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# Carbon Dioxide Treatment on Strawberry Fruit Prep and Its Effect on Shelf Life

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<span id="page-1-0"></span>Carbon Dioxide Treatment on Strawberry Fruit Prep and Its Effect on Shelf Life

Bryan Sterling Dawson

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

Frost Steele, Chair Michael Dunn Laura Jefferies

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# ABSTRACT

#### <span id="page-2-0"></span>Carbon Dioxide Treatment on Strawberry Fruit Prep and Its Effect on Shelf Life

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This research evaluates the effectiveness of using carbon dioxide  $(CO<sub>2</sub>)$  pressurization to extend strawberry fruit prep shelf life for the eventual use in yogurt applications. In this experiment,  $CO<sub>2</sub>$  treatments of 5, 15, and 25 pounds per square inch were used as a processing step to inactivate microorganisms, which in turn could aid in the preservation and maintenance of product quality during storage thus improving consumer acceptance of the yogurt. Microbial levels of the fruit prep treatments were monitored over a six-week period by enumerating aerobic plate counts and yeast and mold levels. The color, pH, and texture of the treatments were also evaluated throughout the duration of the study. Sensory attributes of the product were evaluated by formal sensory panel at the beginning of the study to gather consumer feedback on potential changes introduced by the treatment to the finished product. For sensory analysis, the different CO<sup>2</sup> treatments of fruit prep were mixed with plain yogurt and given to panelists. The different treatments were taken from one homogenous mixture of fruit prep and then were randomly divided into five different treatment groups: a control group, a thermally processed group, and the three different pressure levels of  $CO<sub>2</sub>$ . Results from the experiment showed that carbonation does not negatively impact product overall acceptability. Shelf life results showed that  $CO<sub>2</sub>$ treatments are not effective in maintaining or extending the shelf life of strawberry fruit prep when compared to a thermal treatment.

Keywords: carbon dioxide treatment, strawberry fruit prep, shelf life extension

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Carbon dioxide is a nonpolar gas that, among its different sources and applications, has utility in the food industry. It is most prevalent in its use in the beverage industry where the addition of carbon dioxide gives carbonated beverages their distinct sensory properties. A press release in 2017 by Technomic stated that consumers spend, on average, \$181 billion on soft drinks annually.

Carbon dioxide is introduced to products by one of three main methods. The most common method of introduction is performed by pressurizing the gas in the headspace above a liquid. This forces the gas to dissolve into the liquid and is often used for soft drinks. Another commonly used method for CO<sup>2</sup> introduction is by fermentation where microorganisms metabolize sugars and produce different gasses.  $CO<sub>2</sub>$  is one of the gasses produced by fermentation and is the main mechanism for giving alcoholic beverages like beer and sparkling wines their fizzy properties (Ravindra et al., 2014a). A third method is dissolve gas into a liquid by steeping the gas through the liquid or adding solid carbon dioxide (also known as dry ice) to the product and allowing the gas to dissolve. Despite variations in the method of addition, the mechanism by which carbon dioxide is incorporated into the product, and the results, are similar. All three methods require similar conditions for the carbonated product to form. These conditions include a source of  $CO<sub>2</sub>$ , a sealed container to trap the gasses, and time. When these conditions are met, the non-polar gas will react with water and turn into a more soluble form of carbonic acid (Ferrentino and Spilmbergo, 2011). The solubilized carbonic acid is then able to remain dissolved in the product until opened.

Additional, less utilized, functions of carbon dioxide include its antimicrobial properties and potential to improve the shelf life of products. This area of research is still developing and provides many opportunities to explore the use of carbon dioxide in consumer products as a functional gas (Ferrentino and Spilmbergo, 2011). A functional gas is one that aids in the preservation of a product. For example, potato chips sometimes use modified atmospheric packaging, or MAP, to help preserve the shelf life of a product by using specific gasses to remove oxygen from the packaging (Vermieiren et al., 1999). The same effect can be seen with  $CO<sub>2</sub>$ . The improvement to shelf life by  $CO<sub>2</sub>$  is made possible by several factors. The first factor that allows for  $CO<sub>2</sub>$  to improve shelf life is the production of carbonic acid which lowers the intrinsic pH of a product and which will inhibit microbial production. The second mechanism of shelf life improvement, effective only on select microorganisms, relies on the carbonic acid's ability to denature cell walls and render the microorganism nonviable via nucleophilic attack (Erkmen, 2000). The third mechanism involves the ratio of gasses in the headspace of the product. The introduction of  $CO<sub>2</sub>$  excludes oxygen, and this plays a role in preserving the product like in MAP applications (Daniels et al.,1985). For most products, it is a combination of these mechanisms that has the potential to improve the shelf life of a product without the need to add preservatives to the product. The ability to produce a safe and shelf-stable product is a growing area of interest to help meet consumer demands for products with "clean labels" (Ferrentino and Spilmbergo, 2011).

This literature review focuses primarily on the preserving, sensory, and antimicrobial properties that carbon dioxide has in a food application. The objective was to determine the limitations that current research has presented on  $CO<sub>2</sub>$  applications in food and to identify the possibility of using carbon dioxide as a treatment to improve the shelf life of a product without the addition of preservatives.

#### <span id="page-11-0"></span>**Functions of Carbon Dioxide in Industry**

Something that makes  $CO<sub>2</sub>$  unique is that it can be used in all three stages of matter – solid, liquid, and gas. Solid carbon dioxide is commercially available as "dry ice," named after its resemblance to ice. When dry ice is used to keep a product cold, it has been observed that if the product is in an open container with the dry ice, the product inside can develop similar "fizzy" notes similar to a carbonated soft drink due to the ingress of  $CO<sub>2</sub>$  gas into the product. Another use of  $CO<sub>2</sub>$  is in liquid extractions, specifically supercritical  $CO<sub>2</sub>$  extractions. Typically, extractions are done under conditions of low temperature and high pressure to allow the liquid to behave as a gas for the extraction of a desired compound (Sahena et al., 2009). Supercritical extractions are sought after because, while expensive, they leave no residue in the solvent and can produce a pure product. This outcome is beneficial in nutraceutical applications such as the manufacturing of essential oils where it is used to keep the purity of the final product high. The non-thermal nature of the extraction helps prevent the active compounds from degrading during extraction. The most popular use for carbon dioxide in the food industry uses its gaseous state for the carbonation of beverages (Ferrentino and Spilmbergo, 2011).

