USE OF CITRUS FIBER IN GROUND BEEF MEATBALLS AS A FUNCTIONAL INGREDIENT

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by

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USE OF CITRUS FIBER IN GROUND BEEF MEATBALLS AS A FUNCTIONAL INGREDIENT

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ABSTRACT

In recent years, consumer's food choices have shifted towards healthy foods due to an increased concern about coronary heart disease and diabetes. Food products with high fat and cholesterol content have been associated with these health problems and consumption of processed meat products have been linked to these diseases. Research in developing healthy meat products is becoming more crucial.

Therefore, the objective of this study was to determine the use of citrus fiber in ground beef meatballs as a functional ingredient. The study was conducted in four phases. In the first phase, presence of flavonoid compounds in a citrus fiber was measured by reverse-phase high-pressure liquid chromatography along with total polyphenol content, total carotenoid content and oxygen radical absorbance capacity. Results showed that citrus fiber used in this study had a trace amount of quercetin and kaempferol, and low concentrations of nobiletin, sinensetin, heptamethoxyflavone and tangeretin.

For the second phase of the study, the impact of adding citrus fiber on quality attributes of ground beef meatballs were investigated. For this study, citrus fiber was used at four different levels (0%, 1%, 5% and 10%) in ground beef meatballs and tested over four different days at 0, 3, 6 and 9. While addition of citrus fiber increased cooking yield

and water holding capacity, when it was used at 5% and 10%, the levels caused detrimental change in texture of cooked meatballs and Hunter color L, a, b values of raw ground beef.

The third phase of the study determined the oxidative stability of ground beef meatballs made with (0%, 1% 3% and 5%) citrus fiber levels. The stability was evaluated using Fourier transform infrared spectroscopy. For monitoring lipid oxidation, peaks at 2924 cm^{-1}, 2853 cm^{-1} and 1743 cm^{-1} were used. Results showed that addition of citrus fiber caused a greater decrease in absorbance in comparison to the control (CF 0%) at bands 2924 cm^{-1} and 2853 cm^{-1} due to presence of oxidation products. The band at 1743 cm^{-1} was also a useful indicator. By day 3, a weak band appeared at 1728 cm^{-1} indicating presence of oxidation products such as aldehydes and ketones, and spectra of CF 3% and CF 5% showed a more apparent band than control and CF 1%.

In the final phase of the study, consumer preferences for citrus fiber added meatballs were tested. Four levels (0%, 1%, 3% and 5%) of citrus fiber were used for the meatball formulation. An untrained panel of 164 people tasted ground beef meatballs for flavor, texture and overall likeness. Panelists gave scores based on a hedonic scale of 1 (dislike extremely) to 9 (like extremely). Results showed that consumer acceptance for meatballs made with 1% citrus fiber was not significantly ($P > 0.05$) different than that of control meatballs (CF 0%). Use of citrus fiber at 1% level in ground beef meatballs had been moderately liked with a mean of 6.61 for flavor and 6.56 for overall likeness. In summary, citrus fiber at 1% level can be used in the ground beef meatballs to improve cooking yield and water holding capacity and provide fiber without impacting quality and sensory attributes.

CHAPTER 1: INTRODUCTION

In recent years consumers' food choices have been shifted towards healthy foods due to increased concern with coronary heart disease (CHD), obesity, diabetes, and cancer. It has been estimated that more than 80 million people have been affected by coronary heart disease, stroke, and hypertension. These diseases are the leading causes of morbidity and mortality in the United States (Rosamond and others 2008). It is suggested that CHD risk factors are diet, tobacco use, alcohol abuse, physical inactivity, environment, infection, pollution, and genetic factors. Diet has been found to be the leading contributor for CHD (Kris-Etherton and others 2002). Obesity is another major health concern in the United States that has been linked to comorbidities, such as CHD and diabetes, and increasing its prevalence in past several decades. Increase in obesity has been also observed in children and according to Ogden and others (2002) childhood obesity increased from 5% of the children in the early 1970s to 16% of the children in the 2000s. Due to higher energy intake than expenditure, excess energy is generally stored in the adipose tissues as fat and leading to obesity (Kovacs and Mela 2006).

Foods with high fat concentration especially with high-saturated fats have been associated with obesity and CHD. This impacts also consumer decisions. According to consumer attitudes and the supermarket survey of 2000, consumers' top nutritional concerns are fat content (46%), cholesterol (17%), salt/sodium (17%), sugar (13%), nutritional value (12%), calories (9%), and chemical additives (7%). The same survey also stated that consumers are changing their diet and 68% of the consumers eat more vegetables, 22% of the consumers eat less meat and 23% consumes less fats and oils

(Food Marketing Institute 2000). Micha and others (2010) conducted a systematic review and meta analysis. Results indicated that processed red meat consumption was associated with higher incidence of CHD and diabetes mellitus. Larsson and others (2011) found similar results. The consumption of processed meat had positive correlation with the risk of stroke. Lajous and others (2011) found direct association between processed red meats and type-2 diabetes.

Even though meat is one of the nutritious foods for human consumption that has high quality proteins, is rich in B Vitamins and Vitamin A and minerals like iron (Aberle and others 2001), many studies link processed meat consumption with CHD, stroke and diabetes, due to presence of saturated fats and cholesterol. It is important to produce meat products with significantly less total fat and saturated fat and also provide additional health benefits. Presence of fat in food products impacts the flavor profile, moutfeel, texture and juiciness (Pearson and Gillett 1999). It has been reported that fat in meat products increased tenderness and juiciness. Reduction in the fat content can negatively impact the sensory attributes of the food products particularly flavor (Jimenez Colmenero 2000). Therefore, it is important to investigate fat replacers that can reduce the fat content of meat products without negatively impacting flavor, texture, moutfeel and juiciness of meat products and also provides health benefits. One of those fat replacers is fiber. Dietary fiber is described as polysaccharides and lignin part of the plants that are not digestible by human digestive enzymes (Bermink 1994). Recently dietary fiber definitions also include oligosaccharides, inulin and resistant starches (Jones and others 2006).

Presence of dietary fiber in meat products can provide function of reducing fat content, in addition to creating bulk or replacing fat. Generous consumption of fiber has been associated with reducing the risk of coronary heart disease (Liu and others 1999), stroke (Steffen and others 2003), hypertension (Whelton and others 2005), diabetes (Montenen and others 2003), and obesity (Lairon and others 2005). Furthermore, use of fiber from natural resources such as citrus fiber can also help with reducing oxidation of the product and this may have further antioxidant benefits.

This study will contribute to food industry and consumer health. Use of this ingredient in meat products such as hamburgers, meatballs, and sausages can have multiple benefits for beef producers, byproduct producers, consumers, and for the environment. Since there is a negative marketing trend towards meat products due to their high cholesterol and fat content and having no natural fiber, use of citrus fiber may increase marketability and sale of certain partially processed or processed meat products. Furthermore, citrus peels are the byproducts of juice production, which are mostly underutilized. Use of these byproducts in meat products opens the door for developing new ingredients from natural sources and reduces the environmental waste. Also, use of citrus fiber in meat products supports the clean label policy since they are not additives, which will also attract consumer. Most importantly, use of antioxidant fiber in hamburgers, can provide fiber with antioxidant properties that may provide many health benefits, which will impact consumers' buying decisions. Therefore, the objectives of the study are

1.1. Objectives

1. Determine if citrus fiber have antioxidant properties and identify presence of flavonoids in citrus fiber
2. Evaluate the effects of citrus dietary fiber on physical and chemical properties of ground beef meatballs
3. Evaluate the impact of adding citrus fiber on lipid oxidation of ground beef meatballs during cold storage.
4. Determine the acceptability of the citrus fiber ground beef meatball by a consumer panel.

CHAPTER 2: LITERATURE REVIEW

2.1. Lipids

Lipids are naturally occurring organic compounds that have limited solubility in water. However, they are soluble in organic, non-polar solvents such as hexanes, chloroform, ether and alcohols (Gurr and Harwood 1991; Gruen and Duncan 2007). Lipids that are solid at room temperature are called fats, and liquid ones are called oils. Lipids are classified in three groups: simple lipids, such as triglycerides and waxes; compound lipids, such as glycolipids and phospholipids; derived lipids, such as free fatty acids, sterols and fat soluble vitamins (Ensminger and others 1993; Gruen and Duncan 2007).

Lipids are one of the main energy sources in foods. They provide 9 kcal of energy for per gram of lipid consumed. In addition to providing energy, lipids are the carrier of fat-soluble vitamins A, D, E, K, and provide sterols such as cholesterol and they can impact food quality by influencing the texture, flavor, mouthfeel and color of foods (Kinsella 1988; Gruen and Duncan 2007).

Lipids can come from animal and plant origins. Plant lipids can come from seeds, such as sunflower seed, or they can come from pulp, such as olive. Pigs, beef, mutton, veal, chicken, turkey are sources of animal fats. Fats rendered from animals can come from subcutaneous fats or deposit fats. Subcutaneous fats are found under the skin of animals. Also, there are fat deposits surrounding the internal organs such as kidneys and liver. There are also inter-muscular fat, which located between the muscles and intra-muscular fat, which is located in the muscles.

Both subcutaneous fat, fat deposits around the organs, and removable inter muscular fats can be used in processed meat production. Pig fat and beef fat are more commonly used. Pig fats from jaw and chicks and beef fat from the brisket area are more common. Fat deposits around the organs are not commonly used for processed meat products due to their softness and aroma. Also, fats from older animals and fat from muttons are not commonly used, due to their strong aroma.

According to the USDA (2005) beef fat has a total of 46.1 % saturated fatty acids, 49.5 % mono-unsaturated fatty acids and 4.4% total poly-unsaturated fatty acids. Pork fat has 38.7 % saturated fatty acids, 49.5 % total mono-unsaturated fatty acids, 11.8 % poly-unsaturated fatty acids. Lamb fat has 46.9 % saturated fatty acids, 44.5 % mono-unsaturated fatty acids, and 8.6 % poly-unsaturated fatty acids. Fat content of separable lean for raw beef is 6.16 % and cooked is 9.91 %, for raw pork is 6.75 % and cooked is 13.04 %, for raw lamb is 7 % and cooked is 8.5 % (Aberle and others 2001). Furthermore, cholesterol level in meat products found to be highest in organs, such as brain with 1700 mg/3 oz, kidney with 680 mg/3 oz and liver with 370 mg /3 oz. Red meat such as beef, pork, lamb, veal has 70-85 mg/ 100 g cholesterol. Therefore, processed meat products that include organs can have much higher cholesterol levels. (Aberle and others 2001).

Animal fats in meat products provide nutritional, functional and sensory properties. They are high-density energy sources, can protect products from cooking shrinkage, and also create bulk in processed food products. Also, they are used for transferring heat (ADA 2005). Furthermore, they can provide smoothness, mouthfeel, flavor etc. Meat steaks with high intra-muscular fat, which is called marbling, have been

associated with tenderness. Shorthose and Harris (1991) found a positive correlation between intramuscular fat and tenderness and juiciness. Also, Neely and others (1998) found a strong correlation between tenderness and overall likeness of the product. Shorthose and Harris (1991) also suggested that tender meat can influence the perception of the other factors through a sort of "halo effect". If the meat is tender, consumers see it as juicy and flavorful. Cofrades and others (2000) found correlation between overall likeness of the emulsified type of meat products with presence of fats.

Fats play an important role in processed meat production. Reducing or replacing fat may impact the sensory characteristics of food products and change their functionality. Therefore, fat replacement alternatives should be well investigated.

2.2. Fat Replacers

Fat replacers are ingredients that can replace fat and chemically resemble fats, proteins or carbohydrates. There are four descriptions used for fat replacers. These are fat substitutes, fat analogs, fat extenders and fat mimetics (ADA 2005).

Fat substitutes are macromolecules that physically or chemically resemble triglycerides, but show resistance to digestive enzymes and theoretically replace fat in foods on a weight-by-weight basis. Fat analogs are described as compounds similar to fats but they have altered digestibility and different nutritional values. Fat extenders help with improving functionality of fat in the products in which reduced amount of fat is used (ADA 2005). Fat mimetics are described as ingredients that can imitate organoleptic and physical properties of fats and require water to function. However, they cannot replace fats on a weight-by-weight basis (Jones 1996b; Akoh 1998).

2.2.1. Fat Substitutes

2.2.1.1. Carbohydrate esters and polyesters

Fat substitutes are carbohydrate esters and polyesters that include sucrose fatty acid polyesters and sucrose fatty acid esters. Sucrose fatty acid polyesters are produced by interesterification of sucrose acyl groups with six to eight saturated and unsaturated fatty acids. This molecule cannot be hydrolyzed by digestive lipases. Therefore, it is non-caloric. Kelly and others (1998) investigated the use of sucrose polyester (SPE) as fat replacers in a double blind controlled feeding trial of human volunteers. Volunteers were fed with 20-40 g of SPE daily for three months. Results showed that plasma cholesterol and triglyceride levels were reduced. However, consumption of SPE caused some gastrointestinal problems and also reduced plasma concentrations of Vitamin E and six carotenoids. Weststrate and Van het Hof (1995) also found similar result related to reduction of Vitamin E and beta carotenoid in plasma. The commercial product of this category is called Olestra (The Procter & Gamble Co., Cincinnatti, OH) and the FDA approved Olestra for replacing fat in savory snacks. Consumption of products with Olestra can be beneficial for people with weight issues, but over consumption of Olestra may cause some digestive issues and reduces the absorption of fat-soluble vitamins. Therefore, the FDA requires labeling of products including Olestra (Akoh 1998; Gruen and Duncan 2007).

Another fat substitutes, which are made in a manner similar to sucrose polyesters, are called sucrose fatty acid esters (SFE). Acyl groups of SFEs are esterified with one to three fatty acids (Osipow and others 1956; Akoh 1998). Due to the low degree of

esterification, SFEs can be hydrolyzed by digestive lipases. Thus, they are caloric. SFEs' hydrophilic and lipophilic properties give them surfactant and emulsification qualities. They are also used for lubricants, anticaking agent, thinning agent and as antimicrobials. They have been also used as coating agent to prevent fruit ripening and spoilage in the United States (Harrigan and Breene 1993; Marshall and Bullerman 1994; Akoh 1998).

Another type of synthetic fatty acid ester is polyol fatty acid esters. A minimum four hydroxyl group containing polyols are esterified with fatty acids to synthesize polyol fatty acid esters in the presence of a catalyst (Unilever 1988). Polyols that have at least four-hydroxyl group are sorbitol, trehalose, raffinose, and stachyose polyesters (Akoh 1994). One of the polyol fatty acid esters is Sorbestrin (Cultor Food Science Inc., NY). It is a sorbitol polyester. It has approximately 1.5 Kcal/g caloric value. It is not commercially available yet, however, its intended use is to replace fat in salad dressings and baked goods. Also, due to its heat stable characteristic, it can be used as fat substitute for frying as well (Akoh 1994, Akoh 1998).

2.2.1.2. Structured lipids

Structured lipids are another category of fat substitutes. They are produced by chemical or enzymatic or random transesterification of glycerol with short, medium and long chain fatty acids (Akoh 1998). They are produced for specific reasons, such as reducing calories (Akoh 1995), providing higher oxidative stability and low viscosity (Megremis 1991), being a rapidly utilizable energy source and a resource for fat-soluble vitamins (Gruen and Duncan 2007), providing a range of melting points, hardness and appearance (Kosmark 1996). One of those structured lipids is Medium chain triglycerides

(MCTs). These contain mostly saturated fatty acids of 8 to 10 carbon chain. MCTs are manufactured from vegetable oils such as coconut and palm kernel oil and they are stable under high and low temperatures and don't easily oxidized (Babayan and Rosenau 1991). MCTs provide readily absorbed and rapidly utilizable energy of 8.3 kcal/g (Megremis 1991).

Caprenin (The Procter & Gamble Co.) is another structured lipid. Caprenin is manufactured by esterification of glycerol with caprylic (C8:0), capric (C10:0) and behenic (C22:0) fatty acids, and is technically called a caprocaprylobehenic tryglyceride. Because long chain behenic acid is not completely absorbed and the other two medium chain fatty acids are readily absorbed, caprenin has only 5 kcal/g (Akoh 1998). The intended use of caprenin was to replace cocoa butter in confectionary. The Procter and Gamble Co. filed a generally recognized as safe (GRAS) affirmation petition (CCC 1996) for caprenin as a fat substitute in soft candy and confectionary coatings. Products like M&M bars, Milky Way II were produced with caprenin, however, due to caprenin being difficult to temper, products manufactured with caprenin were pulled from the market (Akoh 2008).

Another structured lipid used as fat substitute is Salatrim. Salatrim is a short and long acyl triglyceride molecule that has at least one short chain fatty acid such as C2:0, C3:0 or C4:0 and at least one long chain fatty acid such as C18:0 randomly attached to glycerol backbone (Akoh 1998). It has only about half of the calories of regular fat with 5 kcal/g. This is because short chain fatty acids having lower caloric values and stearic acid being absorbed incompletely. Commercially Salatrim is known as Benefat. It was developed by Nabisco Food Group, Parsippany, NJ, later was licensed to Cultor Food

Science, New York, NY. Benefat I was developed to replace cocoa butter in confectionary (Akoh 2008). It is well suited for use as chocolate flavor coating, savory dressings, frozen dairy desserts, and cheese (Kosmark 1996; Akoh 1998). However, it cannot be used for deep fat frying of any food products due to the highly volatile nature of short chain fatty acids (Akoh 2008).

2.2.2. Fat Mimetics

Fat mimetics have described as ingredients that can mimic the sensory and physical properties of fats. They are not able to replace fat on a gram-for-gram bases. Fat mimetics are either protein based or carbohydrate based and their caloric value is 0-4 kcal/g (Akoh 1998). Ideal fat mimetics should be safe, physiologically inert, nutritionally equivalent, create the feeling of fat (Stern Hermann-Zaidins 1992) and be able to transfer heat (Jones 1996a).

2.2.2.1. Protein based fat mimetics

Protein based fat mimetics can be obtained from milk, egg, whey, soy, gelatin and wheat gluten. Some of these proteins also can be processed under heat and shear to produce microparticles, which are called microparticulated proteins (MPP). MPPs provide mouthfeel and texture of fat (Akoh 1998). They are also digested as proteins, therefore provide fewer calories and no cholesterol (Singer 1996) but carry the antigenic or allergenic properties of proteins (Gershoff 1995). Microparticulated proteins can be used in pasteurization, retorting etc. But they cannot be used for frying due to lacking

stability against very high heat. MPPs are mostly used in dairy products, cheeses, ice creams, butter, sour cream etc. (Omayma and Youssef 2007).

One of those protein based fat mimetics is Simplesse®. It is a MPP made from whey protein concentrate. It was developed by NutraSweet Co and has been affirmed as GRAS (21 CFR 184.1498) in 1990 for frozen desserts and in 1994 for yogurt, cheese spreads, cream cheese and sour cream (Akoh 1998). Simplesse provides 4 kcal/g on dry, 1 kcal/g on wet basis (Akoh 1998). Another protein-based fat mimetic is Dairy Lo®(Parmalat Ingredients, Canada). It is produced from thermal denaturation of proteins of sweet whey. It provides mouthfeel and texture of fat in low fat foods and affirmed GRAS by FDA (Owusu-Apenten 2005, Omayma and Youssef 2007).

The study conducted by Prindiville and others (2000) investigated the effect of Simplesse and Dairy Lo fat replacers on quality of lowfat and nonfat chocolate ice creams in comparison to milk fat and cocoa butter. Results showed that Simplesse showed similar results to milk fat with a less intense cocoa flavor and resistance to textural changes. Laneuville and others (2005) investigated the effect of protein-carbohydrate complex system based on whey protein and xanthan gum on quality of low fat cake frosting and sandwich cookie filling. Results were promising because low fat products made with fat mimetics were similar to regular products.

In another study, researchers investigated the effect of skim milk co-precipitate on quality of low-fat ground pork patties. According to the results, 7% level skim milk co-precipitate as fat mimetics showed better microbiological stability and sensory properties and lower TBA values in comparison to control under refrigerated storage in air permeable films for 21 days (Kumar and Sharma 2003).

2.2.2.2. Carbohydrate based fat mimetics

The American Dietetic Association (2005) categorizes the carbohydrate based fat mimetics into 8 groups. These are cellulose based (e.g. Avicel and Just Fiber), Dextrins and modified starches (e.g. Stellar, N-Lite-S and Inscosity), fruit based fiber (e.g. dried plum paste, prune paste, WonderSlim), grain based fibers (e.g. Betatrim and Z-trim), Hydrocolloid gums (e.g. Kelgum, Keltrol and Kelcogel), maltodextrin (e.g. Paselle and Maltrin), pectin (e.g. Grindsted and Splendid) and polydextrose (e.g. Gelcarin, Litesse and Sta-Lite). Carbohydrate based fat mimetics have been used for replacing fat in several foods such as low fat cookies, salad dressings, desserts, ice creams, meat products etc. (Akoh 1998). They have been mostly used as thickening agent and for gel formation that provides mouthfeel and texture of fat, but they are not suitable for frying. They generally provide 4 kcal/g, except some of them provide fewer than 4 kcal/g or no calories (ADA 2005).

Cellulose: It is the largest biomass produced on earth. It is found in fruits and vegetables. It is made from β-1-4 linkages of many glucose units (Jane 2007). Cellulose based fat replacers can be found in several forms. Powdered cellulose is obtained by mechanical grinding, microcrystalline cellulose is obtained by chemical depolymerization and wet mechanical disintegration, and sodium carboxymethyl cellulose, cellulose gum, methyl cellulose, modified vegetable gum are obtained by cemical derivatization (Akoh 1998). Microcrystalline cellulose has been used as a fat replacer in many products, such as salad dressings, cheese spreads, ice cream, frozen desserts and processed meat products. It is generally recognized as safe and a non caloric fat mimetic (Akoh 1998). It can be used

from 0.1 % up to 10% level, but optimum usage levels were determined to be 0.4% to 3% (Humphreys 1996, Omayma and Youssef 2007).

Starches: They are one of the major energy sources from plants. They can be found in seeds such as cereal grains, beans, in tubers, roots, or stems, such as potato, tapioca, in fruits, such as green bananas, apples, in leaves, such as tobacco (Stevenson and others 2006; Jane 2007). Starch based fat mimetics can come from corn, waxy maize, potato, wheat, tapioca, rice and waxy rice. Starches used as fat mimetics are mostly modified through acid or enzymatic hydrolysis, oxidation, dextrinization, cross-linking or mono-substitution. Modified starches do well in high moisture products as fat replacers such as emulsified meat products, frostings etc. (Akoh 1998).

Gums: Gums such as xanthan gum, gum Arabic, guar gum, or carrageenan have been used as thickening agents, stabilizers and gelling agents. They are also used in combination with other carbohydrate or protein based fat mimetics to replace fat. Laneuville and others (2005) used whey protein and xanthan gum as fat replacer. Cookie fillings and cake frosting made with combinations of fat mimetics showed results similar to regular products. Kumar and others (2007) investigated the effect of sodium alginate as a fat mimetic on quality of ground pork patties. Reduced fat ground pork patties showed similar results for microbiological, physico-chemical and organoleptic properties and provided reduced calories, total fat and cholesterol.

Maltodextrins: They are obtained from partial hydrolysis of starch particularly from corn or potato. It is defined as a non-sweet, nutritive (4 kcal/g) saccharide that has less than 20 dextrose units combined together mostly with α-1-4 bonds (Akoh 1998). Syed and others (2011) investigated the different maltodextrin levels for fat replacement in cake mix. Results indicated that maltodextrin levels up to 30 % showed the best results for reducing fat levels in cake formulations. Mona and others (2011) investigated the effect of different carbohydrate based fat mimetics (carrageenan, potato starch, carboxymethyl cellulose and maltodextrin) on production of low-fat chicken burgers. Results of their study showed that maltodextrin provided good sensory and physical attributes, followed by potato starch.

Pectin: It is abundantly found in most fruits and vegetables. It is mostly used as a gelling, thickening and stabilizing agent. Pectin is obtained through aqueous extraction of citrus peel, apple pomace and sugar beet pulp (Omayma and Youssef 2007). It is a polysaccharide rich with partial methyl esters of polygalacturonic acid. Based on the degree of methyl esterification, pectins are grouped as low methoxy (LM) pectin and high methoxy (HM) pectin. Low methoxy pectin has less than 50 % degree of esterification that forms a gel in the presence of divalent cations such as calcium. High methoxy pectin on the other hand has more than 50 % degree of esterification that forms gel under high acid or low pH and low water activity conditions (Daniel and others 2007).

Polydextrose: It is another carbohydrate-based fat mimetic that is formed by random polymerization of glucose, sorbitol, and citric or phosphoric acid. It provides 1 kcal/g (Akoh 1998). It has many functions such as bulking agent, humectant, texturizer, thickener etc. Polydextrose is considered a fat mimetic due to its contribution to mouthfeel and creaminess of fat reduced pastry, soft chewy candies, and dressings (Mitchell 1996; Omayma and Youssef 2007). It can cause a laxative effect, therefore any product that has more than 15 g /serving must states that "Sensitive individuals may experience a laxative effect from excessive consumption of this product" (Akoh 1998).

Fiber: It is another carbohydrate based fat mimetics that Trowell described in these word " …fiber consists of remnants of the plant cells resistant to hydrolysis by the alimentary enzymes of the man…" (Trowell 1976). These fiber components are hemicellulose, cellulose, lignin, oligosaccharides, pectins and gums (Rodriguez and others 2006), and the definition of fiber was expanded with the addition of inulin and resistant starch (Jones and others 2006). Fiber is classified into two groups. Soluble dietary fiber is soluble in water that is fermented in the colon (Anderson and others 2009). Pectin, β-glucans, galactomanan gums and are examples of soluble fiber (Rodriguez and others 2006). Insoluble dietary fiber such as lignin, cellulose and hemicellulose has limited solubility in the colon.

Fiber has received increased attention in last couple of decades due to its health benefits and possible use as a functional food ingredient. Researchers observed that people who consume a high fiber diet had lower incidence of atherosclerosis, hemorrhoids and colon cancer in comparison to people consuming a low fiber diet

(Kritchersky 1990; Rodriguez and others 2006). In addition, Gallaher and others (1992) suggested that dietary fiber causes a decrease in total cholesterol and low-density lipoprotein (LDL) in plasma through excretion of bile acids. Schneeman (1987) proposed that soluble fibers are associated with decreasing the cholesterol levels and adsorption of intestinal glucose, on the other hand, insoluble fiber has been linked to regulating bowel movements. Sources of soluble fiber are fruits, vegetables, legumes, soybean and psyllium seeds and oat bran. Insoluble fiber sources are whole grains (Wardlaw and Insel 1996; McKee and Latner 2000). Psyllium and β-glucan are the most commonly used dietary fibers that have been approved by the FDA related to health claims for protection against coronary heart disease (USDHHS 1997; USDHHS 1998). Recommended consumption levels for dietary fiber is 38 g/day for men and 26 g/day for women in United States (Miller 2004, Rodriguez and others 2006).

In recent years, fiber rich products have been developed and fiber has been placed in many food products such as nutrition bars, cereals, desserts, dairy products, drinks and meat products. According to a data collected by the National Center for Health Statistics in between 1999 to 2008, daily dietary fiber intake for adults aged 18 years and older was average 15.78 g/day in the United States (King and others 2012). Expansion of fiber in different products helps to minimize the deficiency of fiber in American diet and also can provide further functional health benefits. In addition to those benefits, fiber has been investigated as a fat replacer for different food products. One of the commercially available products is Oatrim. It was developed by the USDA and licensed to Conagra (Omaha, NR). It is made from partial enzymatic hydrolysis of starch containing part of whole oat or corn flour. It has 5 % β-glucan that has 4 kcal/g on a dry and 1 kcal/g on a

wet basis. This product can be used in production of dairy products, frozen desserts, meat products etc. (Akoh 1998). Another fiber product developed by USDA is Ztrim. It is rich in the insoluble fiber cellulose that has no calories. It is made from hulls of oats, soybeans, rice etc. (Akoh 1998).

Many studies investigated the effect of fiber from cereal grains on quality of meat products. Huang and others (2011) evaluated the effect of wheat, oat fiber and inulin on sensory and physico-chemical properties of Chinese style sausages. Results showed that addition of fiber did not significantly ($p<0.05$) affect the general composition, color and total bacterial count of sausages. However, addition of oat and wheat fibers at 7 % level hardened the sausages. Yang and others (2007) investigated the effect of hydrated oatmeal and tofu on textural and sensory properties of low fat pork sausages. Results showed that increased levels of added oatmeal increased water-holding capacity of the sausages and reduced cooking loss and provided softer texture. Aleson-Carbonell and others (2005) found that addition of β-glucan rich fiber to fresh breakfast sausage reduced cooking loss and increased lightness (L) in cooked sausages. Choi and others (2010) found that replacing pork back fat with vegetable oils and rice bran fiber reduced total fat content, cholesterol and energy values of the product and increased cooking yield. Serdaroglu and others (2005) reported that meatball extended with lentil flour, blackeye bean flour, chickpea flour and rusk had higher cooking yield and water binding capacity. Besbes and others (2008) also reported similar findings with pea and wheat fiber with water on beef burgers. Bilek and Turhan (2009) reported that addition of flaxseed flour to beef patties increased cooking yield of the products and level of α-linoleic acid content increased with increasing level of flaxseed flour.

