QUANTIFYING THE EFFECTS OF CHEMICAL AND PHYSICAL PROPERTIES OF SKIM MILK AND YOGURTS USING STANDARD METHODS AND A NOVEL RAPID DETECTION METHOD

by

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Abstract

This research sets out to determine how varying factors such as electromagnetic fluid conditioning (EFC) and varying protein and sugar contents can influence yogurt and skim milks overall quality. EFC uses magnets to alter the chemical and physical properties of skim milk in these studies. EFC has many different treatment parameters to optimize before this new processing technology can be industrialized. Treatment parameters include voltage, exposure time, flow rate, and magnetic field direction, as studied in this research. Voltage was altered 10 and 30 V for 2 and 10 minutes. This study showed that at 2 minutes that skim milk was not altered, but at both voltages at 10 minutes some changes occurred to surface tension and color properties (L* and a* values) of skim milk. For both voltages at 10 minutes, it was always the negative direction that experienced the most changes. A separate EFC experiment was done that set out to determine if pretreating skim milk with EFC, would have an effect on yogurt's quality post fermentation throughout storage. Results indicated that EFC does alter the yogurt's properties, but not in a desirable manner. Results were undesirable changes to the product's firmness and syneresis when compared to the control sample. Dielectric spectroscopy is a rapid method to determine if varying protein and sugar contents has compromised yogurt's quality. In the dielectric spectroscopy study, this research wanted to determine if varying protein and sugar contents influenced dielectric properties enough to where a model could be developed to predict yogurt's firmness. Both of these methods, EFC and dielectric spectroscopy, are novel technologies to the dairy industry where, both have been very minimally tested on yogurt. This research proved to be a stepping stone to open further doors to research in these areas due to results indicating changes but not pin-pointing exactly what is going on due to these technologies.

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Dedication

My master's work is dedicated to my father who passed away in November 2009, suddenly. He was the one who encouraged me to check out food science in 2009, but never got to see the person I have become by choosing this as my area of study and career path.

Chapter 1 - Literature Review

Magnetic Treatment of Fluids

Little to no research to date, shows efficiency in treating fluid food products (i.e. milk) with magnetic conditioning that could aid in further processing innovations in fermented dairy products (Yoon and Lund, 1994; Grigelmo-Miguel, 2011). Magnetic conditioning has been used in other fields, specifically water treatment, that indicates a positive effect could be observed when treating milk (Benson and others, 2000; Vick, 1991; Abadias and others, 2008). The theories and mechanisms behind how magnetic conditioning work are often studied where computer simulations have theorized how magnetic conditioning can alter a fluid's characteristics (D'Ambrosio and Giordano, 2004; Cowley and Rosensweig, 1967). This research sets out to understand the effects of magnetic conditioning on milk used in further manufacturing processes such as yogurt.

Theories of Magnetism

Magnetic fluids contain several components including solid and or liquid magnetized particles (Chang and others, 2010), which follow the principles of traditional fluid dynamic models. These models are based on the conservation of mass, Newton's first law, and the First Principle of Thermodynamics. Magnetic fluids, however, deviate slightly from Newton's first law and the First Principle of Thermodynamics. These deviations are necessary to account for the magnetic field present in the fluid (D'Ambrosio and Giordano, 2004).

Cost and physical limitations have restricted practical research, so these theoretical assumptions about magnetized fluid properties are expressed through equations. A series of complex mathematical equations that depend on time measurements are used to calculate

magnetic fluid dynamics. Electromagnetic waves are calculated based on the speed of light, whereas acoustic waves are calculated using the speed of sound (D'Ambrosio and Giordano, 2004). Stability of a magnetized fluid has been expressed using mathematical equations as well; magnets can destabilize and polarize a fluid at an interface (Cowley and Rosensweig, 1967). These equations take into account two variables that are parallel and without hysteresis: a uniform magnetic field and a non-linear magnetic force as described in Langevin's classic theory (Cowley and Rosensweig, 1967).

Benson and others (2000) grouped magnetized objects into categories based upon the resiliency or behavior in magnetic fields. Paramagnetism and diamagnetism are two of the most applicable categories of fluid materials. Paramagnetism represents a positive response exhibited by the fluid moving towards the magnetic field, whereas diamagnetism is the negative reaction, with the fluid moving away from the magnetic source (Benson and others, 2000). Aeronautic sub-disciplines have focused on the potential use of magnetic fields in space. Research is still in its infancy, but researchers hope they can control flow-fields by altering electric fields using magnets (D' Ambrosio and Giordano, 2004).

Magnetizing a fluid depends on an interaction where the fluid conducts electricity, resulting in charged particles, mainly electrons and ions, upon magnetization. These particles are subjected to the electromagnetic field by the Lorentz force, which propels particles through the fluid and reduces the net drag of a fluid (Berger and others, 2000). Researchers have found that as a magnetic system's Reynolds number decreases, net drag is reduced and particles flow easier through the magnetized fluid (Berger and others, 2000). According to Gutierrez and Medici (2005), a fluid undergoing magnetic treatment experiences the Lorentz force, causing changes to flow behavior that are directly related to the fluid heat transfer rate.

Horizontal magnetic fields cause electrons to gain excitation because of the Lorentz force (Yang and others, 2008).

Particles in fluids also respond to three types of force other than the Lorentz force: magnetic, van der Waals, and hydrophobic (Macker, 1952). Van der Waal's force relies on the degree to which a particle can be magnetized. A mathematical equation using the Hamaker's constant predicts how much effect van der Waals force can have on a fluid (Scholten, 1983). This equation takes into account aggregation of particles and the distance between a particle and the carrier fluid (Schloten, 1983). The movement of these particles has a direct correlation with the intensity of the electromagnetic field in the fluid (D'Ambrosio and Giordano, 2004). Odenbach (2003) stated that van der Waals forces enables aggregation of magnetic particles. Lebedev and others (1997) described magnetic particle aggregation through their research on how magnetization affects neutrons. They describe the effect due to the polarization of neutron from dipole interactions, which caused particle aggregation in fluids (Lebedev and others, 1997). Scholten (1983) describes magnetic particle attraction as a function of magnetic dipole interactions with the fluid, claiming that stronger magnetic fields slow particle rotation. The surface properties of fluid also affect particle aggregation. Moreover, magnetic poles affect particles within a fluid, indicating that fluid can be saturated magnetically if particle density is less than fluid density (Scholten, 1983). As the magnetic attraction between particles and the thickness of the carrier fluid reach the peak, magnetic saturation of the fluid is reached, where the effects of magnetism has reached a height that no longer has an effect on the fluid. Magnetic saturation depends on particle size, van de Waal's attraction, and dipole moment contributions from the surface (Scholten, 1983).

Units of Measurement

Gauss (G) or Tesla (T) units describe the field strength of the magnetic field. Both of these units were named after Carl Gauss and Nikola Tesla who discovered the units (Gray, 2014). T is used for powerful magnetic fields where 1 T equals 1,000 G. The American Society of Testing Materials officially adopted G as the magnetic field indicator in 1911, replacing the centimeter-gram-second (cgs) (Stratton, 1916). A guassmeter can determine the strength of a magnetic field where larger numbers indicate a stronger magnetic field (Dictionary of Science and Technology, 1992). The Earth's magnetic field strength is 0.5 G, whereas common household magnets are approximately 100 G, and electromagnetics can reach up to 1, 500 G (Gray, 2014).

Agricultural Uses of Magnetic Conditioning

Other positive uses of magnetic conditioning show promising results for justifying that modifications to milk's chemical and physical properties could be seen. One of these uses is the treating of lettuce seeds with magnetic conditioning. Reina and Pascual (2001) investigated the rate of water uptake in lettuce seeds pre-treated by a permanent magnetic field for 10 minutes at 0±10 milliTesla (mT). Their hypothesis was that magnetic treatment altered the ionic concentration in the seed, thus altering the osmotic pressure, which affects the rate that a seed takes up water during germination. Their results showed that germination time decreased (41%) by pretreating the seeds in the magnetic field (Reina and others, 2001). These results coincide with other studies with similar results on different seed types (Vakharia and others, 1991; Alexander and Doijode, 1995; Imimoto and others, 1996.)

Environmentally Friendly Treatment Option

Consumers and environmentalists advocate for less processed and chemically altered products (Vick, 1991; Abadias and others, 2008). Companies implementing magnetically treated products, especially in the water treatment phase, can capitalize heavily on this growing trend (Vick, 1991). Moreover, salts and minerals can cause scale buildup in pipes, which crystallizes water in the pipes, causing a plaque-like substance to form, eventually blocking and damaging the pipes (Vick, 1991). Water treated with magnetism acts differently than chemically treated water; magnetic treatment disrupts the charges of the ions in the water. These ions behave differently, changing the equilibrium of the system. This change in equilibrium means ions do not precipitate in the pipes but in a cooling tank as algae-like material (Vick, 1991). If a positive effect in water can be seen, one would hope changes to milk would occur due to milk's high water and mineral content (Patton, 2004; Farrell and others, 2006).

Magnetic Treatment of Water

Magnetic treatment is an intricate topic with some reports claiming benefits while others do not, possibly due to strategic selection of treatment conditions (Amiri and Dadkhah, 2006). Holysz and others (2002) also claim that results vary so widely that it becomes difficult to compare the benefits of magnetic treatment; as the results vary because of different kinetic systems and chemical and molecular profiles of the fluid (Holysz and others, 2002). According to Vick (1991), only two conclusions from previous magnetic treatment studies can be drawn: the ability to (1) reduce crystal formation and (2) displace charged particles throughout the fluid.

Benson and others (2000) researched solubility changes brought about by magnetic treatment. Minerals were treated by magnetic field for 1 hour at 2,000 G in a permanent magnetic field and compared to untreated samples. Analysis showed no differences in particle size, but solubility of minerals increased markedly. However, Benson and others (2000) did not know if this effect was permanent. Donaldson and Grimes (1988) showed reduced scale buildup at an industrial plant when permanent magnets were installed. Their data could yield clues about the changes in a fluid during magnetic treatment. Observed changes included precipitation of crystals, altered crystal shape, and altered solubility of ions at 0.2-2,500 G. They believed that flow turbulence prevented the buildup of crystals in pipes at a flow rate of 0.5-0.8 m/s (Donaldson and Grimes, 1988). Vick (1991) reported that for maximum efficiency in treating fluids, the turbidity of the fluid must be controlled during fluid treatment. Another possible explanation of how magnetism works is that the charges on a particle's nucleus affect the plane symmetry, thus altering the charges on the surface of the particle. This changes the size and solubility rates (Donaldson and Grimes, 1988).

Bogatin and others (1999) investigated flow rates of 0-1.5 m/s to magnetically treat irrigation water. They tested the pH, color readings, and crystallography of the irrigation water. They assumed that changes to gas availability and carbonic acid production would alter the pH, thus indicating the magnetic treatment worked. Color readings were done to observe fluxes in the H+ concentration. Calcium carbonate crystals formed during magnetic treatment were identified using a microscope. They reported that flow rate is an important parameter to control when treating water magnetically, and flow rate must be optimized for treatment apparatus, set up, and fluid (Bogatin and others, 1999). In a similar study, increased flow rate (300-500 mg l^{-1}) allowed calcium carbonate to precipitate when fluid was treated with a

permanent magnetic system at 0.16T. Treatment time had a significant effect on changing the solubility of calcium carbonate when resonance time was doubled (Alimi and others, 2007). Holysz and others (2002) studied the direction of the magnetic field (North and south combinations) to see how that affected fluid by measuring the zeta potential. The South/South treatment showed a zeta potential between 30 and 70 minutes where treatment effects lasted 4 hours. The North/South treatment had a markedly lower zeta potential than the reference sample, with differences starting to show 5 minutes into treatment. They concluded that treatment times affected how fluid reacts to a magnetic field.

Benson and others (2000) believed the spatial orbitals in magnetic fluids transform because of the heat involved in the process, allowing the atoms to move freely throughout the fluid. Fluids shift toward thermal equilibrium while under magnetization, which influences orbiting particles. Researchers Abu-Aljarayesh and others (2002) reported that as the temperature of the treated fluid rises toward the melting point (85-300°K), entropy increases, causing particles to scatter. When the fluid reaches the freezing point, however, entropy decreases, causing particles to further align and decrease viscosity. They also found that magnetic saturation of a fluid could occur when fluid reaches a maximum entropy level. Thus, a low magnetic field contributed more entropy to a system than higher magnetic fields (Abu-Aljarayesh and others, 2002).

The magnetic Reynolds number (Re_m), which indicates the ability for a fluid to flow while in the presence of a magnetic field, shows the magnetic diffusion potential of a fluid. If Re_m is large, the fluid lacks magnetic diffusion potential, and a wide magnetic field impinges on the fluid, whereas if the Re_m is low, the magnetic diffusion is large, leading to a weak

magnetic field on the fluid (D'Ambrosio and Giordano, 2004). Mekheimer and Abdelmaboud (2008) also stated that a smaller Re_m meant less magnetic potential.

Proximity of Magnetic Field

Computer simulation of the effect of magnetic fields on fluids indicated that proximity of the magnetic field affected flow behavior (Chang and others, 2010). Nearly direct contact with the magnetic field affected flow behavior most. Further studies showed the fluid's particle size affected magnetic potential and flow behavior. Larger particle size meant magnetic treatment affected flow behavior more (Chang and others, 2010). In computer simulations, Ido and others (2011) found that both magnetically and non-magnetically charged particles formed aggregates in the presence of a magnetic field. These particles line up end to end and move freely in the direction of the magnetic field throughout the system. Enomoto and others (2003) noted this aggregation of particles corresponded to a notable increase in viscosity. Odenbach (2003) found that magnetic particles are susceptible to magnetic fields no matter the strength of the field, also noting increased viscosity as the strength of the applied magnetic field increased; viscosity, in other words, can be controlled by the magnetic field (Odenbach, 2003). These results agreed with the results of Beglarian and Grigorian (1990), who reported increased viscosity in magnetically treated milk, and Cai and others (2009), who reported increased viscosity in magnetically treated water. Both observed decreased surface tension. Not only does proximity of the field effect the treatment, but magnetic field length also can affect treatment optimicy where Ciobanu and others (2011) concluded in their study that to see profound results the magnet should encompass the length of the pipe being used to treat the fluid.

Oscillating Magnetic Fields

The use of oscillating magnetic fields as a preservation method in the food industry is relatively new, unlike traditional preservation methods like pasteurization (Grigelmo-Miguel, 2011). Industry strives to meet consumer's demands for less thermally-treated products while retaining the integrity of sensory characteristics, nutritional availability, and product quality (Palamieri and others, 1999). Researchers and industry are exploring other non-thermal methods to aid in preservation of food products such as pulsed electric fields (PEF) and pulsed light (PL) (Palmieri and others, 1999). These three methods either reduce microbial load or inactivate microbial growth (Palamieri and others, 1999, Grigelmo-Miguel and others, 2011). Prior studies on these methods are generally inconclusive, showing they can inhibit, stimulate, or have no effect on bacteria (Palmieri and others, 1999; Grigelmo-Miguel and others, 2011; Barbosa-Canovas and others, 2011). In a study by Yoon and Lund (1994), however, magnetic treatment affected fouling rates in milk depending on surface material. Results showed that varying Teflon strengths of 0.99 to 1.20 did not inhibit milk spoilage and the magnetic treatment did not significantly change either the microbial load or pH of the milk. Moreover, after magnetic treatment, mineral content was reduced (36%) compared with 43% for the control, while protein in the milk remained constant (Yoon and Lund, 1994).

Work done by Yang and others (2008) found that DC voltage could control the degree of oscillation in magnetized fluid, concluding that oscillating waves are reduced as the magnetic field strengthened. The electromagnetic conditioning system operates using the oscillating motion of the charged particle waves and the voltage applied (Yang and others, 2008). Colic and Morse (1999) studied the unpredictable behavior of magnetic fluids and coined the term "magnetic memory of water", in part as an explanation of why, how, and how long magnetic effects last. The researchers hypothesized that magnetic fields affect the gas-

water interface, especially the interfacial water tension. They thought the magnetic field forces the gases in the fluid to relax, creating a weaker gas-water interface (Colic and Morse, 1999).

Oil and Gas Industry uses of Magnetic Conditioning

In recent years, researchers in petroleum engineering have treated gasoline with magnetic fields hoping to create a cleaner fuel (Ciobanu and others, 2012). Magnetic fields cause fuel molecules to rearrange themselves. Petroleum has diamagnetic characteristics, which help in making a cleaner burning fuel. Fuels also exhibit paramagnetic characteristics; these characteristics cause molecules to aggregate upon magnetic treatment (Ciobanu and others, 2011). These changes to fuel molecules parallel the findings of Benson and others (2000), where changes to the spin orbital of electrons directly correlates to the behavior of fluid under magnetic treatment. Lebedev and others (1997) describe the aggregation of particles as a spin orbital correlation where particles aligned with the magnetic field and formed clusters that spin non-symmetrically to the magnetic field's perpendicular plane.

Milk's Composition

Milk's molecular composition has four main parts: colloidal calcium-phosphate, casein micelles, milk fat globules, and lactose (Patton, 2004). Water serves as the main fluid carrier component in milk (Patton, 2004). Dalgleish and Corredig (2012) state, "Casein micelle structures account for 2.5% of milk composition, but occupy 10% of the surface volume." Milk is studied in this research due to it's molecular profile being similar to water, if magnetic conditioning can alter water ions, one would think the results would carry over to changes in milk's chemical and physical characteristics by altering the calcium and phosphate ions.

Casein micelle

Casein micelles are intricate structures (Needs and others, 2000). In 1998, Horne described and illustrated the complex casein micelle structure. His definition involves the intricate balance of electrostatic repulsions and hydrophobic interactions (Horne, 1998). In his model, the hydrophobic bonding sites are the basis for the casein interactions. Casein micelles interlock with calcium phosphate (Farrell and others, 2006), and calcium phosphate is the binder for these interlocking connections (Holt and others, 2003). Woven casein micelles form the structure of fermented dairy products. Energy causes these structures to conglomerate and form intricate bonds (Horne, 1998). The casein becomes fluid because of micelles in the milk, and the micelles solubilize calcium and phosphate ions (Farrell and Thompson, 1988). Milk forms insoluble precipitates because of the calcium and phosphate when milk has less than 2% protein. When calcium and phosphate are removed, milk has a higher viscosity because casein's structure is exposed. Casein colloidal complexes form, combating physical deformities through transporting calcium and phosphate ions (Farrell and others, 2006). Casein contains four phosphorylated proteins: α_{s1}-, α_{s2}-, β-, and κ- (Farrell and others, 2004). κ- casein initiates a colloidal state by stabilizing insoluble calcium caseins and unfolds at the micelle surface (Farrell and others, 2006).

Surface Tension

All fluid dairy products (foams, emulsions, and films) involve surface tension because of the interactions between proteins, free fatty acids, and fat globules and their subsequent effects on surface bulk properties of the fluid (Whitnah, 1959). Surface properties are described in surface tension values, but bulk surface properties also involve the bond between micelles and fat globules (Whitnah, 1959). Traditional methods to test surface tension use a

ring and tensiometer, standard values for water's surface tension at 25° C is 71.99 dynes/cm and skim milk is 47.29 dynes/cm (Vargaftik and others, 1963; Kristensen and others, 1997). The surface tension is determined by strength needed to detach the immersed ring from the liquid (Li and others, 2012). Surface tension decreases as colloid particles occupy more surface area, while surface energy increases (Cho and Lee, 2005). Cho and Lee (2005) reported an 8% decrease in surface tension of magnetically treated water versus an untreated sample of water.

Zhukov's (2011) research linked interface stability of magnetized fluids with surface tension measurements. Cowley and Rosensweig (1967) described an unstable fluid as one with an interface altered because of magnetized particles. Zhukov (2011) and Amiri and Dadkhah (2006) stated that changes in surface tension depend on the strength of the electromagnetic field applied to the fluid (Zhukov, 2011; Amiri and Dadkhah, 2006). A study by Fujimura and Iino (2008) also found that surface tension increases in direct correlation to increases in magnetic field strength. They concluded that the link between increased surface tension and magnetism could be due to hydrogen bonds that stabilize in the presence of a magnetic field, thus causing an increase in Helmholtz free energy. On the other hand, the Lorentz force could have caused surface pressure to drop because electrons emit waves of energy (Fujimura and Iino, 2008).

Yogurt Manufacturing

Yogurt, a fermented dairy product, contains at least one strain of *Streptococcus salaivarius* ssp. *thermophiles* and one strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* (21 CFR $ 131.200a). These strains work symbiotically to transform milk into yogurt (Vedamathu, 1992). Manufacturing of stir-style yogurt requires a step-by step process.

Vedamathu (1992) described the process. First, the yogurt mix is prepared, followed by heating, fermenting, and cooling, and finally, by adding fruits or flavorings. Yogurt quality depends on heat treatment of the mix typically at higher temperatures than 85°C to affect proteins and firmness of the formed gel (Morand, 2012). Setting involves allowing the product to cool to an appropriate temperature for inoculation with starter cultures. The starter cultures transform the milk to yogurt by producing lactic acid from available lactose. Lactic acid production during fermentation can explain why the micelle structure of the caseins destabilize (Remeuf and others, 2003). This lactic acid accumulation also reduces the pH; pH 5.2 is the isoelectric point of denatured whey proteins, while pH 4.6 is the isoelectric point of casein. This causes hydrophobic bonds between the whey proteins and casein (Lucey and Singh, 1995; Remeuf and others, 2003).

Quality Determinates

Success of any product on the market relies on meeting quality standards of consumers (Kroger 1975, Vedamathu, 1992). Yogurt quality has four main categories: body, texture, flavor, and shelf life (Vedamathu, 1992). Kroger (1975) noted that yogurt quality has no set standard because it can vary greatly based on type of yogurt and consumer preference.

Yogurt quality can be confirmed through well-chosen analytical methods or sensory panels. Most yogurt quality evaluations in industry take place 24 hours after production; however, evaluations can occur at any time. Among the quality parameters of overall acceptability of a product are firmness, viscosity, mouth-feel, and syneresis (Ares and others, 2007). Analytical measurements include titratable acidity, pH, compositional analysis, acetaldehyde production, coliform testing, shelf-life testing, and container fill (Kroger, 1975).

Health Benefits

Dairy products are part of a well-rounded diet (Nicklas and others, 2009). Dairy products are a good source of vitamins A, C, D, E, and K as well as the minerals calcium, magnesium, and potassium. A 2010 study released by the Dietary Guidelines notes these vitamins and minerals are often lacking in the diet (USDA, 2010). The nutrition community increasingly recommends low-fat dairy products, but especially yogurt because it has more concentrated protein, vitamins, and minerals than milk (USDA, 2010).

Research has focused on yogurt because of its health benefits, especially for intestinal health and probiotic effects (Vedamuthu, 1992; Peng and others, 2009). *L. delbrueckii* ssp. *bulgaricus* in particular helps establish a proteolytic environment by producing essential amino acids for *S. thermopoilus*, which helps probiotics form. It would be interesting to see if magnetic conditioning had an effect on altering this beneficial bacteria accumulation.

Syneresis

Syneresis is an undesirable defect in yogurt that affects body and texture (Kroger, 1975; Vedamuthu, 1992). Syneresis involves whey expulsion due to broken coagulum during handling, temperature variations, acidity problems, milk defects, low protein (<3.4%) or low fat milk, high mineral composition of the milk, problems with the coagulum during heat treatment and incubation, using rennet, improper acid endpoint, incorrect starter culture, and too much carbon dioxide (Kroger, 1975). Manufacturers try to prevent syneresis because it is unattractive to consumers (Biladeris and others, 1992). To maintain coagulum structure, some prevention techniques include proper homogenization to ensure proper dispersion of milk fat globules, increased protein content in the milk (>3.5%), proper handling throughout storage

and transportation, and avoiding temperature abuse (≥4°C) throughout the manufacturing process (Kroger, 1975).

Color

The micellar structure affects light scattering in milk (Kinsella, 1987). Casein micelle structure is complex; it depends on current conditions as well as changes in the milk. These changes can induce losses in structural stability and can be seen in color value readings. Needs and others (2000) observed significant changes in milk and yogurt treated with high pressure using the L*, a*, and b* values. Lightness (L*) is measured from 0-100, representing the range from black to white; a* represents green to red on a scale of -100 to +100, and b* is blue to yellow, again with values of -100 to +100 (Bakker and others, 1986; Carreno and others, 1995). Overall color differences can be explained by calculating the ΔE, which accounts for differences in L*, a*, and b* in a sample. Changes to these values correlate to changes in the structure of casein micelles due to different treatment effects where L* values increased 17.2%, indicating a more aggregated gel structure in pressure-treated yogurt samples than in heat-treated yogurt samples, with L* values of only 5.4% (Needs and others, 2000). This indicates that processing conditions can influence color properties and casein micelle complex.

Texture

Texture is another quality that contributes to the success of yogurt (Kroger, 1975; Vedamuthu, 1992). Firmness is a desired quality in the final product with minimal syneresis (Kroger, 1975). Properly homogenized milk fat, total solids, starter culture, incubation conditions, and stabilizers all contribute to the overall quality of yogurt (Kroger 1975; Veramuthu, 1992). Texture comprises much of the consumer perception of a product (Tunick,

2000; Paseephol and others, 2008), so producers use both sensory analysis and instrumental data to learn about consumer perceptions of a product's texture (Sodini and others, 2005; Ares and others, 2007). Appearance, mouth-feel, and overall acceptability all help define the texture of a product (Ares and others, 2007). Particularly in dairy products, texture profile analysis is needed to analyze the casein gel matrix (Tunick, 2000). Manufacturers face shifting trends for low fat and no-fat products that still have the same textural components as their full fat counterparts (Paseephol and others, 2008). Companies must develop a desirably firm product with minimal syneresis, to satisfy consumers who want the appropriate texture without the calories (Parnell-Clunies and others, 1986; Kroger, 1975).

Water Holding Capacity

Water holding capacity (WHC) can describe how a product binds water and how that water moves in the product (Hinrichs and others, 2003). Martini and others (2013) described three types of water in products: imbedded water, bulk water, and macromolecular water on the surface. Protein-protein and protein-water interactions control the product's ability to hold water or bind it within a gel system. The bound water is called bulk phase water. Protein denaturation is linked to increase WHC through the gel exposure to charged particles and increased surface area (Fennema, 1977; Kinsella, 1984).

Dielectric Spectroscopy

Dielectric spectroscopy provides information about the electrical properties of food products by providing an idea of its ability to be heated in the microwave and radio frequency range (Ahmed and Luciano, 2009; Motwani and others, 2007; Nelson, 2005). Dielectric spectroscopy has been used to predict quality parameters of foods (Nelson, 2005). When analyzing a product using dielectric spectroscopy, the dielectric constant (ε') and dielectric

loss factor (ε") over multiple frequencies and temperatures are generated. Temperature, as well as chemical and physical properties of the foods can influence dielectric measurements with the ionic concentrations (presence of salts) with water content being the most critical (Nunes and others, 2006; Clerjon and others, 2003; Ahmed and Luciano, 2009).

Once ε' and ε" are obtained, these factors can describe the products capacity for absorbing heat (Ahmed and Luciano, 2009; Motwani and others 2007). Energy storage is represented in the ε'; whereas absorbed energy is represented by ε" (Everard and others, 2006; Foster, 1997). These factors can be used to derive the relative permittivity (ε*) :

$$\varepsilon * = \varepsilon' - j\varepsilon''$$

- j is the square root of -1

Interactions Between a Products Composition and Dielectric Spectroscopy

The dielectric properties of proteins and ion interactions have been an understudied field due to the proteins' intricate relationship with water and the ions side chains (neutral, polar, or charged) (Ahmed and Luciana, 2009). Further complicating this relationship, is that in food systems both bound and free water exist within and between proteins and are affected by the electrostatic interactions and the charges on the proteins. Dielectric properties can help explain the state of water by predicting moisture content in the food (Ahmed and Luciana, 2009; Clerjon and others 2003). Principles behind dielectric spectroscopy detail how ε' and ε" are influenced by the molecular motion of the polar charged particles (water) when exposed to an alternating electric field. Once the molecules reach a state of relaxation and can no longer move at an increased frequency, the relaxation frequency has been reached (Clerjon and others, 2003).

Penetration Depth

Using ε' and ε", the product's penetration depth can be calculated to further describe the microwave heating profile which in turn can ensure uniform heating, where an increase in penetration depth increases the heating consistency of the product (Everard and others, 2006; Wang and others, 2003). Processed cheese, a food that is intended for microwave heating can be evaluated by dielectric spectroscopy to understand how their internal properties react to heating (Everard and others, 2006). The formula for calculating penetration depth is as follows:

$$d_p = \frac{c}{2\sqrt{2\pi} f\{e'_r \left(\sqrt{1 + \left(\frac{e''}{e'}\right)^2} - 1\right)\}^{1/2}}$$

where c is speed of light 3 x 10^8 m/s and f is the frequency.

Temperature's influence on Dielectric Spectroscopy

Ahmed and Luciana (2009) reported positive correlations between dielectric measurements and temperature (20-90°C), β-lactoglobulin concentrations (5-15%), and pH (4-10) by using 2nd order polynomials from the readings taken every 10°C from 915 to 2450 MHz. As the β-lactoglobulin concentration increased, the ε' decreased as less water was available in the system to interact with the proteins. They reported that pH did not influence the model for ε', but temperature and concentration were significant due to the denaturation and unfolding of the proteins. Bircan and Baringer (2002), explained that syneresis in the system caused an increase in the ε" as water freely moved. Increasing the system's temperature until the protein's denaturation temperature resulted in an increase of the penetration depth then the penetration depth decreased. Penetration depth declined as pH decreased from 10.0 to 4.0. Wang and others (2003) tested a 20% whey protein solution and

reported that a state of relaxation occurred at microwave frequencies of 915 and 1800 MHz, whereas the e" increased with increasing temperatures (21-121°C) at the lower frequencies of 27 and 40 MHz. In this study the penetration depth was 4x greater at the lower frequencies when compared to higher frequencies which indicated that whey protein was not a good heat transfer source when using microwave heating but rather radio frequency heating should be used in this application (Wang and others, 2003).

Moisture Content's Influence on Dielectric Spectroscopy

Guo and others (2010) applied dielectric spectroscopy to adulterated milk. Milk samples diluted with water to 70-100% milk concentration were tested at 5% increments. Upon using linear regression, ε" was determined to be the best indicator of the adulteration of milk. Also the ε' decreased as frequency increased from 10 to 4500 MHz (Guo and others 2010). Nunes and others (2006) studied dielectric properties of milk with varying fat contents (skim (0.33%), low fat (1.55%), and whole (3.60%)) from 17-20°C at 1-20 GHz where each fat content had the same dilutions. Researchers found strong correlations between the dielectric constants and loss factors suggested that this technology could be used as a quality predictor. Compiling dielectric spectroscopy to develop predictive models may be useful for determining composition of food products (Nunes and others, 2006).

Sugar's Influence on Dielectric Spectroscopy

Dielectric spectroscopy in the microwave region at 1-20 GHz has been used on store-bought yogurt and prepared yogurt (sugar contents from 0 to 15%) to predict sugar contents (Bohigas and others 2008). Using the results of dielectric spectroscopy in the frequency region of 1-20 GHz a predictive model was built to determine the sugar content of store bought and prepared sugar contents of 0-15%. Bohigas and others (2008) also concluded that

other constituents in yogurt could affect dielectric readings such as protein and carbohydrates, which can alter the dielectric readings. It was also noted that water bound to proteins could decrease the relaxation frequency because molecules are less free to move around and interact with the dipole motions, which increase as frequency increases.

In a similar study by Castro-Gilaraldez and others (2010), dielectric spectroscopy was used to determine ripening stage and prime-eating time of apples by monitoring the malic acid and various sugar contents from 500 MHz to 20 GHz. A positive correlation was found so this method could be used to predict fruit ripening and sugar contents by observing a decrease e" at the relaxation frequencies as sugar content increased (0-15%).

Guo and others (2010)[b] evaluated the adulteration/dilution of honey and reported that values have strong correlations between dielectric properties and total soluble solids and water contents. As water contents increased from 18-42%, the dielectric relaxations decreased proving that sugar and water contents could be determined by using microwave dielectric waves as a quick method for determining adulteration (Guo and others, 2010[b]).

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Chapter 2 - Research Objectives

- To determine if electromagnetic fluid conditioning can alter skim milk's physical and chemical properties to enhance the quality of yogurt derived from skim milk throughout fermentation and storage.
- To determine if conditions (voltage, treatment time, and magnetic field direction) on an electromagnetic fluid conditioner can alter physical and chemical properties of skim milk.
- To determine if varying protein and sugar levels can be used as a firmness predictor in yogurt by using dielectric spectroscopy values.

