Shelf life analysis of cereal products processed with and without BHT

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Samantha D. Baumann

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Joanne Slavin

August 2020

Acknowledgments

I am so very thankful for the abundance of guidance and help that I have received from numerous individuals during this project. Without them, none of this would have ever been possible.

First and foremost, I would like to thank Dr. Joanne Slavin for encouraging and guiding me throughout my study, especially with the added challenge of being a non-traditional student with a limited schedule and a short timeline.

Next, I would like to thank my supervisor, Kevin Meister, for standing behind me in this pursuit and allowing me this opportunity to grow and develop my career, especially as it relates to travel and scheduling restrictions. Additionally, thanks so much to my amazing ICE team for picking up my slack and supporting my crazy schedule.

Thank you to the rest of my Post colleagues for their abundance of knowledge, advice, and laboratory support throughout my project. Shout out to Joel Matasovsky and Kellie Rausch for explaining business needs and the insight needed to organize my experimental design. Thanks also to James Fulton and Jim Waksmonski for talking statistics with me and showing me how to analyze sensory data. Special thanks to Grace Nelson for helping me coat cereal, allowing me to get product into storage quickly.

I also want to thank Post Consumer Brands for making this journey an affordable and realistic option for me. Your funding has allowed me to give all that I can to this project.

Finally, I want to thank my friends and family who have given me nothing but love and support throughout this journey. Thank you Dad, for believing in my abilities and instilling confidence in me during times of doubt. Thank you Mom, for always being proud of my accomplishments and giving me something to work for. Special thank you to Rob, for grounding me during stressful times, looking past my stress-induced moodiness, and for reminding me to enjoy life by giving me the motivation to make room for fun.

Thank you, thank you, thank you!

Dedication

This thesis is dedicated to Doug Hahn. You've always been a mentor to me in my pursuit of Food Science and have given to me what feels like wisdom well beyond my years.

"All you can do is your best, and if you've done your best, then that is all you can do."
- Doug Hahn

Abstract

The use of butylated hydroxytoluene (BHT) has been used in packaging material of consumer packaged goods for decades. Incorporating antioxidants in ready-to-eat cereal products can inhibit oxidation and extend product shelf life. Interest in natural antioxidants has developed over the past few years due to potential risks to consumer health. Despite these risks, BHT remains on the generally recognized as safe (GRAS) list for now, and is a proven way to achieve the desired quality that consumers expect.

The objective of this study was to understand the shelf life reliability of BHT in in four cereal products via application of BHT in two cereal components: the canola oil and the packaging film material. Additionally, this study explored the effects of BHT in cereals prepared using different formulae as well as different processing technology.

Loose sensory testing was performed on four different cereal products with a focus on descriptors that are indicative of lipid oxidation such as cereal rancidity, off flavor/aroma, and flavor intensity. Analysis of variance and t-test statistical analyses were used to determine the significance of sensory data.

BHT was found to affect different cereal products differently. Overall, the use of BHT in packaging material significantly improved the shelf life of breakfast cereal, while the use of BHT in canola oil showed little benefit to product shelf life. The results of this study

will provide a baseline for companies looking to reduce or remove BHT from their cereal products.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Roughly half of the American population regularly consumes breakfast (Lee, Moskowitz, Lee, 2007). Of these regular consumers, breakfast cereal is the number one food of choice for breakfast (Lee et al., 2007). About half of breakfast consumers are “health conscious,” looking for a cereal that consists of high fiber multigrain ingredients, is low in fat and sugar, and is free of artificial colors and preservatives (Lee et al., 2007; Asioli et al., 2017; Nachay, 2017).

Health conscious consumers are motivated to buy healthy breakfast cereals so long as they taste good, but are not willing to pay more than $2.49 for a typical 14 oz box of cereal (Lee et al., 2007). Natural antioxidant ingredients have been shown to be comparable to traditional synthetic antioxidants like butylated hydroxytoluene (BHT) (Onyeneho & Hettiarachchy, 1992; Pryor et al., 1993; Camire & Dougherty, 1998; Sebranek, Sewalt, Robbins, Houser, 2005; Paradiso, Summo, Trani, Caponio, 2007; Estévez, Ramírez, Ventanas, Cava, 2007), but generally add a disapproving cost. While the ingredient cost itself may not be significantly higher, special equipment may be needed for proper application of natural alternatives which may require a hefty capital investment.

Thus, the current study aimed to determine the shelf life reliability of BHT in four cereal products supplied by a major cereal manufacturer by means of utilizing the antioxidant via two cereal components: the canola oil ingredient and the liner packaging material.

Additionally, this study explored the effects of BHT in cereal prepared using different formulae as well as different processing technology. The results of this study will provide a baseline for cereal companies looking to reduce or remove BHT from their cereal products.

Chapter 2: Literature Review

2.1 Breakfast cereal consumption and health

Eating breakfast regularly has been associated with a favorable health status (Smith, 2003; Lee & Lee, 2007). In fact, regular breakfast consumption has been found to increase intake of essential nutrients such as calcium, iron, fiber, and vitamins A, C, and folate (Tietyen & Fleming, 1995; Barton et al., 2005). Greater nutrient intake may instigate healthier eating patterns throughout the day. For those who regularly consume breakfast, ready-to-eat breakfast cereal is the number one food choice (Lee et al., 2007).

It is widely accepted that regularly consuming whole grains can have numerous health benefits. Whole grains are those that contain all parts of the grain: the endosperm, germ, and bran (Slavin, 2010). Specifically, the bran component of cereal grains is known to be an excellent source of fiber (Kamran, Saleem, Umer, 2008). Dietary fiber is an important part of a healthy diet and cereal is known to be a high-fiber food. Ready-to-eat breakfast cereals were actually one of the first deliberately conceived “health foods” as part of the trend toward a whole grain diet (Lee et al., 2007). Fiber consumption is strongly linked to digestive health, heart health, and weight management.

2.1.1 Digestive health

Eating a diet that is high in fiber has been linked to improved digestive health, bringing a reduced risk of constipation, hemorrhoids, diverticulitis, and colon cancer (Burkitt, 1971;

Salvin, 2010). Cereal grains contain significant amounts of fiber, making them suitable high-fiber foods to promote gut health. The link between fiber intake and colorectal cancer, or overall gut health for that matter, was first studied by Denis Burkitt in the 1970s (Burkitt, 1971). Burkitt's study investigated the cause of bowel cancer and other non-infective diseases of the bowel (Burkitt, 1971). He noticed that these illnesses had a strong association with a diet common to areas of the world with significant economic development known as a refined diet, and hypothesized that the removal of dietary fiber may be a causative factor (Burkitt, 1971). Additionally, Burkitt hypothesized that reduced fiber consumption due to a refined diet, and possibly in combination with other dietary factors, was responsible for changes in bacterial flora of the colon and thus suggested that carcinogens were produced in the colon by action of the abnormal flora over time (Burkitt, 1971). Burkitt's work established a relationship between refined grains and bowel-related diseases (Burkitt, 1971).

Much has been discovered since we first began studying the effects of whole grains on gut health. In addition to fiber, whole grains provide resistant starch and oligosaccharides to the diet (Slavin, 2010). All of these components work together to create a healthy gut environment. Fiber promotes normal laxation, which includes a range of gastrointestinal effects like transit time, bloating, constipation, and diarrhea (Slavin, 2010). This occurs through a fermentation process that promotes selective microbial growth, eventually leading to increased water holding capacity of fecal material (Slavin, 2010). Fiber also fuels mucosal cells residing in the colon through the fermentation process by forming short chain fatty acids, which serve as an energy source for the cells (Slavin, 2010). Specifically, insoluble fiber is linked to improved laxation (Slavin, 2010). Fiber, oligosaccharides, and resistant starch are all capable of this fermentation process, making cereal a gut healthy breakfast option.

2.1.2 Heart health

Poor heart health may be indicated by risk factors such as hypertension, high cholesterol, heart attack, diabetes, and obesity (Djoussé & Gaziano, 2007). Many studies have shown a diet consisting of whole grains to be associated with a reduced risk of heart disease (Jacobs, Meyer, Kushi, Folsom, 1998; Keenan, Pins, Frazel, Moran, Turnquist, 2002; Djoussé & Gaziano, 2007). Studies often show a positive correlation with improved blood cholesterol and blood pressure. One such study found a 7.5 mmHg reduction in systolic blood pressure and a 5.5 mmHg reduction in diastolic blood pressure of hypertensive patients after 6 weeks of oat cereal consumption (Keenan et al., 2002). Other studies have focused on the benefits of fiber in protecting against heart disease (Chandalia et al., 2000; Bazzano, He, Odgen, Loria, Whelton, 2003; Krishnan et al., 2007). One study explored the American heart association recommendation of 25 grams of fiber per day and found the risk of heart disease decreased significantly (Bazzano et al., 2003). Specifically, consumption of at least 4.5 g of cereal grain fiber was associated with as much as a 20% reduction in heart disease (Bazzano et al., 2003).

Calcium intake has also been linked to decreased risk of hypertension (Hajjar, Grim, Kotchen, 2003). Milk is known to be a good source of calcium and is also commonly consumed with ready-to-eat breakfast cereal. Thus, cereal is a gateway to adequate calcium consumption. As we already know, fiber is known to benefit heart health. Specifically, soluble fiber has shown cholesterol lowering effects as well as improved glucose response (Slavin, 2010). Paired with the benefits of milk consumption, whole grain cereal makes for a great heart healthy breakfast.

2.1.3 Weight management

One study (n = 2379) found that eating cereal at breakfast was associated with both increased fiber intake and greater physical activity among adolescent girls (Albertson et al., 2008). While it has long been understood that adequate physical activity results in a healthy weight status, the link between weight and fiber consumption is a much newer discovery. High-fiber foods are an important part of the diet for the management of weight and obesity as they are generally less energy dense, take longer to eat, slow down gastric emptying, and thus increase feelings of fullness and satiety (Kamran et al., 2008). Bran and dietary fibers found within whole grains can also reduce a food’s glycemic load, which is defined as the rate at which a food releases sugar into the bloodstream (Kamran et al., 2008). As a result, fiber consumption has been shown to improve glucose tolerance and insulin response, suggesting a link between cereal fiber consumption and the prevention of type 2 diabetes (Fung et al., 2002; Kamran et al., 2008).