In addition to use of cereal grains and legumes and seeds as fiber sources, vegetables and fruits can be great fiber source. Femenia and others (1997) incorporated cauliflower fiber into meat products. Products with cauliflower fiber had higher yields and increased product firmness. Also, slight changes in sensory properties were observed. Grigelmo-Miguel and others (1997) investigated the fiber from peaches on quality of low-fat frankfurters. Results showed that addition of peach fiber caused a decrease in pH, increase in cooking yield and viscosity, and received acceptable sensory scores similar to those of the all-meat controls.

Recently, citrus fiber has been receiving attention for use them as an antioxidant fiber source. Citrus fruits are mostly used for juice making. Left overs, such as peel are either used in animal feed, fertilizer or thrown away. However, some studies suggest that citrus peel is rich in fiber and also, due to presence of phenolic compounds and antioxidants, such as ascorbic acid (Gorinstein and others 2001; Fernandez-Lopez 2004), it can be used in food products to provide further functional benefits. Fernandez-Lopez and others (2004) investigated the influence of mesocarb (Albedo) of cooked and raw lemon and orange on physicochemical and sensory properties of bologna and dry cured salami. Results showed that incorporation of both raw and cooked albedo reduced residual nitrite levels. This indicates that presence of albedo in cured sausage products reduces the possibility of nitrosamine formation. Also they determined that sausage samples with fiber had lower TBA values compared to control samples. This could be linked to the presence of antioxidants in citrus fiber. Yalinkilic and others (2012) reported similar results. The addition of orange fiber decreased the pH and increased the cooking yield of Turkish dry-fermented sausage. Residual nitrite levels decreased. Also,

researchers did not find any significant difference between control and 2 % orange fiber in terms of texture, color, odor taste and general acceptability.

2.3. Oxidation Process

Many foods can undergo oxidation, however, lipids in particular have higher tendency to go through oxidation. In this native state, lipids are generally protected from chemical reactions by native antioxidants such as tocopherol or ascorbic acid. During extraction or processing, these antioxidants can be partially or completely lost (Pratt 1996) so that lipids can under go undesirable reaction called rancidity. In hydrolytic rancidity fatty acid molecules detach from triglyceride molecule and produce –mono, -di glycerides and the glycerol molecule with free fatty acids. Volatile free fatty acid molecules can oxidase and develop uncharacteristic odors. In chemical hydrolysis, fatty acid molecules detach from triglycerides during really high temperatures. Deep fat frying and retort processing may cause chemical hydrolysis of lipids. Enzymatic hydrolysis, caused by enzymes such as lipases naturally found in foods, also cause free fatty acid formation resulting in hydrolytic rancidity. Enzymatic hydrolysis results in “goaty” odors and flavor in milk by short chain fatty acids (Gruen and Duncan 2007; Richards 2007).

Autoxidation of foods is known to be triggered by light, temperature, enzymes, metal and metal-protein (Shahidi and Zhong 2005). It is a free radical chain reaction that consists of three steps. These are initiation, propagation and termination. It is shown at Figure 2.1. Initiation reactions start with unsaturated fatty acids loosing a hydrogen molecule and becoming fatty acid radicals. This fatty acid radical starts the propagation reactions by reacting with oxygen and producing lipid peroxy radicals. This lipid peroxy

radical can react with another lipid and produce more radicals and acids. At the termination stage, these different lipid radicals react with each other and they can produce alcohols, aldehydes, hydroxycarbons, acids causing rancidity (Gruen and Duncan 2007; Richards 2007).

Step 1: Initiation $\quad R\text{-}H + Initiator \Rightarrow R\bullet + H\bullet$

Step 2: Propagation $\quad R\bullet + O_2 \Rightarrow ROO\bullet$

$ROO\bullet + RH \Rightarrow R\bullet + ROOH$

Step 3: Termination $\quad R\bullet + R\bullet \Rightarrow RR$

$ROO\bullet + R\bullet \Rightarrow ROOR$

$ROO\bullet + ROO\bullet \Rightarrow ROOR + O_2$

Figure 2.1. Free Radical Chain Reaction

2.4. Measurement of Lipid Oxidation

Measurement of lipid oxidation is important for determining quality and shelf life of meat products because oxidation causes detrimental effect on meat quality such as warmed over flavor, change in color, rancid flavor, loss of functional properties and nutritional value.

2.4.1. Peroxide value (PV)

Peroxide value is defined as the amount of peroxide oxygen per kg of fat or oil. Standard determination of PV uses iodometric titration method. In this method, saturated solution of potassium iodine is added to oil sample to react with hydroperoxides (Shahidi and Zhong 2005). It is one of the most widely used tests for determining oxidation of oils and fats in its early stages. However, due to procedure being time and labor intensive (Riuz and others 2001) and lack of insensitivity and difficulty to determine titration endpoint (Dobarganes and Velasco 2002), colorimetric method of PV has been developed. In this method, lipid hydroperoxides oxidize ferrous ions (Fe^{2+}), which in complex with thiocyanate, to ferric (Fe^{3+}) thiocyanate. Resulted compound gives strong absorption at 500-510 nm (Dobarganes and Velasco 2002). This method provides more sensitive results in comparison to iodometric method (Dobarganes and Velasco 2002; Shahidi and Zhong 2005).

2.4.2. Thiobarbituric acid test

Thiobarbituric acid test is used for determining secondary oxidation products. Hydroperoxides, primary oxidation products are unstable and they are susceptible to degradation to volatile, nonvolatile and polymeric secondary oxidation products which includes aldehydes, ketones, alchols, hydrocarbons and volatile organic acids etc. (Shahidi and Zhong 2005). In this method, lipid oxidation compound of malonaldehyde reacts with two molecules of thiobarbituric acid and resulting pink compound is measured spectrophotometrically at its absorption maximum at 530-535 nm (Antolovich and others 2002; Shahidi and Zhong 2005). One of the major limitations of TBA test is

that there are other chemicals such as alkanals, 2-alkenals, 2,4-alkadienals, ketones proteins, sucrose, urea, pyridines, which referred as thiobarbuturic acid reactive substances (TBARS) can react with TBA and can contribute to pink color formation. This can lead to over estimation of oxidation. Also, some of protein compounds can react with malonaldehyde and may interfere with pink color formation and causes under estimation of oxidation (Jardine and others 2002; Shahidi and Zhong 2005). Therefore, nowadays the test has been called TBARS test. Despite of some of its limitations TBARS test has been used as one of the main test for lipid oxidation in meat products. One study evaluated the antioxidant potential of grape fiber for delaying lipid oxidation in minced fish. Results showed that samples with grape fiber had lower TBARS values than control fish samples (Sanchez-Alonso and others 2007). Sayago-Ayerdi and others (2009) reported similar results with grape fiber added raw and cooked chickens.

2.4.3. Anisidine value (*p*-AnV)

This methodology measures the secondary oxidation products such as 2-alkenals and 2,4-alkadienals reaction with *p*-methoxyaniline (anisidine). Their reaction under acidic environment produces yellowish products that absorb at 350 nm. This method shows higher sensitivity towards unsaturated aldehydes, because their products absorb more strongly at this wavelength (Gordon 2001; Shahidi and Zhong 2005).

2.4.4. Gas chromatography

Gas chromatography technique has been one of the most common techniques that are used for identifying volatile compounds using gaseous mobile phase (Cserhati and Forgacs 1999). In order to ensure separation, heating of samples are done to provide that they are in gaseous stage. Also, in some cases derivatization reactions need to be performed to prepare sample for gas chromatography analysis. For example, fatty acids cannot be separated in gas chromatography directly, because, they are not volatile. In order for fatty acids to be separated, derivatization reaction such as methylation reaction needs to be performed to convert fatty acids to their volatile fatty acid methyl esters. Then, they can be separated using gas chromatography. Volatile lipid oxidation compounds such as aldehydes, ketones, esters and alcohols can be easily measured using this technique. Gas chromatography uses mostly partition separation principle in most applications. In some applications adsorption (gas-solid) and size-exclusion chromatography are also used (D'Archy 2007). In gas-liquid partition chromatography partition coefficient of the solutes depends on two factors. First one is volatility (boiling point) of each solute. Second one is solubility of each solute in stationary liquid phase. Similar to liquid-liquid partitioning, in order for separation to occur partition coefficients' of volatile solutes need to be different. The degree of separation between solutes can be achieved in two ways. First one is by changing the temperature of the mobile and stationary phase in a GC column. Different solutes have different volatility points that cause different reactions between mobile phase and stationary phase. That leads to production of different partition coefficient. Secondly, type of stationary phase can influence the separation of the analyte. Stationary phase can be polar or non-polar, for

polar analyte to be separated a polar stationary phase is chosen, while for non-polar analyte is separated in non-polar stationary phase (D'Archy 2007).

In this technique, volatile lipid oxidation compounds in a sample vial can be extracted through steam distillation with extraction (SDE), static head-space (HS), dynamic HS and solid phase micro extraction (SPME) HS (Ross and Smith 2006), than they are swept through a column with mobile phase, which can be helium, nitrogen or hydrogen gaseous.

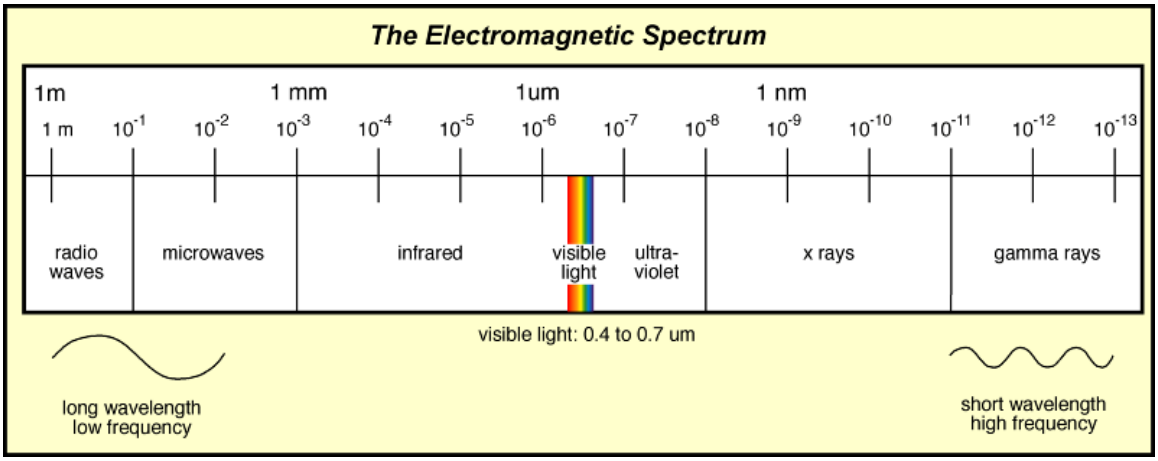
Volatile compounds interact with the column, stationary phase and pass through detector. Time that passes between injection of the sample through column and passing the detector is called retention time. Compounds that interact with stationary phase longer have higher retention times, and compounds with higher volatile (low boiling temperature) leave the column faster have lower retention times. Volatile compounds in foods can be identified by comparing the retention time of pure volatile standards with retention times of volatile compounds in the sample and quantified by using internal standard method. Also, gas chromatography can be coupled with mass spectrometry to identify the volatile compounds in the sample by ionizing samples to generated charged molecules measuring their mass to charge ratio.

Many studies reported high correlation between results of TBARS analysis with gas chromatography. Ang and Young (1985) investigated the lipid oxidation in chicken using static head-space GC. They found pentanal, hexanal, heptanal, octanal, and nonanal oxidation products and found high correlation between their methodologies with TBARS analysis. Lai and others (1995) reported similar results using a dynamic head-space GC and reported presence of hexanal in chicken nuggets. Brunton and others (2000) reported

high correlations between TBARS and SPME-GC for quantifying hexanal and pentanal in cooked turkey. These and many other studies show that use of gas chromatography and GC –MS is a great analytical technique for identification and quantification of volatile lipid oxidation compounds.

2.4.5. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy is one of the analytical tools in chemistry used for identification of compounds. It uses optical device called interferometer. Most interferometers use a beam splitter, which divides the beam of radiation into two beams. One of those beams is reflected on the fixed mirror and pass through reference cell and the other one is reflected on the moving mirror, which pass through sample cell, than both beams reflect back and get back together at the beam splitter creating interfering signals, which is called interferogram. Interferogram allows measurement of all frequencies simultaneously and produces spectra of information for analyte under time domain. By using Fourier transform mathematical technique spectra is decodes the information and converts into frequency domain. Resulting spectra provides information about identification of compounds (Sedman and others 1997).



Source: http://myweb.cwpost.liu.edu/vdivener/notes/electromag_spectrum.htm

Figure 2.2. Electromagnetic Spectrum

Figure 2.2 shows the electromagnetic spectrum. Radio wave is at one end of the spectrum and gamma rays are at the other end of the spectrum. Radio wave has lower frequency and long wavelength in contrast to gamma rays with higher frequency and shorter wavelength. For, Fourier transform infrared spectroscopy, light at infrared region is used. Infrared region subdivided into three regions. These are near, middle and far infrared regions. Near infrared stretches from 12800 to 4000 cm^{-1}, middle infrared region stretches from 4000 to 200 $cm^{-1}$and far infrared region stretches from 200 to 10 cm^{-1}. Middle region of infrared spectroscopy is mostly used for identification of organic compounds. FTIR uses region of 4000 to 500 cm^{-1} for analysis (Bruce 2011). Frequency or wavenumber's of different functional groups and their mode of vibration associated with edible oils and fats have been investigated and shown at Table 2.1. This Table also provides information for identification of bands in beef fat.

In addition, FTIR also has been used qualitatively for identification of lipid oxidation in fats and in oils. In order to determine oxidative stability of edible oils, Guillen and Cabo (2000) used simple methodology of identifying changes in absorbance of different bands and evaluated these bands absorbance's ratios during different stages of oxidation. Same technique applied by the same authors in another study was useful for determining oxidative stability of pork with smoke flavoring (Guillen and Cabo 2004). Recently, FTIR spectral data has been evaluated using principle component analysis (PCA) to determine spoilage of minced beef (Ammor and others 2009). Furthermore, in another study artificial neural network analysis (ANN), a mathematical model has been used to evaluate the oxidative stability of fish oil (Klaypradit and others 2011).

Table 2.1. Wavenumbers of Bands (B) or Shoulders (S) of Edible Oils and Fats in Mid-Infrared Spectra

Wavenumber (cm^{-1})	Functional Group	Mode of Vibration	Intensity
3468 (b)	−C=O (ester)	Overtone	Weak
3025 (s)	=C−H (*trans*-)	Stretching	Very weak
3006 (b)	=C−H (*cis*-)	Stretching	Medium
2953 (s)	−C−H (CH_3)	Asymmetric Stretching	Medium
2924 (b)	−C−H (CH_2)	Asymmetric Stretching	Very strong
2853 (b)	−C−H (CH_2)	Symmetric Stretching	Very strong
2730 (b)	−C=O (ester)	Fermi Resonance	Very weak
2677 (b)	−C=O (ester)	Fermi Resonance	Very weak
1746 (b)	−C=O (ester)	Stretching	Very strong
1711 (s)	−C=O (acid)	Stretching	Very weak
1654 (b)	−C=C− (*cis*-)	Stretching	Very weak
1648 (b)	−C=C− (*cis*-)	Stretching	Very weak
1465 (b)	−C−H (CH_2, CH_3)	Bending (scissoring)	Medium
1418 (b)	=C−H (*cis*-)	Bending (rocking)	Weak
1377 (b)	−C−H (CH_3)	Symmetric Bending	Medium
1238 (b)	−C−O, −CH_2−	Stretching, Bending	Medium
1163 (b)	−C−O, −CH_2−	Stretching, Bending	Strong
1118 (b)	−C−O	Stretching	Medium
1097 (b)	−C−O	Stretching	Medium
1033 (s)	−C−O	Stretching	Very weak
968 (b)	−HC=CH− (*trans*-)	Bending out of Plane	Weak
914 (b)	−HC=CH− (*cis*-)	Bending out of Plane	Very weak
723 (b)	−$(CH_2)_n$−	Bending (rocking)	Medium
	−HC=CH− (cis-)		

Source: Guillen and Cabo (1997).

2.5. Antioxidants

Antioxidants are compounds that prevent oxidation of other compounds. Autoxidation reaction in foods and particularly lipid reach foods such as meat products can be delayed by addition of antioxidants into products. They delay or interrupt the steps of autoxidation by donating its hydrogen atom (Richards 2007). For antioxidant to provide antioxidant benefits they should be effective in low concentrations (0.001-0.02%) capable of surviving processing, does not provide undesirable color, flavor or odor, and it should be cheap (Reische and others 1998; Shahidi and Zhong 2005).

2.5.1. Natural antioxidants

Fruits, vegetables, whole grains and meat are known to be the source of natural antioxidants. Vitamin C (ascorbic acid), Vitamin E (tocopherols), Vitamin A (carotenoids), Lycopene, various polyphenols (flavonoids) and Coenzyme Q10 (Ubiquitin) are examples of natural antioxidants. These antioxidants can have various benefits. Antioxidants help to delay oxidation of food products, improve shelf life, and keep aromas and flavors fresh longer. In addition, due to their radical scavenging activity, they have been associated with reducing the risk of certain illnesses and protect against damage caused by certain reactive oxidative, nitrogen and chlorine species (Shahidi 1997).

2.5.2. Synthetic antioxidants

These compounds are chemically synthesized to help prevention of lipid oxidation. Most widely used synthetic antioxidants are butylated hydroxyanisole (BHA),

butylated hydroxyl toluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Shahidi and Zhong 2005). These antioxidants have been shown to provide greater antioxidant effects on different food products, however, due to possible negative health effects on animal studies, eliminating the use of some of the synthetic antioxidant in food has been considered (Shahidi and Zhong 2005). In addition, synthetic antioxidants have been considered as not label friendly and also, health conscious consumer does not want to buy products that have synthetic antioxidants.

2.6. Antioxidant Components of Citrus

Citrus fruits are commonly produced in the Unites States and around the world in tropical and subtropical regions. It has been reported that due to presence of vitamin C, phenolic acids, carotenoids and flavonoids, citrus fruits can provide antioxidant benefit (Gorinstein and others 2001).

2.6.1. Vitamin C

Vitamin C (ascorbic acid) is a water-soluble vitamin. It is white in crystalline form and odorless with $C_6H_8O_6$ empirical formulation (Cross and Hui 2007). Figure 2.3 shows the formulation of L-ascorbic acid. It has been reported that citrus fruits are important source of ascorbic acid.

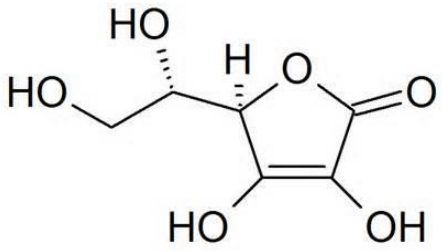


Figure 2.3. L-Ascorbic Acid

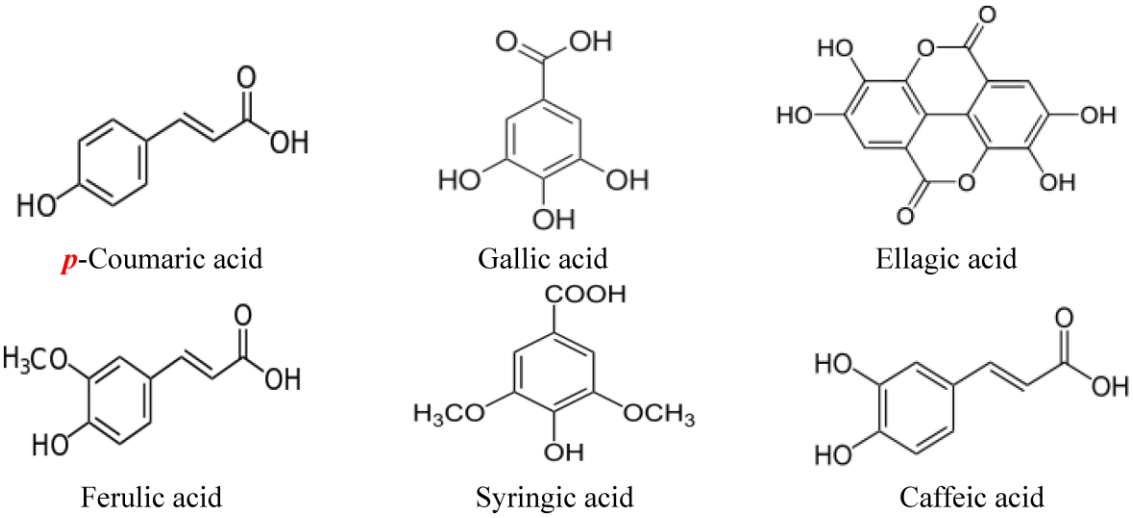
Gorinstein and others (2001) reported that peels of oranges, lemon and grapefruit have higher ascorbic acid content than their peeled fruit counterparts. Ascorbic acid provides antioxidant action through reacting with radical species by donating hydrogen atom. This reaction is shown below. Semidehydroascorbate react with oxidizing radical ($R^{\bullet}$) to produce unreactive tricarbonyl ascorbate free radical.

$$R^{\bullet} + (C_6H_7O_6)^{-} \rightarrow RH + (C_6H_6O_6)^{\bullet}$$

2.6.2. Phenolic acids

Phenolic acids are aromatic secondary plant metabolites found in plants such as fruits and vegetables. Based on carboxylic acid functional group and position of hydroxyl group on the phenol ring creates a variety of phenolic acids (Robbins 2003). Some of the commonly found phenolic acids are gallic acid, cinnamic acid, *0*-coumaric acid, *p*-coumaric acid, caffeic acid, sinapic acid and ferulic acid. Figure 2.4. shows some of the examples of common phenolic acids found in plants. It has been reported by Gorinstein

and other (2001) that the peels of orange, lemon and grape fruit have higher polyphenol content than peeled orange, lemon or grapefruit. They found that highest phenolic acid in all fruit samples was ferulic acid and lowest was caffeic acid. In addition to, their association with color and flavor of foods, it has been determined that phenolic acids can provide antioxidant potential through radical scavenging activity and are useful for delaying lipid oxidation through donating hydrogen atom to free radicals.



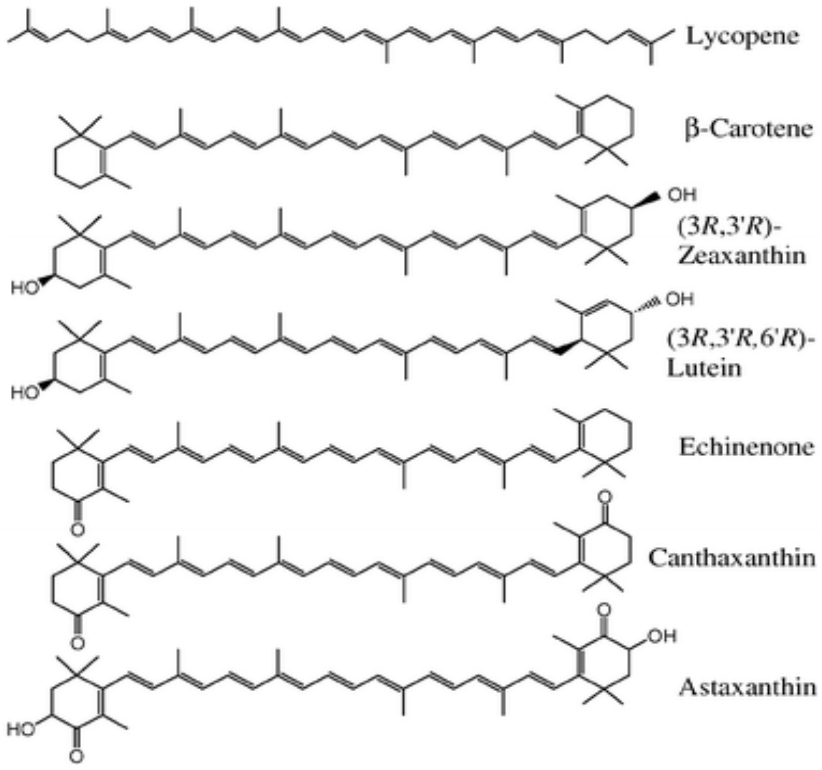
Source: Omotayo and others (2014)

Figure 2.4. Structure of Selected Phenolic Acids

2.6.3. Carotenoids

The carotenoids are commonly found pigments that provide yellow to red color to many fruits and vegetables including citrus fruits. Structures of some of the carotenoids are shown in figure 2.5. Some carotenoids are precursors of Vitamin A. Carotenoids can

be classified into two groups based on their extraction scheme. Carotenoids that are hydrocarbons called carotenes. Examples are β-carotene and lycopene. While oxygenated carotenoids containing alcohol, ester, carboxylic acid functional groups called xanthophyll. Examples are lutein, astaxanthin and canthaxanthin. In addition, carotenoids can be classified in to two groups based on acyclic or cyclic. An example to acyclic carotenoids is lycopene, which does not have any cyclic ring. While, example to cyclic carotenoids is β-carotene. Carotenoids that have at least one un-substituted ring posse's vitamin A precursor. Oxygenated rings do not posses that function (Wrolstad 2007).

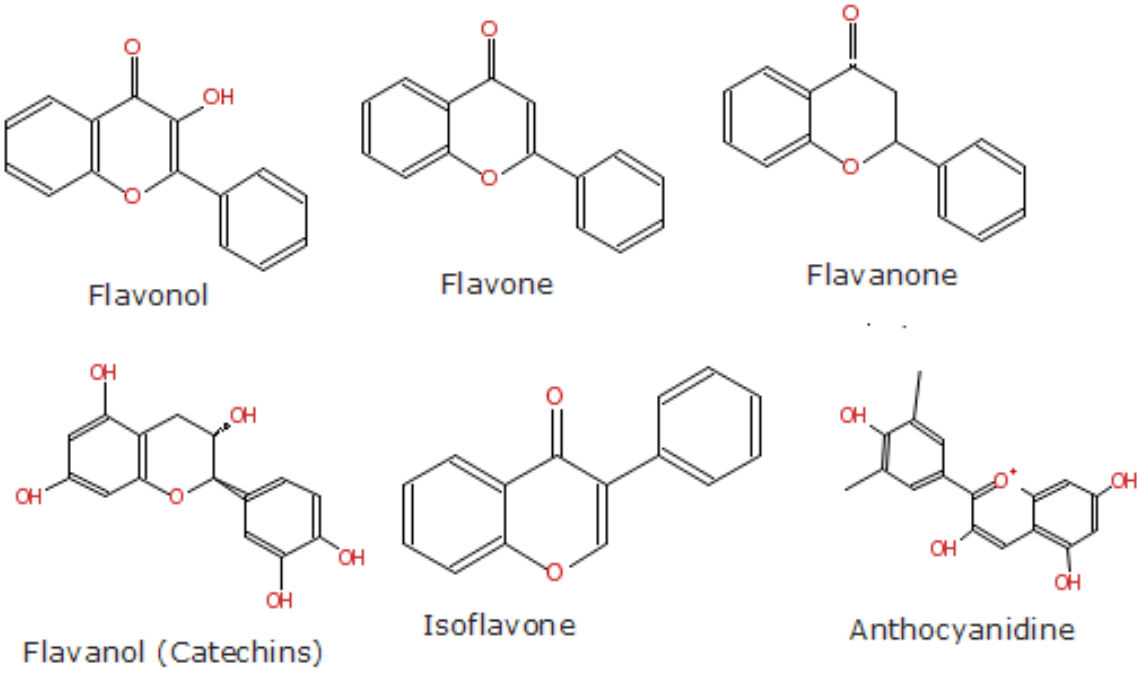


Source: Kopczynski and others (2005)

Figure 2.5. Structure of Selected Carotenoids

2.6.4. Flavonoids

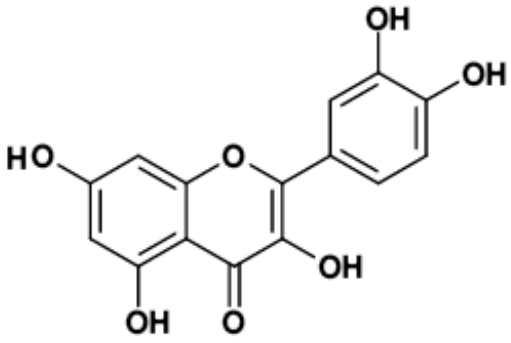
Flavonoids are large family of compounds synthesizes by plants. They are secondary plant metabolites with C6-C3-C6 carbon structure, having two phenyl rings and one heterocyclic ring. Figure 2.6. shows the structure of selected flavonoids. Based on the difference in the structure, they are classified into six classes. These are flavones, flavanones, flavonols, isoflavones, anthocyanidins and flavanols (catechins) (Tripoli and others 2007). It has been reported by Horowitz and Gentili (1977) that more than 60 types of flavonoids were identified from citrus fruits.