## <span id="page-11-1"></span>**Antimicrobial Properties of Carbon Dioxide**

High-pressure carbon dioxide has been shown to effectively kill pathogenic microorganisms such as *Salmonella typhimurium* and *Listeria monocytogenes* as well as common microorganisms such as *Saccharomyces cerevisiae* (Erkmen, 2000). In this study, Erkmen utilized a pressure vessel to pressurize the  $CO<sub>2</sub>$  gas in the headspace to three different pressures: 60 ATM (around 880 PSI), 30 ATM (440 PSI), and 15 ATM (220 PSI). It should be noted that depending on journal requirements and pressures utilized units for reporting pressure include, bar, ATM, PSI, and pascal. In the article by Erkmen, the target microbial inactivation for all the treatments was a seven-log reduction in microbial counts. In all three treatments, seven-log reduction was observed. However, the time required to achieve that level of inactivation across the treatments varied, with the lowest level of pressure taking the longest amount of time to inactivate. The conclusion Erkmen came to is that at lower pressures, more time will be required to inactivate the same level of microorganisms. This understanding is the foundation upon which processes such as high-pressure processing (HPP) exist. HPP utilizes very high pressures (around 87,000 PSI) to pasteurize or sterilize products. Using high-pressure carbon dioxide to pasteurize liquids such as milk and fruit juices has been proven to be effective, but there is a smaller body of research that support the use of high-pressure carbon dioxide to pasteurize solid foods such as fresh fruits. Similar research in the area of solid foods has proven that high-pressure carbon dioxide pasteurization can be achieved with foods such as kimchi, coconut, and carrots (Erkmen, 1997). Further, in the same article by Erkmen, pressurized  $CO<sub>2</sub>$ was able to inactivate *Staphylococcus aureus* in milk and a model broth.

Carbon dioxide's actual mechanism for inhibiting the growth of microorganisms has been greatly hypothesized by Garcia-Gonzalez et al. in a paper published in 2007. The researchers propose several models by which the conversion of carbon dioxide to carbonic acid, and eventually bicarbonate, is effective in damaging several areas of the microorganism. Garcia-Gonzalez et al. outline seven steps by which carbon dioxide in its gaseous state permeates

through a cell membrane resulting in the bactericidal action that the gas may have. According to Garcia-Gonzalez et al. the first step for microbial inactivation is a transition of gaseous  $CO<sub>2</sub>$  into liquid CO2. As the gas is first solubilized, it can interact with other liquids in the system. This is the rate limiting step for the entire process because it is difficult to initially solubilize the gas. Pressure is almost always needed for this to occur because pressurizing the gas will create the conditions favorable for the gas to be converted into carbonic acid by adding the necessary energy to favor the reaction, and the proton exchange will begin to occur. The pressurized system does not catalyze the reaction, but simply adds the energy needed to push the reaction towards the desired outcome.

The second step for microbial inactivation is cell membrane modification. Aqueous  $CO<sub>2</sub>$ will aggregate onto the phospholipid bilayer of the cell wall due to the non-polar properties of both the aqueous  $CO_2$  and phospholipids. The accumulated  $CO_2$  will begin to fluidize the cell membrane and allow for the  $CO<sub>2</sub>$  to permeate through the now weakened cell wall. It is not completely understood why CO<sup>2</sup> permeation is favorable but is postulated to be due to the presence of both bicarbonate (HCO<sub>3</sub><sup>-</sup>) and protein concentrations inside the cell wall. Another postulate is to create an equilibrium of the concentration of  $CO<sub>2</sub>$  on both sides of the cell wall.

The third step for microbial inactivation is intracellular pH decrease. This step outlines the proton exchange necessary to turn  $CO_2$  into carbonic acid ( $H_2CO_3$ ) as an intermediate and eventually bicarbonate. The loss of the proton from carbonic acid to bicarbonate is what causes the intracellular pH to decrease.

The fourth step for microbial inactivation is key enzyme inactivation/cellular metabolism inhibition due to pH lowering. Due to how quickly some enzymes inside the cell react to pH, the

<span id="page-14-0"></span>slightest change in pH from the presence of  $CO<sub>2</sub>$  will cause a loss of activity as shown in Figure 1.



**Figure 1. Mechanism for bactericidal action across a cell wall via CO<sup>2</sup>** Postulated mechanism by which CO<sub>2</sub> gas enters an aqueous phase and can permeate across the cell membrane via fluidization of the cell wall. Once inside the cytoplasm, the formation of carbonic acid and bicarbonate lower the pH of the cytoplasm and decrease the effectiveness of enzymes within the cell wall (Garcia-Gonzalez et al., 2007).

The fifth step for microbial inactivation is a direct inhibitory effect of molecular  $CO<sub>2</sub>$  and bicarbonate on metabolism. This simply states that it is not only the drop in pH responsible for the loss of activity of the enzymes, but also the change in the concentration of substrates and cofactors available.

The sixth step for microbial inactivation is the disordering of the intracellular electrolyte balance. Step six is closely regulated by step five and states how the presence of  $CO<sub>2</sub>$  and

bicarbonate alter the concentrations of cofactors and substrates available. This occurs primarily with interactions between bicarbonate and the electrolyte calcium  $(Ca^{2+})$ .

The last step for microbial inactivation, step seven, is the removal of vital constituents from cells and cell membranes. This step will only occur if the pressure from the system introduced in step one is removed. The decrease in pressure will cause the deformed cells to expel intracellular constituents such as phospholipids and other hydrophobic constituents.

In summary, as  $CO<sub>2</sub>$  moves through the cell membrane, the exchange of protons allow for a drop in pH, which takes microorganisms and enzymes out of their optimal functional range. For this to occur, conditions of pressure and time need to be met. For example, Garcia-Gonzalez et al. (2007) explain how factors such as Henry's Law state how the partial pressure that a gas exerts on a liquid at equilibrium will not be enough to begin interaction at the interface. At ambient pressures the energy at the liquid and gas interface does not overcome the activation energy needed to see any exchange of protons resulting in the gas not dissolving into the liquid. Pressurizing the gas will create the conditions favorable for the gas to be converted into carbonic acid by adding the necessary energy to favor the reaction, and proton exchange will begin to occur. The pressurized system does not catalyze any reaction but simply adds the energy needed to push the reaction towards the desired outcome. The entire process for microbial inactivation is driven by the concentration of  $CO<sub>2</sub>$  both inside the cell wall and outside. For example, if the concentrations of  $CO<sub>2</sub>$  across the cell wall are different enough from each other steps two, three, and four can be bypassed and the gas will enter directly into the cell without first solubilizing. This is directly related to the fact that higher pressures of gas will lead to higher levels of microbial inactivation.

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Looking further at the bactericidal properties that carbon dioxide has on microorganisms; bacterial and fungal spores have also been researched. The viability of *Bacillus subtilis*, *Byssochlamys fulva*, and *Aspergillus niger* under pressurized and heated conditions were observed and researchers found that the microorganisms were completely inactivated (Ballestra and Cuq, 1998). This study, when compared to Erkmen's experiments, was conducted at much higher pressures (around 725 PSI).

