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Accelerated Shelf Life of a Health Bar Contained in Different Bio-Based Packaging Materials

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ACCELERATED SHELF LIFE OF A HEALTH BAR CONTAINED IN DIFFERENT BIO-BASED PACKAGING MATERIALS

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ABSTRACT

Health foods and environmentally safe packaging are ongoing concerns for manufacturers and consumers. The purpose of this study was to compare the shelf life of an intermediate moisture chocolate health bar in its current package to the shelf life of the same health bar in two different bio-based packages. The renewable resources used to create bio-based packaging materials are more environmentally safe than conventional packages used today. This could ultimately reduce costs to manufacturers and lower prices of the health bar for consumers.

The health bar in this study has an actual shelf life of six months. For this study, an accelerated storage of three months was used to observe the property changes of the health bar and its packages. The original packaging material (control) was metallized-oriented polypropylene (Met-OPP). The two bio-based packaging materials (variables) were polylactic acid (PLA) and sugarcane polyethylene (SPE). The two bio-based materials were each laminated to metallized cellophane (Met-Cell). Duplicating the original package, the bio-based packages were created using a double heated bar sealer. Samples were repackaged into these bio-based packages and stored in an environmental chamber at 35ºC, 75% relative humidity (% RH) for 10 weeks. Before all analyses, the seal integrity of all packages was checked. Product analyses included: (1) microbial, (2) percent moisture content, (3) sensory, (4) texture and (5) water activity. Film analyses included: (1) seal-peel, (2) tensile strength, (3) tater vapor transmission rate (WVTR) and (4) oxygen transmission rate (OTR). Before all film analyses, the thicknesses of the film samples were observed.

All samples were analyzed on weeks 0, 2, 4, 6, 8 and 10 in triplicate for each treatment, except for sensory, tensile, seal-peel, WVTR and OTR analyses. Tensile and seal-peel analyses were performed with five replicates for each treatment. WVTR and OTR analyses were performed on weeks 0, 5 and 10 in duplicate for each treatment. Two statistical models were used to analyze the data collected: (1) film and product data were analyzed by Analysis of Variance (ANOVA). This analysis were performed at a 0.05 significance level. Based on these analyses, the PLA package compared best to the original package of the chocolate health bar.

DEDICATION

I dedicate this thesis to my parents, Jerome and Shirley Gibbs.

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INTRODUCTION

Healthier and organic foods are growing in interest among consumers today. These foods are perceived to have a higher quality than highly processed foods. Sustainable packages, including those made from renewable resources, are also perceived as having a higher quality than conventional packages. This is because the production of these sustainable packaging materials causes less harm to the environment than conventional packaging materials. One way to market healthy and organic food could be by packaging them in sustainable packages. The target market for this idea could be for consumers who are concerned about eating healthy and helping the environment in any way they can.

Intermediate moisture foods such as health bars, need to be packaged in the appropriate materials to preserve the food properties. Bio-based packaging can be used to preserve these properties. Used alone, bio-based materials lack the mechanical and moisture barrier properties that are in conventional materials to maintain the quality of food. To improve these properties, different bio-based materials can be combined to create multi-layered food packages. Low moisture permeation and good seal strength of packages help keep intermediate moisture foods from spoiling.

There has been an abundance of research on how perishable foods such as fresh produce behave in bio-based packages. Yet, there is limited research on how intermediate moisture foods behave in bio-based packages. By packaging healthy foods using appropriate bio-based materials, the market for the lifestyle of healthy eating and being environmentally safe can be used to drive bio-based packaging forward.

LITERATURE REVIEW

As consumers have gotten used to longer lasting, high quality foods, their expectations are more demanding for the shelf life of foods. Consumers now expect foods to maintain a high quality from when they purchase it to when they consume it (Kilcast & Subramaniam, 2000). High quality in the food sense means that consumers want their foods to be safe, look good, taste good, and stay fresh for a longer period of time.

Shelf Life

Extending the shelf life of food products is the main purpose of food and food packaging technologies. The shelf life of a food product is the time that its food quality remains acceptable and meets the consumers' expectations (Martins, Lopes, Vicente & Teixeira, 2008). The food should also remain safe throughout its shelf life. A product's shelf life starts from the time the food is manufactured. The length of its shelf life depends on different factors, including the product's ingredients, how it is processed, the type of packaging it is contained in and how it is stored (New Zealand Food Safety Authority [NZFSA] 2005).

In the food industry, all food packages are required to have a shelf life date labeled on them and the storage instructions to meet that shelf life. Anyone who packages and sells food is legally responsible for calculating how long their product will be considered safe and acceptable to consume, without any noticeable changes in quality (NZFSA, 2005). All food product packages are dated, but they are not dated in the same manner. According to the United States Department of Agriculture ([USDA], 2013), there are open dates and "closed" or "coded" dates. Open dates are used on perishable

foods, such as meat and dairy products. “Closed” or “coded” dates are used on shelf stable products, such as canned foods and boxes of food (USDA, 2013).

Food packages can have either of four types of shelf life dates—Sell-By, Best if Used By (or Before), Use-By, and “Closed” or “Coded” dates (USDA, 2013). Sell-By dates tell the store how long to display the product for sell and the consumer should purchase the product before the date expires (USDA, 2013). Best if Used By (or Before) dates tell when the product should be used by for best flavor or quality; these are not purchase or safety dates (USDA, 2013). The Use-By date is the last date recommended to use of the product while at its peak quality (USDA, 2013). “Closed” or “Coded” dates are packing numbers for use by the manufacturer (USDA, 2013). Packing numbers allow manufactures to track their product for stock rotation and for product recalls (Hagan, 1999). Packing codes are usually a series of letters and numbers that tell the dates, times, and place of manufacture of the product (Hagan, 1999).

Shelf Life Testing

Shelf life testing is performed on different food products to determine the shelf life date that will go on the food package. The tests are used to determine the length of time the products will remain acceptable to consume, regarding the food’s safety and quality attributes including flavor, appearance and texture. Consumers necessitate that shelf life tests are performed on food products (Hughes, 2013). Shelf life tests can give important information about the food product to make sure the consumer will have a high quality product for a certain period of time after its manufacture (Sewald & DeVries, n.d.).

Before beginning a shelf life test, the researcher should determine what would cause the food to spoil. Examples of reasons a product could spoil include the product's formulation, water activity, packaging, and storage conditions. The researcher should then decide which tests will be performed on the product to track how it spoils (EMSL Analytical, Inc., 2008). Food can spoil due to sensory, microbiological, chemical and physical changes (EMSL Analytical, Inc., 2008). Next, after deciding which tests to perform, plan the shelf life test (EMSL Analytical, Inc., 2008). This includes determining how long the shelf life test will last, how many samples will be used for each test throughout the entire study and at what storage conditions will the samples be held (EMSL Analytical, Inc., 2008). It is recommended that at least three samples be measured to account for variations in processing (Magari, 2003). Finally, the shelf life test can be performed and at the end of the test, the researcher can determine the shelf life date of the product, which will be shown on the package (EMSL Analytical, Inc., 2008).

Typically, the researcher in a school laboratory would stop the shelf life study here. However, in the food industry, researchers create a working shelf life, which is less than the actual shelf life of the product (EMSL Analytical, Inc., 2008). The working shelf life is created to account for real world factors, such as varying storage conditions throughout distribution. Once the product is in the market, the shelf life is monitored by keeping track of customer complaints and evaluating samples from production and distribution to validate the study results (EMSL Analytical, Inc., 2008). Shelf life tests end when the product is disliked or unsafe for consumption (EMSL Analytical, Inc., 2008).

Shelf Life Test Methods

Depending on the product, there are two methods of performing shelf life tests. They are the direct method and the indirect method. The direct method approach follows the shelf life testing steps above. It is the most commonly used method in the food industry (NZFSA, 2005). Shelf life testing using the direct method involves storing the product at preselected conditions for a period of time longer than the expected shelf life (NZFSA, 2005). The product is checked at regular intervals to see when it begins to spoil (NZFSA, 2005). Perishable food products, usually refrigerated, are typically used for the direct method shelf life testing approach since they usually take less than six months to spoil at their normal storage conditions (Fisch, 2014). Even though the direct method is most commonly used for shelf life testing, it can take up to two years to complete for non-perishable, or shelf stable products. Therefore, the indirect methods, accelerated storage shelf life testing and predictive modeling are used.

The indirect method approach attempts to predict the shelf life of a food product without running a full-length shelf life test (NZFSA, 2005). Typically, accelerated shelf life tests are performed on frozen and shelf stable products that have a shelf life of six months or longer (Fisch, 2014). When testing using accelerated storage, the same steps are used as the direct method. However, the testing period is reduced because the rate of deterioration of the food product is increased (Ling, 2014, Shelf Life Testing section). A food product deteriorates faster by accelerating, or increasing the storage temperature or the relative humidity (%RH).

Relative humidity is the amount of water vapor in the air relative to the amount of water vapor the air can hold (Fondriest Staff, 2010). It is reported as a percentage of the

total amount of moisture that the air can hold. For example, if the air is a quarter saturated with water vapor, the relative humidity is reported as 25% RH.

Temperature levels should be selected based on the nature of the product and its normal storage conditions (Magari, 2003). The increased temperature that is selected for accelerated storage should allow relatively fast degradation of the product. The accelerated storage shelf life test can be shortened to half, or a quarter of the real time storage shelf life study (Ling, 2014). However, it is not recommended to run an accelerated storage test at very high temperatures for a very short period of time because the mechanisms of degradation at very high temperatures may be very different than those at the normal storage temperature (Magari, 2003). The results from the accelerated storage are used to estimate the shelf life of the food product at its normal storage conditions (NZFSA, 2005).

Prediction models are used to predict spoilage and bacterial growth in food products (NZFSA, 2005). Statistical software calculates the rate of deterioration of food properties (NZFSA, 2005) such as water activity and nutrient content. The food package can also help extend shelf life of foods. Properties of packages such as moisture and gas permeation rates are used to calculate the rate of deterioration of foods (NZFSA, 2005). The information from prediction modeling programs should be verified by a shelf life study (NZFSA, 2005). An example of a predictive modeling system is the USDA Pathogen Modeling Program (NZFSA, 2005).

The temperature chosen for an accelerated storage test depends on the type of product and its mode of degradation (Sewald & DeVries, n.d.). To address this, the temperature coefficient, Q_{10} can be used. According to PhysiologyWeb (2014):

> "Q_{10} is a unitless quantity. It is the factor by which the rate increases when the temperature is raised by ten degrees. If the rate of the reaction is completely temperature independent,…the resulting Q_{10} will be 1. If the reaction rate increases with increasing temperature, Q_{10} will be greater than 1. Thus, the more temperature dependent a process is, the higher will be its Q_{10} value….For typical chemical reactions, Q_{10} values are ~2."

Therefore, as the temperature increases by ten degrees, the reaction rate in the product is quicker and causing the product to spoil faster. The equation (Figure 1) below can be used to calculate the Q_{10} value when the estimated shelf life and the temperatures at each estimated shelf life is known (Fisch, 2014):

$$Q10 = \frac{\text{Shelf-life at temperature T (°C)}}{\text{Shelf-life at (T (°C) + 10°C)}}$$

Figure 1: Q_{10} value equation when shelf life and temperatures are known (Fisch, 2014)

Factors Affecting Shelf Life of Foods

In performing a shelf life study for a product, it is important to understand the factors that could affect the product's quality and safety over time. These factors can be intrinsic or extrinsic.

Intrinsic factors are the properties of the food product (U. S. Food and Drug Administration [FDA], 2014) including the product's water activity and moisture content. These factors are influenced by variables including product formulation and structure.

Extrinsic factors are those that involve the product's surrounding environment (FDA, 2014). These factors include storage conditions such as temperature and relative

humidity. As the package is being created, these extrinsic factors influence the properties of the final package (Ricke, Van Loo, Johnson & O'Bryan, 2012).

The interactions between intrinsic and extrinsic factors either prevent or encourage processes that limit the shelf life of a product. These processes are classified as microbial, chemical, physical, or temperature related changes (Kilcast & Subramaniam, 2000).

Intrinsic Factors: Moisture Content and Water Activity

Moisture content is a measure of the amount of water in a product (Carter, 2007), expressed as a percentage. It tells how wet or dry a product is. For example, food with a moisture contents close to 100 percent will be wetter; food with moisture content closer to 0 percent will be drier.

Water activity is defined as “a ratio of water vapor pressure of a material to the vapor pressure of pure water at the same temperature” (Leake, 2006, p.1). It gives information about the safety and quality of food (Carter, 2007). The water activity of a food characterizes the different states in which water can be found, which includes how much water is "bound" in the food, how much water is available to participate in chemical or biochemical reactions, and how much water is available to help the growth of microorganisms (FDA, 2014). Water activity measures if the product has reached the range where spoilage reactions can occur (Mathlouthi, 2001). It can be controlled in foods by different techniques, including adding salt or sugar to the product and physically removing the water by drying or baking (FDA, 2014).

Water activity is measured on a scale of 0.00 – 1.00 with 0.00 being completely

dry food and 1.00 being pure water (FDA, 2014).

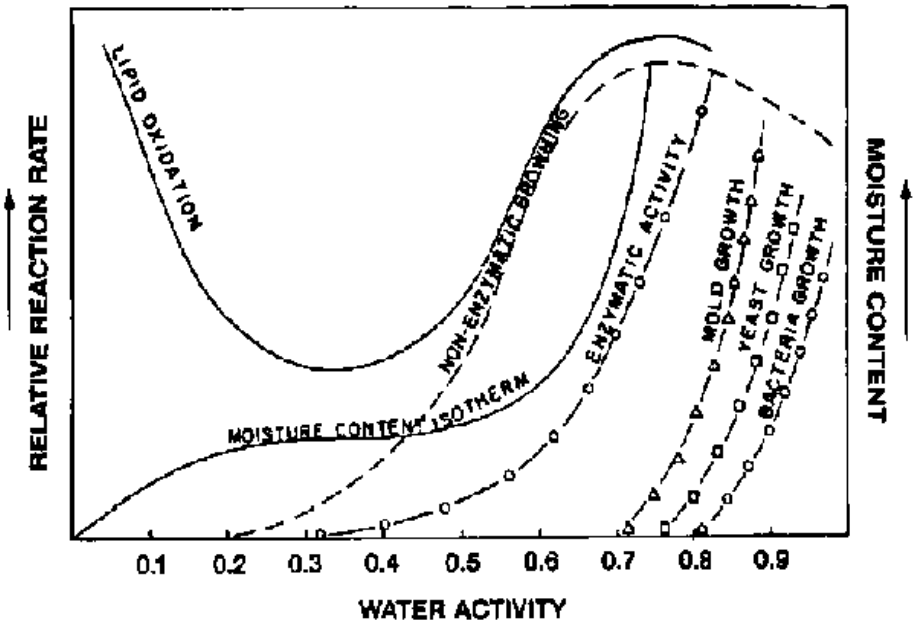


Figure 2: Relationship of Food Deterioration as a Function of Water Activity (Labuza et al., 1972)

Looking at Figure 2 (Labuza et al., 1972) above, in products with a low water activity ranging from 0 – 0.3, the water in the product is "bound" (Cooksey, 2012), meaning that there is little to no water available in the product that would allow most microorganisms to grow and multiply. In this range, there is no microbial growth to cause the deterioration of the food product. Therefore, the shelf life of the product should remain stable.

Food products with water activities between 0.3 and 0.85 have "interstitial" water (Cooksey, 2012), meaning that there is some water available in the product that could allow some microorganisms to grow. Leake (2006, p. 3) quotes Labuza saying that a water activity of 0.6 is a critical point at which there is potential for microbial growth if the moisture content increases. Figure 1 shows that bacterial and yeast and mold growth

begin to increase after the product reaches a water activity greater than 0.7. The reaction rates of lipid oxidation in products with water activities above 0.7 begin to decrease at a high moisture content. This increase in moisture content and water activity causes the chances of microbial growth to increase (Leake, 2006).

In a product with a high water activity, above 0.85, the water in the product is "free" water (Cooksey, 2012), meaning that all the water in the product is available for microorganisms to grow and flourish. A water activity of 0.85 is the critical point for bacterial growth in foods (Leake, 2006). At this water activity and higher, Figure 1 shows that mold, yeast and bacteria growth quickly increase. The increase of the rates of these reactions causes the shelf life of the product to decrease due to microbial spoilage. Controlling water activity in food controls microbial growth, extending the shelf life of food. This allows some products to be safely stored without refrigeration.

The relationship between moisture content and water activity at a given temperature is called the moisture sorption isotherm (Aqua Lab, 2004). This relationship is determined specifically for each product (Aqua Lab, 2004). This is because different products have different interactions with water at different moisture contents, at the same temperature (Labuza, 1984). As shown in Figure 1 above, in most foods, as water activity increases, moisture content increases (Aqua Lab, 2004). This creates a non-linear sigmoidal shape (S-shape). The graph of the sorption isotherm shows the reaction rates in food as a function of water activity (Labuza, 1984). The moisture sorption isotherm of a food product is obtained from the equilibrium moisture contents determined at different water activity levels at a constant temperature (Aqua Lab, 2004). According to the Agriculture, Food, and Rural Development [AFRD] website (2014), the relationship

between water activity and moisture content is "related to the relative humidity of the food and its water content…and that relationship must be determined for each specific food item."