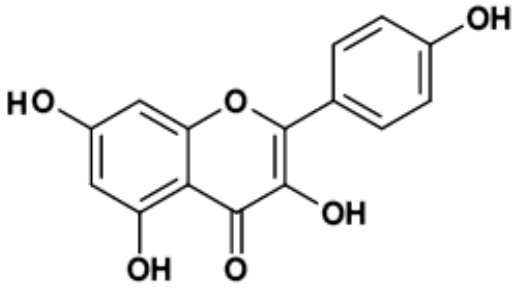
Source: Lakhanpal and Rai (2007)

Figure 2.6. Structure of Major Flavonoid Classes

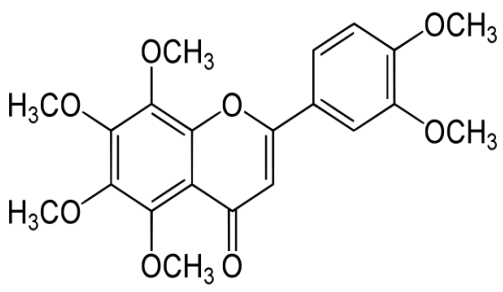
Most identified citrus flavonoids are classified under three groups. These are flavanone, flavone and flavonols. Citrus flavanones are found as glycoside or aglycone forms. Most glycosides form found in citrus are neohesperidosides and rutinosides (Macheix and others 1990; Tripoli and others 2007), while most aglycone form found in citrus are naringenin and hesperitin. Flavonones that consist of neohesperidosides (rhamnosyl-α-1, 2 glucose) has bitter taste, which includes naringin, neohesperidin and neoecitrin. On the other flavonones that has rutinosides such as rutinose (ramnnosyl-α-1, 6 glucose) has no taste, which includes hesperidin, narirutin and didymin. Typical examples to flavones are apigenin and luteolin and to flavonols are quercetin and kaempferol. Both flavones and flavonols have been found in low concentrations in citrus (Tripoli and others 2007). Figure 2.7. shows the structure of flavonols (quercetin and kaempferol) and polymethoxyflovones (nobiletin, sinensetin, tangeretin, heptamethoxyflavone).



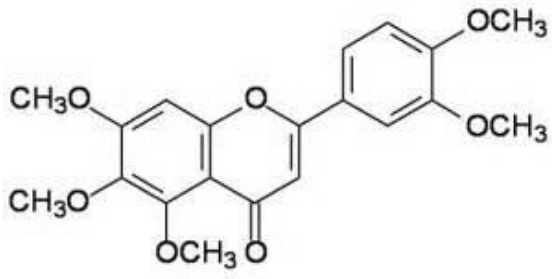
Quercetin



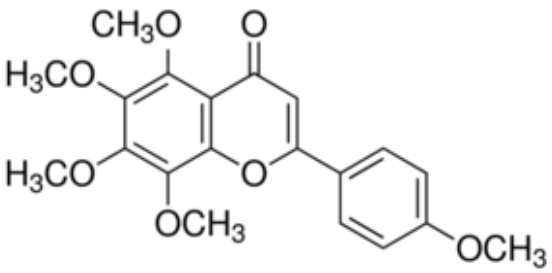
Kaempferol



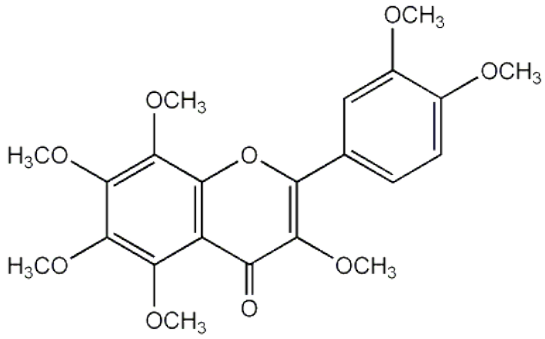
Nobiletin



Sinensetin



Tangeretin



Heptamethoxyflavone

Figure 2.7. Structure of Major Flavonols and Polymethoxyflavones

2.7. Instrumental Analysis of Antioxidants by Liquid Chromatography

Liquid chromatography is very commonly used in food chemistry and due to its separation and detection at ambient temperatures; it is used for thermally label, nonvolatile and highly polar food components. From liquid chromatography techniques, High-pressure liquid chromatography (HPLC) is most commonly used and followed by thin layer chromatography (TLC), and paper chromatography (D'Archy 2007). Separation in liquid chromatography can be obtained through different mechanisms. These are adsorption, partition, ion-exchange, exclusion and affinity.

2.7.1. Separation mechanisms

Absorption chromatography uses liquid mobile phase and solid stationary phase. Mobile phase absorbed on to a stationary solid phase that equilibrium between them provides separation of different solutes. This technique is one of the earliest chromatography techniques and it was developed by Tsweett. This method provides good separation for organic samples (Hurtubise 2005). Most commonly used solid phase or absorbents for this type of chromatography are slica and alumina (Snyder 1968; Hurtubise 2005). Their active sites have polar hydroxyl groups. Mobile phase on the other hand is non-polar. It is suggested that competition between mobile phase and solute for the active sites provides separation. Rate of elution of the solute can be changed by decreasing or increasing the polarity of the mobile phase using mixtures of solvents (D'Archy 2007).

Partition chromatography, separation is obtained mainly through solubility of sample components in stationary phase such in gas chromatography or differences of

solubility's between mobile and stationary phase such in liquid chromatography (Ettre 1993). In liquid-liquid partition chromatography, liquid stationary phase can be coated on surface of solid such as water bonded on to the surface of slica, or permanently bonded to the surface of a porous inert solid by chemical reaction. Solutes can dissolve in mobile or stationary phase in certain ratios that creates partitioning. Ratio of concentration of solute in stationary phase to concentration of solute in mobile phase gives partition coefficient (K). Separation occurs when partition coefficient is different for each solute (Rounds and Gregory III 2003; D'Archy 2007). Based on type of stationary or mobile phase are used there are two types of partition liquid chromatography. These are normal phase and reversed phase partition liquid chromatography. In normal phase chromatography, mobile phase uses non-polar solvent to elute the solute while stationary phase is more polar. The reverse phase chromatography uses non-polar stationary phase consisting C8 or C18 alkane groups attached to the surface of slica and polar mobile phase.

In ion-exchange chromatography separation is based on electrostatic interactions of ionic solutes between aqueous mobile phases and fixed ionic functional groups on the surface of a solid stationary phase. The strength of the electrostatic interactions between the ionic solute and solid stationary phase depend on how quickly ionic solute moves from one active site to another. The strength of the electrostatic interaction as well as elution of the solute from the system can be changed by manipulating the ionic strength and pH of the mobile phase (D'Archy 2007). Stationary phase is resin base and depending on the type of resin it can be cautions or anions. Caution resin interacts with negatively charged molecules, while negatively charged resin interacts with positively (cautions) charged molecules. This method is commonly used for separation of proteins.

Positively charged proteins can be separated by using negatively charged carboxymethyl-cellulose (CM-cellulose) columns, while negatively charged proteins can be separated by using positively charged diethylaminoethyl-cellulose (DEAE-cellulose) columns (Berg and others 2002).

Exclusion chromatography separation is based on differences in molecular size and/or shape or in charge (Ettre 1993). Gel filtration chromatography uses separation based on size. It is also called gel permeation or molecular exclusion chromatography. Columns are mostly made with dextran, agarose gel or polyacrylamide. Small molecules easily penetrate into these beads, while big molecules can't; allowing big molecules to flow through column fast and emerge first (Berg and others 2002).

Separation in affinity chromatography is based on reversible biological interaction between analyte with specific ligand coupled to a chromatographic matrix (lock and key mechanism) (Rounds and Nielsen 2003; D'Archy 2007). This technique can be used for purification of proteins. It uses biological interaction between matrix and the analyte in investigation. These biological interactions can be enzyme, antibody, lectin, nucleic acid, hormone, vitamin receptors etc. (Swamy and others 2011). For example, enzyme inhibitor, a solute can bind to specific enzyme on the solid phase (ligand). This technique is very effective when biological interaction between analyte and ligand is very specific (Berg and others 2002). Interactions between a solute and ligand can be controlled by changing the pH of mobile phase or by adding competitive binding agent (Rounds and Nielsen 2003; D'Archy 2007).

2.7.2. Type of detectors

There are different types of detectors based on type of compounds of interest. These are, UV-Vis, photo diode array, fluorescence, mass spectroscopic, refractive index, electrochemical and light scattering detectors.

UV-Vis detectors detect and identify compounds in food samples based on measuring the samples' adsorption of light at different wavelengths. It cannot be used for detection of compounds that doesn't adsorb light at UV-Vis range (190-700 nm) (Hitachi 2015).

Photo diode array detectors identify analytes based on adsorption of light at different wavelengths as well. However, UV-Vis uses diffracting grating to send particular wavelength of light to be sent to flow cell by adjusting the angle of it. On the other hand, photo diode array directly send the light through flow cell, light pass through flow cell than it goes through diffracting grating separating to different wavelength, which allows monitoring of absorbance at many wavelengths (Hitachi 2015).

Fluorescence detector provides great sensitivity in comparison to UV-Vis and photo diode array detectors and can be used for detection of analytes in trace amounts. When analytes absorb energy to get to higher energy state (excitation wavelength), than emits energy to get back to original state at different wavelength has been called fluorescence. Fluorescence detector detects fluorescence emitted in the direction right angel from exciting light (Hitachi 2015)

Refractive index detector is one of the least sensitive detectors. Any compounds that have refractive index different than reference refractive index can be detected. It can be mostly used for detection of carbohydrates and it is not commonly used for gradient

elution. In this system, flow cell has two sides. These are sample side and reference side. Reference side is filled with mobile phase, when analytes eluted from the column, composition in the flow cell changes, when the light send through the flow cell, it causes change in photorefractive level and change in the amount of light received by light receiving element (Hitachi 2015)

Electrochemical detector is used for detection of electrical current caused by components displaying oxidation-reduction reactions causing electrical current. This is highly sensitive detector. The fact that necessary current to cause oxidation-reduction depends on components makes electrochemical detector to be a highly sensitive one (Hitachi 2015)

Evaporative light scattering detectors can be used for non-volatile compounds. It has better selectivity than refractive index detectors but lower selectivity for low molecular size components. Mobile phase is expose to inert gas to be evaporated into tiny particles. These particles pass through a laser beam. Photodiode detector measures the scattered light (Hitachi 2015).

Mass spectrometric detectors identify the type and amount of components in a sample by measuring mass to charge ratio. Mass spectrometric detectors have three main sections. These are the ion source, the mass analyzer and the detector. Ion source ionize the analytes in interest, then send to mass analyzer through magnetic or electric fields. Types of ionizations are electron and chemical ionizations. In electron ionization molecules are smashed with electrons. It is a harsh process that fragments the molecules. Also, it is mostly used in gas chromatography for gaseous, volatile organic molecules. Chemical ionization is a softer ionization that yields fewer fragments (Bruce 2011).

There are also different types of analyzers. These are magnetic sector analyzer, time of flight analyzer, quadruple analyzer and ion trap analyzer. Magnetic sector analyzer is very precise and accurate analyzer. Different masses uses different road on magnetic field to get to the detector. More charged molecules deflect more. Time of flight analyzer uses electrical field to accelerate ions causing small ions to accelerate faster than bigger ions, reaching detector faster. Quadruple analyzer is commonly used in food science. Ions pass through oscillating quadruple electrical field. Based on the mass, time it takes to pass the channel provides separation. Ion trap analyzer is also common in food science. Ions are trapped in radio frequency field ring than sequentially ejected (Wikipedia 2015)

Electrical conductivity detectors are mostly used in ion chromatography for detection of inorganic ions, organic acids and amines. It measures electrical conductivity of ions in a solution. There is an exposure to constant voltage that changes with eluted ions, which is measured (Hitachi 2015).

2.8. Measurement of Antioxidant Activity by Oxygen Radical Absorbance Capacity Assay

Synthetic and natural antioxidants have been used in fat containing foods to delay lipid oxidation (Shahidi and Zhong 2005). Methodologies have been developed to determine the antioxidant potential of these antioxidants. One of the methods commonly used for determination of antioxidant capacity of these compounds is oxygen radical absorbance capacity (ORAC) assay. The ORAC assay measures the oxidative degradation of fluorescent molecule within the presence of free radical generating AAPH (2,2'-azobis-2- methyl-propanimidamide, dihydrochloride).

The peroxyl radical can oxidize fluorescein to generate product without fluorescence. In the presence of antioxidant, breakdown of AAPH (2,2'-azobis-2-methyl-propanimidamide, dihydrochloride) is suppressed by hydrogen transfer mechanism. Fluorescence signal is proportional to concentration of antioxidant in the system. By measuring fluorescence intensity over time, oxidative radical absorbance capacity of samples can be determined. Trolox (6-hydroxy-2,5,7,8-tetramethylchroma-2-carboxylic acid) has been used as a standard and the ORAC value of an antioxidant has been expressed as μmole Trolox equivalent per gram (Davalos and others 2004).

CHAPTER 3

DETERMINATION OF FLAVONOIDS IN CITRUS FIBER BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND THE IMPACT OF EXTRACTION METHODOLOGY ON TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT, TOTAL CAROTENOID CONTENT AND OXYGEN RADICAL ABSORBANCE CAPACITY

3.1. ABSTRACT

Citrus powder was obtained from peel of citrus fruit, which contains flavonoids, by a washing, drying, and powdering process. This study evaluated the presence of flavonoids in citrus powder using reverse-phase high-pressure liquid chromatography (RP-HPLC). This project investigated the effect of methanol and acetone extraction on total polyphenol content (TPC), total flavonoid content (TFC), oxygen radical absorbance capacity (ORAC) and total carotenoid content (TCC) of citrus powders prepared by regular or hot-washing. Quercetin and kaempferol (flavonols), and sinensetin, nobiletin, heptamethoxyflavone, and tangeretin (polymethoxyflavones) were identified by comparing citrus powder extract retention times with the retention times of pure standards and were quantified by using external standard curves. Nobiletin had the highest concentration with 3.33 mg/g followed by sinensetin (1.96 mg/g) and heptamethoxyflavone (1.24 mg/g), respectively. Extraction with acetone yielded higher ($P < 0.05$) TPC, TFC, and ORAC values than extraction with methanol. The TPC and TFC of citrus powders were unaffected ($P > 0.05$) by the citrus powder production procedure; however, the ORAC values of citrus powder prepared using hot-washing were significantly lower ($P < 0.05$). The carotenoid content was not significantly affected by the washing procedures ($P > 0.05$).

3.2. INTRODUCTION

The United States is a major producer of citrus fruit with Florida being the leading producer followed by California, Texas and Arizona. Main citrus fruits are oranges, grapefruit, lemon, tangelos, tangerines and mandarins. According to a U. S. Department of Agriculture (2013) report approximately 35.5% of the citrus fruits are consumed as fresh fruit while the rest has been processed into juice. Using the majority of the citrus fruits for juice production creates a large amount of waste. The waste products from the peel and seeds are prone to microbial spoilage, making this waste material a candidate for use mostly as animal feed and fertilizer (Fernandez-Lopez and others 2004). However, it has been reported by Saura-Calixto (1998) and Larrauri (1999) that fruit by-products, such as peels, are rich in dietary fiber. Also, due to the presence of flavonoids and carotenoids, they have better nutritional quality than fiber from cereals, as well as a balanced composition of soluble and insoluble fiber and low energy values. Also, Gorinstein and others (2001) and Fernandez-Lopez and others (2004) stated that citrus by-products, such as peel (albedo and flavedo), are not only rich in fiber, but due to the presence of phenolic compounds and antioxidants, such as ascorbic acid, they can be used in food products to provide further functional benefits. Lario and others (2004) stated that fruit fibers, such as citrus, provide good quality soluble and insoluble fiber, as well as functional properties, such as water- and oil-holding capacity. Gorinstein and others (2001) found that the peels of oranges, lemons, and grapefruits had higher total dietary fiber, soluble fiber, and insoluble fiber than these fruits without peel, on both fresh and dry weight bases. Besides these possible functional advantages, citrus fiber can

provide health benefits. It has been reported that consuming a fiber-rich diet is associated with decreased incidence of atherosclerosis, hemorrhoids, and colon cancer (Rodriguez and others 2006). Additionally, the antioxidant potential due to the presence of flavonoids, flavonols and polymethoxyflavones (PMFs) in citrus peel contributes functional and health benefits (Manthey and Grohmann 1996; Bocco and others 1998; Gorinstein and others 2001). In most of those investigations, the citrus peels obtained for the experiments were fresh rather than processed and no study has investigated the presence of these flavonoids in mass-produced citrus by-products. Therefore, the objective of this research was to provide information about the identity and quantity of flavonoids found in mass-produced citrus fiber by reverse-phase HPLC and provide information on the effect of two types of extraction methodologies and processing procedures of citrus fiber on the TPC, TFC, and the antioxidant potential of such powder.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Folin-Ciocalteu's phenol reagent was obtained from MP Biomedicals (Santa Ana, Calif., U.S.A.). Aluminum chloride anhydrous ($AlCl_3$), potassium acetate (CH_3COOK), sodium carbonate (Na_2CO_3), HPLC-grade water, HPLC-grade acetonitrile, HPLC-grade methanol, and glacial m-phosphoric acid were obtained from Fisher Scientific (Waltham, Mass., U.S.A.). Quercetin, kaempferol, and β-carotenoid were obtained (95% > purity by HPLC) from Sigma-Aldrich® Co. (St. Louis, Mo., U.S.A.). Gallic acid was obtained from Fluka® Analytical (St. Louis, Mo., U.S.A.). An ORAC antioxidant assay kit was obtained from Zen-Bio, Inc. (Research Triangle Park, N.C., U.S.A.). Nobiletin, sinensetin, tangeretin, and heptamethoxyflavone were kindly provided by Dr. John Manthey, U.S. Horticultural Research Laboratory, United States Department of Agriculture (USDA), Fort Pierce, Florida. Citrus fibers were kindly provided by Florida Food Products, Inc. (Eustis, Fla., U.S.A.) and Natural Citrus Products Corporation (Fort Pierce, Fla., U.S.A).

3.3.2. Standard Preparation

Stock solutions of quercetin (50 μg/ mL), kaempferol (120 μg/ mL), sinensetin (1060 μg/ mL), nobiletin (540 μg/ mL), heptamethoxyflavone (1900 μg/ mL), and tangeretin (820 μg/ mL) were prepared by dissolving these compounds individually in HPLC-grade methanol. The proper amounts of each standard and cocktail of standards

were made by taking appropriate amounts from each standard and filtering them with a 0.45 μm filter before using them for the HPLC procedure.

3.3.3. Aqueous Acetone Extraction

The methodology of Rodriguez-Saona and Wrolstad (2001) was used with modifications for this study. Ten g of sample was weighed into a beaker. Then, 40 mL of acetone (1:4 ratio of w/v) was measured and placed into the beaker and blended. Using a Buchner funnel, vacuum flask, and Whatman® no. 1 filter paper, the slurry was filtered. While keeping the filtered solution in the dark and under refrigeration, the filtered slurry was placed in a beaker, and 40 mL of 70/30 (v/v) acetone/acidified (0.01% HCl) water solution was added. After sonicating for one hour, the slurry was filtered again with a Buchner funnel, vacuum flask, and Whatman® no. 1 filter paper. This procedure was repeated two more times until a faint yellow color was observed. The collected filtrates were combined, and the liquid extract was placed into a 300 mL round-bottom flask. The acetone and water in the extract were completely evaporated using a rotary evaporator at 50 °C. The residue was dissolved in 10 mL of methanol. This extract was used for the TPC, TFC, and ORAC values as well as HPLC analysis. The methanol extract was filtered with a 0.45 μm filter before being analyzed by HPLC. The extract was kept in the freezer at −18 °C during the experiment.

3.3.4. Methanol Extraction

The methanol extraction procedure, which is similar to the acetone extraction, was adopted from Sun and others (2010). First, 10 g of citrus fiber was mixed with 200 mL of methanol (1:20 w/v). After sonicating for an hour, the slurry was filtered using a Buchner funnel, vacuum flask, and Whatman® no. 1 filter paper and then kept in the dark in a refrigerator. The solid collected from the filtration went through the same procedure two more times until a faint yellow color was observed in the filtrate. All the collected liquids were combined. Using a round-bottom flask and rotary evaporator, methanol in the sample was completely evaporated at 50 °C and the residue was dissolved in 10 mL of methanol. This extract was used for the TPC, TFC, and ORAC analysis.

3.3.5. HPLC Analysis

A Varian ProStar model 410 AutoSampler equipped with Varian 210-218 pumps and a Varian 335 UV detector was used for this study. Separation was achieved by using an Eclipse XDB C_{18} 5 μm (4.6 × 250 mm) column (Agilent Technologies, Santa Clara, Calif., U.S.A.). The mobile phases consisting of 0.1% phosphoric acid in water (A) and acetonitrile (B) were used in a gradient program as follows: 100% (A) for the first 3 min, then 45% (A) for the next 9 min, and finally 5% (A) for the next 13 min. The mobile phase flow rate was 1 mL/min. The column temperature was kept at 30 °C. The methanol solution of the acetone extraction was used for this part of the study. The injection volume was 20 μL. The chromatographic peaks were identified based on their retention times and compared with the retention times of the authentic standards. Flavonoid levels

in the sample were calculated based on the external standard curve of the chosen flavonol and polymethoxyflavones (PMFs) standards.

3.3.6. Determination of Total Polyphenol Content

To determine TPC, 0.1 mL of both methanolic citrus sample extract were mixed with 2.8 mL of deionized water, 2 mL of 2% sodium carbonate (Na_2CO_3), and 0.1 mL of 50% Folin-Ciocalteu reagent. After the solution was incubated at room temperature for 30 min, the reaction mixture absorbance was measured at 750 nm. A deionized water blank was used for zeroing the spectrophotometer. Gallic acid was chosen as a standard, and a six-point standard curve was prepared (0-60 mg/L). The total polyphenol content of the citrus peel powder was expressed as gallic acid equivalents (mg GA/g) for dry powder (Lin and Tang 2007).

3.3.7. Determination of Total Flavonoid Content

To determine TFC, 0.5 mL of both methanolic citrus sample extract were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1M potassium acetate (CH_3COOK), and 2.8 mL of deionized water. After the solution was incubated at room temperature for 30 min, the reaction mixture absorbance was measured at 415 nm. A deionized water blank was used for zeroing the spectrophotometer. Quercetin was chosen as a standard, and a seven-point standard curve was prepared (0-100 mg/L). The total flavonoid content of the citrus peel powder was expressed as quercetin equivalents (mg QUE/g) for dry powder (Lin and Tang 2007).

3.3.8. Determination of Total Carotenoid Content

A slurry of 2.5 g of citrus fiber and 25 mL of n-hexane-acetone-ethanol (v/v/v: 50:25:25) was placed on a shaker for 10 min at 200 rpm at room temperature. Then, it was centrifuged at 4591 g for 15 min at 8 °C. The supernatant was collected and made to a volume of 25 mL with hexane. Absorbance was measured at 450 nm. β-carotene was chosen as standard, and a seven-point standard curve was prepared (0-100 mg/L).

The total carotenoid content was expressed as beta-carotene equivalents (mg β-C/g) of dry powder. This methodology was adopted from Lee (2001), with some modifications.

3.3.9. Oxygen Radical Absorbance Capacity Assay

For this study, instructions from the Zen-Bio kit (Zen-Bio, Inc., Research Triangle Park, N.C., U.S.A.) were followed. A 96-well microplate was used for analysis. The plate reader incubation chamber was set to 37 °C (BioTek® Instruments, Inc., Synergy™ HT, Winooski, Vt., U.S.A.); excitation wavelength was set to 485 nm; and emission wavelength was set to 530 nm. After preparation of the working solution and standard solutions, 150 µL of the working solution was added to each well followed by 25 µL of each sample, standard, or blank. Finally, 25 µL of the AAPH (2,2'-azobis-2- methyl-propanimidamide, dihydrochloride) solution was added to each well and read over 30 min. Trolox was used as the standard, and a five-point (0-50 µM) standard curve was prepared. The ORAC values of the samples were calculated based on Davalos and others (2004) and were expressed as µmole Trolox/g.

3.3.10. Statistical Analysis

Three replications of citrus fiber were evaluated for the TPC, TFC, TCC, and ORAC values. Data was analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of the SAS® Institute Inc. (2011). Means were separated by least significant difference (LSD) when significant ($P < 0.05$) treatment effects were found.

3.4. RESULTS AND DISCUSSION

Figure 3.1 shows the chromatogram of regular-washed citrus fiber. There were two groups of flavonoids found in the citrus samples. The first group was flavonols, and the second group was polymethoxyflavones.

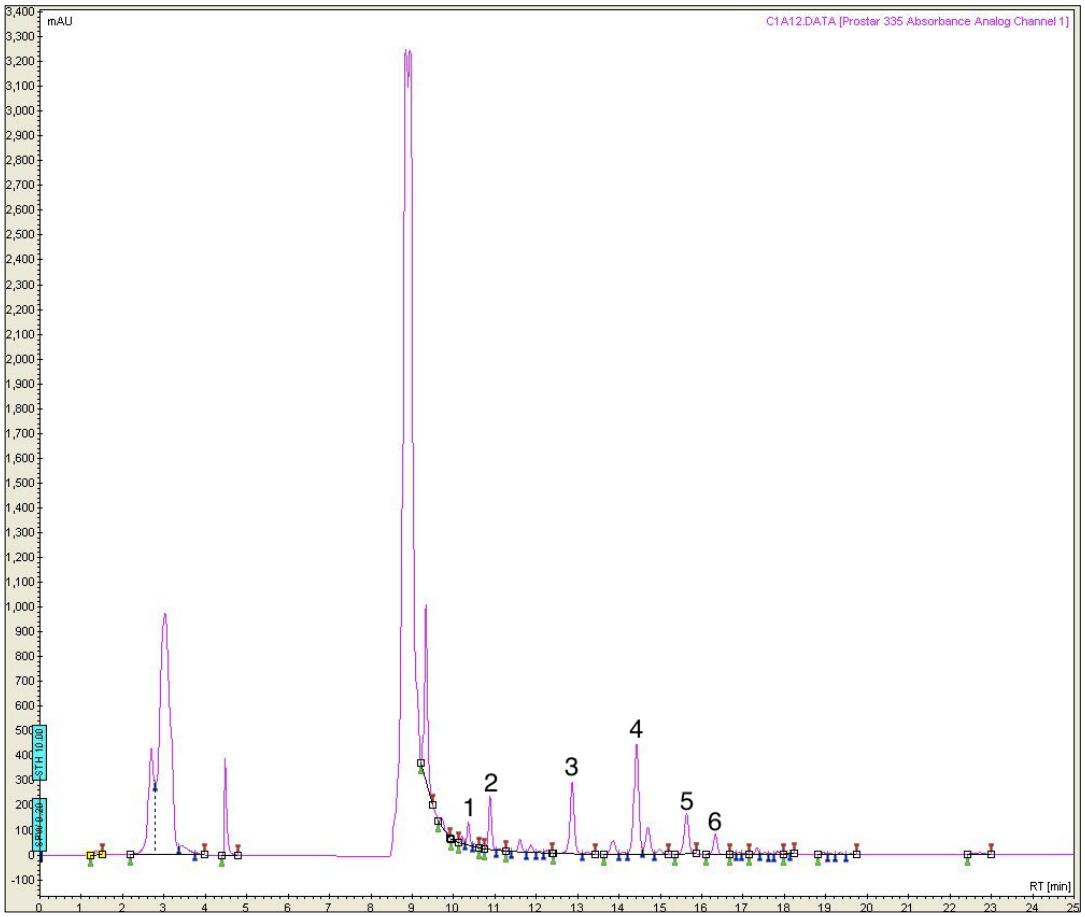


Figure 3.1. Reverse-Phase HPLC Chromatogram of Flavonols and Polymethoxyflavones Extracted From Regular-Washed Citrus Fiber

Peak identifications: 1 - quercetin (QUE), 2 - kaempferol (KAE), 3 - sinensetin (SIN), 4 - nobiletin (NOB), 5 - heptamethoxyflavone (HMF) and 6 - tangeretin (TAN).

There was good separation of four major PMFs and flavonols, which eluted after 10 min. While glycosylated flavonones with an elution window of 8-10 min were also detected based information obtained from running pure standards of glycosylated flavanones (narirutin, hesperidin and isosakuranetinrutinoside), they were insufficiently separated for proper identification or quantitation. The method used in this study provides a fast analysis time for identification of flavonols and PMFs. The quantification of flavonols and polymethoxyflavones were carried out using external standard curves. The results are shown in Table 3.1.

Table 3.1. Flavonols and Polymethoxylated Flavones Concentration in Regular-Washed Citrus Fiber From Florida

Compound	Quantity (mg/g)
Flavonols	
Quercetin	0.0276 ± 0.0107
Kaempferol	1.0945 ± 0.439
Polymethoxylated Flavones	
Sinensetin	1.9608 ± 0.958
Nobiletin	3.3304 ± 1.621
Heptamethoxyflavone	1.2155 ± 0.599
Tangeretin	0.5005 ± 0.251

Each value in the Table is represented as mean ± standard deviation (n=3).