Further research on microbial inactivation, has been investigated on food products with promising results that  $CO<sub>2</sub>$  will improve the shelf life of specific foods. For example, it was observed that cottage cheese shelf life was improved with modified atmosphere package utilizing carbon dioxide to exclude oxygen from the headspace of the product (Mannheim and Soffer, 1996). In this study, the concentration of carbon dioxide used in the headspace of the products was varied. Mannheim and Soffer observed that the most effective method of improving the shelf life was to use pure carbon dioxide to flush the headspace. This indicates that it is not only the removal of the oxygen but the carbon dioxide itself that is influencing the preservation of the product. These results were supported in another study on drinkable dairy products, which also displayed an improvement to shelf life due to a  $CO<sub>2</sub>$  treatment (Ravindra et al., 2014b). These findings are not only specific to pathogenic/harmful microorganisms. In another study, researchers observed that the antimicrobial effects of carbon dioxide treatments could also be observed on potentially beneficial bacteria such as the probiotic microorganism *Lactobacillus helveticus MTCC 5463* (Shah and Prajapati, 2014).

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#### <span id="page-17-0"></span>**Effects of Carbon Dioxide Treatment on The Texture of Products**

When  $CO<sub>2</sub>$  is added to a product or package, textural changes occur. These changes occur as a result of pressurized  $CO<sub>2</sub>$  disrupting cell walls. A review article stated that at high pressures, significant tissue damage was observed in solid fruits and vegetables (Garcia et al., 2007). Harker et al. (2000) observed the changes that occur in strawberries after carbon dioxide treatments. This experiment reported that fruit treated with  $CO<sub>2</sub>$  was no different nutritionally from fruit that received no treatment, but that it had better texture over time. The experiment was conducted at 1 ATM, and the study controlled for differences in  $CO<sub>2</sub>$  concentration in the headspace ranging from 5–40%. The remaining gas in the headspace was nitrogen. Observations from the experiment demonstrate how the tissue of the strawberries changed during storage. Figures 2 and 3 include images taken by scanning electron microscopy (SEM) to demonstrate the changes in texture.

<span id="page-18-0"></span>

**Figure 2. Image of strawberry tissue treated with carbon dioxide**

Image generated using scanning electron microscopy (SEM) showing a rougher texture and a loss of juice after exposure to carbon dioxide. The arrow indicates the area where juice from the strawberry can be seen (Harker et al., 2000).

<span id="page-19-0"></span>

**Figure 3. Image of strawberry tissue not treated with carbon dioxide** Image generated using scanning electron microscopy (SEM) showing smoother texture of fruit tissue and intact juice in the product (Harker et al., 2000). This treatment, in contrast with the  $CO<sub>2</sub>$  treated product, was reported to be softer in texture.

Figure 2 illustrates tissue from the  $CO<sub>2</sub>$ -treated fruit, and Figure 3 illustrates the tissue from the untreated fruit. Harker et al. commented that there was more juice present on the surface of the CO<sub>2</sub>-treated sample that the surface of the untreated samples. The researchers further discuss how soluble pectin in the fruit contributes most to texture and how the presence of juice on the surface may allow for more pectin-to-pectin interaction creating a pseudo gel structure. It is hypothesized that this may be responsible for the perceived increase in firmness of the fruit. Lastly, they observed that the effects of CO<sub>2</sub> treatment were not reversible after the treated fruit samples were removed and stored in ambient conditions. The results of this study

support that  $CO<sub>2</sub>$  treatment could be used as a processing treatment rather than a storage condition due to how the change in texture continues to be seen after a change in storage conditions.

Watkins et al. in 1999 ran a similar experiment, but rather than controlling for different gas ratios, researchers pressurized the gas to different levels up to 3 PSI. They observed the best texture preservation at the highest pressure but did not test for pressures above that level. An additional variable that Watkins et al. explored was the impact that  $CO<sub>2</sub>$  treatments had on the texture of different strawberry cultivars. Figure 4 demonstrates that of the seven cultivars tested, four were firmer in texture after exposure to  $CO<sub>2</sub>$  when compared to untreated cultivars. The other three cultivars were not significantly different. The researchers hypothesized that this was also due to the amount of water soluble pectin found in the different cultivars of fruit. The cultivars containing more pectin were measured to be firmer in texture. The researchers did not report a point of failure at which the tissue of the strawberry was compromised. However, given the delicate texture of strawberries, it can be postulated that little pressure would be required to damage the structural integrity. In summary, pressurized  $CO<sub>2</sub>$  may contribute to an increase in firmness of certain cultivars of strawberry, but too much pressure may damage the tissue of the product.

<span id="page-21-0"></span>



Different cultivars of strawberries exposed to CO2 treatments of 3 PSI for 7 days. Not all cultivars showed improvements to texture. Some cultivars exhibited no change in firmness while others became softer (Watkins et al., 1999).

Another condition that influences the texture of a product when  $CO<sub>2</sub>$  is applied is the rate of decompression after the headspace has been pressurized. Researchers found that when considering the impact that  $CO<sub>2</sub>$  has on both the texture and the microbial lethality in a food product, it is difficult to optimize the conditions where both can be favorably observed. After a product has been pressurized, rapid decompression when compared to controlled decompression of the gas will have the greatest impact on microbial lethality. However, rapid decompression proved to be the most detrimental to texture (Garcia et al., 2007). This is due to the amount of stress that is introduced to the tissue of the product.

#### <span id="page-22-0"></span>**Applications of Carbon Dioxide in Other Food Products**

In recent years, carbon dioxide has been found to be useful in a variety of new food applications in addition to the traditional applications of  $CO<sub>2</sub>$ . New food applications include vegetables, various fruits, grains, seeds, and cocoa powders (Ferrentino and Spilmbergo, 2011). Other products investigated have been in applications such as the carbonation of frozen yogurt (Ogden et al., 2002) and drinkable fermented dairy-based products (Ravindra et al., 2014a). This interest in researching new applications has translated into the consumer market with an increased popularity in carbonated products. One example of a consumer product was the release of carbonated grapes (Espenshade et al., 2007). The objective of this product was to help children eat more fruit while simultaneously being fun to eat.

With all the different product applications for carbonation, a lot of interest has been seen for yogurt-based applications. Research has been conducted to capture the appeal to consumers via sensory testing to understand how such products would be accepted (Chio and Kosikowski, 1985). A study presented by Chio and Kosikowski postulated that sweetened products make the

carbonation preferable due to the "refreshing" flavor profiles that the carbonic acid brings to the overall flavor profile of the products. Researchers that looked at sensory evaluation of dairybased carbonated beverages found that the carbonation process improved the flavor, sweetness, and overall rating of the product (Yau et al., 1989). Looking specifically into strawberry yogurt, the effects of carbonation showed no difference in consumer acceptability, with both carbonated and non-carbonated samples receiving similar hedonic scores (Karagul-Yuceer et al., 1999). The results of these studies are encouraging for the potential use of carbon dioxide as a treatment, given that it does not negatively impact the sensory acceptance of the product.