Chapter 3 - Electromagnetic fluid conditioning and its functionality in manufacturing nonfat yogurt

Abstract

Electromagnetic fluid conditioning (**EFC**) may induce physical and chemical changes to components of skim milk used in manufacturing yogurt. Magnetic fields applied to liquid substances cause temporary changes in the ions in the liquid, which may benefit the dairy industry in processing fluid milk for further applications. In this study, skim milk was used for 3 treatments using EFC. Control had no magnetic charge applied as it went through EFC treatment; negative treatment involved a negative magnetic field in EFC treatment; and positive involved a positive field direction in EFC treatment. EFC treatment was 30 V at 15 minutes. Yogurt samples were processed after treatments were complete. Data were collected throughout fermentation and storage to track quality changes. Water holding capacity, pH, titratable acidity, syneresis, color parameters, firmness, and total solids were assessed to determine changes in yogurt quality from day 1 to day 30. Results show the treated samples did not exceed the quality of the control yogurt although differences were observed during fermentation itself. EFC of skim milk will need further study before the technology can improve the manufacture of yogurt.

Key Words

electromagnetic fluid conditioning, skim milk, yogurt quality

Introduction

Using electromagnetic fluid conditioning (**EFC**) in the food industry, especially in milk products, is a novel processing approach, with little research done to date on milk products. Research in other areas, (e.g., water) demonstrates magnetic conditioning can alter

properties of fluids, indicating that EFC in the food industry may allow processing innovations (Vick, 1991; Bogatin and others, 1999); Donaldson and Grimes, 1988). Magnetically treated water has reduced scale build-up in pipes by altering the net ion charges of water, causing salts and minerals to precipitate in holding tanks instead of pipes (Donaldson and Grimes, 1988). The principle of this treatment relies on molecular polarity where non-polar molecules become polarized due to the reaction to the magnetic treatment (Vick, 1991). Bogatin and others (1999) investigated using magnetic treatment in irrigation water and reported that pH is one of the best indicators of the impact of magnetic treatment because changes in gas content and carbonic acid in water influence pH differences. Magnets cause crystallization centers to form, which in turn cause the carbonic acid and gas concentration to change, altering the pH of the sample.

Other agricultural research has shown the potential of pre-treating seeds with magnetic conditioning. Reina and Pascual (2001) studied the effects of magnetic treatments on germination rates of lettuce seed, reporting that ionic concentrations were altered on the seed's surface, which led to increased osmotic uptake by the seeds. Results showed magnetically-treated seeds had a higher germination rate because water absorption increased in response to changes in osmotic pressure. The effect of magnetism showed higher germination rates for 8 days although the seeds regained their pre-magnetic germination rate by day 25 (Reina and others, 2001).

Donaldson and Grimes (1988) researched salt formation in magnetically treated liquids. Salts in magnetically treated liquids changed shape, precipitation rate, and solubility properties because of the magnetic field. These characteristic changes inhibited scale build-up in pipes (Donaldson and Grimes, 1988). Alimi and others (2007) reported that treatment time

and the flow rate of magnetic conditioning could be significant to the magnetic responsiveness of a fluid. In addition, the proximity of the fluid to the magnet contributed to the strength of the magnetic field. Chang and others (2010) reported that almost direct contact affected the properties of magnetized liquid the most.

Other processing innovations like high-pressure processing are known to alter the physical properties of fluid by inducing chemical alterations. In a study by Needs and others (2000), L*, a*, and b* values were used to describe the alterations to the casein micelle complex due to pressure processing. Needs and others (2000) used this processed milk to manufacture yogurt monitoring color values throughout fermentation. The observed L* values for the pressure-treated samples increased 17.2% due to aggregation of caseins and heat-treated samples by 5.4% due to casein micelle reformation.

In a similar study using L*, a*, and b* measurement to quantify processing effects, Dunkerley and others (1993) found that as temperatures (2-80°C) and dilutions (30-100%) of whole milk increased, the L* values increased by a maximum of 7 units. These changes were attributed to changes in the physical properties of casein micelles. Researchers also assumed that changes to the casein micelle structure and ionic composition of calcium phosphate disrupted the whiteness index of the milk as heating temperatures increased (Dunkerley and others, 1993).

Magnetic treatment of water does alter the properties of minerals found in water (Benson and others, 2000; Donaldson and Grimes, 1988; Bogatin and others, 1995). Alimi and others (2007) reported that calcium carbonate precipitated out of fluid treated under a 0.16 T magnetic field. Similar findings by Bogatin and others (1995) reported that calcium carbonate crystals were observed after magnetic treatment, while Donaldson and Grimes

(1995) reported a marked solubility difference in minerals after treatment. Casein contains minerals like calcium and phosphate that could be altered by magnetic treatment (Patton, 2004; Farrell and others, 2006). Fermented dairy products depend heavily on this interaction between casein proteins, calcium, and phosphate where an interlocking web of these three molecules serves as the gelation base (Farrell and Thompson, 1988; Horne, 1998)

This study will use magnetically-treated skim milk for yogurt manufacturing to see the effects of EFC on milk; changes to the milk as a result of the treatment could carry over, affecting the quality of prepared yogurt. Objectives of this research were 1.) to distinguish differences in yogurt during fermentation and 2.) to distinguish yogurt differences throughout storage of magnetically treated skim milk used in yogurt manufacturing. The methods were chosen to gain an in-depth picture of how magnetic conditioning alters the physical and chemical structure of the milk.

Materials and methods

Chemicals and Reagents

All equipment, chemical, and sample containers were acquired from Fisher Scientific (Pittsburgh, PA, USA) unless otherwise noted. Fat-free skim milk fortified with vitamins A and D and 1% non-fat dry milk solids were obtained from Kansas State University Dairy Processing Plant (Manhattan, KS, USA), and starter culture (YC 495-250, DuPont, New Century, KS, USA) was kindly donated by DuPont.

Electromagnetic Fluid Conditioning (EFC) treatment

Milk was pre-treated by an EFC using two magnetic treatments: Negative (N) and Positive (P); a control (C) treatment was also included. P was exposed to a positive magnetic field whereas the N treatment was exposed to a negative magnetic field, and the control was

run through the conditioner with no magnetic field present. The EFC was set to deliver 30V direct current (VLS-25M, Astron, Irvine, CA) during treatment. The pipe had three magnetic hot spots about 0.0254 cm in diameter, 15.24 cm apart. The EFC design, using a positive displacement pump, allowed milk to be recirculated for the treatment time: 15 min at a constant flow rate of 3L/min in 6.35 mm plastic pipe (Luiquiport © KNF Lab, Trenton, NJ, USA). Each treatment used a sample size of 3.79L of skim milk, and milk treatment was conducted at 25 ± 2°C.

Yogurt Manufacturing

Set-style yogurt was made using the method of Biliaderis and others (1992). Within 30 min of EFC treatment, 3300 mL of milk was heated to 90°C while stirring at 600 rpm on a magnetic stir plate; this sample was typically stirred for 60 ± 5 min. Milk samples were then placed in a 90°C pre-heated water bath (Isotemp 220) for 10 min, cooled to 43°C within 10 min, and then inoculated with yogurt starter culture (usage rate 0.0924 g/L) (YC 495-250, DuPont, New Century, KS, USA). Inoculated milk was poured into containers of different sizes, depending on the requirements of assessment tests. These containers included 120 mL sterile cups (~110 g of mix), 40 mL plastic centrifuge tubes (~20 g mix), and 119 mL plastic disposable vials (~80 g mix). All filled containers were incubated at 42°C (Isotemp, Fisher Scientific) for 5 h ± 10 min. Yogurt was stored at 4 ± 1°C (Kenmore, Chicago, IL, USA).

Fermentation

During fermentation, yogurt mixes were monitored hourly for pH, TA, and color properties (L*, a*, b*) until samples reached 4.6 pH (approximately 5 h ± 10 min). pH was monitored using the methods of Lee and Lucey (2004) with an Accument®, AP63 pH meter

calibrated with standardized buffer solutions (pH 4, 7, and 10 at 42°C) before each measurement.

Titratable acidity used the method described by Hooi and others (2004) using 0.1 N NaOH (Acros Organics, Geel, BE). TA was calculated as:

$$\frac{9\ x\ N\ of\ titrant\ x\ ml\ used}{wt\ of\ sample}$$

A colorimeter (Hunter Lab 4500L, Reston, VA, USA) was used to determine L*, a*, and b* values every hour as described by Needs and others (2000) with modifications for sample containers. The colorimeter was calibrated before each use following the manufacturer's instructions and the black and white tiles provided (X= 80.94, Y= 85.30, and Z= 88.35). Sample readings were taken on 120 mL filled cups at every 90° angle, resulting in four readings per sample on the sample's surface; readings were then averaged. Alternating containers were used during fermentation, so one cup was measured at times 0, 2, and 4 h, while the other cup was measured at times 1, 3, and 5 h.

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$$

Yogurt Quality

Yogurt was assessed on day 1 and then bi-monthly (day 15, 30, and 45) by monitoring the following quality parameters: color properties (L*, a*, and b*), firmness, pH, syneresis, TA, and water holding capacity (WHC). Color, pH, and TA were done as described for the fermentation study, with the test conducted at 25 ± 1°C. On day 1, total solids were measured on all yogurts following the forced air oven method described by Hooi and others (2004) in a 103°C oven (Isotemp) for 24 h. Total solids were calculated as:

$$\frac{After\ weight - crucible\ weight}{sample\ weight} x\ 100$$

Firmness, the force required to penetrate the sample during the first compression cycle, was tested using a texture analyzer (Stable Micro System, Model TZ-XT2, Texture Expert, Surrey, UK) following the method of Magenis and others (2006) with modifications for the container and sample size; this study used 119 mL containers with 80 mL of yogurt. The analyzer was equipped with a 25 mm acrylic probe, and testing parameters were set at velocity = 2.0 mm s^{-1}, time = 5.0 s, and distance = 5.0 mm. Before samples were tested, the equipment was calibrated using a 500 g weight. Firmness values were acquired by macro analysis using the software provided by the manufacturer (Stable Micro System).

Syneresis was done using the method of Amatayakul and others (2006); a 120 mL filled cup of yogurt was held at 4 ± 1°C for 2 h at a 45° angle. Expelled whey was then syphoned off and weighed. Syneresis was expressed as % whey expelled from the samples:

$$\left(\frac{whey\ weight}{(initial\ fill\ weight - prefilled\ cup\ weight)}\right) * 100$$

The method of Parnell-Clunies and others (2006) was used to determine WHC using a centrifuge (Marathon 21000R) set at 13500 x g for 30 min at 4°C. Water holding capacity was expressed as % of expelled whey to total yogurt weight:

$$\left(\frac{(after\ weight)}{(filled\ weight - intial\ tube\ weight)}\right) * 100$$

Experimental Design

This study was a randomized, complete block design with replication as the block and the milks (EFC treated as C, N, and P) and time (h for fermentation and day for storage) as the factors. Three replications were done, and data were analyzed using PROC MIXED in SAS (SAS Institute Inc., v 9.2, Cary, NC, USA). Random effects were repetition, hours, and

repetition × hours for fermentation data, while fixed effects were treatment, hours, and treatment × hours. Random effects of storage data were repetition and repetition × day, while fixed effects were treatment, day, and treatment × day. Least square means were determined at a significance level of $P \leq 0.05$. For significant differences, Bonferroi adjustment was used to separate significant means (Kuehl, 2000).

Results

Fermentation Study

Statistical results showed that TA, pH, and color properties of yogurt samples were significant for hours throughout fermentation, but only pH was a function of the EFC treatment of the milk ($P \leq 0.05$), as seen in Table 3.1. No interactions were significant ($P \geq 0.05$). For the effect of EFC during fermentation, the mean pH averages were C ($5.64^{a} \pm 0.177$), N ($5.58^{b} \pm 0.187$), and P ($5.601^{ab} \pm 0.184$). Figure 3.2 shows the average pH of the yogurt mixes during fermentation. As expected, during fermentation, all yogurts showed a similar downward trend in pH over time ($P \leq 0.05$). Error bars represent differences among hours; h 3 proved to be the most significant with the largest pH decrease observed between EFC-treated milks and control during this time with the N treatment having 3.01% lower pH than the C sample. Total solids did not significantly differ (9.8% ± 0.55) between control and treated samples.

Table 3.1 shows mean values for the color properties (L*, a*, b*), pH, and TA of yogurt mixes during fermentation as a function of time ($P \leq 0.05$). During fermentation, pH significantly decreased every hour ending at pH 4.61, a total decrease of 29.7%. Likewise, TA increased by 73.7% from hour 0 to 5. Comparing hour 0 with 3, L* increased by 0.47%,

indicating a more opaque gel structure during the first three hours of fermentation; however, L* then decreased 6.4% by the end of fermentation indicating a less opaque gel structure. During the entire fermentation, a* decreased by 34.3% with the biggest decrease (22.5%) between hour 2 and 3 indicating a shift from red to green. Throughout the 5 h fermentation, b* increased by 28.8% indicating a shift from yellow to blue. Fermentation time was not significant; all yogurt mixes reached pH $\leq$ 4.6 within 5 $\pm$ 0.67 hours. Also ΔE* was not significant for treatments (C:8.82, N:7.69, and P:7.29) this value can indicate an overall color change throughout fermentation.

Yogurt Quality

Storage data also showed statistically significant differences between EFC-treatment and time; however, no significant interactions occurred $P \leq 0.05$). Non-significant ($P \geq 0.05$) results for L*, a*, b*, and ΔE can be seen in Tables 3.3 and 3.5.

The EFC treatment affected three yogurt quality tests, firmness, pH and syneresis, with the mean differentiations shown in Table 3.2 ($P \leq 0.05$). These means represent the 5 test days. P and N treated samples were significantly less firm with more syneresis than C. N and P gels were 7% less firm with 21% more syneresis ($P \leq 0.05$) than C. pH differed significantly between control and P samples. P decreased 1% less in pH ($P \leq 0.05$) than C, whereas N and C were similar.

Table 3.4 shows the changes during storage for all treatments. Changes in firmness, pH, and syneresis were significant ($P \leq 0.05$). Firmness increased by 13% ($P \leq 0.05$) during the first 30 days of storage and then remained constant thereafter. Syneresis decreased by 21% ($P \leq 0.05$) throughout all 45 days of storage. As syneresis values decreased, firmness

increased, leading to a firmer gel structure. In the first 15 days, pH decreased in all treatments, but during the remaining 30 day, average values remained constant.

Discussion

Results show that EFC conditioning affected certain properties of inoculated milk throughout fermentation and manufacturing for yogurt storage (pH, firmness, and syneresis). However, throughout fermentation, pH, TA, and color values for the samples differed as a time effect as apposed to an EFC effect. A treatment effect for mean pH during fermentation was also significant when comparing all samples ($P \leq 0.05$). During storage, values for firmness, syneresis, and pH were also significant when compared to control ($P \leq 0.05$). These results raise many further questions about using EFC during yogurt processing.

Changes to the ions, ionic concentration, and solubility in the treated samples can be seen in changes in pH during fermentation; these changes differed by hours, with hour 3 significant by treatment. Lee and Lucey (2010) described the acidification process occurring during yogurt fermentation as a highly disruptive environment leading to observed changes in the casein micellar structure. These changes are due to the constant solublization of the colloid calcium phosphate (CCP) as the pH decreases throughout fermentation. EFC may alter the ionic structure within milk because of the salts in milk, which in turn may have altered the CCP bonds, altering the casein micelle complex and leading to differences in pH values in this study. The varying degrees of CCP solublization seemed to affect the quality of the yogurt.

Likewise, TA is an indication of milk's hydrogen ion concentration and acid development, although most apparent TA comes from phosphates in the milk (Sharp and McInerney, 1927). Changes to pH can mean changes in TA as well. The samples in this study

did show increased TA during fermentation due to the production of lactic acid during yogurt manufacturing.

Yogurt firmness can be influenced by several factors, one of which is total solids in the mix and final product (Gastaldi and others, 1997; Penna and others, 1997; Kristo and others, 2003). Total solids did not significantly differ (9.8%) between control and treated samples, so changes to firmness were due to physical changes to the milk through EFC ($P \leq 0.05$). Gel strength due to micellar casein interactions can indicate firmness as well (Hassan and others, 1996). Lower firmness values indicate a weakened gel network with less casein micelle bonding on the surface, so treated samples were 7% less firm than the control throughout storage. If magnetic treatment can alter the calcium and phosphate in water, similar interactions could occur in milk, where these two ions form the bases for the gel structure (Benson and others, 2000; Bogatin and others, 1995; Farrell and Thompson, 1988; Horne, 1998; Alimi and others, 2007). This could be one reason why firmness was affected in this study.

In yogurt, syneresis is linked to firmness during storage; as the gels age, the gels contract because the casein micellar bonds intensify. The gels then release more whey and increase the firmness of the gel (Vargas and others, 2008). Syneresis values provide an idea of the stability of a yogurt's gel formation (Matumoto-Pintro and others, 2011). In this experiment, overall syneresis values decreased throughout storage while yogurt firmness increased. Isleten and Karagul-Yuceer (2006) observed a 19.35% decrease in syneresis during storage of yogurts made with 12% total solids from skim milk powder throughout 12d. Other researchers present similar findings where increased protein interactions that strengthen the gel's network by decreasing pore sizes and cross links may decrease syneresis during storage,

which enhances stability (Puvanenthiran and others, 2002), EFC treatment provided similar results; the EFC treatment could have altered the bonds to create firmer gels over time without added ingredients, but instead decreased these bonds. Indeed, the control sample showed decreased syneresis with increased firmness, whereas the EFC treated samples showed increased syneresis but decreased firmness.

Changes to L*, a*, and b* values in milk depend on processing conditions during treatments and particle aggregation (Needs and others, 2000; Vargas and others, 2008). These changes were apparent throughout fermentation, with increases to L*, a*, and b* values. Magnetic research has shown that EFC causes particles to aggregate in water, which could lead to changes in color during fermentation; indeed, several studies have documented aggregated particles in magnetized fluids (Odenbach, 2003; Ido and others, 2011; Enomoto and others 2003). Color data can indicate an increase in aggregation. They represent the degree of casein aggregation as acidification forms casein micelle structures (Vargas and others, 2008). This data did not show a significant treatment difference in L*, a*, and b* values during fermentation or storage, but a significant effect during fermentation for L*, a*, and b* values due to casein micelle rearrangement was observed. Color values did not change significantly during storage, indicating a stable gel.

Relating these findings to other research to understand the effects of magnetic treatment on milk can be difficult because skim milk has colloidal calcium-phosphate interactions, with milk fat globules and lactose with water (~91%) being the fluid carrier (Patton, 2004). This study did not allow a precise answer as to which physical and chemical structures were altered, although changes to firmness, syneresis, and pH were evident throughout storage in the EFC treated samples. Based on water quality studies that showed

changes to the ionic structure of water, the intricate casein micelle structure may be altered because of changes to the calcium phosphate bonds. Calcium phosphate, an ionic binder, is salt found in milk that binds the casein micelle complex (Farrell and others, 2006; Holt and others, 2003). This structure may have been altered by EFC due to its effect on ions in water.

Magnetic conditioning has many practical applications in the dairy industry if proper processing parameters can be established. More studies could pinpoint EFC processing variables needed to achieve positive effects for industrial applications. Variables that should be tested are the amount of direct current applied, treatment time, pump type, flow rate, temperature, and sample pH. These variables in any combination could reveal optimal processing conditions for the dairy industry. Reduced fermentation times or a magnetically treated yogurt that exceeds the quality of a control sample are possible advantages. Industry could benefit greatly from cutting manufacturing costs if magnetic treatment could decrease fermentation times without affecting quality. In this preliminary study, magnetically treated samples required shorter fermentation times; however, the difference was not significant. Further analysis could reveal EFC processing variables that would yield an optimal treatment process, and another yogurt study could test the process.

Conclusion

Results from this yogurt storage study indicate that physical and chemical changes do occur in magnetic treatment of milk. This study showed changes to the quality of EFC treated samples, but treatment samples did not exceed the quality of control samples. Future research should focus on EFC processing variables that would better explain how EFC alters the physical and chemical com ponents of milk. Further studies could then identify changes in the milk that would optimize the EFC process and improve yogurt-manufacturing practices.

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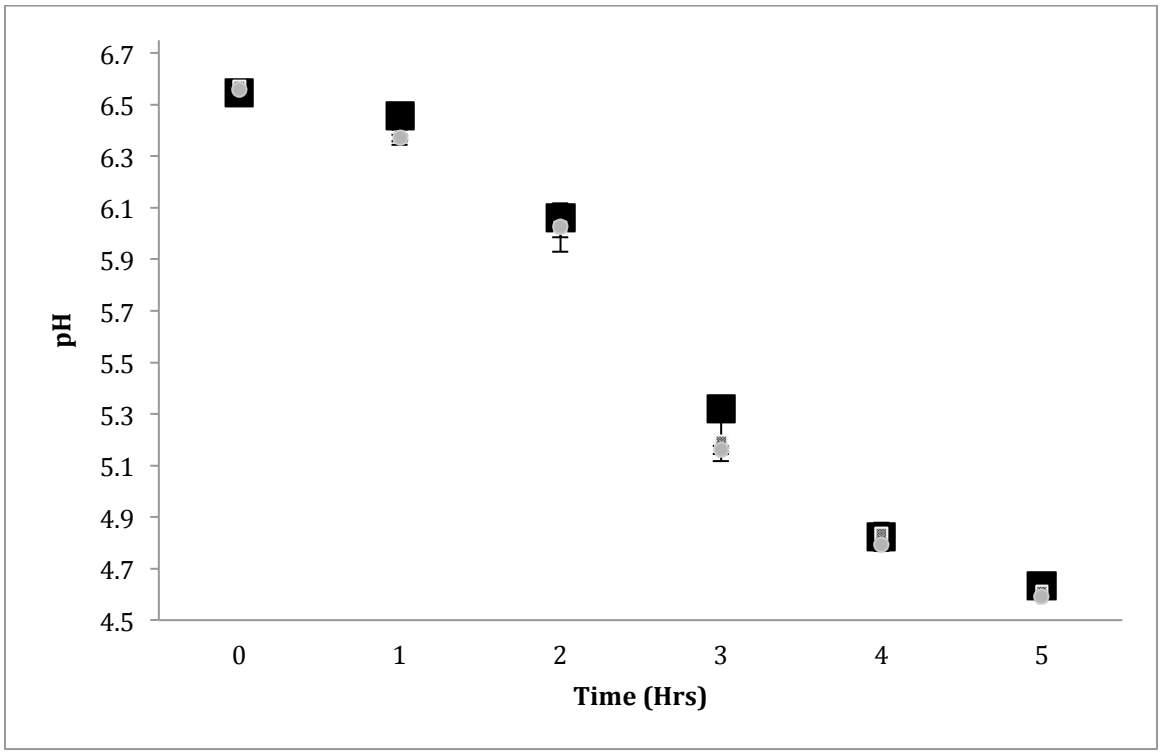
Figure 3.1: Average pH of inoculated yogurt mixes throughout fermentation



■C: control yogurt, ×P: positive yogurt, and •N: negative yogurt

Treatment means (n=9), error bars at hour 3 shows pH differences amongst treatments.

[1]Yogurt mixes include EFC-treated milk without applied magnets (control), EFC-treated milk in the negative direction at 30V for 10 min, and EFC-treated milk in the positive direction at 30V for 10 min

Table 3.1: Color (L*, a*, and b*) properties, pH, and titratable acidity (TA) of inoculated yogurt mixes[1] made from different EFC treated skim milks as a function of fermentation time (means ± standard error)

	Color				
Hours	L*	a*	b*	pH	TA
0	89.93±0.39[a]	-4.19±0.08[b]	7.49±0.13[c]	6.56±0.01[a]	0.216±0.01[d]
1	89.65±0.43[a]	-4.06±0.06[b]	7.43±0.10[c]	6.39±0.02[b]	0.240±0.02[d]
2	90.13±0.39[a]	-3.91±0.10[b]	7.31±0.15[c]	6.03±0.03[c]	0.341±0.01[c]
3	90.36±0.47[a]	-3.03±0.15[a]	8.43±0.26[b]	5.22±0.04[d]	0.590±0.01[b]
4	88.62±1.18[a]	-2.71±0.05[a]	9.97±0.09[a]	4.82±0.02[e]	0.751±0.01[a]
5	84.54±1.43[b]	-2.75±0.10[a]	10.52±0.28[a]	4.61±0.01[f]	0.821±0.02[a]

[a-f] Means (n=9) within a column with different superscripts differ significantly ($P \leq 0.05$).

[1]Yogurt mixes include EFC-treated milk without applied magnets, EFC-treated milk in the negative direction at 30V for 10 min, and EFC-treated milk in the positive direction at 30V for 10 min.

Table 3.2: Firmness, pH, syneresis, titratable acidity (TA), and water holding capacity (WHC) of yogurt made from magnetically treated skim milk throughout the 45 day storage period (means ± standard error).

Treatment	Firmness (g)	pH	Syneresis (%)	TA	WHC
C	128^{a}±2.70	4.31^{b}±0.03	3.99^{b}±0.20	0.963 ± 0.02	17.93 ± 0.39
N	119.0^{b}±3.34	4.34^{ab}±0.03	5.00^{a}±0.29	0.937 ± 0.01	17.16 ± 0.51
P	119^{b}±2.96	4.36^{a}±0.03	5.14^{a}±0.34	0.967 ± 0.02	17.54 ± 0.63

[a,b] Means (n=12) within a column with different superscripts differ ($P \leq 0.05$).

*C: control (no magnetic field) N: negative (negative magnetic field), and P: positive (positive magnetic field)

[1]Yogurt mixes include EFC-treated milk without applied magnets (control), EFC-treated milk in the negative direction at 30V for 10 min, and EFC-treated milk in the positive direction at 30V for 10 min

Table 3.3: Non-significant ($P \geq 0.05$) color (L*,a*, and b*) means (±standard error) (n=12)

Treatment	L*	a*	b*
C	91.72 ± 0.35	-2.12 ± 0.03	10.18 ± 0.40
N	90.85 ± 0.52	-2.591 ± 0.52	9.92 ± 0.04
P	91.23 ± 0.56	-2.639 ± 0.03	9.994 ± 0.54

C: control (no magnetic field) N: negative (negative magnetic field), and P: positive (positive magnetic field)

[1]Yogurt mixes include EFC-treated milk without applied magnets, EFC-treated milk in the negative direction at 30V for 10 min, and EFC-treated milk in the positive direction at 30V for 10 min

Table 3.4: Overall treatment averages of changes in firmness, pH, syneresis, titratable acidity (TA), and water holding capacity (WHC) of yogurt throughout 45 days of storage (means ± standard error).

Day	Firmness (g)	pH	Syneresis (%)	TA	WHC
1	$111.7^{c} \pm 3.17$	$4.48^{a} \pm 0.01$	$5.57^{a} \pm 0.38$	0.902 ± 0.01	16.55 ± 0.42
15	$119.0^{b} \pm 2.64$	$4.29^{b} \pm 0.02$	$4.73^{b} \pm 0.28$	0.971 ± 0.07	18.54 ± 0.78
30	$126.7^{ab} \pm 2.43$	$4.28^{b} \pm 0.02$	$4.42^{bc} \pm 0.27$	0.969 ± 0.01	17.67 ± 0.57
45	$129.2^{a} \pm 3.60$	$4.29^{b} \pm 0.01$	$4.13^{c} \pm 0.37$	0.980 ± 0.02	17.41 ± 0.41

[a-c] Means (n=9) from Control (no magnetic field), Negative (negative magnetic field direction), and Positive (positive magnetic field direction samples averaged over three replications over each storage day 1-45. Means within a column with different subscripts differ significantly ($P \leq 0.05$). Treatment averages include control, negative, and positive.

Table 3.5: Non-significant ($P \geq 0.05$) color (L*, a*, and b*) means (± standard error) (n = 9) of Control (no magnetic field), Negative (negative magnetic field direction) and Positive (positive magnetic field direction) throughout the storage period

Day	L*	a*	b*
1	90.71 ± 0.59	-2.072 ± 0.59	10.30 ± 0.09
15	91.46 ± 0.67	-2.593 ± 0.04	10.11 ± 0.18
30	90.92 ± 0.46	-2.584± 0.04	9.861 ± 0.14
45	91.98 ± 0.46	-2.55 ± 0.03	9.851 ± 0.16

Means (n=9) from Control (no magnetic field), Negative (negative magnetic field direction), and Positive (positive magnetic field direction samples averaged over three replications over each storage day 1-45. Means within a column with different subscripts differ significantly ($P \leq 0.05$). Treatment averages include control, negative, and positive.

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Chapter 4 - Investing the parameters of electromagnetic fluid conditioning to optimize the treatment effects on skim milk

Abstract

Electromagnetic fluid conditioning can be used as a processing innovation to chemically and physically alter skim milk components to create a modified product to be used in further processing applications such as yogurt manufacturing. In order for this to be achieved the processing parameters must be optimized. The parameters tested in this study include voltage applied, magnetic field direction, and treatment time. Parameters are not limited to these 3 other factors include flow rate, pump time, magnetic field type (permanent, oscillating, and electromagnetic), magnetic field proximity, and sample pH to name a few. This study tested 10 and 30 V as the field strength for treatment times of 2 and 10 minutes. Three treatments were distinguished~ Control (C) –received no magnetic field, Negative (N)- negative field direction, and Positive (P)- positive field direction. Once commercial skim milk samples were treated color properties (L*,a*, b*), titratable acidity, pH, viscosity, and surface tension were evaluated. Results proved to be insignificant ($P \geq 0.05$) when skim milk was treated at both 10 and 30 V for 2 minutes for all the variables. When skim milk was treated at 10 V for 10 minutes, results were significant ($P \leq 0.05$) for surface tension only. The N sample exhibited 1.89% greater surface tension when compared to the P treatment. Skim milk treated at 30 V for 10 minutes had significant statistical differences ($P \leq 0.05$) to L* and a* values indicating a overall shift in color change to the samples. For L* values the N sample was 1.18% greater when compared to the C. The P and C samples were statistically the same when compared to each other. N was also the statistically different sample for the variable of a*, where it was 11.10% greater when compared to the control. This data indicates

that there potentially could be an association between increased treatment times due to changes in casein micelle structure despite the voltage strength when optimizing the magnetic treatment.

Introduction

Electromagnetic Fluid Conditioning (EFC) is a novel processing technology being studied for its advantages in the food industry. To take full advantage of EFC, the treatment parameters must be optimal. The water treatment industry has used EFC and has reported changed ion solubility, reduced crystal formation, and inhibited scale buildup (Donaldson and Grimes, 1988; Benson and others, 2000; Amiri and Dadkhah, 2006; Holysz and others, 2002).

Among the parameter settings that yielded these results are flow rate, magnetic field strength, field proximity, and magnetic field direction (Donaldson and Grimes, 1988; Vick, 1991; Bogatin and others, 1999; Holysz and others, 2002; Chang and others, 2010).

Flow rate is one parameter the food industry must investigate and optimize to make EFC more effective (Vick, 1991). Donaldson and Grimes (1988) determined that increasing the flow rate in pipes with permanent magnets created turbulence that inhibited crystal formation. Similar studies concluded that increased flow rates along with magnetic treatment reduced the buildup of calcium carbonate crystals (Bogatin and others, 1995; Alimi and others, 2007; Patton, 2004).

Field strength and proximity of the magnetic field are also important in optimizing EFC treatment. Chang and others (2010) used computer simulations to show that almost direct contact with the magnets yielded disrupted fluid properties. Amiri and Dadkhah (2006) reported that surface tension of calcium carbonate solutions increased as the magnetic field increased. Surface tension is a good measure of the effects of EFC; surface tension is related

to the number of particles on the fluid surface. However, Cho and Lee (2005) reported that magnetic treatment reduced the surface tension of water by 8% indicating that the effects of magnets on fluids is still not fully understood

Color properties (L*,a*,b*) are related to particle aggregation, which makes them a good indicator of the results of magnetic treatment (Needs and others, 2000). Needs and others (2000) reported that L* values increase with more particle aggregation in fluid milk and yogurt. Viscosity can indicate the level of particle aggregation with increases in viscosity showing more particle aggregation. Several studies have shown that magnetic treatment affects particle aggregation and thus increases fluid viscosity (Odenbach, 2003; Enomoto and others, 2003; Beglarian and Grigorian, 1990; Cai and others, 2009).

If magnetic treatments can alter ion properties in water, the same should be true for milk (Vick, 1991; Benson and others, 2000). Milk's composition contains calcium and phosphate ions, both of which could be altered by EFC (Patton, 2004; Farrell and others, 2006). Yoon and Lund (1994) showed that magnetically treated milk had 36% lower mineral content, while protein remained unaltered.

Given the findings in Chapter 1, further evaluations of the processing variables for EFC were required. These variables include treatment time, magnetic direction, and magnetic strength (voltages applied). In this study, two different voltages (10 and 30V) and two different processing times (2 and 10 minutes) were studied to determine the most effective electromagnetic treatment conditions for EFC in testing how EFC changes the properties of skim milk.