Nobiletin had the highest concentration of all the flavones, followed by sinensetin, then heptamethoxyflavone. Similar results were obtained by Green and others (2007). They found that Navel orange peels from the Jamaican cultivars had 3.8 mg/g nobiletin, 3.6 mg/g sinensetin and 2.6 mg/g heptamethoxyflavone. They also found that the majority of the Mexican citrus cultivars had nobiletin as the highest PMF in the citrus peels and nobiletin concentration for sweet orange peels type 1 and type 2 were 5.67 mg/g, 3.96 mg/g, respectively. The presence of flavonoids is influenced by the type of cultivar, as well as by the different tissues (albedo, flavedo, juice vesicles) of citrus fruits (Nogota and others 2006). Our findings were within the range of the results reported in earlier studies that focused on the identification and quantification of flavanone glycosides and PMFs (Manthey and Grohmann 1996; Bocco and others 1998; Green and others 2007; Sun and others 2010). A few studies also found the presence of flavonols, particularly quercetin and kaempferol, in citrus cultivars. Kawaii and others (1999) found quercetin in trace amounts in *Citrus paradisi* (grapefruit) juice extract, while kaempferol was found in trace amounts in *Citrus aurantium* (sour orange) juice extract. Also, Ross and others (2000); Vanamala and others (2006) reported the presence of quercetin in commercially available grapefruit juices. Wang and others (2008) investigated the presence of flavonols in eight citrus species in Taiwan. They found trace amounts of quercetin and kaempferol in the peels of all citrus cultivars. Recently, Shalaby and others (2011) identified the presence of kaempferol in *Citrus aurantifolia* (key lime) leaves, while quercetin was identified in both the leaves and peel of *Citrus sinensis* (sweet orange).

Quercetin has shown some antiviral activity (Kaul and others 1985; Tripoli and others 2007). While it has been reported that polymethoxylated flavones provide antibacterial activity (Tripoli and others 2007).

Table 3.2 shows the comparison of acetone and methanol extraction methodologies and citrus fiber type on the presence of the TPC, TFC, and ORAC values.

Table 3.2. Effect of Extraction Methodology and Type of Citrus Fiber on Total Polyphenol Count, Total Flavonoid Count, and Oxygen Radical Absorbance Capacity of Citrus Fiber

Citrus Samples	Method of Extraction	TPC (mg GAE/g)	TFC (mg QUEE/g)	ORAC Value (μMole Trolox/g)
C1	Acetone	7.959 ± 0.79^{a}	9.621 ± 0.38^{a}	27.834 ± 6.86^{a}
C1	Methanol	3.753 ± 0.49^{b}	2.825 ± 0.008^{b}	10.036 ± 1.94^{b}
C2	Acetone	9.959 ± 1.31^{a}	9.413 ± 0.97^{a}	15.589 ± 4.53^{b}
C2	Methanol	2.503 ± 0.16^{b}	2.518 ± 0.28^{b}	0.318 ± 3.35^{c}

[a, b, c] Different letters in the same column indicate significant difference ($P < 0.05$) analyzed by t-tests (least significant difference).
Each value in the Table is represented as mean ± standard deviation (n = 6).
C1: represents regular-washed samples.
C2: represents hot-washed samples.

The results show that the total polyphenol content, total flavonoid content, and oxygen radical absorbance capacity values were impacted by the extraction methodology.

While the total polyphenol content of citrus fibers did not significantly ($P > 0.05$) differ between hot-washed and regular-washed citrus fibers; extractions using the acetone methodology yielded significantly ($P < 0.05$) higher results than those extracted with methanol. Li and others (2006) found that when using 72% to 85% ethanol, the recovery of lemon peel's total polyphenol content was highest in comparison to using more than 85% or less than 72% ethanol. Our findings also showed that the total flavonoid contents of both regular-washed and hot-washed citrus fibers were significantly ($P < 0.05$) higher for the acetone extraction, which as shown in Table 3.2. Ghasemi and others (2009) reported similar results for the TFC for 13 cultivars of citrus peel including sweet orange, bitter orange, lemon etc., but they reported much higher total polyphenol contents (90.3-396.8 mg GAE/g) than our study found. While TPC and TFC were only affected by the extraction method but not the processing method, the ORAC values were affected by both. Hot-washed samples for either extraction method had lower ORAC values than regular-washed samples. Li and others (2006) reported that frozen lemon peel made from fresh lemons had significantly higher total polyphenol content than oven-dried lemon peel for dried bases. They suggested that a long drying time or temperature might cause a decrease in the total polyphenol content. Based on this information, however the presence of water in the aqueous acetone solvent might increase the extraction of polyphenols and flavonoids more than using 100% methanol. Second, the use of hot water for citrus fiber preparation might lead to a reduction in the antioxidant capabilities of some of the polyphenols and flavonoids, resulting in much lower ORAC values. In the final part of the experiment, the total carotenoid contents of citrus fibers were determined and calculated based on a β-carotenoid standard curve ($R^2 = 0.99947$). There was no

statistical difference ($P > 0.05$) in the total carotenoid content between hot-washed citrus fibers and regular-washed citrus fiber, which as shown in Table 3.3. Wang and others (2008) reported the highest total carotenoid content in *Citrus reticulata Blanco* (tangerine) peel with 2.04±0.036 mg/g, while *Citrus sinensis* L. Osbeck (orange) peel had 0.445±0.008 mg/g, and *Citrus limon* L. Bur peel had 0.110±0.001 mg/g.

Table 3.3. Effect of Type of Citrus Fiber on Total Carotenoid Content of Citrus Fiber

Citrus Samples	TCC (mg β-CE/g)
C1	0.176 ± 0.0046
C2	0.168 ± 0.0046

Each value in the Table is represented as mean ± standard error (n = 6).
C1: represents regular-washed samples.
C2: represents hot-washed samples.

3.5. CONCLUSION

The high-pressure liquid chromatography technique used in this study provided fast results and good separation for polymethoxyflavones and flavonols in citrus fiber. Nobiletin, sinensetin, heptamethoxyflavone, tangeretin, quercetin, and kaempferol were identified. While quercetin was found in trace amounts, polymethoxyflavones were found in much higher quantities in the citrus fiber. Acetone extraction yielded higher total polyphenol and total flavonoid concentrations as well as ORAC values for both types of citrus fibers, while processing, i.e. hot-washing or regular-washing, of citrus fibers did not influence the TPC or the TFC. However the regular-washed citrus samples had a higher antioxidant capacity than the hot-washed citrus fibers. Furthermore, the total carotenoid content of the citrus samples did not significantly ($P > 0.05$) differ for the two types of citrus fibers. Results of our study also indicated that citrus fibers used in this study had relatively lower flavonoid concentration, TPC, TFC and ORAC values than what was found in the literature with fresh citrus peels.

CHAPTER 4

THE EFFECT OF CITRUS FIBER ON THE QUALITY ATTRIBUTES OF GROUND BEEF MEATBALLS

4.1. ABSTRACT

The objective of this study was to determine the effect of different citrus fiber (CP) levels (0%, 1%, 5%, and 10%) on the quality attributes of ground beef meatballs at day 0, 3, 6, and 9. The results showed that treatment and day had a significant ($P < 0.05$) effect on the pH of raw samples, however pH of cooked samples was not significantly ($P > 0.05$) impacted by the addition of citrus fiber. With the addition of citrus fiber the water holding capacity (WHC) increased but there was no significant ($P > 0.05$) difference between treatments. Treatment levels had a significant ($P < 0.05$) effect on the cooking yield (CY) but there was no trt*day effect found. The control samples had the lowest CY, and CY increased with a rise in the CP level. Increasing the CP level was negatively correlated with textural properties in comparison to no citrus fiber added ground beef samples. But, there is no significant ($P > 0.05$) difference found between the control (CF 0%) and the CF 1% for hardness and springiness values. Hunter color *L*, *a*, *b* values decreased with increasing CP levels, and there was no trt*day effect found for Hunter color *L*, *a*, *b* values. Citrus fiber at 1% level can be used in meat products to increase the CY and WHC without detrimental effects on quality.

4.2. INTRODUCTION

There is a growing trend in the food product development area for the inclusion of functional ingredients in food products. These functional ingredients can provide both health benefits as well as improve the safety and/or quality of such products. One of these ingredients is dietary fiber. Dietary fiber has been described by Trowell (1976) as the portions of plant cells that are resistant to digestion by human digestive enzymes. Dietary fiber has been classified into two groups: soluble and insoluble. Soluble dietary fiber has been described by Anderson and others (2009) as the water soluble portion of fiber that is fermented in the colon. In contrast, insoluble dietary fiber does not dissolve in water but provides bulking. Examples of soluble dietary fiber are pectins, β-glucans, and galactomannan gums; insoluble dietary fiber includes lignin, cellulose, and hemicellulose (Rodriguez and others 2006).

Some researchers have investigated the benefits of fiber for human health. Their findings suggest that soluble fibers are linked to decreased cholesterol levels and adsorption of intestinal glucose, while insoluble fibers are associated with regulation of the intestine (Scheneeman 1987). In addition, Gallaher and others (1992), proposed that dietary fiber caused total cholesterol and low-density lipoprotein (LDL) levels to decrease in plasma, through the excretion of bile acids.

In addition to health benefits, dietary fiber has been investigated for its functional properties in food products. Many fiber-rich food products, such as nutrition bars, drinks, and dairy products, have been developed and are commercially available in U.S. markets. There are also opportunities to develop new meat products with dietary fiber to provide

both health and functional benefits. Most studies conducted on the impact of adding dietary fiber to enhance meat product quality showed that adding fiber to various meat products (e.g., sausages, patties, and meatballs) helped to increase their cooking yield and water-binding capacity (Serdaroglu and others 2005; Yang and others 2007; Bilek and Turhan 2009; Choi and others 2010). Most of these studies used whole grains, oat brans, psyllium seeds, and legumes as a source of fiber. However, there is little research related to the use of fruits and vegetables as a source of fiber. Grigelmo-Miguel and others (1997) used peach fiber in low-fat frankfurters; Fernandez-Lopez and others (2004) used citrus albedo in bologna and dry salami; Aleson-Carbonell and others (2005) used citrus fiber in fresh sausage; and Sayago-Ayerdi and others (2009) used grape fiber in chicken hamburgers. According to Gorinstein and others (2001), citrus peel (albedo and flavedo) is rich in soluble fiber and can be used in meat products as a functional ingredient.

Based on this information, the objective of our study was to determine the impact of adding citrus fiber on the quality attributes of beef meatballs. The quality attributes investigated were the pH of both the raw and cooked meatballs, water holding capacity (WHC), cooking yield (%), textural properties, Hunter color *L*, *a*, and *b* values, and proximate composition.

4.3. MATERIALS AND METHODS

4.3.1. Sample Preparation

Beef cattle were slaughtered and their carcasses placed in a cooler for 48 hours. Later, two bottom rounds were collected from the carcass and weighed. After cutting the beef bottom rounds into smaller pieces, they were two-step (course and fine) ground using a LEM™ Products .35 HP stainless steel electric meat grinder (West Chester, OH). Once they were ground, they were separated into four treatment groups and weighed. The treatment group with 0% citrus fiber, in other words control (CP 0%) was made into ground beef meatballs using a 50-mm diameter ice cream scoop; the meatballs were placed onto four Styrofoam® trays for day 0, day 3, day 6, and day 9, and were covered with stretch film and labeled for replication, treatment group, and experimental days. Packages were then placed into a refrigerator. Treatments of 1%, 5%, and 10% citrus fiber were weighed based on the ground beef weight, and the powder was mixed into the ground beef using a KitchenAid® blender. After each mixing, the blender was cleaned before mixing the next treatment group. Later, meat from each group was also made into meatballs using a 50-mm diameter ice cream scoop. The meatballs were placed onto Styrofoam® trays covered with stretch film, and labeled for replication, treatment group, and experimental days. Packages were placed into the refrigerator until their use in the experiment. This procedure was replicated two more times on different slaughtering days to provide three total replications.

4.3.2. pH

A 5 g sample was homogenized with 45 mL distilled water by using a blender. Then, the pH of the slurry was determined by using a Fisher Accumet® model 230A pH/ion meter (Fisher Scientific Inc., Salt Lake City, UT). The pH measurements of both the raw and cooked samples of the three replicates were determined in duplicates.

4.3.3. Water Holding Capacity

The water holding capacity of the samples was determined according to methods reported by (Wierbicki 1958). Between 0.48 and 0.52 g of the raw ground beef treatment was placed on the center of the Whatman® No. 1 filter paper. The paper with the meat sample was placed between two Plexiglas® plates. The plates were pressed 1 minute with 500 psi of pressure using a Carver® press (Laboratory Press Model C, Carver®, Inc., Wabash, IN). After this, the meat and wet area were calculated by using a rib eye area grid transparent sheet. The formula used to calculate the water holding capacity (WHC) is shown below (Price and Schweigert 1987); WHC was determined in triplicate for each treatment.

$$WHC = \left[\frac{\text{Area of free water}}{\text{Area of meat}}\right]$$

4.3.4. Cooking Yield

The cooking yield of the ground beef meatballs was calculated by using the formula shown below (Bishop and others 1993). Weights of the raw meatball treatments were recorded, and the meatballs were placed in a tray on a rack. A temperature probe was placed into one of the meatballs and the tray was placed into an oven. The temperature probe was set at 72 °C (160 °F). Once the ground beef meatballs were cooked, they were taken out of the oven. A couple of minutes later, they were lightly blotted to remove the excess fat on their surface. Later, their cooked weight was recorded, and triplicates of each treatment's cooking yield (%) were calculated.

$$\text{Cooking Yield \%} = \left[\frac{\text{Cooked weight of the product}}{\text{Uncooked weight of the product}}\right] * 100$$

4.3.5. Determination of Moisture, Fat and Protein Content

The moisture and fat content of the meat samples was determined based on the CEM SMART Trac system. This two-step system uses microwave for determining the moisture content of a meat sample. Next, it uses nuclear magnetic resonance (NMR) analysis for determining a fat content of the microwaved sample (Keeton and others 2003). The protein content was determined using bicinchoninic acid (BCA) colorimetric detection and quantitation of the total protein method, according to Smith and others (1985). For this purpose, a Pierce™ BCA Protein Assay Kit (Thermo Scientific™, IL) was used. This kit provides working range of 20-2,000 μg/ml for protein concentration. In two-step reaction, first peptide bonds in proteins reduce cupric ions (Cu^{+2}) to cuprous

ions (Cu^{+}). This reaction is temperature dependent and the reduction in cupric ions is proportional to the amount of protein presents. Next two molecules of bicinchoninic acid (BCA) bind with cuprous ion forming purpled color compound that absorbs light at 562 nm. Bovine serum albumin (BSA) is used as a standard, and a standard curve was made for calculation of protein contents of the samples.

4.3.6. Texture Profile Analysis

After ground beef meatballs were cooked and their weight was recorded for the cooking yield procedure, they were cooled to room temperature before texture profile analysis (TPA). Each meatball was compressed to 50 percent of its original height in two consecutive cycles at a crosshead speed of 50 mm/min by using a TA-TX2 texture analyzer (Stable Micro Systems, Surrey, UK) with a 38 mm diameter probe for the evaluation of the texture profile analysis, as described by Bourne (1978). Triplicates of each treatment were evaluated for hardness, springiness, cohesiveness, gumminess, chewiness, and resilience.

4.3.7. Hunter Color Values

Hunter color *L* (lightness), *a* (redness) and *b* (yellowness) values were evaluated using a Minolta colorimeter (Konica Minolta Chroma Meter CR-410, Minolta Ltd., Milton Keynes, UK). The raw ground beef treatments were placed onto Styrofoam® trays individually, and treatments were spread flat on the tray to provide an even surface for color measurement. The Minolta colorimeter was placed directly on the surface of the ground beef samples. Color values were measured in triplicate for each treatment.

4.3.8. Statistical Analysis

Three replications of ground beef meatballs were evaluated for cooking yields, WHC, pH, TPA, Hunter color values, moisture, fat and protein content. The analysis of variance (ANOVA), using the general linear model (GLM) procedure of the (SAS 2011), was randomized complete block design in which the block was a carcass. The treatments were arranged as a 4×4 factorial (4 levels of citrus fiber, 4 days). Means were separated by the least significant difference (LSD) when significant ($P < 0.05$) treatment effects were found.

4.4. RESULTS AND DISCUSSION

4.4.1. pH

Table 4.1 shows the effect on pH of adding citrus fiber to both raw and cooked ground beef samples. The pH range of the raw treatment ranged between 5.39 and 5.68 during shelf life. During the time of storage, the pH of the raw control treatment (CP 0%) was not significantly ($P > 0.05$) different from other treatments; however, during the time of storage, the pH increased for all the treatments of the raw samples. Also, cooking caused a rise in the pH of all treatments except the CP 10% treatment. Similar results were also observed by Bilek and Turhan (2009). The pH after cooking for all the treatments also significantly increased ($P < 0.05$) over storage time, with the pH ranging between 5.38 and 5.83. Adding 10% citrus fiber caused a significant ($P < 0.05$) change in the pH of the cooked samples. However, the change in the pH of treatments with 1% and 5% citrus fiber was not significant ($P > 0.05$) in comparison to change in the pH of the control. There was no trt*day effect found for both pH of raw and cooked samples.

Table 4.1. Effect of Citrus Fiber on pH of Both Raw and Cooked Ground Beef Meatballs Over Time at Refrigerated Storage

Citrus Fiber	Experimental Days				
Treatment Levels	0	3	6	9	Mean Value[A]
Raw Samples					
0%	5.44	5.66	5.46	5.58	5.54 ± 0.029[ab]
1%	5.56	5.68	5.57	5.66	5.62 ± 0.029[a]
5%	5.55	5.61	5.53	5.67	5.59 ± 0.029[a]
10%	5.41	5.51	5.39	5.57	5.47 ± 0.029[b]
Cooked Samples					
0%	5.63	5.68	5.58	5.72	5.65 ± 0.026[a]
1%	5.64	5.73	5.75	5.83	5.74 ± 0.026[a]
5%	5.59	5.63	5.69	5.73	5.66 ± 0.026[a]
10%	5.38	5.48	5.50	5.60	5.49 ± 0.026[b]

[a, b] Different superscripts in the same column indicates significant difference ($P<0.05$).
[A] Mean value ± standard error (n=24).

4.4.2. Water Holding Capacity (WHC) and Cooking Yield (CY %)

Table 4.2 illustrates the impact of adding citrus fiber on the water holding capacity and cooking yield of ground beef meatball treatments. The addition of citrus fiber boosted both the WHC and cooking yield. There was no trt*day effect found. Besbes and others (2008) reported that an increase in the addition of wheat fiber caused a rise in the water holding capacity of beef burgers in comparison to the control burger

samples. Furthermore, the cooking yield of CP 10% was highest at 92.21, and all the citrus treatments had significantly ($P < 0.05$) higher cooking yields than the control (CP 0%). There was no trt*day effect found for cooking yield (%) of the samples. Serdaroglu and others (2005) found similar results with the use of lentil flours on improving the water holding capacity and cooking yield of low fat meatballs. Cengiz and Gokoglu (2007) also reported that the addition of citrus fiber reduced the cooking loss for frankfurter-type sausages.

Table 4.2. Impact of Adding Citrus Fiber to Ground Beef Samples on Water Holding Capacity (WHC) and Cooking Yield (%)

Quality Attributes	Citrus Fiber Treatment Levels			
	0%	1%	5%	10%
WHC	0.980 ± 0.021	0.981 ± 0.021	0.987 ± 0.021	0.955 ± 0.021
Cooking Yield (%)	71.43 ± 2.47^{c}	78.91 ± 2.47^{b}	86.62 ± 2.47^{a}	92.21 ± 2.47^{a}

$^{a, b, c}$ Different superscripts in the same row indicate significant difference ($P < 0.05$).
Each value in the Table is represented as mean ± standard error (n=36).

4.4.3. Determination of Moisture, Fat and Protein Content

The moisture, fat and protein content of the ground beef treatments are shown in Table 4.3. The moisture content of the control was highest, and an increase in the addition of the dry ingredient—citrus fiber—caused a decrease in the moisture content of all treatments. CP 10% had a significantly ($P < 0.05$) lower moisture content than the control (CP 0%).

Table 4. 3. Moisture, Fat and Protein Content of Ground Beef Meatball Samples

Citrus Fiber Treatment Levels	Moisture Content %	Fat Content %	Protein Content %
0%	60.75 ± 2.51^{a}	21.30 ± 3.01^{a}	14.46 ± 0.69^{d}
1%	60.51 ± 2.14^{a}	20.59 ± 2.76^{ab}	16.49 ± 0.49^{c}
5%	58.49 ± 1.86^{ab}	19.81 ± 2.90^{b}	19.28 ± 0.81^{b}
10%	56.35 ± 3.88^{b}	19.68 ± 3.26^{b}	21.16 ± 0.64^{a}

[a,b,c,d] Different superscripts in the same column indicate significant differences ($P < 0.05$).
Each value in the Table is represented as mean ± standard deviation (n=9).

While the gradual decrease in moisture content was expected due to addition of dry powder in different levels, the major increase in the protein content was not expected. Even with the addition of 6.37% protein coming from citrus fiber, increase in the protein content was normal than higher. This could be due to BCA colorimetric methodology. Smith and others (1985) reported that presence of glucose caused artificially high protein

content values. Kessler and Faneshil (1986) also reported that phospholipids can react with bicinchoninic acid (BCA) that can cause artificially high protein content. Since, citrus fiber has sugars, such as glucose that may interfere with our results and therefore it may cause artificially high protein content. Table 4.4 displays the nutritional facts associated with CitraFiber™ citrus fiber. Huang and others (2011) reported similar results: The addition of wheat fiber into Chinese-style sausages caused a decrease in the moisture content and an increase in the protein content.

Table 4.4. Nutritional Facts About CitraFiber™

Components	Amount
Total Pectin	9390 mg / 100g
Protein	6.37 %
Total Sugars	1.7%
Total Dietary Fiber	82.7%
Soluble Fiber	23.4%
Insoluble Fiber	59.3%
Potassium	453 mg / 100g
Sodium	210 mg / 100g
Calcium	78 mg / 100g
Vitamin A (Beta Carotene)	117 IU / 100g
Vitamin C	0.91 mg / 100g

Source: Natural Citrus Products

4.4.4. Textural Properties

The textural properties of ground beef meatballs made with or without citrus fiber during shelf life are shown at Table 4.5. Hardness was described by Choi and others (2003) as the peak value of the first compression. Hardness has a significant impact on the overall appeal of meat products to consumers (Neely and others 1998), such that if the meat product is tender, consumers tend to perceive it as flavorful and juicy (Shorthose and Harris 1991). Our results showed that the addition of citrus fiber caused a decrease in hardness. The control had the highest hardness values, and there were no significant ($P > 0.05$) difference between the control and CF 1%. However, there were significant ($P < 0.05$) differences between treatments in terms of all of the textural properties. Furthermore, there were no trt*day effect found for all of the textural properties. Yang and others (2007) reported similar results: Adding hydrated oatmeal and tofu caused a decrease in the hardness of low-fat pork sausages. There were also reports of the hardening of meat products with the addition of fiber. Cofrades and others (2000) stated that the addition of soy fiber caused an increase in the hardness of bologna-type sausage. Huang and others (2011) also found hardening in Chinese-type sausages made with wheat or oat fiber.

Springiness was described as the height of the meatball in cm recovered between the end of compression 1 and the start of compression 2 (Choi and others 2003). While springiness slightly decreased with the addition of citrus fiber, the significant difference ($P < 0.05$) was observed between the control and CP 5 and 10%.

Cohesiveness was described as the ratio of the area under compression curve 1 to the area under compression curve 2 (Choi and others 2003). The cohesiveness of ground

beef meatballs made with 0% and 1% citrus fiber was significantly higher ($P < 0.05$) than the meatballs made with 5% and 10% citrus fiber. Samples made with 10% citrus fiber had less cohesiveness and resilience than those of other treatments; this was even visually observable. Meatballs made with 10% citrus fiber were much easier to break using the compression force.

Table 4.5. Effect of Citrus Fiber on Textural Properties of Ground Beef Samples

Citrus Fiber	Textural Properties					
Levels	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
CP 0%	1356.13[a]	0.746[a]	0.553[a]	743.90[a]	563.01[a]	0.228[a]
CP 1%	1088.89[ab]	0.714[a]	0.480[b]	521.59[b]	378.63[b]	0.194[b]
CP 5%	887.17[b]	0.611[c]	0.346[c]	304.06[c]	201.22[c]	0.145[c]
CP 10%	819.69[b]	0.656[d]	0.244[d]	198.62[c]	120.83[c]	0.121[d]

[a, b, c, d] Different letters in the same column indicate a significant difference ($P < 0.05$).
Values are means of three replicates (n=36).

4.4.5. Hunter Color *L, a, b* Values

Results of the Hunter Color *L*, *a*, *b* values were summarized at table 4.6. Results of the study showed that trt*day effect were not found for *L*, *a* and *b* values. While the addition of citrus fiber caused significant ($P < 0.05$) decrease in lightness, redness and yellowness values overall. Only exception, there was no significant ($P > 0.05$) difference found between yellowness values for the control and the CF 10%. The changes in color of treatments were visually apparent and can be seen by the picture 4.1 below. Also, the color of citrus fiber was shown at picture 4.2 that its influence on the color can be observed.

Table 4.6. Effect of Citrus Fiber on Hunter Color *L*, *a*, *b* Values of Raw Ground Beef Samples

Citrus Fiber Levels	Hunter Color		
	L Value	*a* Value	*b* Value
CP 0%	48.24 ± 0.49^{a}	23.36 ± 0.62^{a}	9.98 ± 0.14^{a}
CP 1%	44.42 ± 0.49^{b}	18.64 ± 0.62^{b}	9.35 ± 0.14^{b}
CP 5%	42.92 ± 0.49^{c}	11.89 ± 0.62^{c}	9.39 ± 0.14^{b}
CP 10%	45.61 ± 0.49^{b}	8.23 ± 0.62^{d}	10.29 ± 0.14^{a}

[a, b, c, d] Different letters in the same column indicate a significant difference ($P < 0.05$).
Each value in the Table is represented as mean ± standard error (n=36).

Picture 4.1. Hunter Color Measurement of Raw Ground Beef Treatments

Picture 4.2. Citrus Fiber

Figure 4.1 illustrates the Hunter color lightness values of different treatments during shelf life. Lightness is described as the brightness or darkness of a color. It has scale of 0 to 100, with 0 being black and 100 being white. The control treatment (CP 0%) had the highest lightness values ($P < 0.05$) for all of the storage days in comparison to all of the other treatments. The lightness values of all the treatments increased ($P > 0.05$) during the storage time. Bilek and Turhan (2009) observed similar results, where the addition of flax seed flour caused a decrease in the lightness values of the beef patties made with 20% fat content.

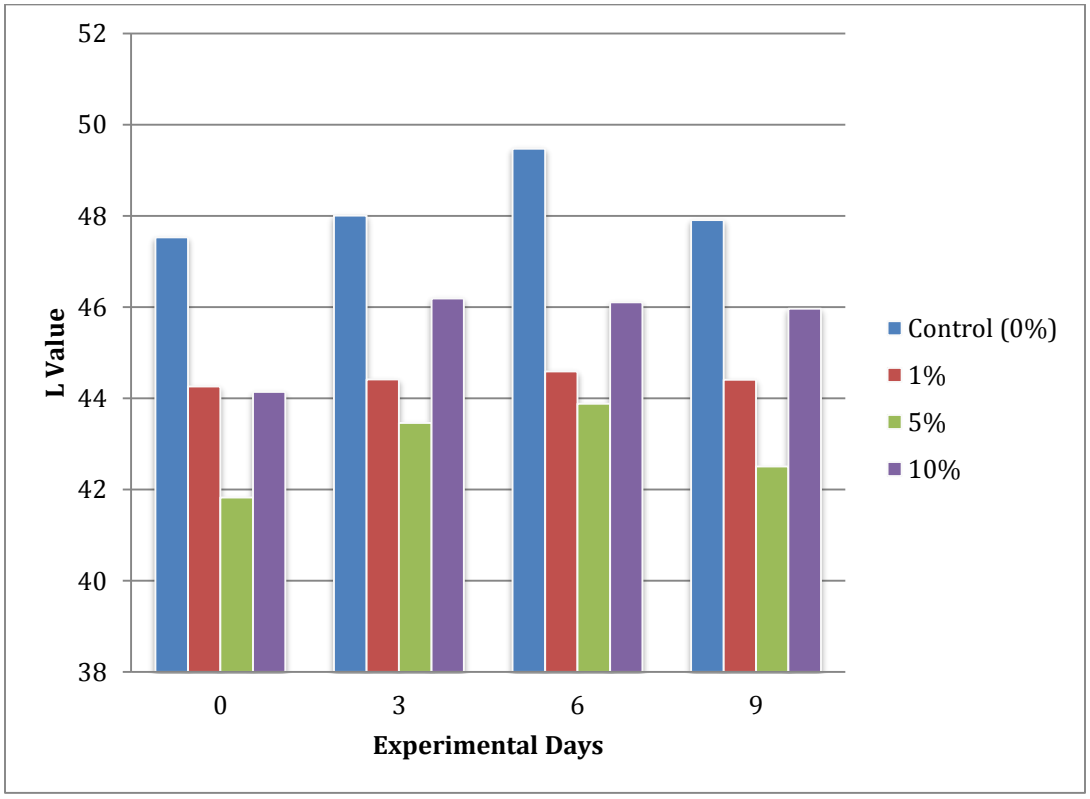


Figure 4.1. Effect of Citrus Fiber on Hunter Color *L* Values of Raw Ground Beef Meatballs

Figure 4.2 details the change in the Hunter color *a* values of raw ground beef with or without citrus fiber during storage time is shown in Figure 2. The Hunter color *a* value describes a scale of redness (a+ = red; a- = green). The results showed that both treatment and storage day had a significant effect ($P < 0.05$) on redness values. However, there was no trt*day effect found for redness values. The control treatment redness values were significantly higher ($P < 0.05$) than all of the other treatments for all of the storage days. The only exception was found at day 9, where there was no significant difference ($P > 0.05$) between the control treatment and CP 1%. The addition of citrus fiber caused a decrease in the redness values for raw ground beef samples. Fernadez-Gines and others (2003) reported an increase in the redness values when citrus fiber were first added to bolognas but a decrease in the redness values during storage time. The difference between our findings and those of prior studies could result from our product being raw and mixed ground beef whereas other studies were conducted with cooked emulsified products.