It is possible for products to have the same moisture content, but have different water activities, such as salami and cooked beef, which both have a moisture content of approximately 60 percent. However, cooked beef has a water activity of approximately 0.98 and salami has a water activity of 0.82 (AFRD, 2014). Isotherms are useful for choosing the package for a product and understanding sensory and chemical changes in foods (Leake, 2006). The sorption isotherm is also important for use in shelf life calculations (Cooksey, 2012). In order to use the isotherm to estimate the shelf life of the product, the slope needs to be calculated where the curve shows the initial uptake of water (swelling) and then that slope would be used as the rate constant for calculation of shelf life (Cooksey, 2012).

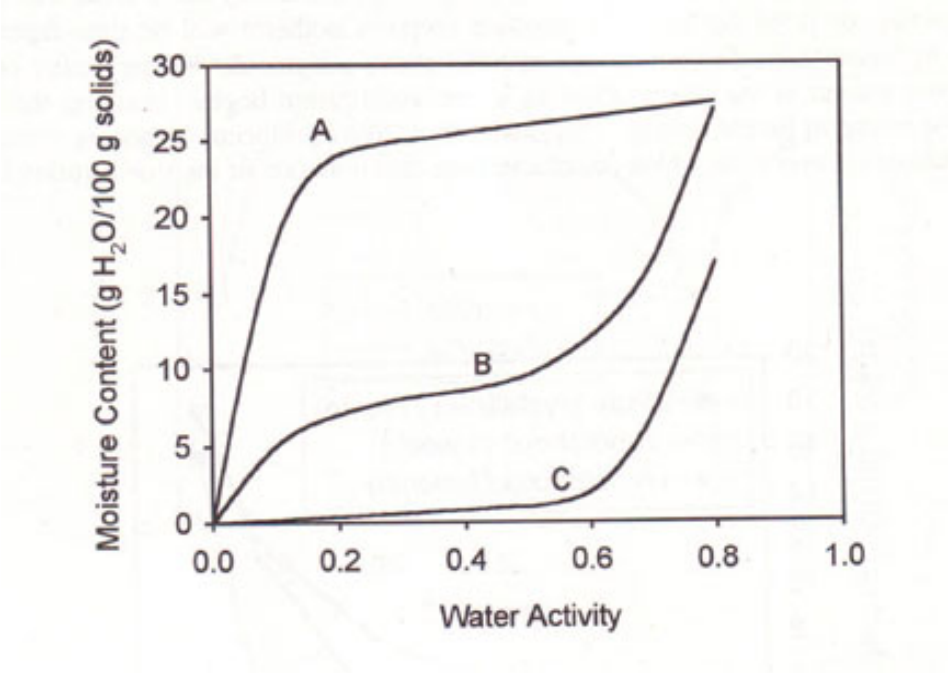


Figure 3: Standard Moisture Sorption Isotherms (Lab Cat, 2006)

Shown in Figure 3 (Lab Cat, 2006) above, there are three types of moisture sorption isotherms. Type A is the typical isotherm for anticaking agents, where the isotherm shows a sharp increase at a low water activity. This increase in moisture content at low water activity occurs because "this type of ingredient adsorbs water onto specific sites, but the binding energy is very large, [reducing] the water activity greatly" (Labuza, 1984, p.10).

Type B is the typical isotherm for dehydrated foods (i.e. dried fruits), where initially, "the dielectric effect of water is not strong enough to break the interactive forces between individual sugar molecules. As the water activity is increased, the overall net water-sugar interaction is enough to cause sugar-sugar dissociation, and thus water begins to penetrate into the crystal, dissolving sugar molecules and exposing new surfaces" (Labuza, 1984, p.10). At this point, the moisture content dramatically increases because a

solution is being made (Labuza, 1984). For intermediate moisture foods, the typical isotherm would be Type B, as these foods are adjusted to their lower water activities. As the water activity increases in intermediate moisture foods, there is more interaction with the water as it becomes available in the product.

Type C is the typical adsorption isotherm for crystalline substances, where there is little moisture increase until the water activity is between 0.7 and 0.8 (Labuza, 1984). This happens because the only effect of water is hydrogen bonding to the –OH groups that stick out on the surface of the crystal (Labuza, 1984).

Extrinsic Factors: Storage Temperature and Relative Humidity

The temperature of storage affects the growth rate of microorganisms that cause food to spoil (NZFSA, 2005). Higher temperatures increase the growth rate and lower temperatures decrease the growth rate (FDA, 2014).

Relative humidity is another storage factor to consider regarding food safety. A high relative humidity causes more moisture in the air. Depending on the product, it can cause a product's water activity to increase due to moisture migration through the package (FDA, 2014).

Packaging

From the initial source to the final consumer, the packaging materials provide physical protection against changes in the environment during distribution (Kilcast & Subramaniam, 2000). The environmental conditions inside a package can also cause a food product to deteriorate. Some foods with lower water activities are subject to having moisture condensing on the surface due to temperature and relative humidity shifts (FDA,

2014). Physical damage to the package such as tears in plastic packages, could allow microorganisms to get into the food and also cause moisture loss from the product (NZFSA, 2005). Mechanical properties of the package such as tensile strength, determine how well a product will be protected in its package (Kilcast & Subramaniam, 2000). If the mode of deterioration of the food is known, it will be easier to determine the type of packaging materials to help control the deterioration (Risch, 2002).

Microbial Growth

Microbial growth in foods can be affected by intrinsic or extrinsic factors. Growth of spoilage microorganisms such as yeast and molds, in a food product cause a food to spoil (NZFSA, 2005). Pathogenic microorganisms (capable of causing diseases) affect the safety of the food product and can lead to food poisoning (FDA, 2014). Yeast and mold spoilage is usually seen on the product whereas pathogenic microorganisms are not seen (Kilcast & Subramaniam, 2000). According to Leatherhead Food research (n.d.), foods with a high water activity will typically spoil because of microbial growth. Foods with lower water activities will more than likely spoil due to sensory changes, such as a food losing its flavor (Leatherhead Food Research, n.d.).

Sensory Characteristics

Some foods lose their sensory characteristics before they become unsafe to consume due to microbial growth. Sensory characteristics such as flavor, texture and aroma, are affected by the deterioration of foods during storage. Given that the food is still safe to consume regarding microbial growth, most changes in sensory quality go unnoticed by consumers (Kilcast & Subramaniam, 2000).

Shelf Stable Foods

Shelf stable foods are products that can be stored at room temperature (USDA, 2014, Shelf-Stable Food Safety section). They are also known as non-perishable foods (USDA, 2014, Shelf-Stable Food Safety section). At room temperature, shelf stable foods are microbiologically safe and acceptable to consume (USDA, 2014, Shelf-Stable Food Safety section). These foods are normally seen on the shelf at the store. Some examples of shelf stable food are jerky, canned and bottled foods, spices, oils and other products that do not require refrigeration until after opening (USDA, 2014, Shelf-Stable Food Safety section). Not all canned foods are shelf stable, such as canned seafood. Foods that are not shelf stable will be labeled "Keep Refrigerated" (USDA, 2014, Shelf-Stable Food Safety section).

To make foods shelf stable, perishable foods can be heated or dried to destroy microorganisms that can cause illness or food spoilage (USDA, 2014). The shelf stable foods are typically packaged in sterile, airtight containers (USDA, 2014). All foods eventually spoil if they are not preserved (USDA, 2014).

Intermediate Moisture Foods

Intermediate moisture foods are foods that are adjusted to 20 – 50% moisture (Cooksey, 2012, Food Preservation Methods). Labuza, as cited by Leake (2006, p. 3), stated that 0.6–0.8 is the water activity range for intermediate moisture foods such as chewy granola bars, or soft, moist pet foods. For intermediate moisture foods in this water activity range, shelf life could decrease as the water activity increases, because of the increased chance of microbial growth in the food product (Leake, 2006). Intermediate moisture foods are considered shelf stable due to the lowered water activity and use of

preservatives in the food (Barbosa-Cánovas et al., 2003). The preservatives help control the spoilage microorganisms that are able to tolerate lower water activities (Barbosa-Cánovas et al., 2003).

The lowered water activity allows intermediate moisture foods not to immediately spoil due to microbial growth, even if the packaging has been damaged before opening (Barbosa-Cánovas et al., 2003). The shelf life of these products, however, is affected by their sensory properties such as development of dry texture, due to moisture loss. For foods that are shelf stable such as intermediate moisture foods, sensory evaluation is the main factor for determining acceptance limit or shelf life (Manzocco & Lagazio, 2009). This means that the food may still be microbiologically safe to consume, but could be rejected by consumers because of the taste, smell, texture or appearance of the food.

Food Packaging

Packaging is an important part of the food industry, as it is a way to control shelf life without changing the components of food by use of more preservatives, or additives. The packaging industry has been relying heavily on conventional packaging materials (made from petrochemical and natural gas) (Johansson et al., 2012). Polymers are the most common packaging materials because of features such as their softness, lightness, and transparency (Siracusa, Rocculi, Romani & Rosa, 2008). Increased use of conventional polymers has raised economic and environmental concerns because they are not biodegradable (Siracusa et al., 2008).

It is important to use renewable raw materials to produce polymers that will give comparable properties as the conventional polymers (Johansson et al., 2012). This could

help slow down the use of the conventional raw materials. Increased use of polymers made from renewable raw materials, would allow the cost of those materials to decrease. When deciding the type of packaging materials to use in the food industry, there is usually a compromise between the ideal barrier and the affordable option for a particular food product or market (Kilcast & Subramaniam, 2000).

Conventional Food Packaging Materials

The overall function of a food package is to protect and preserve the product for as long as possible, helping to extend the shelf life (Kilcast & Subramaniam, 2000). When developing a flexible package for intermediate moisture foods, moisture and gas permeation are the main factors to control.

Moisture migration through the package and into the product could cause moisture gain or loss in the product. If moisture is gained, intermediate moisture foods can become more susceptible to microbial growth, or lead to texture degradation. Moisture loss can be critical for some products such as baked goods, especially if they are packed when warm (Kilcast & Subramaniam, 2000). If moisture is lost, these types of foods become dry. Intermediate moisture foods such as chewy snack cakes, can become unacceptable to the consumer if they dry out. Therefore, moisture permeable and moisture barrier packaging is needed (Kilcast & Subramaniam, 2000).

Gas migration (typically oxygen) into the product from the outside of the package can contribute to oxidation (Kilcast & Subramaniam, 2000). When a product contains fat, it is assumed that the package will need to have an oxygen barrier (Kilcast & Subramaniam, 2000) such as a metallized film layer. If oxygen is increased in food

products, they begin to oxidize any fat components, contributing to rancidity (Kilcast & Subramaniam, 2000). Rancidity can be detected by taste and smell, even at low levels (Kilcast & Subramaniam, 2000). Therefore, oxidation of an intermediate moisture food causes the food to become unacceptable to consume.

Moisture gain or loss and oxidation are temperature dependent factors. As temperature increases from ambient conditions, these factors become more effective (Kilcast & Subramaniam, 2000). As temperature increases, the gas or moisture molecules move faster and permeation through the package is increased (Kilcast & Subramaniam, 2000). The rate of gas and moisture permeation through a package is inversely proportional to the thickness of the material (Kilcast & Subramaniam, 2000).

Some conventional food packaging materials used to package intermediate moisture foods are polypropylene (PP) and polyethylene (PE). They are the most common polymers used in the food packaging industry (Siracusa et al., 2012). Along with other packaging materials, they are used for their relatively low cost, good tensile strength, good barrier to moisture, good abrasion resistance and heat sealing capabilities (Siracusa et al., 2012). PP and PE do not provide sufficient oxygen barriers (Frey, 2009).

Sometimes PP films are oriented by applying force to the softened material either in one direction, or in two directions, and then cooled quickly (Kilcast & Subramaniam, 2000). Orienting PP film improves the moisture and oxygen barrier properties and tensile strength (Kilcast & Subramaniam, 2000), but needs a higher temperature than non-oriented PP to seal (Soroka, 2009). To compensate for this, the oriented PP (OPP) film can be coated or co-extruded to make them heat-sealable at lower temperatures (Soroka,

2009). Snack foods and confectionery products are typically packaged with OPP (Soroka, 2009). Sometimes one OPP film is laminated to another OPP film, which may have been metallized to improve gas barrier properties (Soroka, 2009). Other laminations include biaxially oriented polypropylene (BOPP) films laminated to PE films, where PE has good heat sealing and moisture barrier properties and BOPP has good mechanical and moisture barrier properties (Siracusa et al., 2012).

A good overall barrier in packages containing intermediate moisture foods is a metallized layer made of aluminum. It is often used in film laminations for foods that require barriers to oxygen, light (Chowdhury & Kolgaonkar, 2014) and moisture.

Literature concerning the shelf life of intermediate moisture foods in conventional packages shows mostly modified or controlled atmosphere packaging and irradiation being used to extend shelf life (Waletzko & Labuza, 1976; Lazarides, Goldsmith & Labuza, 1988).

Sustainable Food Packaging

Although sustainable packaging is discussed often, there is still no clear definition of sustainable packaging. There are several different definitions, leaving everyone confused, which could allow companies to market their products as "sustainable" (Robertson, 2014, p. 62). It was determined that the phrase "sustainable packaging" is too broad to be useful at a practical level (Robertson, 2014, p. 62).

According to the U.S. Environmental Protection Agency [EPA], as cited by Robertson (2014, p. 61), "sustainability, or sustainable development, is the ability to achieve continuing economic prosperity while protecting the natural systems of the planet

and providing a high quality of life for its people." This means that the depletion of resources should match their rate of renewal (Robertson, 2014, p. 61). The use of nonrenewable conventional resources (crude oil and natural gas) is unsustainable (Robertson, 2014). Therefore, there has been more interest in using renewable, bio-based raw materials and polymers (Robertson, 2014).

According to Robertson (2014, p. 62), bio-based packaging materials are derived from annually renewable sources. This excludes paper-based materials because trees have a renewal time of 25 – 65 years (Robertson, 2014). "Bio-based plastics are derived from bio-based materials and [can] be biodegradable" (Robertson, 2014). This is because biodegradability depends on the chemical composition and not on the origin of the raw materials (Robertson, 2014).

The following four definitions of the type of sustainable polymers describe how they are sustainable (Darby, 2012, Sustainable Polymers):

- **"Biopolymers**: polymeric materials that are made in nature; formed from saccharides, proteins and nucleotides. Examples are cellulose and chitin."
- **"Bio-based polymers:** polymers that are derived from renewable resources. Examples are PLA, cellophane and sugar-derived [low density PE] LDPE."
- **"Biodegradable polymers:** polymers that can be digested by naturally occurring biological entities in normal environmental conditions, resulting in chemicals including carbon dioxide, methane, and water; PLA and sugar-derived LDPE are not biodegradable. Landfills should not be relied on to biodegrade anything."
- **"Compostable polymers:** polymers that can be digested by naturally occurring biological entities in special environmental conditions like a high temperature

compost; Examples are PLA [depending on the type]; Bio-derived LDPE is not [compostable]."

Environmental packaging can help reduce the environmental impact (Darby, 2012, Sustainable Polymers). This includes (1) lowering energy use and emissions, (2) using less conventional materials and more renewable materials and (3) reducing landfill use (Darby, 2012, Sustainable Polymers). According to Robertson (2014), even though it is not clear what sustainable packaging is, the food industry has become more interested in using bio-based materials for food applications.

Bio-based Food Packaging Materials

There is limited research on how intermediate moisture foods behave in bio-based packaging materials. Some research has been performed on the shelf life of fresh produce (perishable foods) contained in bio-based packaging materials. A study was published in 2009 showing the effect of three bio-based materials on the quality and shelf life of fresh celery (Ifezue, 2009). The control was perforated low-density polyethylene (LDPE) and the three variables were un-perforated compostable packaging materials (EcoFlex®, Mater-Bi and PLA) (Ifezue, 2009). Results showed that Mater-Bi, performed better than the other materials regarding weight loss, water vapor permeation rate, flavor, sensory rank, and tensile testing (Ifezue, 2009). Therefore, Mater-Bi and similar packaging materials could possibly replace LDPE for packaging fresh celery (Ifezue, 2009).

Another study was published in 2011 comparing the shelf life of blackberry fruit in bio-based and conventional containers (Joo, Lewandowski, Auras, Harte & Almenar, 2011). The control container was oriented polystyrene (OPS) and the bio-based container

was oriented polylactic acid (OPLA) (Joo et al., 2011). The blackberries in both containers experienced results including an increase in pH, weight loss, fungal count and a reduction in firmness and anthocyanin content during storage (Joo et al., 2011). The fruit in the OPS container showed better quality over all (Joo et al., 2011). Blackberries in both containers met the "US standard No. 1" grade for commercialization for more than 12 days at 3°C (Joo et al., 2011).

