Evaluating the Effects of Oat Fiber and Modified Corn Starch on the Characteristics of Smoked Sausage Utilizing Mechanically Separated Chicken

by

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Abstract

There is an increasing demand for affordable, all-natural products in the food service industry. This category includes clean label products, which are generally recognized as being minimally processed and having simple and understandable ingredient statements. This study is to evaluate blends of clean label functional ingredients for a low cost, highly extended smoked sausage. Texture profile analysis (TPA), consumer sensory panels, objective color analysis, microbial analysis, and pH analysis were used to evaluate quality attributes of sausages made with three blends of oat fiber (OF) and modified corn starch (CS_m) over a 13-week storage. All sausages were made with mechanically separated chicken (MSC; 0.325% $NaNO_2$, 1.75% salt) in a hog intestine casing. Treatments included a With Phosphate (0.43% sodium phosphate), Without Binders (no sodium phosphate, OF, or CS_m), and three blends of OF and CS_m at 3.5% green weight. Five sausages were selected randomly from each treatment and batch for sensory, color, and pH. One sausage was randomly selected for TPA and one for microbiological analysis. Following cooking and chilling, sausages were vacuum sealed and stored at 2°C in a corrugated box. Three sensory sausages were reheated in an oven to 79.4°C, cut into 2.54 cm segments, and cut in half lengthwise for sensory analysis while the remaining two were evaluated for color and pH. Treatments were given a unique, random 3-digit code. Consumer panelists (n=30) evaluated juiciness, cohesiveness, flavor, texture, and overall acceptability on a 9-point scale. TPA sausages were cut into three 2.54 cm segments, and evaluated using a TA-XT2icon Texture Analyser and 25 mm cylinder press. Data was analyzed using a repeated

measures design with x, y + z as independent variables in the proc mixed procedure of SAS 9.4. Means were separated sing leas squares means with significance set at $P<0.05$. Sensory, texture, pH, and color evaluations were performed every 7 days contingent upon microbial and sensory analysis of spoilage. There was a treatment by week interaction for pH and sensory texture. An overall reduction in pH over extended storage time after week 2 was observed. The with phosphate treatment was similar to an experimental blend for 8 of 9 weeks exhibiting differences when evaluating the texture interaction. The treatments with OF:CS_m were less juicy ($P<0.05$) than those without and were more cohesive sensory analysis. Adding OF at 3.15% and 1.75% had a negative effect on flavor acceptability and overall acceptability. TPA values for hardness were greater ($P<0.05$) in experimental blends than treatments with phosphate or without binders. Adding OF and CS_m made sausages more hard, gummy and chewy ($P<0.05$).

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I. INTRODUCTION

Consumers are, by nature, the largest portion of the food industry. As such they have enormous impact on the direction of food production and strategy. Consumer trends indicate a demand for products with simple ingredients that are clean and free from artificial sweeteners, antibiotics, G.M.O.s, etc. (Watrous, 2016). Though the United States Department of Agriculture (USDA) does not formally recognize a definition for clean label at the time of publishing, for the purpose this paper clean label products are defined as products that exclude ingredients with unfamiliar or complex names and instead focus on simpler ingredients more reminiscent of common kitchen spice racks (Institute of Food Technology, 2015; Sebranek 2009). Recent studies have shown that 64% of millennials believe that fewer ingredients on a label make for a healthier snack (Orlando, 2017). The past several years have shown continuous double-digit growth of organically produced goods (USDA-ERS, 2017). The Food Marketing Institute and the North American Meat Institute found that organic meat sold increased 29.3% by weight in 2015 over 2014 numbers. At 91.6 million pounds, organic meat sales are worth $569 million. Even with that much market penetration, it is important to note the organic meat market is still a niche market, with just 3.8% of meat in 2015 (Sustainable Food News, 2016 March 1). The success of clean label products in stores is due to an increase in consumers' willingness to purchase. According to research by Ingredient Communications in London, England, U.K., 73% of consumers are willing to pay a higher retail price for a food or beverage product made with ingredients they recognize or trust (Nunes, 2017).

One of the principal concerns for further processors involved in producing clean label products is cost of materials. Adopting clean label strategies can become expensive for companies trying to reformulate products, especially when some ingredients considered clean do not perform the same way as conventional ingredients. An example of this alteration in functionality is the use of celery extract in place of sodium nitrite as a curing agent. Another major problem can be the rate of inclusion in final products. Phosphates have a variety of uses in meat products and are included in small percentages. The replacements for phosphates in clean label products are typically not as effective when used alone and must be included in greater amounts. Even though these natural ingredients are available, they often come with higher cost for inclusion than conventional ingredients.

The higher costs mean that further processors must be convinced the niche market will continue to exist and show economic gains despite price differences. More companies are making production decisions because of the consistent double-digit growth indicated earlier in the clean label market. Additionally, further processors are incentivized to meet certain standards to participate in programs with limits on specifically allowable ingredients and inclusion levels. Information is needed on how ingredient substitution affects product acceptance as well as the limits of replacers. Additionally, as margins decrease, further processors are looking for lower cost inputs. If ingredients are more expensive to use, then further processors will be forced to adjust the meat raw materials.

The objective of the current study is to evaluate a blend of clean label functional ingredients for use in an affordable smoked sausage. This study will observe the effects of a blend of oat fiber and modified corn starch at different mixture rates and utilization of a meat block composed entirely of mechanically separated chicken. These effects will be observed

though texture profile analysis, consumer sensory evaluation, microbiological analysis, and objective color evaluation. Yield and cost analysis will further determine the viability of treatments to further processors.

II. REVIEW OF LITERATURE

Meat as a Food Source

Humans have been eating meat since the first hunter-gathers killed animals for sustenance. Meat is a valuable source of zinc, iron, and protein, as well as a variety of vitamins and minerals (Aberle et al., 2012; Romans and Ziegler, 1977). According to a 2017 report compiled by The National Chicken Council (NCC) using data from the United States Department of Agriculture (USDA), Americans consumed 95.6 kg per capita of total red meat and poultry in 2015.

The USDA doesn't list poultry under the umbrella term of meat, which refers to red meat products such as beef, pork, lamb or mutton, and veal (Aberle et al., 2012). Poultry instead is classified as domestic birds including chickens and turkey, among others (Aberle et al., 2012). The 95.6 kg per capita total is partially composed of 40.8 kg of chicken consumption (NCC, 2017). When comparing total chicken consumption to total beef consumption, 24.4 kg, it is evident that Americans consumer almost twice the amount of chicken as beef. From 2011 to 2015, chicken consumption increased 6.76% and is expected to rise another 1.7% from 2015 to 2017. Over that same 5 year period, beef consumption per capita in the United States fell 5.9%. These trends could be due to traditionally lower and more stable chicken prices, higher chicken production, which allows more product placement in the market, and perceived health benefits associated with the low calorie and low fat content of chicken (Hill, 2016; IndexMundi, 2017a,b) as compared to beef. Regardless of the reason, these trends indicate a firm foundation in poultry

further processing could be more valuable than beef further processing to professionals entering the meat industry.

Chickens were not raised commercially for consumption in the United States until the 1930s (Baker and Bruce, 1995). Originally sold as whole birds, a market was developed for pieces and quarters and thus further processing began. Further processing can be defined as the conversion of raw carcasses into value-added, more convenient to use forms such as cut portions, cold cuts, frankfurters, and ready-to-eat (Baker and Bruce, 1995). Further processing led to some new challenges for the poultry industry. Leftover lean on bones was a waste and an efficient method of removing that lean needed to be implemented. Mechanical separation of poultry in the United States began in the 1950s to meet this demand (Field, 1998).

Mechanically Separated Chicken

Definition

Meat removed from bones by machines is generally referred to as Mechanically Deboned Meat (MDM), Mechanically Deboned Tissues (MDT), Mechanically Separated (Species) Meat [MS(Species)M], or Mechanically Recovered Meat (MRM). USDA refers to Mechanically Separated (Species) as any finely comminuted product resulting from the mechanical separation and removal of most of the bone from attached skeletal muscle of livestock carcasses and parts of carcasses and meeting the other provisions of paragraph (a) of Title 9 of the Code of Federal Regulations (CFR), chapter 3, subchapter A, part 319, subpart A, section 319.5 (2017). The following discussion will frequently use these terms interchangeably, being as specific as possible and as precise to current literature as possible.

Production Process

Mechanical deboning is typically broken down into two general categories based on whether the bones are crushed or remain intact, the most relevant of which is mechanical separation. MSM is produced by machines which grind or crush bones and subsequently separate bone, cartilage, ligament, and tendon from soft tissue by forcing the tissues through a sieve to produce a meat paste. This method is most suitable for fish and chicken because the pliable bones are not likely to shatter or produce small fragments that might pass through the sieve. The use of mechanically separated poultry in meat products is currently not limited by regulations (Aberle et al., 2012). Mechanical separation causes cell breakage, protein denaturation, an increase in lipid and heme groups, and poorer mechanical properties (Froning, 1976). The increase in heme groups could have an undesirable impact on a products directed towards consumers seeking white meat products.

Mechanically separated chicken is frequently used in comminuted sausage products. The action of separating meat from bones results in partial emulsification of the product, meaning less comminution is required at later steps (Aberle et al., 2012). This allows for fewer steps in sausage manufacturing, which traditionally begins with grinding raw materials. In many cases, grinding or chopping can be skipped all together in favor of simple mixing.

Effects on Meat Quality

Proximate Analysis

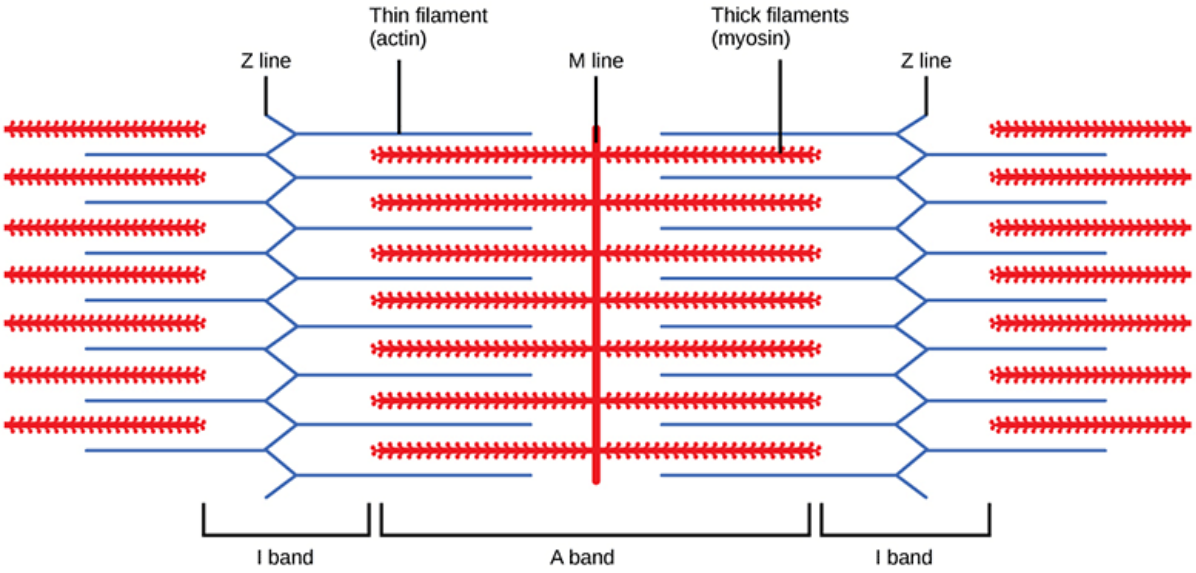
Various research has identified lower protein and higher fat contents in various sources of mechanically deboned poultry meat as compared to that of hand deboned sources (Grunden et al., 1972; Froning, 1970, 1976). Composition varies widely depending on the age, sex, and species of the source. Mechanically deboned products also show a higher moisture content

(Grunden et al., 1972) Research by Froning (1976) indicated protein composition could range from 9.3 to 14.5%, total lipid composition could range from 12.7 to 26.2%, and moisture composition could range from 60.1 to 73.7%, all depending on the anatomical location of MDM pieces. Higher moisture contents are associated with lower fat contents (Froning, 1976). Amino acid composition is similar to hand deboned sources, indicating protein constituents are still valuable (Essary and Ritchey, 1968). Elevated mineral content, particularly calcium and iron, have been noted when compared to hand deboned poultry (CFR, 2017; Grunden et al., 1972; Froning, 1970, 1976). The pH of mechanically deboned chicken varies from 6.2 to 6.6, which is a higher value than the 5.4 to 5.8 pH associated with fresh red meat (Aberle et al., 2012; Grunden et al., 1972). Color standards for mechanically separated chicken are 49.65, 9.95,12.95 for L*, a*, and b* respectively, depending on age, sex, muscle location, lipid content, and pH (Grunden et al., 1972).

Texture

Mechanically separated poultry has numerous textural characteristics that make it a challenge to work with. The high level of protein denaturation and product comminution cause the batter to be viscous and difficult to stuff into casings. Protein denaturation also negatively affects the protein matrix formed during cooking. Yang and Froning (1992) used scanning electron microscopy to review mechanically deboned chicken meat that had undergone surimi processing with multiple different washing solutions. In this study, researchers evaluated an unwashed control and washing solutions of tap water, 0.1M sodium chloride, 0.5% sodium bicarbonate, and sodium phosphate buffer at pH 7.2. Researchers found the unwashed control MDCM had a coarse structure, resulting from large protein aggregates containing semi-continuous fat globules evenly distributed throughout the matrix. Researchers also noticed large

pockets present in the structure, indicating water was held in large amounts in localized regions (Yang and Froning, 1992). Schnell et al. (1974) evaluated the ultrastructure of mechanically deboned poultry meat. Results from Schnell et al. (1974) found a decrease in screen processing size (from 0.1575 cm to 0.0508 cm) caused a loss in myofibrillar structure integrity. Additionally, the smallest screen size destroyed the myofibril structure, resulting in breaks at the Z or M lines of the sarcomere (Schnell et al., 1974).



It is theorized the resulting protein denaturation and sarcomere disruption decreases the available protein matrix binding capabilities (Froning, 1970, Schnell et al., 1974).

Texture Analysis

Texture is frequently analyzed using trained and consumer sensory panels and instrumental analysis, using both subjective and objective measurements. Both sensory and instrumental hardness, springiness, and cohesiveness are primary mechanical parameters that characterize texture properties of smoked sausage (Rongrong et al., 1998). Using an Allo-Kramer shear press to evaluate shear force, Dhillon and Maurer (1975) compared summer sausage made entirely with beef to summer sausage made with 50% mechanically deboned

chicken meat or mechanically deboned turkey meat. Their research reported that incorporating MDCM or MDTM in the blend reduced shear force values compared to the control. Incorporating MDCM also negatively affected the slice-ability of the summer sausage, causing it to become subjectively more crumbly (Dhillon and Maurer, 1975). Smith et al. (1988) compared Allo-Kramer shear force and Texture Profile Analysis (TPA) with broiler breast meat. Smith et al. (1988) found that AlloResults indicated that the correlation of Allo-Kramer shear force to TPA hardness values was more complex that proposed by Dhillon and Maurer (1975) when using whole muscle cuts rather than comminuted sausage. A plethora of research at different institutions agrees as MDP content was increased, a decrease in associated variables such as shear force and tensile strength was observed (Daros et al., 2005; Lyon et al., 1980; Song et al., 2014).

Texture Profile Analysis, a double compression test, calculates hardness, cohesiveness, resilience, chewiness, gumminess, and springiness through variables such as the test's force, area under the curve, and time. Similar to shear and tensile evaluations, Yang and Froning (1992) saw a decreased hardness with increased MDCM in a formula. They also concluded springiness, chewiness, and gumminess decreased as hardness decreased. Results from Yang and Froning (1992) agreed with TPA results from Smith, Lyon, and Fletcher (1988), in that decreases in hardness correlated to decreases in chewiness. Rongrong et al. (1998) evaluated inclusion of wheat protein on the effects of smoked sausage. In concurrence with the work by Yang and Froning (1992), they found that hardness was significantly ($P<0.05$) lower in sausages that contained more MSP (control = no wheat protein added). However, results from Rongrong et al. (1998) did not show a correlation with springiness.

Research by Rongrong et al. (1998) also evaluated the correlation between TPA results and sensory results. Using a trained sensory panel, the researchers were able to correlate hardness ($r = 0.58$, $p = 0.0001$ and $R^2 = 0.3372$) while sensory and instrumental springiness were marginally correlated ($r = 0.26$, $p = 0.05$and $R^2 = 0.0651$), however sensory and instrumental cohesiveness were not correlated ($r = 0.09$, $p = 0.47$ and $R^2 = 0.0086$). Lyon and Lyon (1990) reported instrumental texture and sensory texture measurements do not correlate well in some cases. This is likely due to the fact that the sensory profile encompasses more aspects of meat texture than objective profile, which is based on force-distance measurements (Lyon and Lyon, 1990).

Lipid Oxidation

Mechanical separation can have a variety of effects on the raw material because of the intensity of the separation process. MSC is more prone to oxidative rancidity because it has a higher heme iron and lipid content and because the particles are more exposed to oxygen and light during the separation process, which are key drivers of lipid oxidation (Froning, 1976). Lipid oxidation is most commonly measured using thiobarbaturic acid reactive substances (TBARS or TBA). TBARS protocols are not standardized, however results are commonly reported as a measure of mg malonaldehyde (or malondialdehyde) per kg meat (Esterbauer and Chesseman, 1990). Mielnik et al. (2002) evaluated the effect of stored form of the raw materials (vacuum packed MDPM and air packed skeleton and deboned on day of production), over storage times (6 and 18 weeks) and origin (chicken and turkey neck and frame) on the quality of comminuted sausages. They found that storage form had a greater impact on TBARS values than storage time; where vacuum packed MDPM had on average 0.313 mg less malondialdehyde per kg sample than air packed skeleton. Poultry species was the second most important factor in lipid

oxidation, where turkey sausages showed on average 0.302 mg more malondialdehyde per kg sample than chicken sausages (Mielnik et al., 2002).

Storage Stability

Due largely to the method of manufacturing already discussed, mechanically separated poultry does not have a long shelf life. The propensity to experience lipid oxidation leads to off flavor development fairly quickly with MDM. Jantawat and Dawson (1980) evaluated different storage methods effects on lipid oxidation of mechanically deboned chicken and turkey at -18°C for 4 months. MDCM showed lower total rates of lipid oxidation across all treatments when compared to MDTM. Furthermore, the use of vacuum packaging and nitrogen packaging significantly reduced ($P < 0.05$) TBARS values when compared to carbon dioxide packaging (Jantawat and Dawson, 1980). Mielnik et al. (2003) evaluated the effects of adding antioxidants to mechanically deboned turkey meat and freezing it at -25°C over seven months. They found inclusion of antioxidants could reduce TBARS values nine-fold compared to a control over seven months (2.662 mg malondialdehyde per kg meat) (Mielnik et al., 2003).

Some antioxidants have demonstrated the ability to reduce lipid oxidation in raw MSC and finished products. Herbs of the mint family (*Labiate*) have been shown to be the most effective natural antioxidants (Herrmann, 1981). Research by Mielnik et al. (2003) indicated that antioxidants effectively reduced lipid oxidation of frozen mechanically deboned turkey with free oxygen access over seven months of storage compared to the control.