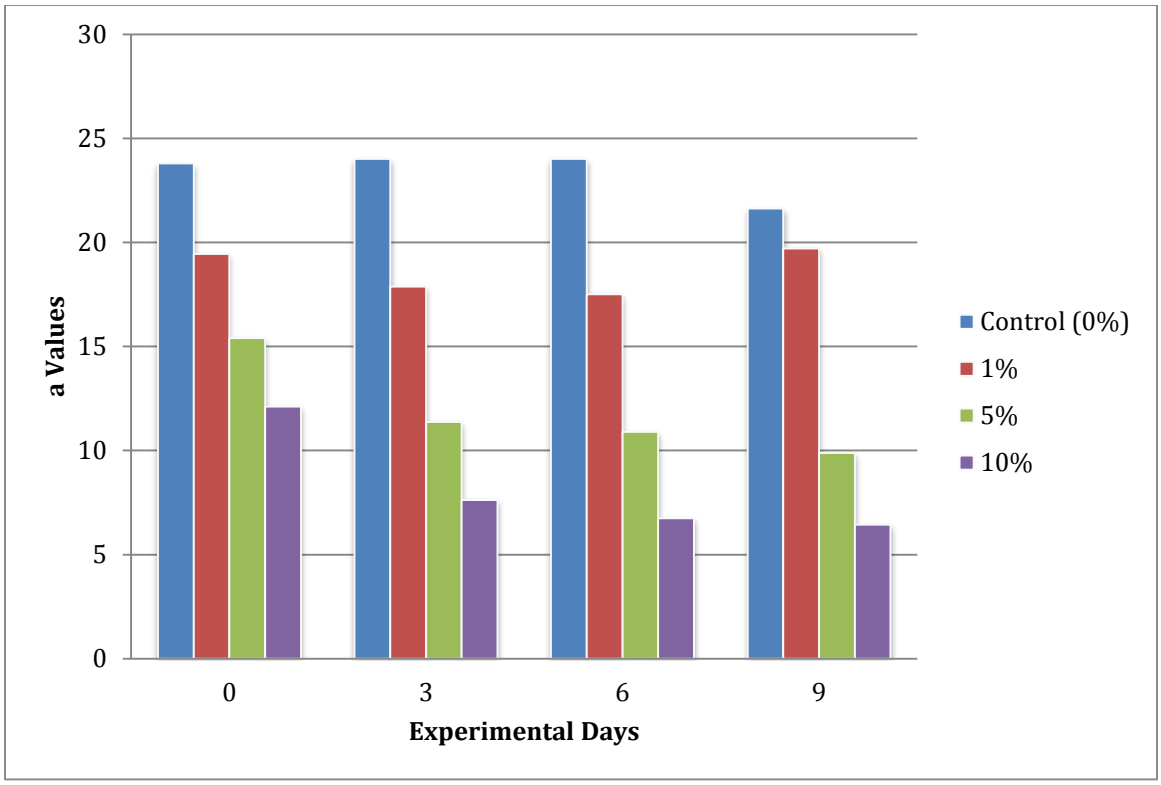


Figure 4.2. Effect of Citrus Fiber on Hunter Color *a* Values of Raw Ground Beef Meatballs

Figure 4.3 shows the impact of adding citrus fiber to raw ground beef on Hunter color *b* values during storage time. Hunter color *b* value is a scale of yellowness (b+ = yellow; b- = blue). The results showed that the addition of citrus fiber to raw ground beef significantly ($P < 0.05$) impacted the *b* values of all treatments. Furthermore, the *b* values of all the treatments slightly increased during their shelf life. Also, trt*day effect was not found for yellowness values. While the addition of citrus fiber at 10% level had the highest yellowness values, it was not significantly ($P > 0.05$) different than the control. Cofrades and others (2000), Fernadez-Gines and others (2003), and Cengiz and Gokoglu (2007) reported similar results: Increasing the addition of fiber caused a rise in *b* values.

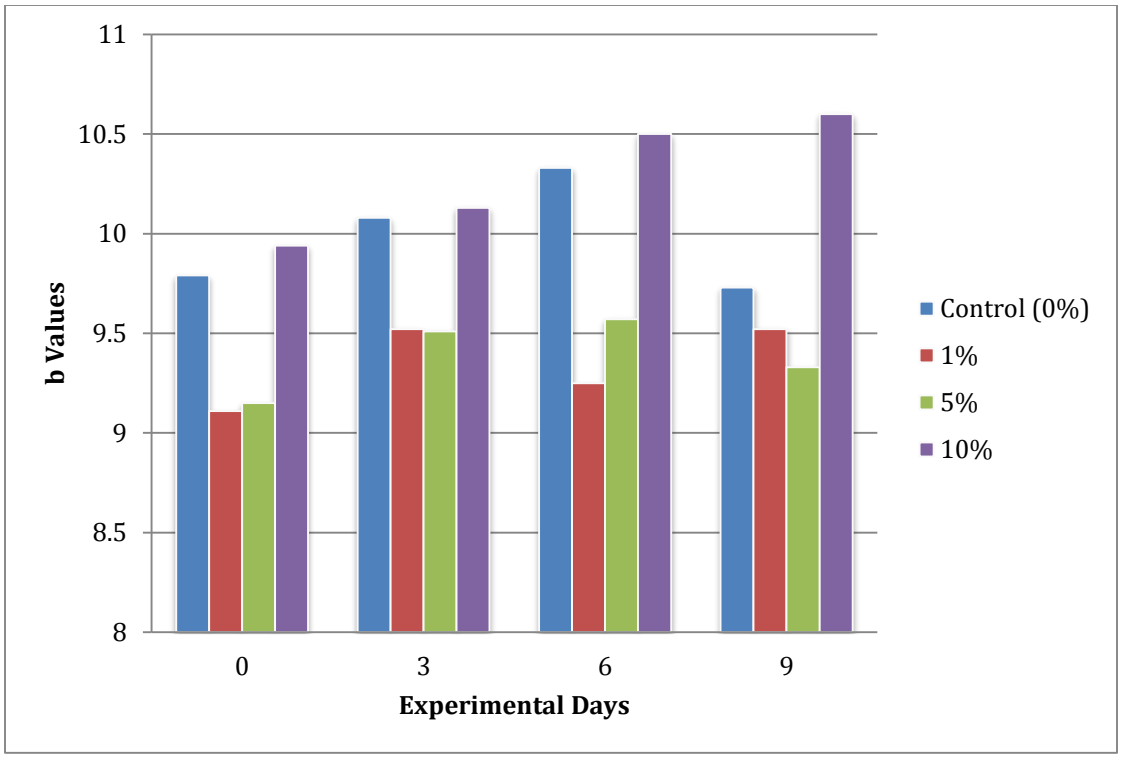


Figure 4.3. Effect of Citrus Fiber on Hunter Color *b* Values of Raw Ground Beef Meatballs

4.5. CONCLUSION

Adding citrus fiber to beef meatballs had a significant effect on the quality attributes. While the addition of citrus fiber did not signifcantly impact the pH of ground beef meatballs, the cooking yield and water holding capacity of these meatballs formulated with citrus fiber were improved. The addition of citrus fiber also had an impact on the texture of the meatballs. Beef meatballs made with citrus fiber had a softer texture than beef meatballs made without citrus fiber. However, beef meatballs formulated with the highest citrus fiber level, CP 10%, had the lowest cohesiveness and resilliance values. There was no statistical difference between samples with no fiber in comparison to samples with 1% citrus fiber for hardness and springiness textural properties. Hunter color *L*, *a*, and *b* values were also impacted by the addition of citrus fiber. While lightness, redness and yellowness values decreased by the addition of citrus fiber, only yellowness of CF 10% was not statistically different than the control. According to these results, citrus fiber can be added to ground beef meatballs to increase the cooking yield and water holding capacity at a 1% level without causing a significant change in overall quality attributes.

CHAPTER 5

PREDICTION OF OXIDATION IN GROUND BEEF MEATBALLS MADE WITH CITRUS FIBER BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

5.1. ABSTRACT

Fourier transform infrared spectroscopy (FTIR) is a rapid and non-invasive technique that provides vibrational information about the compounds that absorb light in the mid infrared region. The FTIR spectral features are unique for each compound and provide fingerprint-like information of the compound. Lipids are organic molecules that give absorbance at 4000-500 cm^{-1}. Therefore, objective of this study was to determine the oxidative stability of ground beef made with different levels of (0, 1, 3, and 5%) citrus fiber during 1, 3, 5, 7 days of refrigerated storage. In addition to FTIR analysis, moisture and fat content of all the treatments were determined.

Triplicates of each solvent-extracted fat sample were measured between 4,000 and 500 cm^{-1} at a resolution of 4 cm^{-1} with 128 scan using a Nicolet 380 spectrometer (Thermo Scientific, U.S.A.). The spectrum of each sample was corrected against the background spectrum of air to present the spectra in absorbance units. Measurements were done in triplicate. Delight software (D-Squared Development Inc., LaGrande, OR, USA) is used for analysis of the FTIR data. Trend analysis with fixed block mean, polynomial subtract (first order) and smoothing (8 cm^{-1}) options were chosen to analyze all the data. For monitoring lipid oxidation, peaks at 2924, 2853, and 1743 cm^{-1} were used. The moisture and fat content of the meat samples was determined based on the CEM SMART Trac system (CEM Corp., North Carolina, U.S.A.).

Results show that the addition of citrus fiber (CF) caused more decrease in absorbance in comparison to the control (CF 0%) at bands 2924 cm^{-1} and 2853 cm^{-1} due to the production of oxidation products. The band at 1743 cm^{-1} was also a useful

indicator. By day 3, weak band appeared around 1728 cm^{-1} indicating the presence of oxidation products such as aldehydes and ketones, and spectra of CF 3% and CF 5% show more apparent band than the control and CF 1%. Moisture content of ground beef was significantly different ($P < 0.05$) between control and CF 3% and CF 5%. Control had the highest moisture content and followed by CF 1%, CF 3% and CF 5%, respectively. There was no significant difference ($P > 0.05$) between treatments for fat content, control having the highest fat content with 4.7% and CF 5% having the lowest fat content with 4.18%. Results of this study demonstrate that the addition of citrus fiber caused oxidation in ground beef. Fourier transform infrared spectroscopy was a useful tool for determination of oxidative stability of ground beef made with different levels of citrus fiber.

5.2. INTRODUCTION

Meat has been one of the major sources of nutrition for human consumption. Beef in particular, due to presence of high quality proteins, as well as presence of B vitamins and minerals such as magnesium, zinc and iron, has been one of the highly consumed meat throughout the World (Aberle and others 2001). In addition to high quality protein content, beef has been known to have high concentration of monounsaturated fats (Aberle and others 2001; Leheska and others 2008). While the high concentrations of monounsaturated fats provide possible benefits to heart health, they have been associated with oxidation of meat products. Oxidation in meat products can cause warmed over flavor, discoloration, rancid flavor and loss of nutritional value (Richard 2007).

Many techniques have been developed to delay the onset of oxidation in meat products. For example, packaging technologies (Benson and Payne 2012) and use of antioxidant ingredients in product formulations as well as in packaging (Brewer 2008) have been successful. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) have been used in food product formulations due to their effectiveness in low concentrations, surviving processing conditions and not providing undesirable color or odor to the food.

However, possible negative side effects found in animal studies, being not label friendly and considered by consumers as not healthy (Shahidi and Zhong 2005; Brewer 2008) have led to search of natural antioxidants. Vitamin A, C and E, and phytochemicals

naturally found in spices, herbs, fruits and vegetables have been shown to provide antioxidant capacities (Shahidi and Zhong 2005; Brewer 2008). Citrus and grape fruit and their by products have been a great interest for researchers due to presence of vitamin C, carotenoids, flavonoids and dietary fiber (Tripoli and others 2007; Saura-Calixto 1998). It has been reported that polyphenolic compounds found in citrus fiber delay the oxidation in bologna sausage (Fernadez-Gines and others 2003) and in dry cured sausages (Fernandez-Lopez and others 2007). Similar results were reported with grape fiber by Sanchez-Alano and others (2007) in minced fish and Sayago-Ayerdi and others (2009) in chicken hamburgers. However, in a recent study Yalinkilic and others (2012) reported that with the addition of citrus fiber in a fermented sausage, lipid oxidation increased. All of these studies reported lipid oxidation in thiobarbituric reactive substances (TBARS) assay. While TBARS assay is commonly used protocol for determining lipid oxidation, it also has some limitations.

The use of simple, rapid, and precise methods such as Fourier transform infrared spectroscopy (FTIR) can be a great tool for evaluation of lipid oxidation of fats and oils (Shahidi and Zhong 2005). Vibrational spectroscopy such as FTIR provides useful information on the vibrational states or motions of molecules in a variety of ways. For example, FT-IR collects mid infrared vibrational spectra based on performing a mathematical Fourier transform on the sample signals which rely on a modification of the intrinsic dipole moment with molecular vibration. In FTIR, all of the infrared frequencies are measured simultaneously using an interferometer and Fourier transform mathematical technique is used for converting interferogram signals to a spectrum. FTIR provides useful information about food samples and it has been used for identification of oils such

as sunflower oil (Guillen and others 2005), olive oil (Rohman and others 2014), and authentication of different oils (Vlachos and others 2006). In addition, FTIR has been used for determining oxidative stability of 10 different edible oils (Guillen and Cabo 2000), extra virgin olive oil (Yildirim 2009) and coconut oil (Lu and Tan 2009). In addition to edible oils, it has been used for identification of animal fats (Man and Mirghani 2001) and authentication of helal meat (Rohman and others 2011). One study specifically studied the fatty acid profile of Wagyu beef using FTIR combination with gas chromatography technique (Hu and others 2010), while in another study, fatty acid composition of different fish fillets were determined using FTIR with chemometric methods (Hernandez-Martinez and others 2013).

In addition, use of FTIR in meat science and meat safety has been useful. It has been used for identification of presence of spinal cord content in beef (Gangidi and others 2003), and presence of *Trichinella spiralis* parasite in different pork muscles (Gomez-De-Anda and others 2012). FTIR also have been used for identification of spoilage by organisms in meat products such as minced beef (Ammor and others 2009; Argyri and others 2013), minced chicken (Vasconcelos and others 2014) and pork (Papadopoulou and others 2011). Also, it has been useful for identification of pathogenic bacteria such as *Escherichia coli* O157:H7 (Davis and others 2010) and *Salmonella enterica* typhimurium (Amamcharla and others 2010) contaminations in beef.

Even though, FTIR has been used in variety of studies for food safety and quality purposes, there are only a few studies investigating the oxidative stability of animal products. Guillen and Cabo (2004) investigated the oxidative stability of smoked pork in comparison to control by FTIR. In another study, FTIR used for determining oxidative

stability of salted and unsalted salmon (Guillen and others 2004). There is no available study using FTIR for determining lipid oxidation in ground beef with antioxidant fiber.

Therefore, the objective of this study was to explore the use of FTIR for predicting the lipid oxidation in ground beef meatballs made with 0%, 1%, 3% and 5% citrus fiber during 7 days of refrigerated storage.

5.3. MATERIALS AND METHODS

5.3.1. Sample Preparation

Beef cattle were slaughtered and their carcasses placed in a cooler for 48 hours. Later, shanks were collected from the carcass and weighed. After cutting the beef shanks into smaller pieces, they were two-step (course and fine) ground using a LEM™ Products .35 HP stainless steel electric meat grinder (West Chester, OH). Once they were ground, they were separated into four treatment groups and weighed. The treatment group with 0% citrus fiber, in other words control (CP 0%) was made into small ground beef meatballs. Treatments of 1%, 3%, and 5% citrus fiber were weighed based on the ground beef weight, and the powder was mixed into the ground beef using a KitchenAid® blender. After each mixing, the blender was cleaned before mixing the next treatment group. Later, meats with citrus fiber treatments were also made into small meatballs. All the meatball treatments were placed onto their individual Styrofoam® trays covered with stretch film, and labeled for replication and treatment group, and date. Packages were placed into the refrigerator until their testing at day 1, 3, 5 and 7. This procedure was replicated two more times by removing shank muscle from two other beef cattle carcasses to provide 3 replicates.

5.3.2. Extraction of Lipids

10 g of ground beef was weighed in a blender jar, 9 mL of chloroform and 18 mL of methanol were measured using graduated cylinder and they were transferred into the jar. The mixture was blended for 2 minutes. Later, 9 mL of chloroform was added and

blended for 30 more seconds. Finally, 9 mL of distilled water added and the mixture was blended for 30 more seconds. Ratio of chloroform, methanol and water were 2:2:1 respectively. The mixture was filtered using Whatman no.1 filter paper, Buchner funnel and a vacuum flask. Filtered liquid was transferred to a graduated cylinder. Later, filtered liquid separated into three layers. The bottom chloroform layer, which has the extracted fat, was kept for further analysis for FTIR (Bligh and Dyer 1959).

5.3.3. Determination of Moisture and Fat Content

The moisture and fat content of the meat samples was determined based on the CEM SMART Trac system (CEM Corp., North Carolina, U.S.A.). This two-step system uses microwave for determining the moisture content of a meat sample. Next, it uses nuclear magnetic resonance (NMR) analysis for determining a fat content of the microwaved sample. (Keeton and others 2003).

5.3.4. FTIR Measurements

Triplicate of each solvent-extracted fat samples were measured between 4,000 and 500 cm^{-1} at a resolution of 4 cm^{-1} with 128 interferogram scan using a Nicolet 380 spectrometer (Thermo Scientific, U.S.A.) with Smart Orbit, which includes diamond plate (30000 – 200 cm^{-1}) and variable pressure swivel tower. The spectra of each samples was corrected against the background spectrum of air to present the spectra in absorbance units. Measurements were done in triplicate. Delight software (D-Squared Development Inc., LaGrande, OR, USA) was used for analysis of the FTIR data. Trend analysis with fixed block mean, polynomial subtract (first order) and smoothing (8 cm^{-1}) options were

chosen to analyze all the data. After, each sampling, surface of the diamond and through insert attachment were cleaned using 70% ethyl alcohol with Kimwipes®.

5.3.5. Statistical Analysis

Three replications of ground beef meatballs were evaluated for moisture and fat content. The analysis of variance (ANOVA), using the general linear model (GLM) procedure of the (SAS 2011), was randomized complete block, in which the block was a carcass. The treatments were arranged as a 4×4 factorial (4 levels of citrus fiber, 4 different days). Means were separated by the least significant difference (LSD) when significant ($P < 0.05$) treatment effects were found.

5.4. RESULTS AND DISCUSSION

5.4.1. Moisture and Fat Analysis

Results of moisture and fat content of ground beef shank muscle made with or without citrus fiber shown in Table 5.1. Results show that there were significant differences ($P < 0.05$) in moisture between control and CF 3% and CF 5%. The control had the highest moisture content, followed by CF 1%, CF 3% and CF 5%, respectively. Also, Table 5.1 shows that there was no significant difference ($P > 0.05$) between treatments for fat content, control having the highest fat content with 4.7% and CF 5% having the lowest fat content with 4.18%.

Table 5.1. Moisture and Fat Content of Ground Beef Shank Muscle Made With or Without Citrus Fiber (CF)

Treatments	Moisture Content (%)	Fat Content (%)
CF 0% (Control)	73.17 ± 0.48^{a}	4.70 ± 0.26
CF 1%	71.97 ± 0.48^{ab}	4.64 ± 0.26
CF 3%	70.42 ± 0.48^{bc}	4.31 ± 0.26
CF 5%	68.88 ± 0.48^{c}	4.18 ± 0.26

[a, b, c] Different superscripts in the same column indicate significant difference ($P < 0.05$).
Each value in the Table is represented as mean ± standard error (n=6).

5.4.2. Results of FTIR Analysis

A typical FTIR spectrum of beef fat (CF 0%) from shank muscle is shown in figure 5.1. The band assignments were done according to Guillen and Cabo (1997) and some of the most significant bands are shown at Table 5.2. Medium band at 3010 cm^{-1} is associated with *cis*-double bond stretching vibration; very strong bands at 2924 cm^{-1} and 2853 cm^{-1} are associated with asymmetric and symmetric stretching of aliphatic CH_2 functional group respectively; very strong band at 1743 cm^{-1} is relate to ester carbonyl bond associated with triglycerides; medium band at 1467 cm^{-1} is relate to aliphatic stretching of CH_2 and CH_3 functional groups; medium bands at 1239 cm^{-1} and 1164 cm^{-1} are associated with ester stretching and CH_2 bending vibrations; medium band at 1097 cm^{-1} is associated with ester stretching, while medium band at 723 cm^{-1} is associated with CH_2 rocking vibration and *cis*-disubstituted olefins.

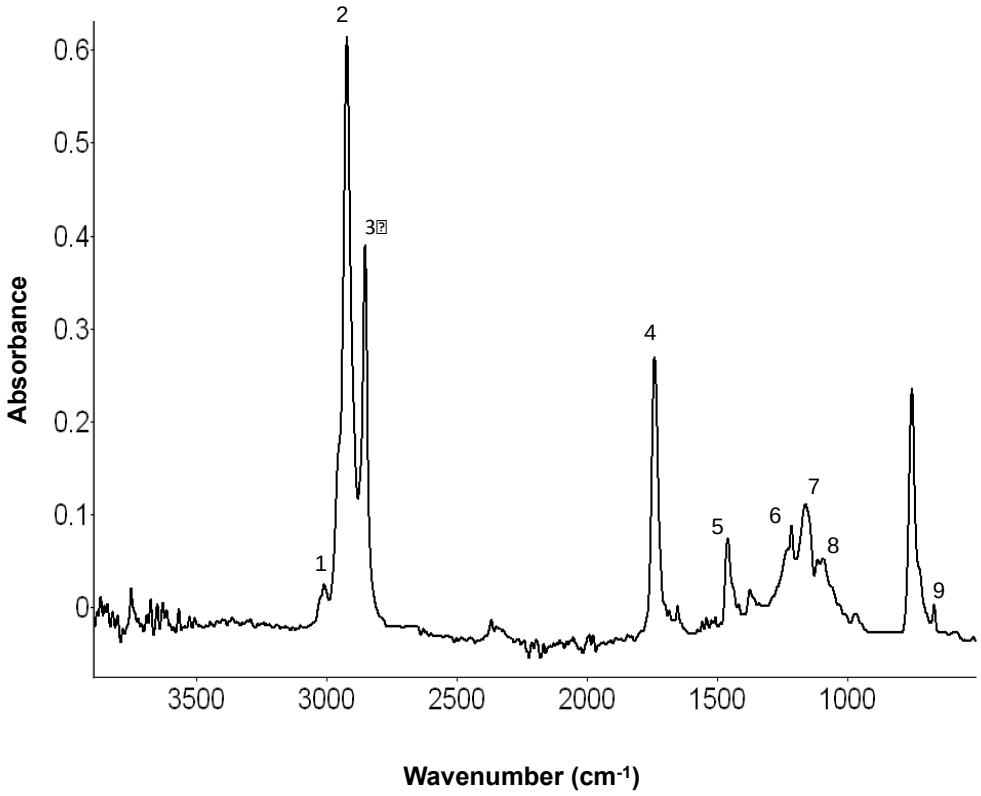


Figure 5.1. FTIR Spectrum of a Beef Fat from Ground Shank Muscle at Day 1

Table 5.2. FTIR Band Assignments for Functional Groups Found in Spectra of Beef Fat from Ground Shank Muscle

No	Frequency (cm^{-1})	Functional Group	Mode of Vibration	Intensity[a]
1	3010	=C–H (*cis*-)	Stretching	m
2	2924	–C–H (CH_2)	Stretching (asym)	vst
3	2853	–C–H (CH_2)	Stretching (sym)	vst
4	1743	–C=O (ester)	Stretching	vst
5	1467	–C–H (CH_2, CH_3)	Bending (scissoring)	m
6	1239	–C–O, –CH_2–	Stretching, bending	m
7	1164	–C–O, –CH_2–	Stretching, bending	st
8	1097	–C–O	Stretching	m
9	723	–$(CH_2)_n$–	Bending (rocking)	m
		–HC=CH– (*cis*-)		

[a]w: weak, m: medium, st: strong, vst: very strong.
According to Guillien and Cabo (1997).

Figure 5.2 shows the spectra of all the treatments at all the storage days. FTIR spectra of beef fat with added fiber at 4 different levels (CF 0%, CF 1%, CF 3% and CF 5%) did not show any difference in peak appearance at day 1, but there were slight difference in absorbance between treatments due to difference in presence of fat content.

Figure 5.2 also shows that band at 3010 cm^{-1}, which is associated with cis-double bond, showed very low absorbance due to location and distribution of unsaturated fat being low in shank muscle. It has been reported that disappearance of cis-double at 3010

cm^{-1} has been associated with advanced oxidation (Guillen and Cabo 2000), in our study this bond disappeared by the seventh day indicating major oxidation, which is shown in Figure 5.2.

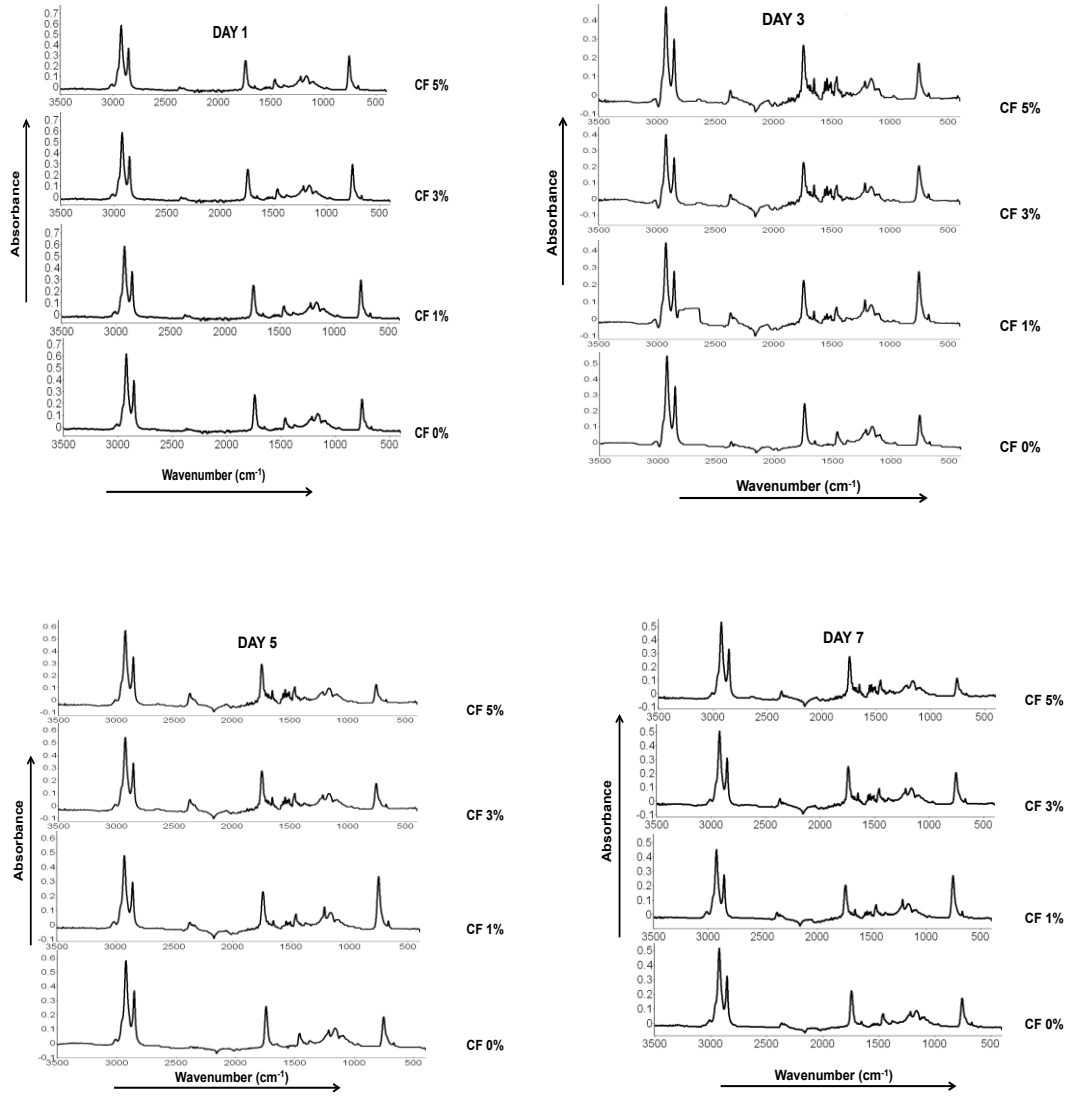


Figure 5.2. FTIR Spectra of Beef Fat (CF 0%, CF 1%, CF 3% and CF 5%) From Ground Shank Muscle at Different Cold Storage Days

In order to investigate the stages of oxidation as well as impact of different treatment levels on the oxidation; peaks at 2924, at 2853 and 1743 cm^{-1} were investigated further.