Bio-based packaging materials used alone lack the moisture and oxygen barrier properties that conventional packages have in order to maintain the quality of food. To make up for the poor moisture and oxygen barrier and properties of bio-based materials alone, literature shows use of coatings, nanocomposites, modified atmosphere packaging (MAP) and oxygen scavengers for barriers (Almasi, Ghanbarzadeh, Dehghannya & Entezami, 2014; Petterson, Bardett, Nilsen & Fredriksen, 2011). A study was published in 2009 where oxygen scavengers were used in PLA trays of refrigerated, ready-to-eat sliced cooked ham (Cerioli, 2009). The trays were under various modified atmospheres (Cerioli, 2009). Results showed that using an oxygen absorber with a carbon dioxide emitter extended the shelf life of the ham in the PLA tray by approximately 10 days at 6 – 8°C. The PLA was found to be a good alternative to traditional packages for packaged slice ham (Cerioli, 2009). There is no research regarding the use of Bio-PE (made from sugarcane) as a food package.

While these technologies are being used to enhance the film barrier properties for bio-based materials, the use of bio-based materials combined with bio-based laminates with barrier properties has limited research. Combining different bio-based materials to create multi-layered food packages is another way to improve these properties for food

application. Using only bio-based laminates instead of conventional laminates could allow food packaging materials to be completely bio-based. A study was published in 2005 on the possibility of biodegradable laminate films derived from naturally occurring carbohydrate polymers that could be used for food packaging (Fang et al, 2005). This study focused on using modified starch and PLA to create laminate films, which were expected to show equal or better performance characteristics compared to existing laminate films (Fang et al, 2005). However, these laminate films would be able to degrade at the end of film life (Fang et al, 2005). Figure 4 below shows the biodegradable

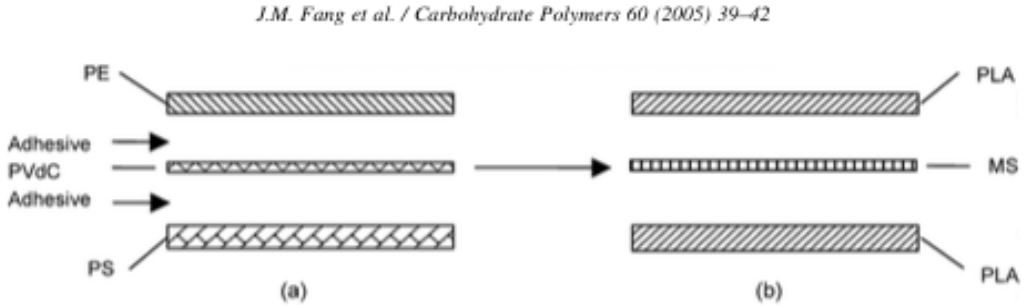


Figure 4: Non-biodegradable and biodegradable laminated structures (Fang et al., 2005) laminated structure (Fang et al, 2005).

While this study shows that bio-based materials can be used as laminates, it does not show how certain foods would behave in packages with bio-based laminates.

Specific Bio-based Packaging Materials

According to BASF The Chemical Company (2013), four categories of polymers are (1) bio-based and compostable, such as PLA, (2) fossil fuel-based (non-renewable) and compostable, such as EcoFlex®, (3) bio-based, but not compostable, such as Bio-Polyethylene (Bio-PE) and (4) fossil fuel-based, such as PP.

Some bio-based materials that are used for food applications are PLA, Bio-PE and cellophane.

Polylactic Acid (PLA)

PLA falls into the category of being a bio-based and compostable polymer (Robertson, 2014). The raw material for lactic acid is typically genetically modified corn, or cornstarch (Robertson, 2014). Other raw materials for lactic acid manufacture could include sugar beets, sugarcane and tapioca (Robertson, 2014).

PLA can be processed using the same machinery as conventional polymers with little modifications and produce plastic that is compostable and recyclable (Johansson et al., 2012). The main application of PLA for food packaging is a rigid bottle or tub, but it can also be used as a film (Robertson, 2014). The main problem with the film is its high water vapor transmission rate (Robertson, 2014) and insufficient gas barrier properties (Johansson et al., 2012). PLA is manufactured in commodity and in specialty grades and the sheets have been thermoformed into trays (Johansson et. al, 2012). However, even with some commercial advances, there is still a need for cost-effective methods to improve PLA's properties, such as its gas and low water vapor barrier, its brittleness and its thermal stability (Johansson et al., 2012).

To make up for these drawbacks, one solution could be to combine PLA films with other bio-based films that decrease moisture and gas migration through the package. Since this is a factor concerning intermediate moisture foods, combining appropriate bio-based materials could help extend the shelf life of the product.

Bio-Polyethylene (Bio-PE)

Bio-PE falls into the category of being bio-based, but not compostable (Robertson, 2014). Ethanol is made from sugarcane (Braskem, 2014). The ethanol is then dehydrated and transformed into ethylene, which is then polymerized to be made into PE plastic (Braskem, 2014). This film has the same properties as PE made from natural gas, or oil feedstocks (Braskem, 2014). It does not require new manufacturing machinery to be processed and it is 100 percent recyclable (Braskem, 2014). Some current applications of Bio-PE are yogurt cups, fruit juice bottles, plastic caps and closures for aseptic paperboard cartons (Robertson, 2014).

As cited by Robertson (2014), Liptow and Tillman found that PE from sugarcane used significantly less fossil fuels. It also has the potential to significantly reduce greenhouse gas emissions (Robertson, 2014). Regarding packaging intermediate moisture foods, this material has the same properties as conventional PE. It has good moisture barrier, but poor oxygen barrier properties (Frey, 2009).

Metallized Cellophane (Met-Cell) (Brand name NatureFlex™ NKME)

Innovia's NatureFlex™ NKME, or metallized cellophane, falls into the category of a bio-based and compostable polymer (Robertson, 2014). Its raw material source is wood pulp, typically sourced from hard wood species such as eucalyptus (Innovia Films, 2014). This film—from inside (near the product) to outside (away from the product)—is made up of a moisture barrier heat-seal coating, a transparent cellulose film, a moisture barrier coating and a metallized surface (Innovia Films, 2014). Properties of this film include good moisture, gas, aroma, and light barriers (Innovia Films, 2014). It is resistant to oils and greases and is heat-sealable (Innovia Films, 2014). Its application is

specifically for lamination purposes to help extend the shelf life of packaged products (Innovia Films, 2014).

Product Analyses

Intermediate moisture foods are mainly affected by its sensory and texture properties.

Sensory Analysis

Sensory analyses are used during shelf life studies to give a subjective view on what happens to a food product over time. The results can be compared to instrumental analyses to see if the average consumer would be able to detect physical and chemical changes in the product. Stone & Sidel, as cited by the Institute of Food Technology [IFT] (2014, p. 55), states that sensory analysis is "a scientific discipline used to evoke, measure, analyze, and interpret…responses to products that are perceived by the senses of sight, smell, touch, and hearing." Sensory panels are used to evaluate the quality or characteristics of foods (Kramer & Szczesniak, 1973). There are two different types of sensory panels—consumer panels and trained panels.

Consumer panels consist of 50 or more untrained, random consumers (Kramer & Szczesniak, 1973). Consumer panelists are people who consume the product or similar products regularly (Moskowitz et al., 2008). A person who consumes the product or similar products regularly is likely to notice small differences in its properties (Moskowitz et al., 2008). Trained panels consist of as few as 5 highly trained, carefully selected consumers who have great sensitivity to different attributes of foods (Kramer & Szczesniak, 1973). The training for a trained panel can take up to six months (Kemp,

Hollowood & Hort, 2009, p. 100). These panelists should be able to detect specific product characteristics such as salt or sugar concentrations. Choosing the right type of panel depends on the questions that will be answered during evaluation of the product (Kramer & Szczesniak, 1973).

Before a sensory analysis is performed, both types of panels (consumer and trained) should understand how to evaluate the product and understand what the researcher is looking for (Meilgaard et al., 2007). To do this, recruited participants attend familiarity sessions, where the participants and the researcher agree on the sensory characteristics of the food product. This is done so that all participants understand the characteristics when evaluating the food product during the sensory analysis (Kramer & Szczesniak, 1973). The panelists should be familiar with the basic test procedures such as how to taste the sample and how the samples will be presented (Meilgaard et al., 2007). They should also understand how to use the sensory ballot and the type of questions to be answered during the evaluation (Meilgaard et al., 2007). Ultimately, the panelists should be prepared enough to complete the sensory test without instruction from the researcher (Meilgaard et al., 2007).

There are several different types of sensory tests that can be used for the evaluation of food products. Sensory tests are grouped into three categories, which are preference or acceptance tests, discriminatory tests and descriptive tests.

A preference test determines which of two or three samples is liked best and answers the question, "*Which sample do you prefer?"* (Kramer & Szczesniak, 1973, p.20). An acceptance test shows if potential consumers would accept a product and answers the question, *"Would you accept this sample?"* (Kramer & Szczesniak, 1973,

p.20). These tests are typically conducted using a consumer panel, as the main purpose is to determine if the product would sell (Kramer& Szczesniak, 1973). In laboratory situations, panelists may need to be selected from a group of available people, excluding those who have any knowledge of the product (Kramer & Szczesniak, 1973). Typically, at least 30 panelists are used on laboratory consumer panels, but even results from 30 panelists are too small to detect trends because of the narrow range of sampling (Kramer & Szczesniak, 1973). Therefore, 50 – 100 panelists are recommended for laboratory consumer tests (Kramer & Szczesniak, 1973).

A discriminatory test determines whether there are detectable differences among samples, but does not indicate how large or what kinds of differences exist (Kramer & Szczesniak, 1973). There are two types of discriminatory tests – threshold tests and difference tests. The absolute threshold is the intensity of a given parameter that can just be perceived by an individual (Kramer & Szczesniak, 1973). The difference threshold is the smallest change in the intensity of an attribute that a given individual can detect as being different (Kramer & Szczesniak, 1973). During a difference test, the panelist identifies the sample that is different from the other, but does not tell how big, or the direction of the difference (Kramer & Szczesniak, 1973).

As cited by Kramer & Szczesniak (1973), the Committee on Sensory Evaluation of the Institute of Food Technologists says that discriminatory tests are typically performed using 3 – 10 trained panelists or 80 or more untrained panelists.

A descriptive test involves "the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects of a product by trained panels of 5 – 100 judges (subjects)" (Meilgaard et al., 2007, p. 173). This test is typically used when

the researcher is interested in the effects of the studied variables on the food (Kramer & Szczesniak, 1973). Descriptive tests answer the questions, *"How much difference is there between samples?"* and *"What is the difference between samples?"* (Kramer & Szczesniak, 1973, p.22). Small panels (5 – 10 panelists) are used for evaluating products already in stores (Meilgaard et al., 2007). Larger panels are used for evaluating products that are produced in large quantities, where small changes are important such as in beers and soft drinks (Meilgaard et al., 2007). The qualitative aspects of a product include the appearance, aroma, flavor, texture, or sound properties (Meilgaard et al., 2007). The quantitative aspect of a product is the degree to which each qualitative aspect is present in the sample (Meilgaard et al., 2007). Three products may have the same qualitative aspect, but they may have different degrees of that aspect in the product (Meilgaard et al., 2007). For example, two potato chips might have the same salty flavor, but one chip might be saltier than the other. When using a descriptive test during a shelf life study, the attributes should represent the most important factors that would affect consumer acceptance (Meilgaard et al., 2007).

An example of a descriptive test is a rating scale (Kramer & Szczesniak, 1973). When using a rating scale, panelists use numbers or words to express the intensity of an attribute (Meilgaard et al., 2007) and it is indicated by a mark on the scale (Kramer & Szczesniak, 1973). On a rating scale, the researcher should use objective terms such as "very hard" and not preference terms such as "much too hard" when defining the scale points (Kramer & Szczesniak, 1973, p.23). One or more attributes can be evaluated at one time (Kramer & Szczesniak, 1973). Using a rating scale requires a trained panel that would be familiar with the attributes of the product being studied (Kramer & Szczesniak,

1973). However, some studies show that consumer panels can be used for descriptive analyses where a rating scale is used to rate the intensities of attributes. Although consumer panels show more variability than highly trained panels, some variability can be reduced even with minimal training of the consumer panels.

A study was published in 2011 where consumer panel evaluations were compared to trained panel evaluations (Ares, Bruzzone & Giménez, 2011). Each panel evaluated the texture of dairy desserts using an rating scale (Ares et al., 2011). Results showed that the consumer and trained panelists had similar discriminative capability for all evaluated texture characteristics (Ares et al., 2011). The consumer panel, however, showed lack of consistency in its evaluations and individual scores, as most consumers were not able to significantly distinguish between samples (Ares et al., 2011). Therefore, even though the average data for attribute intensity from a consumer panel could be valid and comparable to a trained panel, there is still a lot of variability among consumer panels for this type of scale (Ares et al., 2011).

An earlier study was published in 1994 comparing trained and untrained panelists who evaluated sensory attribute intensities and liking of cheddar cheeses (Roberts & Vickers, 1994). Results showed that the trained panelists found larger differences in liking among the cheeses than the untrained panelists (Roberts & Vickers, 1994). The trained panel did not find larger differences among cheeses in the intensity of the attributes than the untrained panel (Roberts & Vickers, 1994). Training improved agreement among panelists on the attribute ratings (Roberts & Vickers, 1994).

An example of a rating scale is an unstructured scale, where the ends of the lines are the extreme intensities of the attributes being evaluated (Kramer & Szczesniak, 1973,

p.24). The descriptors at the ends of these lines are called anchor words and are marked a half-inch from the ends of the scale (Meilgaard et al., 2007). An example of descriptors on an unstructured line scale could be “hard” on the low end and “chewy” on the high end. Panelists place a hash mark on the line, indicating the intensity of an attribute (Meilgaard et al., 2007). Numbers are then assigned to the marks on the scale by measuring the distance from the low end to the panelist’s mark with a ruler (Meilgaard et al., 2007).

Descriptive analysis intensity descriptors can be chosen from a spectrum of intensity scales (Meilgaard et al., 2007). These scales are a group of intensity scale values and descriptors for attributes of different types of products (Meilgaard et al., 2007). When using a descriptive test during a shelf life study, the attributes (i.e. texture, flavor) should represent the most important factors that would affect consumer acceptance (Meilgaard et al., 2007).

A factor affecting the results of rating scales, including unstructured scales, is that the panelists “tend to avoid extremes and confine their ratings to the middle of the scale” (Kemp, et. al., 2009, p.9). This is called the central tendency error (Meilgaard et al., 2007). For example, if a brownie was evaluated for intensity of its chocolate flavor on a scale of 0 - 15, panelists will avoid the numbers 0, 1 and 2. This occurs because of the anticipation of future samples to have very low flavor intensities (Meilgaard et al., 2007). The same happens with the high numbers on the scale, where 13, 14 and 15, would be avoided because of anticipation of future samples to have very high flavor intensities (Meilgaard et al., 2007). This is more likely to occur with an untrained panel (Kemp, et. al., 2009). The central tendency error also applies to the presentation of samples

(Meilgaard et al., 2007). To avoid this error, panelists should be trained to use the scale before participating in the sensory analysis (Kemp, et. al., 2009). The central tendency error may also be reduced because the lines of an unstructured scale extend past the fixed end points (Lawlwss & Heymann, 2010).

Panel conditioning is another factor that can affect results during a sensory analysis for a shelf life study. Panel conditioning can occur when panelists begin to represent the average consumer less as they gain more experience on the panel (Bastian, Eggett & Jefferies, 2014). Moskowitz et al., as cited by Bastian et al. (2014) says, when the same panelists are used to repeatedly evaluate the same product, the panelists become more experienced. Therefore, their ability to detect particular attributes and qualities of a product increase and they begin to view the product differently because of their repeated exposure to it (Bastian et al., 2014). In contrast, there are some studies that claim that panel conditioning does not exist. This suggests that panel conditioning occurs in some areas of research and not in others (Bastian et al., 2014).

Research shows that initial food product judgments change possibly due to factors such as boredom and loss of curiosity (Bastian et al., 2014). A study, as cited by Bastian et al., (2014), for example, showed that panelists frequently changed their product preferences during several laboratory visits over a 2-week period. This study supports Moskowitz's theory, which suggests that repeat exposure to a product changes consumers' acceptability and interest (Bastian et al., 2014).

If data appears to be missing from the sensory evaluation, it should be checked to confirm that it is actually missing (Kemp et al., 2009). Some statistical analyses cannot be performed with missing values (Kemp et al., 2009). If this happens, if possible,

numerical values could be replaced with the mean (Kemp et al., 2009). However, replacing missing values with the mean will impact the statistical analysis (Kemp et al., 2009). Data should be replaced with caution and any modifications should be considered during interpretation of the results (Kemp et al., 2009). In contrast, according to Pripp (2012, p. 13), missing data should not be neglected or replaced with a mean value. Instead, missing data should be investigated to see if there is a pattern to the missing data and try to explain why the data are missing (Pripp, 2012). Technical problems or participants not answering all questions during an evaluation are typical reasons for missing data (Pripp, 2012). Ultimately, it is best to design a study so that the risk for missing data is minimized (Pripp, 2012).

During a sensory analysis, the presentation of the samples plays a role in how the samples are evaluated. Five types of biases might be caused by the order of presentation. They are: (1) contrast effect, (2) group effect, (3) error of central tendency, (4) pattern effect and (5) time error or positional bias (Meilgaard et al., 2007).