The large surface area, the release of cellular fluids, and the heat generated during mechanical separation all combine to enhance bacterial count and growth (Barbut, 2015). As previously mentioned, the pH of MSC exists in an average range from 6.2 to 6.6, which is very close to neutral, thus very beneficial for the growth of bacteria. Bacteria can be either pathogenic

or spoilage bacteria, however the later will contribute more to a decreased storage life. Some examples of spoilage bacteria include Gram-negative genera such as *Enterobacteriacea*, *Pseudomonas*, *Moraxella*, and a Gram-positive genera such as the lactic acid producing *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Weissella* and other Gram-positive genera like *Brochothrix*, and (Cerveny et al., 2009; Wang, 2015). The bacteria can cause purge turbidity in the package, gas production, souring, slime formation, and greening. These are all classic examples of food spoilage (Cerveny et al., 2009; Wang, 2015), and spoilage is considered to occur at 6 $\log_{10}$ Colony Forming Units (CFU) per gram of total plate count (TPC) (Morey et al., 2014).

Palatability

People eat meat because of tradition, nutritive value, availability, satiety value, social status, and flavor. Meat value is ultimately based on consumer acceptability (Aberle et al., 2012). A large part of this acceptability is based off palatability characteristics that make a product agreeable to the eyes, nose, and palate. Three of the biggest drivers of palatability in cooked products are tenderness, juiciness, and flavor (Aberle et al., 2012). Mechanically deboned meats have low hardness and shear force values, so tenderness of the meat is not a negative effect. Mechanically separated poultry, in high usage levels, can lead to mealiness or crumbliness (Dhillon and Maurer, 1975). Juiciness can be a much larger issue when evaluating the palatability of mechanically separated chicken. Mechanically separated chicken experiences protein degradation, which decreases its ability to form a well-structured gel matrix for the binding of water and fat during the cooking process (Yang and Froning, 1992). Because the protein matrix is not well developed, excess water can be lost during cooking or resulting in less

moisture available for palatability. Some possible solutions include incorporating added water, centrifuging raw product, or rinsing raw product as done by Yang and Froning (1992).

Non-Meat Ingredients

Processed meat products are defined as those muscle-derived products in which properties of fresh meat have been modified using one or more procedures contributions to preservation, convenience, appearance, palatability, variety, and/or safety (Aberle et al., 2012). Processed meat products are typically composed of two components, meat and non-meat ingredients. Those non-meat ingredients can further be reduced into functional non-meat ingredients and seasonings and spices. Seasonings and spices are valuable for imparting specific flavor profiles to meat products, however they typically only impact flavor and color. Functional non-meat ingredients more commonly impact characteristics such as moisture, texture, and storage life. Functional non-meat ingredients are a critical part of meat processing. They are used to create unique and distinctive types of processed meats. Yet even as they produce unique products, they are used nearly ubiquitously across the industry for a variety of products.

Conventional Functional Non-Meat Ingredients

Widespread use of functional non-meat ingredients is partially due to their value creating specific outputs, but also because they are typically multifunctional (Sebranek, 2015). Incorporating low amounts of functional non-meat ingredients can have positive effects on a variety of meat quality parameters, such as juiciness, texture, and microbial suppression (Aberle et al., 2012). The most basic functional non-meat ingredients are water, salt, curing agents, cure accelerators, phosphates, and organic acids.

Water

One of the most commonly used ingredients in meat processing is water (Sebranek, 2009; 2015). It plays a role in enhancing products by improving juiciness, tenderness, and yield (Alvarado and McKee, 2007; Sebranek, 2009). According to the *Principles of Meat Science* textbook by Aberle et al. (2012), the normal moisture/protein ratio of comminuted products is considered to be 4:1. As the moisture content begins to exceed this ratio, it is referred to as added water.

Water has a very simple chemical structure composed of two hydrogen atoms separately covalently bonded to one oxygen atom in a V-shape (Ruan and Chen, 1998). The two hydrogen atoms of water are positively charged and the oxygen is negatively charged which creates a dipole meaning that water is polar. This polarity allows hydrogen bonds to form between the hydrogen of one molecule and one of the electron pairs on an oxygen atom of another water molecule. This is significant because water molecules are "held" together within meat structures (Aberle et al., 2012; Sebranek, 2009). Water molecules bind to myofibrillar proteins because the proteins frequently carry a negative charge (i.e. polar) that causes protein repulsion, which allows water to permeate the structure (Aberle et al., 2012).

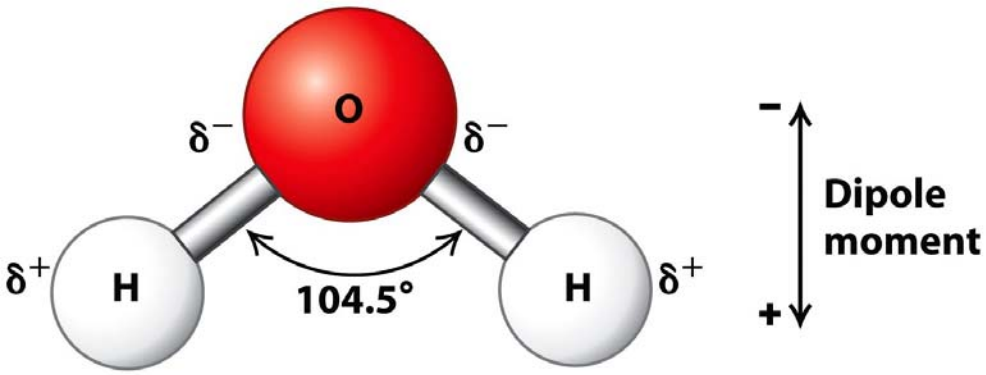


Figure 2-5
Molecular Cell Biology, Sixth Edition
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In addition to being dipolar, water is known as a "universal solvent" because it can dissolve a number of substances into solution (OpenStax, 2015). Among these substances are ingredients frequently used in meat processing, including salt, nitrate, nitrite, sugar, phosphates, and seasonings and spices among others. Because water can bring these ingredients into complete solution, it allows for the uniform distribution of ingredients throughout a meat mixture. The solvent property of water is also essential for meat protein extraction, which is critical to formation of emulsions, binding of restructured products, juiciness of cooked products, and cooked product texture (Aberle et al. 2012; Sebranek 2009; 2015; Tarté and Amundson, 2006; Trout, 1988). Added water in the range of 10% to 20% of the meat weight also plays a synergistic role with salt in protein solubility (Sebranek, 2009). As Sebranek (2009) describes in *Ingredients in Meat Products*, solubilizing meat proteins with salt and added water can create an interfacial protein film around fat globules, stabilizing them during the cooking process.

Research by Hughes, Cofrades, and Troy (1997) evaluated the effects of varying fat content in sausages and replacing fat with carrageenan or oat fiber. Researchers found creating low-fat sausages by increasing water content reduced ($P<0.05$) the emulsion stability, increased the cook loss, and decreased water-holding capacity. There were no differences ($P\geq0.05$) between fat replacers and added water effects on objective color, but that differing levels of fat content (5%, 12%, and 30%) did have an effect on color. Jin et al, (2016) evaluated different raw materials compositions on the quality traits of emulsion-type pork sausages. This study varied protein, fat and water levels in multiple combinations based on protein content. Varying the water content in the product produced no trends on the effect of pH, but did correlate to differences ($P<0.05$) in shear force, objective color (L*, a*, b*), residual nitrite ions, purge loss, and all measured parameters of texture profile analysis (Jin et al., 2016). Researchers found

increasing water content showed greater b* values and had a negative correlation with a* values which agrees with study by Hughes et al. (1997) in terms of fat replacement but not necessarily as an effect of water. Water had a significant effect ($P<0.05$) on the shear force values but the researchers believe it was more a result of excess fat content exceeding the protein matrix's ability to set up optimally. When based on groups classified by fat content, the surface hardness, adhesiveness, and hardness values decreased as the water content was increased (Jin et al., 2016).

Water is also critical when considering how its presence can make a product susceptible to microbial growth, namely brine strength of a processed meat product and water availability, more commonly referred to as water activity (a_w; Coultate, 2009; Sebranek 2009; 2015). Brine strength is defined as the percentage of salt in the total volume of liquid. (Sebranek, 2009; 2015). This means that reformulations, including reduced salt or reduced fat, could negatively impact the ability of a brine to prevent growth of halophilic microorganisms. Water activity is described as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature (Jay et al., 2006). A_w is indicative of the biological and chemical availability of water in a product as it relates to free or "unbound" water (FDA, 2013; Tarté, 2012). Pure water has a_w of 1.0 while most fresh meat products have $a_w \geq 0.98$ (Aberle et al., 2012; Coultate, 2009; Nester et al., 2001). Available water takes into consideration all water binding sites in food, including proteins, salt, sugar, and other ingredients, creating a very comprehensive picture (Sebrank, 2009). A_w can be reduced by adding solutes such as sugar and salt, or by removing unbound water by drying, baking, or cooking food (Aberle et al., 2012; FDA, 2013) Most spoilage bacteria will not grow below a_w of 0.90, however some pathogens and exceptional bacteria, along with certain yeasts and molds, may continue to grow as low as 0.60 (Coultate, 2009; Nester et al., 2001; Sebrank, 2009).

Water is very useful for processers in a variety of ways, however it is closely regulated and, if improperly managed, can have negative effects on product stability and quality. Hard water, or water with a high mineral content, can cause color losses, flavor changes, shorter shelf life, and increased infrastructure maintenance costs (Sebrank, 2009). Added water has labeling requirements in the United States and plays a major role in marketing processed meats as it relates to the protein-fat-free percentages (PFF; Aberle et al., 2012; Sebrank, 2009). Additional limits on water are included in its addition of cooked sausages such as frankfurters, where the added water plus fat content cannot exceed 40% (CFR, 2007). There are numerous regulations on the amount of added water that is allowed into a product so significant consultation with regulatory experts is often beneficial.

Salt

Salt (sodium chloride) is another essential curing ingredient that is frequently overlooked. Salt has the longest history with meat curing of any of the ingredients, dating back more than three millennia to the times of ancient societies (Aberle et al., 2012). It is likely the most commonly used curing ingredient by frequency and quantity so an understanding of how it effects qualities such as flavor, texture, water binding, and shelf life is crucial (Sebranek, 2009; 2015). The functional ability of salt is typically based around its disassociation into sodium (Na^+) and chloride (Cl^-) ions in water (OpenStax College, 2015). Chloride ions with an ionic strength of 0.5 are sufficient to solubilize myofibrillar proteins, swell and disintegrate myofibrils, and depolymerize myosin filaments (Hamm, 1986). Incorporating 2% salt is typically good enough to achieve the necessary ionic strength, but concentrations as low as 0.5% will still increase negative charges of proteins, thus increasing water binding (Sebranek, 2009; 2015). As Sebranek

(2009) points out, the chloride ions are largely responsible for maintaining cooking yields, juiciness, tenderness, and mouthfeel when the product is consumed.

The sodium ion is principally incorporated in flavor development (Sebranek, 2009; 2015). It is not only responsible for the "saltiness" flavor, but also for enhancing the flavor components of other ingredients and overall flavor (Ruusunen and Poulanne, 2005). In June 2016, FDA announced some voluntary sodium reduction targets that U.S. based further processors were encouraged to adopt.

Sodium chloride, as mentioned earlier, plays a crucial role in water activity and protein binding. Reducing sodium in some of the different forms it is incorporated in meat, including sodium nitrite/nitrate, might be an efficient way of replacing sodium but keeping chloride functionality. Salt is classified as GRAS, or generally recognized as safe, and is considered to be self-limiting due to the overwhelming saltiness that occurs with over inclusion (CFR, 2016).

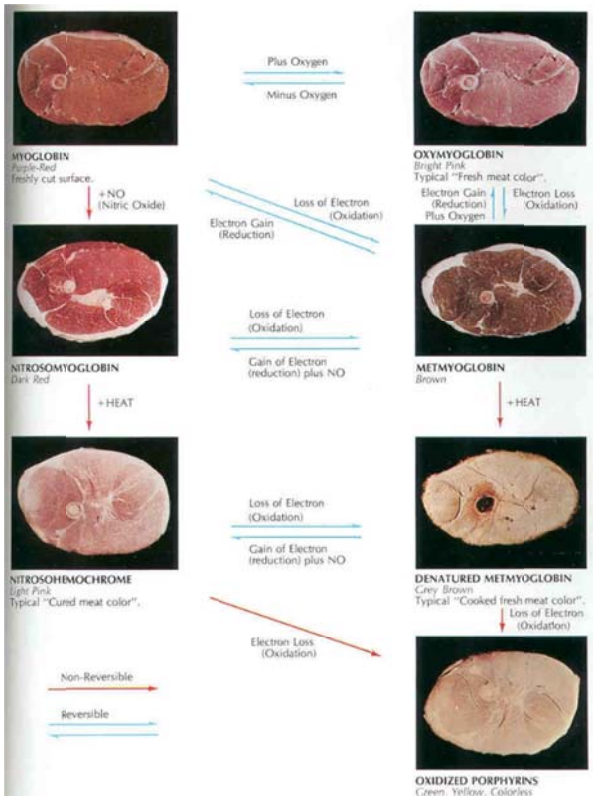
Curing Agents

Curing agents are essential to modern day cured meats because they are responsible for the unique and distinctive properties, including flavor, color development, preservation, and inhibition of bacteria (Aberle et al., 2012; Sebranek, 2009). Curing agents generally refer to nitrate and nitrite. While either can be used, nitrate is only effective as a curing agent if it is first reduced to nitrite, typically by microorganisms and over an extended length of time (Sebranek, 2009).

Nitrate is most commonly observed as either a contaminant of salt or as saltpeter (potassium nitrate) and is typically only including in curing recipes if long term curing is desired or if the target is a natural product (Sebranek, 2009). As a more common ingredient in natural products, they will be covered more extensively later in this review.

Nitrite, either sodium or potassium compounds, is highly soluble in water and is naturally pale yellow in color. Nitrite can be toxic at very low dose for humans though, estimated to be about 1 g (Ellenhorn and Barceloux, 1998). Because of its toxicity, USDA mandates secure storage and written records are maintained for any further processors utilizing nitrites. It is commonly found mixed with sodium chloride at a rate of 6.25% nitrite to 93.75% salt because any potential overuse of nitrite would be rejected by consumers due to saltiness. Another safety precaution taken by further processors is adding a pink color to the nitrite product so that it can be easily distinguished by facility operators. Nitrite is a highly reactive compound that can function as an oxidizing and reducing agent when added to meat (Sebranek, 2009).

Nitrite is effective in very low concentrations, 50-100 ppm, though it is more commonly seen in higher usage concentrations. (Sebranek, 2009; 2015). Nitrite has a variety of functions and they are not all well understood mechanistically, but researchers have identified how nitrite acts on myoglobin to create the unique cured color. Meat color is largely a function of the state of the heme iron of myoglobin. Nitrite produces nitrite ions and sodium ions when dissolved in water (Sebranek, 2009). Nitrite ions then react with hydrogen ions in weakly acidic conditions (pH 5.5 – 6.0) of a meat mixture and form nitrous acid (HNO_2) (Honikel, 2004; Pegg and Shahidi, 2000). Nitrous acid then dissociates to form nitric oxide and



Adapted from Aberle et al. (2009).

nitrogen dioxide. The nitric oxide reacts with the heme-ring of myoglobin to form a dark red color and myoglobin state called nitrosomyoglobin (Aberle et al., 2012). While the nitric oxide is binding to the heme-ring, nitrogen dioxide re-enters the nitric oxide cycle, which produces nitric acid that will dissociate into nitrate (Sebranek, 2009). This explains why residual nitrates can be found in cured products that never had nitrate in the ingredients (Honikel, 2004). After adding heat to meat with nitrite, the myoglobin state is changed to nitrosohemochrome and takes on a pinkish color (Aberle et al., 2012).

Nitrites have a strong antioxidant effect on meats likely as a result of interaction with heme proteins and metal ions, radical chelation activity of nitric oxide, and formation of nitroso- and nitrosyl compounds (Pegg and Shahidi, 2000). Nitrite inclusion at 50 pmm has been shown to reduce rancidity in beef, pork, and chicken by 50-64% (Morrissey and Techivangana, 1985). That same study showed higher concentrations of nitrite could have greater antioxidant effects. An inclusion of 200 pmm nitrite reduced rancidity measures by 87-91% in similar meats.

Nitrite is strongly antagonistic to anaerobic bacteria, most importantly *Clostridium botulinum*, and can help control other pathogenic microorganisms like *Listeria monocytogenes* (Sebranek, 2009). *Clostridium botulinum* produces a potent neurotoxin that, when ingested, can cause severe illness or death (USDA-FSIS, 2013). Nitrate may be effective against *Clostridium botulinum* because of the products of the nitrite reaction sequence, which are heavily dependent, although the exact reasons remain unknown (Tompkin, 2005). Nitrite is not effective for the control of Gram-negative enteric pathogens such as *Escherichia coli* (Pichner et al., 2006).

Though highly effective, nitrite inclusion continues to experience divisions in public perception. Because of nitrites reactivity, in certain conditions with heat present, carcinogenic nitrosamines can form (Sebranek, 2009). The formation of nitrosamines can be reduced or

eliminated by the presences of chemicals that reduce dinitrogen trioxide (N_2O_3), the nitrosating species, to the non-nitrosating nitric oxide (NO) (National Academy of Sciences, 1982). Regulations of nitrate in the United States varies with product. Comminuted products are limited to 156 ppm of nitrite as an upper limit, based on the green weight of the product (USDA-FSIS, 1995). Cure accelerators are recommended in most cured products and act to help reduce nitrite to nitric oxide, probably by serving as one half of the redox reaction with N_2O_3. Examples of cure accelerators include ascorbic acid, and erythorbic acid (Sebranek, 2009).

Phosphates

Phosphates are included in meat formulas to improve the ability of meat proteins to bind and retain water for the improvement of cooking yields, product texture, tenderness and juiciness (Sebranek, 2009, Xiong, 2005). Phosphates are a result of the slats of phosphoric acid and sodium or potassium containing molecules and are typically alkaline. Phosphates typically work by raising the pH of the meat batter, which in turn increases net negative charges myofibrillar proteins resulting in more water binding surface in the sarcomere (Anjaneyulu et al., 1990; Poulanne et al., 2001; Sebranek, 2009; Young, 2005). The purpose of altering pH is to get further away from the isoelectric point of meat, about pH 5.3 (Aberle et al., 2012; Hamm, 1960). Phosphates work similarly to salts in how they impact muscle constituents, including the emulsification of fats into the protein matrix, meaning they can be used to reduce the overall salt content in low-sodium products (Coultate, 2009; Ruusunen and Poulanne, 2005). Like salts, phosphates are water soluble. Phosphates vary widely in pH and solubility, so understanding more about how a phosphate preforms in critical (Molins, 1991). A study by Young et al. (2005) evaluated the effect of raising or lowering the pH of normal or dark cutting beef respectively with acidic or alkaline phosphates. They found initial pH had no effect on color or water holding

capacity (WHC) of the mixes ($P \geq 0.05$). This research also reported incorporating an alkaline phosphate with dark cutting beef (initially high pH) resulted in 17 out of 18 replicates with a WHC of 100% (Young et al., 2005).

Phosphates show some effect as an antioxidant. Phosphates can work to reduce the prooxidant effect of sodium chloride, thereby delaying the onset of lipid oxidation (Huffman et al., 1981). In a study by Huffman et al. (1981), tripolyphosphate was shown to stabilize thiobarituric acid (TBA) values over 31 days (day 0 – day 30) of frozen storage in flaked and formed hamburger patties. Lipid oxidation increased from 0.42 mg malonaldehyde/1000 g meat to 1.27 mg malonaldehyde/1000 g meat over the next 30 days (day 30-60) but that level was not sufficient for a trained sensory panel to detect lipid oxidation (Huffman et al., 1981).

