (0.01% concentration) into one chamber. A second syringe was used to inject 3 mL of deionized water into the second chamber. An initial absorbance reading of deionized water was taken at 650 nm and the absorbance machine was calibrated with deionized water as the baseline. After 3 days, the liquids were observed for visible color change and an absorbance reading of the deionized water was taken. Leakage to the adjoining chamber would have a pink orange appearance, while an absence of leakage would result in a clear color in the adjoining chamber. All results were recorded.

Figure 2: Nafion Initial setup: Left chamber contains 3 mL aqueous bromothymol blue (0.01% concentration). Right chamber contains 3 mL deionized water.

3.1.4.2 Proton Permeability Test

Proton exchange capabilities of Nafion were tested with our required environments. This test was necessary in order to determine the efficacy of using Nafion as a separating barrier. If no protons can be exchanged through the membrane, bromothymol blue will not be able to detect the pH level of the milk, and will ultimately be unable to detect milk spoilage. The same two-chambered well will be used in determining the capabilities of Nafion in proton exchange.

To begin, a Nafion sheet was inserted between the chambers. Using a syringe, a red buffer solution with pH of 4 was slowly and carefully injected into one chamber, and a blue buffer solution of pH 10 into the other. By injecting liquids of contrasting pH (4 and 10), a pH of 7 was expected after time for an equilibrium of protons through the nafion. Over the course of seven days, pH strips were used to measure the pH of the liquids in each chamber. To do this, a small amount of liquid was extracted and excreted onto a pH strip using a micropipette. The pH of the tested buffers were then compared to their expected values (4 and 10). If there is an exchange of protons from one chamber to

the other, then there should be a color change on the pH strips, with the pH values for both being around 7. This color change would indicate that protons are able to pass through the membrane and pH equilibration is occurring.

3.1.4.3 Control Test

A control test evaluated the efficacy of the chamber device by determining whether leakage could occur through a plastic sheet. Theoretically, a plastic sheet should not allow permeability of any substance. To perform this test, a solid plastic sheet was inserted between the chambers. An aqueous pH indicator was injected into one chamber and deionized water into the other. After three day, the liquids were observed for color changes (via absorbance reading). In addition, pH strips were used to identify whether there were pH changes. The observations and results were recorded.

3.1.5 Hydrogel Experiments

After the results of the Nafion experiments were analyzed, we moved onto the task of determining the properties and feasibility of using hydrogels as a medium for the creation of pH-sensitive solid materials. It was hypothesized that the hydrogels were a good medium for the indicator because of their ability to absorb liquids and remain hard when dry (Okay, 2009). The following experiments sought to create hydrogels mixed with pH indicator in order to produce a solid medium sensitive to pH changes. Bromothymol blue was the indicator used in all experiments. Bromothymol blue was the chosen indicator because its pH range for color change (6.0-7.6) most closely matched the pH range of fresh milk to spoiled milk according to literature values (approximately 6.7-4.4; Ostlie et al., 2003).

3.1.5.1 Creation of Gelatin-Based Hydrogel

Gelatin is a protein-based biomaterial that has been used as a medium for cell delivery for medical and pharmaceutical applications (Lai & Li, 2010). Its effective use as an aqueous medium made it an appealing medium for water-soluble bromothymol blue. Using gelatin to create a hydrogel would create a porous material that would allow accessibility of the pH indicator to other liquids. To determine the indicative property of gelatin-based hydrogels, an initial model was created. To do this, a pH-sensitive dye-infused gel with 2 ml of 0.01% bromothymol blue indicator, 13 ml of deionized water, and 0.75g of gelatin (0.75g/15 ml, 5% gelatin mixture) was made and allowed to dry out and harden overnight. A visual comparison of the indicating properties of pH-sensitive dye was performed by pipetting drops of pH 10 buffer solution and pH 4 buffer solution on the gel.

3.1.5.2 Gelatin-based Hydrogel Leakage Test

These tests determined the effectiveness of the hydrogel as a medium for the indicator. They assessed whether the dye-infused gel would leak or disintegrate when in constant contact with milk or other aqueous solutions. In order to perform this test, a gelatin-based hydrogel with 15% composition was made. After the gel dried overnight into a hard disc, it was broken into several small pieces to fit into a test tube. Six ml of milk was poured into a test tube along with a few pieces of the gel. Any noticeable color or consistency changes with the gel were recorded over the course of three days. Four trials of the experiment were run.

3.1.5.3 Polyacrylamide-Based Hydrogel Composition Test

The use of polyacrylamide-based hydrogels was then assessed as another possible medium for bromothymol blue. Acrylamide polymerizes in solution and forms an

insoluble gel when cross-linked with methylenebisacrylamide. Its properties have made it a suitable and supportive medium for gel electrophoresis (Raymond & Wang, 1960). These experiments were set up in order to determine whether a polyacrylamide-based gel would be a more stable and better suited medium for determining milk spoilage. Gelatin disintegrates easily due to its material component properties and uses porcine skin as a base, whereas polyacrylamide is not synthesized from animal parts and maintains more structure in water (Naghash & Okay, 1996). In order to make an 8% polyacrylamide gel solution, 20mL TBE buffer, 8.7g acrylamide, 0.3g N,N-methylenebisacrylamide, 52.7mL DI water, 0.14g ammonium persulfate, and 35uL TEMED were combined in a 200-mL Erlenmeyer flask (Carl Roth, 2012). Afterwards, 15ml of the 8% polyacrylamide gel solution was mixed with 3mL pH indicator to make a 20% pH sensitive polyacrylamide gel. In order to catalyze the reaction, the mixture was heated for 30 seconds and then poured into a petri dish. To let the gel harden, it was left uncovered overnight. Polyacrylamide gels were made in several 1 inch diameter petri dishes with 3 mL of gel solution and 0.0001g of bromothymol blue indicator. Large gels were also made in petri dishes containing 15 mL solution with 0.0001g of indicator. The next day, drops of pH 10 buffer solution and pH 4 buffer solution were pipetted on the gel. Any color changes exhibited were recorded. These procedures were repeated again with 6ml pH indicator and then with 9mL pH indicator.

3.1.5.4 Polyacrylamide-based Hydrogel Leakage Test

Just as it was important to perform leakage tests with the gelatin-based hydrogel, we also performed them on polyacrylamide-based hydrogels. In order to perform this test, a polyacrylamide-based hydrogel solution with 0.002g of bromothymol blue was

made. After the gel dried overnight into a hard disc, it was broken into pieces small enough to fit into a test tube. 0.35 g of the gel was added to a test tube filled with 20ml of either non-spoiled milk or deionized water and then left out at room temperature. Noticeable color or consistency changes in the milk with the gel were recorded for three days. The pH values of four trials of the experiment were run.

3.1.5.5 pH Indicator Comparison

In order to determine which indicator was optimal for indicating milk spoilage we tested several different dyes, each with a different pH range that could potentially show the change in acidity of fresh milk compared to that of spoiled milk. This additional testing sought to compare the effectiveness of the bromothymol blue used with other potential indicators to determine how well our selection matched the pH range of actual milk samples. Furthermore, our prototype testing had thus been conducted on acids and bases, but not on our intended marketable product. This test served to demonstrate the real-world applications of the polyacrylamide gel and determine if it would retain its pH indicating properties.

For this experiment, 250uL of each selected liquid indicator was mixed into two 5mL glass vials of fresh milk, marked trials “a” and “b” to be monitored side by side. Specifically, 2% milk of Cloverfield Farms brand was purchased two weeks before the labeled sell by date to run the experiments on. Seven different types of samples were used, including previously made bromothymol blue indicator acrylamide gels, and a total of 14 vials were marked accordingly (refer to Table 8). Specifically, these were labeled 1a/1b: Bromophenol blue, 2a/2b: Bromocresol purple, 3a/3b: Phenol red, 4a/4b: Bromothymol blue, 5a/5b: Phenolphthalein, 6a/6b: polyacrylamide gel w/ bromothymol

blue indicator (0.002g per 15mL ratio) in milk. A separate set of vials filled with deionized water containing a sample of polyacrylamide gel prototype (0.002g bromothymol blue per 15mL DIH20 ratio) was used as a control to compare to the prototype left in milk. The main milk container was also placed in the same fridge without any addition, acting as an additional control. All of the samples were then refrigerated, emulating the general environment in which milk is kept in. The color changes of the indicators in the samples were recorded daily for 15 days to visualize signs of spoilage and changes in indicator color. These were compared to the expected pH and color ranges obtained from the literature in Table 2 ("Acid base indicators", n.d.). The state of the original milk in the carton was also analyzed through visual and olfactory observations as a control. The expected results were that the samples containing indicators within the range of the milk would change color as the milk carton sample spoiled. The negative control sample in deionized water was expected to have no color change.

Indicator	Range	Color Change
Bromophenol blue	3.0 - 4.6	Yellow → Blue
Bromocresol purple	5.2 - 6.8	Yellow → Purple
Phenol Red	6.8 – 8.0	Yellow → Red
Bromothymol Blue	6.0 – 7.6	Yellow → Blue
Phenolphthalein	8.3 – 10	Clear → Purple

Table 2: Literature pH values

3.2. Market research methodology

3.2.1 Writing the survey

To gauge the interest and marketability of our idea, we conducted surveys to

determine current consumption trends in the market. Our survey's questions emphasized clarity and brevity, and we paid particular focus to categories such as age, gender, and type of milk purchased in order to segment our consumer market.

Simplification of our survey was imperative in order to more effectively engage potential respondents. Originally, we intended to determine a correlation between our proposed product and consumers' income levels. However, after further deliberation we concluded that lines of inquiry into income might trespass privacy levels and deter potential respondents. Questions asking to identify race were also removed because we determined that such characterization was irrelevant and unrelated to our goal of gauging interest in our product.

Data on such factors as milk buying habits, concern about health quality, and concern about global food wastage provided particular points of interest. These factors gauged consumer interest and allowed us to create consumer profiles. For example, a question asking whether consumers typically discard milk before or at the expiration date reveals the extent to which the milk-consuming population wastes milk prior to its expiration. We also asked for the level of consumers' concern over food wastage and health quality. Data from these two questions help further specify our consumer profile, identify which consumers are interested in our product, and determine whether awareness of wastage would affect respondent's interest in our product. Finally, we asked for the level of consumers' interest into purchasing a milk carton that changes color to indicate spoilage. We wanted to correlate this particular question with the previous questions and establish any patterns or connections between different consumer profiles and their

interest in our product (Appendix I).

3.2.2 Determining survey locations

Once we finalized our surveys, we submitted for IRB approval. We qualified for the minimal risk track and planned to conduct surveys during the summer after our sophomore year (U.S. Department of Health & Human Services, 2010). Within the month, we received approval confirmation and were ready to distribute our surveys.

We chose four main locations to distribute our surveys: Silver Spring, Bethesda, Rockville, and College Park. These locations were within driving vicinity and could contribute diverse economic, social, and ethnic backgrounds that would help us achieve a sample representative of the US population (U.S. Department of Commerce, 2010). We targeted multiple types of grocery stores such as supermarkets, specialty grocery stores, and the local farmer's market. Over the course of the summer, we conducted surveys at Magruder's (Silver Spring), Giant (Bethesda), Giant (Rockville), and the Farmer's Market (College Park).

In order to best sample the population, we conducted our surveys in the afternoon through the late afternoon when shopper levels would be highest. We asked each passing shopper to take our quick survey, and asked each to sign a waiver to inform him or her that our surveys were anonymous. While this method of surveying is a form of convenience sampling, we believe the effects of bias are negligible because we asked every single supermarket customer within the time period we surveyed. Over the course of the summer, we collected 295 survey responses, which is a large enough sample to conduct statistical analysis on (Appendix II).

3.2.3 Marketing Assumptions

When conducting our surveys, we made a few assumptions about the market and the people we surveyed. First, we assumed the population in the DC metropolitan area can be generalized to the entire population in the United States. The DC metropolitan area has a very diverse population with people from many different backgrounds with different demographics, similar to that of the United States as a whole (U.S. Department of Commerce, 2010). Our chosen grocery stores, mentioned previously, were in areas with varying income levels, but mainly concentrated on middle income families.

We also assumed any conversations with the respondents and the signs set up at our survey table did not sway respondents to answer differently than they normally would. Especially since we kept our introductions short and formal, this assumption can be rightfully made. We acted friendly enough to receive a high response rate, but also conscientiously enough to avoid moderator acceptance bias (Steward and Shandasini, 1990).

As previously mentioned, our sampling method is considered to be a form of convenience sampling, meaning subjects were chosen based on accessibility and convenience. However, this is negligible because we asked every single consumer that walked in the grocery stores.

Finally, based on personal experience and observation, we assumed a majority of consumers go to their local grocery stores or farmer's markets to buy milk. This was a factor that helped us determine what kind of locations we wanted to distribute our survey.