Body mass index (BMI) has also been found to be significantly lower in cereal eaters versus non-cereal eaters amongst all ages (Barton et al., 2005; Albertson et al., 2008). Numerous mechanisms have been hypothesized to explain the link between cereal consumption and weight management (Albertson et al., 2008). It may be that the nutrients in cereal are conducive to positive health outcomes in general. For instance, consuming cereal for breakfast provides more fiber, iron, folic acid, and zinc and provides less fat, sodium, sugar, and cholesterol than non-cereal breakfasts (Albertson et al., 2008). Cereal is often consumed along with other food products that may be partially responsible for positive health outcomes. For example, cereal is often eaten in milk, which contains calcium and other important nutrients. Not only does calcium provide the body with essential nutrients, but it is predicted that calcium may play a role in regulating body fat as well (Barton et al., 2005). It is possible that the associated food holds some credit for the corresponding health benefit. Being that cereal is considered a "health food," it is also possible that cereal is merely a proxy for other positive health behaviors. A number of other studies have found similar links between cereal consumption, BMI, and weight management amongst all age and gender demographics (Bertrais et al., 2000; Cho, Dietrich, Brown, Clark, Block, 2003; Albertson, Anderson, Crockett, Goebel, 2003; Waller et al., 2004; Song, Chun, Obayashi, Cho, Chung, 2005).

2.2 Sensory evaluation of breakfast cereal

Sensory attributes are arguably the most important tools that can be used to determine product quality as it relates to consumer acceptance (Macedo, Gallagher, Oliveira, Byrne, 2011). Therefore, it is important to measure the sensory characteristics of all new food products to ensure that they meet consumer expectations (Dansby & Bovell-Benjamin, 2003). As cereal products age, the sensory properties are directly affected as the product undergoes both physical and chemical changes (Jacobsen, 1999; Paradiso, Caponio, Summo, Gomes, 2012). As such, it is important to monitor the sensory attributes of a cereal product over time in order to determine its shelf life. Some basic sensory attributes of importance in ready-to-eat breakfast cereal include color, size, shape, flavor aroma, rancid odor, flavor intensity, rancid flavor, hardness, crispness, and overall acceptability (Paradiso et al., 2012).

2.2.1 Measuring sensory attributes

There have been few studies on the sensory properties of ready-to-eat breakfast cereal and therefore few descriptive terms have been used to characterize the flavors of extruded cereals (Dansby & Bovell-Benjamin, 2003). A study conducted by Dansby and Bovell-Benjamin (2003) used descriptive analysis to characterize the main sensory attributes of ready-to-eat breakfast cereal as a means to measure consumer acceptance. Panelists of this study were extensively trained. The main sensory attributes were categorized as belonging to either appearance, flavor, or texture. The most important descriptors for ready-to-eat breakfast cereals in this study were found to be color, glossy, stick-like, bland, sweet, sharp smell, cinnamon aftertaste, crunchy, gritty, hard, chewy, and dry (Dansby & Bovell-Benjamin, 2003).

Another common way to measure sensory attributes is to use a nine-point hedonic liking scale (1 = "dislike extremely", 9 = "like extremely"), as demonstrated by Berglund, Fastnaught, and Holm (1994). Panelists of this study were untrained. Panelists used the scale to evaluate color, flavor, tenderness, crispness, and overall acceptability, and an average was generated for each attribute. A score of 5 was considered to be neutral and scores above 5 were in the "like" range (Berglund et al., 1994). Acceptability of each attribute was compared amongst different formulae by comparing attribute averages. Moisture content, water activity, temperature, relative humidity, peroxide value, anisidine value, headspace analysis, texture, and color are all measurable parameters that can be helpful in quantifying sensory quality of breakfast cereals (Macedo et al., 2011; Paradiso et al., 2012). However, the numeric values of these chemical analyses do not always correlate well with what can be detected by the human senses (Jacobsen, 1999). Additionally, studies have shown that chemical oxidation data may indicate the same degree of oxidation amongst different products, but the sensory impact may still be very different between products (Jacobsen, 1999). Sensory perception is therefore a necessary measurement in determining consumer acceptance and thus product shelf life.

2.2.2 Sensory characteristics of breakfast cereal

The sensory attributes that get the most scrutiny in ready-to-eat breakfast cereal are flavor and texture. Flavor characters of interest include flavor intensity, rancidity, and other off notes. Texture characters of interest include crispness and hardness (Couroux, Chaunier, Della Valle, Lourdin, Séménou, 2005). Other characteristics of ready-to-eat breakfast cereal that may be of importance include those related to appearance such as color, size, and shape. Appearance of breakfast cereals varies widely among type of cereal product and is often subjective to individual preference. It is therefore difficult to find conclusive data on appearance attributes, whereas an undisputed preference for flavor and texture have been well defined.

Sensory perception of bitter off-flavors and rancidity are generally the result of oxidative or hydrolytic degradation, though deterioration of proteins and reactions of phenolic acid compounds may also contribute (Heinio, 2006). Since there are different mechanisms that result in rancidity, the term “rancid” may depend on the food product in question and descriptors may vary (Jacobsen, 1999). Rancid aroma and flavor can be described using descriptors like bitter, acidic, chemical, cardboard, grassy, fishy, painty, sour, soapy, sharp, and pungent (Jacobsen, 1999; Culbertson, 2004).

Crispness perception of breakfast cereals has been positively correlated with descriptors like brittleness, airiness, and lightness of texture, and negatively correlated with cohesiveness and stickiness descriptors (Couroux et al., 2005). Crispness also focuses on the auditory perception in the form of loudness, pitch, and sound duration (Couroux et al., 2005). Hardness can be defined as the force it takes to bite into the cereal product and is rated by degree of intensity (Couroux et al., 2005). One study found that the average consumer was able to identify both crispness and hardness perception as well as a trained descriptive analysis panel (Couroux et al., 2005). Thus, texture is an importance attribute related to consumer acceptability.

It is important to evaluate sensory attributes when the product is dry as well as when the product is in milk, as the texture and flavor profile can change in milk. Ready-to-eat breakfast cereals should ideally have a hard texture so that they hydrate slowly and retain their desired crispness longer when consumed with milk (Chulaluck, Nipat, Waraporn, Pisut, 2011). Specifically, the length of time that cereal can soak in milk without losing texture or becoming soggy is a common attribute to evaluate when determining the shelf life of a cereal product. This process is termed “bowl life” and is an important sensory attribute characteristic to cereal products (Culbertson, 2004). Coating the surface area with a sugar slurry can provide a temporary physical barrier on the surface of the cereal piece until it dissolves in milk, and thus extend its bowl life rather significantly (Culbertson, 2004).

2.3 Degradation of ready-to-eat breakfast cereal

The rate of degradation of cereal products will determine the shelf life of said products. Cereal grains are composed of many chemical compounds, some of which are responsible for and some of which are susceptible to degradation. Too high of moisture levels, too low of moisture levels, light, elevated storage temperatures, and high oxygen concentrations all increase the rate of rancidity development (Heinio, 2006). Modes of deterioration of ready-to-eat breakfast cereal include lipid oxidation, enzymatic degradation, and staleness. During the lipid oxidation process, free radicals, hydroperoxides, and secondary oxidation products are formed (Jacobsen, 1999). Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, and core aldehydes (Jacobsen, 1999). Secondary oxidation products are responsible for the changes in aroma and flavor profiles of a food degraded via lipid oxidation (Jacobsen, 1999). Deterioration of proteins and reactions of phenolic acid compounds may also contribute to off-notes (Heinio, 2006).

2.3.1 Oxidative rancidity

Cereal grains with a high fat content are susceptible to lipid oxidation after the naturally present antioxidants in the grain are damaged during process (Heinio, 2006). Lipid oxidation is a major chemical reaction responsible for the deterioration of foods (Culbertson, 2004). Lipid oxidation proceeds via a free radical mechanism and involves three stages: initiation, propagation, and termination (Figure 2.1) (Velasco, Dobarganes, Marquez-Ruiz, 2010). During initiation, an alkyl radical is formed via the loss of a hydrogen from the unsaturated lipid, equation [1.1]. In step one of propagation, the alkyl radical reacts with atmospheric oxygen to form peroxyl radicals, equation [1.2]. In step two of propagation, the peroxyl radicals react with new lipid molecules creating hydroperoxides as the primary oxidation products and another alkyl radical as a bi-product, which can further promote oxidation, equation [1.3]. During termination, radicals react between each other to form relatively stable non-radical species, equations [1.4]-[1.6]. Hydroperoxides are odorless themselves, but decompose into radicals that follow different pathways to produce a variety of secondary oxidation products (Velasco et al., 2010).

Initiation	RH → R• + H•	[1.1]
Propagation	R• + O2 → ROO•	[1.2]
	ROO• + RH → ROOH + R•	[1.3]
Termination	2ROO• → non-radical products	[1.4]
	ROO• + R• → non-radical products	[1.5]
	2R• → non-radical products	[1.6]

Figure 2.1: Mechanism of lipid autoxidation.

During the induction period, the concentration of hydroperoxides is high and the concentration of secondary oxidation products is low; as the reaction progresses, the concentration of hydroperoxides lessens as the concentration of secondary oxidation products grows (Velasco et al., 2010). Secondary oxidation products are known to be responsible for rancid and other off-notes in food systems (Velasco et al., 2010). Among these compounds, the vinyl ketones as well as the *trans,cis*-alkadienals have the lowest detection threshold (Jacobsen, 1999). In contrast, the hydrocarbon alkanes and alkenes have the highest detection threshold and are therefore considered to be flavor-insignificant (Jacobsen, 1999). In short, lipid oxidation results in the formation of peroxides and the decomposition of peroxides forms a variety of volatile aldehydes and ketones that are responsible for the rancid aroma of cereal products (Culbertson, 2004).

Oxidation of cereal grains will be greater when the concentration of lipids in the grain is high, as well as when the degree of unsaturation is high (Culbertson, 2004). For example, oat based cereal products are at highest risk for oxidation as compared to other grain based extruded products due to their higher lipid content (Viscidi, Dougherty, Briggs, Camire, 2004). Though their lipid content usually does not exceed 6-7% (Paradiso et al., 2012), there are a number of factors that make extruded cereals particularly prone to oxidation. These factors include low moisture content, increased surface area due to expansion, and higher levels of iron, an initiator and catalyst for oxidation, caused by wearing of the screw and barrel during extrusion, as well as from the addition of vitamins during the fortification process (Viscidi et al., 2004).