Phosphates have also been identified as slightly bacteriostatic on some Gram-positive bacteria when used in fresh meats (Dickson et al., 1994; Molins et al., 1987; Sofos, 1985). Research has indicated that trisodium phosphate was an effective sanitizer in controlling *Salmonella spp.* on poultry (Giese, 1992). Carcasses were immersed in solutions with concentrations of 8% to 12% (wt/vol) trisodium phosphate for up to 15 minutes. Results show a reduction to less than 1% after just 15 seconds from 35% positive tests with the control (Giese, 1992).

Organic Acids

Organic acids are used in meat processing to aid in reducing pH for cure acceleration and potential antimicrobial effects due to low pH (Sebranek, 2009). Nitrite reduces to nitric oxide more rapidly in an acidic environment, so a reduction in pH has value for cured meat products (Sebranek, 2009). Organic acids are frequently used as antimicrobials in meat formulas, especially lactic, acetic, and citric acid (Mani-López et al., 2012). Organic acids work though two modes of action: cytoplasmic acidification with subsequent uncoupling of energy production and regulation, and by accumulation of the dissociated acid anion to toxic levels (Taylor et al., 2012). Acetic acid (CH_4O_2) is frequently used in meat products and is generally regarded as safe for miscellaneous and general-propose use (CFR, 2017). Acetic acid is highly soluble in water. It has been shown to be effective against *Salmonella spp., Escherichia coli* O157:H7, *Campylobacter jejuni,* and *Listeria monocytogenes* (Frederick et al., 1994; Entani, et al., 1998; Okrend et al., 1986; Rhee et al., 2003).

Research by Frederick et al. (1994) evaluated the effect of acetic acid as a spray and temperature in reducing *Salmonella enterica* Typhimurium, aerobic plate counts and total coliforms on pork cheek meat. The application of 2% acetic acid reduced ($P < 0.05$) the incidence of *Salmonella*, aerobic plate counts and coliform count (Frederick et al .,1994). Okrend, Johnston, and Moran (1986) utilized a scald water at 52°C with varying concentrations of acetic acid to evaluate death rates of *Salmonella newport, Salmonella Typhimurium,* and *Campylobacter jejuni*. Researchers reduced levels of bacteria by 0.5 to 1.5 $\log_{10}$ CFU, indicating acetic acid could be an effective hurdle for microbial growth on poultry carcasses. Entani et al. (1998) found acetic acid, in concentrations as low as 0.1% with and without sodium chloride, had bacteriostatic effects on 8 strains of *Escherichia coli* O157:H7, *E. coli* O26, *E. coli* O111,

Salmonella enterica Typhimurium, *Salmonella enterica* Enteritidis, *Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus, and Aeromonas hydrophila*. Researchers noted cells in growth phase were demonstrated to have high sensitivity to the vinegar, while those in the stationary phase were more resistant. Rhee et al. (2003) evaluated effects of mustard flour and acetic acid in the inactivation of food-borne pathogenic bacteria at 5°C and 22°C. Researchers found that *Salmonella enterica* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* were all effectively reduced ($P < 0.05$) by as little as 0.5% acetic acid (6.6 $\log_{10}$ CFU after 2 days, 6.6 $\log_{10}$ CFU after 7 days, and 6.6 $\log_{10}$ CFU after 9 days, respectively with growth < 0.3 $\log_{10}$ CFU after 1 day). Acetic acid alone, or in combination with other antimicrobial ingredients, is an effective means of controlling pathogenic and total plate count growth.

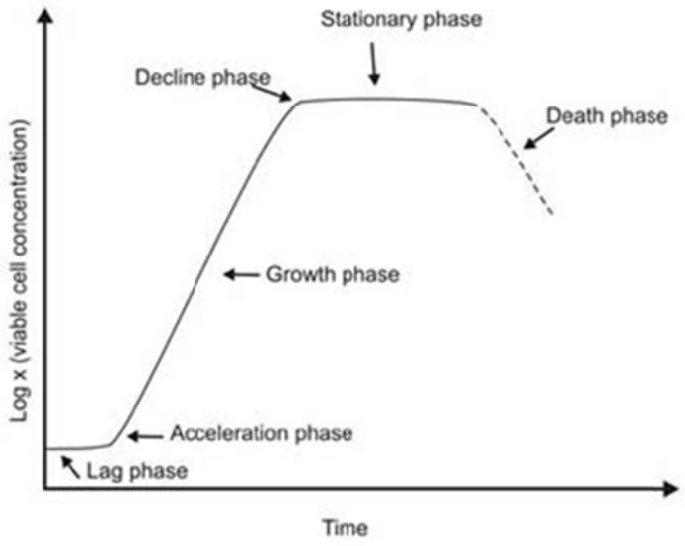


Image adapted from *Biofilms: the Hypertextbook*

Natural Alternative Functional Non-Meat Ingredients

As consumer trends lean towards products that have simpler ingredients statements (i.e. clean label), further processors are incorporating more natural sources of functional ingredients (Myers, 2015). These ingredient replacements are mostly occurring in the five ingredients listed in the Functional Non-Meat Ingredients section above. However they also include food colorants and sweeteners. Historically, meat processing was a function of salt, curing agents, and spices

(Sindelar, 2015). The use of other functional ingredients has developed as food processing became more focused on large scale volume, consistency, and price. These motivations shouldn't be perceived as a negative however, they are merely an adjustment to the market and needs of the consumer.

Processed meat products made with natural alternatives to current functional ingredients offer unique challenges in replacement, product consistency, and profitability. Sodium chloride is still widely used in meat formulations, however many clean label products are incorporating salt in the form of sea salt. Sweeteners such as cane sugar, honey, and Turbinado sugar are becoming popular replacements for dextrose and corn syrup. Natural sources of nitrate, such as celery powder, and bacteria that will work to reduce it to nitrate are becoming more popular alternatives to sodium nitrite in cured products (Sindelar, 2015). Multiple opportunities for replacement exist. One of the largest areas of research recently has been focused on phosphate replacers. Phosphate is a valuable binder in meat and works with water to extend meat products. To date, no direct phosphate replacement has been discovered however the use of a combination of products at different levels can have phosphate like effects.

Food Starches

Starch is well known as water binding ingredient. Starches high carbohydrate content makes them an excellent extender as well, allowing for a more full feeling when eating less (Joly and Anderstein, 2009). Starch is a form of energy reserve in vegetables, typically stored in the grain/seed for use during germination, made up of amylose and amylopectin. Starches can usually be found in two structures in food ingredients: native and modified. Native starches are simple refined starches that show a 'ring' pattern of glucose chains (Joly and Anderstein, 2009). Modified starches are processed to alter the composition of amylose and amylopectin. Starches

are commonly sorted into cook-up and cold swelling categories. Cook-up starches bind water as heat is added. Gelatinization point, the point at which the starch forms a matrix to bind and hold water, is frequently a basis of which starch to use, but these vary greatly depending on the source of the starch and the structure of the starch carbohydrates (Joly and Anderstein, 2009). Cold swelling starches work as water binders in uncooked meats, helping to bind water during the mixing and formulating process and helping with forming and extrusion (Joly and Anderstein, 2009).

Modified dent corn is one of the most popular alternatives when using starches. The modification process consists of soaking the dent corn to separate the germ from the hull (Grain Processing Corporation, personal communication, 5 May 2017). The soaking water is then drained and centrifuged to separate the constituents of the corn kernel. The starch portion, now known as starch milk, can either go into the dryer and come out as native starch or go into the reactor tank for modification. Modifications consist of either substituting or cross linking. Cross linking creates more binding and denser glucose chains, whereas substituting reduces the branching (Joly and Anderstein, 2009; Wurzburg and Szymanski, 1970). Dent corn is 25% amylose and 75% amylopectin normally. The high amylose content means that the glucose chain already has lots of cross linking, so more substitution is preferred in order to prevent the product from swelling and breaking, which would release any bound water (Grain Processing Corporation, personal communication, 5 May 2017).

Beggs et al. (1997) evaluated different levels of modified corn starch and water to determine optimum sensory attributes and physical characteristics for reduced fat turkey frankfurters. Minimum inclusion of starch was 2.379% and the maximum level was 6.621%. Researchers found the level of starch significantly ($P < 0.05$) affected yellowness of turkey

frankfurters, wherein low starch levels produced more yellow frankfurters. As starch levels increased, turkey frankfurters were more resistant to compression. Firmness was maximized at low starch and low water levels, while high starch and high water levels resulted in lower firmness values compared to high starch and low water levels. Starch also affected ($P = 0.11$) cohesiveness, resulting in greater cohesive values in higher starch sausages. Researchers determined optimal level for starch to be 2.3% with a range from 2.3 to 2.5% and the optimal level for water to be 33.6% with a range from 33.6 to 35.5% (Beggs et al., 1997). Seo et al. (2015) evaluated the effect of replacing pork meat with surimi, chicken breast, and corn starch on the quality properties of sausage during storage. The moisture and fat content was greater ($P < 0.05$) in the control compared to the corn starch treatment, but the protein and content was greater ($P < 0.05$) for the corn starch treatment. Sausages with starch were darker ($P < 0.05$) and more yellow (+b*), while sausages without starch were more red (+a*) (Seo et al., 2015). Sausages with starch exhibited greater ($P < 0.05$) TPA values for hardness, cohesiveness, springiness, gumminess, and chewiness. These evaluations agree with conclusions from Beggs et al. (1997) and Pietrasik (1998). Using a trained panel, Seo et al. (2015) found no differences among sensory characteristics between the starch and control sausage.

Dietary Fibers

Dietary fiber acts as a meat extender by increasing water holding capacity (WHC) and as a fat replacer in low fat meat products (Bodner and Sieg, 2009; Claus and Hunt, 1991; Szczepaniak et al., 2005). These dietary fibers serve as a source of fiber in diets, however the USDA does not allow meat products to be labeled as fortified to eliminate marketing claims of fiber in meat products. Fibers are composed soluble and insoluble fractions. Cellulose, hemicellulose, and lignin are insoluble while gums, polyfrustose, pectin, and mucilage are

soluble (Bodner and Sieg, 2009). Fibers can be further classified into native and refined. Native fibers tend to have reduced functional and sensory attributes, imparting flavor, color, and texture differences to meat products (Bodner and Sieg, 2009).

Oat fiber is widely used dietary fiber for meat replacement and water binding. Gould and Gould and Dexter introduced the first patented system for producing a higher absorbing insoluble fiber though alkaline peroxide treatment of hulls (Bodner and Sieg, 2009). In 1991, Ramaswamy patented the application of a process similar to paper making that would remove lignin and silica from oat hulls. This created product that swelled and absorbed more water than less refined forms of the fiber. Oat fiber can absorb 250% to over 800% of its weight in water, depending on the level of refinement.

Hughes et al. (1997) evaluated the impact of different fat, carrageenan, and oat fiber levels on the quality attributes of sausage. At the same fat inclusion level, proximate analysis yielded no differences ($P > 0.05$) in moisture, however at 5% fat, protein levels were higher ($P < 0.05$) in sausages formulated without oat fiber. No difference in protein was seen for treatments formulated from 12 and 30% fat. The use of oat fiber at fat levels of 5 and 12% did reduce cook loss, improve WHC, and increase emulsion stability when compared to controls. No effect was seen when the fat level was at 30%, indicating that oat fiber was most effective when coupled with low fat levels and high water levels. These results are not supported by the results of Claus and Hunt (1991) who determined oat fiber increased ($P < 0.05$) cook loss when compared to high fat controls but found no differences ($P > 0.05$) with low fat controls. Furthermore, research by Hughes et al. (1997) determined that oat fiber had no effect ($P > 0.05$) on objective color measurements or sensory evaluations of sausage. Hughes et al. (1997) determined that low fat formulations can have negative impacts on the characteristics of sausages, however oat fiber can

help to improve some of the quality parameters. Claus and Hunt (1991) measured the effect of added fiber in a low fat (10%) bologna when compared to a low fat control and a standard fat (30%) control. Incorporating fiber created a product with greater ($P < 0.05$) hardness than the low fat control but not as hard as the standard fat control. The oat fiber treatment was less ($P < 0.05$) grainy and less salty than both controls, however was similar ($P > 0.05$) for the traits of after taste and aroma. Cohesiveness of the oat fiber treatment was similar ($P > 0.05$) to that of the low fat control, but lower ($P < 0.05$) than the standard fat control. Claus and Hunt (1991) recommended combinations of fibers and starches be evaluated in order to determine the best possible combination for quality characteristics. Research by Szczepaniak et al. (2005) investigated the effect of fat substitution with varying amounts of wheat and oat fiber in finely comminuted sausages on instrumental texture values, sensory quality, and consumer acceptance. Researchers determined that there was no difference ($P > 0.05$) in protein with the addition of oat fiber when compared to the control, as found by Hughes et al. (1991). However, in contrast to the 1991 study, Szczepaniak et al. (2005) found that moisture was higher in treatments with oat fiber when compared to control. Per Szczepaniak et al. (2005), the investigated types of dietary fiber in the meat batters did not result in any considerable changes in the quality characterizes of the end products. While 7.5% fiber inclusion was not different ($P > 0.05$) than the control, 10% inclusion of oat fiber did increase ($P < 0.05$) shear force and area under the curve when compared to the control. Adding fiber also increased ($P < 0.05$) hardness values for the sausage, while reducing cohesion and elasticity when compared to the control (Szczepaniak et al., 2005).

Alternatives to conventional non-meat functional ingredients can be a viable replacement, especially when considering extending and binding capabilities, however care should be taken to avoid negative sensory attributes such as graininess.

Cost

It is important to understand motivations of meat processers when evaluating new technology. In the meat industry, most of profit is made in volume rather than margins, so the ability to produce a large amount of product efficiently is valued over more time consuming but lucrative ventures. A prime example of this concept is the beef supply chain. The majority of meat sold in in the United States is boneless boxed beef as opposed to a dry aged beef, a higher value but lower yield product (Aberle et al., 2012; Ahnström et al., 2001; Smith et al., 2008). While low volume, higher quality sausages may have been the standard in the past, today's further processing industry is geared towards producing large volumes of meat for consumers (Sindelar, 2015). The sausage making segment of the further processing sector is well positioned to make the switch to more clean label products because, it only requires reformulation as opposed to new technology inputs.

Clean label ingredients on a per pound basis are very comparably priced to conventional ingredients, however usage levels can be much different. A high quality commercial phosphate such as Brifisol 450 costs $3.53 per kg when sold in 22.67 kg bags. The usage rate of phosphate cannot exceed 0.5% in the end product, so the 22.67 kg bag could be utilized in over 4,500 kg of product. According to previous studies, a good rate of use for oat fiber or modified corn starch could be as high as 3% if not higher. Pure Gel B990 modified dent corn starch currently sells for $1.65 per kg. In the same 22.67 kg bag, that would cost approximately $37.40 per bag, a significant price savings per bag when compared to Brifisol 450. When accounting for usage rates of corn starch however, that price becomes much less attractive. A 22.67 kg bag of corn starch would be enough to formulate 755.6 kg of product assuming usage rates of 3%, meaning

that $223 of corn starch would have to be purchased to make the same amount of product as produced using the Brifisol 450 phosphate. Oat fiber can be even more expensive. A typical 22.67 kg bag of oat fiber will cost a processor approximately $100. Making the same amount of product as the phosphate, a 3% inclusion of oat fiber would cost a processor nearly $600. Therefore, the inclusion rates are where some clean label ingredients become economically challenging.

To compensate for these increased ingredient costs, further processors can either cut costs in meat, reduce labor costs, or charge more for products. With as many as 73% of consumers indicating that they are willing to pay more for products made with ingredients they recognize and trust, increasing the market price is an option for further processors (Nunes, 2017). Increasing prices is still risky for further processors, because of unknowns in the survey data and purchase power. A more attractive opportunity is to reduce raw material price. For this reason, many companies are beginning to formulate more products with mechanically deboned meat. MDM can cost between $0.55 and $0.77 per kg while hand cut chicken can cost as much as $2.62 and $2.66 per kg for light and dark meat respectively (USDA-AMS, 2017). This pricing difference can more than make up for the potential negative attributes of MDM. While costs are ultimately not the most prohibitive factor in product development, they still play a very important role in understanding the utility of a product to a further processor.

The objective of the current study is to evaluate a blend of clean label functional ingredients for use in an affordable smoked sausage. This study will evaluate using a blend of oat fiber and modified corn starch at different mixture rates and utilization of a meat block composed entirely of mechanically separated chicken. The resulting produced will be evaluated by texture profile analysis, consumer sensory evaluation, microbiological analysis, and objective

color evaluation. Cost assessment will be performed to evaluate the economic feasibility of each treatment and provide an additional means of separation in case similar meat quality attributes are observed.

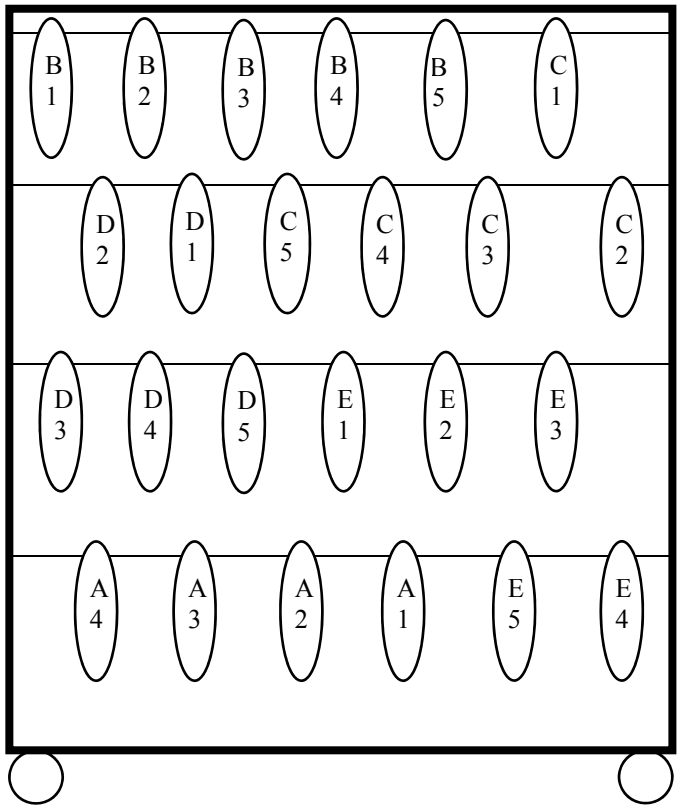
III. Materials and Methods

Experimental Design

Mechanically Separated Chicken (MSC) was obtained from a meat processor in West Alabama and transported to the Auburn University Lambert-Powell Meats Laboratory under refrigerated conditions (0ºC ± 2°C). After arriving at the Lambert-Powell Meats Laboratory, MSC was held for 24 hours (2°C ± 2°C). The MSC came preblended with 0.325% sodium nitrite and 1.75% sodium chloride. Following the 24-hour hold period, MSC was batched into five treatments. The five treatments are as follows: A) with phosphate, B) without binders, C) 90:10, D) 50:50, E) 10:90. Treatments C, D, E refer to the ratio of oat fiber to corn starch composing 3.5% of the raw formulation weight. The sausage formulations can be seen in Table 1. The with phosphate incorporated 0.43% phosphate [(Brifisol® 450 Super) Fibrisol Service Australia, Heatherton, Victoria, Australia]. The without binders did not incorporate any sodium phosphate, oat fiber, or modified dent corn starch (Pure Gel B990 Starch; Grain Processing Corp., Muscatine, IA, USA). The 90:10 treatment was composed of 3.15% oat fiber and 0.35% modified corn starch. The 50:50 blend was composed of 1.75% oat fiber and 1.75% modified corn starch. The 10:90 blend was composed of 0.35% oat fiber and 3.15% modified corn starch. Each treatment also contained 18% added water, 1.7% seasoning (smoked sausage seasoning: SC-16-135-000; A.C. Legg, Inc., Calera, AL, USA), 1.3% vinegar (e(Lm)inate V; Hawkins, Inc., Roseville, MN, USA), and 0.5 % added sodium chloride. To formulate, MSC was batched into 22.6 kg containers and deposited into a Hollymatic® 3000 Mixer / Grinder (Hollymatic

Corporation, Countryside, IL, USA) for mixing dry and wet ingredients. After allowing the meat and ingredients to blend thoroughly, product was deposited (without grinding blades or plates) into separate containers in preparation for stuffing into casings. The order of formulation was: B) without binders, C) 90:10 blend, D) 50:50 blend, E) 10:90 blend, and lastly A) with phosphate.