3.2.4 Best Product Design

Before creating a final prototype, we wanted to find which design would best suit consumer needs. The main factors to consider were: function, shape, and placement of

the indicator. Of the many types of possible spoilage indicators such as pH, gas emissions, bacteria concentration, and protein concentrations, we decided to use pH as the indicator for our product. Compared to the other indicators, detecting changes in pH would be the cheapest and simplest as detecting gas emissions and bacteria count would require heavy electronics and would be difficult to integrate into current production for milk producers. We would have to independently produce separate, far more expensive milk bottles for gas, bacteria, and protein detection (Haugen, Knut, Langsrud, & Bredhold, 2006; Al-Qadiri et al., 2008; Magan, Pavlou, & Chrysanthakis, 2008; Wallach, 2002).

The next step was to choose which form of pH detection would be ideal. We narrowed our choices to either electronic detection or color change through a pH indicator. Electronic detection would also require a separate, independently produced bottle. While it would detect pH changes more accurately, it would also be more expensive and would be a larger transition for consumers. Therefore we decided to use a pH dye that we could integrate into the bottle design. It would be cheap, simple, and would smoothly integrate into current production methods.

We originally considered using a reusable bottle, collapsible bottle design. This would also be independently produced, but would allow consumers to continue reducing food and plastic wastage by using the sample bottle with our pH dye-infused plastic. This design would also allow consumers to extend their milk's shelf life with the collapsible bottle function, which would allow consumers to remove air from the bottle as the consumer drinks the milk. While these functions are health conscious and eco-friendly, the transition to filling their separate reusable carton and high price point of

such a product proved prohibitive.

A regular paperboard carton was also considered based upon its appeal to eco-friendly consumer and its ability to be mass-produced. The paperboard carton is easy to recycle and is biodegradable which coincides with the interests our technology appeals to. However, it would cause higher production costs for our milk producers. Cardboard cannot be infused with a pH dye and would therefore still require the use a plastic strip that could be seen outside of the carton. Unfortunately, mixing cardboard and plastic products would complicate the recycling process, and producers of cardboard cartons would likely lack the machinery to create and integrate such a plastic strip into their bottles. These complications led to the dismissal of the paperboard carton option for our pH sensitive bottle design.

Overall with all the different designs, we felt the most feasible option was using the regular plastic carton design because it would be most feasible in producing and there would be fewer complications in integrating the pH dye. The regular plastic carton could be mass-produced, cheapest for milk producers, and would be the easiest for consumers to transition to. Therefore we created a polyacrylamide-based hydrogel, infused it with bromothymol blue dye, and then allowed it to dry into a thin plastic strip. This plastic strip will be incorporated into the existing bottle design as opposed to integrating the dye throughout the whole carton. The final design decision we needed to make was the location of the pH indicator on the bottle. We have multiple options such as the cap, handle, bottom, or have the pH dye integrated throughout the entire bottle.

4. Results and Analysis

4.1 Science Results

4.1.1 Bacterial/pH Correlation

The pH levels of both the "Off the Shelf" and the "Sterilized" milk samples initially experienced a sharp increase in pH (Table 3). The pH levels then stayed relatively constant for the next 200 minutes. Following, the pH levels of both the "Off the Shelf" and the "Sterilized" milk samples experienced a sharp decline in pH levels back to about the starting pH levels. The pH levels remained relatively the same with slight fluctuations for about the next 450 minutes. There was a slight elevation in pH levels for both the "Off the Shelf" and the "Sterilized" milk samples between the 450 minute and 500 minute mark (Figure 3).

Table 3: pH readings of "Off the Shelf" (OTS) and "Sterile" (ST) milk samples over time

Time (min.)	**0**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**	**330**	**360**	**390**	**420**	**450**	**480**
OTS1	6.64	7.00	7.02	7.02	7.08	7.03	7.03	6.64	6.64	6.68	6.70	6.63	6.68	6.64	6.67	6.73
OTS2	6.62	6.99	7.02	7.04	7.06	7.01	7.02	6.63	6.64	6.67	6.69	6.63	6.67	6.62	6.65	6.81
OTS3	6.65	7.01	7.07	7.03	7.08	7.01	7.02	6.65	6.65	6.65	6.68	6.64	6.68	6.63	6.64	6.70
ST1	6.10	6.69	6.74	6.74	6.77	6.72	6.70	6.37	6.39	6.39	6.41	6.36	6.41	6.34	6.38	6.45
ST2	6.17	6.69	6.75	6.74	6.75	6.76	6.74	6.37	6.38	6.39	6.41	6.37	6.42	6.36	6.39	6.41
ST3	6.18	6.69	6.75	6.74	6.76	6.75	6.75	6.39	6.39	6.39	6.41	6.36	6.41	6.36	6.40	6.44

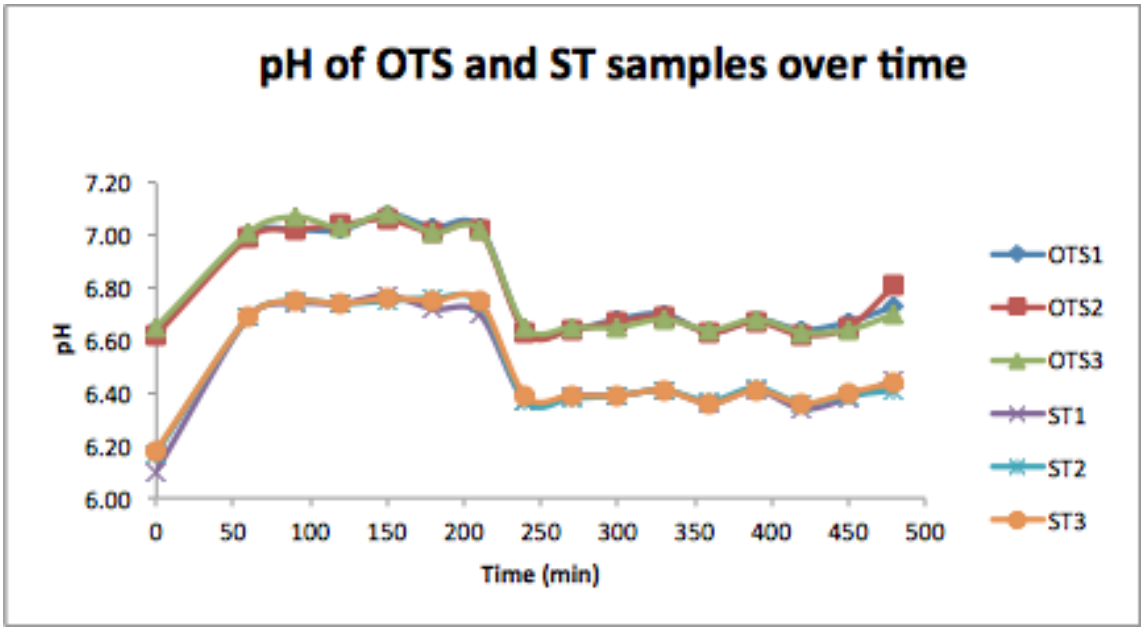


Figure 3: Range of pH recorded of OTS and ST milk samples over time

4.1.2 Heat Treatment

4.1.2.1 Open Flame Variation

When using an open flame, the plastic became soft in areas exposed to the flame. However, long exposures to the flame caused the exposed areas to combust and engulf into flames. After the flames were extinguished, it was observed that the areas exposed to the flame harden in less than a minute. These areas of the plastic were observed to be brittle and burnt. However, areas of the plastic that were not directly exposed to the flame remained hard. When acidic and basic buffers were introduced to the plastic, there was no observed color change in neither the burnt areas nor the non-burnt areas. These effects were seen in all ten trials.

4.1.2.2 Hot Plate Variation

With the hot plate variation, there was noticeable charring on the surface nearest the hot plate which was similar to what was seen during the open flame variation. After the plastic was heated for one hour, it finally softened enough to be prodded with a glass stirring rod. The plastic never achieved a molten-like state, even after being heated for a longer period of time. This small deformation was seen during all ten trials.

When attempting to incorporate either the aqueous or the solid bromothymol blue, neither was able to be stirred into the plastic. Instead, the solid pH indicator coated the outer surface of the plastic while the aqueous version stained the surface. When the acidic buffer was dripped onto the plastic, there was a color change from blue to yellow. The basic buffer presented no color change. These observations were also seen in all ten trials.

4.1.2.3 Precision Plastic Melter Variation

After the milk carton pieces were placed and heated in the LPP at 800 Fahrenheit for one hour, the material had a jelly-like texture. Similar to what occurred with the hot plate variation, the plastic deformed to a small degree and was able to be prodded with a glass stirring rod. However, even after being heated for two hours, the plastic maintained its initial structure and failed to completely melt in all ten attempts.

Since the plastic was unable to be in a molten state, it was unable to be mixed with neither the solid nor the aqueous bromothymol blue versions. As seen with the hot plate variation, the solid bromothymol blue sat on top of the plastic and the aqueous bromothymol blue stained the plastic. A color change from orange to yellow occurred in

both cases when acidic buffer was introduced. No color change was seen when using the basic buffer. These observations were seen during each of the trials.

During the attempts to melt the LDPE beads in the LPP in place of the HDPE beads, similar observations were seen as before. LDPE beads maintained its original structure and thus was unable to have bromothymol blue incorporated into it. There were no noticeable changes between the HDPE trials and the LDPE trials.

4.1.2.4 Oven Test Variation

With the oven test variation, we found that the HDPE beads were able to completely melt at a temperature of around 800 degrees. This variation finally allowed the plastic to reach the soft and miscible consistency needed to effectively mix in the pH indicator homogeneously. There were still difficulties in preventing combustion even in an argon-filled atmosphere. During all ten trials, the plastic became engulfed in flames once the oven door was opened. Argon was used to extinguish the flames.

After melting, charred portions of plastic were observed upon observation. Solid bromothymol blue was able to be stirred into the plastic with a glass stirring rod; however it was not homogenous. Due to the high temperature of the plastic, the aqueous version of the indicator vaporized on contact.

After the plastic cooled at room temperature for ten minutes, it was able to be handled. The plastic stayed its original opaque white color, with some orange areas. No color changes were observed after the addition of acidic and basic buffer solutions onto the cooled and hardened pH indicator-plastic complex. Similar observations were seen in all ten attempts.

Tests were also conducted using LDPE beads. In this case, the beads melted at a

temperature of 600 degrees. Combustion of the melted plastic once place once again as soon as the oven door opened. Charring was again observed and the solid pH indicator was able to be mixed into the plastic, however not homogeneously.

4.1.3 Effectiveness of Microfiltration membranes

As shown in Table 4 below, the samples had an absorbance between that of water and bromothymol blue indicator.

Table 4: Absorbance readings of filtered dye infused deionized water. Pore size of membrane was 0.45µm.

Sample	**Absorbance**
Reference: Clear deionized Water	0
Reference: 0.1% Bromothymol Blue infused water	0.819
Sample 1	0.410
Sample 2	0.434
Sample 3	0.571

4.1.4 Nafion Testing

4.1.4.1 Leakage Test

The initial setup of the Nafion chambers is shown in Figure 4. The Left chamber contained the aqueous version of the bromothymol blue indicator, while the right chamber contained only water.

Figure 4: Nafion Leakage Test Initial setup: Left chamber contains 3 mL aqueous bromothymol blue (0.01% concentration). Right chamber contains 3 mL deionized water.

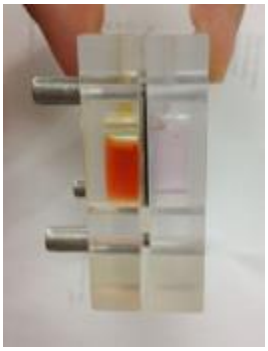


Figure 5: Chambers after day 3. Note the pinkish color in the chamber on the right that previously contained deionized water.

After three days, the absorbance values for the deionized water increased for all trials. A slight pinkish orange hue was observed in the chamber containing the deionized water (refer to Figure 5). In addition, absorbance readings were taken (refer to Table 5) to quantify this color change and compare it to the initial absorbance. From the results it is clear that the indicator passed from the left chamber to the right.

Table 5: Absorbance readings of the deionized water were taken at 650 nm in order to quantify leakage levels.

Trial	Initial Abs	Final Abs
Trial 1	0.00	0.013
Trial 2	0.00	0.005

4.1.4.2 Proton Permeability Test

No observable pH changes were recorded in either buffer after separation by Nafion (refer to Figure 6 and 7).