2.3.2 Hydrolytic rancidity

Hydrolytic rancidity is a result of enzymatic activity on the outer layers of the cereal grain (Heinio, 2006). Through this process, fat degrades into free fatty acids via the work of enzymes and are then subjected to oxidation (Heinio, 2006). As a result, this process is also known as enzymatic oxidation (Velasco et al., 2010). Lipase and lipoxygenase are the enzymes found within cereal grains that are responsible for this (Kamran et al., 2008). The first step involved in enzymatic oxidation is hydrolysis, or the release of fatty acids from the triglyceride molecule by action of the lipase enzyme in the presence of a water molecule (Malekian, 2000; Velasco et al., 2010). The resulting free fatty acids increase the cereal's acidity and reduce pH (Malekian, 2000). Next, lipoxygenase enzymes target these free fatty acids and catalyze the oxidation process (Velasco et al., 2010). The oxidation products of enzymatic oxidation are the same hydroperoxides produced in the lipid autoxidation process, but their relative proportions and stereochemistry are distinctly different because the enzymatic oxidative pathway is stereospecific and regioselective (Velasco et al., 2010).

A soapy flavor and aroma is characteristic of hydrolytic rancidity, and can develop over time if these enzymes are not properly inactivated (Malekian, 2000). Similar to rancidity caused by traditional lipid oxidation, oats are at highest risk of enzymatic degradation due to their higher content of lypolitic enzymes (Viscidi et al., 2004). In fact, hydrolytic enzymes in oats have been found to be 10-15 times more active than those of wheat (Viscidi et al., 2004). Despite this, however, enzymatic oxidation is unlikely the major process of rancidity in ready-to-eat breakfast cereal due to the high temperatures that grains are exposed to during cereal processing, but is still something to be cognizant of.

2.3.3 Moisture content, water activity, and staleness

Moisture content and water activity have been found to be critical quality parameters of breakfast cereals, specifically during storage (Macedo et al., 2011). The moisture content of a cereal product can influence its textural characteristics, chemical stability, and microbial growth rates (Macedo et al., 2011). Moisture gain leads to slight loss of cereal crispness and acceptance between 0 and 0.50 (a_w) or up to 7% moisture content (Sauvageot & Blond, 1991; Culbertson, 2004). Surpassing these ranges lead to very significant staling of ready-to-eat cereal. Of course high moisture and water activity also allow for microbial growth, though this is not commonly an area of concern for ready-to-eat breakfast cereals due to the drying process during manufacturing (Cook & Johnson, 2009). In contrast, too low of moisture level has been associated with higher notes of rancidity, though the exact mechanism for this phenomenon has not been defined (Elder, 1941).

While the effects of moisture on the stability of cereal products have been clearly demonstrated (Sauvageot & Blond, 1991; Berglund et al., 1994; Culbertson, 2004; Macedo et al., 2011), it is not realistic to control this variable to the extent that an exact desired moisture content will be achieved (Elder, 1941). Instead, a range (tolerable upper and lower limits) must be set with texture, oxidation, and microbial considerations in mind so that a product that is reasonably acceptable for all parameters will result (Elder, 1941). The range for moisture content of finished cereals is generally set between 1-3% moisture (Berglund et al., 1994).

2.4 Antioxidants in breakfast cereal

The effectiveness of antioxidants in slowing down the process of oxidation, and thus the onset of undesirable off-flavors, has been widely studied (Anderson, Huntley, Schwecke, Nelson, 1963; Wessling, Nielsen, Leufvén, Jägerstad, 1998; Butt, Ali, Pasha, Hashmi, Dogar, 2003; Paradiso, Summo, Trani, Caponio, 2007). It is well observed that cereal products containing no added antioxidant are considerably less acceptable from a sensory standpoint than those with an added antioxidant (Paradiso et al., 2012). Common antioxidants used to combat oxidation include butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are both synthetic, and tocopherols, which are considered natural (Paradiso et al., 2007). Regardless of natural or synthetic origin, incorporating antioxidants in ready-to-eat cereal products can inhibit oxidation and extend product shelf life.

2.4.1 BHT as a successful antioxidant

The use of BHT has been found to be very effective in combatting oxidation (Anderson et al., 1963; Wessling, Nielsen, et al., 1998; Butt et al., 2003). To display its effectiveness, one study measured the correlation between antioxidant concentration and peroxide concentration throughout shelf life storage of breakfast cereals (Anderson et al., 1963). Peroxide value is an effective way to measure the extent of primary oxidation. It was found that levels of BHT were inversely related to peroxide values, meaning that peroxides developed as the antioxidant was used up, indicating primary oxidation was slowed by means of BHT (Anderson et al., 1963). Interestingly, oat based cereals used in this study were found to utilize the antioxidant nearly twice as fast as wheat based cereals (Anderson et al., 1963).

BHT has been shown to be more effective than the popular natural alternative, alpha-tocopherol (Wessling et al., 1998; Paradiso et al., 2012). This was demonstrated when Wessling et al. studied the migration of BHT as compared to alpha-tocopherol in packaging material (Wessling et al., 1998). Wessling found that BHT was much more mobile and migrated into the cereal much sooner than its counterpart, alpha-tocopherol (Wessling et al., 1998). This was in large part due to alpha-tocopherol being a bigger molecule than BHT, making alpha-tocopherol less mobile and less likely to migrate throughout the product, thus leaving some parts of the cereal unprotected (Wessling et al., 1998).

Antioxidants can be added to food in concentrated form as an ingredient or incorporated into product packaging (Butt et al., 2003). BHT is a volatile, nonpolar compound and therefore does not disperse well as an ingredient in the cereal base formula by itself, and also does not hold up well through the stress of cereal processing (Pasca, Cororian, Socaci, 2018). Therefore, BHT is often incorporated into the packaging material where it can quickly migrate into the product after packaging. Since BHT and the lipid it is trying to protect are both nonpolar molecules, the antioxidant will tend to concentrate where it is most needed (Paradiso et al., 2012).

2.4.2 Link between BHT and consumer health

There is a general tendency among consumers to prefer products, ingredients, and additives of natural origin under the belief that they are healthier (Paradiso et al., 2007).

Despite its proven effectiveness, BHT is often looked at with suspicion due to its synthetic nature and potential risks to consumer health (Paradiso et al., 2007). BHT is listed in the Code of Federal Regulations as a substance that is generally recognized as safe (GRAS), with a usage limit of total antioxidants not to exceed 0.02% of the fat or oil content (Food and Drug, 2019). Despite its GRAS status, there is still debate about BHT's potential hazards to human health (Wang & Kannan, 2019). BHT is known to bioaccumulate and can also be detected in urine (Wang & Kannan, 2019). Toxic effects of BHT including carcinogenicity and reproductive toxicity have been shown in numerous animal studies (Wang & Kannan, 2019). Metabolites of BHT have also been shown to elicit cellular damage, genotoxicity, and carcinogenicity in animal models (Wang & Kannan, 2019). There is some belief that BHT metabolites are even more toxic than BHT itself, but carcinogenic properties of BHT remain controversial and subject to debate (Wang & Kannan, 2019). Fortunately, some studies have shown that BHT is generally consumed well below the recommended daily limit (Botterweck, Verhagen, Goldbohm, Kleinjans, van den Brandt, 2000; Suh et al., 2005). Furthermore, other studies suggest that BHT may actually play a role in the prevention of cancer (Ulland, Weisburger, Yamamoto, Weisburger, 1973; Hocman, 1988). Despite contradicting claims, BHT remains on the GRAS list and is a proven way to achieve the desired quality that consumers expect.

2.5 Packaging features that influence shelf life of breakfast cereal

Textural changes can be avoided to some extent by utilizing suitable packaging material (Heinio, 2006). Packaging material serves to protect the product from undesirable moisture gain, but will often also serve as a delivery method for antioxidants (Culbertson, 2004). Important considerations of packaging materials are water vapor transmission and flavor barrier properties.

2.5.1 Moisture barrier

In general, cereal products are packaged using two different packaging types. The first packaging type serves as a protective barrier to the product and generally takes the form of a polymer liner (Senhofa et al., 2015). The primary packaging is often comprised of multi-layered packaging material to provide light, oxygen, and moisture permeability (Senhofa et al., 2015). Cereal products vary in their need for moisture protection. Cereals with many hygroscopic components such as sugars and other simple carbohydrates may require more substantial packaging materials to protect them from rapid loss of texture due to moisture uptake during storage (Heinio, 2006). This can be accomplished using various layers to create the polymer film structure. Common materials used in cereal film structure include both high and low density polyethylene, polypropylene, and nylon ionomers (Senhofa et al., 2015). Companies generally keep their polymer liner blends proprietary for competitive reasons. The secondary packaging is simply for consumer's ease of use and generally takes the form of a paper carton (Senhofa et al., 2015).

2.5.2 Flavor barrier

Flavor deterioration during storage may result from the volatilization of desired flavor attributes or the development of undesired off-flavors, or both (Heinio, 2006). Many of the naturally occurring and added flavors found in cereal products are volatile. Loss of flavor during storage may be due to absorption of the volatiles by the packaging materials or by their migration through the liner itself. Thus, flavor barrier properties are also a very important function of ready-to-eat cereal packaging.

Multiple packaging products have the ability to trap or otherwise slow down the loss of volatile flavors (Heinio, 2006; Paradiso et al., 2012). The aroma barrier characteristics of cereal packaging are determined with the permeability and solubility of the aromas of interest in mind (Mohney, Hernandez, Giacin, Harte, Miltz, 1988). Specifically, the diffusion coefficient, solubility coefficient, and permeability constant are all necessary data for the prediction of changes in product quality as it relates to loss of desired aroma during storage (Mohney et al., 1988). Essentially, the permeability, or ability of the flavor volatile to diffuse both into and out of the packaging material, is dependent on the volatile’s solubility (Mohney et al., 1988). Once these data are known, the estimated shelf life of a product can be determined.

Chapter 3: Objectives and Hypotheses

Objective: To determine the effectiveness and reliability of BHT as an antioxidant to extend shelf life as it is used in both the packaging material (cereal liner) and ingredients (canola oil) of cereal products.

Hypothesis 1: The use of BHT in packaging material will significantly improve the shelf life of cereal products.

Hypothesis 2: The use of BHT in canola oil will significantly improve the shelf life of cereal products.

Hypothesis 3: The use of BHT in packaging material will improve the shelf life of cereal products more significantly than the use of BHT in canola oil.

Hypothesis 4: The total removal of BHT in packaging material and canola oil will significantly reduce the shelf life of cereal products.

Chapter 4: Materials and Methods

4.1 Participants

Participants of this study were employees of a major cereal company. Panelists were untrained to reflect the average consumer, though their expertise with cereal products does allow for some bias. Panelists evaluated the organoleptic properties of each cereal product with a focus on descriptors that are indicative of lipid oxidation such as cereal rancidity, off flavor/aroma, and flavor intensity. Three panelists were assigned to each of the four cereal products, and tastings were conducted in a group setting. Each set of panelists was designed to be cross-functional, consisting of one lab technician (myself), one product developer, and one packaging engineer. The ability to adhere to this design was dependent upon employee availability, and stand-ins were often needed. Additionally, one participant was replaced midway through experimentation due to resignation from the company. All panelists participated voluntarily.