Materials and Methods

Fat-free skim milk fortified with vitamins A & D and 1% non-fat dry milk solids was obtained from Kansas State University Dairy Processing Plant (Manhattan, KS, USA). All equipment was obtained from Fisher Scientific (Pittsburgh, PA, USA), unless otherwise noted. All measurements were performed in duplicate with three replications with all measurements occurring within 30 minutes. Skim milk samples were analyzed for buffer capacity, titratable acidity (TA), color, viscosity, pH, and surface tension.

Electromagnetic Fluid Conditioning

Skim milk was treated at 10 and 30V, for 2 minutes and 10 minutes using a centrifugal pump with a flow rate of 2L/min. Magnetic direction was also altered in this study for each parameter tested, with a Control (C; no magnetic treatment), Positive direction (P), and Negative direction (N).

Color values

Color was measured using a colorimeter (Hunter Lab 4500L, Reston, VA, USA) to obtain L*, a*, and b* values following the method of Needs and others (2000). Samples were placed in a 120 mL plastic sterile cup (Fisher Scientific), and readings were taken 4 times at every 90° angle on the filled 120 mL plastic sterile cups. The colorimeter was calibrated before each use following the manufacturer's instructions and the black and white tiles provided (X= 80.94, Y=85.30 and Z= 88.35).

pH

pH readings were recorded with an AP63 Accumet® (Fischer Scientific) pH meter before and after EFC treatment on 50 mL of sample placed in a beaker. The pH meter was standardized using pH 4 and 7 purchased standard buffers (Fisher Scientific) at 25°C.

Surface Tension

Before analysis, 50 mL beakers were washed with soap (Dawn, Cincinatti, OH, USA) and water, then allowed to soak overnight in 6N nitric acid (Fisher Scientific), then rinsed with deionized water, and air dried. The tensionmeter was zeroed with the ring attached to the arm by aligning the vernier with 0 on the dial. Beakers were then filled with 20 mL of sample and placed on the sampling stage. Samples were allowed to rest for 20 minutes to establish a surface at 25 ± 1°C. Readings were then taken and recorded in dynes/cm following the method of Adapa and others (1997). Using the following correction factor, the actual surface tension was determined:

$$F = 0.7250 + \sqrt{\frac{0.01452P}{C^2(D-d)}} + 0.04534 - \frac{1.679r}{R}$$

where

F is correction factor

P is visual reading

C is ring circumference (provided on ring box)

D is density of lower phase (sample)

d is density of upper phase (air), assumed d(air)=0g/mL

R is radius of ring

r is radius of ring wire

R/r= Surface tension should be reported as P X F with a unit of measurement dynes/cm.

Titratable Acidity

Titratable acidity (TA) of the milk treatment was done by titrating with 0.1 N NaOH using phenolphthalein as an indicator (Hooi and others, 2004). Samples of 10 mL were

placed in a beaker then titrated to a light pink. Titratable acidity was calculated using the following formula:

$$\frac{9\ x\ 0.1\ NaOH\ x\ ml\ of\ titrant\ used}{wt\ of\ sample}$$

Viscosity

Viscosity readings were taken with a rheometer outfitted with cup and bob (ATS Rheosystem, Bordentown, NJ, USA) using a constant strain test of 1% and shear from 1 to 100 s^{-1} at 25°C (Pollen and others, 2005) with apparent viscosity taken as an average over 10-100s^{-1} shear rate.

Statistical Design

A randomized complete block design was used in these studies. Each voltage and time was treated as a separate experiments. Data generated was analyzed using PROC GLM in SAS (SAS Institute Inc., v 9.2, Cary, NC, USA) as one-way analysis of variance. Least square means was used to determine significant differences at a P value of (≤0.05). If significant differences were found, Bonferroni (Kuehl, 2000) mean adjustment was then used to differentiate between significant means.

Results

When the skim milk was treated at 10V for 2 min, as Table 4.1 shows, the three treatments did not differ statistically (P≤0.05). Table 4.2 shows that when skim milk was treated at 30V, again no statistical differences occurred among the three treatments. This would indicate that treating skim milk at 10 and 30V for 2 minutes did not alter the chemical and physical properties.

The surface tension values, however, did differ statistically ($P \leq 0.05$) when skim milk was treated at 10V for 10 minutes as seen in Table 4.3. The N sample was statistically different from the P milk with a 1.89% greater surface tension. Control was statistically similar to both the N and P samples. None of the other characteristics at these EFC settings were significant ($P \leq 0.05$). Treatments at 30V for 10 minutes also showed differences among the three treatments for L* and a*. The N treatment was statistically different from both the C and P treatments for both L* and a* as seen in Table 4.4. The N treatment showed an L* 1.14% higher and a* 10.89% higher than the C and P treatments. No other statistical differences occurred among the variables.

Discussion

EFC still has many challenges to address before it can be used to magnetically treat milk on an industrial scale to increase the quality of dairy products. Among the questions to answer involves the type of magnet: this experiment used electromagnetic fields, where electricity is applied and the electrons create a magnetic field (Gray, 2014). EFC, as used in this experiment, averages about 2000G whereas permanent magnets, another alternative, can average 6000G, a magnetic field 3x greater.

Research has shown that the magnetic field strength can be a major factor when trying to maximize a fluid's magnetic potential (Zhukov, 2011; Amiri and Dadkhah, 2006). Using a different DC voltage meter to apply more than 30V could strengthen the magnetic field. Another contributing factor, which could explain how few variables were affected by EFC, is the length of the magnetic field (Ciobanu and others, 2011). In this experiment, EFC had 3 magnetic zones within the 0.61m chamber, with each magnet only about 0.3m long. Previous research has shown that longer magnetic fields can better magnetize the fluid (Ciobanu and

others, 2011). In this research, only the 10-minute treatment at both 10 and 30V caused changes in the fluid, possibly because the fluid was exposed to these magnetic zones for more time.

Alimi and others (2007) showed that increased treatment time had a significant effect, changing the solubility of calcium carbonate when resonance time was doubled. In other studies, the flow rate of the magnetic system affected the fluid's ability to magnetize (Vick, 1991; Donaldson and Grimes, 1988; Bogatin and others, 1995), although Vick (1991) concluded that reducing turbulence in the flow had the most impact on optimizing the magnetic treatment. The flow rate for this treatment was 7.6 L/min. Other studies have shown that increasing the flow rate is vital in optimizing the magnetic treatment (Alimi and others, 2007).

When treating skim milk at 10V for 10 min, surface tension seemed to be the only variable affected. The increased surface tension values in the N treatment indicate less particle aggregation along the surface, whereas the P treatment decreased the milks surface tension. Previous research has shown similar conflicting results, where magnetic treatment both increases and decreases the surface tension (Amiri and Dadkhah, 2006; Fujimura and Ino, 2008; Zhukov, 2011) although the results seem to depend on the strength of the magnetic field. If surface tension is altered, viscosity should also change because of changes in particle aggregation on the surface of milk, as well as color properties (Needs and others, 2000). Skim milk treated at 30V for 10 min exhibited changes only to the L* and a* values. The N treatment seemed to cause the largest change. In prior studies, magnetic field direction made a difference in optimizing fluid magnetic potential. Because changes to color values can indicate changes to the casein micelle structure and particle scattering, increases to the L*

values would indicate micelles are rearranging, whereas in this study color properties did not change indicating EFC did not effect the stability of the casein micelle. (Needs and others, 2000).

Conclusion

Given the benefits of magnetic treatment shown in water treatment to reduce sludge and where changes to the ions and minerals reduce scale buildup on pipes (Vick, 1991; Amiri and Dadkhah, 2006; Holysz and others, 2002), skim milk should also respond to this treatment. Magnetic treatment may also be changing ions and minerals, changes not captured by our methods. More in depth research would help in optimizing EFC treatment parameters to fully understand the chemical and physical properties altered by magnetic treatment.

Table 4.1: Chemical and physical properties of EFC-treated skim milk at 10V for 2 min in negative (N) or positive (P) direction compared with a skim milk control (C) run at 0V for 2 min.

	C	N	P	P-Value
L*	88.52 ± 0.28	88.79 ± 0.07	88.42 ± 0.32	0.481
a*	-3.87 ± 0.07	-3.863 ± 0.02	-4.012 ± 0.07	0.157
b*	7.790 ± 0.12	7.827 ± 0.06	7.177 ± 0.33	0.127
pH	6.653 ± 0.01	6.643 ± 0.01	6.667 ± 0.02	0.313
ST (dyne/cm)	47.38 ± 0.21	48.02 ± 0.16	46.57 ± 0.54	0.08
TA (%)	0.210 ± 0.01	0.223 ± 0.01	0.227 ± 0.01	0.049
Vis (mPa·s)	1.230 ± 0.01	1.273 ± 0.02	1.277 ± 0.01	0.156

Means (lsmeans ± standard error), n = 3, of skim milk treated using EFC at 10 V for 2 min for the negative, positive, and control magnetic directions. ST- surface tension; TA- titratable acidity; Vis- apparent viscosity at an average of 10-100 sec^{-1}

Table 4.2: Chemical and physical properties of EFC-treated skim milk at 30V for 2 min in negative (N) or positive (P) direction compared with a skim milk control (C) run at 30V for 2 min.

	C	N	P	P-Value
L*	87.24 ± 0.22	87.31 ± 0.33	87.88 ± 0.18	0.122
a*	-4.086 ± 0.09	-3.813 ± 0.15	-3.793 ± 0.15	0.193
b*	7.490 ± 0.17	6.777 ± 0.17	7.250 ± 0.17	0.524
pH	6.67 ± 0.01	6.643 ± 0.01	6.667 ± 0.01	0.224
ST (dyne/cm)	47.46 ± 0.29	48.02 ± 0.52	46.57 ± 0.29	0.375
TA (%)	0.213 ± 0.01	0.220 ± 0.01	0.220 ± 0.00	0.538
Vis (mPa·s)	1.330 ± 0.04	1.300 ± 0.05	1.363 ± 0.01	0.548

Means (lsmeans ± standard error), n = 3, of skim milk treated using EFC at 30 V for 2 min for the negative, positive, and control magnetic directions. ST- surface tension; TA- titratable acidity; Vis- apparent viscosity at an average of 10-100 sec^{-1}

Table 4.3: Chemical and physical properties of EFC-treated skim milk at 10V for 10 min in negative (N) or positive (P) direction compared with a skim milk control (C) run at 0V for 10 min (mean ± standard error) n = 3.

	C	N	P	P-Value
L*	88.20 ± 0.28	88.44 ± 0.25	88.32 ± 0.14	0.881
a*	-4.143 ± 0.10	-4.157 ± 0.11	-4.227 ± 0.01	0.878
b*	6.123 ± 0.15	6.437 ± 0.19	6.527 ± 0.01	0.163
pH	6.703 ± 0.01	6.700 ± 0.01	6.673 ± 0.01	0.311
ST (dyne/cm)	46.55^{ab} ± 0.29	47.28^{a} ± 0.35	46.39^{b} ± 0.24	0.02
TA (%)	0.227 ± 0.01	0.233 ± 0.01	0.240 ± 0.00	0.124
Vis (mPa·s)	1.310 ± 0.03	1.390 ± 0.01	1.380 ± 0.01	0.096

Means (lsmeans ± standard error), n = 3, of skim milk treated using EFC at 10 V for 10 min for the negative, positive, and control magnetic directions. ST- surface tension; TA- titratable acidity; Vis- apparent viscosity at an average of 10-100 sec^{-1}

[a,b] Means with different superscripts differ within a row ($P<0.05$)

Table 4.4: Chemical and physical properties of EFC-treated skim milk at 30V for 10 min in negative (N) or positive (P) direction compared with a skim milk control (C) run at 0V for 10 min (mean ± standard error) n = 3.

	C	N	P	P-Value
L*	88.20b ± 028	89.26a ± 0.16	88.28b ± 0.21	0.001
a*	-4.143b ± 0.10	-3.683a ± 0.09	-4.123b ± 0.02	0.016
b*	6.123 ± 0.15	5.913 ± 0.24	6.460 ± 0.01	0.312
pH	6.703 ± 0.01	6.716 ± 0.01	6.690 ± 0.01	0.51
ST (dyne/cm)	46.55 ± 0.29	46.96 ± 0.23	46.96 ± 0.60	0.757
TA (%)	0.227 ± 0.01	0.230 ± 0.01	0.220 ± 0.01	0.502
Vis (mPa·s)	1.310±0.03	1.357±0.01	1.347±0.01	0.256

Means (lsmeans ± standard error), n = 3, of skim milk treated using EFC at 30 V for 10 min for the negative, positive, and control magnetic directions. ST- surface tension; TA- titratable acidity; Vis- apparent viscosity at an average of 10-100 sec^{-1}

[a,b] Means with different superscripts differ within a row ($P<0.05$)

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Chapter 5 - Using Dielectric Spectroscopy as a Predicative Model for Determining Yogurt Quality

Abstract

Dielectric spectroscopy was used to determine if dielectric data points (ε' and ε") could be used as variables to predict yogurts firmness. Twenty-five yogurt samples were formulated for increasing sugar content, 0-5%, labeled A-E and increasing protein content, 3.5-10%, labeled 0-4. Proximate analyses were done to determine the true protein content, moisture, total solids, fat, and ash contents of the 25 yogurt samples. Carbohydrate contents were calculated by difference. Firmness measurements conducted by traditional method were compared to the predicted values once models were generated. Using ε' and ε", backwards regression identified which frequencies of the 4 selected (219, 917, 2471, and 6000 MHz) were significant in the firmness predictive model. This model had a R^2 value of 0.60, which is below the "good" category. Predictive models were also generated for protein and moisture content that had much higher correlations with R^2 values of 0.86 and 0.83, respectively. Yogurts (A0, A4, E0, E4, and C2) of the original data set as well as 2 store-bought samples were evaluated to check the goodness-of-fit of the model. Using the 7 samples measured values were compared to the predicted values, the differences ranged from 1.34 to 55.75 for firmness whereas protein content varied ranged from 2.23 to 155.31%. Moisture contents comparing the measured with the predicted, ranged from 1.44 to 18.85%. These data show that there could be potential to use dielectric spectroscopy as a predictive tool, but further research would be needed to determine if ε' and ε" values could accurately predict the product attribute firmness.

Introduction

Dielectric spectroscopy serves as a broad tool to test multiple quality factors of a product, with moisture content and soluble solids being two main factors (Nelson 2005; Castro-Giraldez and others 2010). When measuring dielectric properties, the dielectric constant (ε'), (energy storage potential of the product), and the dielectric loss factor (ε"), (an imaginary coefficient accounting for losses of dipolar relaxation and ionic conduction which represent heat losses), account for the intrinsic properties within the food product (Nelson 2005; Guo and others 2010[ab]). Relative permittivity is represented by e*, and the factors are related by the complex permittivity as noted by the equation $\varepsilon^*=\varepsilon'-j\varepsilon''$, where j is the square root of -1 (Guo and others 2010[ab]). According to the theory of dielectric spectroscopy, foods with various protein and sugar contents impact the ε' and ε" values due to their dipole interactions and ionic concentrations (Nunes and others 2006).

Yogurt quality is defined by many different parameters that include formulations processing conditions (Ares and others 2007). Yogurt firmness is one of the main texture attributes that can alter consumer's perception of yogurt (Ares and others 2007; Kroger 1975; Vedamuthu 1992). Most firmness measurements are done 24 hours after fermentation and reflect the strength of the casein-whey protein gel matrix (Tunick 2000).

Dielectric spectroscopy is not new to the dairy industry. Bohigas and others (2008) have used dielectric spectroscopy to predict sugar content in yogurt, where store bought samples of natural and added sweetener both formulated to contain 9.2% sugar and yogurt manufactured to contain 0 to 15% sugar were used in this experiment. Statistically, differences were observed due to the sugar contents where researchers concluded dielectric spectroscopy could detect sugar differences in the formulations. Discrepancies of less than 5% were observed in this study when comparing measured sugar contents with predicted sugar

contents. These researchers reported that the components of yogurt can influence dielectric readings, especially protein and carbohydrate contents, which gives rise to the question could predictive models be developed as yogurt firmness is dependent on protein content among other factors (Bohigas and others 2008; Damin and others 2009; Salvador and Fiszman 2004). Guo and others (2010[ab]), reported that dielectric spectroscopy can be a rapid tool to determine the water and sugar contents of honey products as well as water content in adulterated milk. Further, dielectric spectroscopy has been used in the dairy industry to determine (water addition, fat content, and temperature effects) of cheese and milk (Guo and others 2010[b]; Nunes and others 2006; Everard and others 2006).

Kudra and others (1992) used electromagnetic readings only at 2450 MHz to build a predictive model for protein content in adulterated milk. This study was conducted due to the researchers prior knowledge that proteins and ions coexist in milk; thusly interacting with each other. Researchers assumed that this would alter the electromagnetic potential of milk by altering dielectric spectroscopy.

Currently, the use of dielectric spectroscopy to predict yogurt quality at the manufacturing level is a novel approach. This work sets out to determine if dielectric spectroscopy can be used as a tool to predict yogurt quality. If successful this analytical test can be used by yogurt manufacturers to ease the quality testing procedures of yogurt, and serve as a predictive tool in the product development process.

Materials and Methods

Yogurt formulation and manufacturing

Skim milk (Kansas State University Dairy Processing Plant, Manhattan, KS, USA) was used as the milk basis for yogurt batches. Twenty-five samples were formulated targeting

increasing protein (3.5-10%) and sugar (0-5%) contents as seen in Table 5.1. As seen in Table 5.1, these are the targeted percentages of protein and sugar in the formulations whereas Table 5.2 contains the actual formulations weight (g). The letters are presented in alphabetical order for increasing sugar content, whereas as the numbers increase the protein content increases. Low-heat nonfat dry milk (NFDM) (Dairy America, Fresno, CA, USA) or MPC 9081 (Kerry Ingredients, Beliot, WI, USA) was used to increase protein contents in these formulations. Granulated sugar (Midwest Country Fare, HyVee, West DeMoines, IA, USA) was the source of sucrose. Yogurt samples were prepared by making the 0 and 4's at each letter, inoculated, and then mixed to make the 1, 2, and 3 samples of each letter. Samples were made with a ratio of 0 to 4 as followed: 1) 75/25, 2) 50/50, and 3) 25/75.

Twenty five hundred mL samples of 0 and 4 mixes were heated to 90°C on a stir plate (Isotemp, Fisher Scientific, Pittsburgh, PA, USA) and then held for 10 minutes in a 90°C water bath (Isotemp 220, Fisher Scientific) using the set-style method as described by Biliaderis and others (1992). After heating, the milk cooled to 43°C in a water bath (Rubbermaid, Wooster, OH, USA), then inoculated with starter culture at a usage rate of (0.014 g per 500 ml) (YC 495-250, DuPont, New Century, KS, USA). Once inoculated, samples were mixed at the ratios to affect protein and sugar content, then placed in 120 mL sterile sample cups (Fisher Scientific) to incubate at 43°C ± 1°C (Isotemp, Fisher Scientific), then stored at 4°C± 1°C for 12 hours (Kenmore, Chicago, IL, USA) Fermentation occurred until a pH of 4.6 was reached.

Validation samples were also prepared using the same formulation provided in Table 5.1 for A0, A4, C2, E0, and E4 to test the applicability of the model generated from dielectric data. Validation was also preformed on two store bought samples to test the applicability of

the model (Fage® total 0% (Fage USA Dairy Industry, Johnstown, NY, USA) (Sample 1) and Kroger blended plain grade A lowfat yogurt (Kroger Company, Cincinatti, OH, USA) (Sample 2), Dillion's Grocery, Manhattan, KS, USA). These 2 samples were chosen based on their protein and sugar contents where Sample 1 was a high protein yogurt and Sample 2 was a low protein high sugar content yogurt.

Measurement of dielectric properties

Broadband dielectric properties of the samples were made using a high-temperature dielectric probe, similar to the technique described in Nunes and others (2006). Using a HP 8753D vector network analyzer (VNA), which generated the data at each frequency and HP 85070A dielectric probe kit (Agilent Technologies, Santa Clara, CA, USA), dielectric measurements were taken the day after yogurt manufacturing. Calibration of the equipment prior to use was conducted with air, metal shorting block, and 25°C deionized water with instruction's provided by manufacturer.

Readings were taken on 2 different yogurt cups and at 2 location sites within each cup for a total of 4 readings per sample. Sample cups were placed on an adjustable stage and raised to the probe for a flush contact surface between the probe and sample, as seen in Figure 5. 1. The probe was inserted to a constant depth ~2 mm beyond the sample surface prior to each measurement. The readings spanned a frequency range of 100 MHz – 6 GHz with 101 intervals with readings conducted at 4°C. Dielectric readings were also taken during fermentation at 43°C every hour on the A0 and A4 samples. Readings were performed in duplicate, averaging 5 seconds per reading.

Penetration depth

Once ε' and ε" were obtained, penetration depth was calculated using the obtained ε' and ε" values to determine the power entering the surface using the equation described by Guo and others (2010[a]):

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon' \left[\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2}-1\right]}}$$

In the equation c represents the speed of light in the free space (3×10^8 m/s) and f is the frequency. The penetration depth was calculated, using Excel (Microsoft Office, Redmond, WA, USA) as a function of frequency of the 25 yogurt samples at varying protein and sugar concentrations.

pH

pH was monitored at 43°C every hour during fermentation, and on day 1 of storage (Lee and Lucey, 2004). Calibration of the pH meter (Accumet®, AP63, Fisher Scientific) was performed prior to use using pH 4 and 7 standard buffer solutions (Fisher Scientific).

Proximate Analysis

Protein contents of the yogurt samples were determined by finding the total nitrogen using a Kjeltec Analyzer 8400 (Eurofins DQCI LLC, Mounds View, MN) (Foss Analytical, Hilleroed, DK) and the nitrogen conversion factor of 6.38 was used to calculate protein (AOAC Int'l 991.20, Gaithersburg, Maryland, USA). Non-protein nitrogen was also determined (AOAC Int'l 991.21) so that true protein was found by difference (AOAC Int'l 991.23).

Using the standardized method of Hooi and others (2004) total solids were determined on the yogurt samples. Samples were dried in a 103°C forced air oven (Isotemp, Fisher Scientific) for 24 hours. Total solids were calculated as

$$\frac{After\ weight - crucible\ weight}{sample\ weight} x\ 100$$

Ash content was determined using the standard method of Hooi and others (2004) where liquid samples were ashed in a muffle furnace (Thermolyne, ThermoScientific, Waltham, MA, USA) at 550°C for 5 hours. Fat was determined by microwave drying followed by nuclear magnetic resonance using the method described by Keeton and others (2003) (CEM Corporation, Matthews, NC, USA). Carbohydrates were calculated by difference (FAO, 2003).

$$\%\ Carbohydrate = Total\ solids - (True\ Protein + Fat + Ash + Non\ Protein\ Nitrogen)$$

Firmness

A texture analyzer (Stable Micro System, Model TZ-XT2, Texture Expert, Surrey, UK) was used to determine firmness of the yogurt on day 1 following a modified method of Magenis and others (2006). Sample containers and sizes were altered from the original method where yogurt was fermented in 120 mL containers (Fisher Scientific) containing 80 mL of inoculated yogurt. Firmness, which corresponds to the initial penetration force of the probe during the first compression of analysis, was obtained by macro analysis using the manufacturer software provided (Stable Micro System). Calibration was performed using a 500 g weight with the analyzer fitted with a 25 mm acrylic probe. Testing parameters were set at velocity = 2.0 mm s^{-1}, time = 5.0 s, and distance = 5.0 mm. Analysis was done in

duplicate on 2 different sample cups and averaged, with readings taking approximately 45 seconds.

Statistical Design

Yogurts were analyzed randomly during this experiment for all analysis. Repeated measures were taken on samples and averaged. Principal Component Analysis (PCA) was conducted using SAS® (version 9.3; SAS® Institute, Cary, NC, USA) to group products on the 2-D space to determine correlations based on product attributes and composition (protein, moisture, firmness, and frequencies).

Proc Mixed (SAS®) was used to determine if protein and sugar contents altered firmness values. Contrasts (Kuehl, 2000) were used to correlate if the 5 varying protein levels were significant to firmness at those levels.

External product mapping, using backwards regression (SAS®) was performed using 4 frequencies (319, 917, 2417.8, 6000 MHz) for ε' and ε", protein, moisture, and firmness to evaluate the relationships between the predictability of dielectric properties on final product firmness. Frequencies selected were based on previous work from Guo and others 2010[ab], where researchers picked their start and end frequencies and 917 and 2450 MHz, due to these frequencies being the commonly used microwave region (Ahmed and Luciano 2009). Kudra and others (1992) described milk characteristics using dielectrics and the frequencies of 2450 MHz.

Finally, the 4 corners plus the middle sample of the experimental design along with 2 store bought samplers were tested to see the applicability of the predictive models to determine if dielectric spectroscopy can be used as an alternative to traditional firmness measurements. When validating the sample, percent variability (mean absolute percent error)

was also determined by taking the absolute value of the difference between the measured and the predicted divided by the measured value. This is to determine the percent difference between samples.

Results and Discussion

Fermentation Study

ε' and ε" throughout fermentation

First, A0 and A4 samples were used to determine if dielectric readings varied during fermentation. Fermentation time varied by protein level where the greater the protein content (A4 10%), required an additional hour (6 vs. 5 hours) to complete fermentation, vs. the lower protein sample (A0 3.5%). Damin and others (2009) reported opposite findings where increased protein contents 0.25-1% decreased fermentation times if adding sodium caseinate, but in the same study increasing whey protein concentrate from 3g to 6g/100 fermentation times increased. Varying fermentation times could be due to the protein sources used where Damin and others (2009), used sodium caseinate and whey protein concentrate, whereas this study used NFDM and MPC which contained a blend of casein and whey proteins.

Results for ε' and ε" during fermentation can be seen in Table 5.3 for samples A0 and A4. As pH decreased throughout fermentation, ε' generally decreased for both samples. As frequency increased from 319 to 6000 MHz, ε' also decreased. The greater protein sample (A4) generally had lower ε' values when compared to the A0 sample, whereas when comparing ε" samples the A4's had greater values compared to A0. As the pH decreased (6.4 to 4.6), ε" values increased, whereas as frequency increased (319 to 6000) ,ε" values decreased.

Penetration depth throughout fermentation

The penetration depth was calculated throughout fermentation time as seen in Figure 5.2, for sample A0, the general trend is that as pH drops (6.4 to 4.6), penetration depth decreased during hours 1-4 except at hour 5, where an increase was observed. Greater protein contents could affect the penetration depth as seen in Figure 5.3, where A4 (10%) samples did not follow the same general decreasing pattern of A0 (3.2%). This could infer that if samples were heated in a microwave, proteins could interfere with uniformity of heating since penetration depth is a measurement of the product's microwave heating profile and microwaves power able to enter the products surface (Guo and others 2010). Hours 2 and 4 had the greatest penetration depths, when compared with hours 3, 5, and 6. Hassan and others (1995) concluded that acidified milk began to gel at pH 5.35 and continued until pH 5 with drastic structural changes to the casein micelle and the liquid system, however changes are less drastic around pH 4.4. The changes in penetration depth were observed between hours 2-4, when the mixes pHs were around 5.35-5.0. Penetration depth is dependent on the free water available; hours 2 and 4 could have had more available free water since the casein micelle gel structure was not formed after 2 hours of fermentation. A previous study by Kudra and others (1992) also indicated that proteins have a higher likelihood then lactose to alter the penetration depth, due to proteins ability to bind with the free water.

Yogurt Study

Proximate Analysis

Results for the proximate analysis are seen in Table 5.4 for the 25 Yogurt Samples. As expected as the numerical code increased, total solids, protein content, and ash, increased. Whereas when the alphabetical code progressed, carbohydrates increased sort of.

Carbohydrate content accounts for all carbohydrates sources and in these samples. Fat was consistent throughout the samples due to skim milk's fat composition. Samples were formulated to contain 3.5, 5.13, 6.75, 8.38, and 10% protein as sample numbers increased from 0-4. As samples progressed from A-E sugar was to remain constant at each letter grouping but increase from 0, 1.25, 2.5, 3.75, and 5% of the formulation per letter grouping. Levels of protein and sugar varied from the targeted numbers generated in the experimental design. Protein followed the targeted values fairly close, where one outlier (sample E3) was reported to have 6.19% protein, and should have been closer to 8.38%. Carbohydrates did not follow any consistent trends where values were not close to the targeted formulations due to a calculation only describing all carbohydrates present in the sample, not just sugar content.

Firmness

Yogurt firmness was measured via the traditional texture analyzer following the method of Magenis and others (2006). When analyzing for a relationship between protein and sugar with firmness, the targeted protein level was significant in this experiment (P value <0.001), whereas targeted sugar content was not (P-value = 0.3398) as seen in Table E.5.1. The average firmness results from the 25 yogurt samples can be seen in Table 5.5. Contrasts determined that the targeted protein levels with measured firmness values were also significant ($P \leq 0.05$) at all 5 levels as seen in Table E.5.2 and E.5.3. In this study, measured firmness increased as protein content increased until protein reached the sample 3's then generally decreased as seen in Figure 5.4. As samples increased in protein A0-A3, firmness increased 47.17% and then decreased 28.44% from A3-A4. In the B samples, B0-B3 firmness increased 39.21%, and then decreased 0.73% from B3-B4. As protein increased in the C samples, firmness increased in C0-C4, 48.62%. The D samples, also exhibited increased

firmness from D0 to D3 where a 59.69% increase was observed, but then a decrease of 18.36% for D3 to D4. As protein increased in the E samples, E0-E4 firmness increased 47.97%. Other studies have reported that increasing protein contents increase firmness values. Damin and others (2009) reported that as sodium caseinate increased from 0.25%-1.00%, firmness increased 62.5%, due to stronger inter-locking bonds formed between the gel networks. Whereas Salvador and Fiszman (2004), reported that an increase in protein content from 3.5 to 4.4g per 100g of yogurt, firmness values increased 24.65% indicating a stronger gel network, more deformable as the probe penetrates the sample. The current study used greater protein contents in the formulations 3.5-10%.

ε' and ε" for yogurt samples

ε' and ε" for the original 25 yogurt samples can be found in Table 5.6 at the 4 selected frequencies (319, 917, 2471, and 6000 MHz). General trends for ε' are as protein and sugar content increase, (moisture decreased), ε' values generally decrease. Values also decreased as frequency increases. Figure 5.5 also shows generally how as protein increases ε' decreased linearly for the E samples whereas the ε" values had a hook shape as seen in Figure 5.6. Guo and others 2010[a], reported that as milk concentrations increased from 70-100%, ε' values generally decreased as well.

Penetration Depth

Penetration depth decreased as protein content increased for all the samples within a letter grouping (A, B, C, D, and E) as seen in Table 5.7. Penetration depth also decreased as frequency increased. Similar findings by Guo and others (2010[a]) have been made that penetration depth was greater in the 70% and 100% diluted milk samples compared with raw milk, and as frequency increased penetration depth increased.

Principal Component Analysis (PCA)

A PCA data set with 25 yogurts and 7 variables (firmness, protein, moisture, and the 4 frequencies: 319, 917, 2471, and 6000 MHz) was conducted separately for ε' and ε". Results for ε', showed that PCA accounted for 96.5% of the total variations for ε' in 2 principal components (PC) PC1 (89.25% variation) and PC2 (7.25% variation). The 4 frequencies 319, 917, 2471, and 6000 MHz were highly correlated with moisture as indicated by their close grouped location on the PCA plot (Figure 5.7). Measured firmness and protein content did not correspond to as many samples but were highly correlated to each other. From observing the data plots it seemed most 3 & 4's were the least correlated to the predictor variables (moisture and frequencies 319, 917, 2741, 6000 MHz) but more correlated to protein and firmness. Samples coded with the 0, 1, and 2's were closer to the predictor variables moisture and the four frequencies. Conclusions from this PCA can not fully indicate if protein is a reliable predictor variable for yogurt characteristics (firmness), whereas moisture content more correlated to the samples.

PCA data for ε" was less correlated on the plots with total variation explained being only 78.13% with PC1 explaining 41.01% and PC2 explaining 37.12%. In this PCA plot, samples were more spread out on the chart as seen in Figure 5.8. The 4 frequencies were dispersed throughout 3 quadrants whereas in ε' they were all in the 1st quadrant. Protein was highly correlated to firmness in this analysis as well. Moisture was the predictive variable furthest away from the tested samples. In this analysis, the high protein content samples 3's and 4's were more correlated to the predictive variables compared with ε', it was the low protein, 1's and 2's, content samples that were more correlated.

Predictive Models

Predictive models were developed for firmness using yogurts protein and moisture contents, ε', ε", and the 4 frequencies (319, 917, 2471.8, and 6000 MHz). Dielectric readings can be almost instantaneous whereas traditional firmness measurements take 45 seconds. Sample predictive models containing ε' and ε" were also developed for protein and moisture contents of the yogurt samples.