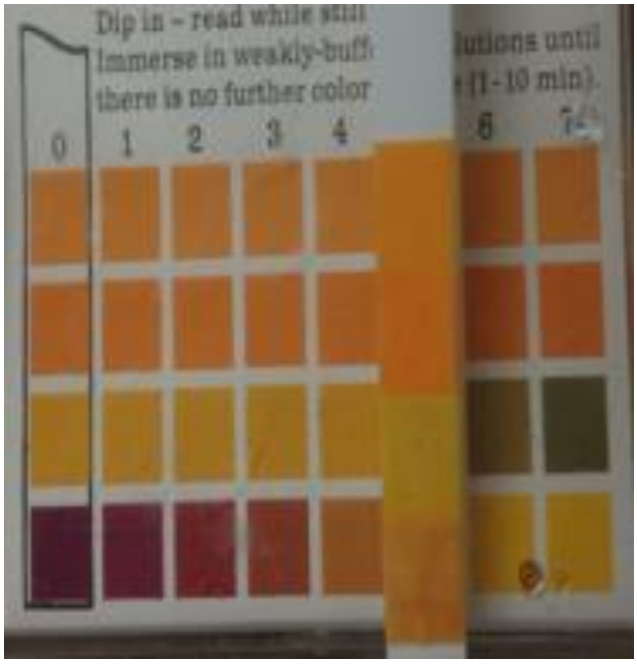


Figure 6: The pH of buffer solution with the initial pH of 10.

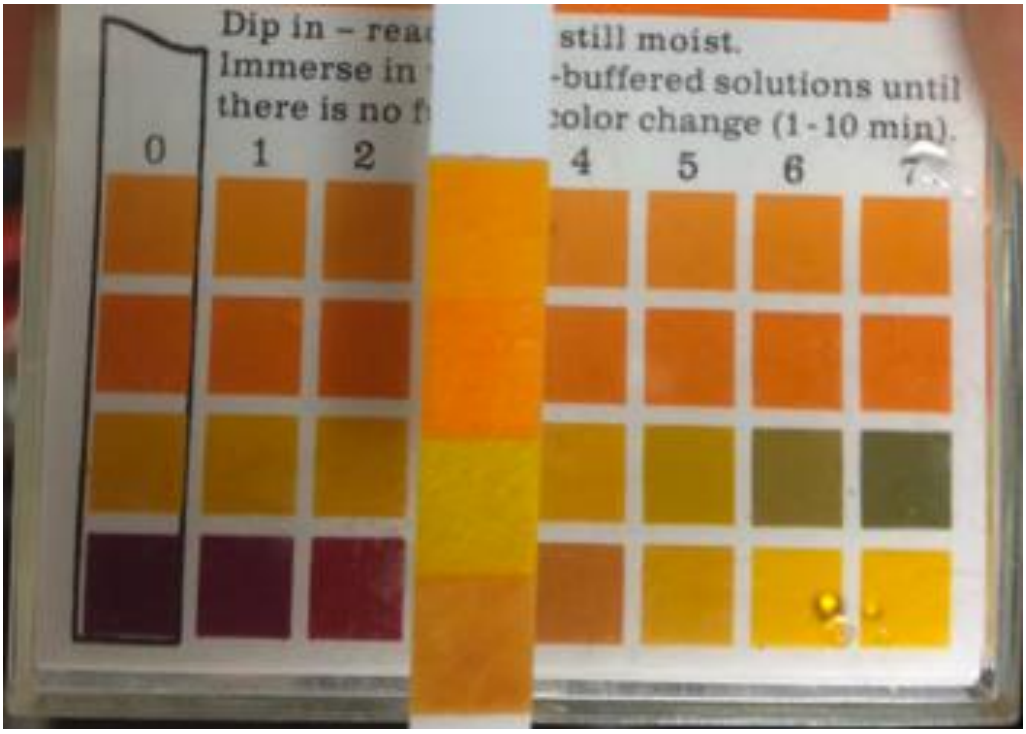


Figure 7: The pH of buffer solution with the initial pH of 4.

4.1.4.3 Control Test

Leakage of bromothymol blue was observed in the deionized water, leaving a slight pink discoloration. The pinkish hue deviated from the clear color of deionized water.

4.1.5 Hydrogel Experiments

4.1.5.1 Creation of Gelatin-Based Hydrogel

Gels solidified into dry, thin, brittle disks which conformed to the shape of the petri dish as desired. Using buffers of different pH, color changes were observed in the gel. Specifically, the color of the dye-embedded gelatin went from yellow to blue when basic solutions were added, and became red when an acidic solution was added (refer to Figure

8). The presence of mold occurred on the gelatin samples that were left out in open atmosphere. The fuzzy greenish brown growth began a few days after creating the gel.

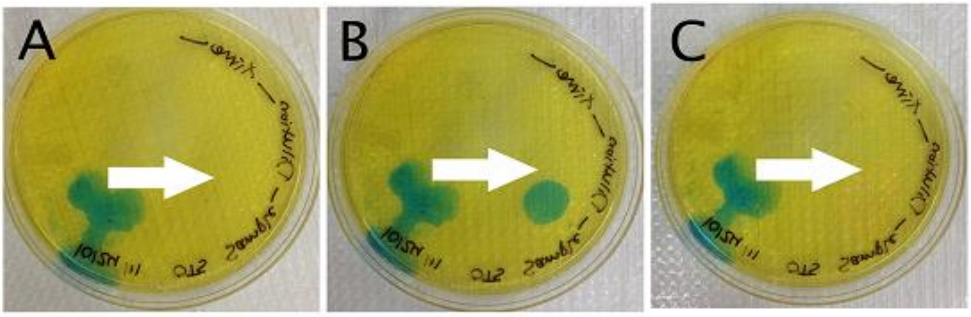


Figure 8: A Visual of the pH Indicating Ability of the Gelatin-Based Hydrogel in Different Buffers. Fig A Gelatin based gel with 2 mL 0.1% liquid bromothymol blue indicator. Arrow indicating area of observation; Fig B Gelatin exhibits gradual color change from yellow to blue within minutes of addition of pH10 buffer solution; Fig C Gelatin exhibits gradual color change from blue to red with addition of pH4 buffer solution after initial exposure to basic solution.)

4.1.5.2 Gelatin-based Hydrogel Leakage Test

A leakage test was conducted on a gel made with 0.75g of gelatin, 13 mL hot water, and 2 mL of 0.1% solution bromothymol blue indicator. The initial color of the solidified gel was yellow when first placed in water.

Table 6: Absorbance Readings of Leakage from Gelatin-based Hydrogel

Sample	**Absorbance Reading (Initial)**	**Absorbance Reading (Day 1)**	**Absorbance Reading (Day 2)**
Blank	0.0	0.0	0.0
Gelatin in H_2O sample 1	0.0	0.010	0.010
Gelatin in H_2O sample 2	0.0	0.018	0.017

In all four of the trials run, the gelatin adsorbed water and increasingly dissolved in the liquid within the span of three days, leaving the surrounding water colored yellow on the second day. All of the gels became blue on the third day of observation. Absorbance readings were taken to quantify the amount of leakage (refer to Table 6) as compared to pure deionized water.

4.1.5.3 Polyacrylamide-Based Hydrogel Composition Test

Polyacrylamide gels were made according to protocol. Calculated amounts of solid bromothymol blue pH indicator were added to 15 mL plates of the gels. Gels were initially liquid and eventually dried out into a hard brittle disc after applying heat or testing. The outside shrunk more, causing the middle portion to rise and forming a round uneven shape. A pH test was conducted on the resulting gels using pH buffer solutions of pH 10 and pH 4 to observe any possible color changes (refer to Table 7). The small gels and gels formed with less than 15 mL were too thin for color detection.

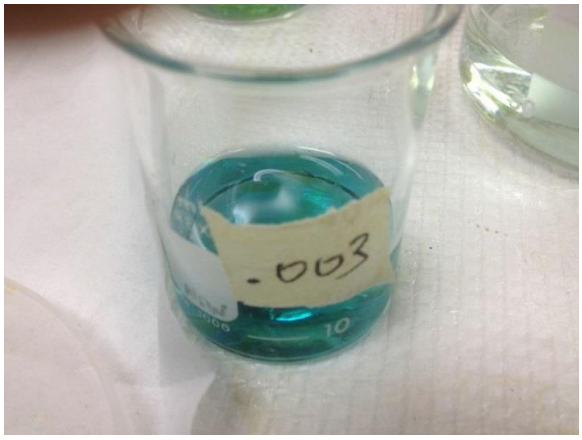
Table 7: Polyacrylamide-based Hydrogel Observations after the Addition of pH Buffers

Amount of pH Indicator Added (in a 15 mL solution of polyacrylamide)	Initial Color	Color after addition of pH10 buffer	Color after addition of pH4 buffer	Color after addition of HCL solution
0.001g of indicator	Light green	Slight change to light blue	Slight change to yellow	Slight change to yellow
0.002g of indicator	Green	Blue	Yellow	Yellow
0.004 g of indicator	Dark Green	Slight change to dark blue	Slight change to green	Yellow

4.1.5.4 Polyacrylamide-based Hydrogel Leakage Test

A leakage test was performed with 0.35 g of each disc in 20 mL of deionized water or milk. After three days, there was a visible difference in the consistency of all of

the gels compared to the initial dry state. The solid disks became more jelly-like and had visibly absorbed water. All milk samples had a faint sour odor, indicating spoilage. The color of the gels turned from green to yellow, and all of the surrounding liquid, whether it was milk or deionized water, had a blue tint (see Figure 9).

a) 

b) 

Figure 9 Polyacrylamide Leakage Test Results
a) Leakage test performed with a piece of the 0.003g indicator in 15mL polyacrylamide gel in deionized water. The indicator leaked out into the surrounding liquid. b) Leakage comparison from left to right between 0.001g indicator gel, 0.002g indicator, and .004g gel. Higher concentrated amounts of dye in the gel had higher amounts of leakage.

4.1.5.5 pH indicator comparison

Table 8 shows the breakdown of the comparison tests. A visual of the milk in the vials starting to change color can be seen in Figure 9. Samples 1a/b containing bromophenol blue had a color change from sky blue to darker blue. During days 7-12., samples 2a/b containing bromocresol purple remained greyish-blue. Samples 3a/b containing phenol red had a slight gradual change from peach to yellow during day 14. The color change in samples 4a to a pale yellow is depicted in Figures 9 and 10. Samples 5a/b remained clear during all 15 days. Samples 6a/b containing the gel prototype had a gradual color change from dark green to yellow over days 5-15. The control sample remained teal throughout the experiment as expected.

Table 8: pH Indicator Comparison Results over Time

Date	1a,1b	2a,2b	3a,3b	4a,4b	5a,5b	6a,6b[5]	Control (H2O)[4]	Comments
Day 1-5: 1/24/13-1/28/13	Layer of Sky blue	Faded grey light blue	Light peach	Light green	Clear	Teal/dark green	Teal/ dark green	No comments
Day 6 1/29/13	Sky blue	Grey light blue	Pale peach	Light green	Clear	Lighter green	Teal	Gas formation in 2b, 3b, and 4b
Day 7-12: 1/30/13-2/03/13*	Sky blue	Grey light blue	Pale peach	Light green	Clear	Yellow green	Teal	Gas formation in 2b, 3b, 4b, 6b
Day 13 2/04/13	2a:Darker sky blue 2b:yellow color on surface	Grey blue	Pale peach	Olive green	Clear	Yellow green	Teal	White milky residue formation on top layers[1]
Day 14 2/05/13	Darker sky blue	Grey blue	3a:Peach 3b:More orange tinge	More yellow, pale chunky white crust	Clear	Yellow green	Teal	White residue still present[2]
Day 15 2/06/13	Darker Sky blue	Grey blue	Light yellow	Darker yellow green	Clear	Mostly yellow	Teal	Control Milk sample has spoiled smell[3,6]

KEY:
1a, 1b: Bromophenol blue, 2a, 2b: Bromocresol purple, 3a, 3b: Phenol red, 4a, 4b: Bromothymol blue, 5a, 5b: Phenolphthalein, 6a, 6b; polyacrylamide gel w/ bromothymol blue indicator (0.002g per 15mL ratio). Control H2O; polyacrylamide gel w/ bromothymol blue indicator (0.002g per 15mL ratio) in deionized water

Note:
[1] Gas still prevalent in 2b, 3b and 6b
[2] 4A and 4B display visible white chunks
gas still prevalent in 2b, 3b and 6b (more gas in 3b)
[3] pH of spoiled milk 6.85.Chunks and froth present in all samples
[4] The gel itself is teal but the surrounding water is also of that color
[5] 6a,6b: The gel itself is a yellow color and the surrounding milk is green. Green dye visibly accumulates on the bottom
[6] The milk obtained was Cloverfield Farms 2% milk from the University of Maryland store with a sell by date of February 6th.
**some days were excluded*

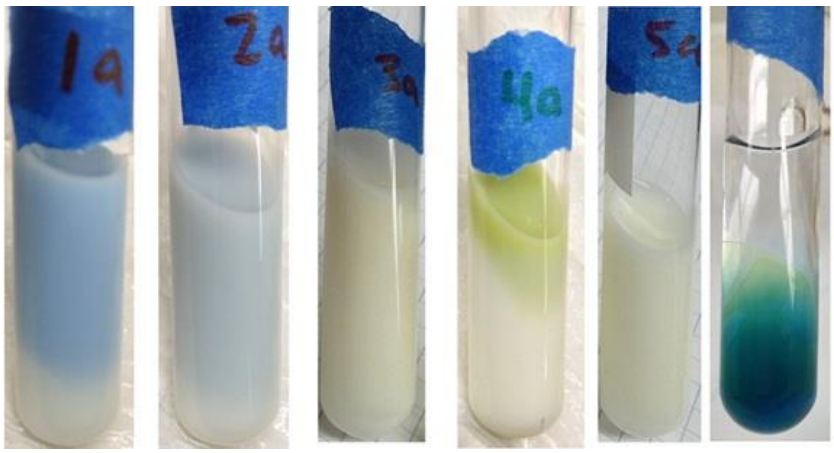


Figure 10: Milk Samples Containing Various pH Indicators

Figure 11: Picture taken after slight mixing of sample 4a on 2/6/13. There is a notable color difference since initial condition. White chunky residue can be seen lining the surface of the test tube.