4.2 Products

4.2.1 Cereal

Four lab-made cereal prototypes were used for this study: multigrain flake, wheat shred, puffed shape, and peanut butter disk. Products were selected to include an array of cereal technology and base formula (Table 4.1). To explain, multigrain flake utilized milled and flaked technology and contained primarily wheat and rice base ingredients. Furthermore, wheat shred utilized shredded technology and wheat base, puffed shape utilized gun puffed technology and oat base, and peanut butter disk utilized direct expansion extrusion technology and corn base. Additionally, shelf life data of peanut butter coated cereal products is lacking in the literature. Therefore, this was a secondary reason for including a peanut butter product as one of the variations.

Table 4.1: Distinguishing characteristics used for cereal product selection.

Cereal Product	**Main Formula Component**	**Processing Technology**
Puffed shape	Oat	Gun puffed
Multigrain flake	Wheat / Rice	Milled and flaked
Wheat shred	Wheat	Shredded
Peanut butter disk	Corn / Peanut butter	Direct expansion

Uncoated cereal base products were sourced from a major cereal manufacturer with factories located across North America. Products were sugar and/or peanut butter coated in a professional cereal laboratory over the span of one week. Coating formulae were loosely based on current proprietary recipes (Appendix A). Each formula used an oil application rate of 1% during the sugar coat process. Peanut butter disk product included additional canola oil usage in the peanut butter application process, for a final application rate of 4.5% (Equation 4.1).

Equation 4.1: Equation used to determine canola oil usage level for peanut butter disk cereal, where the final product is comprised of 70% sugar coated base and 20% peanut butter slurry containing 1% and 19% canola oil, respectively.

$$(\%\ pb\ slurry)(\%\ oil\ in\ pb\ slurry) + (\%\ sugar\ coated\ cereal)(\%\ oil\ in\ sugar\ coat) = total\ \%\ oil$$

$$(20\%)(19\%) + (70\%)(1\%) = 4.5\%$$

Four coating variations were made for each cereal product: Variation A (BHT liner, no BHT oil), Variation B (no BHT liner, BHT oil), Variation C (no BHT liner, no BHT oil), and Variation D (BHT liner, BHT oil). Variation C served as the internal positive control, in which BHT protection is absent from this sample and therefore rancid notes are expected to be present. Variation D served as the gold standard, or "control" reference sample, in which BHT was present in the highest amount and thus protection against oxidation was expected to be highest. Put another way, rancid notes were expected to be minimal for Variation D in comparison to Variations A-C, thus deeming it the gold standard. Finished product was placed into storage conditions on August 19, 2019. Product was placed into accelerated aging conditions (100°F) for eighteen weeks.

Table 4.2: Experimental design.

Cereal product	BHT liner + no BHT oil	No BHT liner + BHT oil	No BHT liner + no BHT oil	BHT liner + BHT oil
Multigrain flake	Variation A	Variation B	Variation C	Variation D (control)
Wheat shred	Variation A	Variation B	Variation C	Variation D (control)
Puffed shape	Variation A	Variation B	Variation C	Variation D (control)
Peanut butter disk	Variation A	Variation B	Variation C	Variation D (control)

4.2.2 Packaging material

Low barrier liners utilizing a 1.75 mm gauge proprietary multi-layer polyethylene film structure with and without BHT were used for multigrain flake and peanut butter disk products. High barrier liners utilizing a 2.25 mm gauge proprietary multi-layer polyethylene film structure with and without BHT were used for wheat shred and puffed shape products. This higher gauge, thicker film was used to avoid pinhole issues caused by the abrasive texture of wheat shreds. Additionally, high barrier liners have high moisture, oxygen, and aroma barrier properties, while low barrier liners only have high moisture barrier properties. Therefore, low barrier was selected for products that were known to contribute off flavors through aging, and high barrier liners were selected for products that have not historically had issues with production of off flavors, but have struggled with oxidation and/or aroma loss. The addition of BHT to the liner structure works to prevent cereal oxidation by the oxygen present in the headspace, including that which may enter through the film structure over time.

4.3 Experimental schedule and storage conditions

The standard shelf life of most ready-to-eat cereal products is twelve months. This aging process can be studied in real-time at ambient temperature, or in an accelerated process at 100°F. As shown in Equation 4.2, the relationship between real-time aging and accelerated aging roughly equates to 4:1, respectively. For example, product aged for four weeks at ambient temperature would be comparable in organoleptic properties to cereal aged for one week at accelerated temperature.

Equation 4.2: Equation used to determine the rate of aging for cereal stored at two different temperatures (Seward & DeVries, 2003).

$$F_2 = F_1 \times Q_{10}^{\Delta(t_2 - t_1)/10}$$

Q_{10} = Rate of change of an attribute (mode of failure) for every 10°C (value of 2 is typical for RTE cereal for lipid oxidation)
F_1 = Time between tasting at the higher temperature storage
F_2 = Time between tasting at the lower temperature storage
Δ = Temperature difference between higher temperature (t_2) and low temperature (t_1) in °C

This study aimed to observe the standard 12 month shelf life of cereal products. Thus, a total of 18 weeks were observed to ensure the shelf life was experienced in its entirety. An accelerated tasting schedule was created for each cereal type (Appendix B). A complete experimental timeline can be seen in Figure 4.1. Products were evaluated at each time increment by assigned panelists.

Figure 4.1: Experimental timeline.

4.4 Tasting procedure

Panelists participated in a total of nine tastings. Only one cereal variety was evaluated at each tasting session, and panelists consistently tasted the same cereal variety except where replacement panelists were necessary. Panelists were provided four cereal samples: Control (Variation D), Test 1 (Variation A), Test 2 (Variation B), and Test 3 (Variation C). Samples were provided in the same order for all panelists. Sample identity was not disclosed to panelists throughout the duration of testing. Panelists were instructed to evaluate each test sample with and without milk for aroma, flavor, and texture attributes as they compared to the control sample. Attributes were those generated by the shelf life tasting ballot template used by a major cereal company (Appendix C). Panelists were asked to rate individual attributes on a -3 to +3 sale, where positive values represented an attribute stronger than the control and a negative value represented an attribute weaker than the control. More specifically, an attribute with a value of (+/-) 1 was determined to be of slight difference, a value of (+/-) 2 was of moderate difference, and a value of (+/-) 3 was of large difference from the control. For example, a sample that was significantly more rancid tasting than control would be marked as +3, while a sample that was significantly less rancid tasting than control would be marked -3. A value of 0 indicated no difference from the control for that attribute. Each attribute category was then rated for overall difference on a 0 to 8 scale, where values 0-2 showed no or slight difference from control, values 3-5 showed moderate difference, and values 6-8 showed large difference. Finally, each sample was rated with a cumulative overall difference score on a 0 to 8 scale.

Tastings were conducted individually, but in a group setting. Once panelists completed their individual ballots, a consensus ballot was created based on group discussion. Thus, the design of having three panelists per test allowed for a clear consensus value to be determined based on majority rule. Consensus data were used to create a composite dataset.

4.5 Data analysis

Quantitative statistical analysis was carried out using Microsoft Excel's "Anova: Single Factor" and "t-Test: Two-Sample Assuming Unequal Variances" features. One-way analyses of variance (ANOVA) tests were used to determine if sensory attribute ratings differed significantly ($p < 0.05$) between Variations A-C for each cereal product (Appendix D). For those attributes found to contain a significant difference, a t-test was conducted to determine which specific test variations differed from which others (Appendix E). Attributes analyzed included rancid aroma, rancid flavor dry, rancid flavor in milk, and overall difference.

Chapter 5: Results

5.1 Multigrain flake

Variation A was found to be statistically different from Variation C for all attributes. Variation A was statistically different from Variation B for the following attributes: overall difference, rancid aroma, and rancid flavor dry. Variation B was not found to be statistically different from Variation C for any attribute (Figure 5.1, Table 5.1, and Table 5.2).

Table 5.1: Mean (n = 9) panelist attribute ratings of multigrain flakes among different test variations for four attributes. F and p-values corresponded to ANOVA analysis between test variations per attribute. Gray shaded rows show attributes containing a statistically significant difference ($p < 0.05$) between test variations.

Attribute	Multigrain Flake			F	p-value
	Variation A	Variation B	Variation C		
Overall difference[a]	1	3	5	7.31	0.003
Rancid aroma[b]	1	2	2	10.56	<0.001
Rancid flavor (dry)[b]	0	1	2	7.6	0.003
Rancid flavor (in milk)[b]	0	1	2	4.39	0.024

[a] Scale values for the overall difference attribute began at “0” on one end of the scale labeled “no difference” and ended at “8” on the other end of the scale labeled “large difference.”
[b] For all other attributes, the scale began at “-3” on the left end labeled “low” and ended at “3” on the right end labeled “high.”

Table 5.2: t-Test analysis between each test variation for attributes containing a statistically significant difference determined by ANOVA (Table 5.1). Grey shaded rows show test variations that differed significantly ($p < 0.05$) among attributes.