Moisture content was found to be the best predictable variable using dielectric spectroscopy in this study, which supported previous studies (Guo and others 2010[ab]; Nunes and others 2006; Ahmed and Luciana, 2009; Clerjon and others 2003).

Considering the 3 predictive models, the coefficient variables for the firmness can be found in Table 5.8. The only variables significant ($P \leq 0.05$) were at ε'319 and ε'917 with a R^2 value of 0.60. The final model is

$$Firmess = -327.66 + 113.53(\varepsilon'319) - 111.50(\varepsilon'917)$$

The significant variables using ε' and ε" to predict protein can be found in Table 5.9. In this model all 4 frequencies, ε', were significant with a R^2 value of 0.86. The final model is

$$Protein = -44.75 + 8.61\ (\varepsilon'319)\ - 10.36(\varepsilon'917) + 2.88(\varepsilon'2417.8)\ - 0.8127(\varepsilon'6000)$$

When ε' and ε" are used to predict moisture content the significant variables can be found in Table 5.10. This model contains only one variable ε' 917 with a R^2 value of 0.83.

Overall, moisture was the best-predicted attribute of yogurt, where previous studies support this finding as and others (Guo and others 2009[b]) reported that dielectric spectroscopy could be an effective method to predict moisture content in adulterated honey. The final model is

$$Moisture = -18.75 + 1.42(\varepsilon'917)$$

Verifying Firmness and Protein

Once the predicted models were compiled new samples were made and the store bought samples were purchased, dielectric spectroscopy was used on new yogurt samples were made (A0, A4, E0, and E4) and middle (C2) sample of the data set as well as on 2 store-bought samples Sample 1 and Sample 2 (Fage Total 0% yogurt (Sample 1) and Kroger Brand Blended grade A low-fat plain yogurt (Sample 2), Dillion's Grocery Store, Manhattan, KS, USA) to test the reliability of the model.

Results obtained from the firmness model varied from the real firmness values obtain from texture analysis by 1.34-55.75%, see Table 5.11. The percent variability shows the difference between the predicted and measured values. In Figure 5.9 the variation can be seen as the 95% confidence interval. At higher proteins (A4, E4) the variability was much closer indicating the model was better at predicting. Sample 1 was the next least variable sample with a measured protein content of 10.88, as seen in Table 5.12. When the protein was closer to 3% the variability was much greater (53.31-155%) as seen in Sample A0 (3.43% protein), and C2 (3.39% protein), with measured values being roughly 50% under predicted. Since protein was determined to be a significant variable in the data set when compared with sugar the prediction model was also used to determine if dielectric spectroscopy could be used to predict other quality attributes of yogurt.

In Table 5.12, it shows the predicted protein content versus the real protein level of the samples. Protein seems to be the most difficult attribute to predict based on these findings where variability ranged from 2-155% variation. The moisture predictive model had the best fit with the measured values being the closest to the predictive values. However, it was much more accurate when predicting the 4 corners and middle sample of the validation set than it was the store bought samples as seen in Table 5.13, with samples ranging from 1.4-18.85%

different although the store bought samples fit within the parameters of the design for protein and sugar contents. The percent variability, which is the variation between the predicted and real values, was much less than in the previous two models. Bohigas and others (2008) were capable of producing a model derived from dielectric spectroscopy to predict sugar content in yogurt if yogurts contained 0 to 15% sugar. They were able to keep all other variables the same (such as protein), therefore their models were successful and sensitive enough (less than 5% variation) to predict sugar contents of store-bought yogurt samples, due to sugar changes in the formulations.

Conclusion

This research proves that dielectric spectroscopy can be a means of a quality and attribute predictor in the yogurt industry; however, more improvements are needed. This would need to be further researched to determine if protein is a variable that can affect dielectric data. Research can be done to see what ingredients effect dielectric readings the most to determine why variations exist in these models. This data altered 2 variables so conclusions can not be drawn to determine which ingredient is altering dielectric data the most.

Table 5.1: Targeted protein and sugar contents of yogurt samples

Targeted yogurt composition		
Sample	Protein (%)	Sucrose (%)
A0	3.5	0
A1	5.13	0
A2	6.75	0
A3	8.38	0
A4	10	0
B0	3.5	1.25
B1	5.13	1.25
B2	6.75	1.25
B3	8.38	1.25
B4	10	1.25
C0	3.5	2.5
C1	5.13	2.5
C2	6.75	2.5
C3	8.38	2.5
C4	10	2.5
D0	3.5	3.75
D1	5.13	3.75
D2	6.75	3.75
D3	8.38	3.75
D4	10	3.75
E0	3.5	5
E1	5.13	5

E2	6.75	5
E3	8.38	5
E4	10	5

Samples read as A-E (increasing sugar content) (0, 1.25, 2.5, 3.75, 5%) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Table 5.2: Theoretical formulations (g) for the yogurt mixes with varying protein and sucrose contents

Sample	Skim Milk (g)	80% Protein MPC (g)	36% NFDM (g)	Sucrose (g)
A0	497.9	0	2.57	0
A1	373.39	10.76	1.61	0
A2	477.41	21.51	1.08	0
A3	467.19	32.27	0.54	0
A4	456.98	43.03	0	0
B0	491.73	0	2.25	6.26
B1	481.4	10.82	1.15	6.25
B2	471.3	21.65	1.01	6.26
B3	450.74	32.47	0.51	6.25
B4	450.42	43.3	0	6.26
C0	484.8	0	2.68	12.5
C1	474.28	10.89	2.01	12.5
C2	464.35	21.79	1.34	12.5
C3	454.13	32.66	0.67	12.5
C4	443.9	43.57	0	12.5
D0	447.9	0	3.33	18.8
D1	467.76	10.96	2.5	18.78
D2	457.63	21.93	1.66	18.77
D3	447.49	32.89	0.83	18.76
D4	437.35	43.85	0	18.75
E0	471	0	3.98	25.03
E1	460.91	11.03	2.98	25

E2	450.88	22.05	1.99	25
E3	440.84	33.08	0.99	25
E4	430.84	44.14	0	25.03

Samples read as A-E (increasing sugar content) (0, 1.25, 2.5, 3.75, 5%) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%). MPC- milk protein concentrate, NFDM- non fat dry milk

Table 5.3: pH , dielectric constant (ε') , and dielectric loss factor (ε") obtained throughout fermentation for samples A0 and A4

Sample	Hour	pH	Permittivity	Frequency (MHz)			
				319	917	2471.8	6000
A0	2	5.9	ε'	71.5±1.43	69.4±1.21	67.4±1.22	62.8±0.58
			ε"	46.5±2.89	19.6±0.73	13.8±0.19	17.7±0.92
A0	3	5.11	ε'	69.8±0.51	67.7±0.39	65.9±0.22	61.4±0.26
			ε"	51.2±2.21	21.6±0.70	15.3±0.14	20.4±1.37
A0	4	4.87	ε'	69.3±0.35	65.3±1.43	628±2.25	58.6±2.49
			ε"	59.2±4.49	23.8±2.67	15.9±0.65	19.9±2.56
A0	5	4.58	ε'	71.0±0.21	68.7±0.14	66.5±0.24	62.1±0.31
			ε"	59.2±1.92	23.8±0.58	15.9±0.01	19.9±0.73
A4	2	5.79	ε'	69.8±0.32	67.1±3.60	64.8±3.67	59.6±0.52
			ε"	45.0±2.37	19.0±0.87	13.7±0.56	17.8±0.20
A4	3	5.38	ε'	69.0±0.86	65.9±1.26	63.3±1.22	58.6±1.08
			ε"	62.4±1.32	24.9±0.52	15.4±2.09	17.5±0.37
A4	4	5.07	ε'	67.8±1.40	64.9±1.99	62.3±2.09	58.5±1.89
			ε"	50.6±1.14	19.8±0.37	12.4±0.33	14.4±0.19
A4	5	4.79	ε'	72.9±1.31	68.9±1.93	66.3±2.04	61.1 ±2.62
			ε"	69.8±1.01	27.5±1.43	16.8±0.14	19.4±0.38
A4	6	4.61	ε'	68.9±2.20	64.6±1.03	61.6±1.74	56.5±1.40
			ε"	60.3±1.36	24.6±0.51	15.3±0.53	17.8±2.99

A0- low protein content (3.5%) and no sugar (0%) yogurt mix, A4- high protein content (10%) and low sugar (0%) yogurt mix

Table 5.4: Proximate analysis (Ash, Total Solid (TS), Fat, True Protein, and Carbohydrate content) of yogurt samples (n=2) (mean±std)

	Ash	TS	Fat	True Protein	Carbohydrates	Moisture
A0	0.86±0.004	11.8±0.010	0.47±0.04	3.12±0.01	7.34	88.2
A1	0.88±0.007	11.4±0.002	0.44±0.01	5.04±0.49	4.99	88.7
A2	0.99±0.008	13.4±0.002	0.43±0.00	6.87±0.04	5.13	86.6
A3	1.14±0.003	15.8±0.001	0.47±0.01	9.57±0.01	4.65	84.2
A4	1.25±0.012	16.7±0.001	0.45±0.04	10.3±0.04	4.67	83.3
B0	0.80±0.007	14.2±0.040	0.47±0.01	3.45±0.02	9.43	85.9
B1	0.95±0.003	12.8±0.004	0.42±0.01	5.12±0.03	6.32	87.2
B2	1.03±0.007	14.7±0.001	0.44±0.01	7.01±0.01	6.24	85.3
B3	1.16±0.003	16.5±0.001	0.38±0.05	8.67±0.01	6.25	83.5
B4	1.26±0.010	18.3±0.001	0.43±0.03	10.58±0.00	5.98	81.8
C0	0.60±0.109	11.3±0.017	0.42±0.00	3.42±0.02	6.87	88.7
C1	0.90±0.002	13.9±0.001	0.47±0.01	5.04±0.01	7.57	86
C2	0.99±0.008	15.7±0.001	0.39±0.04	6.88±0.00	7.47	84.3
C3	1.10±0.037	17.2±0.003	0.47±0.03	8.46±0.02	7.21	82.8
C4	1.24±0.002	19.1±0.003	0.42±0.01	10.3±0.02	7.21	80.8
D0	0.74±0.015	12.3±0.020	0.44±0.00	3.33±0.00	7.75	87.7
D1	0.73±0.002	15.4±0.003	0.45±0.01	5.3±0.01	8.96	84.6
D2	0.91±0.004	17.3±0.002	0.46±0.01	7.09±0.05	8.87	82.7
D3	1.14±0.004	18.6±0.001	0.43±0.01	8.48±0.01	8.53	81.4
D4	1.24±0.004	20.9±0.004	0.47±0.01	10.7±0.01	8.48	79.2
E0	0.76±0.008	15.1±0.002	0.44±0.03	3.33±0.01	10.6	84.9
E1	0.74±0.013	16.8±0.003	0.45±0.02	5.33±0.01	10.3	83.2
E2	0.99±0.025	17.3±0.010	0.42±0.01	6.87±0.00	8.98	82.7
E3	1.17±0.005	19.0±0.020	0.39±0.05	6.19±0.02	11.2	81

E4	1.32±0.108	21.1±0.004	0.38±0.03	10.62±0.01	8.82	78.9

Samples read as A-E (increasing sugar content) (0, 1.25, 2.5, 3.75, 5%) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%). Moisture: 100- Total Solids Carbohydrates: $Total\ solids - (True\ Protein + Fat + Ash + Non\ Protein\ nitrogen)$

Table 5.5: Firmness measurements for all the samples n=2 (±standard deviation)

Sample	0	1	2	3	4
A	127.2±1.36	155.8±14.91	219.5±2.67	240.9±8.94	172.4±2.05
B	139.7±2.89	170.5±5.15	243.2±4.90	229.4±2.22	228.1±29.56
C	129.3±5.31	182.8±4.45	208.7±16.42	200.5±29.41	251.7±7.38
D	110.1±9.49	161.7±9.65	244.7±24.40	273.0±23.19	222.9±14.04
E	140.2±2.18	192.3±4.49	212.6±47.35	255.5±14.25	269.4±10.51

Samples read as A-E (increasing sugar content) (0, 1.25, 2.5, 3.75, 5%) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%). Samples are read in columns.

Table 5.6: ε' and ε" for all yogurt samples taken on day 1 of storage

Sample	Targeted Protein Content	Targeted Sugar Content	Permittivity	Frequency (MHz) 319	917	2471.8	6000
A0	3.5	0	ε'	77.5±1.01	74.7±1.07	69.8±1.12	57.4±1.18
			ε"	33.6±0.95	18.2±0.27	19.4±0.46	28.0±0.86
A1	5.128	0	ε'	76.5±0.48	73.4±0.43	68.5±0.53	56.1±0.90
			ε"	33.6±0.72	18.2±0.09	19.5±0.34	28.0±0.41
A2	6.75	0	ε'	76.8±0.05	73.5±0.17	68.0±0.29	54.7±0.74
			ε"	33.6±0.95	18.3±0.17	19.6±0.24	28.1±0.24
A3	8.375	0	ε'	76.9±1.32	73.6±1.61	68.4±1.95	54.6±0.84
			ε"	33.6±3.34	18.2±0.93	19.5±0.47	27.9±0.48
A4	10	0	ε'	74.5±0.80	70.8±0.97	66.1±0.95	54.2±0.49
			ε"	33.7±0.72	18.2±0.22	19.3±0.62	27.5±0.82
B0	3.5	1.25	ε'	77.9±0.65	75.1±0.97	70.4±0.30	58.3±0.85
			ε"	33.9±0.58	18.4±0.53	19.5±0.88	27.7±1.10
B1	5.128	1.25	ε'	77.1±0.58	74.02±0.53	68.9±0.45	56.3±0.94
			ε"	33.8±1.2	18.5±0.09	19.6±0.64	27.9±0.80
B2	6.75	1.25	ε'	76.4±0.85	73.1±0.91	68.1±0.65	55.6±0.20
			ε"	33.7±1.17	18.5±0.15	19.9±0.56	28.0±0.94
B3	8.375	1.25	ε'	75.4±0.25	71.7±0.25	66.8±0.36	55.1±0.68
			ε"	33.4±0.65	18.6±0.12	20.1±0.13	28.2±0.38
B4	10	1.25	ε'	74.7±5.19	71.3±4.69	66.9±3.98	51.8±4.05
			ε"	35.0±3.72	19.1±1.67	20.4±0.96	27.9±2.37
C0	3.5	2.5	ε'	77.3±0.33	74.4±0.40	69.3±0.29	56.9±0.75
			ε"	37.1±1.00	19.6±0.28	20.5±0.48	27.4±0.62
C1	5.128	2.5	ε'	77.2±0.59	74.2±0.46	69.0±0.20	56.0±1.48
			ε"	37.5±1.85	19.8±0.23	20.5±1.16	27.2±0.87
C2	6.75	2.5	ε'	76.3±0.58	72.9±0.48	67.9±0.18	55.6±0.92
			ε"	38.0±1.38	19.9±0.14	20.5±0.64	27.0±1.00
C3	8.375	2.5	ε'	75.4±0.21	71.9±0.18	66.0±0.20	52.3±0.92

			ε"	37.4±0.48	19.7±0.07	20.0±0.50	26.6±0.65
C4	10	2.5	ε'	74.3±1.35	70.5±1.30	64.4±1.50	50.9±1.10
			ε"	37.3±1.64	19.6±0.47	19.4±0.34	26.0±1.57
D0	3.5	3.75	ε'	76.6±1.30	73.5±1.16	68.7±0.56	56.6±1.30
			ε"	38.2±2.72	19.8±0.25	19.1±1.33	25.5±2.25
D1	5.128	3.75	ε'	76.2±0.10	72.9±0.121	67.7±0.18	55.2±0.15
			ε"	39.5±0.22	20.1±0.05	19.1±0.24	25.1±0.35
D2	6.75	3.75	ε'	75.2±0.07	71.7±0.09	66.5±0.36	54.5±0.67
			ε"	38.2±0.89	19.7±0.27	19.4±0.45	26.2±0.04
D3	8.375	3.75	ε'	73.8±1.74	70.3±1.87	64.9±2.12	52.8±2.25
			ε"	36.4±2.28	19.2±0.68	19.7±0.34	27.4±0.44
D4	10	3.75	ε'	72.9±0.89	68.9±0.94	63.1±1.24	48.9±1.21
			ε"	34.7±0.89	18.5±0.14	19.51±1.30	27.9±4.20
E0	3.5	5	ε'	76.7±0.62	73.7±0.47	68.9±0.35	56.8±1.18
			ε"	32.0±0.69	17.8±0.57	18.7±0.86	27.8±0.83
E1	5.128	5	ε'	74.7±1.15	71.5±1.10	66.8±0.57	56.0±0.63
			ε"	34.4±2.30	18.3±0.33	18.2±1.19	25.6±1.82
E2	6.75	5	ε'	74.9±0.36	71.4±0.44	66.2±0.38	54.3±0.53
			ε"	33.0±0.47	18.3±0.17	18.9±0.20	27.2±0.10
E3	8.375	5	ε'	74.00±2.44	70.3±2.14	64.8±1.24	51.2±1.65
			ε"	32.9±1.36	18.6±0.05	19.9±0.80	28.3±1.53
E4	10	5	ε'	72.4±0.72	68.4±0.90	62.4±1.25	49.2±2.39
			ε"	31.2±0.70	18.2±0.58	19.7±0.82	25.5±0.39

Samples read as A-E (increasing sugar content) (A=0, B=1.25, C=2.5, D=3.75, E=5%) and 0, 1, 2, 3, 4 (increasing protein content (1=3.5, 2=5.13, 3=6.75, 4=8.38, 5=10%). Each alphabet and numerical combination in the table shows the corresponding protein and sugar content in each row

Table 5.7: Calculated penetration depth (mm) for all yogurt samples at the 4 selected frequencies

Sample	Penetration Depth (mm) at each Frequency (MHz)			
	319	917	2471.8	6000
A0	4.00	2.50	0.84	0.22
A1	3.99	2.47	0.83	0.22
A2	3.99	2.46	0.82	0.22
A3	4.00	2.47	0.83	0.22
A4	3.93	2.42	0.82	0.22
B0	3.97	2.47	0.84	0.23
B1	3.98	2.45	0.83	0.22
B2	3.98	2.42	0.81	0.22
B3	3.98	2.40	0.79	0.22
B4	3.79	2.32	0.78	0.21
C0	3.64	2.31	0.79	0.22
C1	3.60	2.29	0.79	0.23
C2	3.54	2.25	0.79	0.23
C3	3.58	2.27	0.79	0.22
C4	3.57	2.26	0.81	0.22
D0	3.53	2.28	0.84	0.24
D1	3.41	2.24	0.84	0.24
D2	3.50	2.26	0.82	0.23
D3	3.63	2.29	0.80	0.22
D4	3.79	2.36	0.77	0.21
E0	4.18	2.53	0.86	0.22

E1	3.87	2.43	0.88	0.24
E2	4.02	2.42	0.84	0.22
E3	4.01	2.36	0.79	0.21
E4	4.17	2.38	0.78	0.23

Samples read as A-E (increasing sugar content) (A=0, B=1.25, C=2.5, D=3.75, E=5%) and 0, 1, 2, 3, 4 (increasing protein content (1=3.5, 2=5.13, 3=6.75, 4=8.38, 5=10%).

Table 5.8: Model coefficients for the prediction of firmness

Variable	Parameter estimate	P-Value
Intercept	-327.7	0.7065
ε' 319	113.53	0.0303
ε' 917	-111.5	0.011

Table 5.9: Model coefficients for the prediction of protein

Variable	Parameter estimate	P-Value
Intercept	-44.75	0.1608
ε' 319	8.612	0.0003
ε' 917	-10.36	0.0001
ε' 2471.8	2.884	0.0032
ε' 6000	-0.8129	0.7844

Table 5.10: Parameter intercepts for the prediction of moisture in the model

Variable	Parameter estimate	P-Value
Intercept	-18.75	0.0651
ε' 917	1.423	<0.001

Table 5.11: Measured and predicted firmness values using the firmness prediction model

Sample	Measured Firmness (g)	Predicted firmness (g)	% Variability
A0	128.93	195.2	33.95
A4	256.76	307.14	16.4
C2	104.39	235.89	55.75
E0	135.89	191.28	28.96
E4	240.5	243.76	1.34
Sample 1	358.74	408.78	12.24
Sample 2	128.11	139.68	8.28

A-E (increasing sugar content) (0-5%) and 0-4 (increasing protein content) (3.5-10%), Sample 1(Fage total 0%) and Sample 2 (Kroger blended plain yogurt). % Variability is found by taking the absolute value of the difference between measured and predicted divided by the measured.

$$Firmness = -327.66 + 113.53(\varepsilon'319) - 111.50(\varepsilon'917)$$

Table 5.12: Measured and predicted protein contents using the protein prediction model

Sample	Measured true protein	Predicted True protein	% Variability
A0	3.43	7.35	53.31
A4	5.88	13.81	57.42
C2	3.39	8.6	60.59
E0	3.42	-6.18	155.31
E4	9.44	9.66	2.23
Sample 1	10.18	33.79	69.87
Sample 2	4.19	11.82	64.55

A-E (increasing sugar content) (0-5%) and 0-4 (increasing protein content) (3.5-10%), Sample 1(Fage total 0%) and Sample 2 (Kroger blended plain yogurt) %Variability is found by taking the absolute value of the difference between measured and predicted divided by the measured

$$Protein = -44.75 + 8.61\ (\varepsilon' 319)\ - 10.36(\varepsilon' 917) + 2.88(\varepsilon' 2417.8)\ - 0.8127(\varepsilon' 6000)$$

Table 5.13: Measured and predicted moisture contents using the moisture prediction model

Sample	Measured moisture	Predicted moisture	% Variability
A0	87.50	89.14	1.444
A4	80.15	85.15	5.874
C2	83.36	85.77	2.808
E0	84.20	77.98	7.976
E4	79.50	86.39	7.974
Sample 1	68.37	84.26	18.85
Sample 2	75.57	84.85	10.94

A-E (increasing sugar content) (0-5%) and 0-4 (increasing protein content) (3.5-10%), Sample 1(Fage total 0%) and Sample 2 (Kroger blended plain yogurt) %Variability is found by taking the absolute value of the difference between measured and predicted divided by the measured

$$Moisture = -18.75 + 1.42(\varepsilon'917)$$

Figure 5.1: Setup of the dielectric spectroscopy system

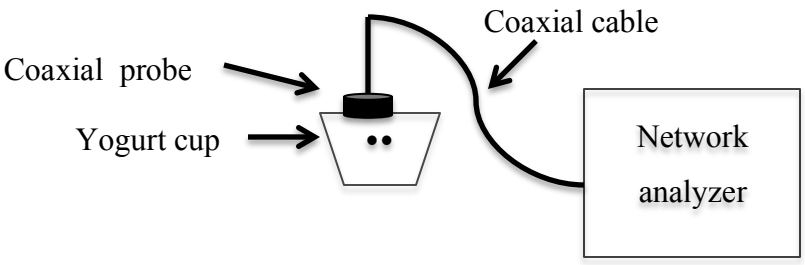
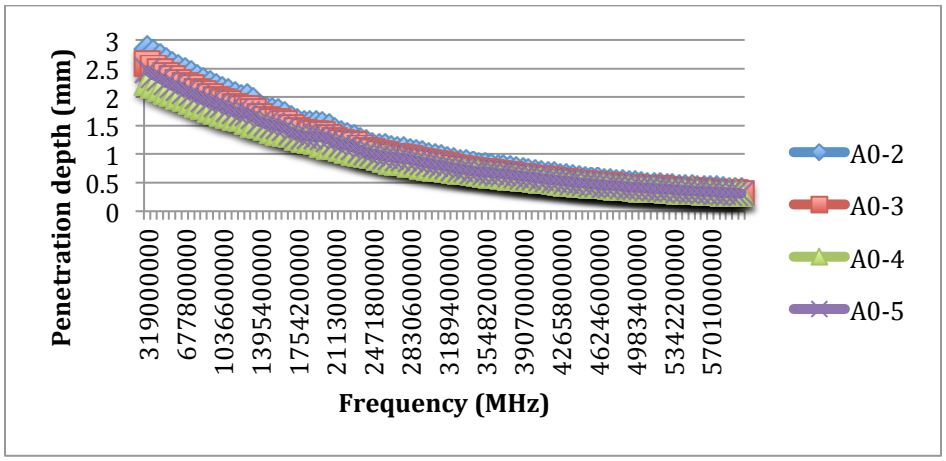
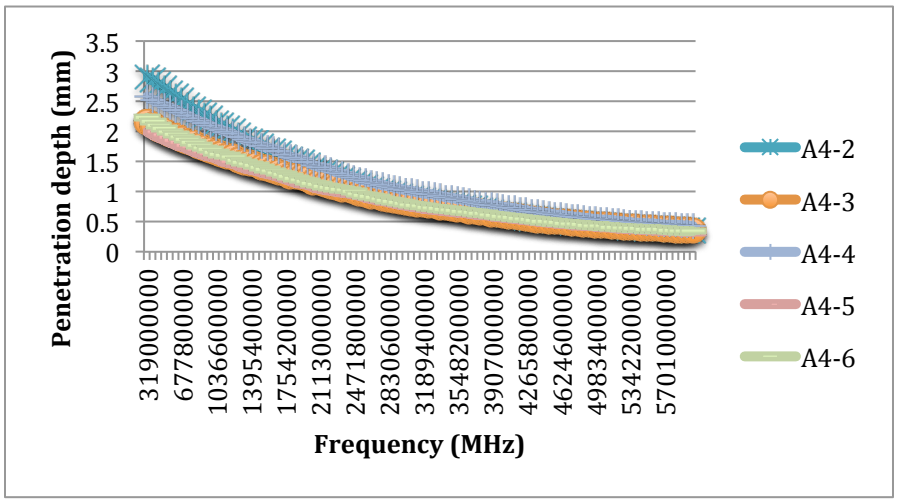


Figure 5.2: Penetration depth for the A0 samples throughout fermentation



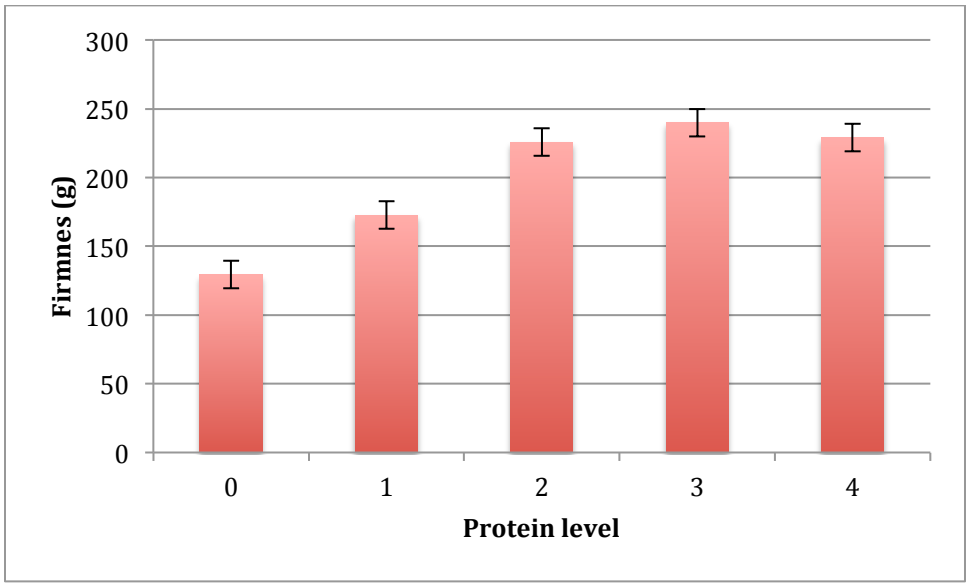
A0- A (sugar content 0%) and 2-5 represents each hour of fermentation

Figure 5.3: Penetration depth (mm) for the A4 samples throughout fermentation



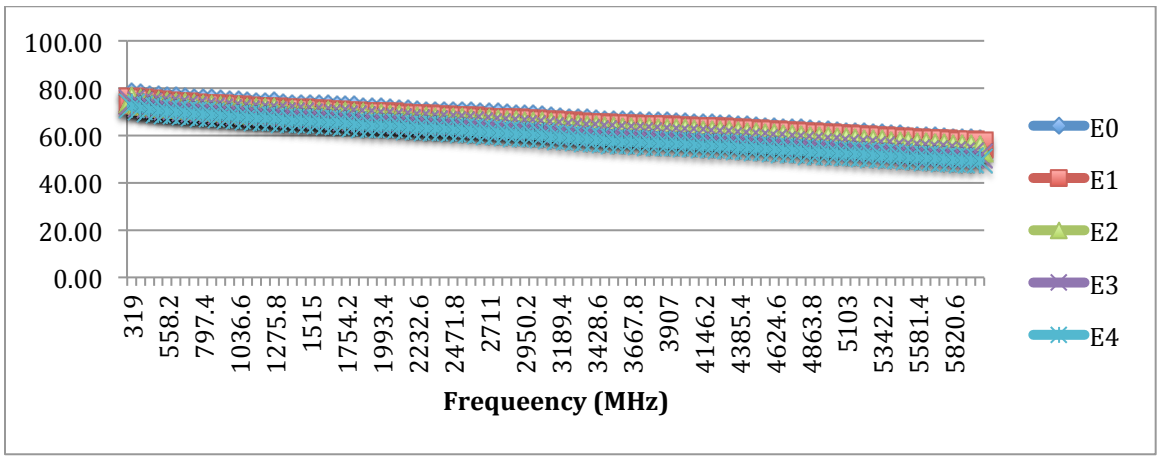
A04- A (sugar content 5%) and 2-6 represents each hour of fermentation

Figure 5.4: Average yogurt firmness values at the 5 targeted protein concentrations



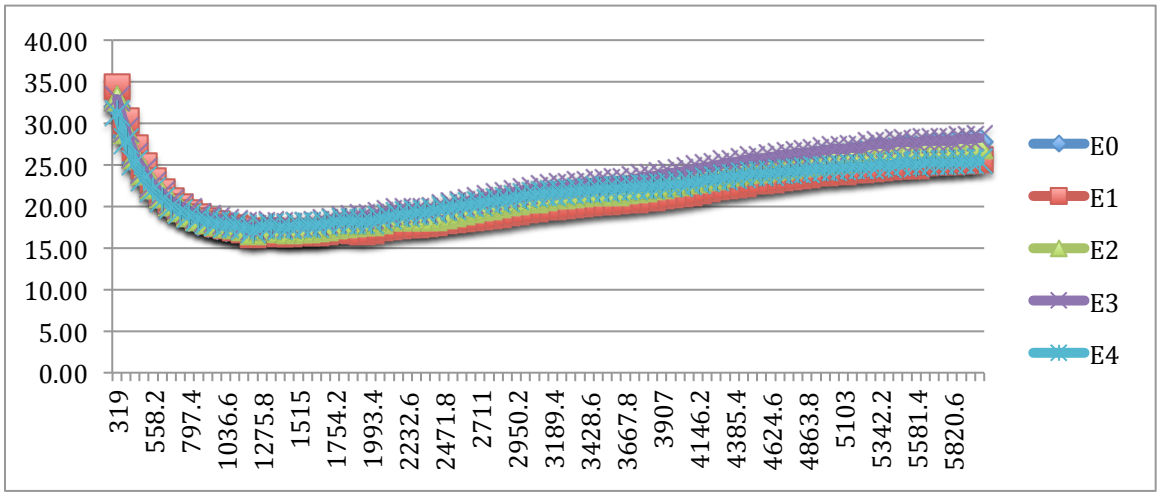
Protein level 1=3.5%, 2=5.13%, 3=6.75%, 4=8.38%, 5=10%