4.2 Science Analysis

4.2.1 Bacterial/pH Correlation

A bacterial count was performed in order to assess the pH at which milk spoils. A parallel linear increase was expected between the number of bacterial colonies grown and

time as the off-the-shelf milk spoiled due to lactic acid build up. This, however, was not observed, as the increase of bacterial colonies was non-linear. No correlation was found between bacterial count and spoilage; therefore, the amount of bacteria was not a useful indicator of the pH at which milk spoils. Correlation was also not found between pH change and spoilage. Although literature values indicate a steady decrease in pH as milk spoils, little variation in pH was observed in the off-the-shelf samples as they spoiled. Among all the off-the-shelf samples, the maximum pH range over 480 minutes was 6.62 to 7.08 (see Table 1). A different pH meter was used after the initial one broke, which explains the sudden drop. However, this change was not the predicted linear decrease as the milk samples spoiled, as the lactic acid by-products of the bacteria involved in spoilage were expected to steadily lower pH over time (Bandler & Barnard, 1984). The sterile samples, as expected, showed no growth of bacterial colonies and a little change in pH, suggesting an effective negative control. This control data does suggest that pH change is linked to the bacteria growth or bacterial activity and byproducts in the milk, even though we were not able to confirm a stable number.

Thus, the premise of sensing pH as the main variable in determining whether the milk is fit for consumption was upheld by the control samples. Overall, this data was unable to confirm the reported literature values of milk pH following spoilage. As a result, subsequent research was conducted using the literature values of pH as a reference. Errors could be attributed to the variances in measurement between pH meters as well as fluctuations before and after calibration, and environmental factors in which the bacteria were cultured, such as agar properties or variances in initial bacteria in the milk samples.

4.2.2 Heat Treatment

4.2.2.1 Open Flame Variation

The use of open flame to melt plastic proved to be unsuccessful in achieving the desired melted plastic consistency. After heat treatment, the plastic was both burnt and brittle. The burnt plastic posed an issue because if the pH indicator had been added and maintained its pH sensitivity, the color changes would not have been able to be seen on the blackened plastic. The brittle aspect of the plastic deemed it too delicate to work with. It was noted that only the areas exposed to the flame melted. Because direct flame contact to melt the plastic was needed, many parts of the plastic remained in a solid state. Thus, using an open flame did not allow for consistent heating throughout. Due to safety concerns and the inability to control the flames, this option for melting plastic was eliminated.

4.2.2.2 Hot Plate Variation

Once again, the charring of the plastic became an issue with regards to the integrity of the plastic. The indirect heating did not supply sufficient heat to melt the plastic. Because of the lack of deformation and thus the inability to homogeneously mix the pH indicator into the plastic, the hot plate variation was ruled out as an effective method of plastic heat treatment.

Because the plastic did not melt completely, properly incorporating the indicator into the plastic could not occur. There was a color change when using the acidic buffer but not the basic buffer because bromothymol blue changes when placed in an acidic condition.

4.2.2.3 Precision Plastic Melter Variation

Using the LPP device resulted in similar results to those of the hot plate variation test. Although the product was advertised to reach a temperature of 800 degrees and have the capability to melt plastic, it was unable to do so in all our attempts. There was not sufficient heat provided to get the milk carton plastic pieces to the ideal molten consistency. Because of the high viscosity, the pH indicator was unable to be homogeneously mixed into the plastic. Instead, superficial coloring occurred on the plastic. In order words, the dye was never able to be fully incorporated into the plastic.

When using the HDPE and LDPE beads, neither proved to have a difference on the outcome of the final product. However because of the faulty equipment, more work using the beads continued when working on other heat treatments. Further experimentation using the LPP was unwarranted because melting the plastic and incorporating the pH indicator was not possible.

4.2.2.4 Oven Test Variation

There were a multitude of reasons for why there was no color change in the plastic after the addition of acidic and basic buffer solutions. As previously mentioned, due to the extreme temperatures, the liquefied plastic burned and darkened in color. Issues also arose when the plastic continued to inflame and burn due to the high temperatures, even after being introduced to an argon-filled atmosphere. Due to this black color change in the plastic, any color change undergone by the pH indicator was unobservable. Another unexpected problem came with the addition of bromothymol blue powder into the melted plastic. Due to the extreme temperature of the plastic, the powder quickly oxidized once it came into contact with the plastic. This oxidation rendered the pH indicator ineffective to sensing pH change. In the end, it was concluded that although the oven provided

enough heat to effectively melt the plastic, this high heat eliminated the efficacy of our pH-sensitive plastic. The realization that introducing high amounts of heat would ruin the end product created the need to conduct more research on alternative methods of creating a pH-sensitive indicator without the use of heat.

4.2.3 Effectiveness of microfiltration membranes

The positive absorbance readings of the filtered liquid indicate that some dye did travel through the membrane pores along with the water. The optical densities were low compared to the reference of the fully dye-infused water, indicating a significant portion of the dye was filtered out of the water.

4.2.4 Nafion Testing

4.2.4.1 Leakage test

From the leakage test, there was a visually observed discoloration and an increase of the absorbance level in deionized water. Since the observation and absorbance values deviated from the original observations and absorbance values, it was concluded that Nafion was unable to prevent leakage between the wells containing liquid bromothymol blue and deionized water. Based on the design of the experiment, there are two explanations for the observed leakage. The first explanation for the leakage involves the structural integrity of the testing chamber. The chamber could have originally been ineffective in separating two liquids, even when separated by a non-permeable membrane. Many possibilities exist for this first hypothesis.

The rubber gaskets that seal each side of the well to the separating material (Nafion, in this case) could contain imperfections that allowed the exchange of the separated solutions. The rubber gaskets could have also been positioned incorrectly to

create a tight seal on the separating material. Another possibility in regards to the design of the testing chamber is that the chamber was never designed to handle a separating material as thin as Nafion, and therefore, the limited amount of force that was applied to create tight seals was not great enough to actually create a necessary tight seal. The second explanation for the leakage is that the solution of bromothymol blue was actually able to permeate and pass through the Nafion wall. However, this explanation can be eliminated since a property of Nafion is that there are no pores for such an exchange. It should not be possible for the bromothymol blue solution to pass through the Nafion. Coupled with the failed test of a control variable, we can then conclude that most likely, the testing chamber utilized in our experiment was not a satisfactory method of displaying the nonporous quality of Nafion.

4.2.4.2 Proton Permeability Test

From the proton permeability test, we had observed an insignificant degree of increase and decrease on the pH scale for the solutions in both wells. The insignificant change in pH indicated that protons were not exchanged through the Nafion membrane. A possible explanation for the failed proton exchange can be derived from an initial failure in understanding the properties of Nafion. While our setup fulfilled the requirement of having two liquids of differing electrochemical potential, there was not an applied energy to drive the protons according to the gradient. In other terms, the buffer solutions existed in a steady-state in which there was no movement of protons across the Nafion membrane. We determined that in order to create a sufficient potential, we would have to apply an electrical voltage to both wells in order to create a significant proton exchange between the wells.

4.2.4.3 Control test

A negative control was performed in order to ensure that any leakage through the chambers was a result of the film between the chambers and not the device itself. Since a plastic sheet should not have been permeable to liquids, any leakage would imply a faulty device. The leakage of dye that was observed ultimately indicated a flaw with the setup of the testing chambers. However, the failure of the control test does not affect our conclusion that Nafion would be an unsuitable material in our application. Since our discovery that an applied energy source would be necessary, most likely through electrical means, our conclusion that Nafion is an unsuitable material remains the same.

4.2.5 Hydrogel Experiments

4.2.5.1 Gelatin-based Hydrogel

During the creation of the gelatin-based hydrogel portion, observations indicated that the bromothymol blue dye still displayed pH indicating properties even after being embedded into the gelatin. When an acidic solution made contact with the gel, the gel turned yellow. When a basic solution was added to the gel, the gel turned blue. As previously mentioned, bromothymol blue turns yellow in acidic conditions and blue in basic conditions ("Acid base indicators", n.d.).

It was also determined that the gel could be used repeatedly and undergo continuous reaction. Contact of pH buffer 10 caused a formerly acidic gel to turn blue, and a subsequent contact of pH buffer 4 at the same location caused the gel to return to its blue hue. In other words, a gel portion that had previously reacted to either a basic or acidic solution could react again with another solution.

In determining whether a hydrogel platform could be used to create a semi-solid pH indicating material, there was success. However, results indicated that after a few days of exposure to an aqueous environment, the once solidified gelatin-based hydrogel turned jelly-like. In addition, the aqueous environment changed colors, revealing that the hydrogel dissolved and the pH dye from the hydrogel leaked out.

Another negative with this approach was the presence of mold. Because gelatin is an organic compound, it was concluded that the gelatin provided nutrients and a surface for the bacteria and mold to attach and grow on. Since the hydrogel maintains much of its gel-like properties and does not hold sufficient structural integrity and provided an ideal medium for mold growth, it was deemed unsatisfactory towards our application.

4.2.5.2 Polyacrylamide-based Hydrogels

After creating the different concentrations of polyacrylamide-based hydrogels, it was determined that the gels with 0.002 g of pH indicator had the most promising results. Gels with more pH indicator were too dark and thus if any color changes occurred, they were difficult to see. Gels with less indicator did not exhibit any noticeable change when acid or basic solutions were added. In order for our product to meet its requirements, it must be able to produce a visible change in color. Thus, .002 g of indicator was selected for use in the gels when conducting further tests.

From the leakage tests, it can be concluded that the polyacrylamide-based hydrogel still displays leakage of the dye after being submerged in a liquid, similar to what was seen with the gelatin-based hydrogel. In addition, the polyacrylamide-based hydrogel reverts back to a more gel-like state, again similar to that of the gelatin-based hydrogel. However this method was deemed better suited due to multiple reasons. Based

on the results of the polyacrylamide-based hydrogel, we were able to confirm that the gel will still allow a change in color with respect to the pH sensitivity of bromothymol blue. After air-drying, the polyacrylamide-based hydrogel also has much greater structural integrity than the gelatin-based hydrogel in regards to its ability to withstand some levels of stress without deformation. Forceful applications of stress will create a clean break in the polyacrylamide-based hydrogel as opposed to the viscous-like deformation found in the gelatin-based hydrogel.

4.2.5.3 pH Indicator Comparison

Indicators were tested based on potential color changes within the range of spoiled milk according to literature values. Most of the samples exhibited little change throughout the span of the trial up until the milk was clearly spoiled, as evidenced by precipitate and pungent odors observed. Bromothymol blue showed the most obvious change compared to the other indicators, making it the most promising for our purposes. Bromophenol blue showed a slight color change around the same time as bromothymol blue; however, the slight change from light to dark blue was a smaller change and was concluded to be not as applicable for our intention, which is to show a clear indication of spoilage. In addition, the gel sample which was placed in a pure milk sample became a yellow color as the milk sample appeared to spoil. Since the indicator ranges from blue to yellow as the pH decreases, this indicates that the gel was able to indicate the milk's pH change and successfully correlated to its usability. Although the gel had some leakage into the milk sample, the indication property of the gel was not affected. Similarly, the dye in the control gel placed in deionized water leaked out as well, but the gel itself remained green. This negative control showed the expected result with no change in

color, indicating that the color change of the other sample was solely due to the milk's pH. All of the milk samples, including the original milk in the carton, showed signs of spoilage well before the sell by date of February 6th, supporting the fact that the dates can be unreliable. In addition, the sell by date is traditionally thought to be about two weeks before the expiration date, which indicates further that this posting is insufficient to accurately inform the consumer of the quality of their purchased milk.

However compelling this evidence, the team also recognizes that the early spoilage of the milk could have been due to a variety of compounding reasons. The fridge the milk was kept in may not been at an optimal temperature, as spoilage causing bacteria grows faster in warmer temperatures. The milk may have also been predisposed to spoiling early due to uncontrollable circumstances such as contaminants.

Overall, this test was an important verification of the usage of bromothymol blue in our experiments as well as a confirmation of the pH indicating properties of the gel prototype created. The tests verified bromothymol blue as an effective indicator of the pH change associated with milk spoilage because, as the data showed, both the indicator alone and the gel sample turned yellow as the milk sample spoiled. This phase also indicated that a better pH indicator could be further tested in order to more correctly match progressive spoilage.