(1) The contrast effect states that presenting a sample of good quality right before a sample of poor quality may cause the second sample to be rated lower than if it had been presented as a single sample (Meilgaard et al., 2007).

(2) The group effect states that one good sample presented in a group of poor samples may be rated lower than if it had been presented as a single sample (Meilgaard et al., 2007).

(3) The error of central tendency states that samples placed near the center of a set are usually preferred over the samples placed at the ends (Meilgaard et al., 2007).

(4) The pattern effect states that panelists will use clues to detect any pattern in the order of presentation (Meilgaard et al., 2007).

(5) The time error or positional bias states that panelists' attitudes undergo small changes over a series of tests, from anticipation or hunger for the first sample, to fatigue or indifference with the last sample (Meilgaard et al., 2007).

To minimize these effects, it is important to randomize samples when panelists are testing multiple samples at one time (Meilgaard et al., 2007). This means that each panelist receives the samples in random order each time they evaluate the product.

In some sensory shelf life studies, the researcher allows panelists to compare a fresh sample to the older samples that are being evaluated (Hough, 2010). In order to do this, the fresh sample would be frozen or refrigerated to slow down or stop the deterioration rate (Hough, 2010). This is only done if the changes in the fresh sample were insignificant compared to the changes in older samples being evaluated (Hough, 2010). All samples should be of the same lot (Hough, 2010).

In some cases, keeping a fresh sample is difficult (Hough, 2010). A study was performed on the shelf life of ready-to-eat lettuce that was stored at 4°C (Araneda, Hough & De Penna, 2008). Consumers evaluated the stored lettuce at different storage times throughout the study (Araneda et al., 2008). For this study, keeping a fresh sample of lettuce would be hard to do and inconvenient (Hough, 2010). Freezing the lettuce would have changed the texture (Hough, 2010, p. 72). If the lettuce was stored at a temperature just above freezing, it may slow the deterioration rate (Hough, 2010). However, the lettuce would not be completely unaffected compared to the samples being tested at different storage temperatures (Hough, 2010).

In other shelf life studies, a fresh sample is unnecessary (Hough, 2010). This could be because of the researcher's experimental design for the sensory analysis, where they want their panelists to only evaluate the current status of the stored samples (Hough, 2010).

Texture Analysis

Chewy and moist are the often desired textural qualities in confectionery items such as brownies. Although some textural attributes of confectionery foods can be evaluated orally and visually, other textural attributes such as the hardness of a brownie, can be measured by texture analysis equipment. Sensory methods of analysis are subject to wide variability, while using instrumental methods to analyze texture offers more controlled conditions (Kramer & Szczesniak, 1973). A texture analysis instrument such as a TA.XT*plus* Texture Analyzer, can be used to measure the changes in hardness and several other attributes of a product during a shelf life study (Stable Micro Systems, 2014). The textural attributes of interest should be identified before performing a texture analysis in order to select the appropriate probe to measure specific textural attributes relevant to the product (Stable Micro Systems, 2014).

A masticometer is a fixture that can be used to measure the firmness of a product over time (Kramer & Szczesniak, 1973). It is equipped with artificial jaws attached to force gauges (Kramer & Szczesniak, 1973). The artificial jaws can simulate bite action and evaluate textural qualities such as crispness, firmness, or softness (Kilcast & Subramaniam, 2000). Another fixture, the 5-diameter cylinder stainless steel probe, is capable of penetrating a sample and calculating the hardness as it goes through (Stable Micro Systems, 2014). It measures the amount of force required to bite into a product

(Stable Micro Systems, 2014). It is also used to assess the softening of a product due to moisture migration (Stable Micro Systems, 2014).

Since some intermediate moisture foods such as a brownie, typically have a chewy and moist texture, higher hardness values indicate that the food product has lost moisture and becomes more firm (Labuza, 1982). The value for this textural attribute is an indication of poor textural quality (Labuza, 1982). Low hardness values indicate that it has either an acceptable amount of moisture, or too much moisture due to moisture migration over time (Labuza, 1982).

Film Analyses

During shelf life studies, in addition to product testing, package testing is performed to see if product deterioration is affected by the changing properties of the package over time. For films, analyses include water vapor and oxygen transmission rate, tensile and seal-peel.

Transmission Rate Analyses

The transmission rate of a package is "the rate at which a permeant goes through a material of a specific area" (Stevens, 2014, p. 3). Transmission rate measurements are specific to the material being tested, the permeant, and the conditions (i.e. temperature and relative humidity) (Stevens, 2014). The most common permeants tested are water vapor and oxygen (Johansson et al., 2012). The transmission rate of flat samples is useful for material evaluations and research and development applications (Stevens, 2014). During transmission rate testing, there is a time to reach equilibrium (Stevens, 2014). That time varies between different materials (Stevens, 2014). Materials with low transmission rates have great barriers and may take weeks to reach equilibrium (Stevens,

2014). Materials with high transmission rates may take a few hours to reach equilibrium (Stevens, 2014).

Relative humidity is an important factor to consider when testing the transmission rate of materials (Stevens, 2014). When testing the water vapor transmission rate (WVTR), the relative humidity should be controlled and monitored because it is the test gas (Stevens, 2014). When testing for oxygen transmission rate (OTR), an increase of moisture causes the oxygen transmission rate to increase (Stevens, 2014). Some other factors to consider during transmission rate testing are material thickness variation, equilibrium time, barometric pressure and proper gas generation (Stevens, 2014).

PLA and Bio-PE materials alone typically have higher water vapor and oxygen transmission rates than conventional materials used for food packaging (Johansson et al., 2012).

Tensile Analysis

Tensile tests are performed to measure properties, including tensile strength at break, break elongation and the modulus of elasticity (E-Modulus) of a film, while observing stress and deformation until the sample breaks (Mechanical Properties of Polymers, 2005). The test method for evaluating tensile strength of plastics is ASTM D882 (Instron: Materials Testing, 2014).

Tensile strength of a material is “the maximum tensile stress which a material is capable of sustaining before rupturing” (Kramer and Szczesniak, 1973, p.35). Tensile strength at break is the maximum about of pulling the sample can take before breaking (ASTM F88, 2009). Break elongation is the elongation of a film sample to the break

point (Instron: Materials Testing, 2014). The E-Modulus is the rate of change of strain as a function of stress in the elastic portion of the curve (Instron: Materials Testing, 2014). It measures the stiffness of the material in the elastic portion of the curve (Instron: Materials Testing, 2014).

A graph of stress vs. strain is produced to show how different packaging materials behave when placed under stress during tensile analysis (Instron: Materials Testing, 2014). Stress is the load of a sample divided by the cross-sectional area (Instron: Materials Testing, 2014). Stress units can be in pounds per square inch (psi), grams per square cemtimeter (g/cm^2), Newtons per square millimeter (N/mm^2), or megapascals (MPa) (Darby, 2012, Deformation Properties). Strain is the amount of deformation or elongation the sample undergoes (Instron: Materials Testing, 2014). Strain is dimensionless, but is often expressed in percent strain (Instron: Materials Testing, 2014). Figure 5 below is an example of a typical stress-strain curve of different types of polymers (Darby, 2012, Deformation Properties).

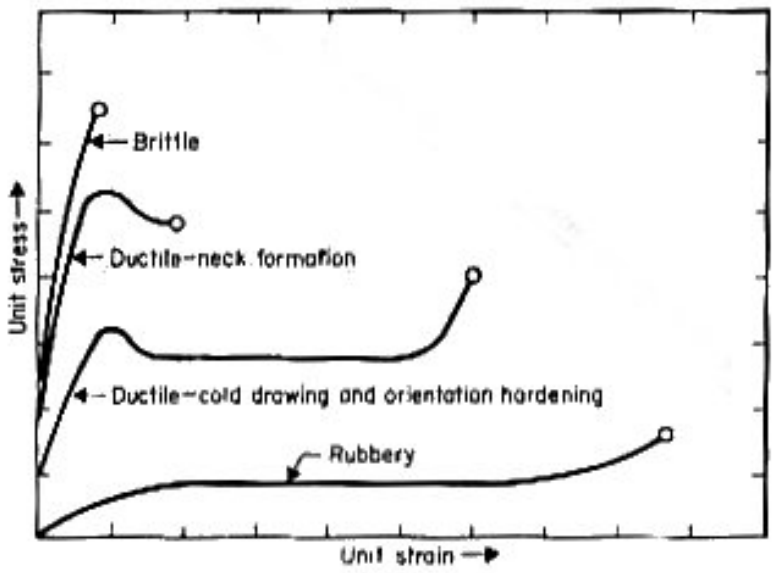


Figure 5: Typical Stress-Strain Curves of Different Types of Polymers (Darby, 2012, Deformation Properties)

As shown in Figure 5, polymers can be brittle, elastic, or rubbery. While stress is measured in force per unit area and expressed in psi, strain is the dimensionless fractional increase in length (Selke, Cutler, & Hernandez, 2004). High tensile strength numbers indicate a strong or rigid material that can resist deformation (Mechanical Properties of Polymers, 2005). Lower tensile strength numbers indicate a material that can easily deform (Mechanical Properties of Polymers, 2005).

Seal-Peel Analysis

The test method for evaluating seal strength of plastics is ASTM F88 (Instron: Materials Testing, 2014). "This method measures the force required to separate a test strip of material containing the seal and identifies the mode of specimen failure" (ASTM International, 2009, ASTM F88). It tests the opening force, package integrity, and the ability of the package system to produce consistent seals (ASTM International, 2009, ASTM F88). A low seal strength may indicate that the seal of a package can be opened easily, which could make products more susceptible to deterioration due to moisture gain or loss, or microbial growth. In contrast, a high seal strength may indicate less susceptibility to deterioration due to moisture gain or loss, or microbial growth. However, the seal could also be hard to break, meaning the package would be hard for consumers to open.

Closing Remarks

Bio-based packaging materials for food applications has a positive perception in target markets where consumers are concerned about being healthy and helping the environment. Finding more applications of bio-based materials will reduce the dependence on the use of conventional materials for food packaging. To do this, the behavior of foods needs to be observed as they are contained in different bio-based materials. The purpose of the current research was to compare the shelf life of an intermediate moisture health bar in its current conventional package to the shelf life of the same health bar in two different bio-based packages.

MATERIALS AND METHODS

Product and Sample Preparation

Brand name chocolate health bars were purchased from Wal-Mart in Central, South Carolina. One hundred boxes of five packages (500 total packages) of product were purchased at the same time. The products were of the same lot number and "Better If Used Before" date. The chocolate health bars were transported to Clemson University's Packaging Science Department, where a shelf life study was performed. This product was chosen because it is a homogenous product, unlike other snack bars that typically have nuts, chocolate chips or raisins in them. Using these homogenous chocolate health bars enabled immediate sensory and instrumental testing.

Materials

The control package was the manufacturer's package. Before analysis, the number and types of layers of the control package were determined in order to make sure the variable packages would be comparable. In order to determine the layers of the control package, two packages of product were used. Both control packages were cut open, removing the sealed ends. Next, the basis weights and thicknesses of both layered films were obtained. Next, one of the layered films was soaked in toluene, between 65.6ºC and 71.1ºC, until the extrudate dissolved, causing the two layers of the film to separate. After that, the separated films were air-dried and the basis weights and thicknesses were obtained for each layer. On the metallized side of the second layered film, the metal in the middle of the film was scrubbed off using acetic acid, diluted to 2%. At this point, the two film layers from the first package and the demetallized second package were analyzed using the Smart Omni-Transmission Infrared Spectrophotometer Model

NICOLET iS10 (Thermo Fisher Scientific, Madison, WI), to confirm the type of polymers. The scan confirmed that the two layers were clear oriented polypropylene and metallized oriented polypropylene (Met-OPP). After consulting with a packaging materials expert, it was determined that the layers of the control package, from outside (away from the product) to inside (closest to the product), is 0.80 mil OPP (coated outside, treated inside)/Ink/0.40 mil white extruded LDPE/0.80 mil Met-OPP (treated outside, coated inside).

The test packages, bio-based films, were EarthFirst® Polylactic Acid (PLA) Film (Plastic Suppliers, Inc., Columbus, OH) and Green Polyethylene™ Film (Sugarcane Polyethylene) (SPE) (Braskem, Pennsylvania, PA), both laminated to the bio-based film, NatureFlex™ NKME (Metallized Cellophane) (Met-Cell) (Innovia Films, Atlanta, GA), to provide a barrier. The bio-based films were delivered to Clemson University as rolls of film, slit to a 14.5-inch web width, on a 3-inch core. The PLA and SPE films were laminated at Clemson University, to the Met-Cell, using Tycel 393 adhesive (Henkel, Gary, NC). The bond strengths were recorded to ensure that the films were sufficiently bonded. After lamination, the structures were allowed to cool for 24 hours minimum.

In preparation for packaging the product, the bio-based films were cut to 5 x 8 inch sheets and sealed into pouches, by hand, using A Sentinel® Brand Machine Model 12-12AS double bar heat sealer (Packaging Industries Group, Inc., Hyannis, MA). The PLA pouches were sealed at 112.7ºC and the SPE pouches were seal at 123.8ºC, both at a pressure of 30 pounds per square inch (psi) with a 1 second dwell time. The sealed bio-based packages duplicated the way the control package was sealed.

Before storage, the number of sample products needed for each analysis was determined for the Met-OPP, PLA and SPE films. During repackaging of the chocolate health bars, 166 of the Met-OPP packages with product were set aside, while the rest of the product in the control packages were used for repackaging in each of the bio-based pouches (166 packages each, one bar per package). After the packages were made, the samples were grouped and labeled by week and analysis method. All of the samples were placed in the Blue M Model FRS-381C-1 environmental chamber (General Signal, Blue Island, IL), which maintained a temperature of 35ºC and a relative humidity of 75%. These settings were determined by using the Q_{10} rule. Using the typical Q_{10} value of 2 for chemical reactions, the original shelf life of the chocolate health bar at 25ºC is 6 months (after purchase), therefore 3 months was required to perform an accelerated shelf life study at 35ºC. The data were collected every other week for 10 weeks (weeks 0, 2, 4, 6, 8 and 10). Prior to all analyses, the seal integrity of all of the packages were checked in the Visual Check machine to ensure that results were due to permeation through the film and not due to faulty seals. Also, each analysis week, the packages were left out for 1 hour, to reach room temperature prior to all analyses. All analyses were performed at room temperature.

Water Activity and Moisture Content Analyses

Water activity was measured in triplicate, using an Aqua Lab Model Series 3TE water activity meter (Decagon Devices, Inc., Pullman, WA). Moisture content was measured using the HR73 Halogen Moisture Analyzer (Mettler Toledo, Switzerland). For moisture content, approximately 0.1 grams of sample was used for each test.

Texture Analysis

Texture of the chocolate health bars were measured on the Stable Micro Systems Texture Analyzer Model TA.XT plus (Texture Technologies Corp., Scarsdale, NY), along with the 5-diameter flat-faced cylinder stainless steel probe attached. Measurements were recorded in triplicate, per sample, and averaged. The probe penetrated three spots in the center of the product all the way through each sample. The measurements provided data on hardness (grams force) of the product.

Microbial Analyses

Total aerobic plate counts (APC) and yeast and mold plate counts were used to measure the microbial quality of the chocolate health bars. Microbial analyses were performed seven days before each sensory analysis under a Class II biosafety cabinet. One chocolate health bar sample was analyzed from each package type. A 1:10 dilution was made for each sample by crushing a 25g sample of product, stomaching it for 1 minute in 250mL of buffered peptone water (BPW), in a sterile stomacher bag, with the Seward Stomacher® 400 Circulator (Seward, England, UK). 3M Petrifilms (3M, St. Paul, MN), were used for enumeration after 48 hours in incubation at 37°C for the APC and 5 – 7 days in incubation at 30°C for the yeast and mold counts. Dilutions from 10^{-1} to 10^{-3} were used to plate the APC and yeast and mold samples. The preferred counting range on a 3M aerobic count plate is between 25 - 250 colonies. The preferred counting range on a 3M yeast and mold count plate is 15 - 150 colonies. All samples were plated in triplicate. Results for total APC were expressed as estimated aerobic plate counts per milliliter

(EAPC/mL). Results for yeast and mold counts were expressed in colony forming units per milliliter (CFU/mL).

Sensory Analysis

The sensory analysis was performed using a computerized 15-centimeter (cm) unstructured line scale sensory ballot. The panelists were recruited from a group of people that frequently participate in a variety of sensory testing performed in the Department of Food, Nutrition and Packaging Sciences at Clemson University. Recruits participated in two familiarity sessions to get familiar with the product and the product descriptors. During these familiarity sessions, panelists were taught how to identify and evaluate the chocolate health bar's sensory characteristics. The descriptors used as the anchor words on the descriptive sensory test were chosen during this time.

Before participating in the sensory analysis for the shelf life study, all participants were instructed to fill out and sign allergy and consent forms. They were also given random 3-digit numbers in order to remain anonymous during result evaluations. A total of 28 panelists participated on the sensory panel. There were 8 men and 20 women; all were 18 – 55 years old.