Lastly, the use and level of carbonation in non-fermented drinkable milk products has been researched (Lederer et al., 1991). In this study, descriptive analysis was conducted using trained panelists, and it was concluded that the more carbonation that a product had, the more difficult it was to pick up on other flavors present. Lederer et al. postulates that this is due to the way that milk proteins interact with taste receptors and weaken the response to bitter flavors. The increased carbonation was perceived on a tactile level and contributed to the overall perception of the flavored milk product, while the increased bitterness from carbonic acid was not observed.

Changes to the level of carbonation in a product were another area of interest for maximizing either flavor or sensory experience. To determine the maximum level of carbon dioxide a product can hold, researchers have investigated the solutes present in acidified drinkable milk products and how the solutes contribute to retention of  $CO<sub>2</sub>$  (Barnes et al., 1992). The study found that an increase in the solute concentration had a measurable difference in the amount of  $CO<sub>2</sub>$  present with less free water in a system allowing for greater levels of  $CO<sub>2</sub>$ .

Additionally, solutes that contributed to a buffer system in an acidified drinkable milk product further improved the amount of  $CO<sub>2</sub>$  a product was able to contain due to the stable pH.

#### <span id="page-24-0"></span>**Carbon Dioxide and Strawberries**

Strawberries and strawberry-flavored products have been considered as a potential product to be treated with carbon dioxide. Reasons for this include the new sensory attributes that the fruit will take on and the potential for improved shelf life. As previously stated, the texture of strawberries can be perceived as firmer due to how juice is expelled from the cells after exposure to carbon dioxide (Harker et al., 2000). However, when looking across different varieties of strawberries, the results differ (Watkins et al., 1999). Watkins et al. further stated that some varieties of strawberries are firmer after carbon dioxide treatments, while other cultivars will exhibit negative effects due to carbon dioxide.

Not only are textural differences shown, but changes in phenolic levels and enzyme activity are observed as well after exposure to  $CO<sub>2</sub>$  (Heimler et al., 2017). Once a strawberry has been processed, it is important to retain as much polyphenol and antioxidant activity as possible to ensure a long shelf life (Aaby et al., 2007). It has also been reported that the soil conditions in which the plant is grown as well as proper treatment of the product during processing have a significant impact on phenolic and antioxidant levels (Heimler et al., 2017). Heimler et al. highlighted the significant impact that the soil conditions will have on the final product concerning phenolic and antioxidant levels. Researchers also found that farming practices had little to no impact on these factors. In conjunction with farming/handling practices, researchers highlighted an additional parameter to consider, the effects that different processing conditions (such as temperature and light exposure) would have on a strawberry puree. (Gossinger et al.,

2009). The relevant finds from that study indicated that the color of the strawberry puree was significantly influenced by different storage conditions and treatments. Gossinger et al. found that both a reduction in pH to two and colder storage temperatures of 4°C were able to preserve the color and give the puree a shelf life of twelve months without the need for preservatives. However, the freezing of the purees did not preserve the structural integrity of the product. Given these observations of shelf life and color, the next step is to understand the effect of carbon dioxide treatments on the color and texture of strawberry products.

#### **MANUSCRIPT**

# <span id="page-26-1"></span><span id="page-26-0"></span>**Introduction**

Carbon dioxide is a versatile gas that impacts both the food industry and its consumers. For example,  $CO<sub>2</sub>$  is most commonly used to carbonate beverages due to the unique sensory properties that the gas imparts to the beverage (Ferrentino and Spilmbergo, 2011). These unique sensory properties have been the source of novel product ideas that have been introduced at the consumer level. One example of this was a carbonated grape product introduction that was intended to make eating fruit a unique experience for children and improve fruit consumption (Espenshade et al., 2007). The sound of opening a carbonated product and its "fizzy" mouthfeel is what makes a carbonated beverage so unique and easily identifiable for consumers. For food industry related applications, carbon dioxide can be used to flush the headspace of meat products to help preserve the red color before the consumer opens the product for the first time (Ferrentino and Spilmbergo, 2011). In research, the use of carbon dioxide has been shown to exhibit antimicrobial properties with significant effectiveness in reducing bacterial and fungal growth. The antimicrobial effects that  $CO<sub>2</sub>$  has on products can improve the shelf life of said products, and thus the use of  $CO<sub>2</sub>$  as a treatment for food products is a growing area of research.

When using carbon dioxide, the type of product chosen has a significant impact on how effective the gas is in extending the shelf life of the product. The mechanism required for  $CO<sub>2</sub>$  to exhibit antimicrobial properties is dependent upon the conversion of carbon dioxide to carbonic acid in the presence of water (Garcia-Gonzalez et al., 2007). The presence of carbonic acid with dissolved carbon dioxide gas is what generates the iconic "fizzy" sensation when carbonated beverages are consumed. A correlation can be drawn between the amount of water a product has

and the amount of carbon dioxide and carbonic acid present in a product. When solid foods such as fruit are carbonated, the level of perceived effervescence of the food is more difficult to detect when compared to beverages, because less gas can dissolve into the product (Yau et al., 1989). In addition to the level of effervescence of a product, the more carbon dioxide gas that can be dissolved into the product, the more significant the antimicrobial properties. This results in the use of pressurized gas to aid in the introduction of carbon dioxide into a food matrix. The pressurized gas can bypass cell walls and enter the cytoplasm resulting in a greater impact on the viability of microorganisms (Garcia-Gonzalez et al., 2007).

In this study, strawberry fruit prep was selected due to findings from previous research and current trends in the food industry. Strawberry fruit prep is a sweet and viscous mixture of fruit, sugar, water, and other ingredients similar in composition and flavor to strawberry jam. The fruit prep is mixed with plain yogurt prior to packaging to create the final flavored yogurt that consumers purchase. Researchers have found that strawberries treated with  $CO<sub>2</sub>$  are firmer in texture which is a desirable trait for fruit pieces mixed in yogurt. Combined with the understanding that  $CO<sub>2</sub>$  can extend shelf life, the selection of strawberry fruit prep is relevant to both the food industry and its consumers (Karagul-Yuceer et al., 1999).