Multigrain Flake			
Attribute	Difference Between	t-statistic	p-value
Overall difference	A & B	2.63	0.021
	A & C	5	<0.001
	B & C	1.89	0.077
Rancid aroma	A & B	2.55	0.027
	A & C	4.38	0.001
	B & C	1.32	0.206
Rancid flavor dry	A & B	2.55	0.027
	A & C	4.38	0.001
	B & C	1.32	0.206
Rancid flavor in milk	A & B	1.86	0.090
	A & C	3.4	0.007
	B & C	1.06	0.305

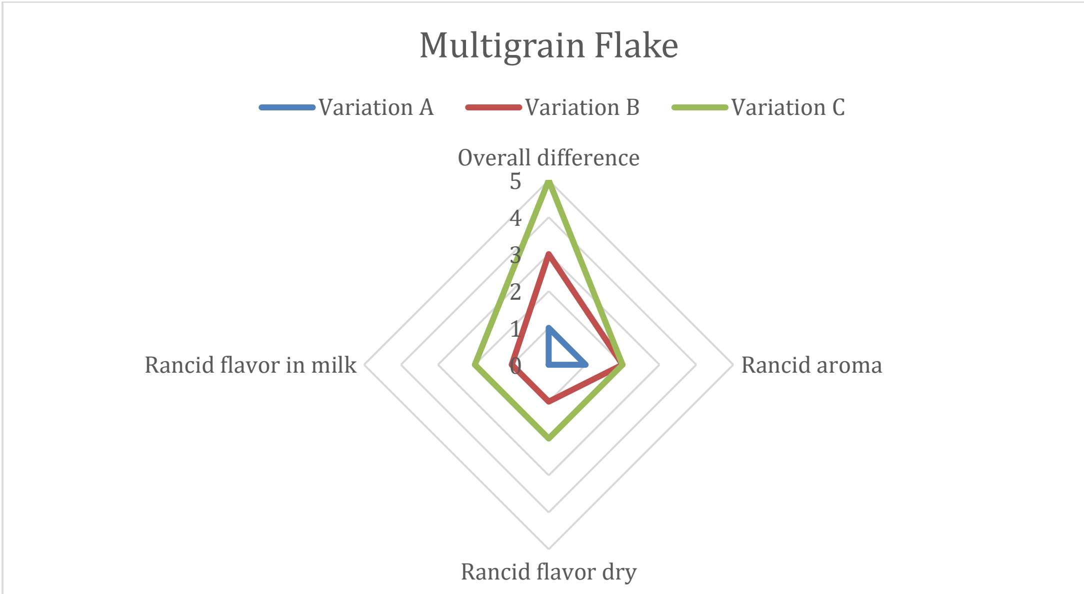

Figure 5.1: Mean (N=9) attribute values of multigrain flake cereal that differed significantly among test variations. Scale values began at "0" on the left end of the scale labeled "no difference" and ended at "8" on the right end of the scale labeled "large difference."

5.2 Wheat shred

Variation A was found to be statistically different from Variation C for the rancid aroma attribute. None of the other test variations differed among attributes (Table 5.3, Table 5.4, and Figure 5.2).

Table 5.3: Mean (n = 9) panelist attribute ratings of wheat shreds among different test variations for four attributes. F and p-values corresponded to ANOVA analysis between test variations per attribute. Gray shaded rows show attributes containing a statistically significant difference ($p < 0.05$) between test variations.

Attribute	Wheat Shred			F	p-value
	Variation A	Variation B	Variation C		
Overall difference[a]	0	1	2	2.44	0.108
Rancid aroma[b]	0	1	1	4.68	0.019
Rancid flavor (dry)[b]	0	0	1	2.21	0.131
Rancid flavor (in milk)[b]	0	0	0	1.75	0.195

[a] Scale values for the overall difference attribute began at "0" on one end of the scale labeled "no difference" and ended at "8" on the other end of the scale labeled "large difference."
[b] For all other attributes, the scale began at "-3" on the left end labeled "low" and ended at "3" on the right end labeled "high."

Table 5.4: t-Test analysis between each test variation for attributes containing a statistically significant difference determined by ANOVA (Table 5.3). Grey shaded rows show test variations that differed significantly ($p < 0.05$) among attributes.

Wheat Shred			
Attribute	Difference Between	t-statistic	p-value
Overall difference	A & B	NA	NA
	A & C		
	B & C		
Rancid aroma	A & B	2.14	0.051
	A & C	2.87	0.017
	B & C	1.32	0.211
Rancid flavor dry	A & B	NA	NA
	A & C		
	B & C		
Rancid flavor in milk	A & B	NA	NA
	A & C		
	B & C		

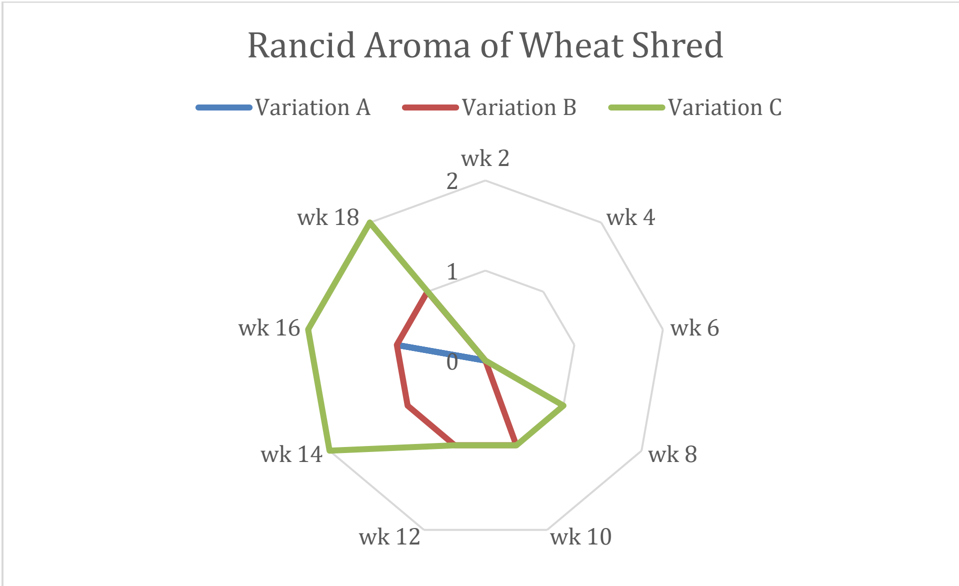

Figure 5.2: Weekly panelist ratings (n = 9) that differed significantly among test variations for the rancid aroma attribute in wheat shred cereal. Scale values began at "0" on the left end of the scale labeled "no difference" and ended at "8" on the right end of the scale labeled "large difference."

5.3 Puffed shape

No statistically significant difference was found among test variations for any attribute (Table 5.5).

Table 5.5: Mean (n = 9) panelist attribute ratings of puffed shape among different test variations for four attributes. F and p-values corresponded to ANOVA analysis between test variations per attribute. Gray shaded rows show attributes containing a statistically significant difference ($p < 0.05$) between test variations.

Attribute	Puffed Shape			F	p-value
	Variation A	Variation B	Variation C		
Overall difference[a]	1	1	1	2.24	0.129
Rancid aroma[b]	0	0	0	0.14	0.868
Rancid flavor (dry)[b]	0	0	0	1.27	0.298
Rancid flavor (in milk)[b]	0	0	0	<0.00	1

[a] Scale values for the overall difference attribute began at "0" on one end of the scale labeled "no difference" and ended at "8" on the other end of the scale labeled "large difference."
[b] For all other attributes, the scale began at "-3" on the left end labeled "low" and ended at "3" on the right end labeled "high."

5.4 Peanut butter disk

Variation A was found to be statistically different from Variation C for the overall difference attribute. None of the other attributes differed among test variations (Table 5.6, Table 5.7, and Figure 5.3).

Table 5.6: Mean (n = 9) panelist attribute ratings of peanut butter disk among different test variations for four attributes. F and p-values corresponded to ANOVA analysis between test variations per attribute. Gray shaded rows show attributes containing a statistically significant difference ($p < 0.05$) between test variations.

Attribute	Peanut Butter Disk			F	p-value
	Variation A	Variation B	Variation C		
Overall difference[a]	1	2	3	5.2	0.013
Rancid aroma[b]	0	0	1	2.38	0.114
Rancid flavor (dry)[b]	0	0	1	2.05	0.151
Rancid flavor (in milk)[b]	0	0	1	1.78	0.191

[a] Scale values for the overall difference attribute began at “0” on one end of the scale labeled “no difference” and ended at “8” on the other end of the scale labeled “large difference.”
[b] For all other attributes, the scale began at “-3” on the left end labeled “low” and ended at “3” on the right end labeled “high.”

Table 5.7: t-Test analysis between each test variation for attributes containing a statistically significant difference determined by ANOVA (Table 5.6). Grey shaded rows show test variations that differed significantly ($p < 0.05$) among attributes.

Peanut Butter Disk			
Attribute	Difference Between	t-statistic	p-value
Overall difference	A & B	1.18	0.258
	A & C	2.64	0.023
	B & C	2.10	0.062
Rancid aroma	A & B	NA	NA
	A & C		
	B & C		
Rancid flavor dry	A & B	NA	NA
	A & C		
	B & C		
Rancid flavor in milk	A & B	NA	NA
	A & C		
	B & C		

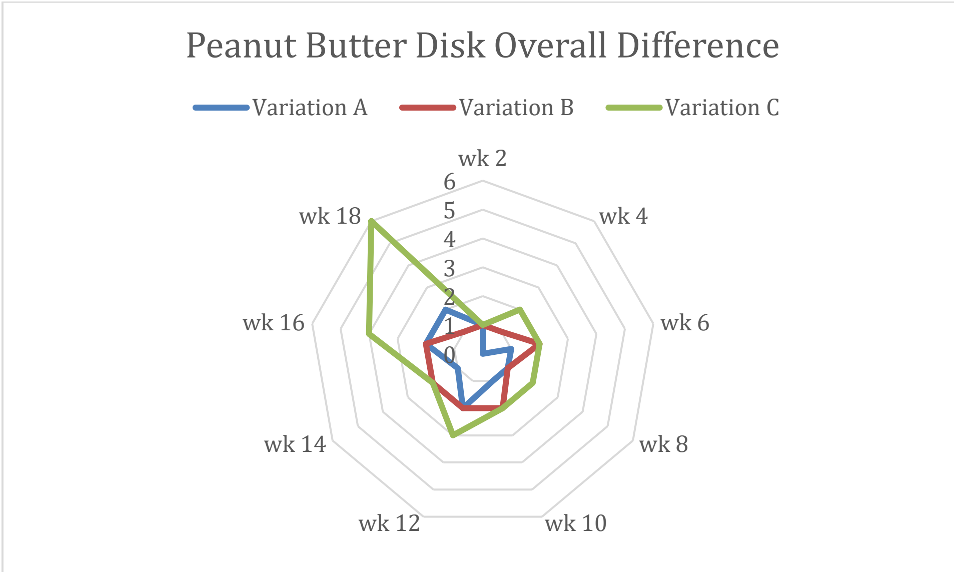

Figure 5.3: Weekly panelist ratings (n = 9) that differed significantly among test variations for the overall difference attribute in peanut butter disk cereal. Scale values began at "0" on the left end of the scale labeled "no difference" and ended at "8" on the right end of the scale labeled "large difference."

Chapter 6: Discussion

6.1 Discussion of results

Multigrain flake results show that numerous differences exist between test variations, while wheat shred and peanut butter disk results show only few differences. Puffed shape results show no differences at all. Results seem disappointing at first glance, however, we do notice two reoccurring trends. First, Variation A trended toward outperforming Variation B and/or Variation C wherever differences occurred. This was to be expected as the use of BHT within packaging material is designed to migrate into the product, providing lasting protection against oxidation within the sealed package (Wessling et al., 1998). In contrast, the use of BHT in oil ingredients is designed to preserve the oil just until use, only stalling the initiation of autoxidation (Yang et al., 2016). Second, Variation C continuously underperformed wherever differences occurred. As expected, the total removal of BHT, as represented by Variation C, resulted in a significantly reduced shelf life for cereal products. Thus, results of this study broadly align with hypotheses 1, 3, and 4.