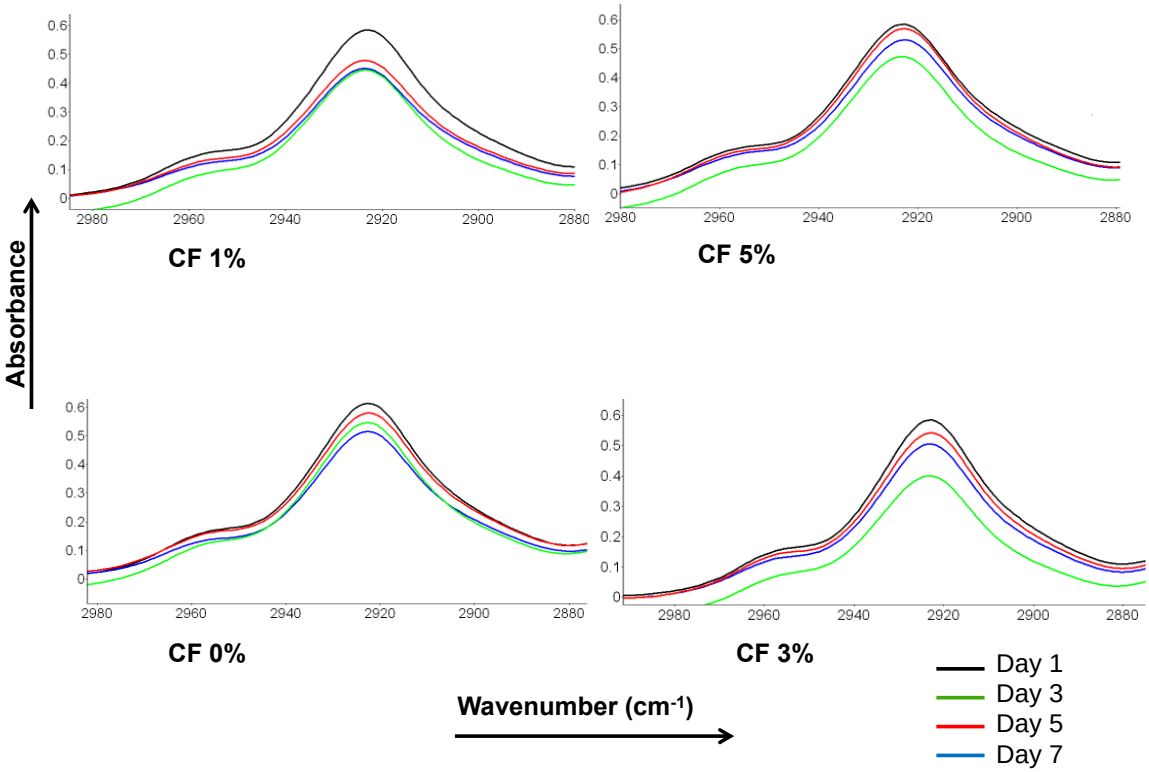


Figure 5.3. FTIR Spectra of Beef Fat From Ground Shank Muscle With and Without Citrus Fiber Between 2980 and 2880 cm^{-1}

Figure 5.3 shows the FTIR spectra of all the treatment levels between 2980 and 2880 cm^{-1}. Results show that decrease in absorbance shows same pattern in all the treatments. First, from day 1 through day 3 absorbance decrease due to production of peroxides, than from day 3 through day 5 absorbance increase due to production of secondary oxidation products, then day 5 through day 7 absorbance start to get decrease due to breakdown of secondary oxidation products. Also, Figure 5.3 shows that all the treatments have the highest absorbance at day 1 and it decreases over time. While the change in the stages of oxidation is same in all treatments, it shows that drop in absorbance from day 1 to day 3 due to possible peroxide formation is much less in control than all the other treatments. The changes in absorbance are shown more apparently in CF 1% and CF 3%. Vlachos and others (2006) tested the impact of heating on oxidation of corn oil spectra. Their result showed that with heating band at 2925 cm^{-1} reduced its absorbance and increase its width. They also tested the addition of oregano in to the corn oil and compare with control corn oil that had no oregano. Results showed that oregano had antioxidant properties and prevented reduction in valley.

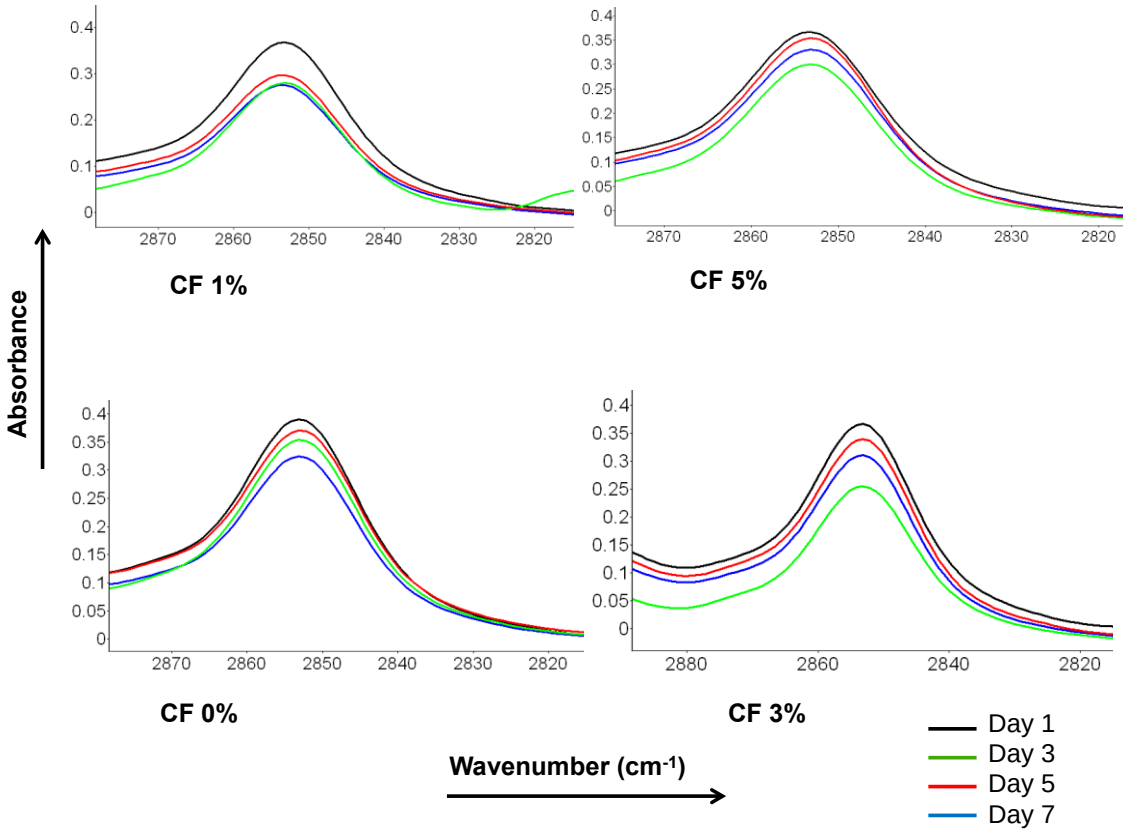


Figure 5.4. FTIR Spectra of Beef Fat From Ground Shank Muscle With and Without Citrus Fiber Between 2870 and 2820 cm^{-1}

Figure 5.4 shows the FTIR spectra of all the treatment levels between 2870 and 2820 cm^{-1}. Similar to a Figure 5.3, control treatment (CF 0%) had much less change in absorbance than all the other treatments and change is more apparent in CF 1% and CF 3%. Guillen and others (2004) investigated the oxidative stability of salted and unsalted salmons. They reported that dry-salting caused oxidation of salmons. In another study, impact of smoke flavorings on oxidative stability of pork adipose tissue investigated. Results showed that liquid smoke flavoring improved oxidative stability (Guillen and Cabo 2004).

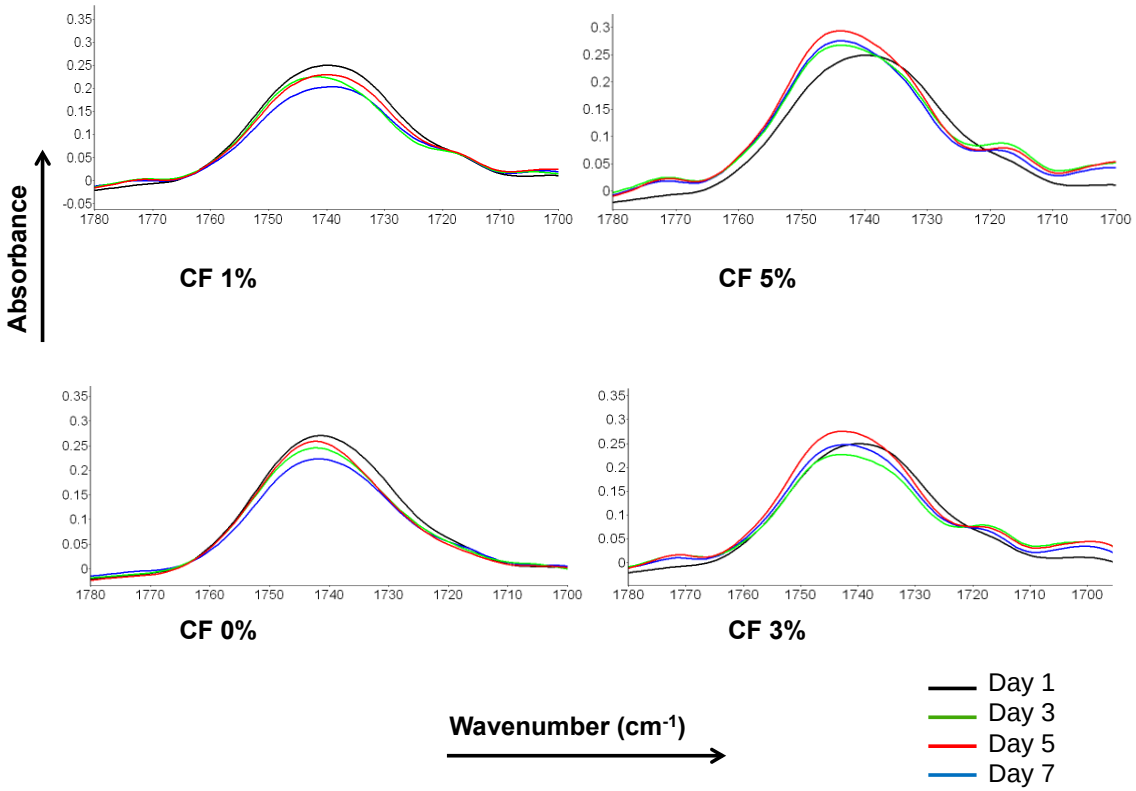


Figure 5.5. FTIR Spectra of Beef Fat From Ground Shank Muscle With and Without Citrus Fiber Between 1780 and 1700 cm^{-1}

Figure 5.5 shows the FTIR spectra of all the treatments between 1780 and 1700 cm^{-1}. Results showed that there is a slight widening in band from day 1 through day 7 for all the treatments. Furthermore, spectra of CF 1%, CF 3% and CF 5% start to develop peak area at 1720-1710 cm^{-1} by day 3. This peak is more apparent for CF 3% and CF 5%. It has been reported by earlier studies that absorbance of aldehydes and ketones at around 1728 cm^{-1} (Guillen and others 2004; Guillen and Cabo 2004).

This could explain that this weak band appears due to oxidation of lipids and production of aldehydes and ketones. While band region at 1780-1700 cm^{-1} provides information relate to oxidation process for our study, it has been reported by Guillen and Cabo (2004) that this band region was not as useful as some of the other bands mentioned earlier for monitoring oxidation process due to weak band around 1728 cm^{-1} overlapping with band around 1743 cm^{-1}.

5.5. CONCLUSION

Use of FTIR spectroscopy has been useful for determining the oxidative stability of ground beef samples made with or without citrus fiber. Peaks at 2924, 2853, and 1743 cm^{-1} were useful for monitoring lipid oxidation. Our results showed that addition of citrus fiber did not delay the oxidation, in contrast caused lipid oxidation.

CHAPTER 6

CONSUMER PREFERENCES FOR GROUND BEEF MEATBALLS MADE WITH CITRUS FIBER

6.1. ABSTRACT

The objective of this study was to determine consumer preferences for ground beef meatballs made with different citrus fiber (CF) levels (0%, 1%, 3% and 5%). An untrained panel of 164 people including students, faculty and staff members of the University of Missouri tasted ground beef meatballs for flavor, texture and overall likeness. Panelists gave scores based on a hedonic scale of 1 to 9, dislike extremely (1) and like extremely (9). Results showed that CF 1% got the highest flavor score with 6.61 followed by Control (CF 0%) with 6.52 with no significance difference between them ($P > 0.05$). Texture values were significantly ($P < 0.05$) influenced by the addition of citrus fiber. CF 5% had the lowest texture scores with 5.46 and was significantly lower than other treatments ($P < 0.05$). Over all likeness was highest for control with 6.69 followed by CF 1% with 6.56, CF 3% with 5.9, and CF 5% with 5.47.

6.1.1. Practical Application

The use of citrus fiber in comminuted meat products could help with increasing fiber consumption through meat products and the development of healthier meat product alternatives with functional properties. Consumers can apply the use of citrus fiber in making tender meatballs that contain less fat than traditional recipes.

6.2. INTRODUCTION

In recent years consumers' food choices have shifted towards healthy foods due to increased incidence of coronary heart disease (CHD), diabetes, obesity and cancer (Rosamond and others 2008). Food products associated with high fat content and high cholesterol have been linked to incidences of CHD (Micha and others 2010), diabetes mellitus (Lajous and others 2011), and risk of stroke (Larsson and others 2011). Processed meat products have been closely linked to these diseases due to their high cholesterol content and saturated fat (Cross and others 2007; Micha and others 2010). New food products have been developed to have high protein content, low fat content as well as high fiber content to provide healthier food alternatives to consumers. Plant based proteins such as legumes (Serdaroglu and others 2005) and soy protein (Singh and others 2008) have been studied as extenders to increase protein content and mimic or replace fats to reduce the use of saturated fat in meat products. Additionally, fiber has been studied for both health and functional benefits. It has been reported that consumption of fiber helps with decreased cholesterol levels, with the absorption of glucose (Scheneeman 1987), and decreased incidence of hemorrhoids and colon cancer (Kritchersky 1990). Also, dietary fiber such as psyllium and β-glucan have been approved by the Food and Drug Administration (FDA) for health claims for protection against coronary heart disease (USDHHS 1997; USDHHS 1998). It has been reported that insoluble fiber such as cellulose has been successfully used as a fat replacement in many food products such as frozen desserts, cheese spreads, salad dressing and processed meat products (Akoh 1998).

Functional properties of processed meat products made with different fiber sources have been studied. Use of peach fiber in low fat frankfurters (Grigelmo-Miguel and others 1997), β-glucan rich fiber in breakfast sausage (Aleson-Carbonell and others 2005), rice bran fiber in reduced fat frankfurters (Choi and others 2010), orange fiber in fermented sausage called Sucuk (Yalinkilic and others 2012) and yellow passion fruit fiber in pork burgers (Lopez-Vargas and others 2014) have been helpful for improving functional properties of meat products.

While there are some studies to show the possible health benefits and functional properties of fiber in meat products, few studies have been conducted to see the consumers' acceptance of meat products made with fiber. Also, there is no existing study on meatballs made with citrus fiber. Therefore, the objective of our study is to determine consumers' acceptance of ground beef meatballs made with citrus fiber.

6.3. MATERIALS AND METHODS

6.3.1. Meatball Manufacture

Ground beef (with 85% meat and 15% fat) and other ingredients were bought fresh from a store the day before the consumer panel. A Turkish köfte recipe was used for the formulation of the meatballs, and this recipe produced approximately 35-40 small meatballs. Table 6.1 shows the formulation of control (CF 0 %) treatment of ground beef meatballs.

Table 6. 1. Ground Beef Meatball Formulation

List of Ingredients	Weight (g) or Quantity
Ground beef	454 g
Onion	240 g (1 medium size onion)
Parsley	12 g
Garlic	3 g (1 and half garlic)
Egg	46 g (1 shelled egg)
Olive oil	15 g
Pepper paste	14 g
Salt	2.3 g
Cumin	2.2 g
Black pepper	1.2 g
Sweet paprika	1 g
Nutmeg	0.8 g
Cinnamon	0.2 g
Total	791.7 g

The rest of the treatments were made the same way with the exception of the addition of citrus fiber in 1%, 3% and 5% levels. After establishing the four ground beef foundations, onion and garlic were peeled and parsley leaves were picked; they were washed, diced and chopped. Ground beef and other ingredients were all mixed together. The meatballs were made using a 36 mm diameter ice cream scoop to make sure that all the meatballs were the same size. Meatballs were placed on a tray with a rack and each rack had a label with the treatment name on it. Picture 6.1 shows the details.

Picture 6. 1. Meatball Samples Before Cooking

Once all the meatballs of a treatment were placed on a rack, the tray was placed in an oven, which was preheated to 190 °C. A probe was placed into one of the meatballs and the temperature was set up for 72 °C. Once the meatballs were properly cooked, the tray was taken out from the oven to cool down. The same procedure was followed for all the treatments. Meatballs were placed into labeled glass containers with lids for each treatment. Because the consumer panel room had only five available seats, the containers were kept in a refrigerator to insure safe handling practices between sets of panels. In order to serve warm meatballs to the panelists, the meatball treatments were placed in individual Crock-Pot slow cookers with tomato sauce. The temperature of the sauce was kept above 60 °C to provide safe and warm meatballs to panelists, and verified by calibrated temperature probes. The recipe of the tomato sauce is shown in Table 6.2. Meatballs were removed from the refrigerator to the Crock-Pots as needed.

Table 6. 2. Tomato Sauce Recipe

List of Ingredients	Weight (g or ml)
Water	1000 ml
Butter	227 g
Tomato paste	120 g
Dry mint flakes	1 g
Black pepper	0.8 g

6.3.2. Sensory Evaluation

Untrained panelists (164) of students, faculty and staff of the University of Missouri volunteered to participate in the consumer taste panel. The consumer panel was carried out at the Food Science Department of the University of Missouri's sensory laboratory, which fulfills the requirement of international standards (ISO 1988). In order to obtain a sufficient number of panelists, the consumer panel was conducted over 3 days. Each panelist evaluated four warm meatball samples. One whole meatball for each treatment was placed into a labeled plastic cup. Each treatment was coded with randomly selected 3-digit numbers, and the four treatments were served to panelists in a randomized order. Panelists were also provided with a glass of water and were instructed to cleanse their pallets before trying the next sample. The rating test employed the hedonic scale of dislike extremely (1) to like extremely (9) (IFT 1981). Panelists were instructed to evaluate the samples based on their degree of likeness for flavor, texture and overall likeness. Hedonic scale results were converted to numerical scores for statistical analysis.

6. 3. 3. Statistical Analysis

The analysis of variance (ANOVA), using the general linear model (GLM) procedure of the (SAS 2011), was randomized complete block design, in which individual person was complete block which they tasted all the treatments. Means were separated by least significant difference (LSD), when significant ($P < 0.05$) treatment effects were found.

6. 4. RESULTS AND DISCUSSION

Consumers' acceptance of ground beef meatballs made with different levels of citrus fiber is shown in Table 6.3. Results showed that meatballs made with 1% citrus fiber (CF 1%) had the highest flavor score with 6.61, followed by the control treatment with 6.52.

Table 6. 3. Consumers' Acceptance of Ground Beef Made With Different Levels of Citrus Fiber

Treatments	Flavor	Texture	Overall Likeness
Control (CF 0 %)	6.52 ± 0.124^{a}	6.69 ± 0.134^{a}	6.69 ± 0.122^{a}
CF 1 %	6.61 ± 0.124^{a}	6.27 ± 0.134^{b}	6.56 ± 0.122^{a}
CF 3 %	5.94 ± 0.124^{b}	5.89 ± 0.134^{c}	5.90 ± 0.122^{b}
CF 5 %	5.49 ± 0.124^{c}	5.46 ± 0.134^{d}	5.47 ± 0.122^{c}

[a, b, c, d] Different letters in the same column indicates significant difference ($P < 0.05$) analyzed by t tests.
Each value in the Table is represented as mean ± standard error (n=164).
CF: Represent citrus fiber.

There was no significant difference ($P > 0.05$) in flavor scores between CF 1% and the control treatment, however, both treatments had significantly ($P < 0.05$) higher flavor scores than CF 3% and CF 5%. Besbes and others (2008) reported similar results. Beef burgers made with pea and wheat fiber received the highest flavor scores. In another study, Yildiz-Turp and Serdaroglu (2010) reported that low fat beef patties made with

10% plum puree received higher flavor scores than the control. On the other hand, Bilek and Turhan (2009) reported that the addition of flaxseed flour to beef patties caused a decrease in flavor scores. Figure 6.1 shows the number of responders' flavor scores for four treatment groups.

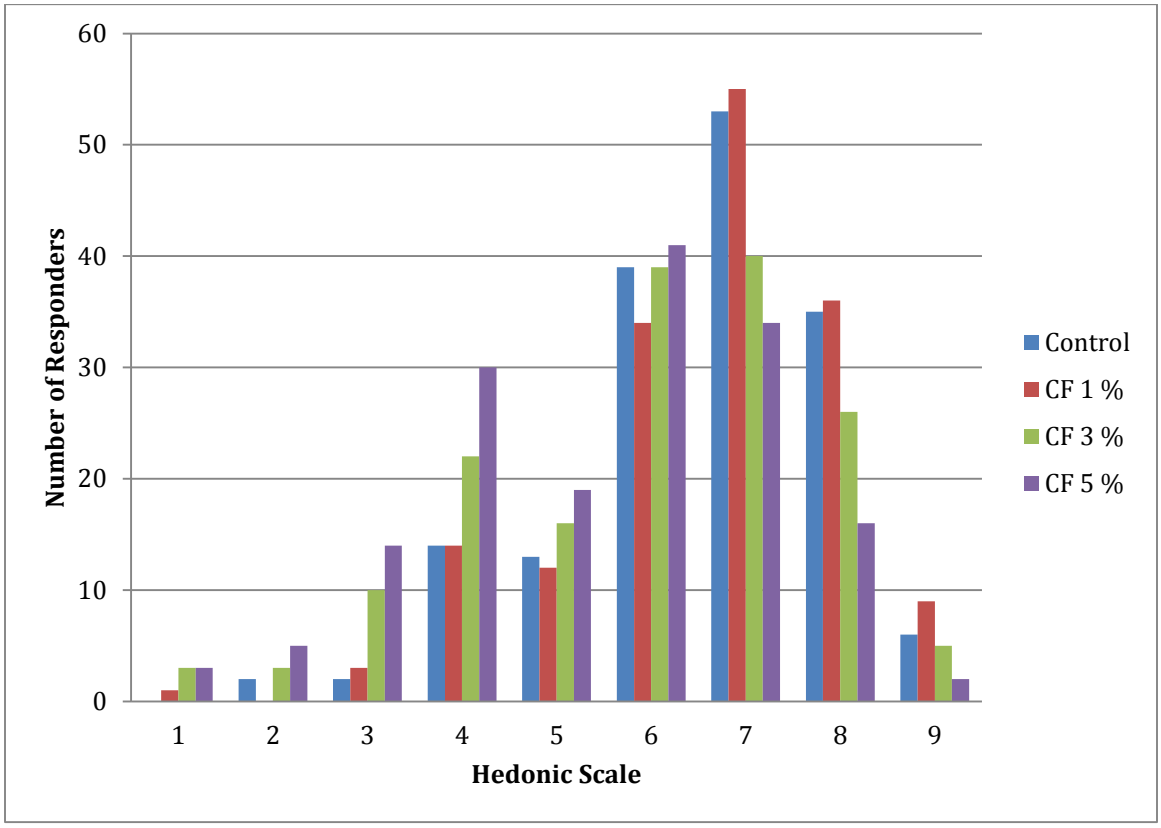


Figure 6. 1. Consumers' Flavor Preferences of Ground Beef Meatballs Made With Different Levels of Citrus Fiber

Results indicate that the majority of the consumers scored mostly for like slightly (6), like moderately (7) and like very much (8) for control, CF 1% and CF 3%. A total of 22 responders gave the score of like extremely (9) for flavor. From those responders, 40.91% of them preferred CF 1% and followed by 27.3% with control, 22.73% with CF 3% and 9.06% with CF 5%, respectively.

Results of consumers' preferences for texture attribute are summarized in Table 6.3. Results showed that texture attribute of ground beef meatballs were significantly ($P < 0.05$) impacted by the addition of citrus fiber. The control meatball treatments received the highest scores of 6.69, followed by the CF 1% treatment with 6.27. Treatments with the highest citrus fiber, the CF 5%, received the lowest score in texture with 5.46, which is like slightly. Besbes and others (2008); Bilek and Turhan (2009) reported similar results: an increase in the fiber levels caused a decrease in texture sensory scores for beef patties. There were also reports of improvements in sensory texture scores for sausage products. Huang and others (2011) reported that Chinese style sausages made with oat fiber received higher scores than the control. Yalinkilic and others (2012) reported that a fermented sausage product called Sucuk made with citrus fiber received slightly higher sensory texture results than the control. Figure 6.2 shows the number of responders' hedonic scale ratings for texture attributes of four meatball treatments. Results showed that 52.44% of the responders gave a score of 6 to CF 5%, while 82.32% of the responders gave a score of 6 to the control.

There are a couple of potential reasons why treatments with high citrus fiber levels received lower sensory scores for texture. First, the meatballs were kept in hot tomato sauce for a while before serving, and due to citrus fiber's high water absorbance

capacity, there is a possibility that the citrus fiber in the meatballs absorbed water from the sauce and they became very soft. Second, for the production of the meatballs, ground beef with 85% meat and 15% fat was chosen. Since ground beef with high fat content tends to produce especially tender meatballs, the combination of these two factors could cause meatballs with citrus fiber to be very soft and it could explain lower texture scores in comparison to the control treatment.

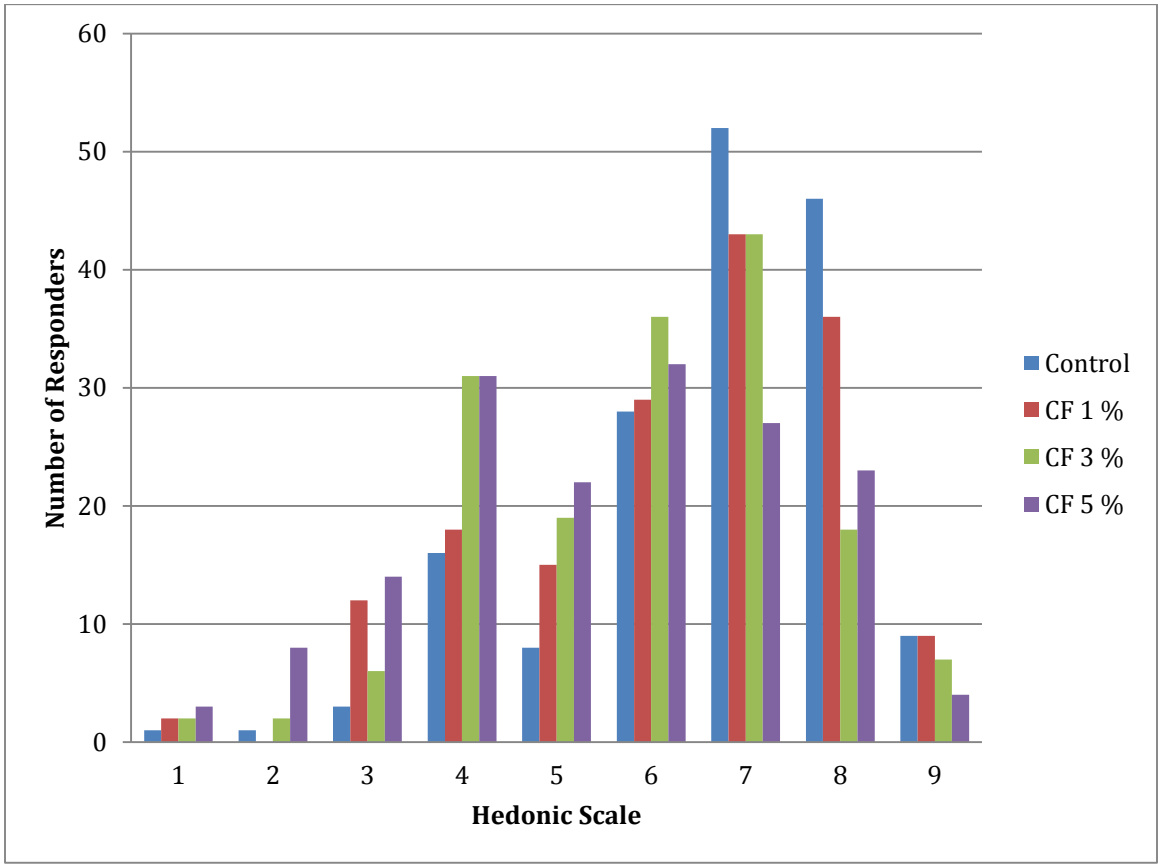


Figure 6. 2. Consumers' Texture Preferences of Ground Beef Meatballs Made With Different Levels of Citrus Fiber

Results of overall likeness for the four treatment groups are shown in Table 6.3. The control has the highest overall likeness scores with 6.69 followed by the CF 1% with 6.56, the CF 3% with 5.9 and the CF 5% with 5.47. There were no significance ($P > 0.05$) difference in overall likeness scores between the control and the CF 1%. However, there were significant ($P < 0.05$) differences between the control with the CF 3% and the CF 5%. Fernadez-Gines and others (2003) reported similar findings. They found that, at the highest concentration, the addition of citrus fiber to bolognas caused a decrease in overall quality scores. Serdaroglu and others (2005) reported that meatballs made with legume flour extenders received high scores (6.8 and above) in overall acceptability. Additionally, in another study low fat pork sausage made with oatmeal or tofu received higher overall acceptability scores than control pork sausages (Yang and others 2007). In a recent study, Tomaschunas and others (2013) reported that low fat Lyon style sausages made with inulin and citrus fiber had similar sensory characteristics to full fat reference. Figure 6.3 shows the number of responders' hedonic scale ratings for overall likeness attributes of the four meatball treatments. Results showed that 82.92 % of the control, 80.49% of the CF 1%, 67.68% of the CF 3% and 53.05% of the CF 5% treatments received a score of 6 or above.