During the sensory analysis for the shelf life study, the panelists were asked to place a hash mark on the computerized line scale, to indicate how they felt about the product. The attributes the panelists evaluated for the chocolate health bar were aroma, texture, flavor, and degree of liking. The following descriptors were used as the anchor words (at the ends of the line scale) for each attribute on the line scale for evaluating the chocolate health bar:

- Aroma: (0cm = No Aroma; 15cm = Intense Aroma)
- Flavor: (0cm = Stale Flavor; 15cm = Fresh Flavor)
- Texture: (0cm = Hard Texture; 15cm = Chewy Texture)
- Degree of Liking: (0cm = Dislike Extremely; 15cm = Like Extremely)

A consumption intent question, "Would you consume the product?" was asked at the end of the sensory ballot and panelists were asked to circle Yes or No. Panelists were also able to give comments on the product. All data were collected using the SIMS 2000 Sensory Evaluation Testing software.

During preliminary sensory evaluations, panelists noticed that the health bar samples were drier on the edges and chewy in the center. This lead to contradicting results of panelists not liking the outside, but still liking the inside of the product sample. Therefore, during preparation for the actual shelf life study, the edges of the chocolate health bar samples were cut off because the researcher wanted the panelists' first bite to be the center of the sample. The middle of the samples was evaluated during sensory analysis to mimic the texture analyzer measuring the hardness of the health bar in the center. Samples were then placed in 2-oz. cups and topped with lids. The sample cups were assigned random 3-digit number labels to prevent biased results from the panelists. Panelists evaluated the samples each week in a quiet sensory booth area, separate from the sample preparation area. The sensory booth area included standard lighting, a water bottle with cups and mini computers. The panelists used the computers to complete the sensory ballots. Samples were presented to panelists with one Met-OPP, one PLA and one SPE sample on a tray. Panelists were reminded each analysis to make sure that the

sample number on sensory ballot matched the sample number on the sample cup before evaluating.

Thickness Analysis of Packaging Materials

Prior to water vapor transmission, oxygen transmission, and tensile testing, the thickness of each packaging material sample was measured according to the ASTM F2251. A Nikon Digimicro stand Model MS-11C micrometer (Excel Technologies, Inc., Enfield, CT) (accurate to the nearest 0.001m) was used to measure the thickness in three locations on the film samples. The measurements were taken on both ends of the sample film and one in the middle for these analyses.

Transmission Rate Analyses

Water vapor transmission rates (WVTR) and oxygen transmission rates (OTR) of the films were measured in duplicate at 23ºC and 100% RH. Each film was tested using the Permatran-W® 3/31 (Mocon, Inc., Minneapolis, MN), according to ASTM F1249 for WVTR, and the Ox-Tran 2/20 Oxygen Permeability Tester (Mocon, Inc., Minneapolis, MN), according to ASTM D3985 for OTR. A preliminary study of five weeks was performed measuring the water activities and moisture contents of the chocolate health bar on weeks 0, 2 and 4. For the 10-week accelerated shelf life study, it was assumed that the transmission rates would not need to be measured on weeks 0, 2, 4, 6, 8 and 10 because there were minimal changes during the preliminary study. Therefore, during the 10-week accelerated storage period, transmission rate analyses were performed only on weeks 0, 5 and 10. For each packaging material, the sealed ends of two samples were cut off to remove the product, and then were cut using a 50-cm^2 template. Samples were

cleaned with soap and water to remove the residue from the film. The average thicknesses of the films were recorded. The bio-based film, PLA, was masked in order to prevent failures during testing for WVTR. The area tested of the masked PLA samples was 5.7645cm^2. No samples were masked for OTR. The samples were then labeled and mounted onto the devices (Permatran-W® 3/31 and Ox-Tran 2/20 Oxygen Permeability Tester). Nitrogen flow rates were adjusted to 10.0sccm for both WVTR and OTR. Oxygen flow rate was set at 20.0sccm for OTR. The samples were conditioned for four hours with nitrogen gas.

Mechanical Analyses

Tensile Analysis

Preparation for tensile testing involved removing the product from the pouch and washing the inside of the package to get rid of residue. One sample of each material was used to cut five 4-inch strips of each film in the cross-machine direction. A taut strip of film was placed between smooth-faced jaws of the Satec-Instron Model T10000 (Satec-Instron, Norwood, MA). Tensile tests were performed according to ASTM D882. Each sample was tested at a rate of 10 inches per minute until break. Tensile strength (MPa), percent break elongation, and the E-Modulus (MPa) were obtained using the BlueHill Materials Testing Software.

Seal-Peel Analysis

Preparation for seal-peel testing involved removing the chocolate health bars from the package and washing the inside of the package to get rid of residue. Using two packages of each package type (control, PLA and SPE), end seals and fin (back) seals were cut into five 1-inch strips. The ends of the film were placed between smooth-faced

jaws of the Satec-Instron Model T10000 (Satec-Instron, Norwood, MA), making sure there was no slack. Seal-peel tests were performed on the end and fin seals according to ASTM F88. Each sample was pulled at a rate of 10 inches per minute until a peel or failure occurred. Measurements of maximum loads per width (gf/25mm) were obtained using the BlueHill Materials Testing Software.

Statistical Analyses

All samples were analyzed on weeks 0, 2, 4, 6, 8 and 10 in triplicate for each treatment, except for sensory, tensile, seal-peel, water vapor transmission rate and oxygen transmission rate analyses. Tensile and seal-peel analyses were performed with five replicates for each treatment. WVTR and OTR analyses were performed on weeks 0, 5 and 10 in duplicate for each treatment. Statistical analyses were performed based on time (week) and material. The data was compared by Analysis of Variance (ANOVA) at a significance level of $\alpha = 0.05$. Significant differences of samples were determined by comparing each sample per week.

RESULTS AND DISCUSSION

The purpose of this study was to compare the shelf life of an intermediate moisture chocolate health bar in its current package (Met-OPP) to the shelf life of the same health bar in two different bio-based packages (PLA and SPE). This section provides the results of the instrumental and sensory analyses of the intermediate moisture chocolate health bars during the 10-week accelerated storage period. Also, this section provides the results of the mechanical analyses of the three different packaging materials during the 10-week accelerated storage period. Results are discussed for each analysis.

Water Activity Analysis

Table 1: Water activities of chocolate health bars stored in three different packaging materials

Week	Met-OPP	PLA	SPE
0	0.638 ±0.00	0.639 ±0.00	0.637 ±0.00
2	0.647 ±0.00	0.665 ±0.01	0.647 ±0.00
4	0.655 ±0.00	0.667 ±0.00	0.670 ±0.00
6	0.643 ±0.00	0.647 ±0.00	0.651 ±0.00
8	0.621 ±0.01	0.638 ±0.01	0.641 ±0.01
10	0.639 ±0.01	0.655 ±0.00	0.649 ±0.01

Table 1 shows the average water activities of the health bars in all of the packaging materials during the 10-week accelerated storage period. Statistical analysis, comparing the data by ANOVA at a significance level of 0.05, indicated that there were no significant differences in the water activities between the products in the control packaging material, compared to the products in the PLA and SPE packaging materials. Since there were no significant differences, Table 1 has no letters shown.

Table 1 shows that the average water activities throughout the accelerated storage period remained between 0.6 and 0.7. Referring to Figure 1 (Reaction rates in food as a

function of water activity), in this water activity range of 0.6 to 0.7, there is no microbial growth expected. A water activity of 0.6 is the critical point at which there is potential for microbial growth if the moisture content significantly increases (Leake, 2006). An increase in water activity, above 0.7, in the health bar would increase the chances of microbial growth.

Microbial Analyses

Aerobic Plate Counts (APC)

Table 2: Aerobic plate counts in chocolate health bars stored in three different packaging materials

	Week 0 (EAPC/mL)	Week 2 (EAPC/mL)	Week 4 (EAPC/mL)	Week 6 (EAPC/mL)	Week 8 (EAPC/mL)	Week 10 (EAPC/mL)
Met-OPP	< 25	< 25	< 25	< 25	< 25	< 25
PLA	< 25	< 25	< 25	< 25	< 25	< 25
SPE	< 25	< 25	< 25	< 25	< 25	< 25

Table 2 shows the results from the aerobic plate count analyses of the health bars during the 10-week accelerated storage period. The preferred counting range on a 3M aerobic count plate is between 25 - 250 colonies. Table 2 shows that throughout 10-week accelerated storage, total aerobic plate counts for all of the products in all three different packaging materials remained below 25 estimated aerobic plate counts (EAPC/mL). As mentioned under Table 1, these results relate to the water activity results stating that there was no microbial growth expected between water activities between 0.6 and 0.7. Bacteria growth begins after the water activity has reached 0.8.

Yeast and Mold Counts

Table 3: Yeast and mold in chocolate health bars stored in three different packaging materials

	Week 0 (CFU/mL)	**Week 2 (CFU/mL)**	**Week 4 (CFU/mL)**	**Week 6 (CFU/mL)**	**Week 8 (CFU/mL)**	**Week 10 (CFU/mL)**
Met-OPP	< 15	< 15	< 15	< 15	< 15	< 15
PLA	< 15	< 15	< 15	< 15	< 15	< 15
SPE	< 15	< 15	< 15	< 15	< 15	< 15

Table 3 shows the results from the yeast and mold plate count analyses of the health bars during the 10-week accelerated storage period. The preferred counting range on a 3M yeast and mold count plate is 15 - 150 colonies. Referring to Table 3, throughout accelerated storage, yeast and mold plate counts for all of the products in all three different packaging materials remained below 15 colony forming units (CFU/mL). As mentioned under Table 1, these results relate to the water activity results stating that there was no microbial growth expected between water activities of 0.6 and 0.7. Yeast and mold growth begins after the water activity of a product has reached 0.7.

According to the literature, intermediate moisture foods are shelf stable, meaning that they are microbiologically safe at room temperature. Their water activities are adjusted through drying processes and are made even more microbiologically safe by using preservatives. A larger increase in water activity, above 0.7, could have caused significant yeast and mold growth. However, the product formulation could prevent microbial growth. Ultimately, these results show that for this product, microbial growth can be controlled in conventional packaging materials and in these PLA and SPE bio-based packaging materials.

Moisture Content Analysis

Table 4: Percent moisture contents of chocolate health bars stored in three different packaging materials

Week	Met-OPP (%)	PLA (%)	SPE (%)
0	7.79[a] ±1.08	8.53[a] ±0.62	8.58[a] ±1.24
2	8.33[a] ±1.01	9.42[a] ±0.71	8.92[a] ±1.70
4	8.25[a] ±0.70	7.80[a] ±0.71	8.47[a] ±0.82
6	8.52[a] ±0.75	7.20[b] ±0.68	7.56[ab] ±0.81
8	7.19[a] ±0.48	7.94[a] ±0.48	8.00[a] ±0.71
10	7.15[a] ±0.77	7.51[a] ±0.31	7.17[a] ±0.79

(Within the weeks, the packaging materials with the same letter are not significantly different.)

Table 4 shows that the average moisture contents of the health bars in all of the packaging materials slightly decreased throughout the 10-week accelerated storage period. The standard deviations show that the moisture content data has more variability, which occurred because of more widely spread measurements to make up the averages. During weeks 0 – 4 and weeks 8 and 10, statistical analysis, comparing the data by ANOVA at a significance level of 0.05, for the moisture contents of each of the products contained in the different films showed no significant differences between the products in the control packaging material, compared to the products in the PLA and SPE packaging materials.

There was a significant difference during week 6, indicating that the moisture content of the product in the control package (8.52% moisture) was significantly greater than the moisture content of the product in the PLA package (7.20% moisture). This significant difference at week 6 may have been caused by how long the PLA samples were sitting out during evaluations. Samples were evaluated in the following order: Control, PLA and SPE. During week 6, the PLA product moisture content measurements

showed that a set of samples were significantly drier (5.6% - 6.8%) than the other two data sets (6.8% - 8.3% moisture). The drying out of the PLA samples may have occurred because the PLA sample was left outside of the package, while waiting for the previous sample to finish being analyzed. The SPE samples were also waiting, but were kept inside the package until they were ready for analysis. According to the literature, PLA has a higher moisture transmission rate than the control package. Moisture migration through the package may have caused moisture loss as well.

Over the 10-week accelerated storage period, the control product had a moisture loss of 0.64% the PLA product had a moisture loss of 1.02 % and the SPE product had the most moisture loss of 1.41%.

Texture Analysis

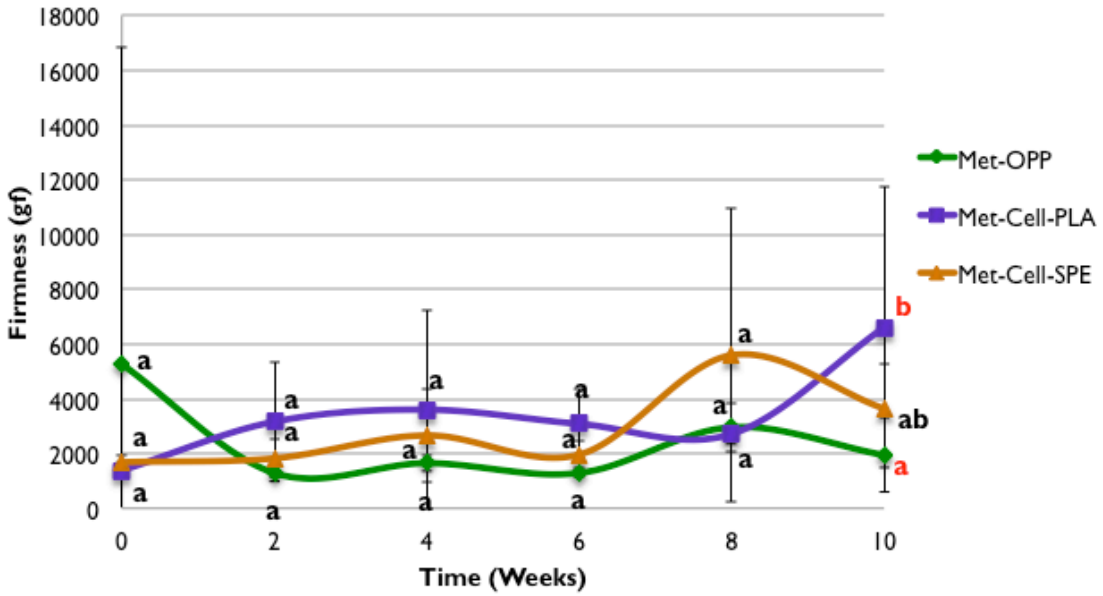


Figure 6: Firmness (gf) of chocolate health bars stored in three different packaging materials (Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 6 shows the standard deviation error bars with letters indicating significant differences. Some standard error bars are not seen due to the number scale. This applies to all line graphs and bar charts from this point forward.

Figure 6 shows the average firmness of the health bars in all of the packaging materials during the 10-week accelerated storage period. The average firmness of the product in the control package decreased over time for the first two weeks. The firmness of the products in the PLA and SPE packages remained relatively unchanged from weeks 0 – 6. Statistical analysis compared by ANOVA, at a significance level of 0.05, showed no significant differences between the products in the control packaging material, compared to the products in the PLA and SPE packaging materials during weeks 0 - 8. This graph shows large error bars, indicating a lot of variability between the samples. Outliers in the measurements caused the variability during testing. These outliers caused the mean of the data sets to increase, as seen in Figure 6, above. The following outliers were:

During week 0, the outlier for 1 control sample out of 3 (~36,049gf). The other control samples ranged from ~1,976gf – 1,991gf. The same happened for:

- Week 0 Control (~36,049gf; Other control samples = ~1,976gf – 1,991gf).
- Week 2 PLA (~8,284gf; Other PLA samples = ~3,074gf – 4,815gf)
- Week 4 PLA (~12,669gf; Other PLA samples = ~3,756gf – 5,247gf)
- Week 8 SPE (~19,597; Other SPE samples = ~3,022gf & 3771gf)
- Week 10 PLA (~10,689 - 15,128gf; Other PLA samples ~1,933gf – 4,670gf)

According to Figure 6, during week 10, a significant difference is seen between the texture of the product in the control package (~1945gf) and the texture of the product in the PLA package (~6614gf). The increase in firmness of the product in the PLA packaging material may have been because PLA is a poor moisture barrier, compared to the control, therefore, causing moisture loss (Johansson et al., 2012). In addition, OPP provides better moisture properties than non-oriented PP, which, according to the literature, still has better moisture barrier properties than PLA (Boa et al., 2006). The outliers during week 10 for the average PLA sample measurements may have also caused the significant difference from the average control sample measurements. Outliers may have occurred because the thickness (height) of the samples did not remain the same over time because the samples were stacked on top of each other in storage. Some samples became more compressed than others, causing the texture analyzer probe to feel a denser product during analysis. A denser product would increase the amount of force it takes to push through the health bar.