The attributes in which Variation A was perceived as superior to other variations was not equivalent across all cereal products. For example, Variation A scored better than Variation C for the rancid aroma attribute of wheat shred cereal, but not of peanut butter disk cereal. Furthermore, Variation A scored better than Variation C for the overall difference attribute of peanut butter disk cereal, but not of wheat shred cereal. Reasons for this variance are unclear, but likely have to do with specific grain composition and the processing conditions each product was subjected to. Cereal products each contain unique precursors and flavor profiles. For example, sorghum and corn grains contain astringent and bitter tannins (Kobue-Lekaleke, Taylor, de Kock, 2007), while oat and wheat grains contain high amounts of rancid-prone unsaturated fatty acids (Heinio et al., 2016). Additionally, flavor and texture changes occur due to process-induced changes of grain biopolymers and flavor-active compounds (Mkandawire, Weier, Weller, Jackson, Rose, 2015). For example, pressure, temperature, and degree of mechanical manipulation will all induce both chemical and physical changes in the food system. The Maillard reaction is one well-studied example of how physical parameters can affect food products differently based on their specific composition. Thus, there are numerous variables that may be responsible for the acceptability of sensory attributes of one cereal but not another.

Variation B was not shown to outperform Variation C in any cereal product. This was unexpected. Though small, Variation B did contain some amount of BHT, and theoretically should have contained some level of protection from oxidation as compared to the complete removal of BHT. If nothing else, the oil used in Variation B should have been less oxidized at time zero, and therefore less progressed in the autoxidation process as compared to the BHT-free oil used in Variation C (Velasco et al., 2010). Fresh ingredients were requested for this project, but it is unrealistic to collect ingredients all having the exact same production date. It is therefore possible that the age of the oils differed enough, to the point where the BHT was no longer functioning as a protective mechanism against oxidation, leaving Variation B to be no different than Variation C. Thus, results of this study do not align with hypothesis 2.

Though not directly studied, it is interesting to see the dramatic difference in results among cereal varieties. For instance, multigrain flakes resulted in significant differences for all attributes, while puffed shapes observed no differences for any attribute. This is unexpected, as oat-based cereals are known to challenge shelf life studies. It is possible that the higher amount of sugar slurry applied to puffed shapes created a stronger barrier to oxygen as compared to the lower amount of sugar slurry used for multigrain flakes. In other words, the surface area of puffed shapes was better shielded from oxygen as compared to the multigrain flakes. It is also possible that the antioxidants naturally present in the oat flour may have contributed towards this protective effect. One study found that in a sample of 30 different oat based breakfast cereals, all of them were of significant polyphenol and antioxidant content (Ryan, Thondre, Henry, 2011). Additionally, oat based cereals have been shown to possess significant free radical scavenging activities and chelating potential, which may protect against lipid oxidation (Yu, Perret, Davy, Wilson, Melby, 2002). These findings further show the highly variable shelf life stability of cereal products.

6.2 Limitations and future research

This study was limited in number of participants (n = 3) for each tasting session. Theoretically, a sample size of 100 or more people would be needed to show true statistical significance. Additionally, a true representation of the cereal consuming population would not include educated employees of a cereal company.

Similarly, this study was limited in number of cereal varieties. Future research should focus on both cereal formulae and cereal processing technology. Replication of cereal products utilizing the same cereal processing technology would be needed to show a true trend for each processing technology. The same is true for cereal formulae; replication of cereal products utilizing the same main grain ingredient would be needed to show a true trend for each grain. Specific combinations of ingredients and technology may also be needed to further investigate the shelf life of cereal products. For instance, direct expansion can utilize a vast array of dry feed formulae, so simply studying the processing technology itself may not be enough. Researching how each grain ingredient responds to direct expansion technology may be necessary to truly represent the trend, and thus predict shelf life expectancy.

Chapter 7: Conclusion

The use of BHT in packaging material significantly improved the shelf life of multigrain flake, wheat shred, and peanut butter disk cereals. This improvement was not seen with puffed shape cereal. The use of BHT directly in canola oil did not significantly improve the shelf life of any of the four cereal products tested. The complete removal of BHT in both packaging material and canola oil significantly reduced the shelf life of multigrain flake, wheat shred, and peanut butter disk cereals. No significant reduction was noticed for puffed shape cereal. Cereal products vary greatly in formulation and processing technology, leaving numerous factors of which determine product acceptability.

Chapter 8: References

Albertson, A. M., Anderson, G. H., Crockett, S. J., Goebel, M. T. (2003). Ready-to-eat cereal consumption: its relationship with BMI and nutrient intake of children aged 4 to 12 years. *Journal of the American Dietetic Association*, *103*(12), 1613–1619. https://doi.org/10.1016/j.jada.2003.09.020

Albertson, A. M., Thompson, D., Franko, D. L., Kleinman, R. E., Barton, B. A., Crockett, S. J. (2008). Consumption of breakfast cereal is associated with positive health outcomes: evidence from the National Heart, Lung, and Blood Institute Growth and Health Study. *Nutrition Research, 28*(11), 7744-752. https://doi.org/10.1016/j.nutres.2008.09.002

Anderson, R. H., Huntley, T. E., Schwecke, W. M., Nelson, J. H. (1963). Disappearance of BHA and BHT in relation to peroxide content in breakfast cereals. *Journal of the American Oil Chemists' Society, 40*(8), 349-352. https://doi.org/10.1007/bf02631556

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Naes, T., Varela, P. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International, 99*, 58-71. http://dx.doi.org/10.1016/j.foodres.2017.07.022

Barton, B. A., Eldridge, A. L., Thompson, D., Affenito, S. G., Striegel-Moore, R. H., Franko, D. L., Albertson, A. M., Crockett, S. J. (2005). The relationship of breakfast and cereal consumption to nutrient intake and body mass index: The national heart, lung, and blood institute growth and health study. *Journal of the American Dietetic Association*, *105*(9), 1383-1389. https://doi.org/10.1016/j.jada.2005.06.003

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., Whelton, P. K. (2003). Dietary Fiber Intake and Reduced Risk of Coronary Heart Disease in US Men and Women. *Archives of Internal Medicine*, *163*(16), 1897. https://doi.org/10.1001/archinte.163.16.1897

Berglund, P. T., Fastnaught, C. E., Holm, E. T. (1994). Physicochemical and sensory evaluation of extruded high-fiber barley cereals. *Cereal Chem, 71*(1), 91-95. https://www.cerealsgrains.org/publications/cc/backissues/1994/Documents/CC1994a18.html

Bertrais, S., Polo Luque, M. L., Preziosi, P., Fieux, B., Torra de Flot, M., Galan, P., Hercberg, S. (2000). Contribution of Ready-to-Eat Cereals to Nutrition Intakes in French Adults and Relations with Corpulence. *Annals of Nutrition and Metabolism*, *44*(5–6), 249–255. https://doi.org/10.1159/000046692

Botterweck, A. A. M., Verhagen, H., Goldbohm, R. A., Kleinjans, J., van den Brandt, P. A. (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study. *Food and Chemical Toxicology*, *38*(7), 599–605. https://doi.org/10.1016/s0278-6915(00)00042-9

Burkitt, D. P. (1971). Epidemiology of cancer of the colon and rectum. *Cancer, 28*(1), 3-13. https://doi.org/10.1002/1097-0142(197107)28:1<3::AID-CNCR2820280104>3.0.CO;2-N

Butt, M. S., Ali, A., Pasha, I., Hashmi, A. M., Dogar, S. (2003). Effect of different antioxidants and packaging materials on the storage stability of breakfast cereals. *Internet Journal of Food Safety 2*, 1–5. https://www.researchgate.net/publication/288262403_Effect_of_different_antioxidants_and_packaging_materials_on_the_storage_stability_of_breakfast_cereals

Camire, M. E., Dougherty, M. P. (1998). Added phenolic compounds enhance lipid stability in extruded corn. *Journal of Food Science*, *63*(3), 516-518. https://doi.org/10.1111/j.1365-2621.1998.tb15776.x

Chandalia, M., Garg, A., Lutjohann, D., von Bergmann, K., Grundy, S. M., Brinkley, L. J. (2000). Beneficial Effects of High Dietary Fiber Intake in Patients with Type 2 Diabetes Mellitus. *New England Journal of Medicine*, *342*(19), 1392–1398. https://doi.org/10.1056/nejm200005113421903

Cho, S., Dietrich, M., Brown, C. J. P., Clark, C. A., Block, G. (2003). The Effect of Breakfast Type on Total Daily Energy Intake and Body Mass Index: Results from the Third National Health and Nutrition Examination Survey (NHANES III). *Journal of the American College of Nutrition*, *22*(4), 296–302. https://doi.org/10.1080/07315724.2003.10719307

Chulaluck, C., Nipat, L., Waraporn, P., Pisut, B. (2011). Optimization of extrusion conditions for functional ready-to-eat breakfast cereal. *Food Science and Technology Research 17*(5), 415-422. https://doi.org/10.3136/fstr.17.415

Cook, F. K, Johnson, B. L. (2009). Microbiological spoilage of cereal products. In: Sperber W., Doyle M. (Eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages* (pp. 223-244). Retrieved from https://www.springer.com/gp/book/9781441908254

Couroux, P., Chaunier, L., Della Valle, G., Lourdin, D., Séménou, M. (2005). Paired comparisons for the evaluation of crispness of cereal flakes by untrained assessors: correlation with descriptive analysis and acoustic measurements. *Journal of Chemometrics*, *19*(3), 129-137. https://doi.org/10.1002/cem.910

Culbertson, J. D. (2004). Grain, cereal: Ready-to-eat breakfast cereals. In J. Scott Smith & Y. Hui (Eds.), *Food processing principles and applications* (pp. 239-248). Retrieved from https://books.google.com

Dansby, M. A., Bovell-Benjamin, A. C. (2003). Sensory characterization of a ready-to-eat sweetpotato breakfast cereal by descriptive analysis. *Food Science*, *68*(2), 706-709. https://doi.org/10.1111/j.1365-2621.2003.tb05736.x

Djoussé, L., Gaziano, J. M. (2007). Breakfast Cereals and Risk of Heart Failure in the Physicians' Health Study I. *Archives of Internal Medicine*, *167*(19), 2080. https://doi.org/10.1001/archinte.167.19.2080