Following formulation, each treatment was stuffed into a hog intestine casing (35/38 Whisker free, tubed hog casings; DeWied International) using a VEMAG Robot 500 (VEMAG Maschinenbau GmbH, Verden, Germany) vacuum stuffer with a 1.27 diameter stuffing horn. Sausages were linked by hand (tied off as needed using food-grade twine), placed onto a metal smokehouse stick, and weighed (raw weight) before being placed on a smokehouse truck. Treatments were placed onto a smokehouse truck in a manner that displayed the negative control at the top; each following treatment was represented in the same formulation order, in a snake pattern down the smokehouse truck ending with the positive control at the bottom. The treatments were split between two smokehouse trucks to create two cooking replications within the same smokehouse (Koch Grand Prize, Kansas City, MO, USA) using an UltraSource CookMaster II (UltraSource LLC., Kansas City, MO, USA). The cooking cycle program was as follows: 1) drying 30 minutes at 48.8ºC and 43% relative humidity (RH), 2) natural smoke 40 minutes at



54.4ºC and 40% RH, 3) drying 15 minutes at 54.4ºC and 0% RH, 4) natural smoke 40 minutes at 60ºC and 50% RH, 5) drying 15 minutes at 65.5ºC and 0% RH, 6) cooking to an internal temperature of 78.8ºC at 87.7ºC and 100% RH, and 7) holding at 60ºC and 100% RH until sausage was removed. Following the completion of the cook cycle, each stick of sausage was weighed, weights were recorded to evaluate cook loss, and sticks were returned to the smokehouse truck.

$$\frac{\text{Raw Weight} - \text{Cook Weight}}{\text{Raw Weight}} \times 100 = \text{Cook Loss}$$

Immediately following the recording of cook weights, the smokehouse truck was placed into a blast cooler at 2°C. After 24 hours in the blast cooler, each stick was weighed, weights were recorded to evaluate chill loss and total percent yield, and sausages were removed from sticks by treatment for packaging.

Following the recording of final weights, each stick of sausage was removed and cut into single link pieces for packaging in vacuum bags (8”x15” 3 mil; Sealed Air, Charlotte, NC, USA)

$$\frac{\text{Raw Weight} - \text{Chill Weight}}{\text{Raw Weight}} \times 100 = \text{Total Yield}$$

using an Ultravac® 2100 Dual Chamber Vacuum Packaging Machine (UltraSource LLC., Kansas City, MO, USA). Thirteen groups of five sausages were randomly selected from each treatment for sensory, pH, and color analysis; thirteen groups of one sausage were randomly selected for microbiological analysis; thirteen groups of one sausage were randomly selected for texture profile analysis. The remaining sausages were packed in sets of four for any additional needs or in case of packaging malfunction. Each bag was given an identity based on treatment, cook cycle, and analysis method, resulting in a three character identity. Following packaging, all

bags were stored by treatment into a corrugated box at 1°C ± 2°C in a blast cooler to mimic storage conditions in a food service institution. Following a 4 day holding period to mimic product shipping from further processor to food service location, initial trials were then conducted every seven days over a ninety day period.

Texture Profile Analysis

Every seven days, starting with week zero (0), one package of each treatment and cook cycle was used to perform a texture profile analysis (TPA). Sausages indicated for TPA evaluation were removed from their package and cut into three (3) 2.54 cm segments and evaluated using a TA-XT2icon Texture Analyser (Texture Technologies Corp. and Stable Micro Systems, Ltd., Hamilton, MA, USA) with a 25 mm cylinder press. The TPA parameters were set to 2 mm/s pre-test speed, 5 mm/s test and posttest speed, compression strain at 35.0%, 0.5 sec between compressions, and auto-trigger force set at 0.005 kg of force.

Proximate Analysis

Sausages (n=2) were randomly selected from the extra products to be completely homogenized using an Osterizer 10 speed blender (Sunbeam®-Oster®, Boca Raton, Florida, USA). FOSS FoodScan™ with ISIscan™ software was used to determine moisture, protein, fat, collagen, and salt content of each sample. Once homogenized, a sample cup [D:140 mm, 14 mm height (FOSS Analytical A/S, Foss Allé 1, DK-3400 Hillerød, 44 Denmark)] was filled completely with sample and pressed into the plate. Each sample weighed approximately 180 g. Samples were packed completely to ensure no air pockets or gaps existed and the surface was even. The ISIscan™ software was initiated and a check cell procedure was run in duplicate to calibrate the device prior to evaluating any sample. Data were exported from ISIscan™ software

into Excel (Microsoft Office, Redmond, WA, USA) and duplicate runs were averaged for each sample for each value.

Color and pH

Two sausages were removed from the five sensory sausages and objective color values were measured (L*, a*, b*) using a Hunter Miniscan XE Plus (Model MSXP-4500C; Hunter Laboratories, Reston, VA, USA) using a D_{65} illuminant with a 10^0 observance angle and a 2.54 cm aperture. The colorimeter was calibrated with HunterLab white and black instrument working standard tiles. Color analysis was measured in duplicate on each sausage for accurate representation, and an average value of L*, a*, and b* was recorded.

Following color analysis, pH was evaluated in duplicate for sausage treatments. The pH of each sausage was assessed via Oakton pH Spear Waterproof Pocket pH Testr™ (OAKTON Instruments, Vernon Hills, IL, USA). The pH was evaluated by cutting both sausages in half, inserting the probe into the open face of each sausage, and observing until equilibrium was reached.

Consumer Sensory Evaluation

The remaining three sausages were utilized for consumer sensory evaluation. A protocol for a consumer sensory panel was reviewed and approved by the Auburn University Institutional Review Board for the Protection of Human Subjects (IRB). A 9-point rating scale was utilized to evaluate five sensory parameters: juiciness, cohesiveness, flavor, texture, and overall acceptability [1= extremely dry, extremely crumbly, dislike extremely (flavor, texture, and overall acceptability); 9= extremely juicy, extremely cohesive, like extremely (flavor, texture, and overall acceptability)]. Panelists were encouraged to write any comments to the side of the row of boxes for each sample.

Each sausage was placed into a full size deep steam table pan (527mm x 325 mm x 85 mm; SAM'S West, Inc., Bentonville, AR, USA). Sausages were reheated in double oven (MET8775XS00; Whirlpool Co., Benton Harbor, MI, USA) at approximately 177°C. The internal temperature of the sausage was monitored with copper constantan thermocouple wire inserted in the geometric center of one sausage in each pan and attached to a hand-held Omega data logger HH309A thermometer (OMEGA® Engineering Inc., Stamford, CT, USA) until the internal temperature reached 79.4°C (ServSafe, 2010). Sausages were removed from the oven, cut into 2.54 cm segments, and further sliced in half lengthwise to reduce potential sensory fatigue. Each treatment was designated with a randomly assigned 3-digit code to ensure no bias, and samples were placed into clear plastic cups with lids. Each panelist (n=30) received one sample of each treatment (n=10), salt-free saltine crackers, and diluted apple juice. Panelists were instructed on how to fill out their survey and to cleanse their palate by consuming a cracker followed by a sip of diluted apple juice between each sample. Panelists were recruited to evaluate samples once a week over 13 consecutive weeks. Panelists were primarily students and faculty of the College of Agriculture at Auburn University.

Storage Life Microbial Study

After the holding period, one package of each treatment was pulled for total plate count microbial analysis. Twenty five grams of each treatment was aseptically removed and placed into a sterile Whirl-Pac sample bag (1627 mL Filter Bag; Nasco Whirl-Pac, Atkinson, WI, USA,) with 225 mL of 0.1% peptone water (BPW; Bacto™ Peptone; Becton, Dickinson and Company, Sparks, MD, USA), and stomached for 2 minutes at 300 rpm (Stomacher® 400 Circulator Paddle Blender; Seward LTD, London, England, UK). After stomaching, serial dilutions were created by pipetting 1 mL from the Whirl-Pac bag (dilution 0) into 9 mL of BPW, and then mixing for 5

seconds using an analog vortex mixer (VWR International, Radnor, PA, USA). This process was repeated until all dilutions had been created. The number of dilutions utilized was sufficient to observe clearly enumerable colonies based on a variety of factors including raw material base, length of storage, and subject matter expert advice. After all dilutions were complete, 100 µL of each dilution was pipetted onto the surface of agar in petri plates (100 x 15 mm; VWR International, Radnor, PA, USA) in duplicate and spread with a disposable L-shaped culture spreader (VWR International, Radnor, PA, USA) for either aerobic or anaerobic growth. Aerobic agar (Difco™ Plate Count Agar, Standard Methods; Becton, Dickinson and Company, Sparks, MD, USA) plates were incubated for 24 hour at 35 ± 1°C. Anaerobic agar (Difco™ Anaerobic Agar; Becton, Dickinson and Company, Sparks, MD, USA) plates were incubated inside a sealed container (MGC AnaeroPack® System, Rectangular Jar 7.0 L; Mitsubishi Gas Chemical Co., Inc., Chiyoda, Tokyo, Japan) with oxygen scavenger packs (GasPak™ EZ; Becton, Dickinson and Company, Sparks, MD, USA) for 48 hours at 35 ± 1°C. All plates were enumerated after incubation and results were reported in CFU/g of meat.

Statistical Analysis

The experiment utilized completely random design. Statistical analysis was performed using the mixed procedure of SAS 9.4 with repeated measures design and fixed effects of treatment (SAS Inst. Inc., Cary, NC, USA). Meats for treatment were separated using least squares means procedure with P set at 0.05 for significance. Orthogonal contrasts were used to look at linear, quadratic, and cubic effects of dependent variables over time (Friendly, 2008; Hays, 1994). Cook yield for both batches exiting the smokehouse was evaluated first with treatment and smokehouse as fixed effects. Following yield analysis, consumer sensory panel,

texture profile analysis, color and pH, proximate analysis, and microbiological storage study were analyzed.

IV. Results and Discussion

Formulation

During the formulation process, working with the mechanically separated chicken meat was difficult. The raw material was very sticky and clung to equipment, making it difficult to effectively blend all the ingredients into the mixture. Every container utilized to weigh chicken had to be scraped clean to get all the raw material into the mixer/grinder. After the sausage batter was mixed and went back into the containers, the whole process had to be repeated to get the sausage batter into the vacuum stuffer. Mechanically separated chicken was a difficult product to use in sausage manufacturing in a pilot plant and could be a serious problem for a further processor attempting to run large volumes of the product with no other meat sources to give the MSC more bulk.

When stuffing the product, there were severe product losses with the phosphate treatment. Unlike the experimental blend treatments that had experienced some apparent reduced viscosity with the addition of oat fiber and modified corn starch, the with phosphate treatment slid into the vacuum stuffer's screw conveyor and proceeded in the wrong direction, towards the vacuum pump. This is a serious flaw for further processors attempting to use a vacuum stuffer as it could clog up the pump, lead to severe microbial contamination, and create hours of down time trying to repair stuffers. The stuffing observation was not a critical flaw in the processing portion of this study, but represents a serious concern for further processors.

Cook Yield

Cook yield analysis was performed to measure the impact of phosphate and three blends of oat fiber and modified dent corn starch (Table 2). Yield analysis allowed researchers to determine if any variations in batch one and batch two occurred. Type three tests of cook yield revealed no interaction ($P = 0.1045$) for treatment by smokehouse. The positive control (A) had a lower ($P < 0.05$) yield than all other treatments, which were similar ($P \geq 0.05$) (Table 2). This result contradicts previous studies that indicate incorporating phosphate into a meat block will increase water binding capacity and increase cook yields (Knipe et al., 1985; Young et al., 2005). The effect of oat fiber and modified corn starch to increase yields are supported by this data, although not to the extent that other studies have observed (Beggs et al., 1997; Claus and Hunt, 1991; Seo et al., 2015)

Interaction – pH

There was a treatment by week interaction for pH ($P < 0.0001$) (Table 3). There was a cubic response in the data (Figure 1). There was no definitive week by treatment trends that could be determined from the data, however the data can be interpreted as an overall reduction in pH value occurring over extended storage time. When comparing treatments among each other in the same week (Week X Treatments A- E), with phosphate had the lowest ($P < 0.05$) or similar to the lowest ($P \geq 0.05$) pH value 11 of 13 weeks. Conversely, the without binder treatment (B) had the greatest ($P < 0.05$) or similar to the greatest ($P \geq 0.05$) pH value 10 of 13 weeks. While not conclusive, this difference in pH could have a role in the total yield (Table 2) seen in the current experiment as pH greatly affects WHC (Aberle et al., 2012; Beggs et al., 1997; Sebranek, 2009; Young et al., 2005). Experimental blends of 90:10 (C) and 50:50 (D) were similar ($P \geq 0.05$) 10 of 13 weeks and were more frequently paired together than either treatment with the

blend 10:90 (E) in any combination. When within the same treatment by week (treatment X week 0-12) week 2 always had the greatest pH value and no other weeks were similar to week 2. Week 12 had the lowest ($P < 0.05$) pH value in 4 of 5 treatments, and was similar ($P = 0.8557$) to the lowest in treatment A. Week 11 was the next to lowest value, or similar to the next to lowest value, in all 5 treatments over weeks. The data indicate statistically significant figures, however the pH values are between 6.1 and 6.4 primarily, which are within the average range of MDM so the differences are not biologically noteworthy (Grunden et al, 1972).

Interaction - Texture of Consumer Sensory Evaluation

There was a treatment by week interaction for texture during the consumer sensory panel evaluation (P 0.0133) (Table 4). There was a cubic response from data (Figure 2). Clear, overarching trends are difficult to determine within the data set, but some data stand out. When evaluating all treatments within a week (Week X Treatments A-E) with phosphate treatment (A) and 10:90 blend (E) always had similar ($P \geq 0.05$) texture values and were the greatest in all weeks that exhibited significant differences (n=9), with the exception of week 12 when A was greater ($P = 0.0248$) than E. The 50:50 blend (D) and 90:10 blend (C) were similar ($P \geq 0.05$) in 8 of 9 significant weeks. When evaluating all weeks within a treatment, without binder (B) had a steady numerical decline in texture values following week 6 of the study till the conclusion, from 6.5 (slightly acceptable) to 5.36 (neutral) ($P = 0.001$). Week 12 in all treatments had the lowest or similar to the lowest texture value. With the exception of with phosphate (A) in week 12, all sausages experienced decreased texture values for weeks 10 through 12. Panelist noted that treatments with oat fiber included at 1.75% or more (C and D) sausages began to have a graininess to them.

Proximate Analysis

Proximate analysis values can be found in Table 5. Collagen levels were lowest in treatment 10:90 blend (E), and greatest in without binder (B). Lipid levels were similar and numerically highest in treatments with phosphate (A) and without binder (B). The 90:10 blend (C) and 50:50 (D) had similar ($P \geq 0.05$) lipid levels, and 10:90 blend (E) had the least lipid content ($P = 0.0046$). Previous research on low fat sausages indicates that flavor can become more spicy, salty, and the sensory characteristics negatively altered as fat is decreased with binders (Hughes et al., 1997; Beggs et al., 1997). Moisture levels were higher in with phosphate (A) and without binders (B); B had the greatest moisture levels ($P > 0.05$). The 90:10 blend (C) and the 50:50 blend (D) were similar, and 10:90 (E) had the lowest moisture values. Protein values were highest in A; treatments B and C, and C and D shared similarity ($P \geq 0.05$). Treatment E had the numerically lowest protein values in the study. No differences in salt content were observed. Week 1 had the nest to greatest pH, or similar ($P \geq 0.05$) to the next to greatest pH in all treatments.

Objective Color Analysis

Objective color evaluations are found in Figures 3 and 4 and Table 6. There were no differences ($P \geq 0.05$) for L* by treatment or week. This corresponds to data from Hughes et al. (1997) who found that incorporating oat fiber or carrageen in low fat sausages did not alter the objective color values of pork sausages. Conversely, Pereira et al. (2011) found that incorporating mechanically deboned poultry meat into the meat block reduced L* values (more dark) because of the higher myoglobin content. When evaluating a*, there was an effect for treatment but not for week. The with phosphate treatment (A) had a more positive a* value ($P = 0.0004$) than all other treatments. Treatment A had more MSC than other treatments by

percentage, which supports studies suggesting that an increase in MSC results in higher a* values due to increased heme iron content (Froning, 1981; Mielnik et al., 2002; Pereira et al., 2011). Without binders (B) was the least red ($P < 0.05$). All experimental blends were similar ($P \geq 0.05$). An effect for treatment and week was observed for b*. Treatment A had more positive b* values ($P < 0$ .05) than all other treatments. The 90:10 blend (C) and without binders (B) shared similarities ($P \geq 0.05$), as did treatments B and 50:50 blend (D), and D and 10:90 blend (E). The incorporation of modified corn starch as a fat replacer and extender may have some impact on the yellowness (b*) of sausage although no definite associations can be made at this time. There was no obvious trend among weeks for b* (Figure 4).

Consumer Sensory Evaluation

Consumer Sensory Evaluation means can be found in Figures 5 and 6 and Table 7. Effects were observed for treatment and week, however no interaction was observed. Perceived Juiciness had a linear response (Figure 7). Juiciness was greatest ($P = 0.0048$) in the treatment without binders (B). This is likely because less water is being bound by either phosphate or binders, meaning that more water is available for the sensory evaluation. This correlates to work by Rongrong et al. (1998) who found that higher inclusion of water increased sensory values for juiciness. Water is constant in the current study, however the action of functional ingredients for with phosphate (A), 90:10 blend (C), 50:50 blend (D), and 10:90 blend (E) had an effect ($P < 0.05$) on juiciness. Juiciness of treatments decreased significantly for A, E, D and C respectively, though no treatments were deemed unacceptable. When evaluating the change in juiciness over weeks, the data indicate that treatments became more juicy as storage time increased, perhaps indicating a break down in the water binding abilities of the sausages.

Cohesiveness values were greatest for experimental sausages. Cohesiveness yielded a quadratic data response (Figure 8). The 90:10 (C) and 50:50 (D) had the greatest cohesiveness values and were similar ($P = 0.1394$), while the with phosphate had the lowest ($P < 0.05$) cohesiveness value. These results are similar to previous sensory studies where the addition of starch and dietary fiber has increased the cohesiveness of products (Beggs et al., 1997; Troutt et al., 1992). Though not steadily, cohesiveness values declined over time after week 4 (week 4 to week 12 = $P < 0.0001$).

Flavor had a quadratic response but was tending towards a cubic reaction (Figure 9). Flavor values were highest for treatment A; treatments B and E and treatments D and C were similar. Flavor values were volatile over weeks, but never scored below 'slightly acceptable' by panelists.

Overall acceptability data had a quadratic response (Figure 10). Overall acceptability was greatest for treatment A ($P = 0.0001$), followed by the similar ($P \geq 0.05$) pair of without binders (B) and 90:10 blend (E) and the pair of 50:50 (D) and 90:10 (C). Overall acceptability improved from week 1 to week 2, and was similar ($P \geq 0.05$) until declining in week 12. It's important to note that overall acceptability of all treatments was generally scored as slightly acceptable or better by panelists. This indicates that while one treatment may receive greater approval from the public, all are generally well received.

