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PHYSICOCHEMICAL AND SENSORY PROPERTIES OF AUTUMNBERRY AND APPLICATION IN BREAD

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PHYSICOCHEMICAL AND SENSORY PROPERTIES OF AUTUMNBERRY AND APPLICATION IN BREAD

By

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ABSTRACT

PHYSICOCHEMICAL AND SENSORY PROPERTIES OF AUTUMNBERRY AND APPLICATION IN BREAD

By

Aileen Diana Tanojo

Autumnberry is a low-cost underutilized fruit with potential for highly valuable antioxidant/nutraceutical applications. Spectrophotometric and Oxygen Radical Absorbance capacity ($ORAC_{FL}$) methods were used to test the lycopene content and ORAC-value of both pureed and freeze-dried autumnberry (in dried weight): 2.90±0.04 mg/g lycopene and 144.14±4.86 µmol TE/g ORAC-value, and after freeze-drying, 0.70±0.00 mg/g lycopene and 102.26±3.27 µmol TE/g ORAC-value. A sensory trained panel (n=12) participated in descriptive analysis to evaluate freeze-dried autumnberry fortified bread at 0%, 3%, 6%, and 9% levels. A general linear mixed model was fitted using the mixed procedure of SAS. At $p<0.05$, the significant differences were detected among all breads in term of crumb color and autumnberry flavor, but not in yeasty flavor. The significant differences in crust color and firmness were detected at 6% and 9% level; and at 0% and 9% for crumb cell uniformity. The 3%-fortified bread was preferred and the closest to the control in terms of flavor and physical characteristics. The Principal Component Analysis (PCA) demonstrated that the bread control had distinct yeasty flavor, while the 9% fortified bread was strongly related to crumb cell uniformity. Lycopene in autumnberry appeared to be easily degraded by freeze-drying (75.86%) and only small quantity retained after baking (7-9%).

DEDICATION

To my beloved Mom and Dad, Oei Giok Koen and Kardi Tanojo, for your unconditional love, endless prayers, and supports. You have taught me to be resilient, independent, and optimistic. Thank you for believing in me and always being there for me every step of the way. To my older brother, Mustika Utomo Tanojo a.k.a. Koko Bear, you are the best brother anyone could ask for, a best friend, a role model. Thank you for your patience, emotional and financial support, and your brotherly advices. Especially to my dearest Dad, you have given us strength, inspiration, and the sweetest of memories that we will carry for the rest of our lives. We miss you very much and will always keep you in our thoughts and in our heart every single day. I cannot thank you enough for your selfless sacrifice and tremendous dedication to your family that have shaped me into the person that I am today. My wish is that one day, we will happily be together again.

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CHAPTER 1: INTRODUCTION

Carotenoids are naturally available in fruits and vegetables. Lycopene, one of many other classifications in carotenoid family, is a natural orange-to-red pigment mostly present in tomato, guava, rosehip, watermelon, and pink grapefruit (Holden 1999). Lycopene has high levels of antioxidant properties that have a high rate of quenching reactive singlet oxygen ($^1O_2$), which causes cell damage leading to cell death, deoxyribonucleic acid (DNA) damage or mutation, and protein damage and/or its functional alteration. It is also known as the most efficient antioxidant among other carotenoids (Di Mascio and others 1989; Chadwick and others 2003).

Various positive effects of lycopene on human health have been reported in the literature. Due to its antioxidant characteristics, lycopene may also protect against chronic degenerative diseases such as inflammation in arthritis and atherosclerosis (Schmidl and Labuza 2000). The normal human body has defenses against the free radicals. However, people under stress, high exposure to cigarette smoke, pollution, illness and dietary deficiencies, are more prone to having impaired antioxidants function. Epidemiological studies suggest that people who consume diets rich in tomato and tomato products have a lower risk of certain types of cancer, especially prostate, lung, and stomach cancers (Weisburger 1998). Additionally, lycopene is also known to have a preventative function towards cardiovascular diseases (Rao and Rao 2007).

As more scientific data on lycopene's beneficial health effects become available, food manufacturers seek more avenues to incorporate a natural source of lycopene into daily food products to produce value-added or functional food products. In addition, consumers have a growing interest in the use of "natural" ingredients in food products, such as lycopene, that are perceived as safer and healthier than the synthetic counterpart. The utilization of natural ingredients has also attracted many food manufacturers striving for a "clean label" (Fletcher 2006). Some popular applications of lycopene mostly extracted from tomato include natural food colorant in juice and nutritional beverages, smoothies and yogurt, and snack foods; as well as in the form of dietary supplements (Danzig and Hartal 2001; USDA Food and Nutrition Information Center 2007).