4.3 Business Results

The business methodology sought to answer the question, "Would a milk spoilage detection product for individual consumers be successful in the US market?" through 1) an original consumer survey, 2) archival research of market trends, and 3) hypothetical market strategy and financial projections of how a company could sell such a product.

For the purposes of the hypothetical market strategy and financial projections, the company ColorCarton was assumed.

4.3.1 Survey Results

A detailed quantitative analysis of survey data can be found in Appendix II, but the important results are outlined in the following section. The purpose of the survey was to determine if there is significant consumer interest in a milk spoilage detection product for individuals. The main goal of our survey was to determine consumer interest in a color-changing milk quality detector.

Of the 295 total respondents to our survey, we found that 19 respondents failed to answer every question in the set. To eliminate variance and lack of full consumer profiles, we removed those 19 responses which left us with a total sample size of 276 for analysis.

To begin, we analyzed the demographic distribution of our survey respondents. Of those who completed our survey, 57 percent were female and more than 50 percent of participants fell in the age group of 35-54. We found that of 276 respondents, 44.5 percent throw away milk before or at the expiration date. Two hundred and fifty nine (94 percent) respondents showed concern for food wastage, 88 percent were concerned/very concerned about health quality, and 66 percent of respondents showed interest in our product (refer to Table 9). We also found that 22 percent of respondents regularly buy organic milk, 38 percent regularly buy non-organic milk, and 9 percent buy both (refer to Figure 11). Overall these results show that there are consumer concerns that need to be addressed and our product may be able to address those concerns.

Table 9: Survey results

Survey Question	Percent of respondents
Throw away milk before or at expiration date	44.50%
Concern for food wastage	94.00%
Concerned about health quality	88.00%
Interest in color-changing milk quality detector	66.00%

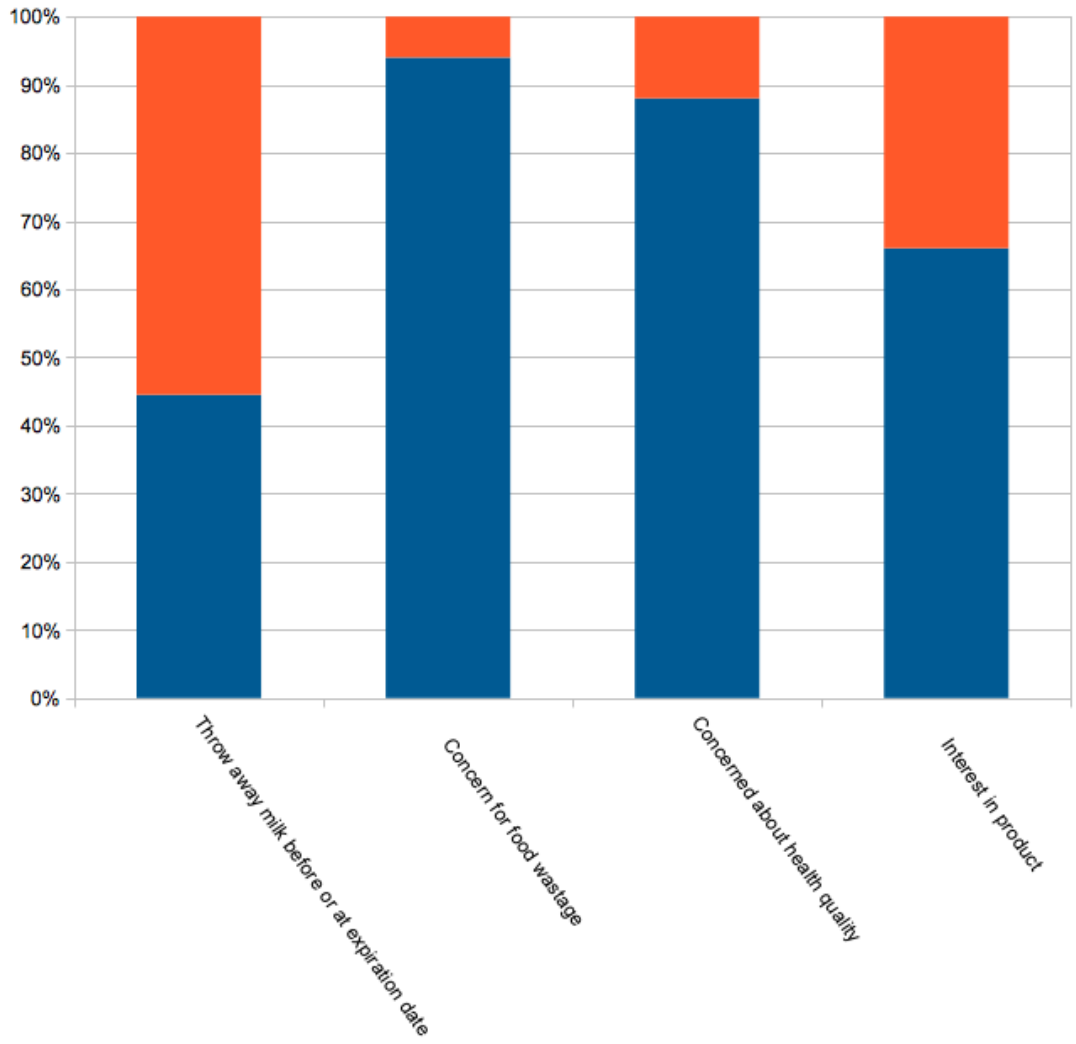


Figure 11: Survey Results
Red: Total Respondents, Blue: Percent of Respondents

Using this data, we conducted preliminary analysis on the correlation of responses between certain questions. Our initial theory was that there will be a significant correlation between an expressed desire to purchase our product and those who identified themselves as members of the younger, health-conscious, market segment. By determining the specific consumer profile of interested customers, we can establish a marketing strategy that would be most effective for the type of consumer that would be likely to purchase a color-changing milk quality detector.

We converted each survey answer into numerical data by assigning each potential answer (i.e. “strongly disagree,” “disagree,” “neutral,” “agree,” “strongly agree”) a numerical value (1 to 5, with 1 signifying “strongly disagree”). Our main goal was to cross-link the question about interest in our product with every other survey question. To analyze relationships between the questions from our survey, we conducted various chi-square tests for independence between the different questions asked in the survey. This test tells us how significantly correlated two questions from our survey are.

A chi-square test for independence starts with the null hypothesis that there is no statistically significant relationship between the variables, which can be rejected if certain conditions are met. The chi-square tests produce test statistics, which are then compared against a predetermined confidence level. We used a high confidence level of 99% to ensure that all results were valid and not the result of chance. Therefore, if a test statistic was smaller than 1-.99=.01, then the null hypothesis can be rejected and the variables can be deemed statistically dependent.

The chi-square tests for independence produced some statistically significant

results based on our survey data. The chi square test for independence between the response for concern about food wastage and interest in purchasing our product produced a test statistic of 0.0004008. This shows that there is a statistically significant relationship between concern about food wastage and interest in our product, which is consistent with our preliminary hypothesis. Unfortunately, we were unable to produce statistically significant conclusions regarding the relationship between consumer demographics such as age and gender with interest in our product. It is possible that such relationships do exist, but require a larger sample size in order to be statistically proven.

In terms of observational analysis of our data, 96 percent of respondents who showed concern about food wastage also showed interest in our product. We also found that, in particular, 76 percent of people who only buy organic milk showed interest in our product. These relationships were not proven to be statistically significant based on our results alone, but the data gives some credence to identifying an initial target market of organic milk purchasers from the ages of 35 to 54 who throw away milk once it reaches the expiration date for our product. In addition, we found that there was a very even distribution among gender and their concern for food quality.

Of the participants who responded that they were concerned about food wastage, 41 percent still threw away milk before or at the expiration date. Such results indicate that while consumers want to see a reduction in food wastage, there is obvious misinterpretation as to what expiration dates convey. Many consumers believe milk spoils at the expiration date and will waste milk even though they show concern for food wastage. It is also interesting to note that there is a significant relationship between those who are concerned about wastage and their interest in purchasing our product. These

findings were taken and applied in the sections below to determine the marketability and feasibility of creating a consumer product using this technology.

4.3.2 Results and Application of Market Research

Market research on trends in the milk and food packaging industries shows interest in eco-friendly packaging, particularly among bulk distributors (DuPont Packaging, 2012). This section projects the influence of industry trends on ColorCarton, a hypothetical company that creates a plastic milk carton that indicates milk quality through a color change.

4.3.2.1 Target Market

The U.S. milk industry in 2011 amounted to 530.93 million cwt of fluid milk (where 1 cwt = 100 lbs.), generating $10.7 billion in fluid milk sales annually. Of total milk sales, 3.9 percent ($417 million) were generated from organic milk sales (United States Department of Agriculture, 2012). Typically, this kind of food packaging product can be licensed as a design to bulk distributors, who package food from large quantities into smaller units (IBISWorld, 2012). Thus, the direct consumers of this product would be milk distributors, who package milk from large bulk vats to smaller units such as gallons or pints. The milk distributors, in turn, serve mainly household consumers and high-volume consumers (IBISWorld, 2012).

Based on our market survey of household consumers, 64.5 percent of all milk drinkers showed strong interest in buying a color-changing milk quality detector. Organic milk drinkers showed the most interest, as 76 percent expressed interest in such a product (Appendix II). Thus, any efforts to market such a device should initially target organic milk drinkers from the household market segment. After establishing popularity of the

product among organic milk consumers, a company's marketing efforts can expand to conventional milk. High-volume consumers purchase mainly conventional milk in large quantities, so for them, a company with a milk quality-indicating product should focus first on gaining a foothold among conventional milk suppliers.

4.3.2.2 Household Consumer Segment Analysis

Household consumers are defined as typical consumers who purchase milk from the grocery store for personal use. Among household consumers, those that purchase organic milk show the most interest in using high-tech milk packaging to detect spoilage (Appendix II**)**. Studies have shown that organic milk drinkers are typically white, high-income, and well-educated (Dimitri et al. 2007). Organic milk drinkers have also stated in multiple surveys they choose to buy organic products for environmental and health benefits—our product addresses both of these concerns (Williams, 2005). Milk distributors can use health- and waste-conscious organic milk drinkers as an initial target market.

4.3.2.3 High-Volume Consumer Segment Analysis

High-volume consumers include food services institutions, such as schools, hospitals, and prisons, which provide milk for large quantities of people. Quality control is the main factor of interest for high-volume consumers—because they buy in such great quantities and serve so many people, the consequences of poor milk quality can be severe. Color-changing milk quality detector technology would ensure more consistent food quality by eliminating the ambiguity of expiration dates. In addition, high-volume consumers of this technology could lower costs: instead of throwing away milk based on the expiration date, these consumers would know specifically when to throw away milk

and thus reduce costs from wasted milk.

4.3.3 Industry Analysis

Based on Porter's Five Forces, a framework for business industry analysis, a company that designed color-changing milk quality detector would be subject to medium to high bargaining power of buyers, low bargaining power of suppliers, low threat of new entrants, medium rivalry from existing firms, and medium threat of substitute.

Bargaining Power of Buyers – **medium to high**

The milk industry heavily focuses on affordability, which makes buyers price-sensitive to any changes in the milk price caused by a product. During the beginning stages of marketing such a packaging product, any licensing contract should extend only to a few milk distributors; a small number of buyers thus have relatively higher buyer power. However, buyer power is greatly reduced by the fact that such a product would be the only differentiating, viable alternative to inaccurate expiration dates. Overall, the buying power of milk distributors would be mitigated by this product's singularity in the milk spoilage detection industry.

Bargaining Power of Suppliers – **low**

After the initial investment, this product could avoid supplier costs by adopting a licensing strategy. The company would not need to manufacture the product; rather, they could license the design to interested packaging manufacturers.

Threat of New Entrants – **low**

Low initial capital and research and design costs create only small barriers to entry, but the company should file for patent protection for its product design within the three years of operation. Patent protection will create major legal obstacles for potential rivals who seek to enter the industry.

Rivalry Among Existing Firms – **medium**

Intelligent packaging is a major trend in food technology today. Various rival firms are developing products that also seek to replace standard milk packaging, but no similar quality-detection products are currently available to consumers.

Threat of Substitutes – **medium**

While there are virtually no formal milk spoilage detection methods directly available to consumers, there are other ways to determine spoilage including expiration dates, smelling the milk, and observing the visible appearance of the milk.

4.3.4 Competitive Advantage

A successful quality-indicating milk carton design could provide milk producers with differentiation and brand development. The product could provide consumers with more information about their milk purchase, reduce fluid milk and plastic waste, and improve food quality control.

Eco-friendly: Currently there are packaging products that promote environmental consciousness by using less or recycled materials in order to reduce waste.

Spoilage detection: Competitive products use various methods to detect food spoilage,

such as the detection of gas emissions or microbial by-products.

Cost effective: Most intelligent packaging designs add a substantial price increase due to the production cost of incorporating a detection system.

Other competitors only focus on eco-friendliness or spoilage detection—but there is a market niche available for a product that combines both advantages while maintaining a relatively low cost.