Yogurt is a healthy product, but recently there has been shift driven by consumers to cleaner-labeled products, which includes the desire to remove preservatives and artificial ingredients (Ferrentino and Spilmbergo, 2011). The use of preservatives has contributed greatly to improvements in food safety and quality to feed the growing population. The removal of preservatives would drastically increase the risk of foodborne illness if the proper safety measures are not taken. Given the potential antimicrobial properties that carbon dioxide

provides, it is hypothesized that a CO<sup>2</sup> treatment would allow for the removal of antimicrobial ingredients while maintaining a shelf life similar to a product with preservatives. Strawberry yogurt is a popular flavor, and the fruit prep that goes into yogurt can include a commonly used industry antimicrobial agent, potassium sorbate. Each yogurt manufacturer will use a different method for addition of potassium sorbate due to trade secrets that manufactures may have regarding how they produce their respective yogurt. The purpose of this study was to observe the potential effects that a carbon dioxide treatment would have on the microbial levels, shelf life, and sensory attributes of a strawberry fruit prep. Methods were selected from a combination of methods found in the literature, available equipment, and preliminary research conducted.

#### <span id="page-28-0"></span>**Materials and Methods**

Fruit prep samples were prepared from fresh strawberries purchased from a local supermarket and processed into fruit prep using good manufacturing practices. The fruit prep was homogenized and then randomly divided into five different treatment groups: a control group, a thermal treatment, and  $CO<sub>2</sub>$  treatments of 5, 15, and 25 PSI. Samples of fruit prep were prepared with a provided formulation in which the potassium sorbate had been removed. The fruit prep was stored in refrigerated conditions in sealed containers. Sensory evaluation of the fruit prep was conducted at week one of the study in order to gather consumer insight regarding the different treatments applied. The samples were prepared by mixing the fruit prep with plain yogurt. Separate samples were also evaluated for microbial growth, color, pH, and texture. These attributes were monitored to determine the effects that the carbon dioxide treatment had on the fruit prep.

# <span id="page-29-0"></span>*Preparation of Fruit Prep*

Berries were purchased from a local supermarket, and the grower was contacted for variety and pick date. The grower confirmed that the variety was Monteverde, which is part of the Cavendish cultivar, and that the strawberries were picked on the same day and from the same farm in Watsonville, CA. Once purchased, the strawberries were washed with water and a mild detergent. After washing, the stems were removed and approximately 450 g portions of berries were drained on a number 32 sieve for two minutes. The fruit prep was comprised of two different phases. The first phase is a stabilizer phase containing activated pectin, sugar, and water. The second phase was a fruit phase that contained additional sugar, sodium citrate, calcium chloride, and citric acid. As the strawberries dried on the sieve, the pectin phase of the fruit prep was prepared. The strawberry phase was then prepared in the same bowl. Once the two phases of the fruit prep had been separately prepared they were combined using a Thermomix TM5 (Thermomix, Thousand Oaks, CA).

The pectin phase was prepared by first mixing 16.2 g of Grindsted Pectin YF 357 (DuPont, Wilmington, DE) and 101.25 g of sugar to prevent the pectin from creating a lumpy texture. This mixture was then added to 222.75 g of filtered water. Next, the solution was heated to 85°C with a mixing speed of 3 in the Thermomix with a butterfly whisk TM5 attachment (Thermomix, Thousand Oaks, CA). The pectin phase was held at 85°C for 3 minutes with constant stirring and then the pectin phase was removed from the bowl of the Thermomix to cool. The material of design for the Thermomix mixing bowl allowed for rapid cooling of the mixing bowl so little to no thermal shock was introduced to the strawberry phase during preparation.

The strawberry phase was then prepared in the same bowl of the Thermomix. To prepare the strawberry phase, 1012.5 g of whole strawberries were mixed with 490.05 g of sugar, 4.05 g sodium citrate, 2.01 g calcium chloride, and 8.1 g of citric acid. Like the pectin phase, the sodium citrate, calcium chloride, and citric acid were dry mixed with the sugar before addition to the strawberries to allow for even dispersal. The entire mixture was mixed in the Thermomix on a reverse setting at mixing speed setting number three for three minutes. The reverse mixing direction allowed the blunt side of the mixing blades to give the overall fruit prep a rough chop rather than a smooth blend to keep the integrity of the fruit pieces.

After the three minutes of mixing, the pectin phase was added to the fruit phase and the mixing speed was reduced from three to two. The reverse direction of the blade was still used, and the entire mixture was stirred together for 5 minutes. In total, the fruit prep was made in 2025 g batches and the overall fruit prep was formulated to a pH of 3.43, a  $A_w$  of 0.961, and a brix of 35.6. See Appendix A for a table of ingredients and their respective levels of use.

Once individual batches were prepared, they were added to a clean mixing vat, and the total mixture was manually mixed for 15 minutes to ensure homogeneity. The mixture was stored overnight in the sealed mixing vat to allow the mixture to cool. The temperature of the fruit prep was 3.5°C before the mixture was divided into the individual treatments.

The thermally processed treatment was heated to 85°C in the Thermomix on the reverse mixing direction on speed setting 1.5 and the temperature was verified with a thermocouple. Once the desired temperature had been achieved, the mixture was held at that temperature for 5 minutes and then cooled to 3.5°C over the course of 1.5 hours using an ice bath. Once cooled, the thermal treatment was poured into storage containers. All treatments were individually stored in cleaned 3.5 oz polypropylene cups with polypropylene snap-on lids (Gygi Company, South Salt Lake, UT).

The three carbon dioxide treatments were each processed in a Cornelius Keg style beer keg (Beverage Factory, San Diego, CA). Once the sample was in the canister, CO<sub>2</sub> was steeped through the fruit prep for 5 minutes at 5 PSI to evacuate oxygen from the headspace of the canister. The canisters were then sealed and pressurized to the treatment pressures of 5, 15, and 25 PSI. Next, the canisters were stored for three hours at refrigeration temperatures  $(2-3\degree C)$  and poured into storage containers.

#### <span id="page-31-0"></span>*Experimental Design*

Fruit prep treatments were prepared from one homogenous mixture of fruit prep and randomly divided into five different treatment groups. The first was a control group with no treatment method prescribed to the samples. The second group was a thermally processed group with the fruit prep heated to 85<sup>o</sup>C and held at that temperature with slow agitation for 5 minutes then cooled to 3.5°C using an ice bath over the course of an hour and a half. The remaining three treatment groups were  $CO_2$  treatments at different pressures of  $CO_2$ . The  $CO_2$  treatments were flushed with  $CO<sub>2</sub>$  for five minutes to evacuate the oxygen prior to pressurization. The canisters were the pressurized to their respective pressures of 5, 15, and 25 PSI, and held at that pressure for 3 hours at 3°C. Temperature and pressure were monitored throughout the duration of the three-hour period. Once each treatment was complete, the fruit prep was weighed into 240 g portions and stored in clean one-time use 3.5 oz plastic containers with snap-on lids to ensure that samples were not contaminated during storage. Each sample was labeled with a three-digit blinding code and stored under refrigerated conditions. Sample order was randomized each week and the individuals performing the testing were not aware of the treatment given to each sample. Each of the five treatment methods were further separated into three sample groups resulting in a total of 15 samples measured each week.