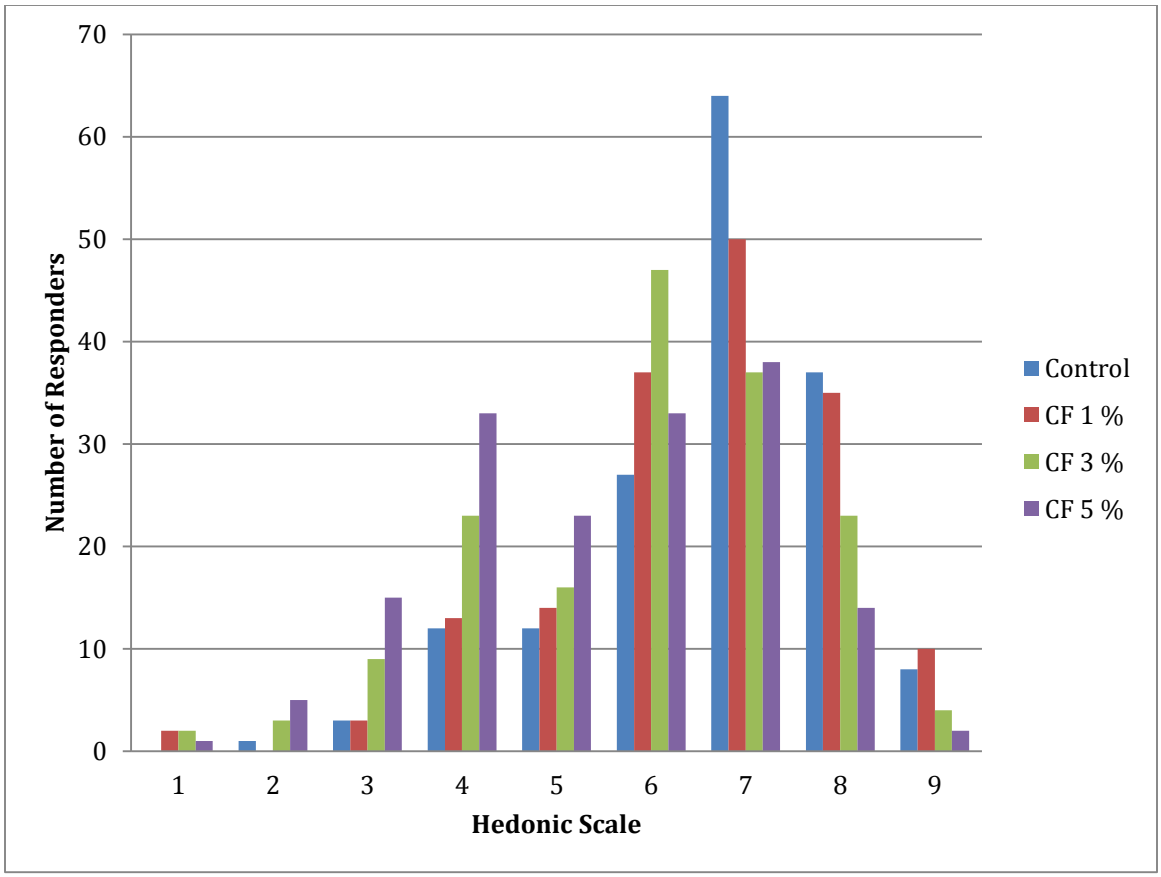


Figure 6. 3. Consumers' Overall Likeness Preferences of Ground Beef Meatballs Made With Different Levels of Citrus Fiber

6.5. CONCLUSION

Results of this study shows that citrus fiber in lower concentrations can be used in comminuted meat products and can have high acceptability by the consumer. The results of this study also showed that the addition of citrus fiber causes an increase in the tenderness of the meatballs. Both industry and consumers can benefit from using citrus fiber in meat products. Citrus fiber is a great, functional ingredient and can help with decreasing the use of saturated fat in comminuted meat products.

CHAPTER 7: CONCLUSION

In this study, presence of flavonoids and antioxidant potential of citrus fiber has been investigated. Also, impact of adding citrus fiber into ground beef meatballs on quality attributes, lipid oxidation stability and consumer acceptance have been evaluated.

In first part of the study, the presence of flavonoids in citrus fiber using reverse-phase high-pressure liquid chromatography (RP-HPLC) was evaluated. Also, the effect of methanol and acetone extraction on total polyphenol content (TPC), total flavonoid content (TFC), oxygen radical absorbance capacity (ORAC) and carotenoid content of citrus fibers prepared by regular or hot-washing were investigated. Quercetin and kaempferol (flavonols), and sinensetin, nobiletin, heptamethoxyflavone, and tangeretin (polymethoxyflavones) were identified by comparing citrus fiber extract retention times with the retention times of pure standards and were quantified by using external standard curves. Nobiletin had the highest concentration with 3.33 mg/g followed by sinensetin (1.96 mg/g) and heptamethoxyflavone (1.24 mg/g), respectively. Extraction with acetone yielded higher ($P < 0.05$) TPC, TFC, and ORAC values than extraction with methanol. The TPC and TFC of citrus fibers were unaffected ($P > 0.05$) by the citrus fiber production procedure; however, the ORAC values of citrus fiber prepared using hot-washing were significantly lower ($P < 0.05$). The carotenoid content was not significantly affected by the washing procedures ($P > 0.05$). Results of this study showed that extraction efficiency of acetone method was better than methanol method. Also, citrus fiber prepared through regular washing had higher antioxidant capacity and total flavonoid content than hot washed citrus fiber.

Second part of the study, the effect of different citrus fiber levels (0%, 1%, 5%, and 10%) on the quality attributes of ground beef meatballs at day 0, 3, 6, and 9 of refrigerated storage was investigated. Quality attributes tested were pH of both raw and cooked samples, water holding capacity of raw samples, cooking yield, texture parameters of cooked samples, Hunter color *L*, *a*, *b* values of raw samples, moisture, fat and protein contents. The results showed that treatment and day had a significant ($P < 0.05$) effect on the pH of raw samples, but there was no trt*day effect ($P > 0.05$) found for both pH of raw and cooked samples. Increase in citrus fiber levels caused increase water holding capacity, but there was no trt*day effect ($P > 0.05$) found. Treatment levels had a significant ($P < 0.05$) effect on the cooking yield. The control samples had the lowest cooking yield, and cooking yield increased with a rise in the citrus fiber level. Increasing the citrus fiber level was negatively correlated with desirable textural properties in the ground beef samples. With increase in the level of citrus fiber in the ground beef meatballs caused decrease in hardness, cohesiveness and resilience. However, for overall hardness and springiness values between the control and the CF 1% were not significantly ($P > 0.05$) different. Hunter color *L*, *a* and *b* values decreased with increasing citrus fiber levels. Citrus fiber at 1% can be used in meat products to increase the cooking yield and water holding capacity without detrimental effects on quality.

Third part of the study, the oxidative stability of ground beef meatballs made with different levels of citrus fiber was investigated by Fourier transform infrared spectroscopy (FTIR). This spectroscopy technique provides fast and accurate information for compounds that absorbs light at infrared region. The spectral bands created by the FTIR machine are unique for each compound and provides information about functional

groups found in the compound. Lipids are organic molecules that give absorbance at 4000-500 cm^{-1}. Therefore, objective of this study was to determine the oxidative stability of ground beef made with different levels of (0%, 1%, 3% and 5%) citrus fiber during 1, 3, 5, 7 refrigerated storage days. Addition to FTIR analysis, moisture and fat content of the all the treatments were determined. For monitoring lipid oxidation, peaks at 2924 cm^{-1}, 2853 cm^{-1} and 1743 cm^{-1} were used. Results showed that addition of citrus fiber caused higher decrease in absorbance in comparison to control (CF 0%) at bands 2924 cm^{-1} and 2853 due to production of oxidation products. The band at 1743 cm^{-1} was also a useful indicator. By day 3, weak band appeared around 1728 cm^{-1} indicating presence of oxidation products such as aldehydes and ketones, and spectra of CF 3% and CF 5% showed more apparent band than control and CF 1%. Result of this study shows that addition of citrus fiber caused oxidation in ground beef.

Final part of the study, consumer preferences for citrus fiber added meatballs were tested. Four levels of (0%, 1%, 3% and 5%) citrus fiber used for the meatball formulation. An untrained panel of 164 people including students, faculty and staff members of the University of Missouri tasted ground beef meatballs for flavor, texture and overall likeness. Panelists gave scores based on a hedonic scale of 1 to 9, dislike extremely (1) and like extremely (9). Results showed that CF 1% got the highest flavor score with 6.61 followed by Control (CF 0%) with 6.52 with no significance difference between them ($P > 0.05$). Texture values were significantly ($P < 0.05$) influenced by the addition of citrus fiber. CF 5% had the lowest texture scores with 5.46 and was significantly lower than other treatments ($P < 0.05$). Over all likeness was highest for control with 6.69 followed by CF 1% with 6.56, CF 3% with 5.9, and CF 5% with 5.47.

Results showed that consumers acceptance for meatballs made with 1% citrus fiber was not significantly ($P > 0.05$) different than control meatballs (CF 0%).

In conclusion, citrus fiber used in this study did not have significant antioxidant potential. Presence of quercetin, kaempferol, nobiletin, sinensetin, heptamethoxyflavone and tangeretin were relatively lower concentrations in comparison to what has been cited in the literature. Furthermore, addition of citrus fiber at 3% and 5% caused lipid oxidation. However, when citrus fiber was used at 1% level did help with improving cooking yield and water holding capacity without significant impact on texture and color. Acceptability of ground beef meatballs made with 1% citrus fiber was moderately liked by the consumers. Results showed that citrus fiber should be used in ground beef meatballs up to a 1% level.

APPENDIX A: FIGURES

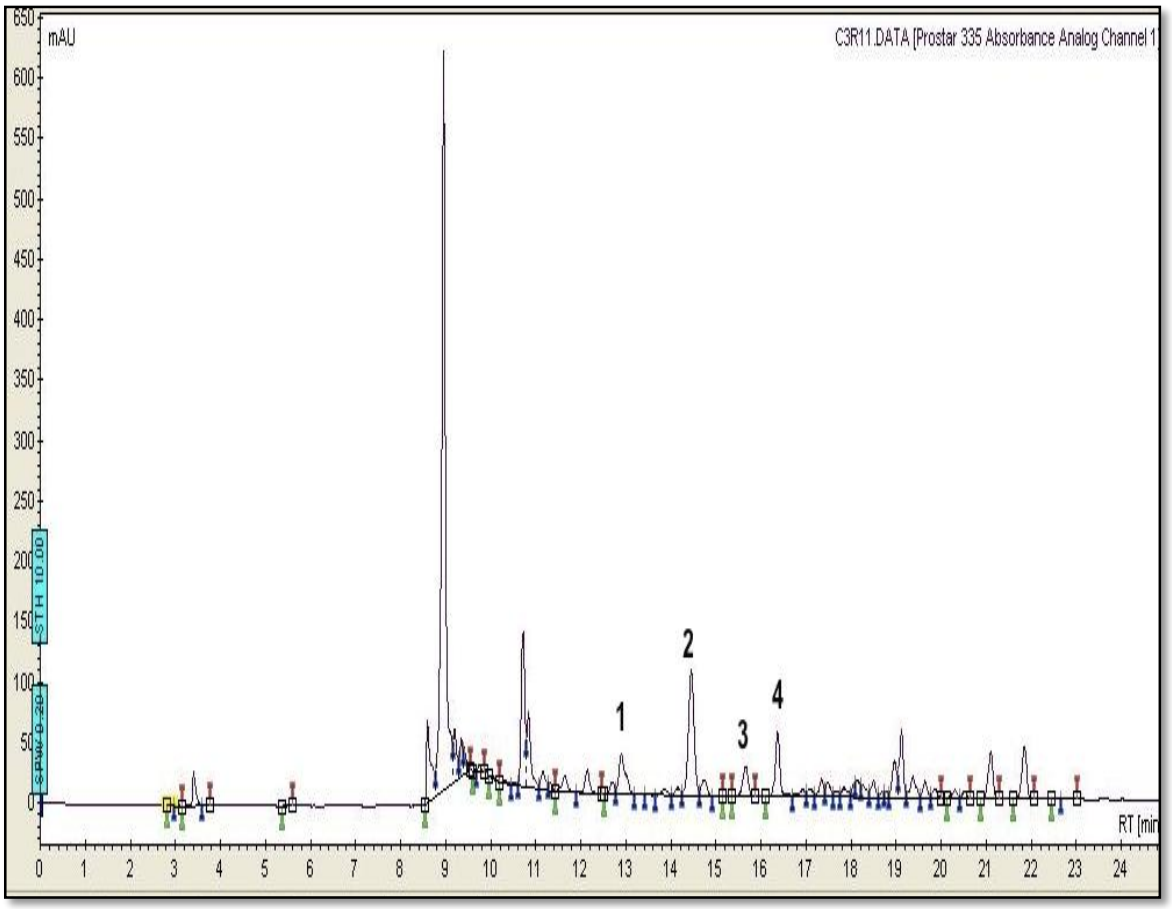


Figure A.1. Reverse-Phase HPLC Chromatogram of Polymethoxyflavones Extracted

From Hot Washed Citrus Fiber

Peak identifications: 1 - sinensetin (SIN), 2 - nobiletin (NOB), 3 - heptamethoxyflavone (HMF) and 4 - tangeretin (TAN).