Sensory Analysis

Aroma

Table 5: Aroma of chocolate health bars stored in three different packaging materials

Week	Met-OPP (cm)	PLA (cm)	SPE (cm)
0	8.28 ±2.79	8.13 ±2.34	8.13 ±2.57
2	7.97 ±3.40	8.32 ±2.93	8.58 ±3.62
4	8.10 ±3.15	9.18 ±2.40	8.73 ±3.08
6	8.25 ±2.99	9.10 ±2.58	8.43 ±3.00
8	8.93 ±2.73	8.28 ±3.60	7.93 ±3.36
10	7.76 ±2.82	8.81 ±3.90	7.83 ±3.41

Aroma scale: 0cm = No Aroma; 15cm = Intense Aroma

Table 5 shows the average aroma intensities of the health bars in all of the packaging materials during the 10-week accelerated storage period. Aroma was rated on a scale of no aroma to intense aroma. Referring to Table 5, statistical analysis compared by ANOVA, at a significance level of 0.05, showed that there were no significant differences between the products contained in all three packaging materials during the 10-week accelerated storage. The product in the PLA package increased from 8.13 to 8.81 in aroma intensity, while the products in the control and SPE packages decreased from 8.28 to 7.26 and 8.13 to 7.83, respectively, over time. Panelists rated the aroma for all products in each film as mild during the 10-week accelerated storage period. In Table 5, since all measurements remained above 7.5, the products in all of the packaging materials remained acceptable to the panelists during the 10-week storage period. Results from Table 5 shows that the aroma sensory properties of this product will not significantly change in the control and the PLA and SPE packages during accelerated storage.

Flavor

Table 6: Flavor of chocolate health bars stored in three different packaging materials

Week	Met-OPP (cm)	PLA (cm)	SPE (cm)
0	8.76 ±2.85	7.51 ±3.62	7.74 ±3.34
2	7.22 ±3.10	8.35 ±3.93	7.81 ±3.84
4	8.45 ±3.48	8.30 ±3.27	9.04 ±3.15
6	7.60 ±3.82	8.72 ±3.30	7.69 ±3.15
8	7.94 ±4.32	8.48 ±3.49	8.14 ±3.40
10	8.06 ±3.12	7.27 ±4.21	7.25 ±3.16

Flavor scale: 0cm = Weak flavor; 15cm = Strong flavor

Table 6 shows that average flavor intensities of the health bars in all of the packaging materials during the 10-week accelerated storage period. Flavor intensity was rated on a scale of weak flavor to strong flavor. Referring to Table 6, statistical analysis compared by ANOVA, at a significance level of 0.05, of the flavor values over time showed no significant differences between the products contained in all three packaging materials. The strength of the flavor in the products did not change much over time for any of the products in each film. The flavor intensity in the control product decreased from 8.76 to 8.06. The flavor intensity of the PLA product decreased from 7.51 to 7.27. The flavor intensity of the SPE product decreased from 7.74 to 7.25. Table 6, therefore, shows indicates that the flavor sensory properties of the health bars will not significantly change in the conventional and the PLA and SPE packages during accelerated storage.

Texture

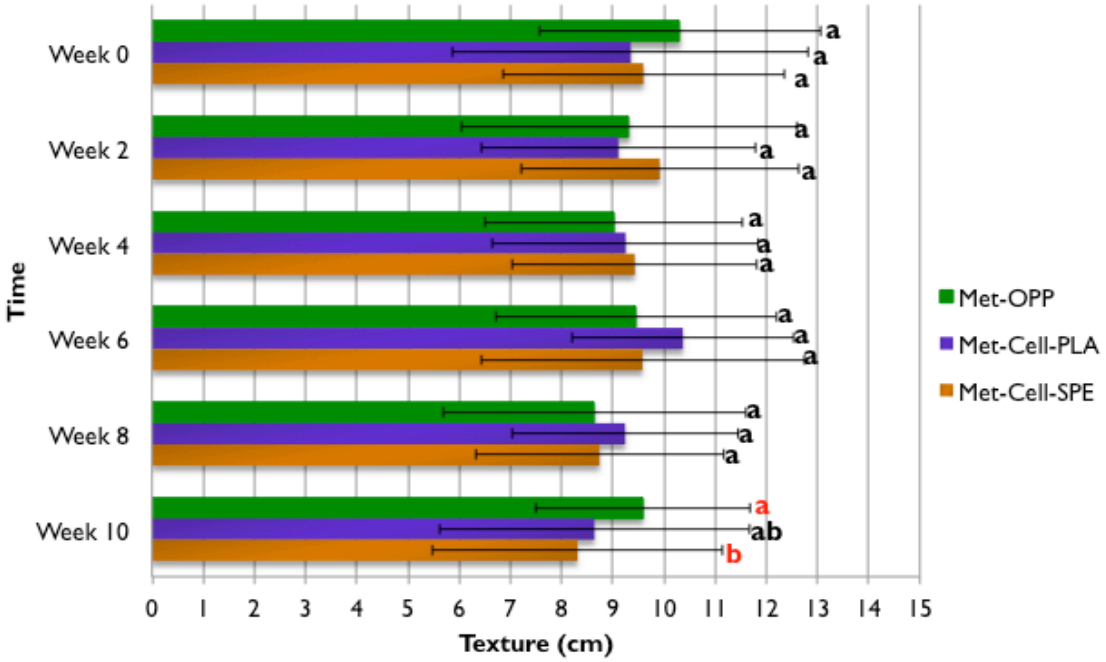


Figure 7: Texture (cm) of chocolate health bars stored in three different packaging materials
Texture scale: 0cm= Hard texture; 15cm = Chewy texture
(Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 7 shows the average texture intensities of the health bars in all of the packaging materials during the 10-week accelerated storage period. Texture intensity was rated on a scale of hard texture to chewy texture. The big error bars indicate a lot of variability. This variability may have been reduced if the fixed ends of the line scales on the computerized sensory ballot were moved in a half inch (Lawlwss & Heymann, 2010). Panelists evaluated the center of the samples. No measurements fell below 7.5, indicating that the texture of the products in all three packaging materials remained chewy throughout the 10-week accelerated storage period. Statistical analysis compared by ANOVA, at a significance level of 0.05, of the texture of the products contained in all three packaging materials indicated no significant differences during weeks 0 – 8. During week 10, the texture of the product in the control package was significantly more chewy (9.58cm) than the SPE product (8.31cm).

Referring to the moisture content results (Table 4) on week 10, for all products in all packaging materials, there was no significant difference in moisture loss. If the texture of the product in the SPE package was significantly more firm than the product in the control package, the percent moisture content of the SPE product should have been significantly lower during week 10. During week 6, for the moisture content analysis, the PLA product had more moisture loss than the control. But the panelists indicated in Figure 7 above that the PLA product was more chewy (10.37cm) than the control (9.45cm) on week 6. Therefore, relating both the texture analyzer results and the sensory texture results to the moisture content results, the texture analyzer detected the moisture loss in PLA at week 6, but the panelists did not.

Referring to the results of the instrumental analysis for texture (Figure 6) during week 10, the texture of the control product and the texture of the SPE product were not significantly different. The texture of the PLA product and the texture of the SPE product were not significantly different. However, Figure 7 shows that the SPE product is more firm than the control product on week 10. This means that the loss of moisture was noticed more through instrumental texture analysis for the PLA product than for the SPE product on week 10. The panelists noticed the loss of moisture more in the SPE product on week 10. However, both the texture analyzer and the panelists noticed a loss of moisture in the products in all three packaging materials by week 10, according to the following comments:

Comments on Week 0:

- "Has a good flavor and texture."
- "It is a bit chewy for my liking."

Comments on Week 10:

- "It was a bit dry, but overall had a nice chewy texture."
- "This seemed a little hard and stale, but the flavor was still good."
- "Very dry and bitter."

The panelists indicated the samples were dry and slightly more firm, but still ranked the samples as more chewy. These contradicting results may have been due to the central tendency error, where panelists tend to avoid extremes and confine their ratings to the middle of the scale (Meilgaard et al., 2007).

Figure 7 and the panelists' comments above show that the texture sensory properties of this product will become significantly less chewy by week 10 in the PLA

and SPE packages. The product in the control package will not vary significantly by week 10.

Degree of Liking

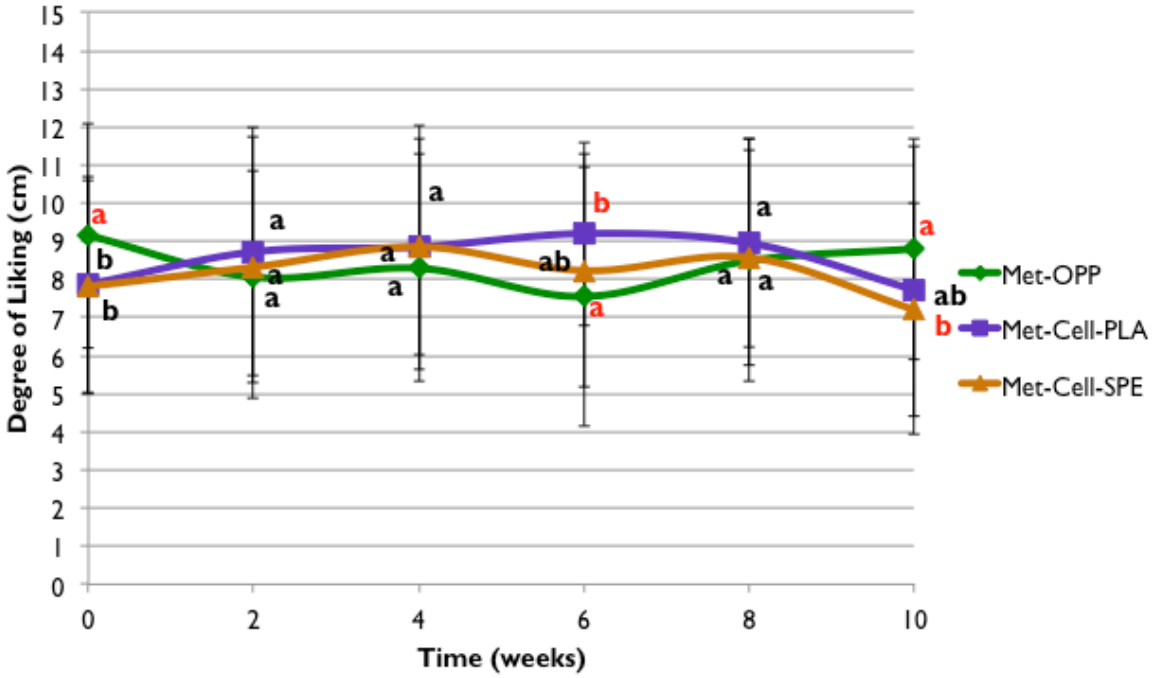


Figure 8: Degree of Liking (cm) of chocolate health bars stored in three different packaging materials
Degree of Liking scale: 0cm = Dislike extremely; 15cm = Like extremely
(Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 8 shows the average degree of liking intensities of the health bars in all of the packaging materials during the 10-week accelerated storage period. The degree of liking was rated from dislike extremely to like extrememly. Referring to Figure 8, statistical analysis compared by ANOVA, at a significance level of 0.05, of the degree of liking indicated no significant differences between the health bars packaged in all three packaging materials during weeks 2, 4 and 8. At week 0, the degree of liking of the product in the control package (9.15) is significantly more liked than products in the PLA (7.86) and SPE (7.81) packages. These results for week 0 were unexpected because the

health bar samples were all the same. The significant difference at week 0 may have occurred due to the panelists looking for a difference between the samples. However, all samples were randomized for each panelist during evaluations. According to the literature, panelists can become more experienced panelists because of repeated exposure to the food product and the same test (Bastian et al., 2014). Therefore, it is concluded that as the panelists became more experienced, they evaluated the product more effectively.

Referring to Figure 8, on week 6, the degree of liking of the health bars in the control package (7.55) was significantly less liked than the health bars in the PLA package (9.20). This significant difference may have been because of the significant increase on moisture in the product on week 6, compared to the PLA product, as seen in Table 4 for moisture content. The panelists' comments relate to the moisture gain in the control product, saying that the product felt chewy, but crumbled as soon as it reached the mouth. The crumbliness may have been due to the water interacting with the water binding ingredients in the food product. Those ingredients may have broken down because of the moisture gain. The PLA product was described by the panelists as dry, indicating the significant difference in moisture loss compared to the control product. In addition, since the health bars were removed from the control packages to be repacked in the bio-based packages, panelists could have detected a difference between the products at week 0. The control product was rated ~7.5, which is the rejection point.

Referring to Figure 8, on week 10, the degree of liking of the product in the control package was significantly more liked than the product in the SPE package. The panelists' comments indicated that the SPE products were not as tasty as the rest of the samples and stated being able to taste the "staleness" of the SPE product. The panelists

rated the SPE product 7.2, which is below the rejection line 7.5 on week 10.

It was expected that the PLA product liking would significantly decrease based on the panelists' comments. The contradicting results regarding the PLA product at week 10, suggests the central tendency error, where panelists tend to avoid extremes and confine their ratings to the middle of the scale (Meilgaard et al., 2007). The PLA product remained in the acceptable range for the degree of liking throughout the whole 10-week accelerated study. This means that even though the PLA product showed a lot of variability from the texture analyzer, and there was a significant loss of moisture at week 6 in the PLA product, the PLA product was still rated above 7.5 on the liking scale.

Ultimately, the results from Figure 8 show that the panelists' still found the health bars acceptable in all of the packaging materials by the end of the 10-week accelerated storage.

Transmission Rate Analyses

Water Vapor Transmission Rate (WVTR) Analysis

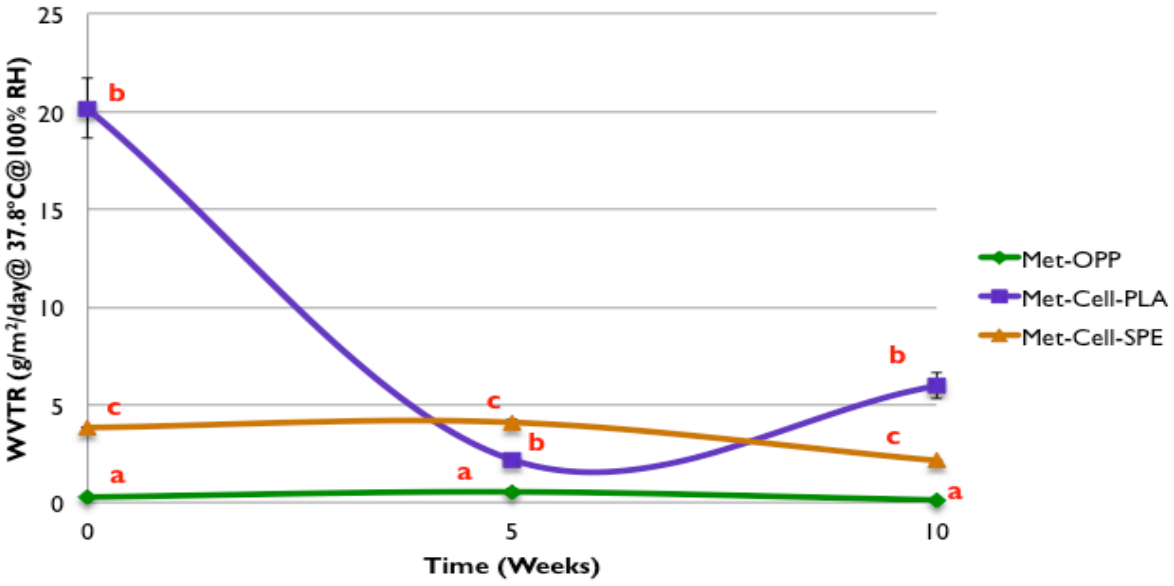


Figure 9: Water Vapor Transmission Rates (WVTR) (g*mil/m^2/day @37.8ºC@100%RH) of three different packaging materials stored over time. (Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 9 shows the average water vapor transmission rates of the control, PLA and SPE packaging materials during the 10-week accelerated storage period. PLA samples were masked. The control and the SPE materials were not masked. Referring to Figure 9, statistical analysis compared by ANOVA at a significance level of 0.05, indicated that there are significant differences for the transmission rates between all of the packaging materials for all of the weeks.

The WVTR for the control and SPE packages remained low (between 0 and 0.55 g/m^2/day @37.8ºC@100%RH, and between 2.00 and 5.00 g/m^2/day @37.8ºC@100%RH, respectively), during the 10-week accelerated storage period. This indicates that the control and SPE packages provided good moisture barriers. The PLA WVTR varied from 2 to 21 g/m^2/day @37.8ºC@100%RH, which indicated that moisture can travel through the PLA package easier. However, even though the PLA packages were poor moisture barriers, the health bars still remained acceptable to the panelists and remained microbiologically stable. The moisture barrier of the SPE material was expected to be better than the PLA material because polyethylene provides a good moisture barrier (Frey, 2009). The control, Met-OPP, is one of the best moisture barriers compared to other typical packaging materials used for intermediate moisture foods (Kilcast & Subramaniam, 2000).