#### <span id="page-32-0"></span>*Statistical Analysis*

The 15 fruit prep samples that went into the five different treatments were randomly selected and placed in storage containers with three-digit blinding codes until testing. Microbial samples were tested with a serial dilution out to 1:100. Color and pH samples were tested in triplicate and the averages were taken. Texture samples were the most invasive test performed, thus each sample was only tested once. One hundred panelists were screened and recruited from the Brigham Young University Sensory Lab database. Microbial counts, pH, and color values were analyzed using a pairwise comparison test with a pseudo-Bonferroni correction at a 99% confidence level and an alpha set at 0.01. Sensory data was analyzed using a Tukey's HSD test for significance at a 95% confidence level and an alpha set at 0.05. All statistical tests were performed on the statistical software SAS JMP 14 (SAS, Cary, NC) provided by the BYU Department of Statistics.

### <span id="page-32-1"></span>*Enumeration of Microorganisms*

Before testing for other changes in the samples, a sample was taken from each of the 15 sample cups for microbial testing. Each container was aseptically opened, and the contents were stirred for five seconds to ensure homogeneity. A 25 g sample was removed each container and placed into a sterile homogenizer bag. 225 g of 0.1% sterile peptone broth was then added to the bag and the contents were further homogenized with a paddle mixer for 60 seconds. A 1 ml

portion was plated onto 3M APC film and 3M Y/M respectively (3M, Maplewood, MN). The APC film was incubated at 38°C for 48 hours, and the Y/M film was incubated at 24°C for 72 hours followed by enumeration of colony-forming units.

## <span id="page-33-0"></span>*Preparation of Samples for Color and pH Testing*

The 15 samples were removed from storage and allowed to warm to room temperature before color and pH determinations. The order of testing of the samples was randomized to avoid bias and the colorimeter and pH meter were calibrated as needed. A Hunter colorimeter (Hunter Lab, Reston, VA) was used to determine  $L^*$  a\* and  $b^*$  color values. The sample was placed in a clean sample container for measuring color. Once the colorimeter had been calibrated according to the manufacturer's directions, readings were taken in triplicate and the averages were recorded. The sample cup containing the fruit prep was rotated approximately 120 degrees between readings. Special care was taken to ensure that no air bubbles were present at the bottom of the sample during determinations to ensure accurate readings. In a few instances, some condensation was found on the sides of the container, and it was immediately wiped off.

For pH determinations, a Five Easy<sup>TM</sup> Plus pH meter (Metler Toledo, Columbus, OH) was used and calibrated as needed using pH 4 and pH 7 buffers. During calibrations, a minimum slope of 96 was allowed. This model of pH meter accounts for temperature differences given the product being tested does not change. With that in mind, the samples warmed up to room temperature before pH determinations were taken and it was determined that small changes in temperature did not negatively affect the results.

# <span id="page-34-0"></span>*Preparation of Samples for Analysis of Texture*

A TA-XT2 texture analyzer fitted with a TA-65A multi-puncture probe (Stable Micro Systems, Surrey, United Kingdom) attachment was used to measure the firmness of the fruit pieces and gel mixture after the five different treatments were applied and to record any changes that occurred during storage. The TA-XT2 was set to measure the fruit prep to a depth of 50 mm, and the probe moved at a rate of 5 mm/second. The instrument was calibrated as needed. Each sample was measured immediately after removal from the storage refrigerator and the measurement was taken as close to 3°C as possible. Due to the invasive nature of the testing, each sample was only tested once.

# <span id="page-34-1"></span>*Preparation of Samples for Sensory Analysis*

Strawberry fruit prep by itself is very sweet and is designed to be mixed into plain yogurt. Sensory analysis on the fruit prep by itself would have produced biased results and would not have been representative of how the product is used by consumers. For sensory analysis, each treatment of fruit prep was mixed into yogurt at a concentration representative of commercial strawberry yogurt. Containers of plain yogurt were purchased at a local grocery store and the fruit prep was added the morning of the sensory panel to produce strawberry flavored yogurt. The fruit prep was whisked into the plain yogurt at a level of 31% total formula by weight for 5 minutes in 5000 g batches to ensure that the fruit prep was properly incorporated into the plain yogurt. Once the smaller batches were prepared, they were combined into one large container and mixed for an additional 10 minutes. Each of the five treatments were allowed to set in the refrigerator for four hours before the sensory panel took place. Panelists were recruited from the

BYU Sensory Lab database and were screened to ensure that their preferences would align with consumers who consume strawberry yogurt on a regular basis. One hundred total panelists were recruited from different age demographics with equal gender representation between men and women. All panelists were served the strawberry yogurt samples side-by-side, but the order in which the samples were presented to the panelist was randomized. Each sample was assigned a three-digit blinding code so as not to bias the panelists into inaccurately favoring one sample over another. Samples were ranked on both a seven-point hedonic scale and a five point just about right (JAR) scale. The sensory analysis was conducted at the BYU Sensory Lab.

#### <span id="page-35-0"></span>**Results & Discussion**

Throughout the study, in addition to the response variables, the overall visual appearance of the fruit prep samples was monitored. It was observed that visible spoilage occurred on the non-thermally treated samples before the thermally treated samples showed any signs of visible spoilage. The samples appeared to have a white mold growing on the surface of the product and black spots forming around the edges. Visible spoilage indicated that the fruit prep had reached the end of its shelf life and would not be consumable at that point. This was the first indication of the effectiveness of the thermal treatment compared to the  $CO<sub>2</sub>$  treatments.

# <span id="page-35-1"></span>*Aerobic Plate Count, Yeast, and Mold Results*

Visible spoilage on the samples (except for the thermal treatment) was observed at the week five determination. This was sooner than anticipated and did not align with the findings of Gossinger et al. (2009), which stated that the product should last over a year after  $CO<sub>2</sub>$  treatment. While the products studied by Gossinger et al. are similar to fruit prep, differences in the fruit
prep formulation may be the reason for the difference in shelf life. The thermal treatment had significantly lower plate counts compared to the other treatments after week two determinations with a p-value of  $\leq 0.001$ . Figure 5 shows the differences in plate counts between the treatments. It was observed that the thermal treatment had an average of 22 fewer colonies than the other treatments. Over time, the thermal treatment was significantly different from certain treatments. At week five, the thermal treatment had a significantly lower plate count than the 25 PSI treatment and the control treatment  $(p=0.02)$ . At week six, the thermal treatment had a significantly lower plate count than the control treatment ( $p=0.048$ ).





Thermal treatment had significantly lower plate counts at weeks 2 and 3 when compared to all other samples. At weeks five the thermal treatment had significantly lower plate counts than the 25 PSI and control treatments, and in week six the thermal treatment was significantly lower in plate counts than the control group. Values shown with pseudo-Bonferroni corrected 95% confidence intervals with p=0.048, n=90.