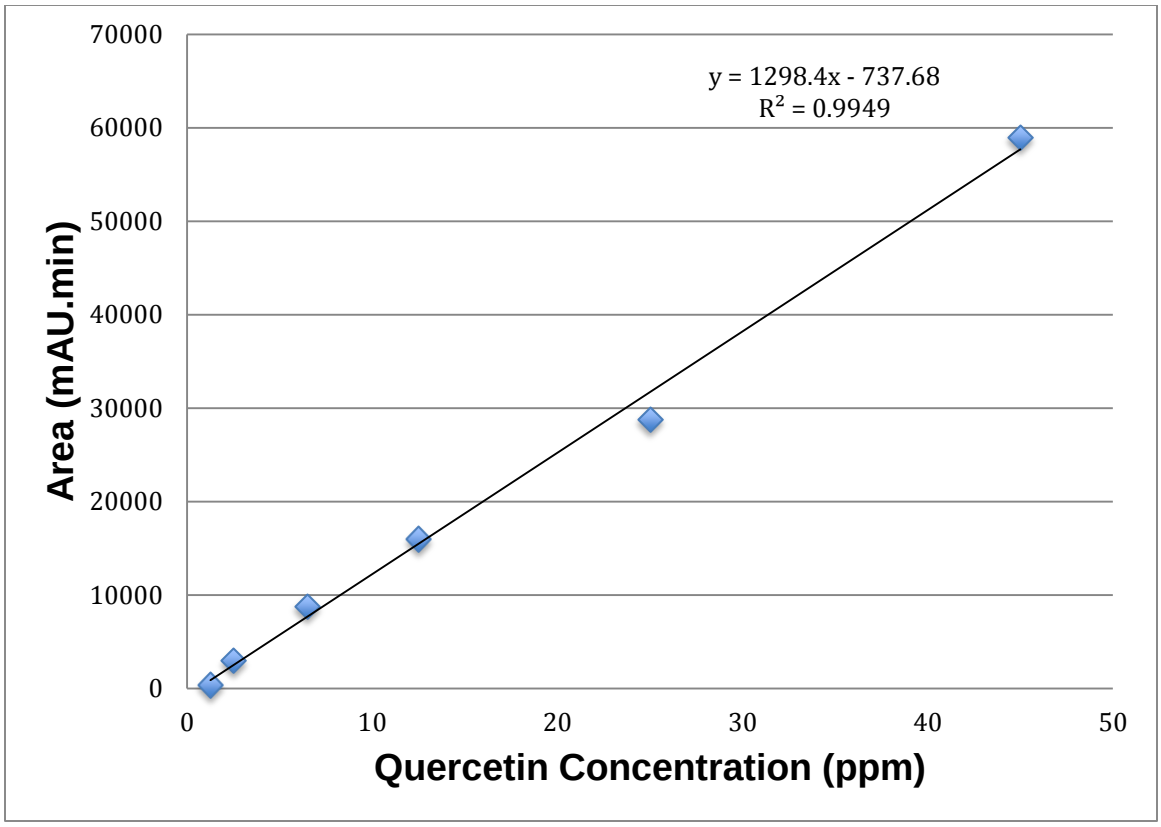


Figure A.2. Quercetin Standard Curve for HPLC Analysis

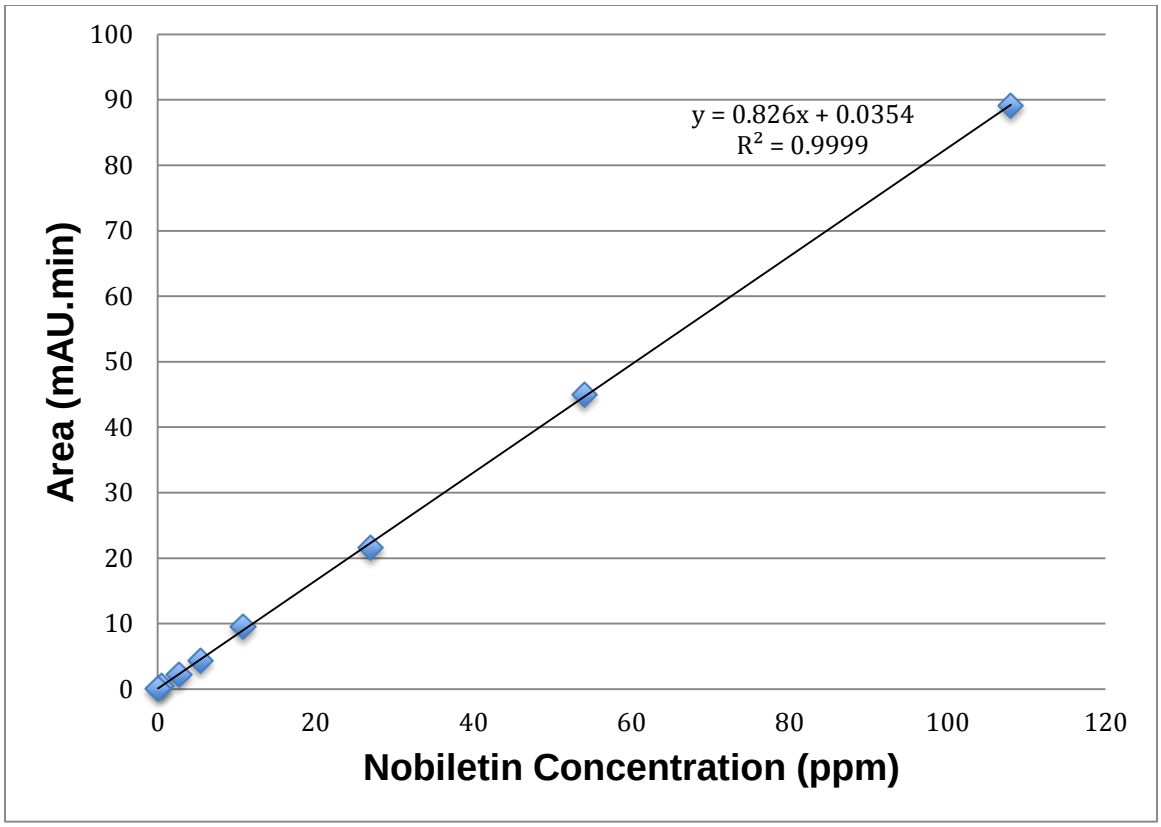


Figure A.3. Nobiletin Standard Curve for HPLC Analysis

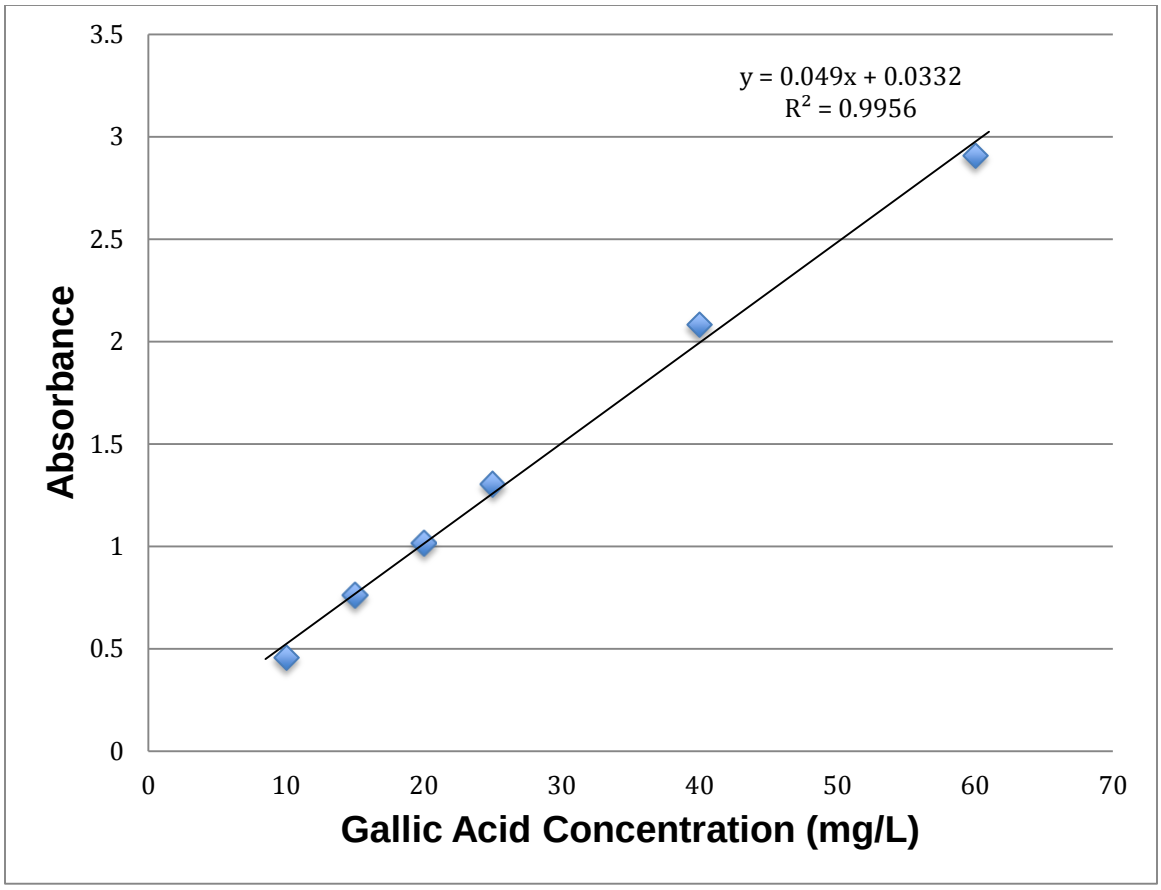


Figure A.4. Gallic Acid Standard Curve For Total Polyphenol Content Analysis

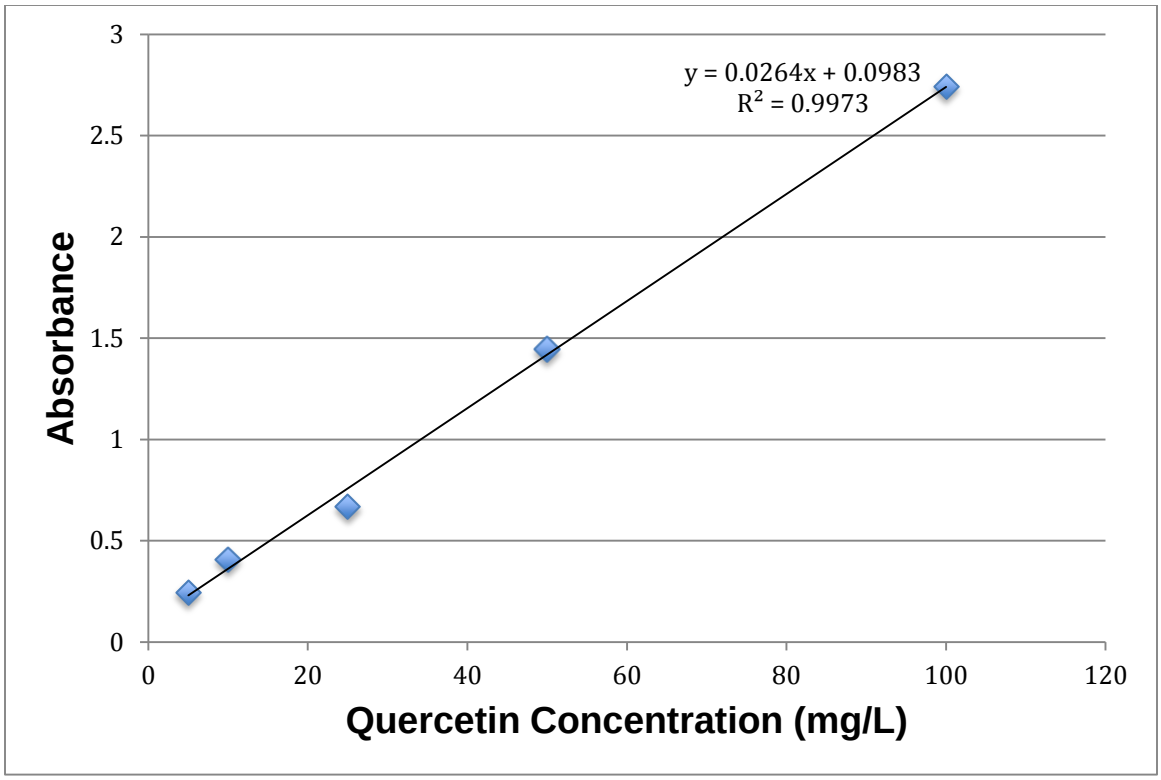


Figure A.5. Quercetin Standard Curve For Total Flavonoid Content Analysis

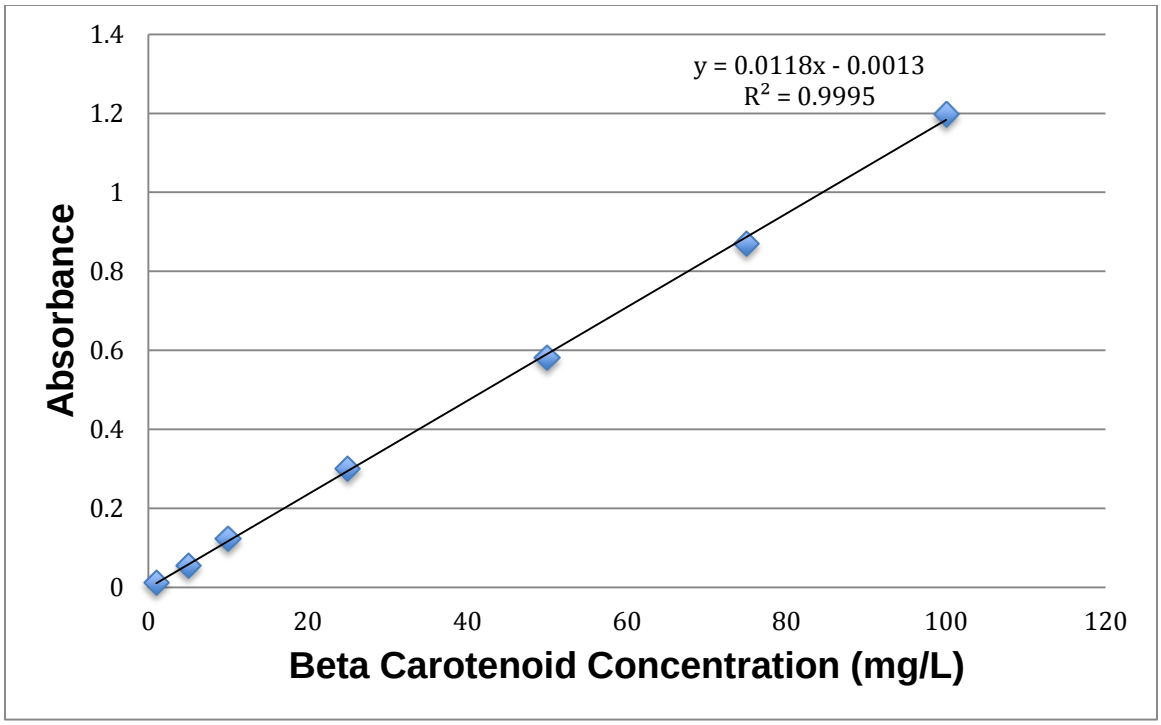


Figure A.6. β-Carotenoid Standard Curve For Total Carotenoid Content Analysis

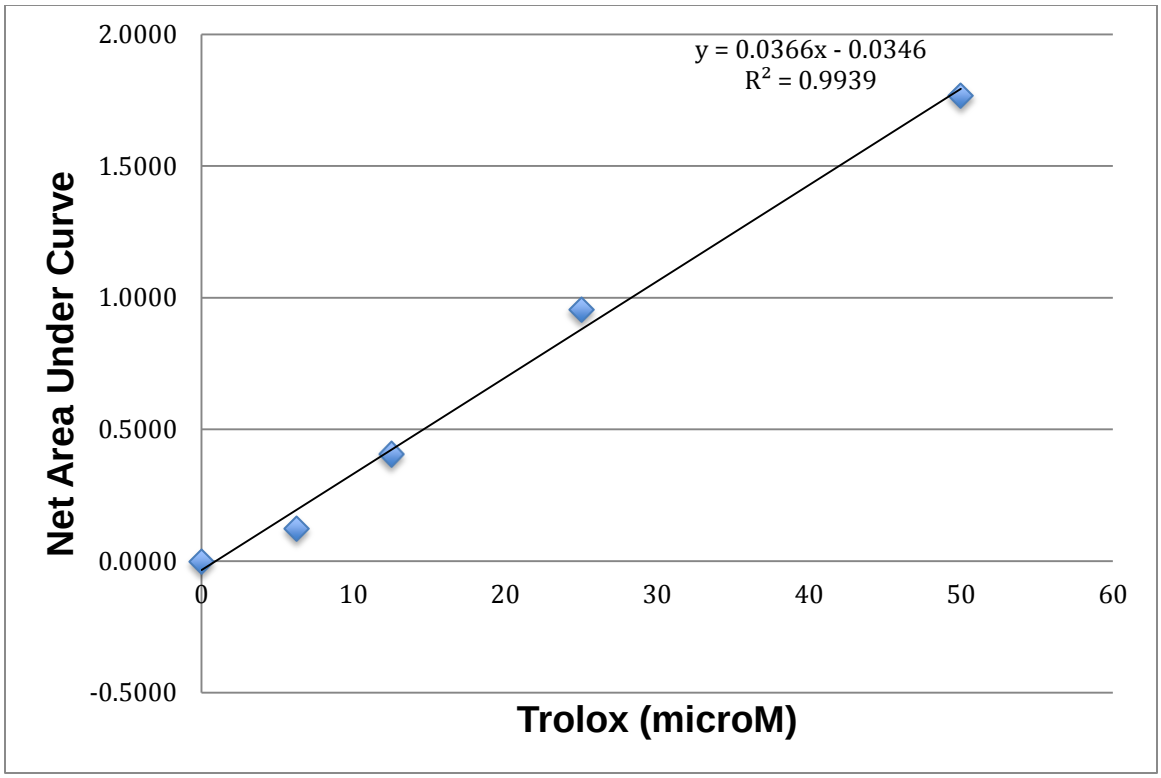


Figure A.7. Oxygen Radical Absorbance Capacity Assay

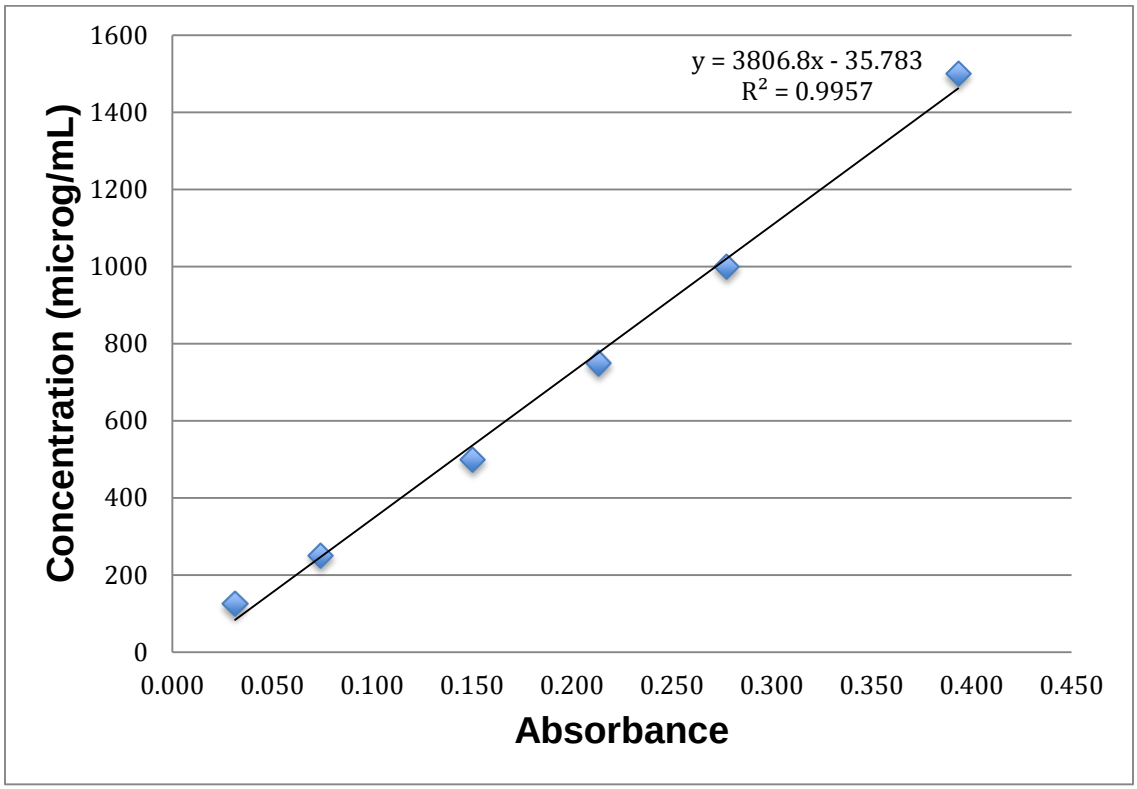


Figure A.8. Bicinchoninic Acid Standard Curve For Protein Assay

APPENDIX B: Results of Texture Profile Analysis

Table B.1. Effect of Citrus Fiber on Textural Properties of Ground Beef Samples During Shelf Life at Refrigerated Storage

Citrus Fiber Levels	Textural Properties					
	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
CP 0%						
Day 0	1798.73^{a}	0.752^{a}	0.513^{abc}	920.54^{a}	707.74^{a}	0.214^{abc}
Day 3	941.21^{bcd}	0.748^{a}	0.570^{a}	537.1^{bc}	407.06^{bcd}	0.234^{a}
Day 6	1212.64^{bcd}	0.749^{a}	0.567^{a}	687.18^{ab}	514.78^{abc}	0.234^{a}
Day 9	1471.94^{ab}	0.735^{ab}	0.563^{ab}	830.77^{a}	622.44^{ab}	0.229^{ab}
CP 1%						
Day 0	1422.42^{abc}	0.711^{abc}	0.468^{c}	655.33^{ab}	477.09^{abc}	0.189^{c}
Day 3	873.61^{cd}	0.719^{abc}	0.492^{bc}	429.73^{bcde}	311.92^{cdef}	0.199^{bc}
Day 6	930.7^{bcd}	0.708^{abc}	0.486^{c}	460.2^{bcd}	329.01^{cdef}	0.194^{c}
Day 9	1128.86^{bcd}	0.716^{abc}	0.473^{c}	541.12^{bc}	396.47^{bcde}	0.191^{c}
CP 5%						
Day 0	1135.69^{bcd}	0.667^{abcd}	0.323^{d}	353.25^{cdef}	235.19^{def}	0.133^{def}
Day 3	762.25^{d}	0.645^{cd}	0.352^{d}	275.28^{cdef}	180.37^{def}	0.148^{de}
Day 6	807.25^{d}	0.651^{bcd}	0.354^{d}	290.46^{cdef}	192.21^{def}	0.154^{d}
Day 9	843.46^{d}	0.659^{bcd}	0.356^{d}	297.23^{cdef}	197.08^{def}	0.147^{de}
CP 10%						
Day 0	1095.88^{bcd}	0.600^{d}	0.216^{e}	225.24^{def}	132.71^{f}	0.107^{f}
Day 3	792.54^{d}	0.641^{cd}	0.312^{d}	255.02^{def}	161.68^{ef}	0.142^{def}
Day 6	666.17^{d}	0.599^{d}	0.242^{e}	163.57^{ef}	97.54^{f}	0.122^{ef}
Day 9	724.16^{d}	0.603^{d}	0.206^{e}	150.64^{f}	91.38^{f}	0.112^{f}

$^{a, b, c, d, e, f}$ Different letters in the same column indicate a significant difference ($P < 0.05$).
Values are means of three replicates (N=9).

APPENDIX C: SENSORY SURVEY

Sensory Evaluation of Ground Beef Meatballs made with Citrus Fiber

You will be given 4 samples of ground beef meatballs. Please taste each sample in the order listed and rate the FLAVOR, TEXTURE and OVERALL liking of the product on the scale given. Please select only one degree of liking for each sample.

Sample _____

FLAVOR

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

TEXTURE

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

OVERALL LIKEING

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Sample _____

FLAVOR

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

TEXTURE

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

OVERALL LIKEING

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Sample _____

FLAVOR

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

TEXTURE

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

OVERALL LIKEING

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Sample _____

FLAVOR

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

TEXTURE

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

OVERALL LIKEING

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

APPENDIX D: THE GLM PROCEDURES OUTPUT

PROJECT 1

Dependent Variable: Gallic Acid

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	233.6804901	21.2436809	6389.01	<.0001
Error	12	0.0399004	0.0033250		
Corrected Total	23	233.7203905			

R-Square	Coeff Var	Root MSE	GAE Mean
0.999829	0.954137	0.057663	6.043488

Source	DF	Type I or III SS	Mean Square	F Value	Pr > F
rep	2	1.7791447	0.8895724	267.54	<.0001
cp	1	0.8434125	0.8434125	253.66	<.0001
am	1	204.0004505	204.0004505	61352.8	<.0001
cp*am	1	15.8536641	15.8536641	4767.97	<.0001
rep(cp*am)	6	11.2038183	1.8673030	561.59	<.0001

Tests of Hypotheses Using the Type III MS for rep(cp*am) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cp	1	0.8434125	0.8434125	0.45	0.5266
am	1	204.0004505	204.0004505	109.25	<.0001
cp*am	1	15.8536641	15.8536641	8.49	0.0268

Dependent Variable: Quercetin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	287.3949226	26.1268111	50821.0	<.0001
Error	12	0.0061691	0.0005141		
Corrected Total	23	287.4010918			

R-Square	Coeff Var	Root MSE	QUEE Mean
0.999979	0.372036	0.022674	6.094483

Source	DF	Type I or III SS	Mean Square	F Value	Pr > F
rep	2	1.8167770	0.9083885	1766.97	<.0001
cp	1	0.3987650	0.3987650	775.66	<.0001
am	1	281.1597451	281.1597451	546902	<.0001
cp*am	1	0.0146619	0.0146619	28.52	0.0002
rep(cp*am)	6	4.0049735	0.6674956	1298.39	<.0001

Tests of Hypotheses Using the Type III MS for rep(cp*am) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cp	1	0.3987650	0.3987650	0.60	0.4689
am	1	281.1597451	281.1597451	421.22	<.0001
cp*am	1	0.0146619	0.0146619	0.02	0.8870

Dependent Variable: ORAC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2595.004367	235.909488	14.79	<.0001
Error	12	191.451726	15.954311		
Corrected Total	23	2786.456094			

R-Square	Coeff Var	Root MSE	ORAC Mean
0.931292	29.70920	3.994285	13.44460

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	2	111.966221	55.983111	3.51	0.0631
cp	1	723.490872	723.490872	45.35	<.0001
am	1	1640.280271	1640.280271	102.81	<.0001
cp*am	1	9.570130	9.570130	0.60	0.4536
rep(cp*am)	6	109.696873	18.282812	1.15	0.3943

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	2	111.966221	55.983111	3.51	0.0631
cp	1	723.490872	723.490872	45.35	<.0001
am	1	1640.280271	1640.280271	102.81	<.0001
cp*am	1	9.570130	9.570130	0.60	0.4536
rep(cp*am)	6	109.696873	18.282812	1.15	0.3943

Tests of Hypotheses Using the Type III MS for rep(cp*am) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cp	1	723.490872	723.490872	39.57	0.0008
am	1	1640.280271	1640.280271	89.72	<.0001
cp*am	1	9.570130	9.570130	0.52	0.4966

Dependent Variable: β-Carotenoid

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Citrus Type	2	1 2

Number of Observations	
Number of Observation Read	12
Number of Observation Used	12

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	0.00044699	0.00008940	1.45	0.3291
Error	6	0.00036992	0.00006165		
Corrected Total	11	0.00081691			

R-Square	Coeff Var	Root MSE	pH Mean
0.547170	4.574848	0.007852	0.171633

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Rep	2	0.00000193	0.00000096	0.02	0.9845
Ct	1	0.00018881	0.00018881	3.06	0.1307
Rep (Ct)	2	0.00025625	0.00012812	2.08	0.2062

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	2	0.00000193	0.00000096	0.02	0.9845
Ct	1	0.00018881	0.00018881	3.06	0.1307
Rep(Ct)	2	0.00025625	0.00012812	2.08	0.2062

Test of Hypothesis Using the Type III MS for Rep(Ct) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Ct	1	0.00018881	0.00018881	1.47	0.3487

PROJECT 2

Dependent Variable: pH-Raw

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	47	1.53238333	0.03260390	15.25	<.0001
Error	48	0.10260000	0.00213750		
Corrected Total	95	1.63498333			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.937247	0.832342	0.046233	5.554583

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	0.14773333	0.07386667	34.56	<.0001
Trt	3	0.32060833	0.10686944	50.00	<.0001
Day	3	0.39877500	0.13292500	62.19	<.0001
Trt*day	9	0.06456667	0.00717407	3.36	0.0029
Rep(try*day)	30	0.60070000	0.02002333	9.37	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	0.32060833	0.10686944	5.34	0.0046
Day	3	0.39877500	0.13292500	6.64	0.0014
Trt*Day	9	0.06456667	0.00717407	0.36	0.9459

Dependent Variable: pH-Cooked

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	142856.6442	3039.5031	1.00	0.5015
Error	48	146097.2384	3043.6925		
Corrected Total	95	288953.8826			

R-Square	Coeff Var	Root MSE	pH Mean
0.494393	489.7622	55.16967	11.26458

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	6153.76713	3076.88356	1.01	0.3715
Trt	3	8973.64316	2991.21439	0.98	0.4088
Day	3	9048.40123	3016.13374	0.99	0.4051
Trt*day	9	27354.67623	3039.40847	1.00	0.4541
Rep(try*day)	30	91326.15644	3044.20521	1.00	0.4897

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	8973.64316	2991.21439	0.98	0.4141
Day	3	9048.40123	3016.13374	0.99	0.4104
Trt*day	9	27354.67623	3039.40847	1.00	0.4624

Dependent Variable: Water Holding Capacity

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	47	0.52562396	0.01118349	1.12	0.3473
Error	48	0.478855000	0.00997604		
Corrected Total	95	1.00447396			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.5232283	10.23209	0.099880	0.976146

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	0.07300833	0.03650417	3.66	0.0332
Trt	3	0.01411146	0.00470382	0.47	0.7036
Day	3	0.03467813	0.01155938	1.16	0.3352
Trt*day	9	0.08273437	0.00919271	0.92	0.5151
Rep(try*day)	30	0.32109167	0.01070306	1.07	0.4058

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	0.01411146	0.00470382	0.44	0.7264
Day	3	0.03467812	0.01155937	1.08	0.3725
Trt*day	9	0.08273437	0.00919271	0.86	0.5705

Dependent Variable: Cooking Yield

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	145

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	47	17877.03053	380.36235	78.48	<.0001
Error	97	465.25647	4.84642		
Corrected Total	144	18342.28700			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.974635	2.675168	2.201459	82.29236

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	104.289376	52.144688	10.76	<.0001
Trt	3	8871.127058	2957.042353	610.15	<.0001
Day	3	2239.468914	746.489638	154.03	<.0001
Trt*day	9	57.669692	6.407744	1.32	0.2357
Rep(try*day)	30	6604.475490	220.149183	45.43	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	8871.127058	2957.042353	13.43	<.0001
Day	3	2239.468914	746.489638	3.39	0.0307
Trt*day	9	57.669692	6.407744	0.03	1.000

Dependent Variable: Hardness

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	159221601.64	338757.48	17.47	<.0001
Error	48	930806.63	19391.80		
Corrected Total	95	16852408.28			

R-Square	Coeff Var	Root MSE	pH Mean
0.944767	13.41603	139.2545	1037.970

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	53143.137	26571.569	1.37	0.2638
Trt	3	4180953.602	1393651.201	71.87	<.0001
Day	3	3886159.584	1295386.528	66.80	<.0001
Trt*day	9	803713.621	89301.513	4.61	<.0002
Rep(try*day)	30	6997631.700	233254.390	12.03	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	4180953.602	1393651.201	5.97	0.0026
Day	3	3886159.584	1295386.528	5.55	0.0037
Trt*day	9	803713.621	89301.513	0.38	0.9341

Dependent Variable: Springiness

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	0.43674649	0.00929248	7.41	<.0001
Error	48	0.06022550	0.00125470		
Corrected Total	95	0.49697199			

R-Square	Coeff Var	Root MSE	pH Mean
0.878815	5.197531	0.035422	0.681510

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	0.00565158	0.00282579	2.25	0.1162
Trt	3	0.26164328	0.08721443	69.51	<.0001
Day	3	0.00205361	0.00068454	0.55	0.6535
Trt*day	9	0.00845659	0.00093962	0.75	0.6628
Rep(try*day)	30	0.15894142	0.00529805	4.22	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	0.26164328	0.08721443	16.46	<.0001
Day	3	0.00205361	0.00068454	0.13	0.9420
Trt*day	9	0.00845659	0.00093962	0.18	0.9950

Dependent Variable: Cohesiveness

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	1.55862096	0.03316215	27.67	<.0001
Error	48	0.05752800	0.0019850		
Corrected Total	95	1.61614896			

R-Square	Coeff Var	Root MSE	pH Mean
0.964404	8.531751	0.034619	0.405771

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	0.01507108	0.00753554	6.29	0.0038
Trt	3	1.36807321	0.45602440	380.50	<.0001
Day	3	0.03390846	0.01130282	9.43	<.0001
Trt*day	9	0.02652663	0.00294740	2.46	0.0215
Rep(try*day)	30	0.11504158	0.00383472	3.20	0.0002

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	1.36807321	0.45602440	118.92	<.0001
Day	3	0.03390846	0.01130282	2.95	0.0487
Trt*day	9	0.02652663	0.00294740	0.77	0.6456

Dependent Variable: Gumminess

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	6524516.750	138819.505	23.10	<.0001
Error	48	288517.794	6010.787		
Corrected Total	95	6813034.544			

R-Square	Coeff Var	Root MSE	pH Mean
0.957652	17.53894	77.52927	442.0408

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	7380.461	3690.231	0.61	0.5454
Trt	3	4217809.266	1405936.422	233.90	<.0001
Day	3	379599.794	126533.265	21.05	<.0001
Trt*day	9	377009.097	41889.900	6.97	<.0001
Rep(try*day)	30	1542718.132	51423.938	8.56	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	4217809.266	1405936.422	27.34	<.0001
Day	3	379599.794	126533.265	2.46	0.0819
Trt*day	9	377009.097	41889.900	0.81	0.6070

Dependent Variable: Chewiness

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	4488903.863	95508.593	27.77	<.0001
Error	48	165090.766	3439.391		
Corrected Total	95	4653994.628			

R-Square	Coeff Var	Root MSE	pH Mean
0.964527	18.56378	58.64632	315.9180

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	2534.092	1267.046	0.37	0.6938
Trt	3	2788810.632	929603.544	270.28	<.0001
Day	3	215196.796	71732.265	20.86	<.0001
Trt*day	9	222532.365	24725.818	7.19	<.0001
Rep(try*day)	30	1259829.979	41994.333	12.21	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	2788810.632	929603.544	22.14	<.0001
Day	3	215196.796	71732.265	1.71	0.1864
Trt*day	9	222532.365	24725.818	0.59	0.7957

Dependent Variable: Resilience

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	96

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	0.19842883	0.00422189	26.14	<.0001
Error	48	0.00775300	0.00016152		
Corrected Total	95	0.20618183			

R-Square	Coeff Var	Root MSE	pH Mean
0.962397	7.397963	0.012709	0.171792

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	0.00267152	0.00133576	8.27	0.0008
Trt	3	0.16618992	0.05539664	342.97	<.0001
Day	3	0.00549125	0.00183042	11.33	<.0001
Trt*day	9	0.00232133	0.00025793	1.60	0.1432
Rep(try*day)	30	0.02175481	0.00072516	4.49	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	0.16618992	0.05539664	76.39	<.0001
Day	3	0.00549125	0.00183042	2.52	0.0765
Trt*day	9	0.00232133	0.00025793	0.36	0.9471

Dependent Variable: Color *L* Value

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	145

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	912.279849	19.410210	7.67	<.0001
Error	97	242.942533	2.530651		
Corrected Total	144	1155.222383			

R-Square	Coeff Var	Root MSE	pH Mean
0.789701	3.512119	1.590802	45.29465

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	37.7785722	18.8892861	7.46	0.0010
Trt	3	544.8341243	181.6113748	71.76	<.0001
Day	3	47.0522076	15.6840692	6.20	0.0007
Trt*day	9	22.1914729	2.4657192	0.97	0.4661
Rep(try*day)	30	260.4234722	8.6807824	3.43	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	544.8341243	181.6113748	20.92	<.0001
Day	3	47.0522076	15.6840692	1.81	0.1672
Trt*day	9	22.1914729	2.4657192	0.28	0.9741

Dependent Variable: Color *a* Value

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	145

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	47	5864.298827	124.772315	24.68	<.0001
Error	97	485.268867	5.054884		
Corrected Total	144	6349.567694			

R-Square	Coeff Var	Root MSE	pH Mean
0.923575	14.47699	2.248307	15.53021

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	83.823229	41.911615	8.29	0.0005
Trt	3	4952.031124	1650.677041	326.55	<.0001
Day	3	235.425424	78.475141	15.52	<.0001
Trt*day	9	178.969434	19.885493	3.93	0.0003
Rep(try*day)	30	414.049615	13.801654	2.73	0.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	4952.031124	1650.677041	119.60	<.0001
Day	3	235.425424	78.475141	5.69	0.0033
Trt*day	9	178.969434	19.885493	1.44	0.2154

Dependent Variable: Color *b* Value

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT
Day	4	0 3 6 9

Number of Observations	
Number of Observation Read	145
Number of Observation Used	145

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	47	56.93237500	1.21132713	7.66	<.0001
Error	97	15.17360000	0.15805833		
Corrected Total	144	72.10597500			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.789565	4.076027	0.397566	9.753750

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	6.09306667	3.04653333	19.27	<.0001
Trt	3	22.88755833	7.62918611	48.27	<.0001
Day	3	3.38336944	1.12778981	7.14	0.0002
Trt*day	9	3.35620278	0.37291142	2.36	0.0188
Rep(try*day)	30	21.21217778	0.70707259	4.47	<.0001

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	22.88755833	7.62918611	10.79	<.0001
Day	3	3.38336944	1.12778981	1.60	0.2112
Trt*day	9	3.35620278	0.37291142	0.53	0.8429

Dependent Variable: Moisture

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT

Number of Observations	
Number of Observation Read	12
Number of Observation Used	12

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	87.06790833	17.41358167	10.80	0.0058
Error	6	9.67158333	1.61193056		
Corrected Total	11	96.73949167			

R-Square	Coeff Var	Root MSE	pH Mean
0.900024	2.151014	1.269618	59.02417

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Rep	2	49.10101667	24.55050833	15.23	0.0045
Trt	3	37.96689167	12.65563056	7.85	0.0168

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	2	49.10101667	24.55050833	15.23	0.0045
Trt	3	37.96689167	12.65563056	7.85	0.0168

Dependent Variable: Fat

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT

Number of Observations	
Number of Observation Read	12
Number of Observation Used	12

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	73.60418333	14.72083667	30.77	0.0003
Error	6	2.87088333	0.47848056		
Corrected Total	11	76.47506667			

R-Square	Coeff Var	Root MSE	pH Mean
0.962460	3.399687	0.691723	20.34667

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Rep	2	68.46031667	34.23015833	71.54	<.0001
Trt	3	5.14386667	1.71462222	3.58	0.0859

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	2	68.46031667	34.23015833	71.54	<.0001
Trt	3	5.14386667	1.71462222	3.58	0.0859

Dependent Variable: Protein

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 10% CF 5% CONT

Number of Observations	
Number of Observation Read	12
Number of Observation Used	12

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	5	81.69325000	16.33865000	107.51	<.0001
Error	6	0.91185	0.15197500		
Corrected Total	11	82.60510000			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.988961	2.184588	0.389840	17.84500

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	2.63655000	1.31827500	8.67	0.0170
Trt	3	79.05670000	26.35223333	173.40	<.0001

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	2.63655000	1.31827500	8.67	0.0170
Trt	3	79.05670000	26.35223333	173.40	<.0001

PROJECT 3

Dependent Variable: Moisture

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 3% CF 5% CONT

Number of Observations	
Number of Observation Read	24
Number of Observation Used	24

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	11	85.87898333	7.80718030	37.03	<.0001
Error	12	2.53000000	0.21083333		
Corrected Total	23	88.40898333			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.971383	0.645705	0.459166	71.11083

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Rep	2	15.12330833	7.56165417	35.87	<.0001
Trt	3	62.51115000	20.83705000	98.83	<.0001
Trt*day	6	8.24452500	1.37408750	6.52	0.0030

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	62.51115000	20.83705000	15.16	0.0033

Dependent Variable : Fat

Class Level Information		
Class	Levels	Values
Replication	3	1 2 3
Treatment	4	CF 1% CF 3% CF 5% CONT

Number of Observations	
Number of Observation Read	24
Number of Observation Used	24

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	11	42.94993333	3.90453939	11.29	0.0001
Error	12	4.15120000	0.34593333		
Corrected Total	23	47.10113333			

R-Square	Coeff Var	Root MSE	pH Mean
0.911866	13.19240	0.588161	4.458333

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Rep	2	39.44973333	19.72486667	57.02	<.0001
Trt	3	1.14770000	0.38256667	1.11	0.3848
Trt*day	6	2.35250000	0.39208333	1.13	0.4002

Test of Hypothesis Using the Type III MS for Rep(trt*day) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Trt	3	1.14770000	0.38256667	0.98	0.4639

PROJECT 4

Dependent Variable : Flavor

Class Level Information		
Class	Levels	Values
Treatment	4	CF 1% CF 3% CF 5% CONT

Number of Observations	
Number of Observation Read	656
Number of Observation Used	656

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	3	136.804878	45.601626	17.93	<.0001
Error	652	1658.292683	2.543394		
Corrected Total	655	1795.097561			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.076210	25.97294	1.594802	6.140244

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	136.8048780	45.6016260	17.93	<.0001

Dependent Variable: Texture

Class Level Information		
Class	Levels	Values
Treatment	4	CF 1% CF 3% CF 5% CONT

Number of Observations	
Number of Observation Read	656
Number of Observation Used	656

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	3	134.907012	44.969004	15.29	<.0001
Error	652	1917.810976	2.941428		
Corrected Total	655	2052.717988			

R-Square	**Coeff Var**	**Root MSE**	**pH Mean**
0.065721	28.20453	1.715059	6.080793

Source	**DF**	**Type I or III SS**	**Mean Square**	**F Value**	**Pr>F**
Trt	3	134.9070122	44.9690041	15.29	<.0001

Dependent Variable: Overall Likeness

Class Level Information		
Class	Levels	Values
Treatment	4	CF 1% CF 3% CF 5% CONT

Number of Observations	
Number of Observation Read	656
Number of Observation Used	656

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	3	160.516768	53.505589	21.90	<.0001
Error	652	1592.932927	2.443149		
Corrected Total	655	1753.449695			

R-Square	Coeff Var	Root MSE	pH Mean
0.091543	25.39920	1.563057	6.153963

Source	DF	Type I or III SS	Mean Square	F Value	Pr>F
Trt	3	160.5167683	53.5055894	21.90	<.0001

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