Tomato (*Lycopersicon esculentum*) is the best known source of lycopene. Fresh tomatoes and processed tomato products such as tomato sauces, pastes, canned tomatoes, ketchup, and juice are the primary sources of daily lycopene intake (0.5-5mg/day) (Chug-Ahuja 1993). In the United States, the tomato source of lycopene account for 81% of all lycopene intake (Plummer 1999; Fordham and others 2001; Grolier and others 2007). In recent years, the average rate of increase in quantity in tomato and tomato products consumption is 3% yearly. Together with this constant growth consumption, consumers not only demand higher quality of tomato products but also edible convenience as an important factor in fulfilling their daily lycopene intake (Business Wire 2007).

The United States Department of Agriculture (USDA) Fruit Laboratory has recently discovered autumn olive berry, or better know as autumnberry, that once

was identified as a fruit of an invasive plant, autumn olive plant (*Elaeagnus umbellata*), which was believed to possess high lycopene content. The USDA researchers found that typical autumnberry has up to 17 times the lycopene content (30-70 mg/100 g wet weight) as compared to fresh tomato (0.88 - 4.20 mg/100 g wet weight) (Bramley 2000; Boileau, 2002; Strax, 2006). This discovery has shed a possibility of autumnberry being a better source of lycopene than tomato. The pronounced tartness and slightly sweetness of the berry makes it suitable to be processed into jams and jellies (Fordham and others 2001). However, other edible convenience food applications have not been explored.

"Let food be thy medicine and medicine be thy food," a theory promoted nearly 2,500 years ago by Hippocrates, father of modern medicine, has recently regained more and more interest. According to the "2008 Food & Health Survey: Consumer Attitudes toward Food Nutrition & Health" conducted by the International Food Information Council (IFIC), 67% of Americans are now switching their diet toward healthfulness and wholesomeness (International Food Information Council Foundation 2008). Additionally, 80% of US population are currently consuming, or would be interested in consuming, specific healthful foods or beverages (Hasler 1998; Tan 2002; Foster 2008; International Food Information Council Foundation 2008). Practical applications of functional ingredients, in this case, lycopene, into staple food products such as fortification of bread are an excellent approach to accommodate recent consumer demand. Examples of some existing fortified or enriched breads are whole grain breads,

breads with increased fiber, protein, vitamins, and minerals, but there are no breads enriched with lycopene. The bread fortification trend is not only gaining popularity in the US, but also worldwide, especially in Japan and France. The global increase in health consciousness and the awareness of health benefits of functional ingredients are resulting in a large array of nonstandard functional bread products (Kubomura 2007; Foster 2008). This increased interest has become the inspiration to create autumnberry-fortified bread, or so called lycopene bread.

Lycopene in general is an open-chained carotenoid that is also highly unsaturated due to its eleven conjugated double bond. This characteristic of lycopene makes it considerably reactive with light, heat, oxygen and acid, which can cause problems during food processing (Nguyen and Schwartz 1998). Typically, the production of a fruit powder involves heat that evaporates the water from the fruit juice or puree, and a grinding mechanism that converts the dried product to smaller particles form. These processing steps easily diminish the lycopene content in autumberry pureed; therefore, the freeze-drying technique is proposed. Freeze-drying is the superior drying method, widely used in food manufacturing, for producing fruit product from its liquid state to dehydrated fruit powders. The freeze-dried fruit powders have the highest quality in term of nutrient retention, chemical stability, and convenience, as compared to other drying methods (Barbosa-Casanovas and others 2005). In addition, the autumnberry powder, rather than the pureed form, is a better form to use for an optimum bread fortification since its powder form is the same as the flour.

Although many studies have emphasized the physical and chemical properties of other lycopene-rich foods, little is known about this recently known fruit, autumnberry, and its physicochemical and sensory characteristics. Because of the promising properties of autumnberries as a good source of lycopene, they are attractive for the applications in foodstuffs, for example in fortified bread.

The objectives of this research were:

1) To analyze the physicochemical properties of autumnberry pureed as well as the freeze-dried autumnberry powder.
2) To determine the fate of nutraceuticals of autumnberry (lycopene and antioxidant capacity) after freeze-drying and baking process.
3) To fortify yeast-raised bread with freeze-dried autumnberry powder and to analyze some of its physical, physicochemical, and sensory attributes.