Concerning yeast and mold results, the thermal treatment inactivated all spores and plate counts were recorded as zero throughout the duration of the study. It is important to note that with recorded values of zero, the thermal data was not incorporated into the statistical analysis but is shown in Figures 6 and 7. The data shows that the thermal treatment was more effective at inactivating yeast and mold spores and the potential to form colonies on their respective plates. Comparing the yeast and mold results across the other treatments, there were no results that were statistically significant over time. Figures 6 and 7 show enumeration of the yeast and mold colonies. The 15 PSI and control treatments showed higher plate counts, but this was not statistically significant with p-values of 0.5735 and 0.2529 respectively. Additionally, it was observed that the yeast and mold colonies grew over time, indicating that the microorganisms were replicating, but not at the same rate that would indicate visible growth. It was hypothesized that more recorded growth would be necessary before visible growth could be seen. Next steps would be to conduct additional studies to determine if the matrix of the fruit prep impacts the ability to enumerate.





Thermal treatment was recorded at zero CFU/g throughout the duration of the study. At week six, the 15 PSI treatment was higher than the other treatments. The thermal data is shown, but was not included in the analysis given its value of zero. Values shown with pseudo-Bonferroni corrected 95% confidence intervals with p=0.5735, n=90.



#### **Figure 7. Average recorded mold colonies**

Thermal treatment was recorded at zero CFU/g throughout the duration of the study. There was a general increase in the levels of mold throughout the study indicating growth. Week five is when visible spoilage was observed on all the samples except for the thermal treatment. The thermal data is shown, but was not included in the analysis given its value of zero. Values shown with pseudo-Bonferroni corrected 95% confidence intervals with p=0.2529, n=90.

### *pH Testing Results*

Over time, results from measuring the pH of the fruit prep showed that the pH of the thermal treatment increased slightly, while the other treatments decreased in pH. The decrease in pH indicates that there is microbial spoilage occurring within the products as they age. As bacteria reproduce, lactic acid is one of the byproducts and will lead to a decrease in pH (Ferrentino and Spilmbergo, 2011). When the samples were treated with  $CO<sub>2</sub>$ , there was an expectation that by the addition of carbonic acid would decrease the pH of the fruit prep. The fruit prep was formulated to a pH of 3.43 and the initial pH after treatment was 3.33 at 25 PSI, 3.32 at 15 PSI, and 3.30 at 5 PSI. The reason for such a small change in pH after treatment with  $CO<sub>2</sub>$  is a possible combination of two factors. The first factor is that the pH of the system is already low so the presence of a weak acid such as carbonic acid or bicarbonate will only contribute minimally to a change in pH (Garcia-Gonzales et al., 2007). If the initial pH of the fruit prep were higher, a greater change in  $pH$  after the  $CO<sub>2</sub>$  treatment may have been observed. The second factor that may explain the small change in pH is a result of the formulation of the product. The inclusion of sodium citrate and citric acid in the formulation created a buffer system due to how the weak acid reacted with its conjugate salt. The presence of the buffer system is critical in maintaining product attributes throughout the shelf life but would decrease the sensitivity to changes in pH by the addition of  $CO<sub>2</sub>$  or bacterial proliferation. Despite these factors, the pH of the thermal treatment was significantly higher than the rest of the treatments in weeks five and six of the study with a p-value  $\leq 0.01$  (see Figure 8).



#### **Figure 8. Average recorded pH of samples**

The thermal treatment had a significantly higher pH in weeks 5 and 6 of the study. The general decrease in pH in the other samples indicates growth of microorganisms. Values shown with pseudo-Bonferroni corrected 95% confidence intervals with  $p = 0.01$ ,  $n = 90$ .

#### *Color Measurement Results*

Color determinations are produced using three scales of measurement:  $L^*$ ,  $a^*$ , and  $b^*$ , which measure light to dark, red to green, and blue to yellow respectively. Looking at the L<sup>\*</sup> values for the different treatments, the thermal treatment was consistently lighter than the other groups week to week with an average difference of 4.504. Concerning a\* values, the thermal group and the control group were significantly redder than the carbonation treatments. The average difference in a\* for the thermal group was 3.387, while the average difference for the control group was 2.57. Lastly, regarding the b\* color values, the thermal treatment was significantly more yellow than the rest of the treatments with an average difference of 3.86. Despite these measurable differences, during testing there was no reported visual difference.

#### *Analysis of Texture Results*

The texture of the different samples did not significantly differ from week to week or between the different treatments. The pseudo-Bonferroni test indicated that the thermal treatment was less firm than the 5 PSI at the end of the study but was not statistically significant with a pvalue of 0.7574. Figure 9 shows a representation of the firmness of the different fruit prep samples.





Recorded average force applied to the fruit prep across different treatments. Smooth lines indicate soft fruit pieces and rough lines indicate firmer fruit pieces. The 5 PSI treatment had the firmest fruit while the 25 PSI had the softest fruit. Values shown with pseudo-Bonferroni corrected 95% confidence intervals with p=0.7574, n=90.

#### *Sensory Analysis Results*

The results from the sensory test showed that panelists reported no difference in

acceptability between the different treatments as shown in Table 1. This finding indicates that the

carbon dioxide treatment neither positively nor negatively impacted the acceptability of the yogurt samples. Hedonic ratings of the different samples showed that the treatments did not negatively affect the properties of the samples, nor did they have a positive effect. The samples scored highly with an average hedonic rating of around 7.3, which means that the product was very well liked. This is consistent with findings of other studies that stated that fruit purees treated with carbon dioxide score no differently than their untreated counterparts (Karagul-Yuceer et al., 1999). Questions using a JAR notation were ranked on a Just About Right scale with averages scoring near three considered to be ideal. All the treatments were stated to be just about right for fruit piece firmness with an average around 3.1. The thermal treatment ranked as too firm but with a p-value of 0.2411, it was not significant.