Oxygen Transmission Rate (OTR) Analysis

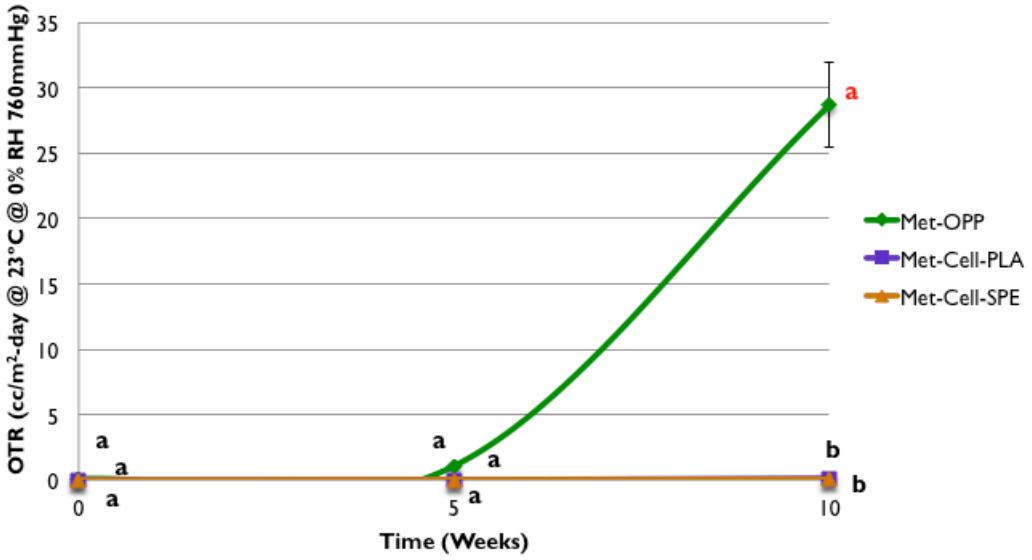


Figure 10: Oxygen Transmission Rates (OTR) (cc*mil/m^2/day @23ºC@0%RH 760mmHg) of three different packaging materials stored over time
(Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 10 shows the average oxygen transmission rates of the control, PLA and SPE packaging materials during the 10-week accelerated storage period. Referring to Figure 10, statistical analysis compared by ANOVA at a significance level of 0.05, indicated that there were no significant differences between transmission rates of all of the materials for weeks 0 and 5. For week 10, the control material OTR (28.6 cc*mil/m^2/day @23ºC@0%RH 760mmHg) is significantly greater than the PLA and SPE materials OTR (~0.13 and ~0.05 cc*mil/m^2/day @23ºC@0%RH 760mmHg, respectively). OTR for the PLA and SPE materials remained consistently low throughout the entire study. The OTR of the control film slowly increased from weeks 0 – 5, and then greatly increased from week 5 – 10.

The OTR of the PLA and SPE packages remained low (between 0 and 0.15 cc*mil/m^2/day @23ºC@0%RH 760mmHg and 0 and 0.05, respectively), which indicates

that the PLA and SPE packages provided good oxygen barriers. The control package's OTR started low at ~0.1 cc*mil/m^2/day @23ºC@0%RH 760mmHg and increased significantly to ~ 28.6 cc*mil/m^2/day @23ºC@0%RH 760mmHg by week 10. The OTR value of 28.6 OTR for the control material is an outlier in the data that caused the variability to increase for the control at week 10. Typically, Met-OPP films provide good oxygen barriers (Chowdhury & Kolgaonkar, 2014), so this increase was unexpected. Panelists did not indicate that the health bars became rancid on week 10. Therefore, this increase in oxygen may have been due to improper mounting of the film onto the machine, causing an increased oxygen flow. Distribution of the control packages may have caused very small pinholes in the package, causing the material to have an increased OTR by the end of the shelf life study.

Mechanical Analyses

Tensile Strength at Break

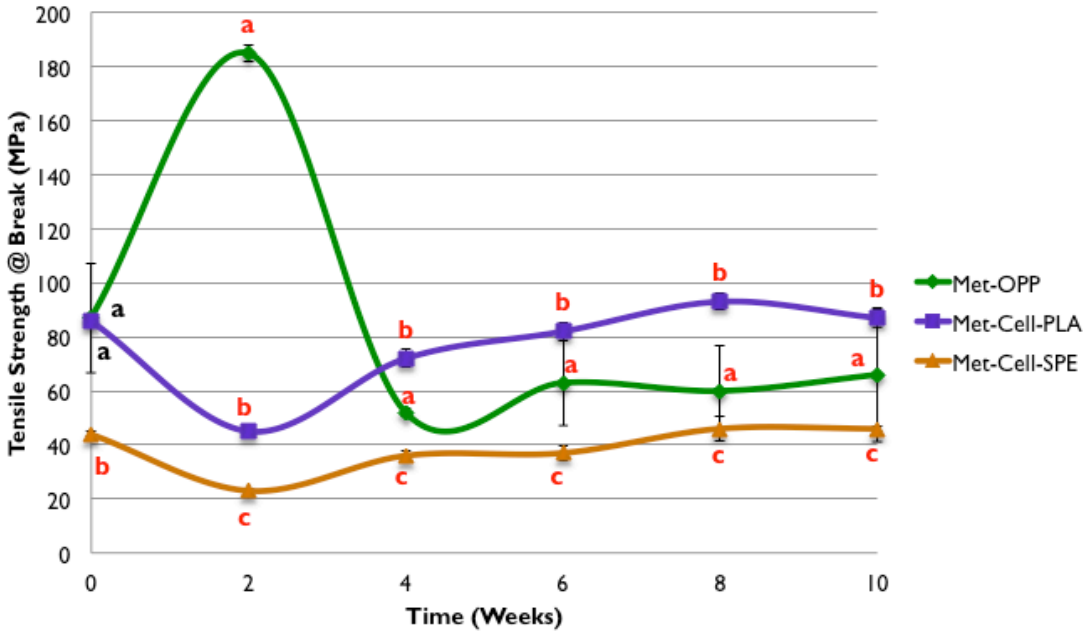


Figure 11: Tensile strength at break (MPa) of three different packaging materials stored over time (Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 11 shows the average tensile strengths at break of the control, PLA and SPE packaging materials during the 10-week accelerated storage period. During testing of the control materials, some samples slipped through the smooth-faced jaws on the Satec. Therefore, different control packages that had been sitting out with the initial control samples were used for analyzing the tensile strength data. The use of the different packages causes more variability in the data measurements for weeks 0, 6, 8 and10. For the other average tensile strength data with very small error bars, those measurements were taken from the same pouch.

Referring to Figure 11, the tensile strength at break of the PLA and SPE packaging materials initially decreased from 86 to 46MPa and 44 to 23MPa, respectively, at week 2. The tensile strength at break of the control packaging material greatly increased at week 2. After week 2, the control material was not as strong by week 10, as it was at week 0. The PLA and SPE materials ended up with tensile strengths at break that were about the same by week 10 as the tensile strengths at break at week 0. Referring to Figure 11, statistical analysis compared by ANOVA, at a significance level of 0.05, indicated for week 0, that the tensile strength of the SPE material was significantly less than the control and PLA materials.

For weeks 2 – 10, the control material was significantly greater than SPE and less than PLA; PLA was significantly greater than the control and SPE; and the SPE was significantly less than the control and PLA.

During testing for week0 and weeks 4 – 10, as the control materials stretched, they delaminated and then broke. During week 2, control samples did not delaminate, but they stretched a lot more before breaking. This is what caused the tensile strength to

significantly increase. The same package was used to record the measurements for the average tensile strength. If another sample control package was used to record the data, the data for week 2 may have been between 20 – 40 MPa, instead of around 180 MPa. During testing of the PLA and SPE materials for all of the weeks, the materials broke without delamination and no significant stretching.

The PLA material showed a higher tensile strength than the control and SPE materials, indicating the PLA material is tougher. The SPE material was softer than the control and PLA materials; therefore, it deformed easily and had a lower tensile strength. Throughout the 10-week accelerated storage period, the PLA and SPE materials maintained their average tensile strengths at break.

End Seal-Peel Analysis

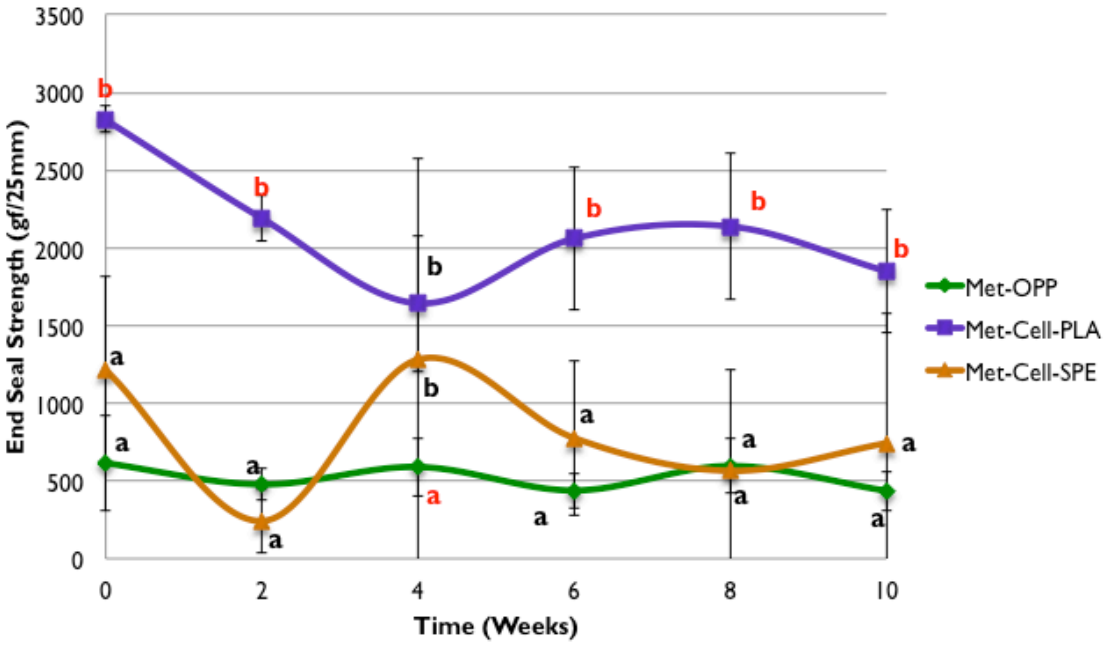


Figure 12: End seal strength max loads (gf/25mm) of three different packaging materials stored over time **(**Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 12 shows the average end seal strengths of the control, PLA and SPE packaging materials during the 10-week accelerated storage period. The data shows the maximum force it took to separate the seals. The large error bars indicate high variability, which occurred because different packages were used for most of the end seal peel strength measurements. Referring to Figure 12, statistical analysis compared by ANOVA, at a significance level of 0.05, for weeks 0, 2 and weeks 6 - 10 showed that the end seal strength for the PLA packaging material was significantly (1849 gf/25mm – 2827 gf/25mm) than the control (436 – 612 gf/25mm) and SPE materials (565 – 1282 gf/25mm). During week 4, the control material was significantly weaker (590 gf/25mm) was significantly weaker than the PLA (1643 gf/25mm) and SPE (1282 gf/25mm) materials. The control end seal strength stayed about the same throughout the accelerated study.

During the end seal peel testing of all of the packaging materials, the control material's end seal peeled cohesively without delamination, or breaks of the film throughout the entire study. The consistency in the peels of the end seals of the control material can be a reason for the consistency shown on the graph above. During testing, throughout the study, the PLA packaging materials either both delaminated without peeling cohesively and stretched until the film broke at the seal, or the PLA material just broke at the seal without delamination, or being stretched. The stretching of the materials is what caused the max load value to be higher than the control and SPE materials.

During testing of the SPE films, in weeks 0, 2 and weeks 6 - 10, almost all of the SPE materials peeled cohesively, while in week 4, the SPE materials delaminated and stretched, instead of peeled, causing the max load value to increase. These modes of

failure of the PLA and SPE materials could also mean that the seals were too strong to peel.

Fin Seal-Peel Analysis

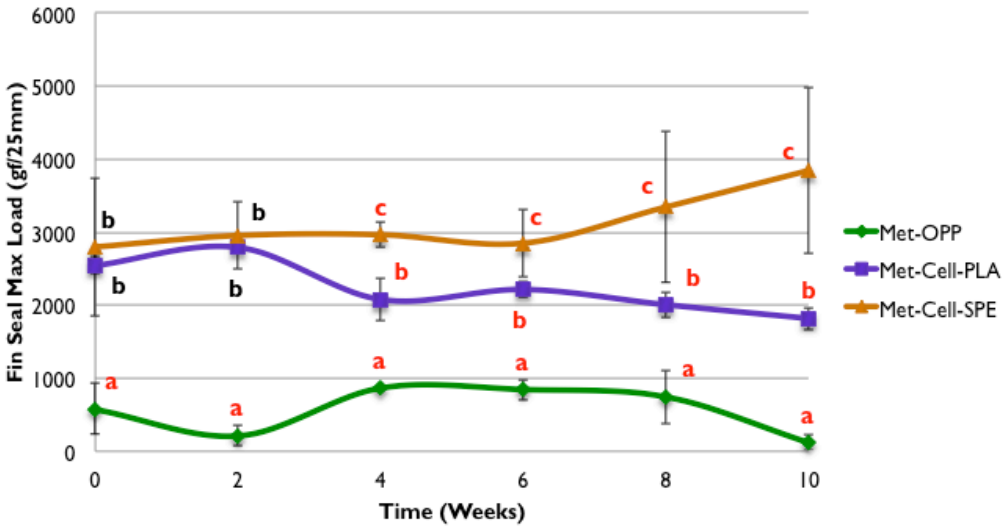


Figure 13: Fin seal strength max loads (gf/25mm) of three different packaging materials stored over time
(Within the weeks, the packaging materials with the same letter are not significantly different.)

Figure 13 shows the average fin seal strengths of the control, PLA and SPE packaging materials during the 10-week accelerated storage period. According to Figure 13, statistical analysis compared by ANOVA at a significance level of 0.05, indicated that for weeks 0 and 2 the fin seal strength for the control material was significantly weaker (582 & 211 gf/25mm) than the PLA (436 – 612 gf/25mm) and SPE materials (565 – 1282 gf/25mm). During weeks 4 – 10, each film was significantly different.

- Control = (117 - 866 gf/25mm)
- PLA = (1819 - 2216 gf/25mm)
- SPE = (2850 - 3846 gf/25mm)

Overall, the control was significantly weaker than PLA and SPE; PLA was significantly less than SPE and significantly greater than the control.

During the fin seal peel testing of all of the packaging materials, the control material's fin seal peeled cohesively without delamination, or breaks of the film throughout the entire study. During weeks 0 - 6, the PLA and SPE packaging materials both delaminated without peeling cohesively and stretched until the film broke at the seal. During weeks 8 and 10, the PLA material just broke at the seal without delamination, or being stretched. The stretching of the materials is what caused the max load values of the PLA and SPE materials to be higher than the control material. These modes of failure of the PLA and SPE materials could also mean that the seals were too strong to peel.

Comparing the end seal peel strengths to the fin seal peel strengths, the fin seal strengths for all packaging materials were stronger than the end seal strengths over the 10-week storage period.

CONCLUSIONS

The purpose of this accelerated shelf life study was to compare the shelf life of an intermediate moisture chocolate health bar in its current package (Met-OPP) to the shelf life of the same health bar in two different bio-based packages (PLA and SPE).

The chocolate health bars in the PLA package remained microbiologically safe to consume by the end of product shelf life by maintaining water activities between 0.6 and 0.7. This was expected, as intermediate moisture foods are formulated to prevent microbial growth (Barbosa-Cánovas et al., 2003). The formulation of the chocolate health bar included syrups, acacia gum and soy lecithin, which are ingredients that bind water and therefore help keep the product in tact when in high moisture conditions (International Food Informational Service, 2009; Functionality of Soy Ingredients, 2010; Mitchell, 2009).

The moisture contents of the products showed significant differences only in week 6, where the product contained in the PLA was significantly drier than the control product. The sensory properties—aroma, flavor, texture, and degree of liking—of the chocolate health bars in all of the packaging materials on a 15-cm unstructured scale were acceptable throughout the 10-week accelerated study.

The tensile strength of the PLA package became tougher than the control package during the accelerated storage period after week 4. This indicates that the PLA package is stronger than the control package. PLA alone is brittle, but when laminated with the Met-Cell, it became a stronger material than the control and SPE packages. The variation in end and fin seal-peel strengths for the PLA and SPE materials compared to the control seal-peel strengths occurred because the bio-based seals were hand-made, while the

control seals were machine-made. The control fin and end seals cohesively peeled each week, showing minimal variation over the 10-week accelerated storage. The PLA and SPE fin and end seals did not cohesively peel each week, but delaminated, or broke at the seal when being pulled. These modes of failures indicated that the fin and end seals for the bio-based materials were too strong to peel.

Based on the results from the WVTR, the control and SPE packages provided good moisture barriers. The PLA package WVTR results indicated that moisture could travel through the PLA package easier. Even though the PLA package had a poor moisture barrier, the chocolate health bar still remained acceptable to consumers and microbiologically safe.

Based on the OTR results, the PLA and SPE packages provided good oxygen barriers. The control package provided a good oxygen barrier until the end of the 10-week accelerated study, which was unexpected. The increase in oxygen at week 10 may have been cause by distribution or small creases in the film when mounted to the machine. Increased oxygen in the product would have caused oxidation, which leads to product rancidity. But the panelists did not indicate that the control samples became rancid at week 10.