Figure 5.5: ε' values for E yogurt samples throughout 101 frequencies



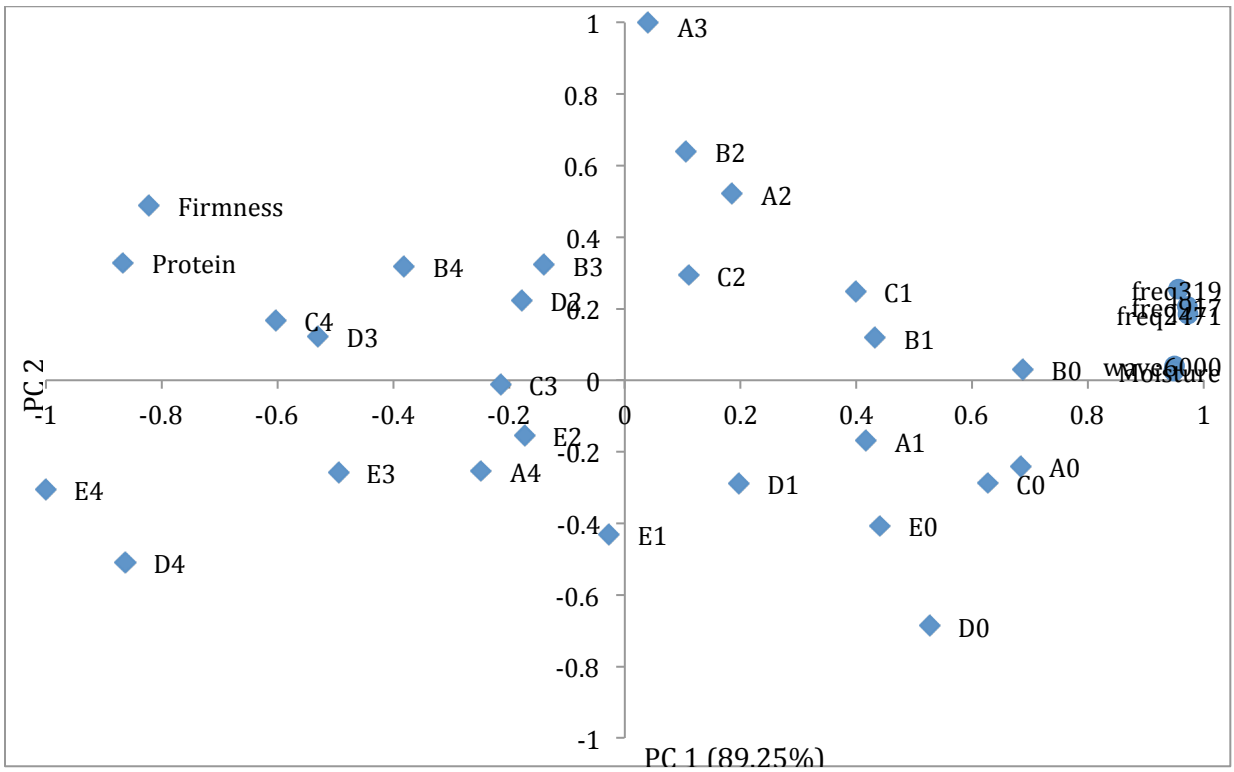
E (amount of sugar) (5%) and 0-4 (increasing protein content) (3.5-10%)

Figure 5.6: ε" values for all E yogurt samples throughout 101 frequencies



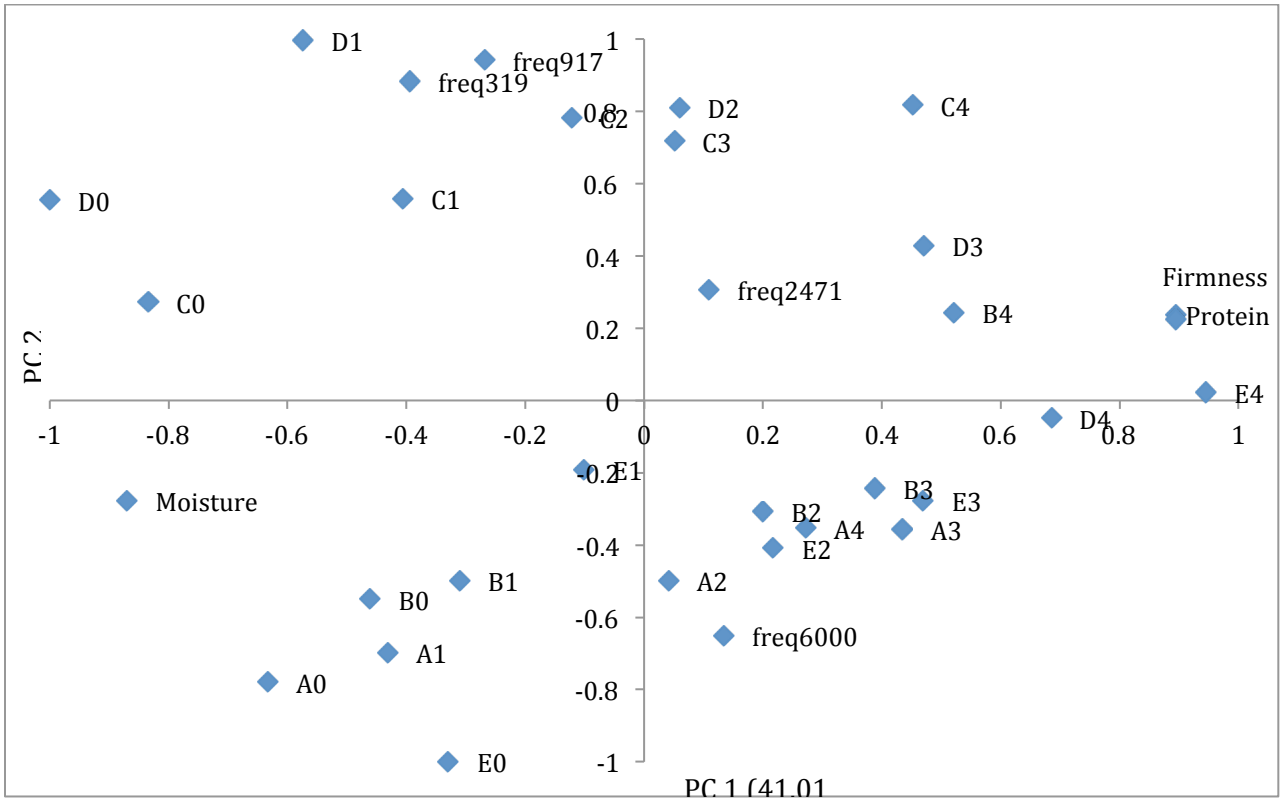
E (amount of sugar) (5%) and 0-4 (increasing protein content) (3.5-10%)

Figure 5.7: Principle Component Analysis for ε'



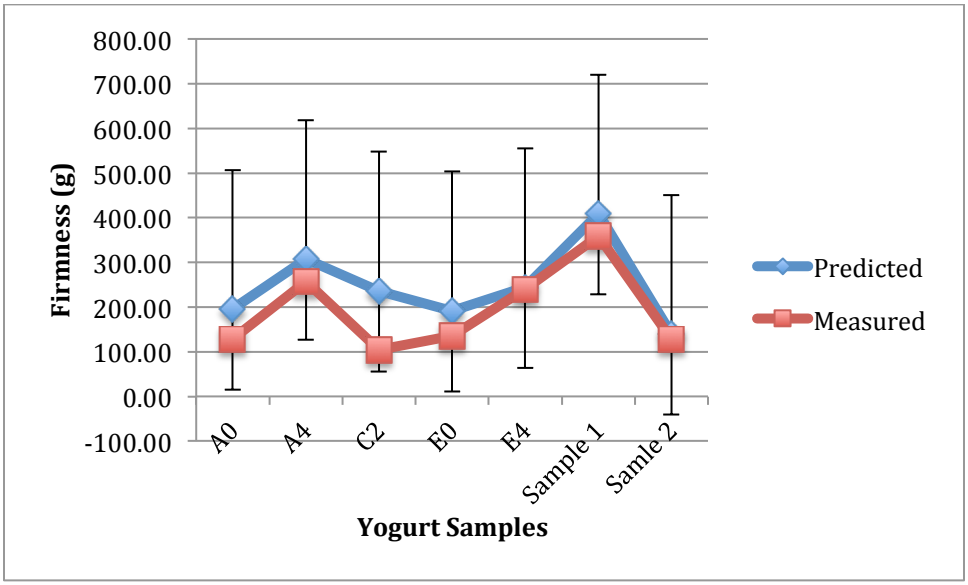
● Variables, ◆Yogurt Samples

Figure 5.8: Principle component analysis for ε"



● Variables, ◆Yogurt Samples

Figure 5.9: Firmness (g) comparison of measured and predicted values to determine fit of the model fitted with 95% Confidence Intervals for each sample



A's (0% sugar) C's (2.5% sugar), E's (5% sugar), 0's (3.5% protein), 2's (6.75% protein), 4's (10% protein), Sample 1 (Fage Total 0% yogurt), Sample 2 (Kroger blended plain low fat yogurt)

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Chapter 6 - Research Summary

In Chapter 3, electromagnetic fluid conditioning (EFC), indicated changes occurred in milkwhich altered the chemical and physical properties of yogurt, but these changes were not desired. The EFC treated skim milk, negative (N) and positive (P) samples when compared with the control (C) produced yogurts that had significantly less firmness and more syneresis ($P \leq 0.05$). The N and P gels were 7% less firm and had 21% more syneresis than the C gels. This indicates that EFC somehow altered milk gel structure, but did not give a clear indication as to what properties were altered. This study also hinted that fermentation time could also be reduced by pretreating the milk with EFC. Determined to figure out what properties of milk EFC alters, further studies on milk were conducted.

To optimize the EFC treatment where many treatment combinations could be tested (altering voltage, time, direction, and flow rate). In this study, skim milk was treated at 10 and 30 V for 2 and 10 minutes, these treatment combinations were chosen to have a minimal and maximal extreme exposure to the magnets. Also magnetic direction was tested in this study: control (C), negative (N), and positive (P). When treating skim milk at 10 and 30 V for 2 min, all variables tested were insignificant ($P \geq 0.05$). Treating skim milk at 10 minutes for 10 V, surface tension was significant ($P \leq 0.05$). The N direction had the greatest effect, where milk had 1.89% greater surface tension, compared with milk undergoing the P treatment. When treating skim milk at 30 V for 10 minutes, color properties were altered. The L* and a* values increased in the N sample, 1.18% compared to the C sample, and N was 11.10% greater for a* when compared with the C samples respectively. The P and C samples were

statistically ($P \geq 0.05$) similar to one another. This data shows that it is possible to alter skim milk with EFC treatment. It needs to be further investigated which design settings result in an optimized treatment. Treatment parameters should be fully investigated to determine if and how EFC changes skim milk to further aid the dairy processing industry because of the numerous combinations of voltages, treatment times, flow rate, and magnetic direction.

Finally dielectric spectroscopy was used to generate predictive models for yogurt quality and composition. Twenty-five yogurts were formulated for increasing sugar contents, 0-5%, (A-E) and increasing protein contents, 3.5-10%, (0-4) and analyzed for ε' and ε". Once the model for firmness was generated with an R^2 of 0.60 indicating not a great model. In a validation study predicted values varied from the actual firmness measurements preformed by traditional testing. Predictive models for protein and moisture had R^2 values of 0.86 and 0.83 respectively. More work needs to be done to determine which frequencies are important to focus on when building a predictive model. It is also important to further study which formulation additions, like protein and sugar, affect the model the most. In this study since both were altered you could not pinpoint which one was doing the changing to make the model not as accurate.

Appendix A - Raw Data for Chapter 3

Raw Data for Chapter 3

Table A.1: Fermentation data (Repetition (Rep), Treatment (Trt), pH, titratable acidity (TA), L*, a*, b*) for SAS

Rep	Trt	Hour	pH	TA	L*	a*	b*
1	c	0	6.61	0.21	88.42	-4.03	7.2
1	c	1	6.43	0.22	88.96	-3.98	7.09
1	c	2	6.1	0.31	90.6	-3.2	7.53
1	c	3	5.38	0.58	89.05	-2.73	9.89
1	c	4	4.92	0.83	81.74	-2.65	10.21
1	c	5	4.7	0.85	80.47	-2.59	10.76
1	hp	0	6.6	0.22	87.76	-4.77	7.06
1	hp	1	6.38	0.22	91.12	-4.35	7.34
1	hp	2	6.15	0.37	90.28	-4.24	7.21
1	hp	3	5.22	0.65	89.9	-4.11	7.17
1	hp	4	4.86	0.77	92	-2.81	9.66
1	hp	5	4.61	0.88	84.48	-2.8	10.11
1	hn	0	6.55	0.24	90.85	-4.18	7.41
1	hn	1	6.33	0.23	89.78	-4.11	7.28
1	hn	2	5.98	0.36	90.125	-3.99	7.24
1	hn	3	5.13	0.59	91.91	-3.01	8.35
1	hn	4	4.8	0.77	85.23	-2.68	9.87
1	hn	5	4.58	0.82	83.1	-2.66	10.46
2	c	0	6.51	0.22	90.71	-4.05	7.85
2	c	1	6.47	0.24	91.37	-4.09	7.98
2	c	2	5.99	0.34	90.92	-3.91	7.73
2	c	3	5.26	0.59	91.85	-3.19	8.28
2	c	4	4.82	0.77	92.04	-2.66	9.75
2	c	5	4.61	0.89	84.04	-2.93	11.07
2	hp	0	6.56	0.23	90.35	-3.99	7.91
2	hp	1	6.42	0.23	86.98	-3.85	7.42
2	hp	2	6.08	0.32	89.53	-3.87	7.78
2	hp	3	5.31	0.59	88.06	-2.92	7.81
2	hp	4	4.85	0.74	90.3	-2.88	10.28

2	hp	5	4.62	0.87	76.7	-3.33	12
2	hn	0	6.58	0.22	89.61	-4.07	7.92
2	hn	1	6.36	0.23	89.43	-3.83	7.69
2	hn	2	5.99	0.37	90.8	-3.87	7.66
2	hn	3	5.18	0.53	90.49	-2.76	8.62
2	hn	4	4.8	0.72	90.48	-2.69	10.26
2	hn	5	4.6	0.77	90.45	-2.51	9.55
3	c	0	6.52	0.23	90.74	-4.16	7.78
3	c	1	6.47	0.36	89.32	-4.11	7.54
3	c	2	6.09	0.36	87.29	-3.88	7.28
3	c	3	5.32	0.64	89.24	-2.76	8.62
3	c	4	4.73	0.72	85.79	-2.68	9.9
3	c	5	4.59	0.75	85.6	-2.74	10.28
3	hp	0	6.55	0.18	91.09	-4.25	7.33
3	hp	1	6.32	0.21	89.6	-4.08	7.43
3	hp	2	5.84	0.34	90.55	-4.02	6.39
3	hp	3	5.05	0.53	90.79	-2.68	9.02
3	hp	4	4.8	0.75	89.55	-2.45	9.6
3	hp	5	4.6	0.8	89.78	-2.24	9.35
3	hn	0	6.54	0.19	89.84	-4.22	6.93
3	hn	1	6.37	0.22	90.32	-4.22	7.06
3	hn	2	6.11	0.3	91.04	-4.22	6.96
3	hn	3	5.17	0.61	91.91	-3.09	8.07
3	hn	4	4.78	0.69	90.42	-2.92	10.18
3	hn	5	4.6	0.76	86.26	-2.905	11.13

*c- Control Treatment, hn- negative treatment, hp- positive treatment

Table A.2: Raw data for Storage study over 45 days (Repetition (Rep), Treatment (Trt), Day, pH, Titratable Acidity (TA), L*, a*, b*, Syneresis, Water Holding Capacity (WHC), Firmness

Rep	Trt	Day	pH	TA	L*	a*	b*	Syneresis	WHC	Firmness
1	c	1	4.49	0.9	92.74	-2.73	10.67	4.8	15.32	120.8
1	hp	1	4.44	0.97	89.1	-2.75	9.81	7.57	15.43	114.98
1	hn	1	4.47	0.89	89.5	-2.73	10.32	5.91	16.31	111.15

2	c	1	4.47	0.88	92.01	-2.6	10.35	4.23	18.71	121.96
2	hp	1	4.53	0.88	92.01	-2.62	10.64	5.73	16.84	119.52
2	hn	1	4.46	0.89	92.11	-2.63	10.28	4.27	18.21	116.23
3	c	1	4.47	0.87	89.94	2.65	10.09	4.76	16.96	105.19
3	hp	1	4.54	0.9	87.52	-2.66	10.08	6.25	16.05	99.63
3	hn	1	4.49	0.94	91.42	-2.58	10.44	6.57	15.15	95.68
1	c	15	4.28	1.08	89.8	-2.6	9.9	4.97	18.3	126.13
1	hp	15	4.25	0.97	93.31	-2.74	10.33	4.98	21.8	118.73
1	hn	15	4.23	0.97	89.67	-2.54	9.78	5.62	19.4	125.54
2	c	15	4.2	0.97	92.28	-2.38	10.7	4.06	20.4	126.05
2	hp	15	4.32	0.98	93.29	-2.7	11.01	3.54	20.01	118.69
2	hn	15	4.29	0.92	89.23	-2.49	9.85	4	18.97	123.41
3	c	15	4.33	0.97	93.37	-2.49	10.16	4.06	18.11	126.77
3	hp	15	4.37	0.97	89	-2.63	9.2	6.01	15.02	108.07
3	hn	15	4.38	0.91	93.2	-2.77	10.06	5.3	14.85	106.05
1	c	30	4.25	0.99	88.67	-2.51	9.75	4.25	19.18	127.52
1	hp	30	4.33	0.92	90.1	-2.64	9.9	5.22	19.72	118.77
1	hn	30	4.18	0.92	90.63	-2.4	10.3	4.67	19.18	126
2	c	30	4.25	0.99	93.01	-2.62	10.5	3.72	16.78	138.52
2	hp	30	4.31	0.99	93.08	-2.48	9.1	4.18	19.39	126.74
2	hn	30	4.32	0.95	90.25	-2.58	10.05	4.08	16.57	128.75
3	c	30	4.23	1.01	90.85	-2.5	9.62	3.12	17.15	136.25
3	hp	30	4.29	0.98	90.89	-2.77	9.91	4.69	15.94	119.75
3	hn	30	4.32	0.97	90.87	-2.76	9.62	5.87	15.14	118.17
1	c	45	4.27	0.97	91.13	-2.49	9.8	4.07	18.47	129.74
1	hp	45	4.33	0.97	92.31	-2.72	9.86	4.62	16.12	117.68
1	hn	45	4.31	0.98	89.77	-2.53	9.06	5.07	16.3	138.7
2	c	45	4.29	0.97	94.5	-2.63	10.75	2.78	17.1	139.45
2	hp	45	4.33	1.13	92.96	-2.56	10.32	3.44	18	142.94
2	hn	45	4.32	0.98	91.01	-2.51	9.87	3.01	19.32	127.68
3	c	45	4.22	0.96	92.33	-2.54	9.82	3.11	18.68	133.95
3	hp	45	4.28	0.94	91.22	-2.4	9.77	5.39	16.18	121.01
3	hn	45	4.25	0.92	92.61	-2.57	9.41	5.68	16.55	111.16

*c- Control Treatment, hn- negative treatment, hp- positive treatment

Appendix B - SAS Code for Chapters 3

SAS Codes for Chapter 3

SAS Codes for Chapter 3: Fermentation Study

```
data storage;
input rep  trt $ day ph        ta        l        a        b ;
cards;
1      c      0      6.61   0.21   88.42 -4.03 7.2
1      c      1      6.43   0.22   88.96 -3.98 7.09
1      c      2      6.1    0.31   90.6  -3.2  7.53
1      c      3      5.38   0.58   89.05 -2.73 9.89
1      c      4      4.92   0.83   81.74 -2.65 10.21
1      c      5      4.7    0.85   80.47 -2.59 10.76
1      hp     0      6.6    0.22   87.76 -4.77 7.06
1      hp     1      6.38   0.22   91.12 -4.35 7.34
1      hp     2      6.15   0.37   90.28 -4.24 7.21
1      hp     3      5.22   0.65   89.9  -4.11 7.17
1      hp     4      4.86   0.77   92    -2.81 9.66
1      hp     5      4.61   0.88   84.48 -2.8  10.11
1      hn     0      6.55   0.24   90.85 -4.18 7.41
1      hn     1      6.33   0.23   89.78 -4.11 7.28
1      hn     2      5.98   0.36   90.125      -3.99 7.24
1      hn     3      5.13   0.59   91.91 -3.01 8.35
1      hn     4      4.8    0.77   85.23 -2.68 9.87
1      hn     5      4.58   0.82   83.1  -2.66 10.46
2      c      0      6.51   0.22   90.71 -4.05 7.85
2      c      1      6.47   0.24   91.37 -4.09 7.98
2      c      2      5.99   0.34   90.92 -3.91 7.73
2      c      3      5.26   0.59   91.85 -3.19 8.28
2      c      4      4.82   0.77   92.04 -2.66 9.75
2      c      5      4.61   0.89   84.04 -2.93 11.07
2      hp     0      6.56   0.23   90.35 -3.99 7.91
2      hp     1      6.42   0.23   86.98 -3.85 7.42
2      hp     2      6.08   0.32   89.53 -3.87 7.78
2      hp     3      5.31   0.59   88.06 -2.92 7.81
2      hp     4      4.85   0.74   90.3  -2.88 10.28
```

```
2       hp      5       4.62    0.87    76.7    -3.33 12
2       hn      0       6.58    0.22    89.61 -4.07 7.92
2       hn      1       6.36    0.23    89.43 -3.83 7.69
2       hn      2       5.99    0.37    90.8    -3.87 7.66
2       hn      3       5.18    0.53    90.49 -2.76 8.62
2       hn      4       4.8     0.72    90.48 -2.69 10.26
2       hn      5       4.6     0.77    90.45 -2.51 9.55
3       c       0       6.52    0.23    90.74 -4.16 7.78
3       c       1       6.47    0.36    89.32 -4.11 7.54
3       c       2       6.09    0.36    87.29 -3.88 7.28
3       c       3       5.32    0.64    89.24 -2.76 8.62
3       c       4       4.73    0.72    85.79 -2.68 9.9
3       c       5       4.59    0.75    85.6    -2.74 10.28
3       hp      0       6.55    0.18    91.09 -4.25 7.33
3       hp      1       6.32    0.21    89.6    -4.08 7.43
3       hp      2       5.84    0.34    90.55 -4.02 6.39
3       hp      3       5.05    0.53    90.79 -2.68 9.02
3       hp      4       4.8     0.75    89.55 -2.45 9.6
3       hp      5       4.6     0.8     89.78 -2.24 9.35
3       hn      0       6.54    0.19    89.84 -4.22 6.93
3       hn      1       6.37    0.22    90.32 -4.22 7.06
3       hn      2       6.11    0.3     91.04 -4.22 6.96
3       hn      3       5.17    0.61    91.91 -3.09 8.07
3       hn      4       4.78    0.69    90.42 -2.92 10.18
3       hn      5       4.6     0.76    86.26 -2.905        11.13
;
proc print data=storage;
run;


proc mixed data=storage;
class rep trt day;
model ph= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;
```

```
proc glimmix data=storage;
class rep trt day;
model ph= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
class rep trt day;
model ta= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model ta= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
class rep trt day;
model l= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model l= trt day trt*day/ddfm= satterth;
```

```
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
class rep trt day;
model a= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model a= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
class rep trt day;
model b= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model b= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;
```

SAS Codes for Chapter 3: Storage Study

```
data storage;
input rep  trt $ day ph        ta        l        a        b        Syn
WHC        Firm        Consist        Cohesiv        Index@@;
cards;
1      c      1      4.49  0.9   92.74 -2.73 10.67 4.8   15.32 120.8 227.24
       -33.98       -7.25
1      hp     1      4.44  0.97  89.1  -2.75 9.81  7.57  15.43 114.98
       212.49       -32.59       -7.2
1      hn     1      4.47  0.89  89.5  -2.73 10.32 5.91  16.31 111.15
       212.51       -31.3 -6.53
2      c      1      4.47  0.88  92.01 -2.6  10.35 4.23  18.71 121.96
       231.715      -36.7 -8.37
2      hp     1      4.53  0.88  92.01 -2.62 10.64 5.73  16.84 119.52
       223.56       -34.73       -7.15
2      hn     1      4.46  0.89  92.11 -2.63 10.28 4.27  18.21 116.23
       229.79       -33.84       -7.02
3      c      1      4.47  0.87  89.94 2.65  10.09 4.76  16.96 105.19
       199.48       -30.73       -6
3      hp     1      4.54  0.9   87.52 -2.66 10.08 6.25  16.05 99.63 194.7 -
27.87 -5.72
3      hn     1      4.49  0.94  91.42 -2.58 10.44 6.57  15.15 95.68 177.34
       -22.4 -3.43
1      c      15     4.28  1.08  89.8  -2.6  9.9   4.97  18.3  126.13
       245.65       -35.63       -7.89
1      hp     15     4.25  0.97  93.31 -2.74 10.33 4.98  21.8  118.73
       230.68       -29.37       -6.98
1      hn     15     4.23  0.97  89.67 -2.54 9.78  5.62  19.4  125.54
       232.13       -28.09       -5.14
2      c      15     4.2   0.97  92.28 -2.38 10.7  4.06  20.4  126.05
       237.95       -26.51       -6.75
2      hp     15     4.32  0.98  93.29 -2.7  11.01 3.54  20.01 118.69
       225.79       -27.8 -5.93
2      hn     15     4.29  0.92  89.23 -2.49 9.85  4     18.97 123.41
       236.58       -31.195      -7.13
3      c      15     4.33  0.97  93.37 -2.49 10.16 4.06  18.11 126.77
       245.46       -28.59       -6.11
```

```
3       hp      15      4.37    0.97    89      -2.63 9.2     6.01    15.02 108.07
        207.43          -21.97          -4
3       hn      15      4.38    0.91    93.2    -2.77 10.06 5.3     14.85 106.05
        212.51          -27.155         -5.89
1       c       30      4.25    0.99    88.67 -2.51 9.75    4.25    19.18 127.52
        241.25          -28.19          -5.71
1       hp      30      4.33    0.92    90.1    -2.64 9.9     5.22    19.72 118.77
        227.74          -31.45          -6.71
1       hn      30      4.18    0.92    90.63 -2.4    10.3    4.67    19.18 126     236.3 -
31.19 -6.63
2       c       30      4.25    0.99    93.01 -2.62 10.5    3.72    16.78 138.52
        264.54          -30.15          -7.01
2       hp      30      4.31    0.99    93.08 -2.48 9.1     4.18    19.39 126.74
        244.96          -29.99          -6.07
2       hn      30      4.32    0.95    90.25 -2.58 10.05 4.08    16.57 128.75
        232.77          -29.69          -5.91
3       c       30      4.23    1.01    90.85 -2.5    9.62    3.12    17.15 136.25
        248.68          -30.73          -6.33
3       hp      30      4.29    0.98    90.89 -2.77 9.91    4.69    15.94 119.75
        225.68          -24.4 -5.15
3       hn      30      4.32    0.97    90.87 -2.76 9.62    5.87    15.14 118.17
        221.85          -24.65          -5.12
1       c       45      4.27    0.97    91.13 -2.49 9.8     4.07    18.47 129.74
        254.54          -35.16          -8.54
1       hp      45      4.33    0.97    92.31 -2.72 9.86    4.62    16.12 117.68
        211.17          -24.72          -4.38
1       hn      45      4.31    0.98    89.77 -2.53 9.06    5.07    16.3    138.7 262.23
        -24.22          -4.33
2       c       45      4.29    0.97    94.5    -2.63 10.75 2.78    17.1    139.45
        259.19          -28.52          -6.19
2       hp      45      4.33    1.13    92.96 -2.56 10.32 3.44    18      142.94
        267.77          -34.94          -7.1
2       hn      45      4.32    0.98    91.01 -2.51 9.87    3.01    19.32 127.68
        247.12          -30.29          -6.32
3       c       45      4.22    0.96    92.33 -2.54 9.82    3.11    18.68 133.95
        247.49          -31.69          -6.9
3       hp      45      4.28    0.94    91.22 -2.4    9.77    5.39    16.18 121.01
        222.79          -22.48          -3.87
```

```
3      hn     45     4.25   0.92   92.61 -2.57 9.41   5.68   16.55 111.16
       211.67       -25.87       -5.36
;
proc print data=storage;
run;


proc mixed data=storage;
class rep trt day;
model ph= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model ph= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
class rep trt day;
model ta= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model ta= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
```

```
run;


proc mixed data=storage;
class rep trt day;
model l= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model l= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
class rep trt day;
model a= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model a= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;


proc mixed data=storage;
```

```
class rep trt day;
model b= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model b= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;

proc mixed data=storage;
class rep trt day;
model syn= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model syn= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;

proc mixed data=storage;
class rep trt day;
model whc= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
```

```
run;

proc glimmix data=storage;
class rep trt day;
model whc= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;

proc mixed data=storage;
class rep trt day;
model firm= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model firm= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;

proc mixed data=storage;
class rep trt day;
model consist= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model consist= trt day trt*day/ddfm= satterth;
random rep rep*day;
```

```
lsmeans trt*day/pdiff adjust=bon lines;
run;

proc mixed data=storage;
class rep trt day;
model Cohesiv= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model Cohesiv= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;

proc mixed data=storage;
class rep trt day;
model Index= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt/pdiff adjust=bon;
lsmeans day/pdiff adjust=bon;
lsmeans trt*day/pdiff adjust=bon;
run;

proc glimmix data=storage;
class rep trt day;
model Index= trt day trt*day/ddfm= satterth;
random rep rep*day;
lsmeans trt*day/pdiff adjust=bon lines;
run;
```

Appendix C - SAS code with Raw data for Chapter 4

SAS Code for Chapter 2: 10 V at 2 minutes

```
data s;
input rep$ trt$  l a b ta vis ph ST;
cards;
1       c       87.99  -3.99  7.55   0.2    1.23   6.67   47.4624
2       c       88.92  -3.74  7.93   0.22   1.24   6.65   46.9817
3       c       88.66  -3.88  7.89   0.21   1.22   6.64   47.7029
1       hp      87.81  -4.135 6.87   0.22   1.27   6.7    45.5082
2       hp      88.88  -3.91  6.83   0.23   1.26   6.65   47.222
3       hp      88.59  -3.99  7.83   0.23   1.3    6.65   46.9817
1       hn      88.84  -3.83  7.91   0.22   1.31   6.64   47.7029
2       hn      88.65  -3.87  7.72   0.22   1.26   6.65   48.1843
3       hn      88.88  -3.89  7.85   0.23   1.25   6.64   48.1843

;
proc glm data=s;
class rep trt;
model l = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model a = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model b = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ta = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/pdiff adjust=bon;
```

```
run;
proc glm data=s;
class rep trt;
model ph = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ST = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glimmix data=s;
class rep trt;
model l= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model a= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model b= trt;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ta= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ph= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
```

```
class rep trt;
model ST= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
      run;
```

SAS Code for Chapter 2: 30 V at 2 minutes

```
data s;
input rep$ trt$  l a b ta vis ph ST;
cards;
1     c     86.86 -4.27 7.17  0.21  1.26  6.67  47.9435
2     c     87.24 -4    7.55  0.21  1.37  6.65  47.7029
3     c     87.63 -3.99 7.75  0.22  1.36  6.67  48.6665
1     hp    87.48 -4.13 7.84  0.22  1.37  6.7   48.4112
2     hp    87.91 -3.73 7.07  0.22  1.39  6.65  48.6661
3     hp    88.25 -3.52 6.84  0.22  1.33  6.64  48.1843
1     hn    87.14 -3.95 7.25  0.23  1.34  6.71  46.5025
2     hn    86.71 -4.05 7.53  0.22  1.37  6.68  48.6661
3     hn    88.09 -3.44 5.55  0.21  1.19  6.67  47.222
;
proc glm data=s;
class rep trt;
model l = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model a = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model b = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ta = trt rep;
lsmeans trt/pdiff adjust=bon;
```

```
run;
proc glm data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ph = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ST = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glimmix data=s;
class rep trt;
model l= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model a= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model b= trt;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ta= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
```

```
model vis= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ph= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ST= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
      run;
```

SAS Code for Chapter 2: 10 V at 10 minutes

```
data s;
input rep$ trt$  l a b ta vis ph ST;
cards;

1      c      88.1   -4.32  5.82   0.23   1.23   6.69   46.47405855
2      c      88.83  -4.19  6.45   0.23   1.34   6.69   45.99091647
3      c      87.66  -3.92  6.1    0.22   1.36   6.73   47.19963926
1      hp     88.5   -4.25  6.51   0.24   1.37   6.68   46.95766362
2      hp     87.98  -4.22  6.53   0.24   1.38   6.66   45.99091647
3      hp     88.48  -4.21  6.54   0.24   1.39   6.68   46.23242953
1      hn     88.63  -3.9   5.98   0.24   1.38   6.73   47.92625611
2      hn     87.85  -4.24  6.76   0.24   1.4    6.69   46.47405855
3      hn     88.84  -4.33  6.57   0.22   1.39   6.68   47.44173003

;
proc glm data=s;
class rep trt;
model l = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model a = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
```

```
proc glm data=s;
class rep trt;
model b = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ta = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ph = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ST = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glimmix data=s;
class rep trt;
model l= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model a= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model b= trt;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
```

```
model ta= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ph= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ST= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
```