<b>Attribute</b>	Control	<b>Thermal</b>	<b>5 PSI</b>	<b>15 PSI</b>	<b>25 PSI</b>	p-value	<b>Conclusions</b>
Overall Acceptability/Ranking/Purchase Likelihood							
<b>Overall</b> Appearance <b>Acceptability</b>	7.16a	7.13a	7.39a	7.43a	7.2a	0.0499	No difference
Preference <b>Ranking</b>	330 a	316 a	302a	282 a	300a	0.276	N <sub>o</sub> difference
Purchase Likelihood	6.11 a	6.18a	6.37a	6.49a	6.39a	0.3627	No difference
<b>Attribute Acceptability</b>							
Appearance	7.41a	7.39a	7.55a	7.63a	7.33a	0.0825	No difference
Color	7.74a	7.74a	7.85 a	7.81 a	7.82 a	0.3643	N <sub>o</sub> difference
Aroma	6.79a	7.00a	6.67a	7.00a	6.89 a	0.0639	N <sub>o</sub> difference
<b>Flavor</b>	6.74a	6.78 a	7.02a	7.00a	6.75a	0.3452	N <sub>o</sub> difference
<b>Texture</b>	6.63a	6.80a	6.96 a	6.73a	6.87a	0.2007	N <sub>o</sub> difference
<b>Aftertaste</b>	6.17 a	6.30a	6.40a	6.57a	6.47 a	0.1494	N <sub>o</sub> difference
<b>Attribute Ideality</b>							
<b>Firmness/Soft</b> ness Level <b>Ideality</b>	3.13 <b>JAR</b>	3.25 Too firm	3.11 <b>JAR</b>	3.10 <b>JAR</b>	3.22 <b>JAR</b>	0.2684	Thermal too firm

**Table 1. Summary of sensory data**

Almost every attribute measured in the sensory paned showed that the  $CO<sub>2</sub>$  treatment of the fruit prep had no significant difference when compared to each other. Lowercase letters next to averages indicate significant differences if any. The same letter indicates that the sample was not significantly different. The one difference observed was in the firmness of the fruit where the thermal treatment was ranked as too firm. Values shown with a Tukey's HSD corrected 95% with  $p = 0.2411$ ,  $n = 100$ .

### **Conclusions**

Carbon dioxide treatment of fruit prep is not as effective as thermal processing for preserving fruit prep. The  $CO<sub>2</sub>$  treatments, including the control group, showed signs of visible spoilage before the thermal treatment at five weeks. This is consistent with other data collected such as plate counts and pH data which show that the thermal treatment had lower plate counts and had a more stable pH. Additional findings show that there is no difference between samples in appearance and that the 5 PSI treatment produced the firmest fruit prep as measured by the texture analyzer. Sensory data, on the other hand, states that the thermal treatment was slightly too firm. This discrepancy between observations may be due to the fact that data was collected on the fruit prep while each panelist would be able to identify and evaluate individual fruit pieces in the yogurt. Additional studies could prove beneficial in gathering more information about how additional changes to the formulation of the fruit prep would respond to  $CO<sub>2</sub>$  treatment. For example, removal of the buffer system in the fruit prep could potentially allow for a greater impact on pH, which may in turn have a greater antimicrobial effect. The levels of other ingredients could also be altered to further explore the possibilities of maximizing or minimizing the sensory properties that the CO2 treatment could have on the final product.

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### Appendix A: Preparation of Fruit Prep

## **Strawberry Fruit Prep Procedure**

- 1. Hydrate pectin in hot water (85 C) with strong agitation (work up to level 3) for 5 minutes, pour into bowl and set aside
- 2. Using the REVERSE mixing rotation (3) on the Thermomix, mix together strawberries, calcium citrate, sodium citrate, sugar and remaining water and mix for 3 minutes (verify with thermocouple)
- 3. Add pectin solution to the fruit while stirring (REVERSE mode 2) and mix for 5 minutes
- 4. Fill into airtight containers
- 5. Transfer to fridge to store

### **Strawberry Fruit Prep Formulation**



### Appendix B: Complete Sensory Information

### **Copy of Sensory Ballot**

Name Signature

(sign after reading consent form)

Welcome to the Food Science Sensory Laboratory. A copy of the form titled "Consent to Be a Research Subject" is posted in each booth. Please read it carefully before continuing. By signing your name above, you acknowledge that you have read and understand the consent form, and desire of your own free will and volition to participate in this study. Please inform the receptionist if you wish to withdraw.

In this session, you will evaluate **FIVE** samples of **STRAWBERRY YOGURT**, side by side.

Please read all instructions and questions carefully.

\* What is your age category?



\* What is your gender?



- \* What is your attitude about **YOGURT**?
	- Positive
	- Neutral
	- o Negative

# \* What is your attitude about **STRAWBERRY FLAVOR**?

○ Positive ○ Neutral o Negative

# \* When was the last time you **PURCHASED YOGURT**?

○ Less than a day ago ○ Less than a week ago ○ Less than a month ago ○ Less than 3 months ago ○ More than three months ago

- \* When was the last time you **CONSUMED YOGURT**?
	- Less than a day ago
	- Less than a week ago
	- Less than a month ago
	- Less than three months ago
	- More than three months ago

Locate the set of lights to the right of the computer screen and press the red button next to the green "READY" light to indicate that you are ready to receive your samples. Please be patient; they should arrive shortly.

Don't taste your samples yet. You will first evaluate the **APPEARANCE** and **AROMA**. Evaluate the samples from left to right, in the order presented on the tray.

If at any time during the test you need more sample or any other help, **press the button by the** "HELP" LIGHT to the right of the screen.

Please keep in mind that you will be asked to **RANK** the samples in order of preference.

**DON'T TASTE THE SAMPLES YET.** Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you.

\* How much do you like or dislike the **APPEARANCE** of each sample? Sample #'s (please write in the numbers)



\* How do you feel about the **COLOR** in each sample?



**Please smell the samples before answering the next question**, but don't taste them yet.

\* How much do you like or dislike the **AROMA** of each sample?



**NOW TASTE THE SAMPLES**. Use a bite of cracker and a sip of water between samples to refresh your sense of taste.

# \* **EVERYTHING CONSIDERED**, how do you feel about the **OVERALL ACCEPTABILITY** of each sample?



\* How much do you like or dislike the **FLAVOR** of each sample?



\* How much do you like or dislike the **OVERALL TEXTURE** of each sample? Sample #'s (please write in the numbers)



\* How do you feel about the **FIRMNESS/SOFTNESS** of the fruit pieces in each sample? Sample #'s (please write in the numbers)



\* How much do you like or dislike the **AFTERTASTE** of each sample? Sample #'s (please write in the numbers)



\* How likely or unlikely would you be to **PURCHASE** each product if it was priced comparable to other similar products at the grocery store?



# **\* RANK** the samples in order of **PREFERENCE** by writing the sample code in the appropriate space below.



You are finished. Please place the sample and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "FINISHED" LIGHT**. Please give this questionnaire to the receptionist. **THANK YOU!**

# Appendix C: Sample Blinding Code Key



## **Table 2. Sample blinding code key**

# Appendix D: Plate Count Data

# **Microbial Data**













# Appendix E: pH, Color, and Texture Values

# **Collected Data**












# Appendix F: Statistical Outputs

# **Aerobic Plate Count Outputs**







### **Yeast Data Output**





#### **Mold Data Output**





### **pH Output**







# **L\* Color Output**







### **a\* Color Output**







### **b\* Color Output**









#### **Texture Data Output**