Elder, L.W. (1941). Rancidity in flaked breakfast cereals. *Oil and Soap*, *18*(2), 38-42. https://doi.org/10.1007/bf02544238

Estévez, M., Ramírez, R., Ventanas, S., Cava, R. (2007). Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *LWT - Food Science and Technology*, *40*(1), 58–65. https://doi.org/10.1016/j.lwt.2005.07.010

Food and Drug, 21 C.F.R. § 182.3173 (2019)

Fung, T. T., Hu, F. B., Pereira, M. A., Liu, S., Stampfer, M. J., Colditz, G. A., Willett, W. C. (2002). Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *The American Journal of Clinical Nutrition*, *76*(3), 535–540. https://doi.org/10.1093/ajcn/76.3.535

Hajjar, I. M., Grim, C. E., Kotchen, T. A. (2003). Dietary Calcium Lowers the Age-Related Rise in Blood Pressure in the United States: The NHANES III Survey. *The Journal of Clinical Hypertension*, *5*(2), 122–126. https://doi.org/10.1111/j.1524-6175.2003.00963.x

Heinio, R. L. (2006). Sensory attributes of bakery products. In Y. Hui, H. Corke, I. De Leyn, W. Nip, N. Cross (Eds.), *Bakery products science and technology* (pp. 293). Retrieved from https://books.google.com

Heinio, R. L., Noort, K. K., Alam, S. A., Sozer, N., de Kock, H. L., Hersleth, M., Poutanen, K. (2016). Sensory characteristics of wholegrain and bran-rich cereal foods – A review. *Trends in Food Science & Technology, 47,* 25-38. https://doi.org/10.1016/j.tifs.2015.11.002

Hocman, G. (1988). Chemoprevention of cancer: Phenolic antioxidants (BHT, BHA). *International Journal of Biochemistry, 20*(7), 639–651. https://doi.org/10.1016/0020-711x(88)90158-9

Jacobsen, C. (1999). Sensory impact of lipid oxidation in complex food systems. *European Journal of Lipid Science and Technology 101*(12), 484-492. https://doi.org/10.1002/(SICI)1521-4133(199912)101:12<484::AID-LIPI484>3.0.CO;2-H

Kamran, M., Saleem, N., & Umer, Z. (2008). Ready-to-eat (RTE) wheat bran breakfast cereals as a high-fiber diet. *Food Processing and Preservation, 32*(5), 853-867. https://doi.org/j.1745-4549.2008.00218.x

Keenan, J. M., Pins, J. J, Frazel, C., Moran, A., Turnquist, L. (2002). Oat ingestion reduces systolic and diastolic blood pressure in patients with mild or borderline hypertension: A pilot trial. *Journal of Family Practice, 51*(4), 369-369.

Kobue-Lekalake, R. I., Taylor, J. RN., de Kock, H. L. (2007). Effects of phenolics in sorghum grain on its bitterness, astringency and other sensory properties. *Journal of the Science of Food and Agriculture, 87,* 1940-1948. https://doi.org/10.1002/jsfa.2940

Krishnan, S., Rosenberg, L., Singer, M., Hu, F. B., Djoussé, L., Cupples, L. A., Palmer, J. R. (2007). Glycemic Index, Glycemic Load, and Cereal Fiber Intake and Risk of Type 2 Diabetes in US Black Women. *Archives of Internal Medicine*, *167*(21), 2304. https://doi.org/10.1001/archinte.167.21.2304

Lee, C. M., Lee, S.-Y. (2007). Consumer insights on healthy breakfast cereal – A focus group research. *Journal of Sensory Studies*, *22*(4), 417-432. https://doi-org.ezp3.lib.umn.edu/10.1111/j.1745-459X.2007.00116.x

Lee, C. M., Moskowitz, H. R., Lee, S-Y. (2007). Expectations, needs and segmentation of healthy breakfast cereal consumers. *Journal of Sensory Studies, 22*(5), 587-607. https://doi-org.ezp3.lib.umn.edu/10.1111/j.1745-459X.2007.00127.x

Macedo, I. S. M., Gallagher, M. J., Oliveira, J. C., Byrne, E. P. (2011). Shelf-life aspects of toasted breakfast cereal with and without fruit. *Italian Journal of Food Science, 23*, 178-181. Retrieved from https://www.researchgate.net/publication/292068709_Shelf-life_aspects_of_toasted_breakfast_cereal_with_and_without_fruit

Malekian, F. (2000). *Lipase and lipoxygenase activity, functionality, and nutrient losses in rice bran during storage* (Report no. 293). Retrieved from LSU Agricultural Experiment Station Reports website: http://digitalcommons.lsu.edu/agexp/293

Mkandawire, N. L., Weier, S. A., Weller, C. L., Jackson, D. S., Rose, D. J. (2015). Composition, in vitro digestibility, and sensory evaluation of extruded whole grain sorghum breakfast cereals. *LWT – Food Science and Technology*, *62*(1), 662-667. https://doi.org/10.1016/j.lwt.2014.12.051

Mohney, S. M., Hernandez, R. J., Giacin, J. R., Harte, B. R., Miltz, J. (1988). Permeability and solubility of d-limonene vapor in cereal package. *Journal of Food Science*, *53*(1), 253-257. https://doi.org/10.1111/j.1365-2621.1988.tb10222.x

Nachay, K. (2017, November 1). Clean Label Approaches to Food Safety. *IFT, 71*(11). https://www.ift.org/news-and-publications/food-technology-magazine/issues/2017/november/columns/ingredients-that-provide-food-safety-food-preservation

Onyeneho, S. N., Hettiarachchy, N. S. (1992). Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry, 40*(9), 1496-1500. https://doi.org/10.1021/jf00021a005

Pasca, C., Cororian, A., Socaci, S. (2018). Risks and benefits of food additives – review. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Animal Science and Biotechnologies*, *75*(2), 71-79. http://doi.org/10.15835/buasvmcn-asb: 2018.0026

Paradiso, V. M., Caponio, F., Summo, C., Gomes, T. (2012). Influence of some packaging materials of natural tocopherols on the sensory properties of breakfast cereals. *Food Science and Technology International, 20*(3), 161-170. https://doi.org/10.1177/1082013213476074

Paradiso, V. M., Summo, C., Trani, A., Caponio, F. (2007). An effort to improve the shelf life of breakfast cereals using natural mixed tocopherols. *Journal of Cereal Science*, *47*(2), 322-330. https://doi.org/10.1016/j.jcs.2007.04.009

Pryor, W. A., Cornicellie, J. A., Devall, L. J., Tait, B., Trivedi, B. K., Witiak, D. T., Wu, M. (1993). A rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants. *Journal of Organic Chemistry, 58*(13), 3521-3532. https://doi.org/10.1021/jo00065a013

Ryan, L., Thondre, P. S., Henry, C. J. K. (2011). Oat-based breakfast cereals are a rich source of polyphenols and high in antioxidant potential. *Journal of Food Composition and Analysis*, *24*(7), 929–934. https://doi.org/10.1016/j.jfca.2011.02.002

Sauvageot, F., Blond, G. (1991). Effect of water activity on crispness of breakfast cereal. *Journal of Textural Studies, 22*(4), 423-442. https://doi.org/10.1111/j.1745-4603.1991.tb00502.x

Sebranek, J. G., Sewalt, V. J. H., Robbins, K. L., Houser, T. A. (2005). Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Science*, *69*(2), 289–296. https://doi.org/10.1016/j.meatsci.2004.07.010

Senhofa, S., Straumite, E., Sabovics, M., Klava, D., Galoburda, R., Rakcejeva, T. (2015). The effect of packaging type on quality of cereal muesli during storage. *Agronomy Research*, *13*(4), 1064-1073. https://www.researchgate.net/publication/281731804_The_effect_of_packaging_type_on_quality_of_cereal_muesli_during_storage

Sewald, M., DeVries, J. Shelf life testing (2003). *Medallion Laboratories Analytical Progress*. http://foodnutritionhaccp.tripod.com/sitebuildercontent/sitebuilderfiles/shelf-life-studies-guidelines.pdf

Slavin, J. (2010). Whole grains and digestive health. *Cereal Chemistry*, *87*(4), 292-296. https://doi.org/10.1094/CCHEM-87-4-0292

Smith, A. P. (2003). Breakfast cereal consumption and subjective reports of health by young adults. *Nutritional Neuroscience*, *6*(1), 59-61. https://doi.org/10.1080/1028415021000042820

Song, W. O., Chun, O. K., Obayashi, S., Cho, S., Chung, C. E. (2005). Is Consumption of Breakfast Associated with Body Mass Index in US Adults. *Journal of the American Dietetic Association*, *105*(9), 1373–1382. https://doi.org/10.1016/j.jada.2005.06.002

Suh, H.-J., Chung, M.-S., Cho, Y.-H., Kim, J.-W., Kim, D.-H., Han, K.-W., Kim, C.-J. (2005). Estimated daily intakes of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) andtert-butyl hydroquinone (TBHQ) antioxidants in Korea. *Food Additives and Contaminants*, *22*(12), 1176–1188. https://doi.org/10.1080/02652030500195288

Tietyen, J. L., Fleming, K. H. (1995). Nutrient intatke of breakfast vs. non-breakfast eaters. *Journal of the American Dietetic Association, 95*(9), A-55. https://doi.org/10.1016/S0002-8223(95)00537-4

Ulland, B. M., Weisburger, J. H., Yamamoto, R. S., Weisburger, E. K. (1973). Antioxidants and carcinogenesis: butylated hydroxytoluene, but not diphenyl-p-phenylenediamine, inhibits cancer induction by N-2-fluorenylacetamide and by N-hydroxy-N-2-fluorenylacetamide in rats. *Food and Cosmetics Toxicology*, *11*(2), 199–207. https://doi.org/10.1016/s0015-6264(73)80486-9

Velasco, J., Dobarganes, C., Marquez-Ruiz, G. (2010). Oxidative rancidity in foods and food quality. In L. Skibsted, J. Risbo, & M. Anderson (Eds.), *Chemical deterioration and physical instability of food and beverages* (pp. 3-28). Retrieved from https://books.google.com

Viscidi, K. A., Dougherty, M. P., Briggs, J., Camire, M. E. (2004). Complex phenolic compounds reduce lipid oxidation in extruded oat cereals. *LWT – Food Science and Technology, 37*(7), 789-796. https://doi.org/10.1016/j.lwt.2004.03.005