SAS Code for Chapter 2: 30 V at 10 minutes

```
data s;
input rep$ trt$  l a b ta vis ph ST;
cards;
1     c     88.1  -4.32 5.82  0.23  1.23  6.69  46.47405855
2     c     88.83 -4.19 6.45  0.23  1.34  6.69  45.99091647
3     c     87.66 -3.92 6.1   0.22  1.36  6.73  47.19963926
1     hp    88.27 -4.08 6.45  0.21  1.32  6.72  45.50823906
2     hp    88.73 -4.18 6.48  0.22  1.34  6.68  47.92625611
3     hp    87.85 -4.11 6.45  0.23  1.38  6.67  47.44173003
1     hn    89.22 -3.86 6.44  0.23  1.34  6.71  46.47405855
2     hn    89.61 -3.71 5.87  0.22  1.34  6.73  46.95766362
3     hn    88.94 -3.48 5.43  0.24  1.39  6.71  47.44173003
;
proc glm data=s;
class rep trt;
model l = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
```

```
proc glm data=s;
class rep trt;
model a = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model b = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ta = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ph = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glm data=s;
class rep trt;
model ST = trt rep;
lsmeans trt/pdiff adjust=bon;
run;
proc glimmix data=s;
class rep trt;
model l= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model a= trt rep;
```

```
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model b= trt;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ta= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model vis= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ph= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
proc glimmix data=s;
class rep trt;
model ST= trt rep;
lsmeans trt/ pdiff adjust=bon lines;
run;
```

Appendix D - ANOVA Tables with P-Values for Chapter 4

Table D.3: 10 V at 2 minutes for the variable L*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.212	0.106	0.88	0.4811
Rep	2	0.622	0.311	2.59	0.1902
Error	4	0.481	0.120		

Table D.4: 10 V at 2 minutes for the variable a*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.042	0.021	3.05	0.1570
Rep	2	0.032	0.016	2.29	0.2173
Error	4	0.028	0.007		

Table D.5: 10 V at 2 minutes for the variable b*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.800	0.400	3.62	0.1266
Rep	2	0.305	0.153	1.38	0.3496
Error	4	0.442	0.110		

Table D.6: 10 V at 2 minutes for the variable titratable acidity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.005	0.0002	7.00	0.0494
Rep	2	0.002	0.0001	3.00	0.1600
Error	4	0.001	0.00003		

Table D.7: 10 V at 2 minutes for the variable viscosity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.004	0.002	3.05	0.1568
Days	2	0.004	0.0002	0.35	0.7243

Error	4	0.003	0.0006		

Table D.8: 10 V at 2 minutes for the variable pH

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.0008	0.0004	1.57	0.3131
Rep	2	0.0011	0.0006	2.21	0.2254
Error	4	0.0010	0.0003		

Table D.9: 10 V at 2 minutes for the variable surface tension

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	3.182	1.591	5.06	0.0803
Rep	2	0.888	0.444	1.41	0.3438
Error	4	1.258	0.315		

Table D.10: 30 V at 2 minutes for the variable L*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.731	0.366	3.73	0.1217
Rep	2	1.199	0.600	6.12	0.0606
Error	4	0.392	0.098		

Table D.11: 30 V at 2 minutes for the variable a*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.161	0.081	2.55	0.1928
Rep	2	0.330	0.165	5.24	0.0764
Error	4	0.126	0.032		

Table D.12: 30 V at 2 minutes for the variable b*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.079	0.395	0.76	0.5235
Rep	2	0.949	0.475	0.92	0.4698
Error	4	2.069	0.517		

Table D.13: 30 V at 2 minutes for the variable titratable acidity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.0001	0.00004	0.73	0.5378
Rep	2	0.00002	0.00001	0.18	0.8403
Error	4	0.0002	0.0001		

Table D.14: 30 V at 2 minutes for the variable viscosity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.006	0.003	0.70	0.5482
Rep	2	0.011	0.005	1.24	0.3800
Error	4	0.017	0.004		

Table D.15: 30 V at 2 minutes for the variable pH

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.001	0.001	2.23	0.2238
Rep	2	0.002	0.001	4.55	0.0934
Error	4	0.001	0.0002		

Table D.16: 30 V at 2 minutes for the variable surface tension

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	1.426	0.713	1.27	0.3750
Rep	2	0.794	0.397	0.70	0.5467
Error	4	2.253	0.563		

Table D.17: 10 V at 10 minutes for the variable L*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.089	0.044	0.13	0.8813
Rep	2	0.054	0.027	0.08	0.9246
Error	4	1.362	0.340		

Table D.18: 10 V at 10 minutes for the variable a*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.012	0.006	0.13	0.8783
Rep	2	0.008	0.004	0.08	0.9201
Error	4	0.179	0.045		

Table D.19: 10 V at 10 minutes for the variable b*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.269	0.134	2.95	0.1631
Rep	2	0.348	0.174	3.83	0.1179
Error	4	0.182	0.046		

Table D.20: 10 V at 10 minutes for the variable titratable acidty

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.003	0.0001	4.00	0.1111
Rep	2	0.0002	0.0001	3.00	0.1600
Error	4	0.0001	0.00003		

Table D.21: 10 V at 10 minutes for the variable viscosity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.011	0.006	4.44	0.0964
Rep	2	0.005	0.003	1.97	0.2533
Error	4	0.005	0.001		

Table D.22: 10 V at 10 minutes for the variable pH

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.002	0.0008	1.59	0.3109
Rep	2	0.001	0.0003	0.67	0.5595
Error	4	0.002	0.001		

Table D.23: 10 V at 10 minutes for the variable surface tension

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	1.339	0.669	3.68	0.1241
Rep	2	1.611	0.810	4.42	0.9699
Error	4	0.729	0.182		

Table D.24: 30 V at 10 minutes for the variable L*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	2.078	1.039	60.62	0.0010
Rep	2	1.244	0.622	36.27	0.0027
Error	4	0.069	0.017		

Table D.25: 30 V at 10 minutes for the variable a*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.406	0.203	13.61	0.0164
Rep	2	0.102	0.051	3.43	0.1357
Error	4	0.060	0.149		

Table D.26: 30 V at 10 minutes for the variable b*

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.456	0.228	1.58	0.3123
Rep	2	0.135	0.067	0.47	0.6575
Error	4	0.578	0.144		

Table D.27: 30 V at 10 minutes for the variable titratable acidity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.0001	0.0001	0.82	0.5017
Rep	2	0.0001	0.00004	0.47	0.6553
Error	4	0.003	0.0001		

Table D.28: 30 V at 10 minutes for the variable viscosity

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.004	0.002	1.95	0.2561
Rep	2	0.010	0.005	5.19	0.0775
Error	4	0.017	0.001		

Table D.29: 30 V at 10 minutes for the variable pH

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.001	0.0005	0.80	0.5102
Rep	2	0.0001	0.00003	0.05	0.9518
Error	4	0.003	0.001		

Table D.30: 30 V at 10 minutes for the variable surface tension

Source	DF	Type 1 SS	Mean Square	F-Value	Pr>F
Trt	2	0.325	0.163	0.29	0.7596
Rep	2	2.274	1.137	2.06	0.2428
Error	4	2.208	0.552		

Appendix E - Tables and Figures for Dielectric Data

Table E.31: Statistical Results to determine if protein and sugar are significant to the formulation

Effect	Number DF	Den DF	F Value	P- Value
Sugar	4	16	1.22	0.3398
Protein	4	16	20.86	<0.001

Table E.32: Real test using contrast to determine if targeted protein content (%) (0-4) is significant to firmness when compared to a different level

Label	Estimate	Standard Error	DF	t-Value	P-value
0 vs 1	-21.66	7.44	20	-2.91	0.0086
1 vs 2	-26.57	7.44	20	-3.57	0.0019
2 vs 3	-21.66	7.44	20	-2.91	0.0086
3 vs 4	-26.57	7.44	20	-3.57	0.0019

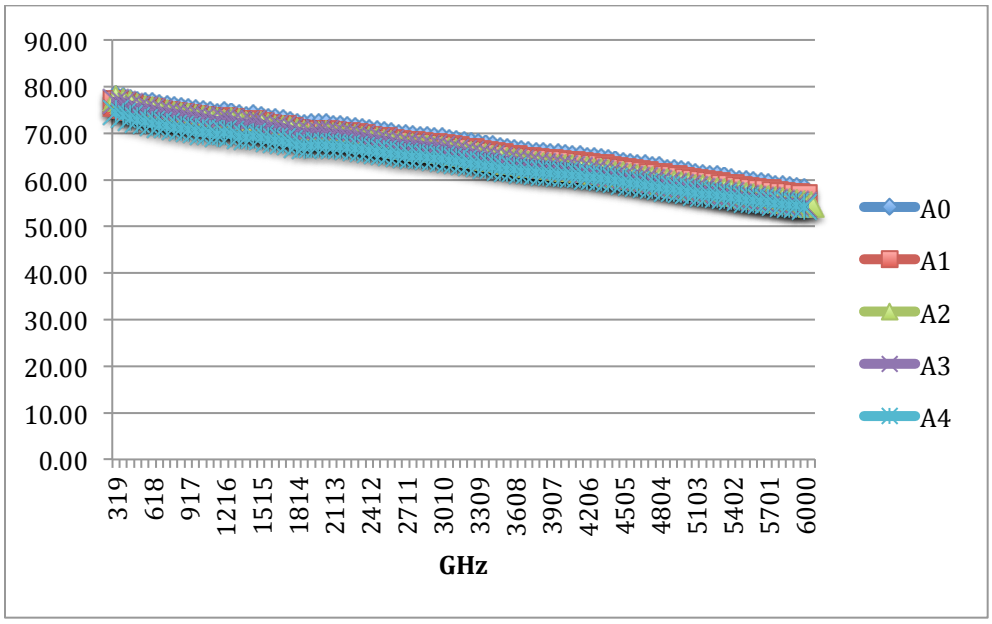
1=3.5%, 2=5.13%, 3=6.75%, 4=8.38%, 5=10%).

Table E.33: Estimate of firmness to determine if protein level is significant using measured firmness

Protein level	Estimated firmness	Standard error	DF	t Value	P Value
0	129.28	10.52	20	12.29	<0.0001
1	172.61	10.52	20	16.41	<0.0001
2	225.75	10.52	20	21.46	<0.0001
3	239.90	10.52	20	22.81	<0.0001
4	228.87	10.52	20	21.76	<0.0001

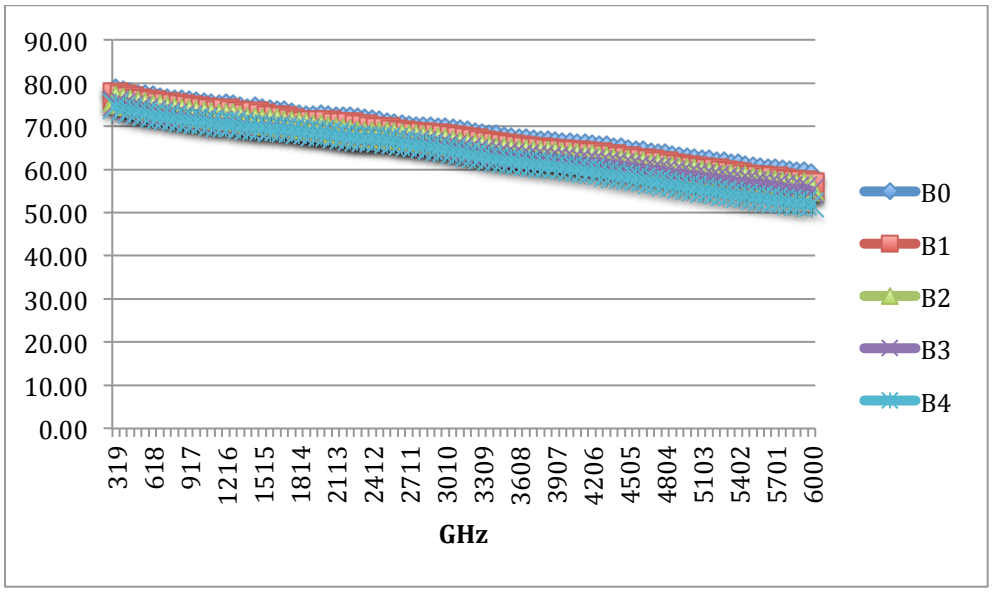
1=3.5%, 2=5.13%, 3=6.75%, 4=8.38%, 5=10%).

Figure E.1: Samples A0-A4 ε' spectra



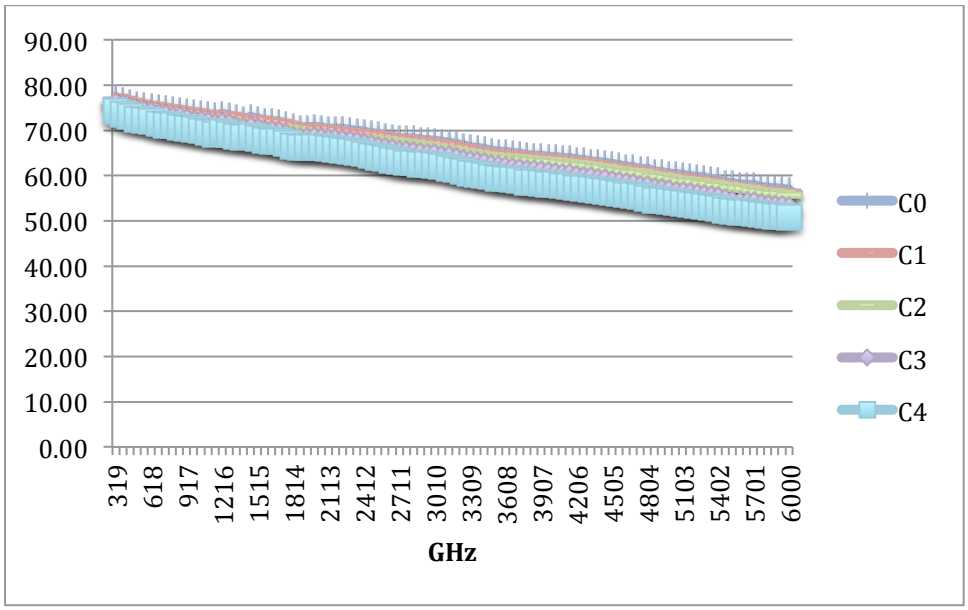
Samples read as A sugar content= 0 and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.2: Samples B0-B4 ε' spectra



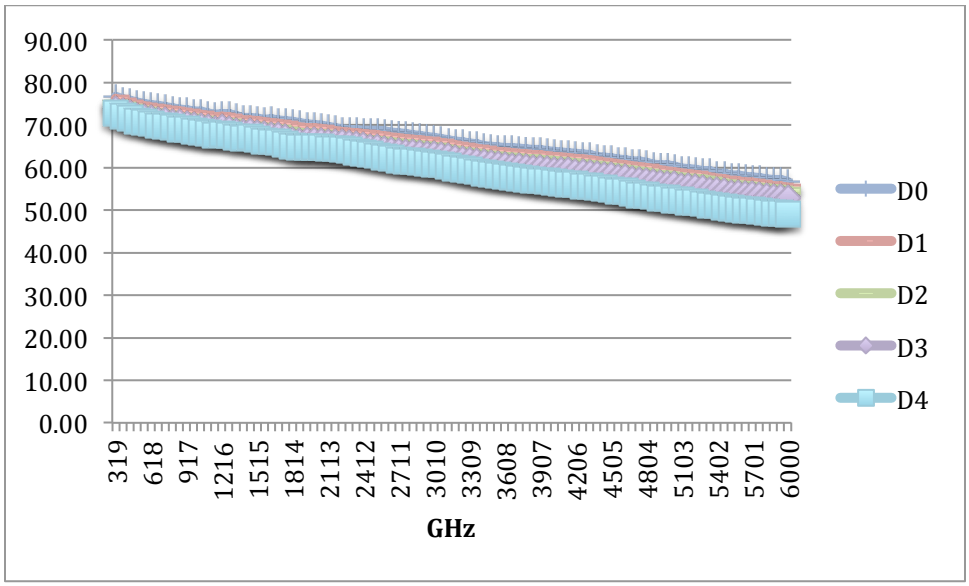
Samples read as B (sugar content= 1.25)and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.3: Samples C0-C4 ε' spectra



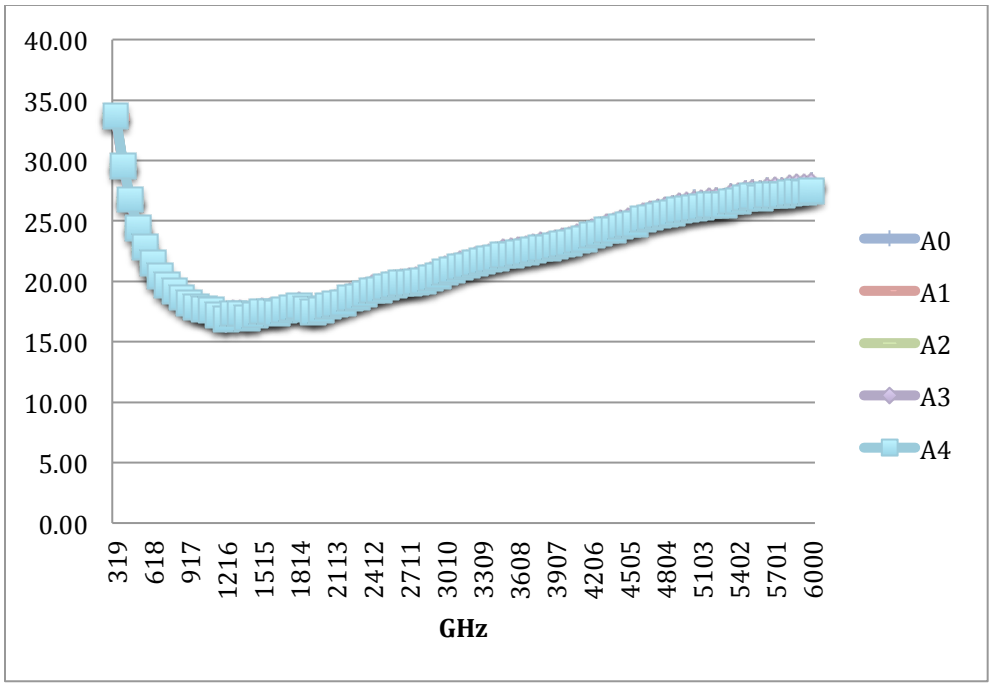
Samples read as C (sugar content- 2.5) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.4: Samples D0-D4 ε' spectra



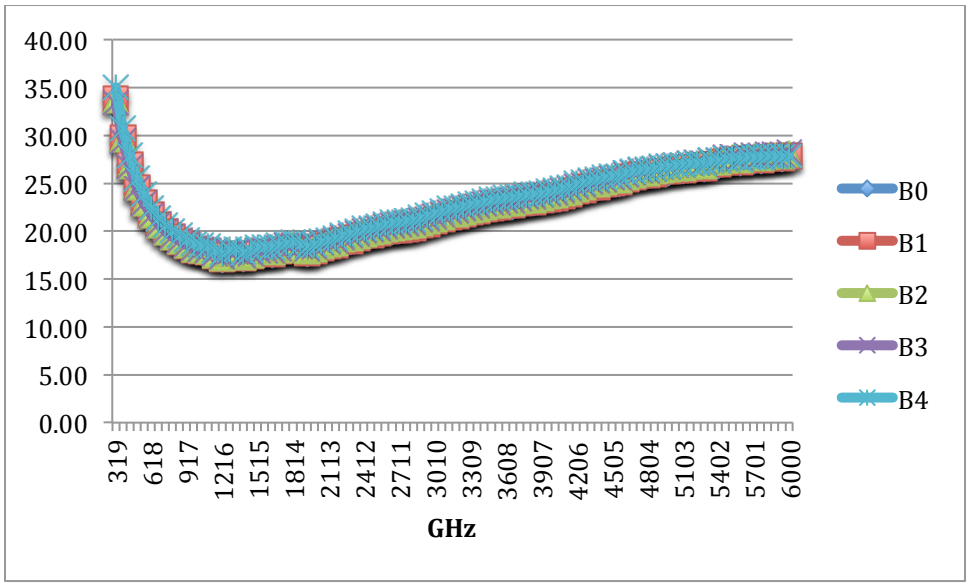
Samples read as D (sugar content=3.75) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.5: Samples A0-A4 ε" spectra



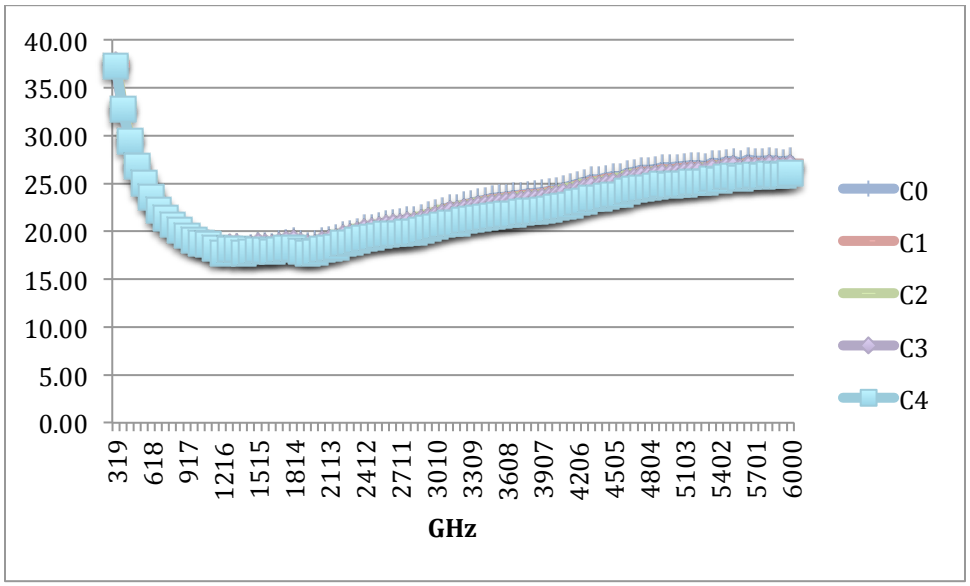
Samples read as A (sugar content=0) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.6: Samples B0-B4 ε" spectra



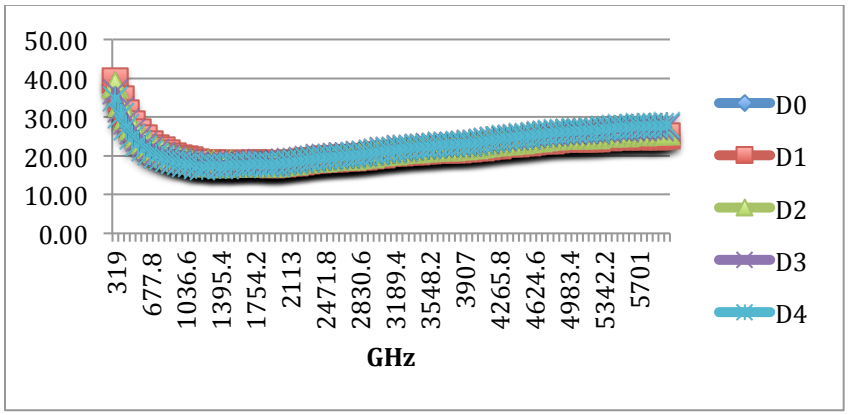
Samples read as B (sugar content=1.25) and 0, 1, 2, 3, 4 (increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.7: Samples C0-C4 ε" spectra



Samples read as C (sugar content=2.5) and 0, 1, 2, 3, 4 increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Figure E.8: Samples D0-D4 ε" spectra



Samples read as D (sugar content=3.75) and 0, 1, 2, 3, 4 increasing protein content (3.5, 5.13, 6.75, 8.38, 10%).

Appendix F - Step Wise Regression Results

A different type of statistical analysis was preformed to include all 101 frequencies for ε' and ε" in total for 202 frequencies and approximately 2,000 linear models developed to determine if any of the frequencies would be significant in developing a predictive model. By doing this, the limit of power of the model decreases since a guessing game has occurred, and extreme significance levels were used when developing the model. A sle of 0.4 was used which means any frequencies was a P value of less than 0.4 was included in the model, and sls of 0.3 was used which means any frequencies with a P value of less than 0.3 was to stay in the model. When a lower sls of 0.15 values was chosen only 2 frequencies variables remained in all the models for firmness, moisture and protein, therefore this limit was raised to 0.3. Step-wise regression was used to perform this analysis to determine predictive models for firmness, moisture, and protein. There are limitations to this method and one is that there is not guaranteed to have the best model when finished with the analysis.

This stepwise analysis produced a model with the same R^2 value for firmness of 0.59 as did with the backward selection. It is interesting to note that it included different frequencies, though.

$$Firmness = 853.9 + 230.8(\varepsilon' 5282) - 244.5(\varepsilon' 5402)$$

As for protein and moisture prediction the R^2 was much higher at 0.95 for both models. These are very high R^2 indicating that protein and moisture are very highly correlated to the frequencies .

$$Protein = -42.72 + 0.41(\varepsilon' 0.0002) + 1.12(\varepsilon' 136) - 8.64(\varepsilon' 5402) + 7.41(\varepsilon' 5940)$$

$$Moisture = 18.75 + 17.05(\varepsilon'5103) - 14.43(\varepsilon'5282) - 11.10(\varepsilon'5461) + 9.44(\varepsilon'5521)$$
$$+ 2.83(\varepsilon"3667) - 18.69(\varepsilon"5402) + 16.20(\varepsilon"5581)$$

Differences between predicted and real firmness values can be seen in Table F.1. The % variability still varied a lot due to the low R^2 value of the firmness predictive model. Protein discrepancies between predicted and measured values can be seen in Table F.2. Even though the R^2 was higher for the model, the model is no more accurate than the firmness model. Moisture results obtained from the predictive model can be seen in Table F.3. The predicted results were much closer together than the previous 2 models for firmness and protein.

It is interesting to note in both the backwards and stepwise results that the ε' samples were always included in the model whereas some of the ε" was only included in the moisture model. A future area to further study would be why some of these same frequencies reappear in all the models in the step-wise selection method, e.g. frequencies ε'(5282) and ε'(5402) appear in all three models. Doing step-wise selection for this data set to develop models resulted in frequencies that are at the ends of the frequency spectra where you would usually expect to find noise. If a future study were to be conducted, protein and sugar contents should be investigated separately as to define which variable affects dielectric properties. In this study it is hard to tell which variable is to blame for not achieving a significant model to predict firmness.

Once the models were developed using step-wise regression PCA was performed to determine correlations and groupings between samples and the variables (firmness, protein, moisture, and the 7 frequencies discussed in the model development). PCA accounted for 92.54% of the total variability with PC1 describing 68.85 and PC2 describing 23.69%. The

frequencies were highly correlated to them selves in 2 separate groupings as seen in Figure F.1. Moisture was highly correlated to 1 of these clusters. Protein and firmness was highly correlated to each other but not correlated with the frequencies. The samples were also grouped together by protein content where 3's and 4's were on the left side of the map and 0's, 1's, 2's, and 3's were on the right side of the map.

Table F.34: Measured and predicted firmness (g) values using the firmness model generated

Sample	Measured Firmness (g)	Predicted firmness (g)	% Variability
A0	128.93	195.16	33.94
A4	256.76	261.2	1.7
C2	104.39	273.49	61.83
E0	135.89	141.88	4.22
E4	240.5	276.55	13.04
Sample 1	358.74	227.51	57.68
Sample 2	128.11	200.43	36.08

A-E (increasing sugar content) (0-5%) and 0-4 (increasing protein content) (3.5-10%), Sample 1(Fage total 0%) and Sample 2 (Kroger blended plain yogurt) %Variability is found by taking the absolute value of the difference between measured and predicted divided by the measured

$$Firmness = 853.9 + 230.8(\varepsilon' 5282) - 244.5(\varepsilon' 5402)$$

Table F.35: Measured and predicted protein contents using the protein model generated

Sample	Measured protein	Predicted protein	% Variability
A0	3.43	6.97	50.81
A4	5.88	17.36	66.14
C2	3.39	11.56	70.69
E0	3.42	14.43	76.3
E4	9.44	7	34.84
Sample 1	10.18	16.2	37.18
Sample 2	4.19	15.3	72.62

A-E (increasing sugar content) (0-5%) and 0-4 (increasing protein content) (3.5-10%), Sample 1(Fage total 0%) and Sample 2 (Kroger blended plain yogurt) %Variability is found by taking the absolute value of the difference between measured and predicted divided by the measured

$$Protein = -42.72 + 0.41(\varepsilon' 0.0002) + 1.12(\varepsilon' 136) - 8.64(\varepsilon' 5402) + 7.41(\varepsilon' 5940)$$

Table F.36: Measured and predicted moisture content (%) using the moisture-generated model

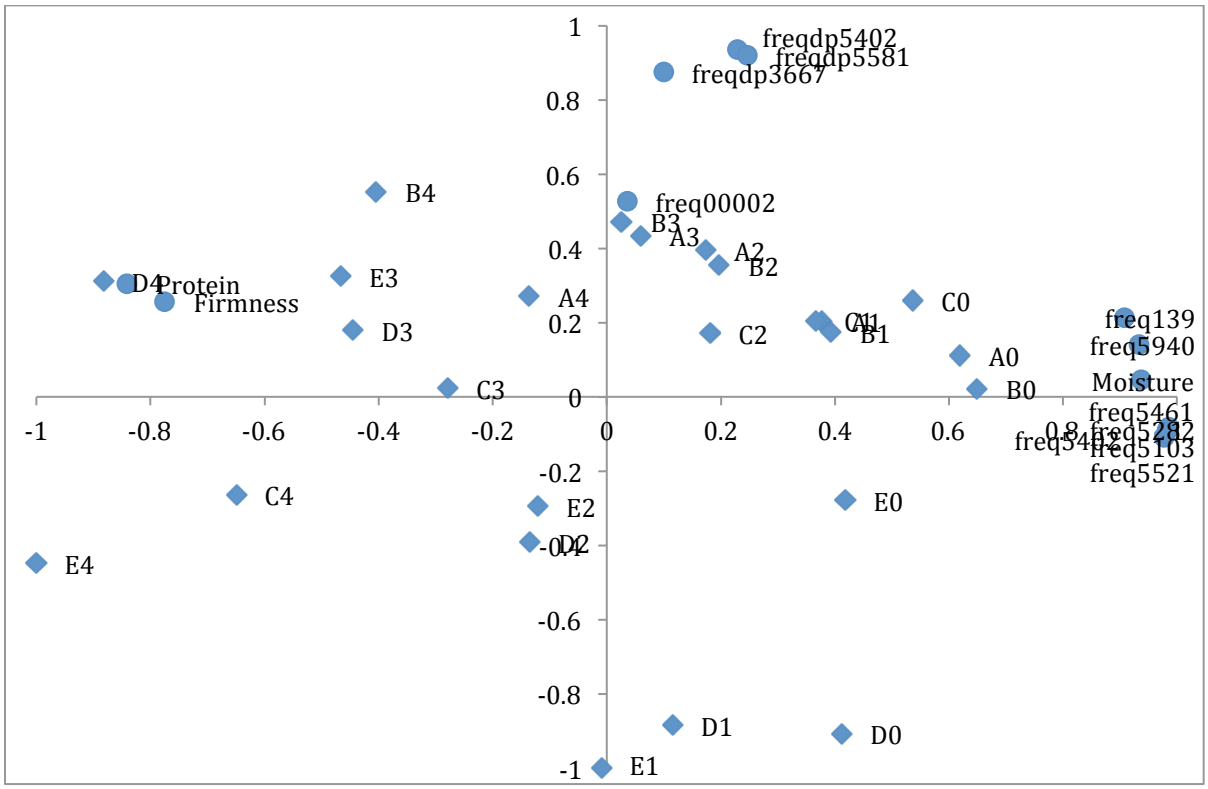
Samples	Measured moisture (%)	Predicted moisture (%)	% Variability
A0	87.85	86.6	1.45
A4	80.15	88.18	9.1
C2	83.36	81.54	2.23
E0	84.2	85.41	1.42
E4	79.5	82.13	3.2
Sample 1	68.37	89.16	23.3
Sample 2	75.57	83.54	9.54

A-E (increasing sugar content) (0-5%) and 0, 2, 4 (increasing protein content) (3.5, 6.75, & 10%), Sample 1(Fage total 0%) and Sample 2 (Kroger blended plain yogurt). % Variability is found by taking the absolute value of the difference between measured and predicted divided by the measured value

$$Moisture = 18.75 + 17.05(\varepsilon'5103) - 14.43(\varepsilon'5282) - 11.10(\varepsilon'5461) + 9.44(\varepsilon'5521)$$

$$+ 2.83(\varepsilon"3667)\text{-}18.69(\varepsilon"5402) + 16.20(\varepsilon"5581$$

Figure F.9: Principal Component Analysis data plot for the significant frequencies that made up the firmness, moisture, and protein predictive models.