When analyzing consumer sensory evaluations, researchers attempted to identify if any categories of the evaluation could be linked, either negatively or positively, to overall acceptability. Researchers concluded that it might be possible to gain insight into the sensory parameters that consumers desire when evaluating a smoked sausage product. Flavor, when treatments were evaluated with all weeks compressed as seen in Figure 5 and Table 7, was

directly correlated to differences in the overall acceptability. The greatest values for texture correlated to two of the three highest scores of overall acceptability. Cohesiveness and juiciness could not definitively be associated with better overall acceptability, although a lower cohesiveness value and a higher juiciness value associated with both the controls, which were evaluated as more acceptable than treatments containing 1.75% or 3.15% oat fiber. It is therefore concluded flavor and texture acceptability were most closely tied to overall acceptability in this study and further research on what drives smoked sausage acceptability could be of interest for future studies. These results are supported by previous studies evaluating products categorized as sausages (Morey et al., 2012; Rongrong et al., 1998; Szczepaniak et al., 2005).

Texture Profile Analysis

Texture Profile Analysis values can be found in Figures 11-14 and Table 8. No effect was observed for the interaction of treatment and week, however both treatment and week had an effect. Hardness had a cubic response (Figure 13). Hardness was similar and least ($P < 0.05$) for with phosphate (A) and without binders (B), and greatest for experimental 90:10 blend (C) ($P = 0.0323$). Hardness was volatile over weeks, and showed no clear trends. Hardness substantially decreased in week 12 following a mostly stable pattern, indicating product degradation.

Springiness had quadratic response from the data (Figure 14). Springiness was similar and least for 4 treatments, with phosphate (A), without binders (B), 90:10 blend (C), and 50:50 blend (D), and for treatments D and 10:90 blend (E) were similarly greatest. Springiness over time increased from weeks 0 to 5. A decrease occurred for two weeks and then spiked ($P < 0.05$) in week 8. After week 8, springiness declined.

Cohesiveness had quadratic response (Figure 14). Cohesiveness was lowest ($P < 0.05$) for treatments C and D. As seen in Table 8, sensory cohesion did not follow this same pattern. The

highest sensory cohesion values correlate to the lowest TPA cohesion values. This is supported by Rongrong et al. (1998) and Lyon and Lyon (1990) who both reported variations in instrumental and sensory texture measurements. Treatment E was similar ($P \geq 0.05$) to treatment B, which was similar to treatment A. Cohesiveness of all treatments was volatile over storage time, but exhibited three clear episodes of increasing and decreasing at weeks 6, 10, and 12. Cohesiveness had a general trend towards decreasing over time.

Gumminess had cubic response (Figure 13). Gumminess values were lowest ($P < 0.05$) for treatments A and B and greatest ($P < 0.05$) in treatments D and E. Gumminess values over time were similar for weeks 0 through 4 when the values began to increase before reducing ($P = 0.0001$).

Chewiness had a cubic response (Figure 13). Chewiness and Gumminess are similar to Hardness because they are both calculated off of Hardness. Chewiness was lowest for the with phosphate (A) and without binders (B), which were similar ($P \geq 0.05$), and greatest ($P < 0.05$) for 50:50 blend (C). Chewiness values were similar for weeks 0 through 4, before beginning to increase through week 11 where values fell sharply ($P < 0.0001$).

Resilience had a quadratic response (Figure 14). Resilience was least and similar for treatments C and D, while treatments A and B were similarly more resilient than treatment E. As storage extended, there were three major decreases in resilience values, from week 5 to 6 ($P = 0.0003$), week 9 to 10 ($P < 0.0001$), and week 11 to 12 ($P < 0.0001$). It is unclear why the significant decreases occurred.

Storage Stability

Results from the microbiological study are found in Figures 15 and 16 and Table 9. No interaction for treatment by week was observed for total plate count growth for either aerobic or

anaerobic incubation. Type 3 Test for aerobic plate count growth indicated week and treatment effects and week effects for anaerobic plate count growth. Aerobic plate count was lowest in treatment A ($P = 0.0384$). Treatments B, E, and C, were similar ($P \geq 0.05$) while C and D were similar ($P \geq 0.05$) and lowest for aerobic plate count growth. When analyzing all treatments by week, values were lowest ($P < 0.05$) at week 0 before increasing weeks 1 through 6 then deceasing weeks 7 through 12. The average of colony forming units observed did not exceed 4.5 $\log_{10}$ aerobic growth at any point in the study, indicating spoilage did not occur. Anaerobic plate count growth was lowest ($P < 0.05$) at week 2 although week 0 was similar ($P \geq 0.05$) to the next to least total plate count observed. With the exception of week 2, there was gradual increase weeks 0 through 6 then gradual decrease from weeks 7 through 12. No spoilage was detected during evaluation of anaerobic total plate count growth.

Cost

Cost of formulations can be found in Tables 10-15. Mechanically separated meat was the most expensive raw ingredient because of the inclusion rate. Oat fiber was more expensive per kg than phosphate, which was more expensive than modified corn starch. Treatment B had the lowest cost followed by Treatments A and C respectively. Treatment C was the most expensive because of a high inclusion of oat fiber.

V. Conclusions

Proximate analysis of the different treatments utilized in this study indicate that incorporating fiber and starches decreased moisture and lipid content. When analyzing consumer sensory evaluations, incorporating oat fiber and modified corn starch in a blend of 3.5% does not cause consumer overall acceptability to become dislikable. Incorporating oat fiber and corn starch does cause a sausage to become or nearly become moderately cohesive. Sausages without oat fiber and corn starch were considered moderately juicy by panelists while sausages with fiber and starch received slightly juicy or neutral scores. Texture profile analysis indicated that hardness and chewiness of treatments containing oat fiber and corn starch could be higher. Anaerobic and aerobic plate count analysis indicated no product spoilage occurred during 13 weeks of storage. The negative control was the least cost choice, however the positive control and the blend 10:90 were priced comparably.

If a processor wanted to remove phosphates from a formulation for a more clean label product, then the best recommendation based on this study would to incorporate 3.15% modified corn starch and 0.35% oat fiber to replace the effects of phosphate.

VI. Implications

The best blend of oat fiber and modified corn starch used when evaluating for consumer acceptance is 0.35% oat fiber and 3.15% modified corn starch. The 10:90 blend of corn starch was marginally less cost effective than the positive control, meaning that it remains economically viable. If a meat processor wanted to remove phosphates from a formulation for a more clean label product, then the best recommendation based on this study would to incorporate 3.15% modified corn starch and 0.35% oat fiber.

This product is recommended for use in restaurants and other food serving locations where customers receive food either by employee or buffet style. This product is not recommended for use in retail sales or consumer choice situations due to potential consumer discrimination against mechanically separated chicken.

Incorporating emulsion stability and viscosity measurements to this study would have added value. Additional research evaluating varying levels of water inclusion and more alternative functional ingredients, such as celery and cherry powder, is needed to determine processing changes resulting in more clean label alterations.

Table 1. Sausage formulations as a percent of raw weight.

	Treatment				
	With phosphate	Without binders	Blend 90:10	Blend 50:50	Blend 10:90
	A	B	C	D	E
Ingredient[1]					
Water	18%	18%	18%	18%	18%
Phosphate	0.43%	-----	-----	-----	-----
Oat Fiber	-----	-----	3.15%	1.75%	0.35%
Modified Corn Starch	-----	-----	0.35%	1.75%	3.15%
Salt	2.00%	2.00%	2.00%	2.00%	2.00%
Sodium Nitrite	0.0625%	0.0625%	0.0625%	0.0625%	0.0625%
e(Lm)inate V	1.3%	1.3%	1.3%	1.3%	1.3%
Spices	1.7%	1.7%	1.7%	1.7%	1.7%

[1] All treatments are based on 54.4 kg of mechanically separated chicken.

Table 2. Least Squares Analysis of Total Yield.

Treatment	*Total Yield*	*SEM*
With Phosphate (A)	84.06[b]	0.250
Without Binders (B)	85.44[a]	0.224
90:10 Blend (C)	85.55[a]	0.2377
50:50 Blend (D)	84.95[a]	0.224
10:90 Blend (E)	85.20[a]	0.224

[ab] Means within the same column with the same superscripts are similar ($P \geq 0.05$)

Table 3. Interaction between Week and Treatment Least Squares Means[1] for pH.

	Treatment					
Week	A[2]	B[2]	C[2]	D[2]	E[2]	T*W[3]
0	6.28^{c,xy}	6.33^{bc,w}	6.29^{c,x}	6.28^{c,xy}	6.26^{ef,y}	S
1	6.31^{bc,x}	6.35^{b,w}	6.35^{b,w}	6.34^{b,w}	6.31^{bc,x}	S
2	6.37^{a,x}	6.40^{a,w}	6.39^{a,wx}	6.40^{a,w}	6.39^{a,wx}	S
3	6.21^{d,y}	6.26^{d,w}	6.25^{d,wx}	6.25^{g,wx}	6.23^{g,xy}	S
4	6.29^{c,x}	6.32^{c,w}	6.30^{c,wx}	6.28^{cd,wx}	6.30^{cd,x}	S
5	6.17^{e,x}	6.23^{e,w}	6.23^{de,w}	6.22^{fg,w}	6.24^{fg,w}	S
6	6.28^{c,xy}	6.32^{c,w}	6.29^{c,x}	6.26^{de,y}	6.29^{de,xy}	S
7	6.20^{d,x}	6.22^{ef,wx}	6.23^{de,w}	6.23^{fg,w}	6.24^{fg,w}	S
8	6.29^{c,x}	6.33^{bc,w}	6.33^{b,w}	6.33^{b,w}	6.33^{b,w}	S
9	6.33^{b,w}	6.22^{ef,x}	6.21^{ef,x}	6.20^{g,x}	6.22^{g,x}	S
10	6.31^{bc,w}	6.27^{d,y}	6.28^{c,xy}	6.28^{cd,xy}	6.30^{cd,wx}	S
11	6.13^{f,y}	6.20^{fg,x}	6.18^{fg,x}	6.19^{fg,x}	6.24^{fg,w}	S
12	6.14^{f,x}	6.18^{g,w}	6.16^{g,wx}	6.16^{h,wx}	6.16^{h,wx}	S

[1] SEM = 0.00968

[2] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[3] Interactions are listed as significant if $P < 0.05$.

^{abcdefgh} Means within the same column with common superscripts are not different ($P \geq 0.05$).

^{wxy} Means within the same row with common superscripts are not different ($P \geq 0.05$).

Table 4. Interaction between Week and Treatment Least Squares Means[1] for Sensory Texture.

	Treatment					
Week	A	B	C	D	E	T*W[2]
0	$6.37^{b,w}$	$6.30^{a,w}$	$6.10^{abc,w}$	$5.93^{ab,w}$	$6.21^{bc,w}$	NS
1	$6.51^{ab,w}$	$6.00^{abcd,w}$	$6.00^{abc,w}$	$6.21^{ab,w}$	$6.18^{bc,w}$	NS
2	$6.66^{ab,w}$	$6.55^{a,wx}$	$5.40^{d,y}$	$6.03^{ab,x}$	$6.38^{ab,wx}$	S
3	$6.56^{ab,w}$	$6.36^{a,wx}$	$6.21^{abc,wx}$	$6.00^{ab,x}$	$6.63^{ab,w}$	S
4	$6.60^{ab,w}$	$6.18^{a,w}$	$6.30^{ab,w}$	$6.23^{ab,w}$	$6.56^{ab,w}$	NS
5	$6.46^{ab,w}$	$6.13^{ab,wx}$	$5.76^{bcd,x}$	$5.93^{ab,wx}$	$6.63^{ab,w}$	S
6	$6.96^{a,w}$	$6.50^{a,wx}$	$5.93^{bcd,y}$	$6.16^{ab,xy}$	$6.68^{ab,w}$	S
7	$6.48^{ab,wx}$	$6.15^{ab,x}$	$5.98^{bc,x}$	$6.41^{a,wx}$	$6.81^{a,w}$	S
8	$6.53^{ab,w}$	$6.05^{abc,w}$	$6.55^{a,w}$	$6.28^{ab,w}$	$6.31^{ab.w}$	NS
9	$6.66^{ab,w}$	$5.45^{de,x}$	$6.30^{ab,w}$	$6.33^{ab,w}$	$6.61^{ab,w}$	S
10	$6.75^{ab,w}$	$5.61^{bcde,y}$	$6.16^{abc,wy}$	$6.45^{a,wx}$	$6.68^{ab,wx}$	S
11	$6.03^{b,wxy}$	$5.56^{cde,y}$	$5.95^{bcd,wy}$	$6.28^{ab,wx}$	$6.51^{ab,w}$	S
12	$6.31^{b,w}$	$5.36^{e,x}$	$5.71^{cd,x}$	$5.8^{b,wx}$	$5.68^{c,x}$	S

[1] SEM = 0.194

[2] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[3] Interactions are listed as significant if $P < 0.05$.

[abcde] Means within the same column with common superscripts are not different ($P \geq 0.05$).

[wxy] Means within the same row with common superscripts are not different ($P \geq 0.05$).

Table 5. Least Squares Means of Proximate Analysis.

	Collagen	*Fat*	*Moisture*	*Protein*	*Salt*
A[1]	0.92^{ab} ± 0.0591	11.56^{a} ± 0.0777	69.15^{b} ± 0.122	13.28^{a} ± 0.111	1.78 ± 0.0632
B[1]	1.05^{a} ± 0.0512	11.41^{ab} ± 0.0673	69.78^{a} ± 0.106	12.89^{b} ± 0.0968	1.72 ± 0.0547
C[1]	0.82^{bc} ± 0.0512	11.29^{bc} ± 0.0673	68.25^{c} ± 0.106	12.64^{bc} ± 0.0968	1.85 ± 0.0547
D[1]	0.95^{ab} ± 0.0512	11.15^{c} ± 0.0673	67.94^{c} ± 0.106	12.50^{cd} ± 0.0968	1.84 ± 0.0547
E[1]	0.75^{c} ± 0.0512	10.83^{d} ± 0.0673	67.30^{d} ± 0.106	12.25^{d} ± 0.0968	1.83 ± 0.0547

[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[abcd] Means within the same column with common superscripts are not different ($P \geq 0.05$).

Table 6. Least Squares means of Objective Color.

	*L**	*a**	*b**	*pH*
Treatment				
A[1]	44.25	16.19[a]	21.83[a]	6.25[c]
B[1]	42.45	14.49[c]	20.88[bc]	6.28[a]
C[1]	41.17	15.25[b]	21.23[b]	6.27[b]
D[1]	42.75	15.40[b]	20.39[cd]	6.26[b]
E[1]	43.05	15.10[b]	20.03[d]	6.27[b]
SEM	*0.7656*	*0.1485*	*0.1939*	*0.00268*
Week				
0	44.77	15.11	21.79[a]	6.29[c]
1	40.53	14.85	20.21[de]	6.33[b]
2	42.34	15.12	20.84[bcde]	6.39[a]
3	41.71	15.38	21.08[abcd]	6.24[d]
4	42.07	15.01	20.51[cde]	6.29[c]
5	43.07	15.96	21.42[ab]	6.22[e]
6	43.53	15.27	20.80[bcde]	6.29[c]
7	43.02	15.25	20.14[e]	6.22[e]
8	44.79	15.55	20.84[bcde]	6.32[b]
9	42.69	15.59	21.05[abcde]	6.23[d]
10	43.22	15.46	20.87[bcde]	6.28[c]
11	43.69	15.33	21.31[abc]	6.19[f]
12	40.11	14.84	20.48[cde]	6.16[g]
SEM	*1.234*	*0.2394*	*0.3127*	*0.00433*

[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[abcde] Means within the same column and independent variable with common superscripts are not different ($P \geq 0.05$).

Table 7. Least Squares Means of Consumer Sensory Evaluation[1].

	Juiciness	*Cohesiveness*	*Flavor*	*Texture*	*Overall Acceptability*
Treatment					
A[2]	7.10^{b}	6.64^{c}	6.91^{a}	6.53^{a}	6.76^{a}
B[2]	7.34^{a}	5.99^{d}	6.63^{b}	6.01^{b}	6.32^{b}
C[2]	5.33^{e}	6.97^{ab}	6.24^{c}	6.02^{b}	5.97^{c}
D[2]	5.50^{d}	7.07^{a}	6.27^{c}	6.16^{b}	6.10^{c}
E[2]	6.12^{c}	6.87^{b}	6.62^{b}	6.45^{a}	6.45^{b}
SEM	*0.0593*	*0.0469*	*0.055*	*0.054*	*0.0550*
Week					
0	5.90^{e}	6.73^{cd}	6.28^{d}	6.18^{b}	6.18^{b}
1	6.09^{de}	6.85^{bcd}	6.54^{bc}	6.18^{b}	6.27^{ab}
2	6.09^{de}	6.82^{bcd}	6.37^{cd}	6.20^{ab}	6.24^{ab}
3	6.27^{cd}	7.05^{ab}	6.62^{abc}	6.35^{ab}	6.41^{ab}
4	6.37^{bc}	7.18^{a}	6.67^{ab}	6.37^{ab}	6.47^{a}
5	6.31^{bcd}	6.66^{def}	6.46^{bcd}	6.18^{b}	6.32^{ab}
6	6.21^{cd}	6.90^{bc}	6.67^{ab}	6.45^{a}	6.49^{a}
7	6.30^{bcd}	6.79^{cd}	6.38^{cd}	6.37^{ab}	6.33^{ab}
8	6.19^{cd}	6.43^{e}	6.86^{a}	6.34^{ab}	6.43^{ab}
9	6.57^{ab}	6.30^{e}	6.84^{a}	6.27^{ab}	6.46^{a}
10	6.25^{cd}	6.77^{cd}	6.50^{bcd}	6.33^{ab}	6.36^{ab}
11	6.65^{a}	6.65^{d}	6.52^{bcd}	6.07^{b}	6.30^{ab}
12	6.42^{abc}	6.09^{f}	6.27^{d}	5.77^{c}	5.92^{c}
SEM	*0.0956*	*0.0757*	*0.0889*	*0.871*	*0.0887*

[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.

[2] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[abcde] Means within the same column and independent variable with common superscripts are not different ($P \geq 0.05$).

Table 8. Least Squares Means of Texture Profile Analysis

	Hardness (kg)	*Springiness*	*Cohesiveness*	*Gumminess (kg)*	*Chewiness (kg)*	*Resilience*
Treatment						
A[1]	5.87[a]	0.937[a]	0.792[c]	4.65[a]	4.37[a]	0.523[c]
B[1]	6.00[a]	0.928[a]	0.789[bc]	4.74[a]	4.41[a]	0.524[c]
C[1]	8.15[d]	0.911[a]	0.756[a]	6.15[c]	5.61[c]	0.459[a]
D[1]	7.63[c]	0.917[ab]	0.751[a]	5.74[b]	5.28[b]	0.454[a]
E[1]	7.05[b]	0.919[b]	0.783[b]	5.53[b]	5.09[b]	0.506[b]
SEM	*0.167*	*0.00607*	*0.00294*	*0.133*	*0.128*	*0.00393*
Week						
0	6.79[bc]	0.907[ab]	0.772[cd]	5.24[bc]	4.75[bc]	0.477[b]
1	6.74[bc]	0.922[bcd]	0.775[de]	5.23[b]	4.81[bc]	0.494[bcde]
2	6.62[a]	0.924[bcd]	0.791[f]	5.21[b]	4.83[bc]	0.512[ef]
3	6.65[a]	0.919[bcd]	0.788[ef]	5.23[bc]	4.80[bc]	0.507[cdef]
4	6.52[a]	0.924[bcd]	0.779[def]	5.08[b]	4.68[b]	0.499[cde]
5	7.21[bcd]	0.943[d]	0.785[def]	5.64[bcd]	5.32[cd]	0.511[def]
6	7.49[cd]	0.935[cd]	0.756[b]	5.64[bcd]	5.26[bcd]	0.477[b]
7	7.76[d]	0.915[bc]	0.777[def]	6.02[d]	5.50[d]	0.493[bc]
8	7.04[bcd]	0.983[e]	0.778[def]	5.46[bcd]	5.35[cd]	0.504[cdef]
9	6.60[a]	0.921[bcd]	0.787[ef]	5.19[b]	4.78[bc]	0.518[f]
10	7.65[d]	0.902[ab]	0.762[bc]	5.83[cd]	5.27[bcd]	0.479[b]
11	7.80[d]	0.916[bcd]	0.776[de]	6.04[d]	5.53[d]	0.494[bcd]
12	5.34[a]	0.881[a]	0.741[a]	3.49[a]	3.47[a]	0.456[a]
SEM	*0.268*	*0.00979*	*0.00474*	*0.214*	*0.206*	*0.00633*

[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[abcde] Means within the same column with common superscripts and independent variable are not different ($P \geq 0.05$).