Based on the results from the 10-week accelerated storage period, it is concluded that the PLA package would compare best to the manufacture's film. The water activities and the moisture contents of the health bars in the PLA packages kept them microbiologically safe. The sensory properties were acceptable to the panelists by the end of the 10-week accelerated storage period. The PLA packaging material maintained good tensile strength and good seal strength. It provides a good oxygen barrier. Even though

the moisture barrier was poor, the health bars still remained acceptable throughout the 10-week study. PLA's renewable source, modified cornstarch, is known to be more readily available than other bio-based materials sources. Since PLA is a more commercialized material, it is possible that is has the lowest cost of all of the bio-based materials and it can be processed on standard film lines with minimal modifications. PLA could be a bio-based alternative for these intermediate moisture chocolate health bars.

FUTURE RESEARCH RECOMMENDATIONS

During the sensory analyses, there was an inconsistency of the number of panelists that participated each week, therefore, leading to missing data and incomplete ballots. It is recommended in future research to use trained panelists and to check the sensory ballots before panelists are dismissed. This would allow for more valid statistical data.

In this study, an unstructured scale was used to evaluate the shelf life samples. This scale left room for unanswered questions during the result analysis. For instance, if the panelists were able to see the neutral point on the scale, the results may have been different.

Future research should look into laminating oriented PLA and oriented Bio-PE to metallized bio-based laminate films. Orienting bio-based films will most likely improve the moisture barrier and mechanical properties, which will allow the product to last longer, thus, extending the shelf life.

APPENDICES

Appendix A: Abbreviations

Full Name	Abbreviations
Aerobic Plate Count	APC
Agriculture, Food, and Rural Development	AFRD
Analysis of Variance	ANOVA
Biaxially Oriented Polypropylene	BOPP
Bio-Polyethylene	Bio-PE
Buffered Peptone Water	BPW
Centimeter	cm
Colony Forming Units per milliliter	CFU/mL
Estimated Aerobic Plate Count per milliliter	EAPC/mL
Grams force per 25 square meters	gf/25mm
Grams per square cemtimeter	g/cm^2
Low-Density Polyethylene	LDPE
Megapascals	MPa
Metallized Cellophane	Met-Cell
Metallized Oriented Polypropylene	Met-OPP
Modified Atmosphere Packaging	MAP
Modulus of Elasticity	E-Modulus
New Zealand Food Safety Authority	NZFSA
Newtons per square millimeter	N/mm^2
Oriented Polylactic Acid	OPLA
Oriented Polypropylene	OPP
Oriented Polystyrene	OPS
Oxygen Transmission Rate	OTR
Polyethylene	PE
Polylactic Acid	PLA
Polypropylene	PP
Pounds Per Square Inch	psi
Relative Humidity	RH
Sugarcane Polyethylene	SPE
U. S. Environmental Protection Agency	EPA
U. S. Food and Drug Administration	FDA
United States Department of Agriculture	USDA
Water Activity	A_w
Water Vapor Transmission Rate	WVTR

Appendix B: Product Analyses Data Tables

Texture Analysis Results (gf)

Week	Control (gf)	PLA (gf)	SPE (gf)
0	5274.01[a] ±11551.43	1382.04[a] ±273.71	1704.15[a] ±229.61
2	1290.79[a] ±302.45	3167.46[a] ±2177.09	1828.33[a] ±708.68
4	1650.23[a] ±286.74	3606.74[a] ±3630.33	2665.61[a] ±1705.45
6	1293.23[a] ±135.51	3088.08[a] ±1255.60	1964.75[a] ±504.94
8	2959.36[a] ±897.32	2716.45[a] ±364.68	5598.02[a] ±5358.38
10	1945.63[a] ±1343.70	6614.22[b] ±5123.73	3642.34[ab] ±1661.08

(Within the weeks, the packaging materials with the same letter are not significantly different.)

Sensory Analysis: Texture Results (cm)

Week	Control (cm)	PLA (cm)	SPE (cm)
0	10.31[a] ±2.75	9.35 ±3.48[a]	9.60 ±2.75[a]
2	9.32[a] ±3.28	9.11 ±2.68[a]	9.92 ±2.71[a]
4	9.01[a] ±2.52	9.25 ±2.59[a]	9.43 ±2.38[a]
6	9.45[a] ±2.75	10.37[a] ±2.16	9.58 ±3.16[a]
8	8.64[a] ±2.96	9.24[a] ±2.21	8.74 ±2.43[a]
10	9.58[a] ±2.10	8.64[ab] ±3.03	8.31 ±2.83[b]

Texture scale: 0cm = Hard Texture; 15cm = Chewy Texture (Within the weeks, the packaging materials with the same letter are not significantly different.)

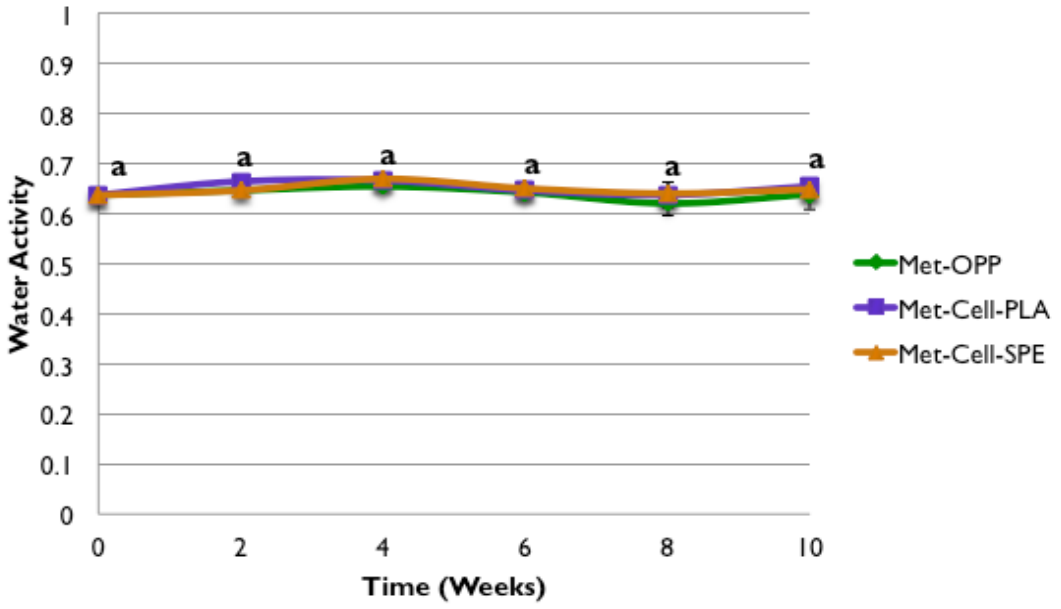
Sensory Analysis: Degree of Liking Results (cm)

Week	Control (cm)	PLA (cm)	SPE (cm)
0	9.15[a] ±2.95	7.86[b] ±2.85	7.81[b] ±2.81
2	8.06[a] ±2.77	8.72[a] ±3.26	8.32[a] ±3.44
4	8.29[a] ±2.98	8.85[a] ±2.83	8.85[a] ±3.21
6	7.55[a] ±3.40	9.20[b] ±2.41	8.23[ab] ±3.07
8	8.49[a] ±3.18	8.95[a] ±2.74	8.57[a] ±2.82
10	8.80[a] ±2.90	7.72[ab] ±3.79	7.20[b] ±2.79

Degree of Liking scale: 0cm = Dislike extremely; 15cm = Like extremely (Within the weeks, the packaging materials with the same letter are not significantly different.)

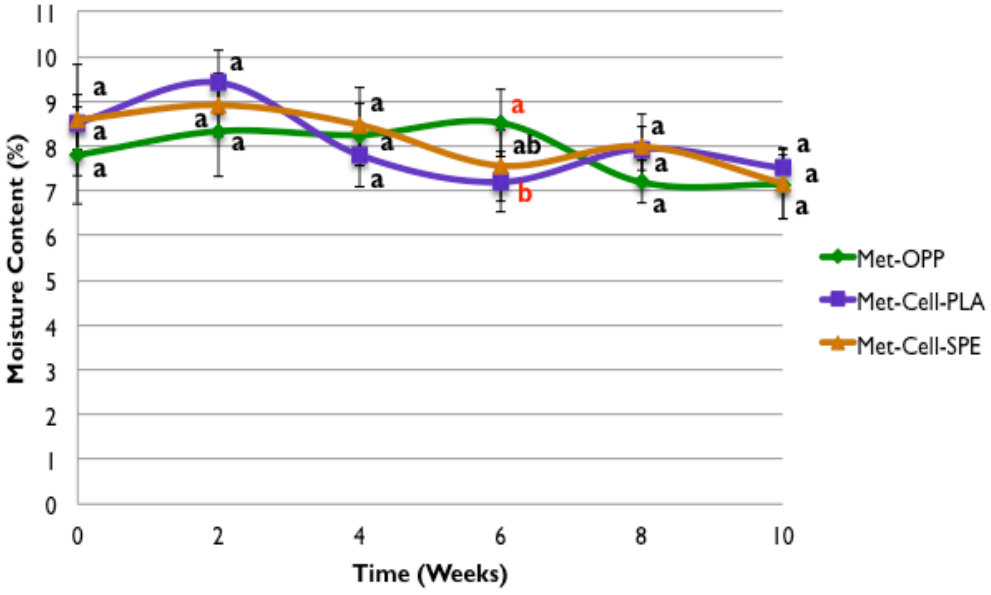
Appendix C: Product Analyses Data Figures

Water Activity Analysis Results



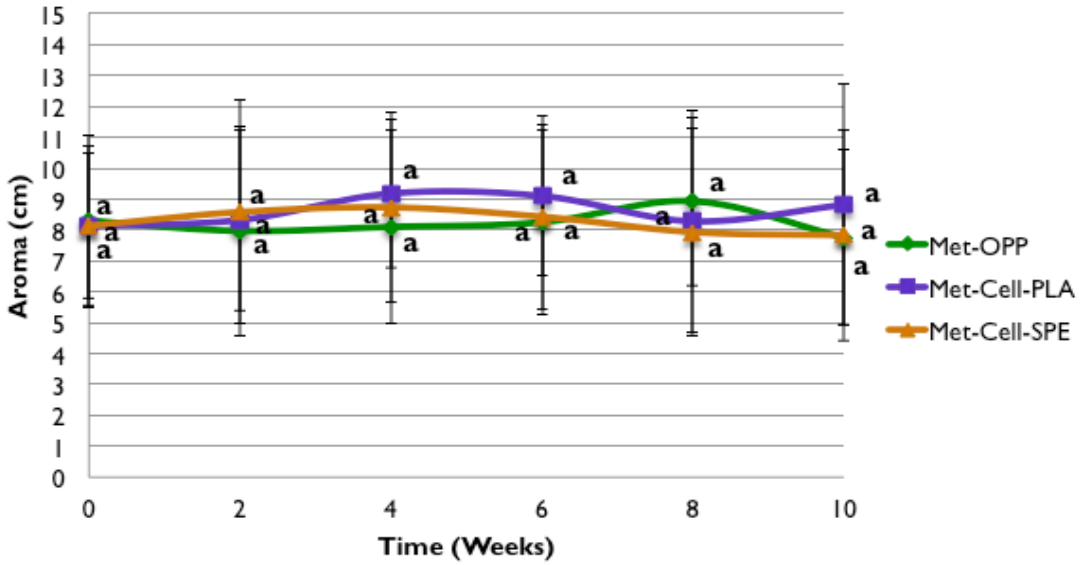
(Within the weeks, the packaging materials with the same letter are not significantly different.)

Moisture Content Analysis Results (%)



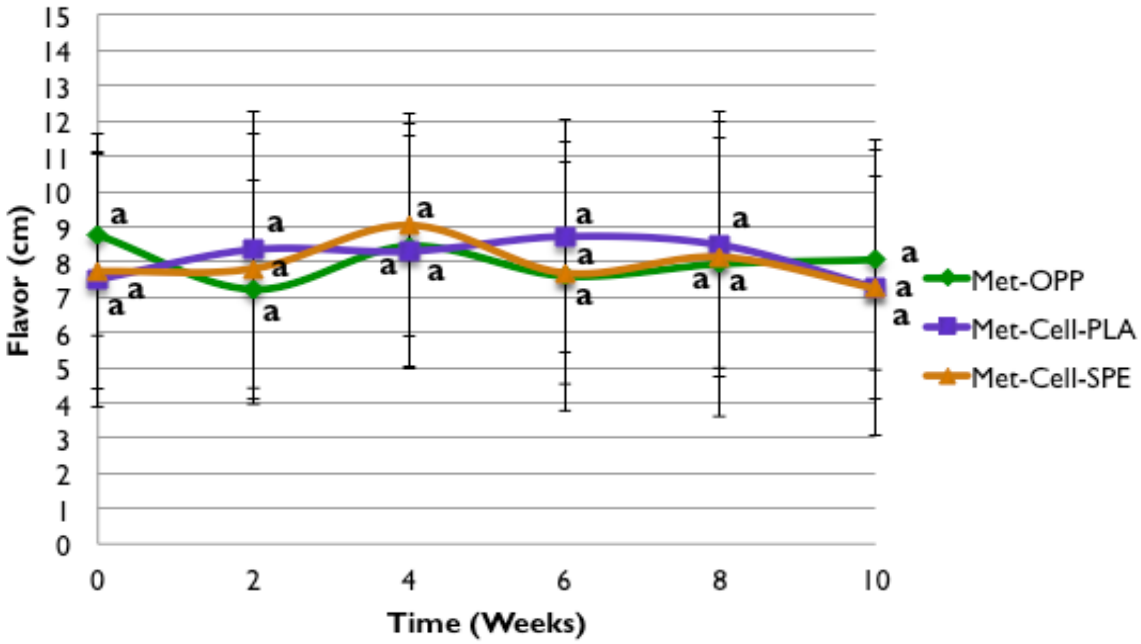
(Within the weeks, the packaging materials with the same letter are not significantly different.)

Sensory Analysis: Aroma Results (cm)



(Within the weeks, the packaging materials with the same letter are not significantly different.)

Sensory Analysis: Flavor Results (cm)



(Within the weeks, the packaging materials with the same letter are not significantly different.)

Appendix D: Film Analyses Data Tables

Tensile Strength at Break Analysis Results (MPa)

Week	Met-OPP (MPa)	PLA (MPa)	SPE (MPa)
0	87[a] ±20.29	86[a] ±1.64	44[b] ±0.84
2	185[a] ±3.11	45[b] ±0.89	23[c] ±0.89
4	52[a] ±1.48	72[b] ±3.44	36[c] ±2.05
6	63[a] ±15.80	82[b] ±3.21	37[c] ±2.68
8	60[a] ±16.92	93[b] ±3.03	46[c] ±4.56
10	66[a] ±24.83	87[b] ±3.39	46[c] ±0.84

(Within the weeks, the packaging materials with the same letter are not significantly different

End Seal-Peel Analysis Results (gf/25mm)

Week	Control	PLA	SPE
0	612[a] ±308.30	2827[b] ±86.48	1220[a] ±599.57
2	478[a] ±105.81	2190[b] ±145.23	242[a] ±205.43
4	590[a] ±186.65	1643[b] ±438.59	1282[b] ±1298.27
6	439[a] ±112.97	2060[b] ±458.30	776[a] ±496.29
8	596[a] ±175.19	2138[b] ±474.62	565[a] ±654.59
10	436[a] ±126.91	1849[b] ±394.18	743[a] ±840.64

(Within the weeks, the packaging materials with the same letter are not significantly different.)

Fin Seal-Peel Analysis Results (gf/25mm)

Week	Control	PLA	SPE
0	582[a] ±345.49	2550[b] ±114.00	2798[b] ±944.20
2	211[a] ±136.42	2791[b] ±51.03	2955[b] ±454.95
4	866[a] ±30.09	2079[b] ±287.43	2966[c] ±171.17
6	846[a] ±142.05	2216[b] ±103.95	2850[c] ±465.29
8	744[a] ±363.50	2007[b] ±172.64	3346[c] ±1032.27
10	117[a] ±103.68	1819[b] ±152.83	3846[c] ±1143.00

(Within the weeks, the packaging materials with the same letter are not significantly different.)

Water Vapor Transmission Rate Analysis Results ($g/m^2/day$ @37.8ºC@100%RH)

Week	Met-OPP	PLA	SPE
0	0.2920^{a} ±0.04	20.1795^{b} ±1.55	3.8453^{c} ±0.02
5	0.5415^{a} ±0.12	2.1852^{b} ±0.17	4.0968^{c} ±0.19
10	0.1244^{a} ±0.01	5.9571^{b} ±0.67	2.1437^{c} ±0.04

(Within the weeks, the packaging materials with the same letter are not significantly different.)

Oxygen Transmission Rate Analysis Results ($cc/m^2/day$ @23ºC@0%RH 760mmHg)

Week	Met-OPP	PLA	SPE
0	0.1056^{a} ±0.01	0.0009^{a} ±0.00	0.0011^{a} ±0.00
5	1.0687^{a} ±0.14	0.0180^{a} ±0.01	0.0210^{b} ±0.00
10	28.6872^{a} ±3.25	0.1320^{a} ±0.01	0.0460^{b} ±0.02

(Within the weeks, the packaging materials with the same letter are not significantly different.)

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