● Variables ◆ Yogurt

Appendix G - SAS code for Chapter 5

SAS Code for Backwards Selection

```
data yogurt;
input obs sugar $ prot $ sugar_prot $ e1_319 e1_917 e1_2471 e1_6000 e2_319 e2_917
e2_2471 e2_6000 protein moisture firmness;
datalines;
1 A 0 A0 77.45 74.67 69.83 57.41 33.63 18.15 19.42 28.00 3.12 88.2
  127.235
2 A 1 A1 76.48 73.43 68.50 56.08 33.56 18.19 19.50 27.99 5.04 88.7
  155.75
3 A 2 A2 76.82 73.45 68.02 54.70 33.63 18.27 19.62 28.07 6.87 86.6
  219.485
4 A 3 A3 76.86 73.57 68.44 54.58 33.56 18.23 19.51 27.86 9.57 84.2
  240.855
5 A 4 A4 74.49 70.80 66.06 54.23 33.66 18.23 19.31 27.49 10.3 83.3
  172.325
6 B 0 B0 77.85 75.14 70.42 58.33 33.89 18.39 19.48 27.74 3.45 85.9
  139.665
7 B 1 B1 77.05 74.02 68.96 56.28 33.81 18.45 19.62 27.86 5.12 87.2
  170.505
8 B 2 B2 76.41 73.06 68.05 55.58 33.67 18.53 19.86 28.02 7.01 85.3
  243.215
9 B 3 B3 75.35 71.73 66.83 55.06 33.39 18.60 20.13 28.22 8.67 83.5
  229.74
10 B 4 B4 74.65 71.31 66.89 51.80 35.02 19.08 20.39 27.87 10.58 81.
8 228.06
11 C 0 C0 77.26 74.38 69.30 56.85 37.09 19.64 20.51 27.42 3.42 88.7
  129.305
12 C 1 C1 77.23 74.22 69.04 56.04 37.49 19.78 20.52 27.17 5.04 86
```

182.795
13 C 2 C2 76.28 72.90 67.90 55.61 37.98 19.91 20.47 26.99 6.88 84.3
208.695
14 C 3 C3 75.44 71.87 66.02 52.33 37.40 19.67 20.00 26.56 8.46 82.8
200.445
15 C 4 C4 74.34 70.52 64.40 50.86 37.26 19.55 19.39 26.03 10.3 80.8
251.68
16 D 0 D0 76.57 73.52 68.65 56.62 38.24 19.76 19.13 25.45 3.33 87.
7 110.045
17 D 1 D1 76.17 72.89 67.66 55.22 39.50 20.05 19.10 25.12 5.3 84.6
161.675
18 D 2 D2 75.20 71.74 66.47 54.46 38.24 19.73 19.38 26.23 7.09 82.
7 244.71
19 D 3 D3 73.81 70.27 64.90 52.76 36.41 19.22 19.73 27.36 8.48 81.
4 273.01
20 D 4 D4 72.90 68.90 63.14 48.90 34.65 18.50 19.51 27.92 10.66 79
.2 222.88
21 E 0 E0 76.69 73.73 68.87 56.83 32.01 17.80 18.74 27.75 3.33 84.9
140.16
22 E 1 E1 74.72 71.50 66.76 55.97 34.40 18.30 18.21 25.62 5.33 83.2
192.33
23 E 2 E2 74.89 71.43 66.22 54.25 33.00 18.32 18.93 27.21 6.87 82.7
212.63
24 E 3 E3 73.98 70.30 64.83 51.21 32.91 18.64 19.91 28.26 6.19 81
255.465
25 E 4 E4 72.41 68.37 62.36 49.21 31.22 18.23 19.73 25.45 10.62 78.
9 269.4

;
run;
proc print data=yogurt;

```
run;
*backward selection to find the model for e1 e2 to predict firmness;
proc reg data = yogurt;
backwardselect: model firmness = e1_319 e1_917 e1_2471 e1_6000 e2_319 e2_917 e2_2471
e2_6000 /selection=backward aic slstay = 0.05;
run;
*result is : e1_319, e1_917;
*backward selection to find e1 e2 to predict protein;
proc reg data = yogurt;
backwardselec: model protein = e1_319 e1_917 e1_2471 e1_6000 e2_319 e2_917 e2_2471
e2_6000 /selection=backward aic slstay = 0.05 ;
run;
*result is e1_319 e1-917 e1-2471 e1-6000;
*backward selection to find e1 e2 to predict moisture;
proc reg data = yogurt;
backwardselec: model moisture = e1_319 e1_917 e1_2471 e1_6000 e2_319 e2_917 e2_2471
e2_6000 /selection=backward aic slstay = 0.05 ;
run;
*result is e1_-917;


*model to see relationship between firmness and sugar and protein catergorical data;
proc means data=yogurt mean std min max;
class sugar prot;
var firmness;
run;
proc mixed data=yogurt;
class sugar prot sugar_prot;
model firmness=sugar prot/ ddfm=kr outp=diagnostics residual;
run;
*sugar is not significant, only use protein;
```

```
proc mixed data=yogurt;
class sugar prot sugar_prot;
model firmness=sugar prot/ ddfm=kr;
run;
proc mixed data=yogurt;
class sugar prot sugar_prot;
model firmness=prot/ ddfm=kr;
lsmeans prot;
estimate '0 vs 1' prot 1 -1 0 0 0/divisor=2 e;
estimate '1 vs 2' prot 0 1 -1 0 0/divisor=2 e;
estimate '2 vs 3' prot 1 -1 0 0 0/divisor=2 e;
estimate '3 vs 4' prot 0 1 -1 0 0/divisor=2 e;
run;
*they are sinificant different from each others;
symbol1 value=dot color=blue interpol=join;
data estimated_prot;
input prot estimate;
datalines;
0    129.28
1    172.61
2    225.75
3    239.9
4    228.87
;
run;
proc gplot data=estimated_prot;
plot estimate*prot;
run;
```

SAS Code using Stepwise Regression

```
data yogurte1;
> input obs sugar $ prot $ sugar_prot $
e1_1  e1_2  e1_3  e1_4  e1_5  e1_6  e1_7  e1_8  e1_9  e1_10
> e1_11
e1_12  e1_13  e1_14  e1_15  e1_16  e1_17  e1_18  e1_19  e1_20  e1_21  e1_22  e1_23
 e1_24
> e1_25
e1_26  e1_27  e1_28  e1_29  e1_30  e1_31  e1_32  e1_33  e1_34  e1_35  e1_36  e1_37
 e1_38
> e1_39
e1_40  e1_41  e1_42  e1_43  e1_44  e1_45  e1_46  e1_47  e1_48  e1_49  e1_50  e1_51
 e1_52
> e1_53
e1_54  e1_55  e1_56  e1_57  e1_58  e1_59  e1_60  e1_61  e1_62  e1_63  e1_64  e1_65
 e1_66
> e1_67
e1_68  e1_69  e1_70  e1_71  e1_72  e1_73  e1_74  e1_75  e1_76  e1_77  e1_78  e1_79
 e1_80
> e1_81
e1_82  e1_83  e1_84  e1_85  e1_86  e1_87  e1_88  e1_89  e1_90  e1_91  e1_92  e1_93
 e1_94
> e1_95 e1_96  e1_97  e1_98  e1_99  e1_100  e1_101  protein moisture firmness;
> datalines;
>
1  A  0  A0  109.96 82.31  79.69  78.49  77.92  77.45  77.02  76.60  76.18  76.
06  75.81  75.44  75.29  75.07  74.87  74.67  74.39  74.18  73.93  73.60  74.00  73.71
73.22  72.97  73.31  72.95  72.60  72.38  72.31  71.94  71.47  71.24  71.32  71.45  71.3
1  71.07  70.98  70.86  70.56  70.41  70.14  69.83  69.67  69.41  69.17  68.95  68.82  6
```

8.76 68.58 68.46 68.31 68.05 67.83 67.57 67.35 67.05 66.78 66.64 66.30 66.06 65.88 65.66 65.43 65.29 65.15 65.02 64.87 64.80 64.60 64.50 64.22 64.08 63.91 63.66 63.42 63.15 62.89 62.72 62.55 62.24 62.14 61.62 61.54 61.34 61.08

60.80 60.51 60.39 60.18 59.90 59.59 59.31 59.06 58.93 58.74 58.59 58.22 58.04 57.82 57.70 57.41 3.12 88.2 127.235

>

2 A 1 A1 110.10 81.96 78.99 77.53 76.94 76.48 75.92 75.52 75.04 74.94 74.60 74.25 74.07 73.88 73.69 73.43 73.16 72.95 72.73 72.39 72.72 72.49 71.98 71.78 72.03 71.64 71.26 71.06 70.98 70.55 70.09 69.83 69.95 70.03 69.92 69.69 69.63 69.52 69.22 69.06 68.79 68.50 68.30 68.04 67.83 67.59 67.44 67.41 67.19 67.11 66.93 66.70 66.48 66.18 65.94 65.66 65.37 65.24 64.90 64.67 64.49 64.26 64.05 63.90 63.72 63.61 63.48 63.40 63.18 63.13 62.84 62.67 62.53 62.26 62.00 61.76 61.53 61.35 61.15 60.83 60.73 60.28 60.17 59.91 59.63

59.46 59.12 58.99 58.79 58.46 58.26 57.85 57.72 57.49 57.33 57.22 56.86 56.64 56.50 56.39 56.08 5.04 88.7 155.75

>

3 A 2 A2 110.82 82.56 79.47 78.03 77.41 76.82 76.27 75.76 75.27 75.11 74.76 74.37 74.12 73.88 73.71 73.45 73.17 72.94 72.67 72.33 72.66 72.38 71.84 71.60 71.90 71.51 71.11 70.89 70.79 70.36 69.87 69.60 69.67 69.79 69.59 69.36 69.25 69.10 68.78 68.62 68.33 68.02 67.82 67.54 67.28 67.04 66.87 66.80 66.57 66.41 66.27 66.02 65.77 65.48 65.22 64.89 64.67 64.48 64.11 63.89 63.69 63.43 63.21 63.09 62.89 62.73 62.60 62.49 62.27 62.19 61.92 61.71 61.46 61.22 60.98 60.72 60.46 60.27 60.06 59.72 59.59 59.14 58.99 58.73 58.49

58.24 57.93 57.81 57.51 57.25 57.00 56.60 56.42 56.23 56.02 55.88 55.56 55.32 55.11 54.99 54.70 6.87 86.6 219.485

>

4 A 3 A3 112.92 82.57 79.75 78.14 77.48 76.86 76.38 75.84 75.34 75.24 74.87 74.49 74.25 74.06 73.82 73.57 73.36 73.15 72.82 72.56 72.95 72.65

72.19 71.97 72.25 71.85 71.38 71.19 71.13 70.72 70.22 69.97 70.03 70.20 70.0
4 69.82 69.73 69.57 69.27 69.09 68.79 68.44 68.27 67.99 67.70 67.47 67.39 6
7.26 67.00 66.90 66.70 66.38 66.17 65.80 65.59 65.22 64.93 64.77 64.38 64.13
63.90 63.61 63.42 63.23 63.02 62.88 62.67 62.59 62.37 62.25 61.91 61.72 61
.53 61.19 60.96 60.68 60.34 60.13 59.91 59.57 59.42 58.94 58.82 58.57 58.24

57.96 57.67 57.45 57.38 56.97 56.85 56.38 56.23 56.00 55.76 55.74 55.32 55.1
9 55.01 54.91 54.58 9.57 84.2 240.855
>
5 A 4 A4 117.35 81.54 77.72 75.91 75.10 74.49 73.87 73.27 72.80 72.
55 72.17 71.77 71.58 71.27 71.06 70.80 70.52 70.31 70.04 69.75 70.07 69.83
69.34 69.10 69.40 69.04 68.69 68.48 68.39 68.04 67.55 67.29 67.41 67.53 67.3
8 67.17 67.12 66.96 66.74 66.57 66.30 66.06 65.82 65.63 65.38 65.18 65.03 6
5.00 64.74 64.67 64.51 64.24 64.03 63.76 63.53 63.23 62.99 62.88 62.53 62.31
62.14 61.93 61.73 61.62 61.44 61.34 61.23 61.12 60.90 60.88 60.56 60.45 60
.25 60.02 59.80 59.51 59.30 59.14 58.89 58.61 58.48 58.07 58.00 57.76 57.46

57.26 56.97 56.85 56.69 56.39 56.22 55.92 55.69 55.53 55.28 55.28 54.96 54.7
8 54.60 54.49 54.23 10.3 83.3 172.325
>
6 B 0 B0 109.59 82.33 79.74 78.71 78.23 77.85 77.45 76.94 76.54 76.
53 76.21 75.86 75.71 75.50 75.38 75.14 74.86 74.68 74.39 74.11 74.46 74.11
73.66 73.45 73.81 73.47 73.10 72.91 72.84 72.44 71.95 71.69 71.74 71.90 71.7
2 71.54 71.50 71.44 71.15 70.98 70.68 70.42 70.23 69.93 69.67 69.43 69.33 6
9.33 69.12 69.01 68.95 68.71 68.50 68.20 67.86 67.60 67.33 67.18 66.83 66.65
66.47 66.34 66.16 66.00 65.80 65.73 65.50 65.44 65.18 65.11 64.96 64.78 64
.64 64.37 64.21 63.97 63.71 63.46 63.23 62.93 62.88 62.42 62.24 61.99 61.87

61.61 61.34 61.13 61.04 60.68 60.52 60.10 59.78 59.59 59.51 59.43 59.14 58.9
5 58.73 58.70 58.33 3.45 85.9 139.665
>

7 B 1 B1 109.35 82.19 79.46 78.05 77.47 77.05 76.56 76.08 75.59 75.
53 75.15 74.80 74.67 74.46 74.29 74.02 73.76 73.53 73.31 72.96 73.26 72.99
72.50 72.30 72.59 72.17 71.80 71.60 71.51 71.06 70.56 70.29 70.41 70.53 70.3
9 70.17 70.08 70.01 69.69 69.54 69.25 68.96 68.76 68.47 68.23 67.99 67.80 6
7.79 67.58 67.48 67.34 67.11 66.85 66.55 66.29 66.00 65.72 65.55 65.21 65.00
64.82 64.60 64.41 64.21 64.06 63.93 63.80 63.70 63.48 63.40 63.14 62.97 62
.82 62.59 62.31 62.09 61.87 61.67 61.38 61.07 60.96 60.49 60.33 60.13 59.84

59.68 59.39 59.27 59.08 58.75 58.53 58.01 57.91 57.67 57.49 57.49 57.13 56.9
0 56.72 56.64 56.28 5.12 87.2 170.505
>
8 B 2 B2 108.50 82.01 79.33 77.93 76.78 76.41 75.93 75.38 75.07 74.
59 74.35 74.10 73.82 73.55 73.31 73.06 72.82 72.71 72.35 72.05 72.10 72.14
71.59 71.26 71.23 71.12 70.88 70.88 70.63 70.45 70.29 69.95 69.63 69.53 69.3
4 69.00 68.61 68.38 68.24 68.14 68.13 68.05 68.06 67.86 67.64 67.40 67.33 6
7.08 66.83 66.56 66.36 66.06 65.78 65.42 65.19 64.98 64.77 64.40 64.29 64.11
63.93 63.88 63.66 63.57 63.46 63.38 63.15 63.02 62.83 62.54 62.53 62.26 62
.15 61.84 61.62 61.36 61.14 60.96 60.63 60.57 60.26 59.94 59.69 59.54 59.29

58.94 58.77 58.65 58.21 58.12 57.69 57.67 57.21 57.11 56.91 56.66 56.42 56.1
2 55.92 56.02 55.58 7.01 85.3 243.215
>
9 B 3 B3 108.53 81.67 78.49 77.02 75.77 75.35 74.70 74.15 73.82 73.
36 73.09 72.80 72.48 72.23 71.98 71.73 71.49 71.34 70.91 70.69 70.70 70.75
70.24 69.87 69.82 69.74 69.51 69.54 69.27 69.12 68.96 68.70 68.39 68.28 68.1
7 67.81 67.50 67.20 67.08 66.97 66.91 66.83 66.78 66.64 66.37 66.18 66.07 6
5.81 65.63 65.36 65.18 64.93 64.64 64.32 64.11 63.88 63.76 63.35 63.22 63.06
62.91 62.87 62.67 62.60 62.44 62.44 62.20 62.07 61.91 61.69 61.60 61.42 61
.24 60.90 60.76 60.49 60.35 60.08 59.81 59.80 59.44 59.11 58.96 58.80 58.51

58.22 58.01 57.91 57.50 57.41 56.97 56.95 56.62 56.53 56.32 56.11 55.83 55.5

6 55.39 55.46 55.06 8.67 83.5 229.74
>
10 B 4 B4 107.96 80.70 77.55 76.26 75.02 74.65 74.05 73.59 73.24 72.
78 72.56 72.29 72.05 71.75 71.54 71.31 71.02 70.98 70.59 70.40 70.48 70.53
70.01 69.65 69.65 69.52 69.33 69.41 69.17 69.05 68.89 68.66 68.35 68.36 68.1
8 67.93 67.57 67.30 67.19 67.08 66.99 66.89 66.80 66.52 66.18 65.91 65.75 6
5.43 65.11 64.82 64.56 64.22 63.85 63.46 63.17 62.92 62.63 62.21 62.02 61.83
61.60 61.44 61.18 61.04 60.79 60.68 60.41 60.18 59.95 59.61 59.47 59.20 58
.93 58.55 58.34 58.06 57.82 57.51 57.18 57.07 56.65 56.34 56.05 55.84 55.58

55.22 54.97 54.80 54.48 54.31 53.85 53.80 53.40 53.28 53.11 52.88 52.61 52.3
4 52.13 52.24 51.80 10.58 81.8 228.06
>
11 C 0 C0 108.15 81.67 79.34 78.19 77.67 77.26 76.78 76.26 75.82 75.
76 75.50 75.18 74.96 74.74 74.59 74.38 74.10 73.94 73.62 73.26 73.62 73.33
72.92 72.67 72.95 72.56 72.18 71.99 71.93 71.46 70.92 70.64 70.68 70.82 70.6
8 70.43 70.40 70.34 70.07 69.90 69.62 69.30 69.18 68.82 68.55 68.29 68.18 6
8.18 67.92 67.83 67.71 67.49 67.26 66.91 66.62 66.28 66.07 65.88 65.50 65.29
65.22 64.97 64.80 64.63 64.46 64.35 64.18 64.08 63.80 63.79 63.53 63.38 63
.21 62.97 62.80 62.56 62.30 62.06 61.80 61.50 61.44 60.91 60.77 60.50 60.30

60.19 59.85 59.72 59.55 59.21 59.05 58.53 58.32 58.11 57.97 57.96 57.53 57.3
9 57.16 57.11 56.85 3.42 88.7 129.305
>
12 C 1 C1 105.39 82.10 79.68 78.44 77.70 77.23 76.78 76.30 75.97 75.
69 75.42 75.10 74.88 74.66 74.47 74.22 73.95 73.79 73.48 73.18 73.38 73.18
72.73 72.44 72.55 72.27 71.94 71.85 71.65 71.30 70.89 70.61 70.48 70.49 70.3
5 70.09 69.90 69.75 69.55 69.38 69.25 69.04 68.91 68.66 68.37 68.13 68.00 6
7.87 67.65 67.46 67.27 67.02 66.76 66.44 66.09 65.83 65.58 65.33 65.01 64.85
64.68 64.52 64.37 64.22 64.06 63.97 63.77 63.65 63.47 63.26 63.14 62.95 62
.77 62.50 62.26 62.05 61.75 61.54 61.24 61.10 60.84 60.41 60.26 60.05 59.77

59.48 59.27 59.10 58.82 58.60 58.26 58.02 57.73 57.55 57.37 57.20 56.87 56.7
0 56.45 56.48 56.04 5.04 86 182.795
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13 C 2 C2 105.84 81.51 79.09 77.62 76.53 76.28 75.70 75.19 74.82 74.
43 74.20 73.94 73.64 73.28 73.08 72.90 72.60 72.44 72.13 71.83 71.89 71.88
71.39 71.02 71.01 70.92 70.68 70.70 70.42 70.27 70.09 69.81 69.49 69.40 69.2
2 68.92 68.54 68.27 68.11 68.01 67.96 67.90 67.86 67.69 67.43 67.25 67.15 6
6.90 66.66 66.42 66.21 65.97 65.67 65.32 65.09 64.86 64.69 64.32 64.21 64.01
63.88 63.81 63.56 63.51 63.38 63.34 63.08 62.96 62.81 62.52 62.47 62.27 62
.10 61.80 61.66 61.33 61.19 60.94 60.64 60.54 60.27 59.95 59.75 59.63 59.25

58.95 58.78 58.64 58.27 58.17 57.70 57.72 57.31 57.20 57.01 56.78 56.45 56.2
5 56.01 56.12 55.61 6.88 84.3 208.695
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14 C 3 C3 108.62 80.83 78.22 76.79 76.05 75.44 74.83 74.35 73.83 73.
65 73.32 72.88 72.62 72.34 72.16 71.87 71.55 71.34 71.04 70.67 70.91 70.58
70.10 69.85 70.08 69.68 69.25 69.03 68.89 68.45 67.87 67.62 67.70 67.80 67.6
4 67.41 67.36 67.26 66.93 66.68 66.35 66.02 65.73 65.40 65.07 64.78 64.63 6
4.57 64.31 64.18 64.01 63.77 63.52 63.15 62.87 62.50 62.27 62.05 61.65 61.38
61.25 61.05 60.83 60.64 60.44 60.30 60.12 59.92 59.66 59.60 59.32 59.10 58
.93 58.66 58.45 58.17 57.91 57.65 57.42 57.11 56.98 56.50 56.30 56.07 55.84

55.69 55.36 55.27 55.02 54.73 54.48 54.08 53.77 53.53 53.49 53.35 53.06 52.8
7 52.71 52.66 52.33 8.46 82.8 200.445
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15 C 4 C4 109.19 80.46 77.33 75.72 74.93 74.34 73.67 73.09 72.54 72.
32 72.01 71.55 71.26 71.04 70.78 70.52 70.17 69.97 69.63 69.24 69.51 69.15
68.66 68.45 68.71 68.28 67.88 67.66 67.54 67.06 66.54 66.24 66.29 66.39 66.2
0 65.97 65.84 65.73 65.36 65.11 64.77 64.40 64.15 63.79 63.41 63.13 62.93 6
2.86 62.55 62.41 62.23 61.96 61.68 61.34 60.97 60.64 60.34 60.15 59.73 59.51

59.32 59.13 58.91 58.67 58.49 58.34 58.15 57.98 57.75 57.66 57.36 57.22 57
.01 56.74 56.52 56.30 56.01 55.80 55.57 55.29 55.14 54.66 54.54 54.33 54.12

53.92 53.64 53.51 53.30 52.97 52.82 52.42 52.10 51.94 51.83 51.75 51.48 51.3
0 51.12 51.08 50.86 10.3 80.8 251.68
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16 D 0 D0 103.49 81.36 78.91 77.92 76.82 76.57 76.09 75.66 75.33 74
.92 74.68 74.47 74.18 73.86 73.70 73.52 73.23 73.11 72.74 72.47 72.57 72.58

72.08 71.72 71.69 71.53 71.29 71.30 71.06 70.92 70.76 70.46 70.16 70.07 69.9
1 69.60 69.26 69.00 68.85 68.75 68.73 68.65 68.63 68.44 68.20 67.99 67.88 6
7.64 67.41 67.20 66.98 66.72 66.48 66.14 65.90 65.67 65.47 65.10 64.97 64.80
64.63 64.59 64.38 64.30 64.10 64.11 63.87 63.77 63.58 63.34 63.26 63.07 62
.90 62.54 62.41 62.14 61.92 61.74 61.51 61.41 61.09 60.77 60.53 60.50 60.20

59.85 59.67 59.52 59.14 59.14 58.65 58.64 58.21 58.09 57.88 57.75 57.51 57.2
4 57.02 57.07 56.62 3.33 87.7 110.045
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17 D 1 D1 104.89 81.27 78.64 77.31 76.45 76.17 75.64 75.09 74.66 74
.45 74.13 73.81 73.60 73.34 73.12 72.89 72.61 72.45 72.11 71.79 71.96 71.82

71.31 71.02 71.15 70.89 70.55 70.46 70.27 70.01 69.61 69.35 69.23 69.24 69.0
8 68.80 68.57 68.40 68.20 68.05 67.89 67.66 67.59 67.34 67.05 66.85 66.73 6
6.62 66.37 66.19 66.02 65.77 65.50 65.17 64.86 64.64 64.42 64.13 63.90 63.70
63.56 63.42 63.24 63.11 62.93 62.89 62.68 62.56 62.34 62.15 62.03 61.86 61
.69 61.37 61.21 60.98 60.75 60.54 60.28 60.08 59.92 59.45 59.28 59.10 58.85

58.63 58.41 58.25 57.91 57.70 57.40 57.20 56.80 56.72 56.52 56.49 56.15 55.9
1 55.64 55.62 55.22 5.3 84.6 161.675
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18 D 2 D2 106.58 80.57 77.85 76.30 75.53 75.20 74.60 74.08 73.63 73

.31 73.04 72.71 72.52 72.15 71.97 71.74 71.40 71.21 70.93 70.58 70.73 70.66

70.08 69.84 69.92 69.64 69.33 69.25 69.10 68.77 68.39 68.17 68.02 68.10 67.8
9 67.68 67.42 67.25 67.05 66.89 66.75 66.47 66.44 66.16 65.90 65.67 65.55 6
5.44 65.19 65.04 64.86 64.61 64.36 64.03 63.76 63.52 63.31 63.04 62.79 62.61
62.48 62.35 62.19 62.05 61.96 61.84 61.64 61.56 61.34 61.24 61.05 60.89 60
.71 60.41 60.28 59.97 59.78 59.54 59.31 59.10 58.94 58.48 58.32 58.20 57.96

57.67 57.39 57.35 57.18 56.88 56.53 56.30 56.06 55.89 55.75 55.59 55.29 55.0
7 54.95 54.82 54.46 7.09 82.7 244.71
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19 D 3 D3 110.01 79.92 76.67 75.07 74.43 73.81 73.17 72.67 72.17 72
.05 71.70 71.22 71.02 70.79 70.57 70.27 69.96 69.75 69.46 69.12 69.41 69.12

68.61 68.41 68.63 68.25 67.85 67.65 67.58 67.09 66.55 66.28 66.35 66.40 66.2
9 66.06 66.07 65.94 65.66 65.50 65.20 64.90 64.70 64.37 64.11 63.80 63.69 6
3.64 63.41 63.31 63.13 62.92 62.64 62.35 62.01 61.75 61.46 61.34 60.96 60.77
60.65 60.46 60.30 60.15 59.99 59.86 59.70 59.56 59.33 59.31 59.07 58.89 58
.69 58.50 58.33 58.12 57.85 57.67 57.38 57.07 57.00 56.54 56.37 56.21 56.01

55.79 55.53 55.39 55.24 54.90 54.70 54.34 54.14 53.95 53.83 53.82 53.51 53.3
5 53.17 53.14 52.76 8.48 81.4 273.01
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20 D 4 D4 108.08 79.47 76.04 74.27 73.39 72.90 72.06 71.52 70.97 70
.75 70.39 69.94 69.67 69.37 69.16 68.90 68.58 68.34 68.00 67.67 67.90 67.58

67.09 66.89 67.09 66.71 66.29 66.07 65.94 65.53 65.00 64.73 64.79 64.89 64.7
2 64.49 64.43 64.29 64.00 63.77 63.45 63.14 62.85 62.50 62.14 61.81 61.63 6
1.55 61.26 61.11 60.91 60.62 60.38 59.95 59.62 59.28 59.03 58.78 58.38 58.15
57.98 57.77 57.55 57.34 57.11 56.94 56.79 56.60 56.36 56.25 55.96 55.78 55
.57 55.27 55.08 54.83 54.56 54.32 54.03 53.63 53.57 53.12 52.91 52.68 52.41

52.25 51.98 51.83 51.52 51.23 51.06 50.68 50.39 50.23 50.02 49.94 49.65 49.4
9 49.32 49.24 48.90 10.66 79.2 222.88
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21 E 0 E0 102.33 81.58 79.10 77.90 76.94 76.69 76.21 75.80 75.49 75.
08 74.88 74.68 74.41 74.18 73.94 73.73 73.45 73.35 73.00 72.73 72.76 72.84
72.28 71.95 71.92 71.81 71.55 71.56 71.27 71.13 70.97 70.66 70.31 70.27 70.0
5 69.76 69.38 69.15 69.01 68.95 68.95 68.87 68.86 68.68 68.41 68.24 68.11 6
7.89 67.60 67.39 67.20 66.90 66.59 66.30 66.02 65.85 65.62 65.31 65.17 65.00
64.83 64.76 64.53 64.47 64.29 64.34 64.07 63.96 63.83 63.51 63.49 63.29 63
.10 62.80 62.67 62.37 62.14 61.96 61.70 61.67 61.38 60.99 60.79 60.68 60.38

60.04 59.89 59.70 59.34 59.39 58.81 58.89 58.43 58.31 58.15 57.99 57.65 57.3
7 57.20 57.25 56.83 3.33 84.9 140.16
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22 E 1 E1 105.40 79.82 76.98 75.80 75.12 74.72 74.15 73.70 73.25 73.
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70.05 69.75 69.89 69.61 69.36 69.32 69.17 68.88 68.53 68.29 68.20 68.27 68.1
1 67.85 67.64 67.53 67.29 67.12 66.96 66.76 66.68 66.47 66.20 65.99 65.89 6
5.77 65.61 65.45 65.31 65.10 64.88 64.60 64.35 64.14 63.91 63.72 63.50 63.33
63.17 63.13 62.92 62.83 62.68 62.64 62.49 62.37 62.20 62.04 61.92 61.76 61
.65 61.32 61.18 60.96 60.75 60.56 60.30 60.21 60.00 59.63 59.51 59.35 59.06

58.88 58.73 58.58 58.30 58.12 57.83 57.65 57.38 57.22 57.12 56.94 56.68 56.5
6 56.33 56.33 55.97 5.33 83.2 192.33
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23 E 2 E2 103.65 80.22 77.78 76.14 75.26 74.89 74.33 73.81 73.39 72.
98 72.69 72.49 72.18 71.89 71.66 71.43 71.15 71.00 70.58 70.34 70.36 70.33
69.84 69.49 69.42 69.33 69.04 69.10 68.82 68.65 68.45 68.17 67.84 67.77 67.6
0 67.27 66.89 66.60 66.45 66.34 66.30 66.22 66.17 65.99 65.72 65.56 65.42 6
5.20 65.00 64.71 64.52 64.27 63.95 63.67 63.38 63.17 62.96 62.61 62.48 62.30

62.15 62.08 61.88 61.79 61.67 61.67 61.41 61.30 61.13 60.91 60.80 60.66 60
.47 60.15 60.01 59.75 59.54 59.35 59.07 59.00 58.69 58.40 58.17 58.06 57.76

57.44 57.30 57.14 56.81 56.64 56.22 56.19 55.85 55.74 55.57 55.37 55.12 54.8
7 54.60 54.69 54.25 6.87 82.7 212.63
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24 E 3 E3 103.05 79.71 76.91 75.35 74.33 73.98 73.28 72.73 72.39 71.
98 71.67 71.39 71.11 70.77 70.55 70.30 69.99 69.86 69.46 69.20 69.21 69.19
68.68 68.30 68.26 68.11 67.80 67.83 67.57 67.38 67.18 66.85 66.54 66.45 66.2
6 65.96 65.58 65.29 65.15 65.04 64.96 64.83 64.80 64.56 64.25 64.01 63.90 6
3.63 63.37 63.14 62.87 62.60 62.29 61.93 61.65 61.43 61.21 60.84 60.68 60.48
60.31 60.19 59.95 59.86 59.68 59.57 59.37 59.20 59.03 58.69 58.60 58.41 58
.19 57.84 57.66 57.36 57.15 56.90 56.59 56.54 56.17 55.87 55.63 55.47 55.12

54.80 54.59 54.44 54.06 53.91 53.45 53.44 53.01 52.84 52.64 52.46 52.17 51.8
6 51.67 51.67 51.21 6.19 81 255.465
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25 E 4 E4 105.15 78.71 75.63 74.02 72.97 72.41 71.67 71.12 70.63 70.
28 69.91 69.54 69.24 68.97 68.68 68.37 68.08 67.83 67.48 67.17 67.27 67.07
66.61 66.27 66.30 66.00 65.63 65.52 65.29 65.01 64.60 64.31 64.12 64.14 63.9
3 63.66 63.48 63.26 63.01 62.84 62.61 62.36 62.20 61.90 61.56 61.26 61.10 6
0.89 60.61 60.41 60.17 59.86 59.57 59.18 58.86 58.60 58.32 58.03 57.76 57.56
57.37 57.22 57.01 56.83 56.66 56.55 56.33 56.19 55.96 55.76 55.61 55.44 55
.27 54.95 54.79 54.52 54.32 54.12 53.83 53.63 53.43 53.08 52.86 52.71 52.47

52.21 52.01 51.85 51.63 51.37 51.08 50.93 50.58 50.49 50.32 50.21 49.92 49.6
9 49.52 49.55 49.21 10.62 78.9 269.4
> ;
> run;
>
> **********Stepwise secletion**********;