4.3.5 Competitor Matrix

A competitor matrix (refer to Table 10) clearly shows the areas in which the ColorCarton has a market. From the table it can be seen that other comparable products do not satisfy as many targets as the ColorCarton.

Table 10: Comparison of ColorCarton to Competitors

Product	Company Name	Milk-specific	Eco-friendly	Detects Spoilage	Cost-Effective	High-volume consumer	Household consumers
FreshTag	COX Technologies		x	x	x		x
Green Bottle	Pure & Simple Solutions, LLC		x				x
infini bottle	Nampak Plastics	x	x		x		x
Biodegradable bag-pack	Daylesford	x	x				x
Good Milk	StudioIN	x	x				x
Electronic Nose	MDPI			x	x		
ColorCarton	**The ColorCarton Group**	x	x	x	x	x	x

4.3.6 Marketing Strategy and Sales Plan

4.3.6.1 Strategy for Household Consumers

Among distributors to household consumers, organic milk distributors would find this technology most attractive and would benefit the most from using our technology. Our market surveys show that among all milk consumers, organic milk consumers are particularly interested in using our design. Survey respondents expressed approval of the ColorCarton's potential to address two major grocery shopping concerns: reduction of food wastage and improvement in food health quality. Market trends show that organic milk often costs almost double the price of conventional milk. The consumers that spend this extra money to buy organic milk—usually citing the environmental or health benefits of organic farming—also demonstrate a willingness to pay higher-than-conventional prices for other products marketed as eco-friendly or health-conscious (Venezia, 2012).

Thus, we plan to penetrate the milk industry by convincing organic milk distributors that increased consumer interest would outweigh the minimal added cost associated with incorporating our technology. Consumers would prefer to buy milk in ColorCarton packaging and would be willing to pay slightly higher prices in order to enjoy our unique technology's health and environmental benefits.

4.3.6.2 Strategy for High-Volume Consumers

In addition, we will market our plastic design to milk distributors who specialize in bulk milk sales. Fundamentally, our technology is a plastic that detects and indicates milk spoilage without damaging milk quality – our plastic can be molded into any shape. We will encourage bulk distributors to purchase the license to our plastic design and production method with the understanding that they do not require the plastic to be in the

carton shape. In our marketing approach to these bulk distributors, we will focus on the benefits of our technology to food services institutions such as cafeterias and restaurants. Our technology solves food wastage and quality control problems, which are compounded for institutions that regularly serve milk in mass quantities.

4.3.6.3 Target Distributors

Consolidation and concentration are changing the milk industry as only a few large operations dominate each segment of the milk market. We aim to target the large, dominant milk distributors instead of small, peripheral firms. Large companies can take advantage of economies of scale and produce at a lower cost per unit. This advantage makes it more likely that large operations will implement our technology. The fixed cost required to incorporate our technology would be less significant for large operations.

We hope to establish a foothold in organic milk and bulk milk production by highlighting the effectiveness, environmental friendliness, and valuable savings potential of the ColorCarton. Once industry professionals recognize our track record of providing a competitive advantage to organic milk products and bulk milk sales, our sales force will encourage other milk suppliers to invest in our technology and adopt the ColorCarton as the new standard in milk packaging.

We will demonstrate to milk distributors that our product has strong potential for establishing brand recognition. With user-friendly visibility and bright color changes, the ColorCarton will be instantly recognizable to consumers. Distributors that package their milk in the ColorCarton will thus enjoy heightened consumer attention and a subsequent boost in sales.

4.3.6.4 Pricing

The ColorCarton derives revenue by granting non-exclusive licenses to milk suppliers. It currently costs $.24 for us to create our product with non-bulk pricing (Appendix V). However, we believe with large-scale manufacturing and streamlined optimization, these costs could be brought down to $.05. A non-exclusive license would cost approximately $0.05 per carton and we estimate that use of the ColorCarton would increase production costs for the milk supplier by $0.10 per carton. Market trends show that consumers are willing to spend more for eco-friendly or health-conscious product. We expect that consumers would be prepared to pay $0.20 more for our carton—a conservative estimate, considering that our target household consumers regularly pay double the price of conventional milk for organic milk. Milk distributors who adopt our product will thus generate at least an additional $0.05 profit per carton of milk sold.

4.3.7 Financial Plan

4.3.7.1 Costs

An estimated initial investment of $100,000 would be required to support the first two years of a company attempting to commercialize this research. These cost projections include $7,000 to support patent filing and legal costs, continued research and development, and marketing towards milk distributors. The first two years of development would focus on finalizing the product design, specifically in regards to integration with the current milk carton design. Major costs consist of a plastic injection molding machine ($15,000) and a milk packaging machine ($40,000). In 2015 Q2, a small sales force should be recruited to begin marketing to milk distributors. A mapped

out income statement can be found in Appendix III.

4.3.7.2 Revenues

The projected revenues are based on the number of distributors contracted to use the non-exclusive license. With penetration focused on organic milk and bulk distributors, the initial business will expand marginally; however growth thereafter will be exponential as the technology expands into conventional milk markets and establishes itself as the industry standard for milk cartons.

4.3.7.3 Pricing

The license pricing is not a fixed number; rather it is calculated based as a percentage of the current price of milk. As milk prices rise, the price of the license will rise, and similarly, as milk prices fall, the price of the license will fall. This pricing strategy will give the business more flexibility to change pricing depending on how the pricing model is established with the distributor. It will also encourage distributors to ally themselves with the product, even if milk prices decrease.

The calculations for the revenue projections for the next ten years were conducted assuming charging an average of $0.05 per carton for each license. If distributors adopt the new technology, they will incur an additional $0.10 manufacturing cost, but can charge a premium of $0.20 per carton at retail price. Assuming milk prices remain stable, milk distributors can earn an extra $0.05 profit per carton.

4.3.7.4 Burn Rate and Breakeven Point

Based on projections of Cash Spent to Cash Earned, a business could expect to reach a breakeven point in 2015 Q4. The burn rate and breakeven point projections, based

on quarters, are shown in Table 11.

Table 11: Burn Rate and Breakeven Point

Year (Quarters)	**Cash Spent**	**Cash Earned**	**Yearly Retained Earnings (Cash Earned Net Cash Spent)**	**Accumulated Cash**
2013 Q1	$2,000	$0	-$2,000	-$2,000
2013 Q2	$5,000	$0	-$7,000	-$7,000
2013 Q3	$8,000	$0	-$8,000	-$15,000
2013 Q4	$35,000	$0	-$35,00	-$50,000
2014 Q1	$40,000	$0	-$40,000	-$90,000
2014 Q2	$3,000	$0	-$3,000	-$93,000
2014 Q3	$3,000	$0	-$3,000	-$96,000
2014 Q4	$4,000	$0	-$4,000	-$100,000
2015 Q1	$30,000	$0	-$30,000	-$130,000
2015 Q2	$32,000	$25,000	-$7,000	-$137,000
2015 Q3	**$34,000**	**$65,000**	**$31,000**	**-$106,000**
2015 Q4	**$34,000**	**$120,000**	**$86,000**	**$117,000**
2016 Q1	$60,000	$145,000	$85,000	$202,000
2016 Q2	$100,000	$160,000	$60,000	$262,00
2016 Q3	$140,000	$180,000	$40,000	$302,000
2016 Q4	$200,000	$215,000	$15,000	$317,00

4.3.7.5 Financial Projections

For the first few years, no revenue is projected as research is completed. Once the company starts to penetrate the market, the contracts with a few corporations will result in a small positive net profit. As the company expands into conventional milk markets, the revenue growth will be exponential.

4.3.8 Feasibility

The results of our surveys and market research indicate the ColorCarton's marketing, sales, and financial plan would be feasible in the US milk industry. Market trends show eco-friendly products that increase product quality and reduce wastage are gaining momentum in the US market.

Our survey results support our hypothesis that a milk spoilage detection product for individual consumers would be successful in the US milk industry, revealing that 94 percent of respondents showed concern for food wastage, 88 percent were concerned/very concerned about health quality, and 66 percent of respondents showed interest in our product. This demonstrates the clear need for a milk spoilage detection product for individuals that is not yet being met in the market. Organic milk drinkers showed the most interest, with 76 percent expressing interest in a color-changing milk spoilage detection product. Our strategy of penetrating the market through first approaching organic milk distributors is practical as organic milk purchasers spend nearly double the amount on milk compared to purchasers of conventional milk and would be likely to spend more on a product that increases product quality and reduces food wastage. The likelihood of milk distributors being interested in our product is also high as a non-exclusive license would cost around $0.05 per carton and the use of the ColorCarton would increase production costs for the milk supplier by only $0.10 per carton. Through charging an additional $0.20 per carton with our technology, the milk distributors would earn an added $0.05 per carton, increasing the incentive for milk distributors to purchase our technology.

4.3.9 Key Risks

A layout of the proposed mitigation strategies for each foreseeable risk is illustrated in Table 12.

Table 12: Risks and Mitigation Strategies

Risk	**Mitigation Strategy**
Product is not FDA-compliant	The ColorCarton Group has been in contact with FDA staff throughout design process and is aware of all relevant regulations.
Patent is not approved	The ColorCarton Group has conducted preliminary patent research and is now working with the UMD Office of Technology Commercialization.
Milk distributors do not adopt product	Government subsidies will encourage use of our product due to the environmental benefits of spoilage detection.
Required investment is not met	The government and nonprofit organizations have historically supported environmentally friendly technologies through grants.

5. Conclusion

Milk plants often use rudimentary methods such as standard plate count (SPC) to determine and detect bacterial concentrations in post-pasteurized milk. These methods provide no information to the end consumers of the product other than an inaccurate prediction of freshness. We hypothesized that a method of detection utilizing pH sensors would provide the end consumers with this valuable information at a non-prohibitive cost.

Various metrics, including simplicity of procedure, speed and range of detection, accuracy of results, and cost of equipment, were critical in determining the optimal detection method for milk spoilage. Of the potential methods of detection outlined in this thesis, pH sensors were determined to be the best fit for household consumers. Our laboratory results have identified a polyacrylamide-based hydrogel integrated with pH sensitive dyes as the best pH detection method as the dye maintains its detection properties after introduction to the hydrogel. Additionally, our preliminary testing indicates bromothymol blue to be the most accurate indicator for milk spoilage, but this has yet to be thoroughly vetted through multiple trials testing for accuracy.

Our survey and market analysis paired with our cost estimates indicate that there is potential for a business entity to exploit this methodology for sustainable profits in both niche milk markets and in mass production of standard pasteurized milk. However, the methodology for creating pH sensitive hydrogels is flexible and the potential exists to expand this technology to different food packaging systems by using different pH dyes.

6. Future Directions While we have not fully developed a usable prototype that we envisioned at the beginning of the project, we do see strong potential for a product to be completed by future Gemstone research. Research thus far has generated myriad results. Survey analysis has confirmed preliminary research on an environmentally-friendly trend and has thus revealed that there is strong consumer interest in our environmentally-friendly product. Through our financial analysis, we have determined that milk distributors who adopt our product will generate at least an additional $0.05 profit per carton of milk sold. Through our market analysis, we have determined that our business model is financially viable and can generate positive cash flows.

However, there are still complications that should be addressed.

1) Firstly, our survey of two hundred and ninety four respondents restricts our market analysis to a limited geographical range based solely in Maryland. In order for this product to be integrated in a nation-wide manner, we should first expand our surveys and market research to a larger geographical area that will represent a sample of the nation-wide population. Different regions throughout the nation have very different perceptions. Thus, we must expand our market analysis to include these differences so that we can better tailor our product to a wide range of consumers.

2) Further data on consumer preferences will also need to be gathered. While surveys only helped us gauge initial consumer interest in our product to give us the impetus to go through with it, focus groups will help us establish and narrow specific consumer preferences that specifically help us satisfy the desires of our consumers. For example, whereas on our survey we questioned whether they would be interested in such a product as the ColorCarton, we would use focus groups to determine specific characteristics of

the product, such as color, placement of indicator, and type of packaging material that would be consistent with our team's mission statement.

Our product is food packaging, which is a food contact substance and, more generally, a food additive. New types of food additives must undergo FDA approval before they can be marketed. Thus, FDA approval would be required before milk producers could use our bottle design for their product. The approval process generally takes about six months.

During the product development process, we can maximize our chances for eventual FDA approval by using mainly substances classified as REG, FS, PS, or GRAS. Upon completion of product development, we would submit electronically to the FDA Form No. 3480, "Notification for New Use of a Food Contact Substance under Premarket Notification."

In this vein, more research is necessary to fully understand the government regulations that apply to our product. FDA approval is the first and most obvious hurdle, but many states and municipalities have their own regulations regarding food packaging and labeling. More detailed analysis of existing regulations—as well as an examination of relevant interest groups and the political alliances of the milk industry—would resolve legal questions surrounding widespread adoption of our product.