Table 9. Least Squares Means of Storage Stability Evaluation[1].

	Aerobic Total Plate Count	*Anaerobic Total Plate Count*
Treatment		
A[1]	3.914[a]	3.803[a]
B[1]	3.983[b]	3.771[ab]
C[1]	4.045[b]	3.892[b]
D[1]	4.058[bc]	3.897[b]
E[1]	3.985[c]	3.838[ab]
SEM	*0.0232*	*0.0344*
Week		
0	3.493[a]	3.716[b]
1	4.257[de]	3.973[def]
2	4.041[c]	3.505[a]
3	4.267[de]	4.024[ef]
4	4.166[d]	4.092[f]
5	4.195[d]	4.026[ef]
6	4.350[e]	3.908[cde]
7	3.990[c]	3.695[b]
8	3.810[b]	3.803[bc]
9	3.790[b]	3.785[bc]
10	3.808[b]	3.781[bc]
11	4.002[c]	3.776[bc]
12	3.824[b]	3.833[bcd]
SEM	*0.0374*	*0.0555*

[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend.

[abcdef] Means in the same column and independent variable with similar superscripts are similar ($P < 0.05$

Table 10. Cost of ingredients per kg in US dollars.

Ingredient	Ingredients cost/kg
Mechanically separated chicken	$0.77
Salt	$0.31
Modified corn starch	$1.65
Oat fiber	$4.44
Sodium phosphate	$3.53
Vinegar	$3.52
Seasoning	$9.90

Table 11. Formulation and Costing of Treatment With Phosphate (A).

Ingredient	Inclusion % by raw weight	Ingredients in product (kg)	Ingredients cost/ kg	Cost of inclusion
Mechanically separated chicken	-	58.9	$0.77	$45.35
Water	18	10.602	0	$0.00
Salt	0.5	0.2945	$0.31	$0.09
Modified Corn Starch (B990)	0	0	$1.65	$0.00
Oat Fiber (HF251)	0	0	$4.44	$0.00
Sodium Phosphate (B450)	0.43	0.25327	$3.53	$0.89
Vinegar (EV)	1.7	1.0013	$3.52	$3.52
Seasoning	1.7	1.0013	$9.90	$9.91
Total Formulation weight (kg)		72.05237		
Total Cost				$59.78
Cost per kg				$0.83

Table 12. Formulation and Costing of Treatment Without Binders (B).

Ingredient	Inclusion % by raw weight	Ingredients in product (kg)	Ingredients cost/ kg	Cost of inclusion
Mechanically separated chicken	-	58.9	$0.77	$45.35
Water	18	10.602	0	$0.00
Salt	0.5	0.2945	$0.31	$0.09
Modified Corn Starch (B990)	0	0	$1.65	$0.00
Oat Fiber (HF251)	0	0	$4.44	$0.00
Sodium Phosphate (B450)	0	0	$3.53	$0.00
Vinegar (EV)	1.7	1.0013	$3.52	$3.52
Seasoning	1.7	1.0013	$9.90	$9.91
Total Formulation weight (kg)		71.7991		
Total Cost				$58.88
Cost per kg				$0.82

Table 13. Formulation and Costing of Treatment 90:10 Blend (C).

Ingredient	Inclusion % by raw weight	Ingredients in product (kg)	Ingredients cost/ kg	Cost of inclusion
Mechanically separated chicken	-	58.9	$0.77	$45.35
Water	18	10.602	0	$0.00
Salt	0.5	0.2945	$0.31	$0.09
Modified Corn Starch (B990)	0.35	0.20615	$1.65	$0.34
Oat Fiber (HF251)	3.15	1.85535	$4.44	$8.24
Sodium Phosphate (B450)	0	0	$3.53	$0.00
Vinegar (EV)	1.3	0.7657	$3.52	$2.70
Seasoning	1.7	1.0013	$9.90	$9.91
Total Formulation weight (kg)		73.625		
Total Cost				$66.63
Cost per kg				$0.90

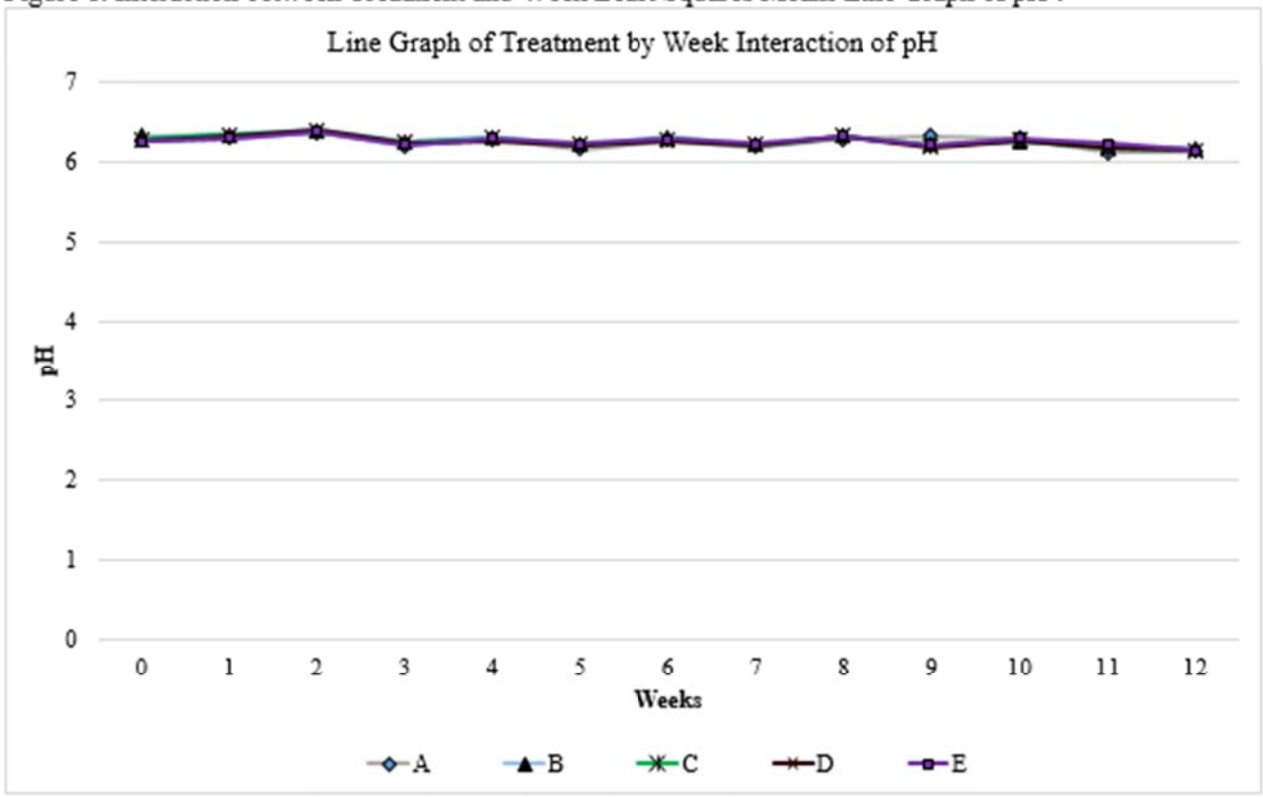
Table 14. Formulation and Costing of Treatment 50:50 Blend (D).

Ingredient	Inclusion % by raw weight	Ingredients in product (kg)	Ingredients cost/ kg	Cost of inclusion
Mechanically separated chicken	-	58.9	$0.77	$45.35
Water	18	10.602	0	$0.00
Salt	0.5	0.2945	$0.31	$0.09
Modified Corn Starch (B990)	1.75	1.03075	$1.65	$1.70
Oat Fiber (HF251)	1.75	1.03075	$4.44	$4.58
Sodium Phosphate (B450)	0	0	$3.53	$0.00
Vinegar (EV)	1.3	0.7657	$3.52	$2.70
Seasoning	1.7	1.0013	$9.90	$9.91
Total Formulation weight (kg)		73.625		
Total Cost				$64.33
Cost per kg				$0.87

Table 15. Formulation and Costing of Treatment 10:90 Blend (E).

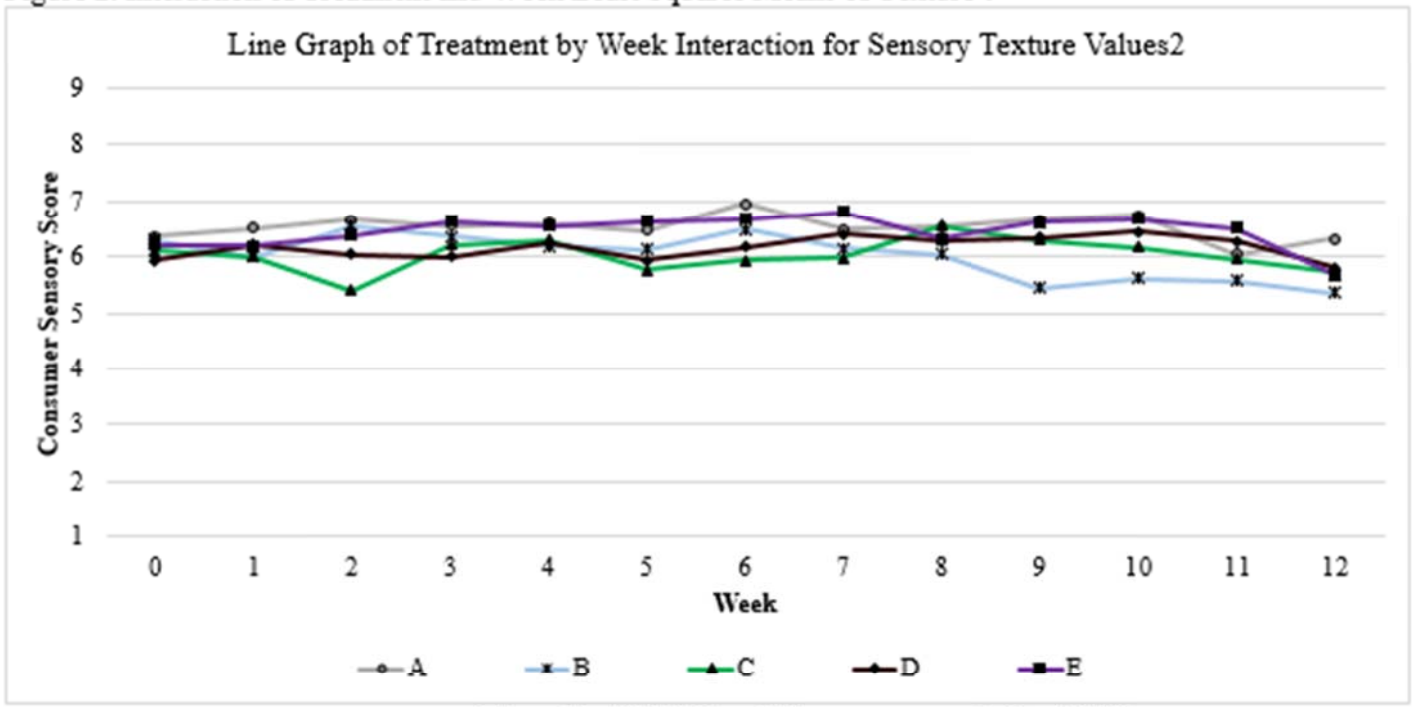
Ingredient	Inclusion % by raw weight	Ingredients in product (kg)	Ingredients cost/ kg	Cost of inclusion
Mechanically separated chicken	-	58.9	$0.77	$45.35
Water	18	10.602	0	$0.00
Salt	0.5	0.2945	$0.31	$0.09
Modified Corn Starch (B990)	3.15	1.85535	$1.65	$3.06
Oat Fiber (HF251)	0.35	0.20615	$4.44	$0.92
Sodium Phosphate (B450)	0	0	$3.53	$0.00
Vinegar (EV)	1.3	0.7657	$3.52	$2.70
Seasoning	1.7	1.0013	$9.90	$9.91
Total Formulation weight (kg)		73.625		
Total Cost				$62.03
Cost per kg				$0.84

Figure 1. Interaction between Treatment and Week Least Squares Means Line Graph of pH[1].



[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend

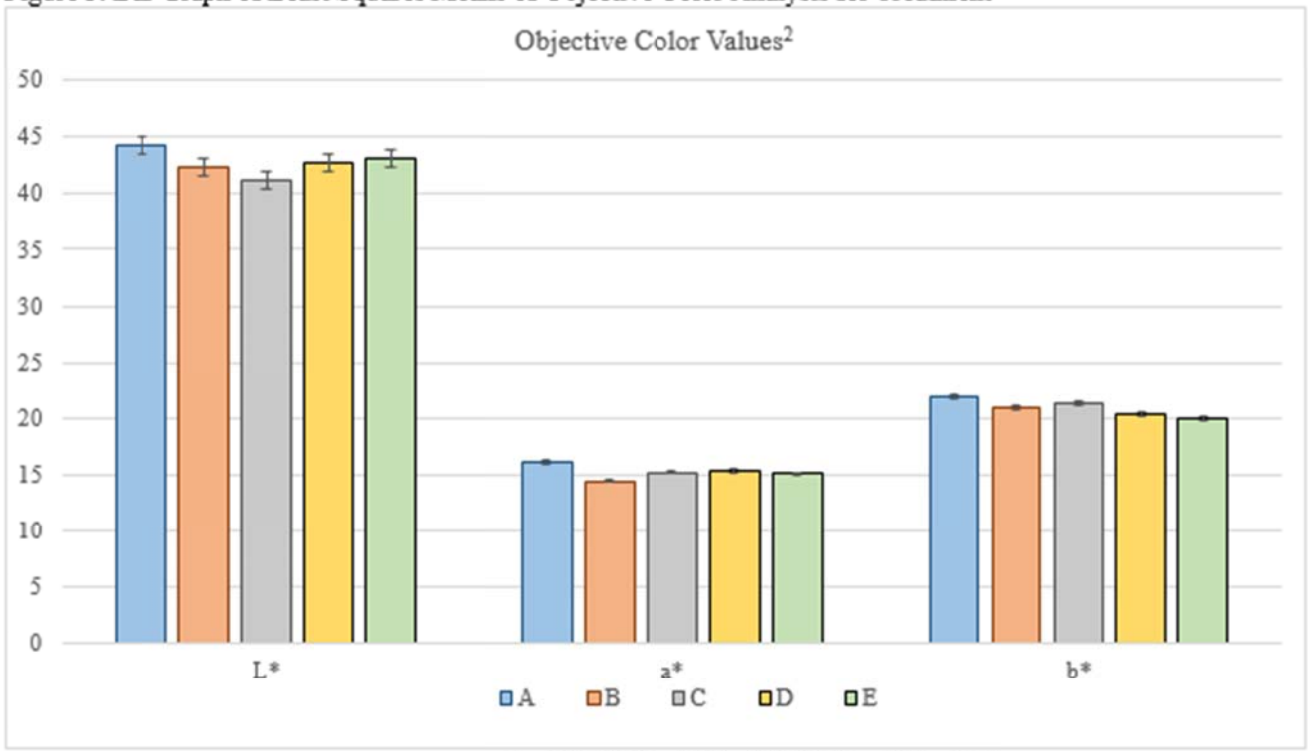
Figure 2. Interaction of Treatment and Week Least Squares Means of Texture[1].



[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend

[2] Consumer sensory values assessed on a 9 point scale (1 = Dislike Extremely; 5 = Neutral; 9 = Like Extremely).

Figure 3. Bar Graph of Least Squares Means of Objective Color Analysis for Treatment[1]



[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend
[2] SEM L* = 0.765; SEM a* = 0.148; SEM b* = 0.193

Figure 4. Line Graph of Least Squares Means of Objective Color Analysis for Week.