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>
>
*****************************firmness*************************************
*****************
>
*************************************************************************
*****************;
> proc reg data = yogurte1;
> stepwisee1: model firmness = e1_1--e1_101 /selection=stepwise  sle=0.4 sls=0.3 aic ;
> run;
>
> *******VARIABLES e1_2
e1_34  e1_35  e1_39  e1_40  e1_41  e1_42  e1_43  e1_48  e1_54  e1_57
> e1_58
e1_61  e1_73  e1_78  e1_80  e1_89  e1_90  e1_91  e1_92  e1_96  e1_100*****;
> *******IF SLS<0.3, THERE ARE ONLY e1_89 AND
e1_91*****************************************;
>
> data yogurte2;
> input obs sugar $ prot $ sugar_prot $
e2_1   e2_2   e2_3   e2_4   e2_5   e2_6   e2_7   e2_8
>
e2_9 e2_10  e2_11  e2_12  e2_13  e2_14  e2_15  e2_16  e2_17  e2_18  e2_19  e2_20
> e2_21
e2_22  e2_23  e2_24  e2_25  e2_26  e2_27  e2_28  e2_29  e2_30  e2_31  e2_32
> e2_33
e2_34  e2_35  e2_36  e2_37  e2_38  e2_39  e2_40  e2_41  e2_42  e2_43  e2_44
> e2_45
e2_46  e2_47  e2_48  e2_49  e2_50  e2_51  e2_52  e2_53  e2_54  e2_55  e2_56
> e2_57
e2_58  e2_59  e2_60  e2_61  e2_62  e2_63  e2_64  e2_65  e2_66  e2_67  e2_68
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> e2_69
e2_70 e2_71 e2_72 e2_73 e2_74 e2_75 e2_76 e2_77 e2_78 e2_79 e2_80
> e2_81
e2_82 e2_83 e2_84 e2_85 e2_86 e2_87 e2_88 e2_89 e2_90 e2_91 e2_92
> e2_93 e2_94 e2_95 e2_96 e2_97 e2_98 e2_99 e2_100 e2_101
> Protein Moisture Firmness;
> datalines;
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1 A 0 A0 467.57 117.54 69.06 50.30 39.91 33.63 29.47 26.67 24.27 22
.70 21.40 20.33 19.61 19.04 18.63 18.15 17.82 17.62 17.50 17.16 16.77 17.25

17.32 16.91 17.12 17.39 17.41 17.35 17.58 17.79 17.88 17.70 17.44 17.64 18.0
2 18.16 18.32 18.69 18.82 19.06 19.38 19.42 19.64 19.78 19.90 19.93 20.07 2
0.17 20.42 20.63 20.91 21.12 21.36 21.39 21.69 21.85 22.02 22.19 22.36 22.42
22.59 22.65 22.82 22.96 23.00 23.13 23.31 23.48 23.63 23.77 24.11 24.27 24
.51 24.62 24.85 25.02 25.06 25.41 25.62 25.79 25.90 26.07 26.28 26.29 26.54

26.48 26.69 26.77 26.74 27.08 27.04 27.36 27.47 27.35 27.63 27.61 27.60 27.8
0 27.83 27.77 28.00 3.12 88.2 127.235
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2 A 1 A1 465.34 116.82 68.75 50.17 39.79 33.56 29.40 26.63 24.25 22
.69 21.41 20.37 19.63 19.07 18.65 18.19 17.87 17.68 17.55 17.24 16.84 17.32

17.37 16.96 17.17 17.45 17.44 17.37 17.59 17.80 17.92 17.74 17.47 17.69 18.0
7 18.22 18.37 18.77 18.91 19.17 19.48 19.50 19.71 19.86 19.99 20.01 20.16 2
0.25 20.49 20.70 20.98 21.19 21.41 21.46 21.75 21.90 22.08 22.22 22.44 22.48
22.65 22.72 22.87 23.04 23.05 23.23 23.37 23.53 23.71 23.86 24.20 24.33 24
.62 24.68 24.92 25.09 25.11 25.48 25.68 25.83 25.98 26.11 26.32 26.35 26.58

26.54 26.75 26.79 26.80 27.10 27.09 27.37 27.46 27.40 27.63 27.64 27.61 27.8
0 27.89 27.80 27.99 5.04 88.7 155.75

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3 A 2 A2 464.76 116.81 68.84 50.19 39.90 33.63 29.48 26.70 24.32 22
.76 21.46 20.45 19.70 19.15 18.71 18.27 17.93 17.75 17.63 17.32 16.91 17.43

17.45 17.08 17.27 17.55 17.57 17.50 17.73 17.91 18.05 17.82 17.57 17.75 18.1
5 18.29 18.47 18.86 19.00 19.27 19.56 19.62 19.82 19.97 20.10 20.13 20.26 2
0.36 20.60 20.79 21.12 21.29 21.54 21.60 21.86 22.03 22.16 22.32 22.54 22.59
22.74 22.82 22.97 23.14 23.14 23.34 23.47 23.60 23.80 23.96 24.29 24.46 24
.69 24.81 25.03 25.18 25.25 25.59 25.77 25.92 26.11 26.23 26.41 26.50 26.65

26.62 26.86 26.86 26.86 27.18 27.22 27.47 27.54 27.47 27.70 27.75 27.71 27.9
0 28.01 27.92 28.07 6.87 86.6 219.485
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4 A 3 A3 462.09 116.26 68.55 49.97 39.84 33.56 29.43 26.67 24.30 22
.77 21.43 20.43 19.68 19.12 18.66 18.23 17.90 17.72 17.63 17.28 16.88 17.40

17.40 17.05 17.25 17.55 17.55 17.47 17.69 17.88 18.02 17.79 17.53 17.74 18.1
0 18.24 18.39 18.79 18.90 19.17 19.45 19.51 19.72 19.86 20.00 20.04 20.16 2
0.27 20.50 20.67 21.01 21.19 21.45 21.52 21.75 21.91 22.05 22.19 22.43 22.47
22.62 22.68 22.84 23.01 23.02 23.19 23.33 23.46 23.67 23.81 24.12 24.29 24
.53 24.61 24.88 25.03 25.09 25.44 25.61 25.75 25.92 26.03 26.22 26.32 26.42

26.44 26.67 26.67 26.62 26.99 27.04 27.26 27.33 27.26 27.52 27.54 27.54 27.7
1 27.80 27.75 27.86 9.57 84.2 240.855
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5 A 4 A4 462.08 116.62 68.81 50.17 40.00 33.66 29.58 26.77 24.40 22
.85 21.50 20.46 19.70 19.18 18.69 18.23 17.88 17.70 17.61 17.25 16.86 17.34

17.34 16.99 17.22 17.52 17.51 17.42 17.64 17.82 17.96 17.71 17.46 17.62 17.9
8 18.12 18.23 18.63 18.73 18.99 19.26 19.31 19.54 19.68 19.81 19.85 19.96 2
0.06 20.30 20.46 20.80 20.97 21.22 21.30 21.52 21.68 21.82 21.97 22.18 22.23

22.37 22.44 22.57 22.72 22.74 22.89 23.04 23.15 23.36 23.52 23.81 23.97 24
.24 24.28 24.55 24.70 24.75 25.11 25.27 25.43 25.58 25.68 25.90 25.96 26.07

26.11 26.30 26.28 26.28 26.63 26.67 26.90 27.02 26.89 27.14 27.14 27.13 27.2
8 27.42 27.36 27.49 10.3 83.3 172.325
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6 B 0 B0 463.10 117.19 69.15 50.40 40.22 33.89 29.80 26.92 24.54 22.
98 21.63 20.60 19.84 19.29 18.80 18.39 18.01 17.84 17.74 17.37 16.98 17.47
17.48 17.11 17.34 17.66 17.64 17.56 17.78 17.99 18.13 17.88 17.63 17.77 18.1
5 18.28 18.39 18.78 18.89 19.13 19.41 19.48 19.70 19.84 19.98 20.02 20.13 2
0.23 20.48 20.62 20.97 21.13 21.41 21.49 21.68 21.83 21.99 22.17 22.34 22.40
22.56 22.59 22.77 22.89 22.92 23.07 23.22 23.34 23.52 23.71 24.00 24.17 24
.45 24.48 24.74 24.90 24.95 25.32 25.47 25.67 25.77 25.87 26.12 26.13 26.28

26.30 26.48 26.53 26.48 26.83 26.85 27.11 27.27 27.12 27.38 27.39 27.34 27.5
1 27.64 27.57 27.74 3.45 85.9 139.665
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7 B 1 B1 460.60 116.80 68.92 50.30 40.10 33.81 29.72 26.93 24.56 22.
99 21.69 20.63 19.89 19.34 18.86 18.45 18.08 17.91 17.80 17.44 17.09 17.52
17.55 17.17 17.41 17.75 17.74 17.64 17.87 18.11 18.24 18.01 17.78 17.96 18.3
1 18.44 18.55 18.93 19.05 19.27 19.56 19.62 19.82 20.00 20.11 20.16 20.25 2
0.34 20.60 20.75 21.05 21.25 21.50 21.60 21.80 21.94 22.11 22.29 22.46 22.53
22.69 22.74 22.92 23.04 23.08 23.23 23.37 23.52 23.69 23.86 24.18 24.31 24
.63 24.64 24.88 25.04 25.09 25.43 25.62 25.81 25.87 26.00 26.25 26.25 26.41

26.43 26.62 26.67 26.61 26.97 26.99 27.27 27.42 27.26 27.54 27.52 27.45 27.6
4 27.73 27.67 27.86 5.12 87.2 170.505
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8 B 2 B2 455.31 115.63 68.30 50.01 39.82 33.67 29.64 26.83 24.55 22.
97 21.67 20.65 19.92 19.42 18.95 18.53 18.18 18.02 17.89 17.57 17.21 17.64
17.68 17.30 17.54 17.88 17.87 17.80 18.04 18.28 18.40 18.17 17.95 18.13 18.4

9 18.63 18.75 19.13 19.27 19.49 19.77 19.86 20.04 20.25 20.34 20.39 20.51 2
0.60 20.86 21.02 21.29 21.50 21.75 21.82 22.05 22.20 22.37 22.53 22.72 22.75
22.90 22.98 23.15 23.28 23.31 23.47 23.60 23.76 23.94 24.12 24.43 24.56 24
.88 24.91 25.10 25.28 25.33 25.67 25.82 26.05 26.11 26.24 26.47 26.48 26.62

26.65 26.84 26.89 26.84 27.18 27.19 27.46 27.61 27.45 27.73 27.72 27.65 27.8
3 27.92 27.81 28.02 7.01 85.3 243.215
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9 B 3 B3 449.49 114.38 67.53 49.49 39.50 33.39 29.41 26.73 24.44 22.
91 21.62 20.67 19.94 19.45 19.00 18.60 18.24 18.10 18.00 17.67 17.34 17.78
17.83 17.44 17.69 18.02 18.05 17.98 18.23 18.45 18.57 18.35 18.15 18.34 18.7
2 18.86 19.00 19.38 19.52 19.75 20.04 20.13 20.31 20.50 20.63 20.67 20.79 2
0.89 21.13 21.32 21.58 21.79 22.03 22.11 22.34 22.49 22.66 22.81 23.00 23.02
23.17 23.25 23.43 23.55 23.62 23.74 23.90 24.06 24.22 24.42 24.72 24.86 25
.16 25.22 25.40 25.59 25.64 25.93 26.11 26.35 26.37 26.51 26.71 26.75 26.88

26.89 27.08 27.14 27.11 27.45 27.46 27.70 27.80 27.67 27.95 27.95 27.89 28.0
6 28.07 28.03 28.22 8.67 83.5 229.74
>
10 B 4 B4 478.74 121.16 71.41 52.16 41.56 35.02 30.75 27.91 25.47 23
.83 22.45 21.38 20.60 20.05 19.56 19.08 18.68 18.51 18.39 18.02 17.68 18.09

18.15 17.76 18.03 18.33 18.36 18.27 18.52 18.72 18.86 18.62 18.41 18.59 18.9
7 19.10 19.26 19.63 19.76 19.99 20.29 20.39 20.57 20.77 20.88 20.95 21.09 2
1.16 21.42 21.64 21.86 22.10 22.35 22.41 22.66 22.81 22.97 23.13 23.31 23.36
23.48 23.59 23.71 23.83 23.90 24.00 24.17 24.31 24.49 24.66 24.94 25.09 25
.37 25.42 25.62 25.76 25.80 26.08 26.24 26.45 26.46 26.59 26.77 26.80 26.88

26.93 27.10 27.10 27.04 27.38 27.37 27.60 27.64 27.49 27.80 27.71 27.70 27.8
1 27.79 27.75 27.87 10.58 81.8 228.06
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11 C 0 C0 514.78 129.62 76.26 55.46 44.15 37.09 32.48 29.37 26.73 24
.92 23.43 22.25 21.39 20.72 20.21 19.64 19.18 18.98 18.83 18.41 18.01 18.43

18.47 18.07 18.34 18.60 18.62 18.50 18.73 18.90 19.06 18.81 18.58 18.73 19.1
0 19.23 19.39 19.77 19.90 20.12 20.42 20.51 20.71 20.89 21.00 21.08 21.27 2
1.32 21.58 21.83 22.04 22.27 22.56 22.58 22.84 23.00 23.13 23.33 23.47 23.53
23.66 23.74 23.84 23.95 24.02 24.09 24.26 24.40 24.57 24.74 24.98 25.15 25
.40 25.44 25.65 25.78 25.78 26.09 26.21 26.43 26.41 26.52 26.69 26.68 26.76

26.82 26.93 26.94 26.83 27.16 27.11 27.32 27.34 27.21 27.49 27.35 27.35 27.4
2 27.40 27.34 27.42 3.42 88.7 129.305
>
12 C 1 C1 519.11 130.99 77.10 56.03 44.64 37.49 32.79 29.68 26.96 25
.14 23.68 22.43 21.56 20.85 20.34 19.78 19.30 19.06 18.94 18.51 18.11 18.50

18.55 18.15 18.42 18.67 18.67 18.52 18.75 18.91 19.07 18.82 18.59 18.71 19.1
1 19.23 19.40 19.78 19.93 20.15 20.44 20.52 20.71 20.87 20.98 21.05 21.25 2
1.26 21.53 21.77 22.01 22.21 22.49 22.52 22.77 22.92 23.04 23.23 23.37 23.44
23.57 23.63 23.74 23.83 23.91 23.96 24.14 24.27 24.45 24.58 24.82 25.00 25
.25 25.28 25.49 25.59 25.57 25.89 26.04 26.24 26.22 26.34 26.50 26.46 26.55

26.62 26.70 26.72 26.62 26.94 26.87 27.14 27.13 26.99 27.21 27.12 27.09 27.1
7 27.12 27.09 27.17 5.04 86 182.795
>
13 C 2 C2 524.94 132.34 77.99 56.70 45.09 37.98 33.22 29.95 27.23 25
.40 23.92 22.62 21.72 21.03 20.49 19.91 19.40 19.16 19.03 18.59 18.16 18.54

18.61 18.20 18.46 18.71 18.67 18.52 18.76 18.91 19.07 18.81 18.57 18.65 19.0
6 19.18 19.35 19.72 19.88 20.10 20.39 20.47 20.65 20.81 20.90 20.98 21.16 2
1.18 21.45 21.67 21.88 22.10 22.39 22.40 22.65 22.80 22.91 23.12 23.24 23.33
23.44 23.50 23.59 23.71 23.77 23.82 23.98 24.10 24.31 24.42 24.67 24.83 25

.09 25.12 25.33 25.43 25.39 25.77 25.90 26.07 26.07 26.18 26.36 26.30 26.39

26.46 26.56 26.58 26.45 26.79 26.68 26.99 27.00 26.81 27.06 26.98 26.92 27.0
3 26.98 26.91 26.99 6.88 84.3 208.695
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14 C 3 C3 512.24 129.77 76.60 55.80 44.37 37.40 32.80 29.55 26.90 25
.12 23.64 22.34 21.47 20.78 20.26 19.67 19.20 18.94 18.80 18.37 17.94 18.33

18.37 17.95 18.17 18.41 18.37 18.23 18.47 18.60 18.72 18.43 18.19 18.27 18.6
6 18.76 18.91 19.27 19.42 19.64 19.90 20.00 20.17 20.32 20.41 20.48 20.62 2
0.65 20.92 21.10 21.31 21.51 21.76 21.80 22.02 22.15 22.28 22.44 22.57 22.63
22.75 22.81 22.92 23.04 23.05 23.16 23.29 23.43 23.62 23.73 24.00 24.15 24
.38 24.45 24.64 24.77 24.76 25.09 25.25 25.42 25.43 25.54 25.74 25.70 25.77

25.83 25.96 25.96 25.89 26.22 26.11 26.43 26.49 26.25 26.49 26.47 26.41 26.5
4 26.49 26.42 26.56 8.46 82.8 200.445
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15 C 4 C4 504.79 128.84 76.08 55.65 44.14 37.26 32.73 29.44 26.82 25
.05 23.53 22.23 21.37 20.69 20.15 19.55 19.07 18.78 18.63 18.19 17.75 18.14

18.13 17.70 17.89 18.09 18.04 17.89 18.11 18.22 18.29 17.97 17.72 17.78 18.1
6 18.24 18.35 18.72 18.84 19.07 19.29 19.39 19.52 19.66 19.73 19.77 19.86 1
9.91 20.16 20.31 20.52 20.67 20.90 20.92 21.14 21.26 21.38 21.51 21.65 21.66
21.78 21.84 21.98 22.09 22.07 22.20 22.32 22.48 22.63 22.75 23.05 23.19 23
.42 23.48 23.67 23.81 23.84 24.16 24.35 24.48 24.55 24.67 24.87 24.85 24.94

24.98 25.14 25.18 25.16 25.48 25.38 25.68 25.77 25.58 25.80 25.86 25.77 25.9
4 25.89 25.87 26.03 10.3 80.8 251.68
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16 D 0 D0 521.54 133.05 78.53 57.29 45.40 38.24 33.61 30.13 27.44 2
5.59 23.99 22.67 21.73 21.03 20.44 19.76 19.26 18.95 18.76 18.28 17.81 18.21

18.15 17.70 17.87 18.07 18.00 17.84 18.05 18.17 18.23 17.90 17.63 17.71 18
.03 18.13 18.18 18.53 18.62 18.83 19.05 19.13 19.26 19.39 19.46 19.50 19.59

19.64 19.89 20.04 20.22 20.41 20.62 20.62 20.84 20.96 21.08 21.20 21.33 21.3
4 21.44 21.52 21.61 21.74 21.70 21.84 21.93 22.10 22.23 22.35 22.65 22.78 2
3.01 23.08 23.26 23.37 23.44 23.72 23.91 24.03 24.09 24.21 24.39 24.40 24.45
24.52 24.68 24.71 24.65 24.97 24.85 25.12 25.23 25.06 25.31 25.32 25.21 25
.40 25.33 25.28 25.45 3.33 87.7 110.045
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17 D 1 D1 543.15 138.55 81.60 59.36 47.06 39.50 34.59 31.05 28.20 2
6.25 24.55 23.15 22.20 21.41 20.77 20.05 19.56 19.20 18.95 18.47 17.98 18.33
18.25 17.80 17.94 18.13 18.04 17.87 18.05 18.22 18.24 17.91 17.62 17.75 18
.05 18.13 18.19 18.49 18.60 18.80 19.00 19.10 19.19 19.33 19.40 19.44 19.53

19.59 19.83 19.99 20.18 20.38 20.59 20.57 20.81 20.92 21.03 21.12 21.28 21.2
7 21.41 21.45 21.54 21.66 21.62 21.75 21.85 22.00 22.13 22.26 22.56 22.67 2
2.91 22.95 23.12 23.24 23.31 23.57 23.73 23.86 23.91 24.02 24.20 24.20 24.22
24.33 24.44 24.47 24.39 24.71 24.60 24.84 24.93 24.75 25.02 25.01 24.88 25
.05 25.00 24.95 25.12 5.3 84.6 161.675
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18 D 2 D2 527.39 134.01 78.77 57.31 45.48 38.24 33.45 30.11 27.35 2
5.50 23.88 22.59 21.66 20.97 20.36 19.73 19.25 18.90 18.70 18.27 17.82 18.19
18.15 17.70 17.86 18.08 18.00 17.88 18.06 18.27 18.32 18.03 17.76 17.90 18
.22 18.32 18.42 18.75 18.87 19.07 19.28 19.38 19.47 19.60 19.71 19.74 19.86

19.93 20.19 20.35 20.58 20.79 21.03 20.98 21.23 21.38 21.49 21.62 21.77 21.7
9 21.92 22.00 22.11 22.20 22.22 22.32 22.43 22.59 22.74 22.92 23.20 23.31 2
3.62 23.65 23.84 23.95 24.02 24.30 24.47 24.61 24.72 24.82 25.01 25.04 25.08
25.22 25.30 25.37 25.29 25.65 25.52 25.80 25.89 25.73 26.05 26.11 25.97 26
.10 26.11 26.06 26.23 7.09 82.7 244.71
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19 D 3 D3 504.22 127.46 74.89 54.37 43.28 36.41 31.89 28.81 26.20 2
4.47 22.96 21.79 20.94 20.34 19.78 19.22 18.81 18.51 18.36 17.98 17.56 17.97
17.99 17.56 17.74 18.04 17.99 17.91 18.12 18.35 18.44 18.21 17.94 18.11 18
.48 18.59 18.71 19.04 19.18 19.39 19.63 19.73 19.84 19.99 20.13 20.19 20.33

20.44 20.71 20.88 21.14 21.38 21.63 21.63 21.88 22.02 22.17 22.30 22.47 22.5
3 22.68 22.77 22.89 22.99 23.01 23.14 23.25 23.38 23.55 23.76 24.04 24.19 2
4.49 24.54 24.75 24.91 24.94 25.24 25.40 25.58 25.68 25.79 25.98 26.03 26.10
26.26 26.31 26.35 26.27 26.68 26.59 26.85 26.96 26.79 27.14 27.19 27.07 27
.22 27.26 27.15 27.36 8.48 81.4 273.01
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20 D 4 D4 482.01 121.27 71.13 51.66 41.14 34.65 30.36 27.45 24.98 2
3.35 21.93 20.82 20.04 19.51 19.01 18.50 18.12 17.87 17.75 17.41 17.01 17.45
17.50 17.10 17.27 17.60 17.58 17.52 17.73 17.95 18.05 17.83 17.58 17.77 18
.15 18.28 18.44 18.80 18.95 19.15 19.41 19.51 19.66 19.80 19.94 20.01 20.15

20.28 20.54 20.73 21.00 21.24 21.49 21.53 21.76 21.91 22.09 22.24 22.41 22.5
0 22.69 22.75 22.91 23.00 23.04 23.18 23.33 23.41 23.62 23.87 24.14 24.33 2
4.60 24.70 24.91 25.10 25.10 25.43 25.59 25.81 25.92 26.02 26.25 26.32 26.43
26.56 26.63 26.66 26.60 27.00 26.97 27.23 27.38 27.19 27.62 27.66 27.61 27
.72 27.81 27.71 27.92 10.66 79.2 222.88
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21 E 0 E0 424.64 111.73 66.26 47.76 38.19 32.01 28.37 25.25 23.47 21
.76 20.69 19.77 19.13 18.56 18.09 17.80 17.50 17.29 17.15 16.97 16.55 16.97

17.03 16.89 16.73 16.93 16.96 17.05 17.23 17.45 17.62 17.75 17.83 17.83 18.0
4 18.32 18.46 18.36 18.42 18.32 18.43 18.74 18.95 19.23 19.48 19.74 19.95 2
0.17 20.38 20.63 20.76 20.93 21.11 21.30 21.39 21.45 21.63 21.68 21.84 21.92
21.90 22.03 22.15 22.32 22.53 22.72 22.87 23.02 23.28 23.49 23.66 23.85 24
.13 24.21 24.49 24.73 24.75 24.93 25.15 25.23 25.37 25.50 25.72 25.86 26.03

25.95 26.26 26.31 26.71 26.73 26.66 26.90 27.06 26.99 27.22 27.31 27.30 27.3
7 27.55 27.58 27.75 3.33 84.9 140.16
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22 E 1 E1 467.63 120.90 71.03 51.58 41.04 34.40 30.30 27.11 24.90 23
.14 21.80 20.70 19.95 19.28 18.73 18.30 17.88 17.61 17.47 17.14 16.64 17.01

17.06 16.74 16.71 16.88 16.82 16.80 16.93 17.14 17.18 17.09 17.00 17.07 17.3
9 17.56 17.67 17.80 17.84 17.94 18.08 18.21 18.35 18.48 18.64 18.75 18.88 1
9.00 19.20 19.34 19.53 19.69 19.88 19.92 20.07 20.16 20.32 20.39 20.54 20.60
20.68 20.74 20.87 21.02 21.10 21.23 21.35 21.50 21.68 21.84 22.12 22.28 22
.54 22.58 22.80 23.06 23.06 23.22 23.41 23.52 23.66 23.76 23.96 24.04 24.22

24.18 24.36 24.39 24.56 24.73 24.74 24.92 24.96 24.96 25.24 25.29 25.31 25.4
1 25.55 25.51 25.62 5.33 83.2 192.33
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23 E 2 E2 427.47 113.14 67.37 48.85 39.10 33.00 29.10 26.06 24.17 22
.45 21.36 20.42 19.75 19.13 18.69 18.32 17.98 17.78 17.63 17.42 16.96 17.34

17.40 17.20 17.04 17.22 17.21 17.32 17.48 17.69 17.83 17.94 17.99 18.03 18.2
9 18.58 18.68 18.64 18.64 18.60 18.67 18.93 19.13 19.33 19.56 19.81 19.99 2
0.18 20.36 20.60 20.73 20.87 21.07 21.20 21.32 21.34 21.51 21.59 21.69 21.75
21.72 21.86 22.01 22.15 22.30 22.49 22.66 22.81 23.10 23.19 23.45 23.55 23
.90 23.97 24.21 24.39 24.45 24.64 24.77 24.88 25.10 25.10 25.36 25.48 25.66

25.60 25.83 25.90 26.24 26.30 26.21 26.49 26.57 26.51 26.68 26.84 26.83 26.8
4 27.11 27.10 27.21 6.87 82.7 212.63
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24 E 3 E3 421.58 111.98 66.70 48.65 39.01 32.91 29.26 26.10 24.33 22
.63 21.56 20.64 19.99 19.42 18.93 18.64 18.36 18.14 18.02 17.82 17.43 17.81

17.93 17.78 17.65 17.81 17.83 17.93 18.11 18.31 18.52 18.63 18.74 18.76 19.0

1 19.33 19.47 19.43 19.49 19.47 19.60 19.91 20.13 20.36 20.63 20.86 21.06 2
1.28 21.46 21.70 21.87 22.02 22.22 22.38 22.51 22.56 22.76 22.80 22.97 23.03
23.04 23.10 23.31 23.50 23.62 23.83 23.98 24.17 24.41 24.59 24.79 24.93 25
.24 25.27 25.52 25.73 25.78 25.96 26.12 26.22 26.41 26.48 26.64 26.73 26.91

26.91 27.07 27.10 27.48 27.57 27.46 27.75 27.78 27.70 27.91 27.96 27.90 27.9
2 28.14 28.19 28.26 6.19 81 255.465
>
25 E 4 E4 394.44 103.91 62.26 45.75 36.84 31.22 27.88 25.25 23.41 21
.96 20.84 19.99 19.36 18.93 18.54 18.23 17.93 17.77 17.72 17.47 17.15 17.57

17.67 17.43 17.49 17.73 17.74 17.74 17.93 18.13 18.25 18.21 18.15 18.20 18.5
6 18.74 18.96 19.09 19.21 19.33 19.53 19.73 19.89 20.09 20.23 20.40 20.57 2
0.70 20.87 21.05 21.29 21.37 21.53 21.61 21.72 21.75 21.88 21.94 22.05 22.10
22.11 22.20 22.27 22.37 22.40 22.52 22.62 22.72 22.84 22.98 23.20 23.32 23
.55 23.59 23.74 23.90 23.89 24.00 24.11 24.23 24.28 24.37 24.55 24.56 24.62

24.69 24.76 24.76 24.85 25.05 24.93 25.14 25.20 25.06 25.30 25.32 25.24 25.3
4 25.36 25.39 25.45 10.62 78.9 269.4
> ;
> run;
> proc reg data = yogurte2;
> stepwisee2: model firmness = e2_1--e2_101 /selection=stepwise sle=0.4 sls=0.29 aic ;
> run;
> *******VARIABLES e1_2
e1_34 e1_35 e1_39 e1_40 e1_41 e1_42 e1_43 e1_48 e1_54 e1_57
> e1_58
e1_61 e1_73 e1_78 e1_80 e1_89 e1_90 e1_91 e1_92 e1_96 e1_100*****;
> *******IF SLS<0.3, THERE ARE ONLY e2_37 AND
e2_50**;
> ***********ATTENTION: THE R^2 IS JUST

```
0.075**********************************************;
> data combine;
>    set yogurte1;
>    set yogurte2;
> run;
> proc print data=combine;
> run;
> proc reg data = combine;
> stepwisecombine: model firmness =
e1_2      e1_34  e1_35  e1_39  e1_40  e1_41  e1_42  e1_43  e1_48  e1_54  e1_57  e1_5
8
> e1_61 e1_73  e1_78  e1_80  e1_89  e1_90  e1_91  e1_92  e1_96  e1_100
e2_7    e2_8   e2_11  e2_12  e2_20  e2_22
> e2_25
e2_31  e2_33  e2_34  e2_37  e2_49  e2_50  e2_70  e2_77  e2_79  e2_85  e2_86  e2_90
 e2_91  e2_94  e2_97
> e2_101
> /selection=stepwise  sle=0.5 sls=0.2 aic ;
> run;
>
> ***********E1-91,E1-89******************;
>
>
**************************************************************************
**************************************************
>
***********************************Protein*********************************
************************
>
**************************************************************************
************************;
```

```
>
> proc reg data = yogurte1;
> Proteinstepwisee1: model protein = e1_1--e1_101 /selection=stepwise  sle=0.3 sls=0.15 aic
;
> run;
> ***************1,3,91,100 for
e1************************************************;
>
> proc reg data = yogurte2;
> Proteinstepwisee2: model protein = e2_1--e2_101 /selection=stepwise  sle=0.5 sls=0.4 aic ;
> run;
>
**********************e2_2    e2_36   e2_40   e2_41   e2_43   e2_58   e2_59   e2_62*****
*********
> *******************more serious setting will cause no varibale be
slected**************;
>
> proc reg data = combine;
> Proteinstepwisecombine: model protein = e1_1  e1_3    e1_91   e1_100
e2_2    e2_36   e2_40   e2_41   e2_43   e2_58   e2_59   e2_62
> /selection=stepwise  sle=0.5 sls=0.2 aic ;
> run;
>
>
*****************************e1_1    e1_3    e1_91   e1_100*********************
**********
>
>
*****************************************************************************
**************************************************
>
```

```
**********Moisture**********
**********
>
**********************************************************************
**********;
> proc reg data = yogurte1;
> Moisturestepwisee1: model moisture = e1_1--e1_101 /selection=stepwise  sle=0.4 sls=0.3
aic ;
> run;
> *********e1_2 e1_16  e1_59  e1_64  e1_66  e1_71  e1_72  e1_73  e1_75  e1_77
> e1_78 e1_79  e1_80  e1_81  e1_82  e1_83  e1_86  e1_88  e1_89  e1_92  e1_93
> e1_96 e1_97***************;
>
> proc reg data = yogurte2;
> Moisturestepwisee2: model moisture = e2_1--e2_101 /selection=stepwise  sle=0.4 sls=0.3
aic ;
> run;
>
> ******e2_1   e2_16  e2_18  e2_22  e2_23  e2_32  e2_33  e2_34  e2_49
> e2_51 e2_62  e2_71  e2_73  e2_75  e2_77  e2_78  e2_85  e2_89  e2_91  e2_92
> e2_94
e2_97  e2_98  **********************************************************;
>
> proc reg data = combine;
> Proteinstepwisecombine: model moisture = e1_2
e1_16  e1_59  e1_64  e1_66  e1_71  e1_72  e1_73  e1_75  e1_77
> e1_78 e1_79  e1_80  e1_81  e1_82  e1_83  e1_86  e1_88  e1_89  e1_92  e1_93
> e1_96 e1_97 e2_1    e2_16  e2_18  e2_22  e2_23  e2_32  e2_33  e2_34  e2_49
> e2_51 e2_62  e2_71  e2_73  e2_75  e2_77  e2_78  e2_85  e2_89  e2_91  e2_92
> e2_94 e2_97  e2_98
> /selection=stepwise  sle=0.5 sls=0.2 aic ;
```

```
> run;
>
> proc reg data = combine;
> Proteinstepwisecombine: model moisture =e1_86
e1_88   e1_89   e1_92   e1_93   e2_62   e2_91   e2_94 /selection=backward  aic slstay = 0.05 ;
> run;
>
> **********e1_86
e1_89    e1_92   e1_93   e2_62   e2_91   e2_94**********;
```