5) While we have created a polyacrylamide gel that would serve as a medium for our spoilage detection system, we have yet to decide how to best integrate our design into milk packaging. This not only includes where to integrate the detection system, but also how manufacturers can most efficiently integrate this system.

6) We have established consumer interest in our product, but we must more solidly gauge industry interest. A pH-sensitive detector-indicator has the potential to introduce both advantages and disadvantages to adoptees of our technology. On the positive side, our product offers individual milk producers opportunity for brand differentiation—a significant number of consumers may choose to buy milk from a particular company because they like the packaging, and if this packaging preference becomes a key enough sales factor, this style of packaging may become the new industry standard. However, during this period of transition and early adoption, milk companies will have several concerns: first, if consumers can see that milk lasts longer, they will buy milk less frequently than before; and second, producers may find prohibitive the costs of incorporating the plastic material into current manufacturing processes. More conversation with industry representatives would help us better understand the milk industry's perspective; indeed, industry support is key to the success of this product.

After these complications are addressed, work and development on the prototype can continue. The first steps that will need to be taken is to finalize the manufacturing and product design in a way such that problems described above are not only addressed but that the product itself is manufactured in the most cost-effective way while also maintaining its usability. Once the design and product are finalized, the product can be presented to milk distributors. We will use trade shows and exhibitions to initially establish our product and target manufacturers willing to collaborate with us. Upon forming a partnership, focus will be concentrated on streamlining the manufacturing processes and controlling costs.

Concurrently, further research will be carried out in relevant fields to adapt our

detection system into all different kinds of food products such as fruit juices and wines. This will require different pH indicators and further experimentation to determine spoilage characteristics of different food products. Collaboration with our manufacturing partners will further determine the extent to which a nation-wide marketing campaign should be carried out in order to familiarize consumers with our product.

Appendix I: Survey Questionnaire

Team Milk Survey

I have thrown away milk as soon as the expiration date is reached.

Yes No

I am concerned about food wastage.

Yes No

I am _____ about the health quality of my food.

Not concerned Slightly concerned Concerned Very concerned

I buy this type of milk. Circle all that apply:

Non-organic Milk Organic Milk Soy Milk/Lactose Free Milk Other

I would prefer to purchase a milk carton that changes color to indicate spoilage.

Yes No

Age (in years):

18-24 25-34 35-44 45-54 55-64 Over 64

Gender:

Male Female

Appendix II: Survey Data

			Key: 1=not concerned, 2=slightly concerned, 3=concerned, 4=very concerned	Key: 1=non-organic, 2=organic, 3=soy/lactose-free, 4=other		Key: 1=18-24, 2=25-34, 3=35-44, 4=45-54, 5=55-64, 6=64+	
SURVEY NUMBER	**Thrown away milk at expiration date (Y/N)**	**concern about food wastage (Y/N)**	**concern about health quality**	**Buy this type of milk**	**Purchase a milk carton that changes color? (Y/N)**	**Age Group**	**Gender (M/F)**
1	no	yes	3	2 and 3	yes	1	f
2	no	yes	4	1	yes	5	m
3	yes	yes	4	1	yes	4	f
4	no	yes	4	1 and 2	no	3	m
5	yes	no	4	1	no	2	m
6	no	no	4	1	no	5	f
7	yes	yes	4	1	yes	3	f
8	no	yes	2	1	yes	2	m
9	no	yes	1	1 and 4	no	4	f
10	NR	yes	3	4	no	6	f
11	yes	yes	2	1	yes	6	NR
12	yes	yes	4	3	no	6	f
13	yes	yes	2	1	yes	1	f
14	no	yes	4	1 and 2	no	4	f
15	yes	no	4	1	no	6	f
16	no	yes	3	3	yes	6	f
17	yes	yes	3	2	no	5	f
18	yes	yes	4	2	yes	4	f
19	yes	yes	4	1	yes	4	m
20	yes	yes	4	3	yes	5	f
21	no	yes	2	1 and 2 and 3	yes	1	m
22	no	yes	3	2	yes	1	f
23	no	yes	2	2 and 3	yes	5	m
24	yes	yes	3	1 and 2 and 4	yes	4	m
25	no	yes	3	1	don't know	5	m
26	yes	yes	4	2	yes	2	f
27	yes	yes	3	2	yes	3	m
28	yes	yes	3	1 and 2	yes	5	m
29	yes	yes	2	3	no	5	f
30	yes	yes	3	1	yes	6	f
31	no	yes	3	1	no	6	f
32	yes	yes	3	4	yes	2	f
33	no	yes	3	1	yes	4	m
34	no	yes	4	1	no	6	m
35	no	yes	4	3 and 4	yes	5	m
36	no	yes	4	1 and 2 and 4	yes	3	f
37	no	no	4	1	yes	2	f
38	no	no	3	1 and 3	no	3	f
39	no	yes	4	3	yes	2	m
40	no	yes	2	1	yes	4	f
41	yes	no	3	1 and 2	yes	3	m

42	no	yes	3	1	yes	4	f
43	no	yes	3	1 and 2	doesn't matter	4	m
44	no	yes	4	1	no	6	m
45	yes	yes	3	1	yes	4	f
46	yes	yes	2	4	yes	underage	f
47	no	yes	3	4	yes	4	m
48	yes	yes	3	1 and 2	yes	3	m
49	no	yes	2	1 and 2	yes	3	m
50	no	yes	3	1	no	5	m
51	no	yes	4	2	yes	2	m
52	yes	yes	3	1	no	3	f
end of Giant (Arlington Rd)							
53	no	yes	3	1	yes	6	f
54	no	yes	4	1	no	6	m
55	no	yes	1	1	no	4	m
56	yes	yes	3	1	yes	3	f
57	yes	yes	4	1 and 3	yes	6	m
58	no	yes	4	4	yes	6	f
59	no	yes	2	1	yes	5	f
60	no	yes	3	4	yes	6	m
61	no	yes	3	1 and 3	yes	4	m
62	NR	yes	3	NR	no	6	f
63	yes	yes	4	1 and 3	no	3	m
64	no	yes	4	3	no	5	m
65	yes	yes	4	2 and 4	no	2	f
66	no	yes	3	2	?	6	m
67	yes	yes	4	1 and 2	no	5	f
68	yes	yes	4	4	yes	5	f
69	yes	no	4	1	yes	2	f
70	no	yes	3	1 and 2	yes	5	f
71	no	yes	4	4	no	6	f
72	no	yes	3	1 and 2	no	4	m
73	yes	yes	3	4	yes	6	f
74	no	yes	3	1 and 2 and 3	yes	6	m
75	yes	yes	3	1	yes	6	f
76	yes	no	2	1	no	6	m
77	yes	yes	3	1	yes	5	m
78	no	yes	3	1	yes	2	f
79	no	yes	3	1	yes	4	m
80	no	yes	3	2 and 3	yes	1	f
81	no	yes	3	1	no	5	f
82	yes	yes	4	4	yes	5	f
83	yes	yes	3	1	yes	6	f
84	no	yes	3	1	no	2	f
85	no	yes	4	1	no	5	m
86	yes	yes	2	1	no	2	m
87	no	yes	4	2 and 3 and 4	no	4	f
88	yes	yes	3	1	yes	5	m
89	no	yes	4	1	yes	4	f
90	yes	yes	4	2 and 3	yes	2	m

91	yes	yes	4	3	yes	6	f
92	no	yes	3	1	yes	6	f
93	yes	yes	4	4	yes	5	f
94	no	yes	4	2 and 3	yes	3	f
95	no	yes	2	1 and 2	yes	3	f
96	yes	yes	4	2 and 3	no	3	m
97	yes	yes	NR	3	no	3	m
98	no	yes	4	1	no	3	f
99	no	yes	4	1	yes	4	f
100	no	yes	4	4	yes	6	m
101	no	yes	3	1	yes	4	m
end of Magruders 8/6							
102	no	yes	3	1	no	2	m
103	yes	yes	3	4	yes	6	m
104	no	yes	4	1 and 2 and 3	yes	4	m
105	yes	yes	4	3 and 4	yes	4	m
106	no	yes	4	1	no	5	f
107	yes	yes	4	3	no	6	f
108	no	yes	3	1 and 2	yes	4	m
109	yes	yes	4	1	yes	6	m
110	yes	yes	4	3	yes	5	m
111	yes	yes	4	3	no	5	m
112	yes	yes	4	3	yes	6	f
113	yes	yes	3	1	no	6	m
114	yes	yes	4	1	no	5	f
115	yes	yes	3	2 and 3	no	6	f
116	no	yes	1	2	yes	6	m
117	no	yes	4	2	yes	6	m
118	yes	yes	3	2	no	5	NR
119	no	no	4	NR	no	5	m
120	yes	yes	3	1	yes	6	f
121	yes	yes	2	3	yes	5	m
122	no	yes	3	1 and 2	yes	5	f
123	yes	yes	2	2	no	1	m
124	yes	yes	3	2	yes	1	m
125	yes	yes	4	1 and 3 and 4	yes	1	m
126	yes	yes	4	3	no	2	f
127	no	yes	4	1	no	1	f
128	no	yes	3	3	yes	5	m
129	no	yes	2	1	no	6	f
130	yes	yes	3	4	yes	NR	m
131	yes	yes	4	1 and 2 and 3	NR	6	f
132	yes	yes	4	4	yes	6	f
133	yes	yes	4	2	yes	5	f
134	no	yes	2	1	yes	1	m
135	no	yes	3	1 and 2 and 3	no	2	m
136	no	yes	4	1	no	4	f
137	no	yes	3	1	yes	4	m
138	no	yes	3	1	yes	6	m
139	no	yes	4	1	no	5	m
140	no	yes	4	4	no	6	f
141	yes	yes	4	2	yes	2	m

142	no	yes	3	1	yes	6	m
143	yes	yes	2	2	yes	4	m
144	no	no	2	1	no	4	m
145	no	yes	2	1	no	6	m
146	no	yes	4	3	no	6	f
147	no	yes	4	1	no	5	f
148	yes	yes	4	1	yes	4	f
149	no	yes	3	1	no	5	f
150	yes	yes	2	1	no	1	m
151	yes	yes	3	2 and 3	no	NR	f
152	yes	yes	3	1	yes	6	m
153	no	yes	3	2 and 3	no	1	f
154	no	yes	3	1	yes	6	f
155	no	yes	4	3	no	5	m
156	no	yes	3	1 and 2 and 3	yes	4	f
157	yes	yes	3	1	yes	1	m
158	no	yes	4	1	yes	6	f
159	yes	yes	4	1	yes	6	f
160	no	yes	4	1	doesn't matter	5	f
161	yes	yes	4	2	yes	4	f
162	no	yes	4	2 and 3	yes	4	f
163	no	yes	3	4	yes	5	f
164	yes	yes	3	1	yes	6	f
165	no	yes	3	1 and 3	yes	6	m
166	no	yes	2	1 and 2 and 3	depends on cost	3	f
167	yes	yes	4	3	yes	5	f
168	no	yes	3	4	yes	6	f
169	yes	yes	3	2	no	3	m
170	no	yes	4	2	yes	6	f
171	no	yes	3	1	yes	3	f
172	yes	yes	3	3	yes	5	f
173	no	yes	2	1 and 2 and 3	maybe if cost doesn't increase more than 5 cents per container	4	m
174	yes	yes	4	1 and 2	no	4	f
175	yes	yes	4	1	yes	4	m
176	yes	yes	2	4	yes	5	f
177	yes	yes	3	1	yes	6	m
178	no	yes	3	1	yes	5	m
179	yes	yes	4	1	yes	5	f
180	yes	yes	4	3	yes	6	f
181	no	yes	3	1 and 2	yes	1	m
182	yes	yes	4	2	yes	3	f
183	no	yes	4	1	yes	6	f
184	no	yes	4	1	no	4	f
185	no	yes	1	3	yes	6	f
186	no	yes	3	1	no	5	m

187	no	yes	4	2 and 3	yes	4	f
188	no	yes	3	1	yes	1	m
189	yes	yes	3	1	no	4	f
190	yes	yes	4	1	yes	4	f
end of giant (Wootton pkwy)							
yes/1	86	180	4		118	15	85
no/2	102	10	24		65	17	103
3			78			20	
4			83			38	
						43	
						54	