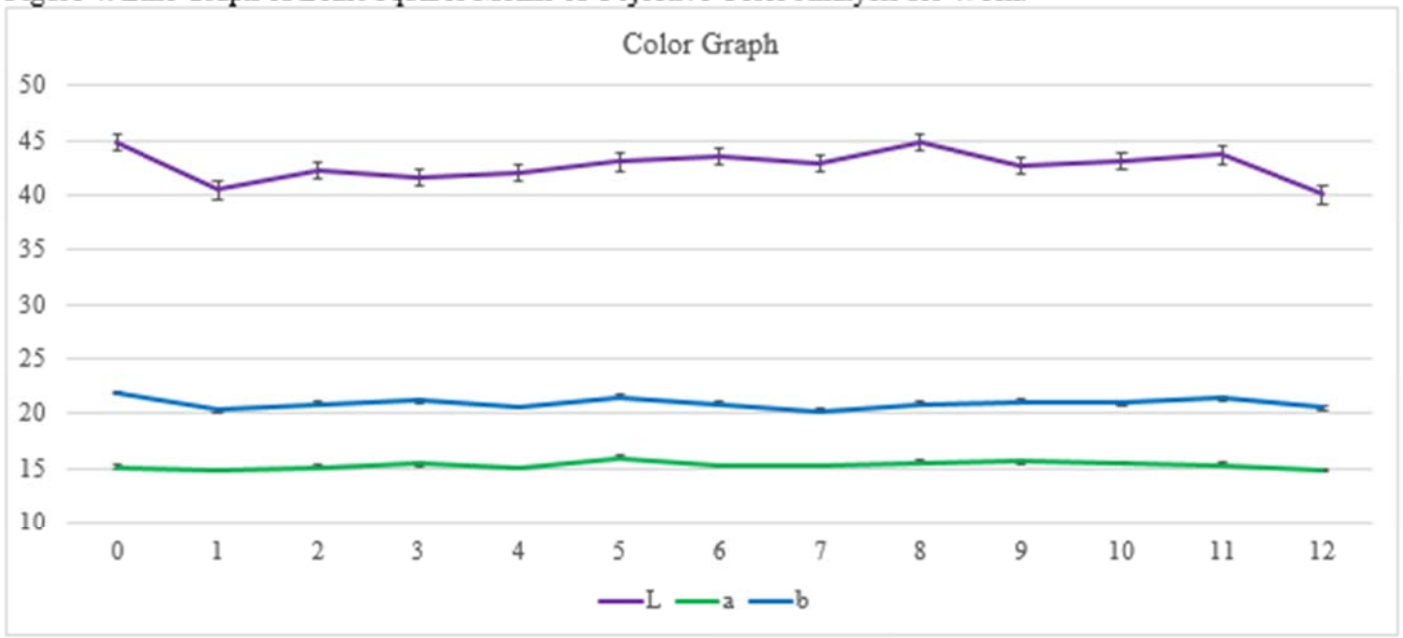
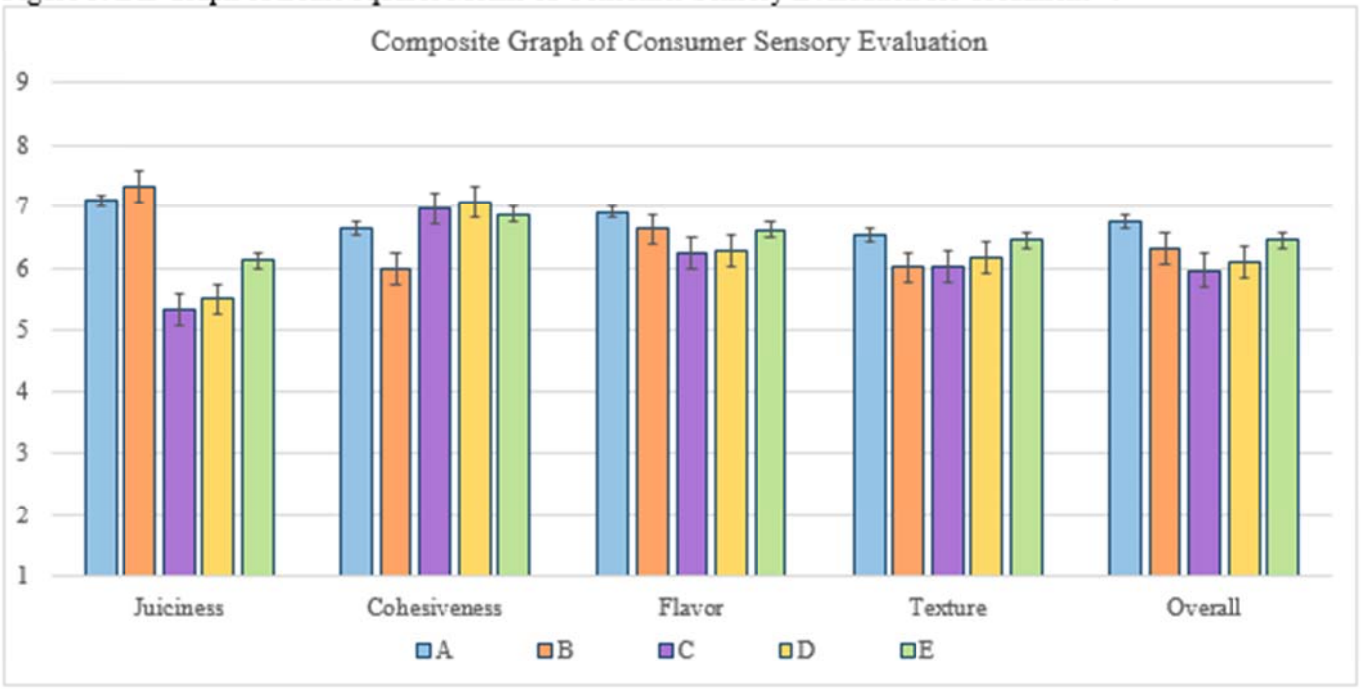


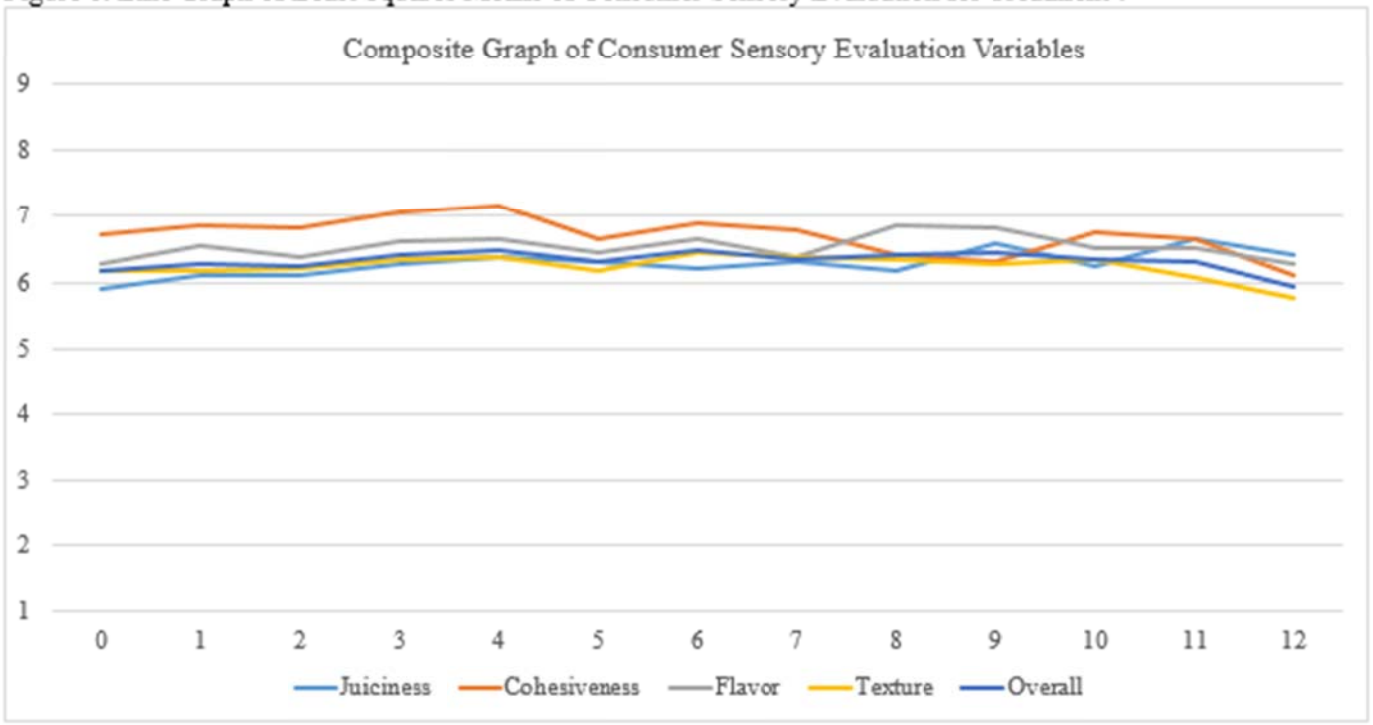
Figure 5. Bar Graph of Least Squares Means of Consumer Sensory Evaluation for Treatment[1,2].



[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.

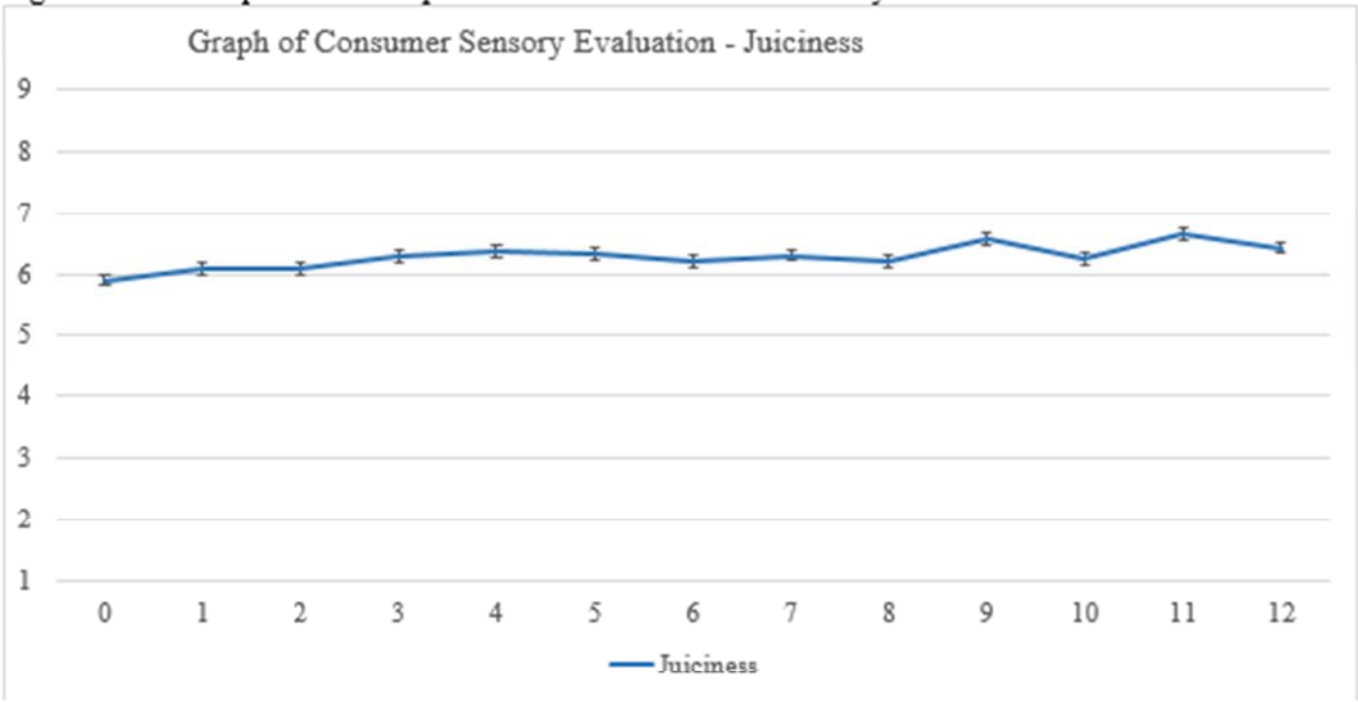
[2] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend

Figure 6. Line Graph of Least Squares Means of Consumer Sensory Evaluation for Treatment[1].



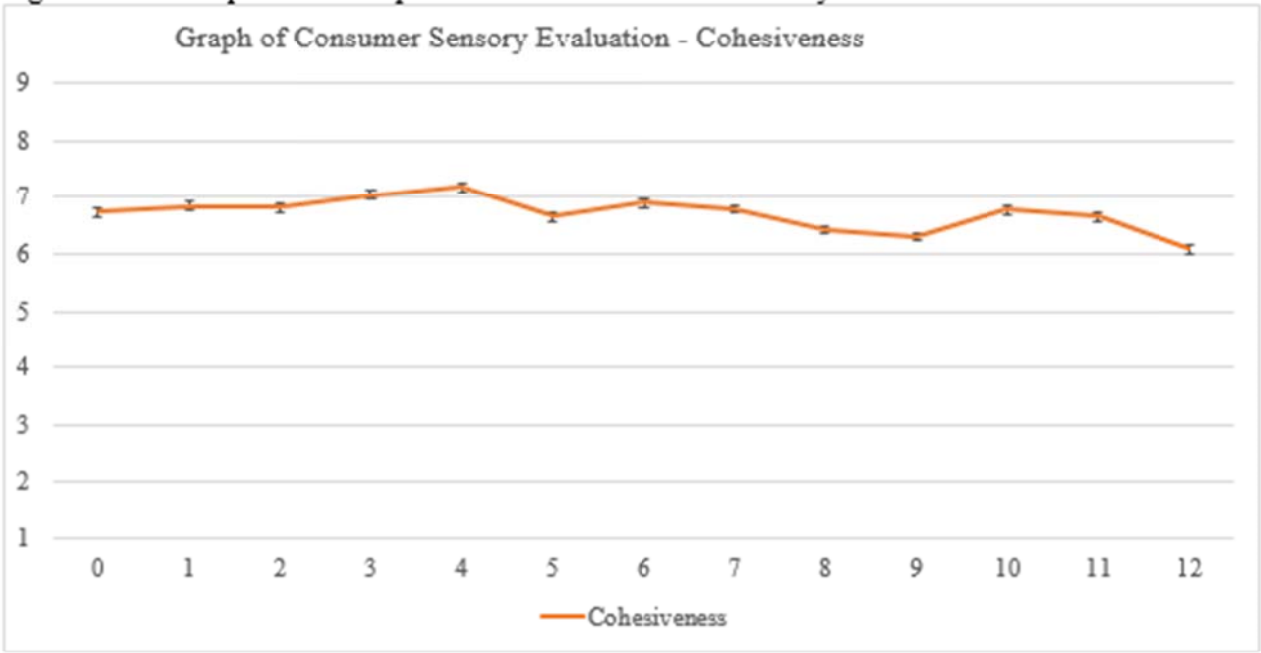
[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.

Figure 7. Line Graph of Least Squares Means for Consumer Sensory Variable – Juiciness[1,2].



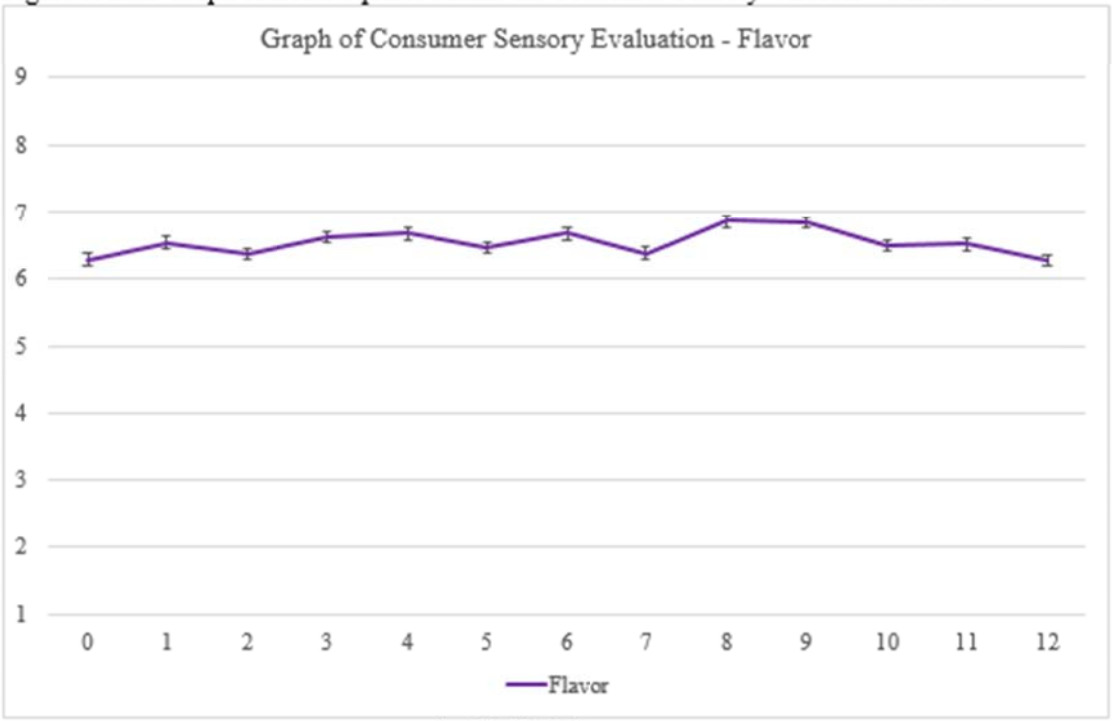
[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.
[2] SEM = 0.0956

Figure 8. Line Graph of Least Squares Means for Consumer Sensory Variable – Cohesiveness[1,2].



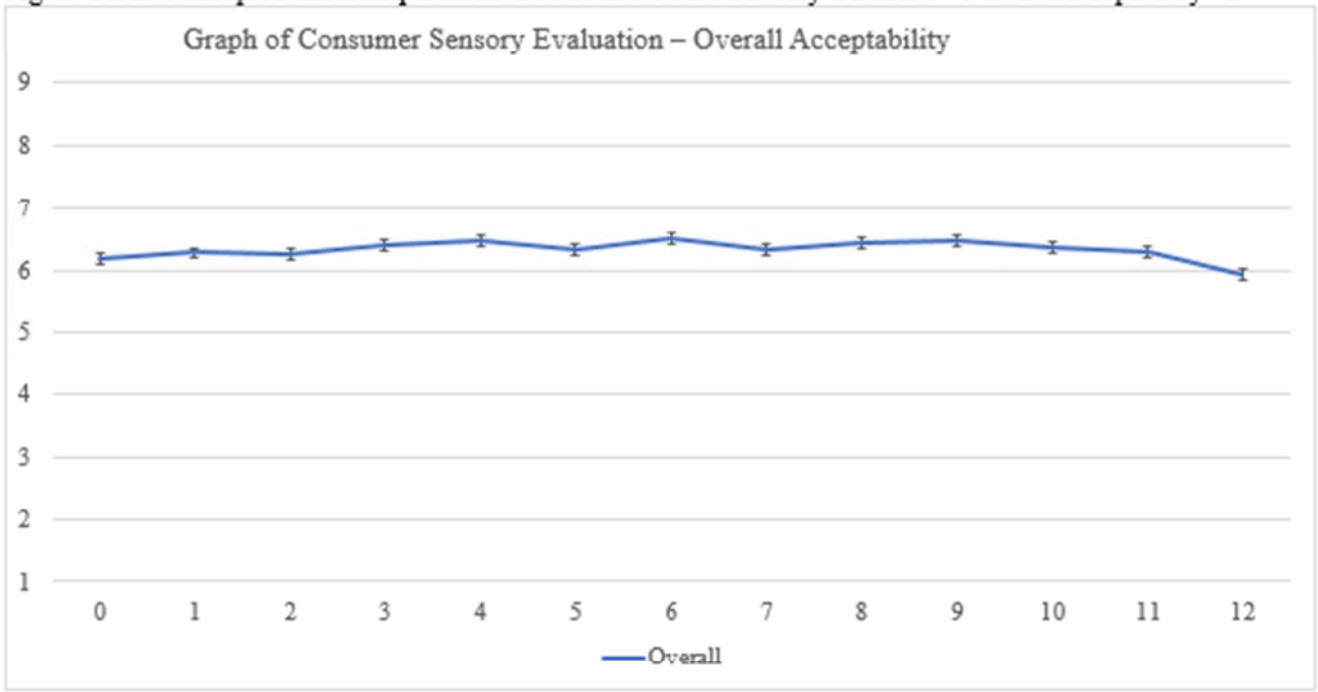
[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.
[2] SEM = 0.0757

Figure 9. Line Graph of Least Squares Means for Consumer Sensory Variable – Flavor[1,2].