Waller, S. M., Vander Wal, J. S., Klurfeld, D. M., McBurney, M. I., Cho, S., Bijlani, S., Dhurandhar, N. V. (2004). Evening Ready-to-Eat Cereal Consumption Contributes to Weight Management. *Journal of the American College of Nutrition*, *23*(4), 316–321. https://doi.org/10.1080/07315724.2004.10719374

Wang, W, Kannan, K. (2019). Quantitative identification of exposure to synthetic phenolic antioxidants, including butylated hydroxytoluene, in urine. *Environment International*, *128*, 24-29. https://doi.org/10.1016/j.envint.2019.04.028

Wessling, C., Nielsen T., Leufvén, A., Jägerstad, M. (1998). Mobility of α-tocopherol and BHT in LDPE in contact with fatty food simulants. *Food Additives & Contaminants, 15*(6), 709-715. http://doi.org/10.1080/02652039809374701

Yang, Y. Song, X., Sui, X., Qi, B., Wang, Z., Li, Y., Jiang, L. (2016). Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. *Industrial Crops and Products*, *80*, 141-147. https://doi.org/10.1016/j.indcrop.2015.11.044

Yu, L., Perret, J., Davy, B., Wilson, J., Melby, C. L. (2002). Antioxidant Properties of Cereal Products. *Journal of Food Science*, *67*(7), 2600–2603. https://doi.org/10.1111/j.1365-2621.2002.tb08784.x

Chapter 9: Appendices

Appendix A: Cereal coating formulae

Project:	Thesis	Date:	8/2/2019
Description:	Multigrain Flake Coating		
Label:	Sam Baumann | Thesis Coating | Multi flake		
Dry Temp/Time:	275 degrees, 10 min		

Part 1:
Sugar coating

Recipe: Multigrain flake	
Ingredient	**% Batch**
Cereal base	85%
SUGAR SYRUP/SLURRY	14%
Canola oil (with/without BHT)	1%
TOTAL	100%

SUGAR SYRUP/SLURRY	
Ingredient	**% Batch**
Water	25%
Sugar, white	75%
TOTAL	100%

PROCEDURE/SPECIAL INSTRUCTIONS

1 Place base in enrobing drum
2 Make sugar syrup
3 Add sugar syrup to base cereal in drum
4 Scrape sides of drum
5 Add oil, let tumble for 2 minutes
6 Spread on tray and dry at temp and time listed above
7 Store in cereal liner bag

Project:	Thesis	Date:	8/4/2019
Description:	Wheat Shred Coating		
Label:	Sam Baumann | Thesis Coating | Wheat shred		
Dry Temp/Time:	265 degrees, 10 min		

Part 1:
Sugar coating

Wheat shred	
Ingredient	**% Batch**
Cereal base	88%
SUGAR SYRUP/SLURRY	11%
Canola oil (with/without BHT)	1%
TOTAL	100%

SUGAR SYRUP/SLURRY	
Ingredient	**% Batch**
Water	30%
Sugar, white	70%
TOTAL	100%

PROCEDURE/SPECIAL INSTRUCTIONS

1. Place base in enrobing drum
2. Make sugar syrup
3. Add sugar syrup to base cereal in drum
4. Scrape sides of drum
5. Add oil, let tumble for 2 minutes
6. Spread on tray and dry at temp and time listed above
7. Store in cereal liner bag

Project:	Thesis	Date:	8/6/2019
Description:	Puffed Shape Coating		
Label:	Sam Baumann | Thesis Coating | Puffed shape		
Dry Temp/Time:	200 degrees, 30 min		

Part 1:
Sugar coating

Recipe: Puffed shape	
Ingredient	**% Batch**
Cereal base	63%
SUGAR SYRUP/SLURRY	36%
Canola oil (with/without BHT)	1%
TOTAL	100%

SUGAR SYRUP/SLURRY	
Ingredient	**% Batch**
Water	30%
Sugar, white	70%
TOTAL	

PROCEDURE/SPECIAL INSTRUCTIONS

1. Place base in enrobing drum
2. Make sugar syrup
3. Add sugar syrup to base cereal in drum
4. Scrape sides of drum
5. Add oil, let tumble for 2 minutes
6. Spread on tray and dry at temp and time listed above
7. Store in cereal liner bag

Project:	Thesis	Date:	8/8/2019
Description:	Peanut Butter Disk Coating		
Label:	Sam Baumann | Thesis Coating | Peanut butter disk		
Dry Temp / Time:	200 degrees, 30 min		

Part 1:
Sugar coating

Recipe: Peanut butter disk	
Ingredient	**% Batch**
Cereal base	62%
SUGAR SYRUP/SLURRY	37%
Canola oil (with/without BHT)	1%
TOTAL	100%

SUGAR SYRUP/SLURRY	
Ingredient	**% Batch**
Water	33%
Sugar, white	56%
Corn syrup	11%
TOTAL	100%

Part 2:
Peanut butter coating

Recipe: Peanut butter disk	
Ingredient	**% Batch**
Sugar coated cereal	70%
PEANUT BUTTER SLURRY	20%
Dextrose	10%
TOTAL	100%

PEANUT BUTTER SLURRY	
Ingredient	**% Batch**
Peanut butter	80%
Salt	1%
Canola oil (with/without BHT)	19%
TOTAL	100%

PROCEDURE/SPECIAL INSTRUCTIONS

1. Place base in enrobing drum
2. Make sugar syrup

3 Add sugar syrup to base cereal in drum
4 Scrape sides of drum
5 Add oil, let tumble for 2 minutes
6 Spread on tray and dry at temp and time listed above
7 Let cool
8 Return base to enrobing drum
9 Make peanut butter slurry
10 Add peanut butter slurry to sugar coated base cereal in drum
11 Let tumble for 1 minute
12 Add dextrose, let tumble for 2 minutes
13 Store in cereal liner bag

Appendix B: Shelf life testing pull schedule

Sample Variables	Storage Conditions	Week 0 8/19/2019	Week 2 9/2/2019	Week 4 9/16/2019	Week 6 9/30/2019	Week 8 10/14/2019	Week 10 10/28/2019	Week 12 11/11/2019	Week 14 11/25/2019	Week 16 12/9/2019	Week 18 12/23/2020
Control	Elevated Temp. (100 °F)	N/A	1	1	1	1	1	1	1	1	1
Test 1 (A)	Elevated Temp. (100 °F)	N/A	1	1	1	1	1	1	1	1	1
Test 2 (B)	Elevated Temp. (100 °F)	N/A	1	1	1	1	1	1	1	1	1
Test 3 (C)	Elevated Temp. (100 °F)	N/A	1	1	1	1	1	1	1	1	1

Appendix C: Shelf life ballot template

Product/Project:	Cereal Product/Thesis	Name:	
Storage Condition:	Accelerated/Ambient	Shelf Life Pull:	Mo/Wk
Sample Code:	Test 1 (A)/Test 2 (B)/Test 3 (C)	Date:	

Dry Evaluation

Aroma:									
Deviation From Control		-3	-2	-1	Control	+1	+2	+3	
Rancid Aroma	Low								High
Other Off-Aroma	Low								High
Flavor Aroma	Low								High
Chemical Notes	Low								High
Diff From Control	No/ Slight Difference			Moderate Difference			Large Difference		
	0	1	2	3	4	5	6	7	8
Comments:									

Flavor Dry:									
Deviation From Control		-3	-2	-1	Control	+1	+2	+3	
Rancidity	Low								High
Off-Flavor	Low								High
Flavor Intensity	Low								High
Chemical Notes	Low								High
Diff From Control	No/Slight Difference			Moderate Difference			Large Difference		
	0	1	2	3	4	5	6	7	8
Comments:									

Texture:									
Deviation From Control		-3	-2	-1	Control	+1	+2	+3	
Hardness	Low								High
Crispness	Low								High
Diff From Control	No/Slight Difference			Moderate Difference			Large Difference		
	0	1	2	3	4	5	6	7	8
Comments:									

Evaluation In Milk

Flavor in Milk:									
Deviation From Control		-3	-2	-1	Control	+1	+2	+3	
Rancidity	Low								High
Off-Flavor	Low								High
Flavor Intensity	Low								High
Chemical Notes	Low								High

Difference From Control	No Difference Slight Difference			Moderate Difference			Large Difference		
	0	1	2	3	4	5	6	7	8
Comments:									

Texture:									
Deviation From Control		-3	-2	-1	Control	+1	+2	+3	
Hardness	Low								High
Crispness	Low								High

Difference From Control	No Difference Slight Difference			Moderate Difference			Large Difference		
	0	1	2	3	4	5	6	7	8
Comments:									

Overall Difference From Control	**No Difference Slight Difference**			**Moderate Difference**			**Large Difference (Failure)**		
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Comments:									

Appendix D: Sample Excel analysis of variance testing

ANOVA Testing (overall difference)			
Multigrain flake	Variation A	Variation B	Variation C
wk 2	0	0	1
wk 4	1	1	2
wk 6	0	1	2
wk 8	1	2	3
wk 10	1	3	6
wk 12	2	5	8
wk 14	1	5	7
wk 16	1	6	7
wk 18	2	6	8
Average	1	3	5

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	9	1	0.5
Column 2	9	29	3.222222222	5.444444444
Column 3	9	44	4.888888889	8.111111111

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	68.5185	2	34.2592	7.3122	0.00331	3.4028
Within Groups	112.444	24	4.6851			
Total	180.962	26				

Appendix E: Sample Excel t-test testing

t-Test: Two-Sample Assuming Unequal Variances

Between A&B

	Variable 1	*Variable 2*
Mean	0.666666667	1.555555556
Variance	0.25	0.777777778
Observations	9	9
Hypothesized Mean Difference	0	
df	13	
t Stat	-2.630383797	
P(T<=t) one-tail	0.010386125	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	0.020772251	
t Critical two-tail	2.160368656	

t-Test: Two-Sample Assuming Unequal Variances

Between A&C

	Variable 1	*Variable 2*
Mean	0.666666667	2.333333333
Variance	0.25	0.75
Observations	9	9
Hypothesized Mean Difference	0	
df	13	
t Stat	-5	
P(T<=t) one-tail	0.000121477	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	0.000242954	
t Critical two-tail	2.160368656	

t-Test: Two-Sample Assuming Unequal Variances

Between B&C

	Variable 1	*Variable 2*
Mean	1.555555556	2.333333333
Variance	0.777777778	0.75
Observations	9	9
Hypothesized Mean Difference	0	
df	16	
t Stat	-1.887759615	
P(T<=t) one-tail	0.038666085	
t Critical one-tail	1.745883676	
P(T<=t) two-tail	0.07733217	
t Critical two-tail	2.119905299	