Balance Sheet								
Year	2014 Q1	2014 Q2	2014 Q3	2014 Q4	2015 Q1	2015 Q2	2015 Q3	2015 Q4
Current Assets	$11,300	$12,300	$13,300	$14,300	$15,300	$16,300	$17,300	$18,300
Cash	$300	$0	$0	$0	$0	$0	$0	$0
Account Receivables	$0	$0	$0	$0	$0	$0	$0	$0
Inventory	$0	$0	$0	$0	$0	$0	$0	$0
Total Current Assets	**$11,600**	**$12,300**	**$13,300**	**$14,300**	**$15,300**	**$16,300**	**$17,300**	**$18,300**
Fixed Assets	$10,000	$10,000	$10,000	$10,000	$10,000	$10,000	$10,000	$10,000
Net Plant, Property, and Equipment	$65,000	$65,000	$65,000	$65,000	$95,000	$95,000	$95,000	$95,000
Total Fixed Assets	**$75,000**	**$75,000**	**$75,000**	**$75,000**	**$105,000**	**$105,000**	**$105,000**	**$105,000**
Total Assets	**$86,600**	**$87,300**	**$88,300**	**$89,300**	**$120,300**	**$121,300**	**$122,300**	**$123,300**
Short-term liabilities	$15,000	$15,000	$15,500	$15,500	$15,500	$5,500	$0	$0
Accounts Payable	$0	$0	$0	$0	$0	$0	$0	$0
Total Current Liabilities	**$15,000**	**$15,000**	**$15,500**	**$15,500**	**$15,500**	**$5,500**	**$0**	**$0**
Long-Term Liabilities	$51,000	$51,000	$51,500	$51,500	$60,000	$31,000	$28,000	$28,000
Capital Lease Obligations	$0	$0	$0	$0	$2,500	$2,500	$2,500	$2,500
Total Liabilities	**$66,000**	**$66,000**	**$67,000**	**$67,000**	**$78,000**	**$39,000**	**$30,500**	**$30,500**
Shareholder and Investor Capital	$20,600	$21,300	$21,300	$22,300	$42,300	$82,300	$91,800	$92,800
Retained Earnings	$0	$0	$0	$0	$0	$0	$0	$0
Dividends	$0	$0	$0	$0	$0	$0	$0	$0
Total Equity	**$20,600**	**$21,300**	**$21,300**	**$22,300**	**$42,300**	**$82,300**	**$91,800**	**$92,800**
Total Liabilities and Equity	**$86,600**	**$87,300**	**$88,300**	**$89,300**	**$120,300**	**$121,300**	**$122,300**	**$123,300**

Income Statement										
Year	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Revenues										
Grants/Rewards	$2,600	$600	$0	$0	$0	$0	$0	$0	$0	$0
Investments	$0	$0	$0	$0	$0	$0	$0	$0	$0	$3,000,000
License Fee	$0	$0	$210,000	$700,000	$1,820,000	$4,200,000	$8,400,000	$14,000,000	$22,400,000	$32,000,000
Total Revenue	**$2,600**	**$600**	**$210,000**	**$700,000**	**$1,820,000**	**$4,200,000**	**$8,400,000**	**$14,000,000**	**$22,400,000**	**$35,000,000**
Expenses										
Lab Fee	$0	$0	$10,000	$10,000	$60,000	$210,000	$560,000	$1,200,000	$2,000,000	$2,500,000
Patent	$10,000	$0	$0	$0	$0	$0	$0	$0	$10,000	$0
Materials	$10,000	$4,000	$4,000	$5,000	$15,000	$38,000	$160,000	$280,000	$730,000	$1,690,000
Equipment	$25,000	$40,000	$30,000	$100,000	$150,000	$600,000	$900,000	$1,900,000	$2,800,000	$4,600,000
Depreciation	$3,750	$6,000	$4,500	$15,000	$22,500	$90,000	$135,000	$285,000	$420,000	$690,000
Labor	$0	$0	$20,000	$200,000	$940,000	$1,500,000	$2,000,000	$2,000,000	$2,000,000	$2,000,000
Administrative	$0	$0	$0	$0	$80,000	$130,000	$480,000	$640,000	$860,000	$1,530,000
Marketing	$0	$0	$56,500	$160,000	$300,000	$953,000	$2,100,000	$3,440,000	$5,900,000	$9,430,000
Travel	$0	$0	$5,000	$10,000	$45,000	$175,000	$420,000	$490,000	$700,000	$1,020,000
Misc	$1,250	$0	$0	$0	$50,000	$60,000	$70,000	$90,000	$130,000	$160,000
Total Expenses	**$50,000**	**$50,000**	**$130,000**	**$500,000**	**$1,662,500**	**$3,756,000**	**$6,825,000**	**$10,325,000**	**$15,550,000**	**$23,620,000**
Net Income	**-$47,400**	**-$49,400**	**$80,000**	**$200,000**	**$157,500**	**$444,000**	**$1,575,000**	**$3,675,000**	**$6,850,000**	**$11,380,000**

Appendix IV: The Milk Industry Report (Mintel, 2012)

In 2011, total milk sales are estimated at $18 billion. Milk is a mature market, so growth is likely to either come from a growth in population or by increasing volume consumption from its current levels among households that already drink milk. There has been an increase in dollar sales for milk, but after looking at dollar/volume sales and price trends, this increase is mainly related to price increases and not a growth in volume. During recent years, 2006 to 2011, milk volume has stayed either depressed or flat.

FIGURE 16: FDMx trend in dollar/volume sales and price of milk*, 2006-16

Year	Dollar sales		Volume sales		Price	
	$million	% change	million gallon	% change	$/gallon	% change
2006	11,158		3,299		3.38	
2007	12,439	11.5	3,227	-2.2	3.85	14.0
2008	12,785	2.8	3,137	-2.8	4.08	5.7
2009	10,816	-15.4	3,165	0.9	3.42	-16.1
2010	11,113	2.7	3,094	-2.2	3.59	5.1
2011	11,924	7.3	2,983	-3.6	4.00	11.3

* excludes powdered/condensed/evaporated milk

SOURCE: Mintel/based on SymphonyIRI Group InfoScan® Reviews

FIGURE 28: Volume sales of milk, by segments and organic/non-organic, 2010 and 2011

	2010	% of total	2011	% of total	% change
	million pounds		million pounds		2010-11
Non-organic milk (total)	52,161	96.5	51,033	96.1	-2.2
Whole milk	14,118	26.1	13,690	25.8	-3.0
Flavored whole milk	552	1.0	523	1.0	-5.3
Reduced-fat milk (2%)	18,537	34.3	18,190	34.3	-1.9
Low-fat milk (1%)	7,051	13.0	7,097	13.4	0.6

Fat-free milk (skim)	8,001	14.8	7,717	14.5	-3.6
Flavored fat-reduced milk	3,902	7.2	3,816	7.2	-2.2
Organic milk (total)	1,916	3.5	2,070	3.9	8.0
Organic whole milk	434	0.8	499	0.9	14.9
Organic reduced-fat milk (2%)	564	1.0	598	1.1	6.0
Organic low-fat milk (1%)	401	0.7	425	0.8	6.0
Organic fat-free milk (Skim)	432	0.8	458	0.9	6.0
Organic flavored milk	85	0.2	90	0.2	6.0
Total milk (non-organic and organic)	54,077	100.0	53,103	100.0	-1.8

SOURCE: Mintel/AMS/USDA

Challenges in the market

There are a few challenges milk processors are currently facing:

- The dominance of private labels (56% of total market sales in 2011)

- Consumer concerns on growth hormones in non-organic milk

- Commodity price fluctuations

- Milk's label as a “commodity”

- Supply shortages for organic milk

- Increasing consumer concern on the general safety of animal product

Although consumers continue to portray milk's nutritional benefits, these challenges prevent the milk market from experiencing great growth opportunities.

Trends in the market

Organic milk, while still only accounting for a small percentage of total volume sales (3.9%) of the total cow's milk, has experienced a great amount of growth (8%) during 2010-2011. A large portion of this increase stems from parents' concerns about the growth hormone rBST in non-organic milk and the growing awareness of the benefits of consuming organic and natural foods and beverages. These parents do not mind spending

twice as much to buy organic milk for their children.

The aging population also provides concerns for the industry, since they exhibit lower-than-average interest in drinking milk. This group is significantly more likely (43% vs. 30%) to find 1-gallon milk packaging inconvenient compared to other groups. This is likely due to the packaging design; it holds too much milk for them to drink before the expiration. Those who consume the most milk are Hispanics and black households. The number of households with children has also been decreasing, which is detrimental to the market growth. Households with more people were more likely to purchase more milk.

Innovation in the market

Although private labels beat sales of branded milk in 2011, branded milk processors accounted for over 71% of total new product count. The new milk products are focused on health contributions, with many innovations coming from rice/nut/grain/seed-based drinks.

In addition to new milk products, producers are attempting to make milk more functional – by adding attributes like heart health, post-workout recovery, etc. - can bring milk sales up to equal other beverages that make similar functional claims.

Packaging and attitudes

Nearly 1/3 of milk drinkers find 1-gallon milk packaging to be inconvenient, with adults aged 65+ significantly more likely to feel this way. ¼ of all milk drinkers say they find milk packaging boring. Since milk is seen as a commodity, milk manufacturers don't find it cost effective to invest in making the packaging more attractive.

One-third of all milk drinkers show interest in milk with functional attributes (heart health, etc.). One-third are likely to buy milk that is good for digestive health. 45%

of consumers favor “green” or recyclable milk cartons to one that can't be recycled.

FIGURE 75: Attitudes toward milk, December 2011

“For each of the following statements, please tell us how much you agree or disagree.

Select one response per row.”

	Any agree	Neither agree nor disagree	Any disagree
Base: 1,887 respondents aged 18+ who personally drink or buy milk for household	%	%	%
Packaging			
I favor “green” or recyclable milk cartons to the ones that cannot be recycled	45	39	16
1-gallon milk packaging is inconvenient to use	30	27	43
Milk packaging is boring	25	55	20
Aseptic packaging is better for milk because I can take milk on the go (single-serve) and/or store it at home for a longer period of time (full or half gallon)	23	54	22
If the milk packaging provided recipes of food and beverages that use milk, I am more likely to buy it	19	40	40
If the packaging of single-serve flavored milk was more exciting, I would drink or buy it more often	13	36	51
Milk origin and health-related attitudes			
I prefer to buy locally or regionally produced milk	44	43	13
Flavored milk has too much sugar	42	41	17
I would buy more milk if it lasted longer before spoiling	42	31	26
Flavored milk should not be served in schools because it has too much sugar	36	39	25
Raw milk is healthier than pasteurized milk	18	46	36
Milk-based drinks			
I’m interested in “functional” milk drinks that might contain nutrients that help with heart health, stress reduction, or have other benefits	35	39	26
I’m interested in “probiotic” milk drinks that are beneficial for digestive health	33	38	29
I’m interested in milk-infused drinks such as Starbucks Frappuccino®	27	27	46
I prefer “energy” milk drinks that contain added vitamins and/or minerals	18	36	45

SOURCE: Mintel

FIGURE 76: Attitudes toward milk (any agree), by age, December 2011

"For each of the following statements, please tell us how much you agree or disagree.

Select one response per row."

Any agree:	All	18-24	25-34	35-44	45-54	55-64	65+
Base: all respondents aged 18+ who personally drink or buy milk for household	1,887	248	343	349	370	268	309
	%	%	%	%	%		%
Packaging:							
I favor "green" or recyclable milk cartons to the ones that cannot be recycled	45	48	51	47	42	40	41
1-gallon milk packaging is inconvenient to use	30	31	29	26	24	31	43
Milk packaging is boring	25	33	34	25	23	24	14
Aseptic packaging is better for milk because I can take milk on the go (single-serve) and/or store it at home for a longer period of time (full or half gallon)	23	29	27	26	21	23	15
If the milk packaging provided recipes of food and beverages that use milk, I am more likely to buy it	19	30	28	21	18	14	7
If the packaging of single-serve flavored milk was more exciting, I would drink or buy it more often	13	24	21	13	10	8	4
Milk origin and health-related attitudes							
I prefer to buy locally or regionally produced milk	44	43	43	44	41	46	49
Flavored milk has too much sugar	42	45	40	42	38	37	49
I would buy more milk if it lasted longer before spoiling	42	52	48	47	39	40	30
Flavored milk should not be served in schools because it has too much sugar	36	37	36	32	30	36	47
Raw milk is healthier than pasteurized milk	18	27	22	19	17	12	12
Milk-based drinks							
I'm interested in "functional" milk drinks that might contain nutrients that help with heart health, stress reduction, or have other benefits	35	42	43	31	35	35	26
I'm interested in "probiotic" milk drinks that are beneficial for digestive health	33	40	43	38	28	29	21
I'm interested in milk-infused drinks such as Starbucks Frappuccino®	27	45	41	30	23	16	8

I prefer “energy” milk drinks that contain added vitamins and/or minerals	18	26	27	18	15	15	11

SOURCE: Mintel

Appendix V: Cost of the ColorCarton with Non-Bulk Pricing

Cost of Acrylamide: $20/25 grams = $0.80/gram

SOURCE: MP Biomedicals

(http://www.mpbio.com/Marketing%20Documents/Promotions/2013-BiochemMailerUS.pdf)

To make the ColorCarton according to our protocol we need:

8.7g Acrylamide * (25 ml per plate / 100 ml total) = 2.175 g per plate

2.175 g per plate * (1 plate / 9 pieces) = **$0.24 per piece**

We divided by 9 pieces because that is about 870 mm^2 of area, which is suitable for an indicator size on a carton. The cost of bromothymol blue (powder) dye per piece is negligible because minuscule amounts of the powder are used per plate, and would cost less than one cent to incorporate. The same argument applies to TBE buffer and Ammonium persulfate.

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