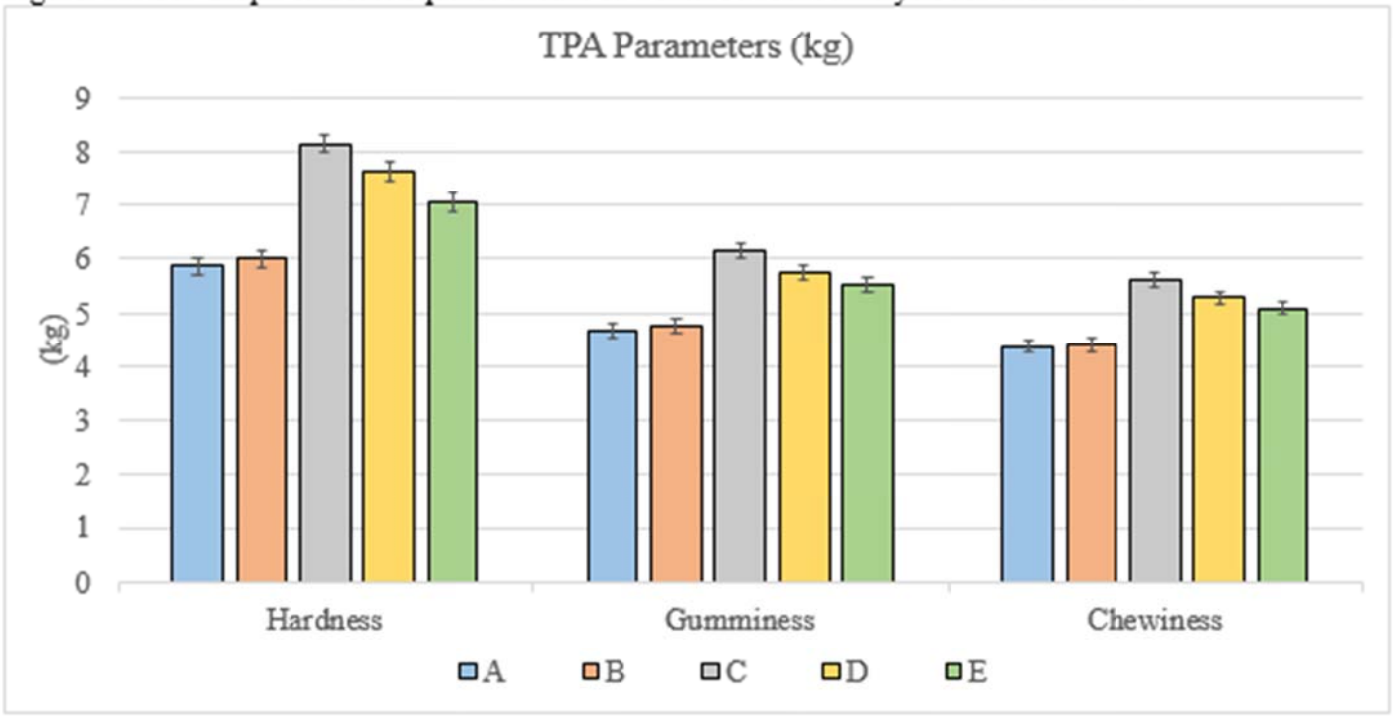
[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.
[2] SEM = 0.0889

Figure 10. Line Graph of Least Squares Means for Consumer Sensory Variable – Overall Acceptablity[1,2].



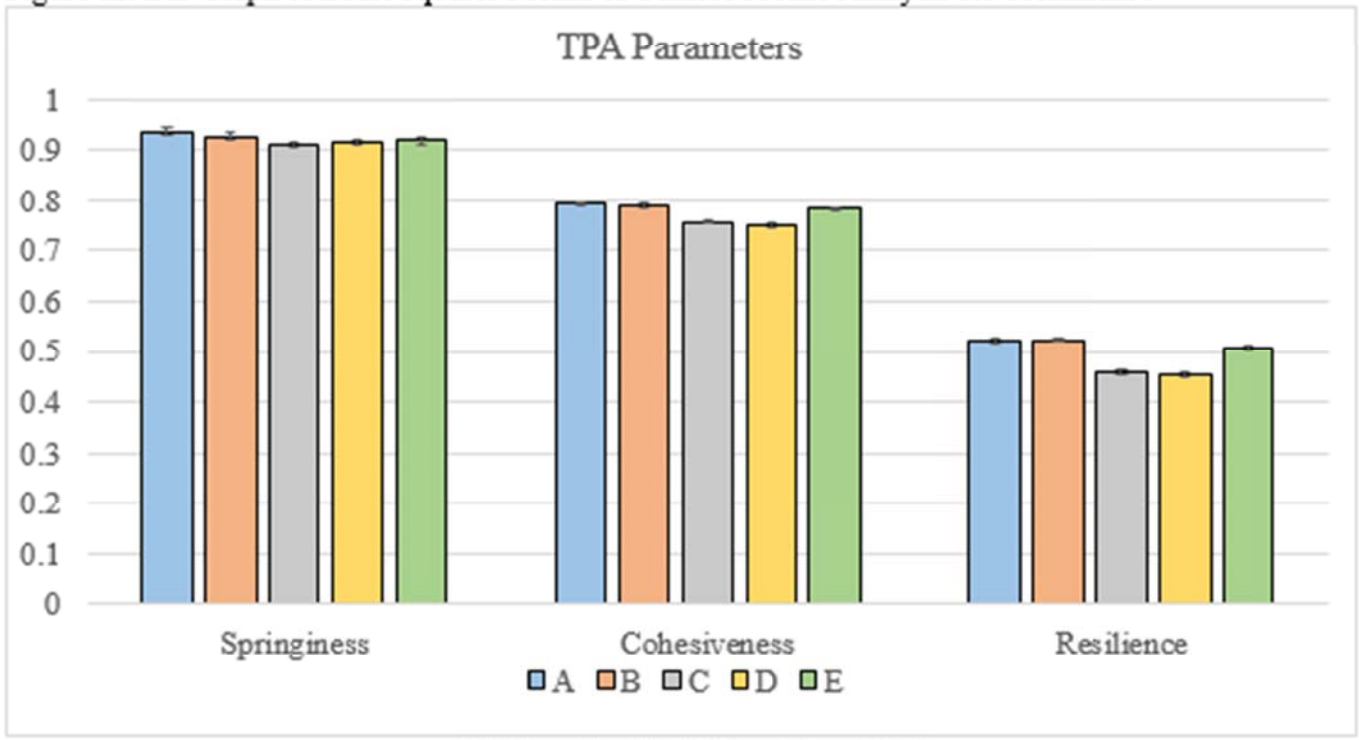
[1] 0 = Extremely Dry, Extremely Crumbly, Dislike Extremely; 5 = neutral; 9 = Extremely Juicy, Extremely Cohesive, Like Extremely.
[2] SEM = 0.0887.

Figure. 11. Bar Graph of Least Squares Means of Texture Profile Analysis for Treatment[1].



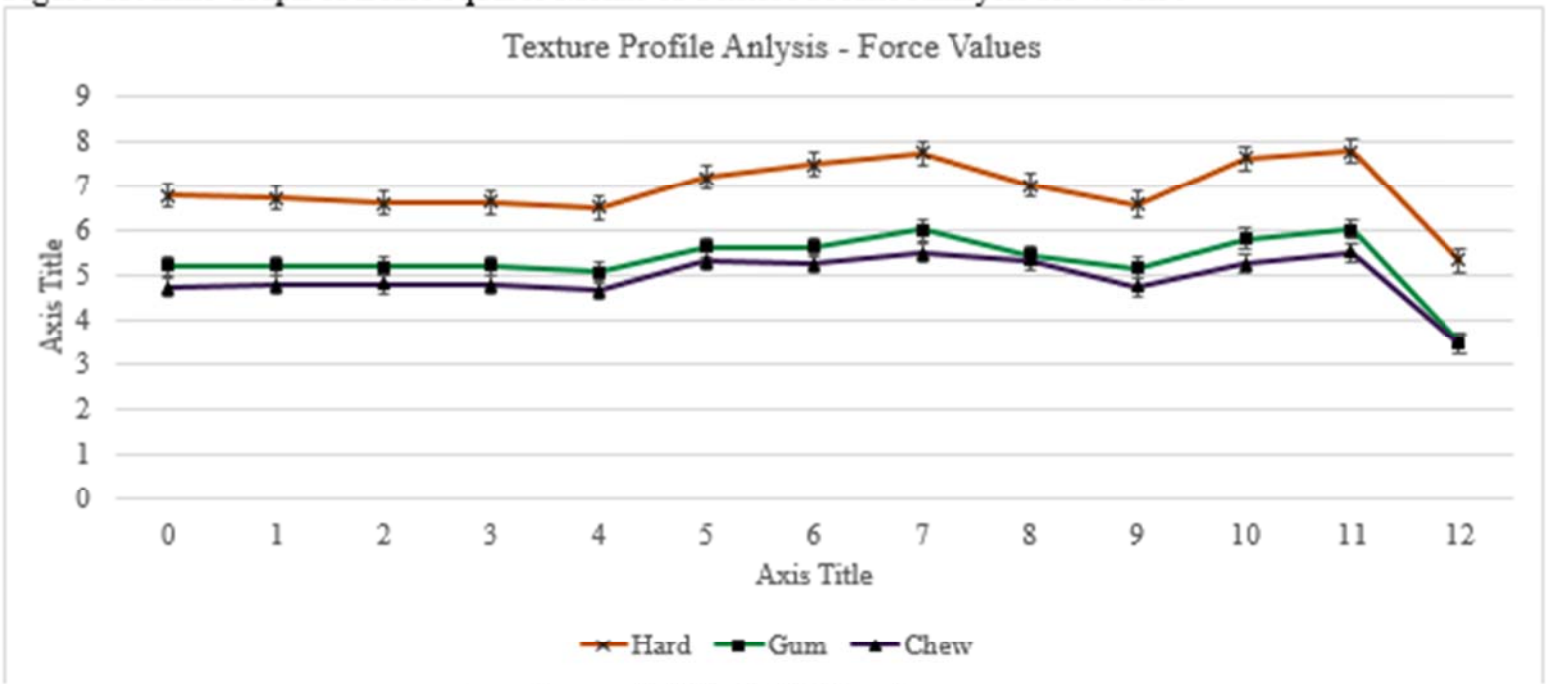
[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend

Figure 12. Bar Graph of Least Squares Means of Texture Profile Analysis for Treatment[1].



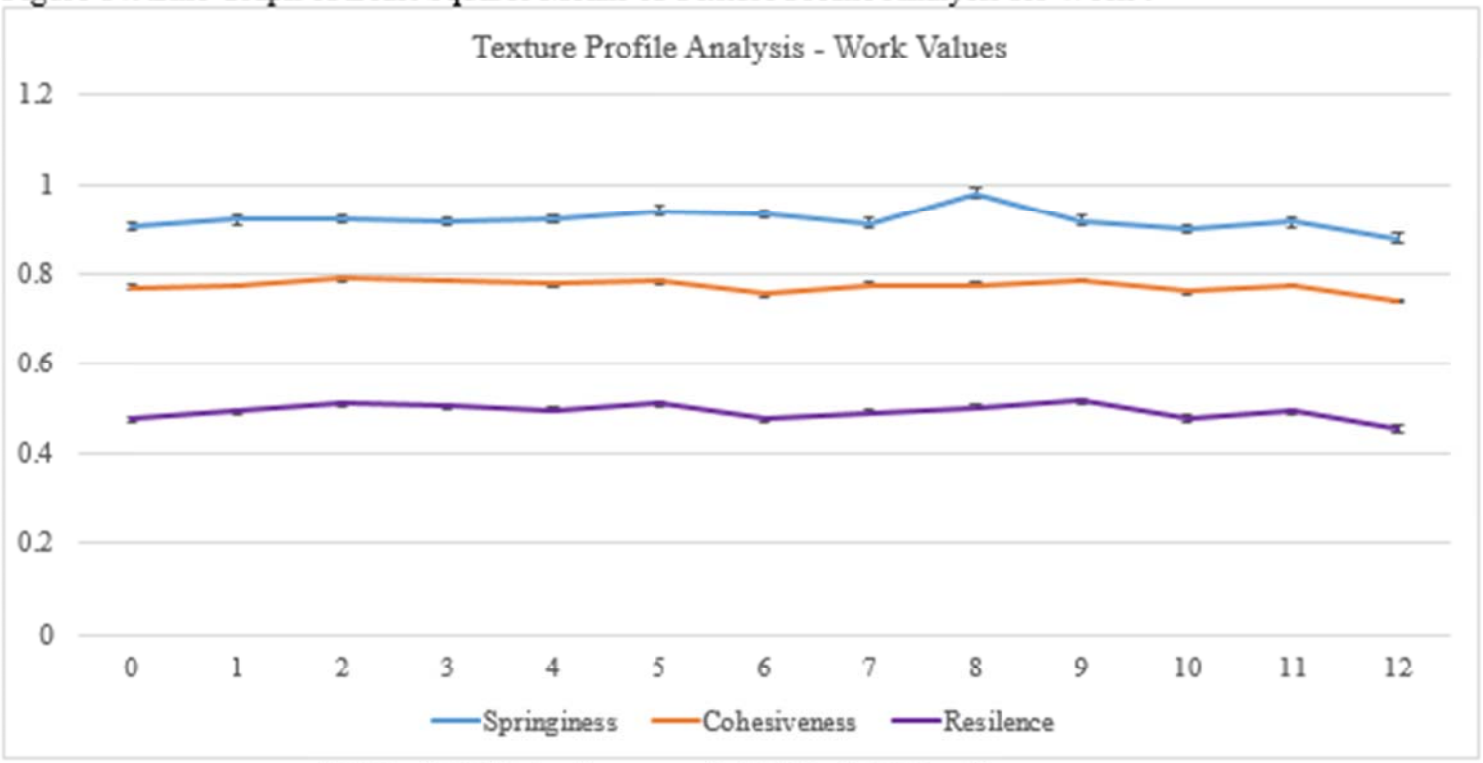
[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend

Figure 13. Line Graph of Least Squares Means of Texture Profile Analysis for Week[1].



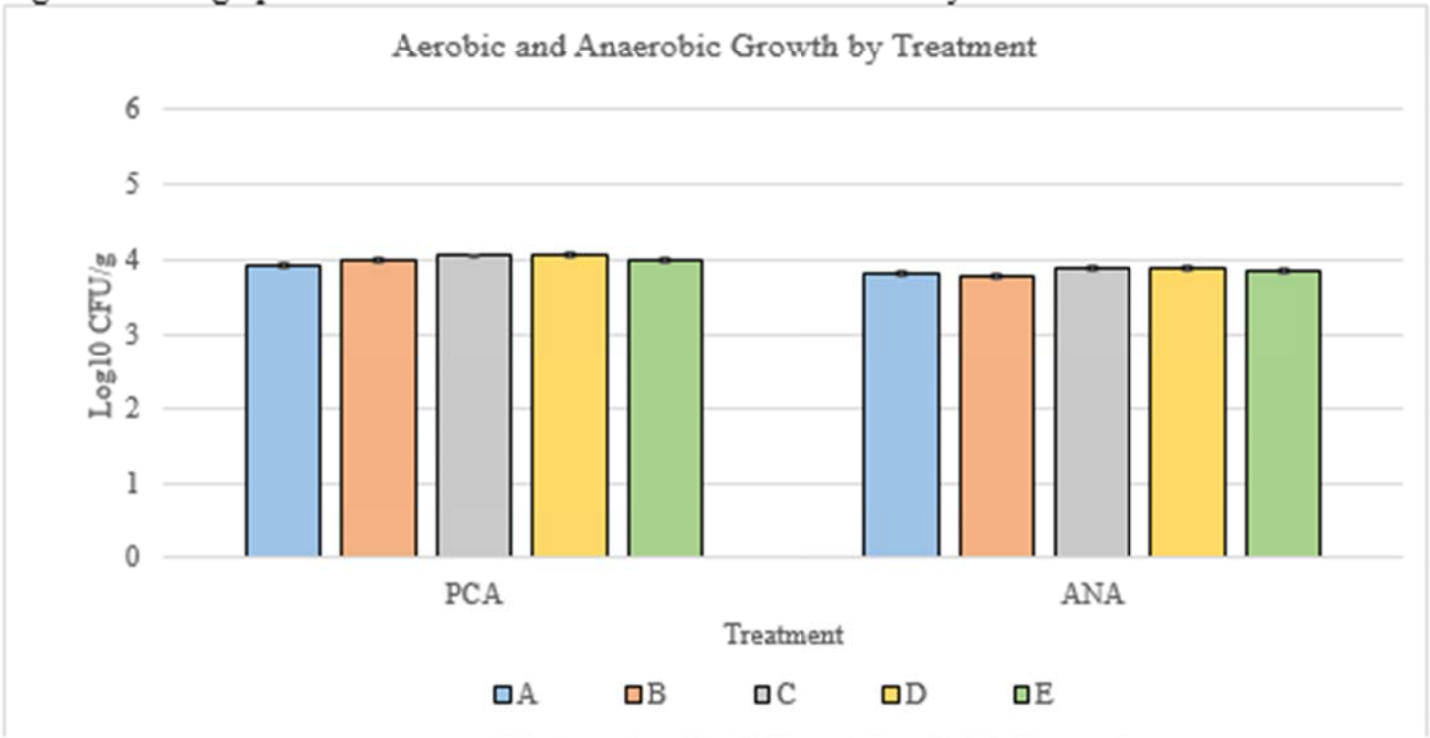
[1] SEM Hardness = 0.167; SEM Gumminess = 0.133; SEM Chewiness = 0.128.

Figure 14. Line Graph of Least Squares Means of Texture Profile Analysis for Week[1].



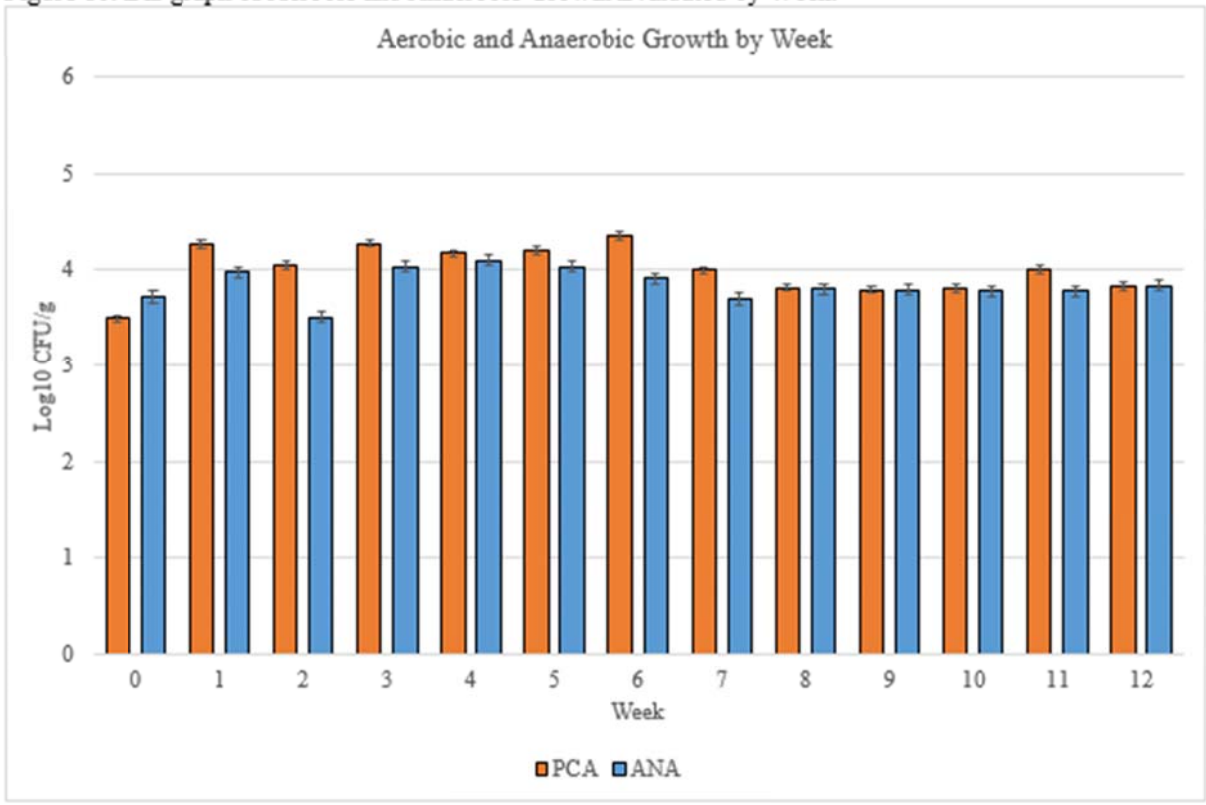
[1] SEM Springiness = 0.00979; SEM Cohesiveness = 0.00474; SEM Resilience = 0.00633.

Figure 15. Bar graph of Aerobic and Anaerobic Growth Evaluated by Treatment[1].



[1] A – With Phosphate, B – Without Binders, C – 90:10 Blend, D – 50:50 Blend, D – 10:90 Blend

Figure 16. Bar graph of Aerobic and Anaerobic Growth Evaluated by Week.



VII. References

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