CHAPTER 2: LITERATURE REVIEW

2.1. Nutraceuticals and Functional Foods as Food Ingredients

Nutraceuticals and functional foods are closely related to one another and the terms have been used interchangeably, however, they have not been clearly defined. Nutraceuticals are often defined as products manufactured as dietary supplements, such as those in pill or powder form, whose ingredients offer medical or health benefits for disease prevention and/or treatment. Functional foods, on the other hand, are food products in form of conventional foods consumed in regular daily meal, for example fortified energy drinks or nutritionally enhanced snack bars (Iisakka 2003).

The extensive growth of nutraceuticals and functional foods has appeared to be the channel towards consumers' healthier lifestyle worldwide. Globally, this industry corresponds to approximately $75.5 billion in 2007 with growth projections to $167 billion by 2010 (Basu and others 2007). In the United States alone, the worth of this industry was $21.3 billion in 2006 and continues to grow, thus placing the United States as the largest and fastest expanding nutraceutical and functional food market in the world. In addition, 50% of the United States multi-million dollar food market is related to the application of nutraceuticals and functional food products (Datamonitor 2007).

Fortification has become more and more popular approach to incorporate nutraceuticals and functional ingredients into food products. Initially, fortification is the simplest and oldest method used to replenish nutrient lost in particular

staple foods after processing, mainly vitamins and mineral fortifications. Recently, fortification has become more robust involving wide range of food stuffs, various nutrients, and phytochemicals which now become more well accepted for their proven positive health benefits and disease-preventing properties in many clinical studies and researches (Myers 2005).

The projected sale of fortified food products in 2004 was $23.4 billion, which is 3% increase over 2003 sales. Despite the broad availability of fortified food products in the market, 27% of the consumers feel that they are deficient in antioxidants (Sloan 2004). Carotenoids, especially lycopene, along with essential fatty acid, and phytonutrients are among the top in the list of ingredients for food fortification. Fortifying waters, energy/sport drinks, and hot beverages with antioxidants such as lycopene is one of the most recent notable trends in food industry (Myers 2005). Taken together, consumers' escalating demand and interest in fortified food product makes the fortification of staple foods, such as bread, with lycopene-rich autumnberry a viable yet more accessible channel to fulfill their needs.

2.2. Lycopene and Human Health

Lycopene is by far considered as the most effective antioxidant among all other dietary carotenoids. Unlike β-carotene, lycopene does not get converted into vitamin A after it is digested and metabolized. The conversion of carotenoids into vitamin A actually weakens the antioxidant capacities. The acyclic structure, the numerous conjugated double bonds, and high hydrophobicity of lycopene are

the characteristics of lycopene that contribute greatly to its antioxidant benefits (Clinton 1998). Therefore, lycopene is believed to be a more powerful antioxidant, thus, the most efficient quencher of singlet oxygen in biological systems that has protective effects against certain tumors and cancers such as prostate, lung, and stomach cancers (American Cancer Society 2007).

The proposed mechanisms by which lycopene could decrease certain cancer risks are directly related to its antioxidant activities. Oxygen is very crucial to sustain life; however, it can also be toxic due to its potential to unleash free radicals. The unstable and highly reactive free radicals have unpaired electrons around them. These free radicals always try to capture electrons from nearby stable molecules in order to gain stability. However, the molecule whose electron was taken becomes a free radical and further starts a chain reaction, a process that finally ends in undesirable disruption or damage of the cells. Research studies have proven that oxidation through the free radicals processes is associated with reduced body capabilities to fight serious illnesses such as cancer and atherosclerosis (Chadwick 2003). Natural antioxidants lycopene have the ability to neutralize these free radicals in our body by donating an electron without loosing their own stability. Singlet-oxygen quenching reactions of lycopene are summarized as follows:

$$^{1}O_2 + \text{Lycopene} \rightarrow {}^{3}O_2 + {}^{3}\text{Lycopene}$$

$$^{3}\text{Lycopene} \rightarrow \text{Lycopene} + \text{Heat}$$

The sunlight and other chemical actions can convert ground-state oxygen ($^3O_2$) to extremely reactive singlet oxygen ($^1O_2$). These reactive singlet oxygen molecules can be quenched by the reaction with the lycopene to produce triplet-excited lycopene, which would decays exothermically (Wildman 2001).

The ingested dietary lycopene possess ability to increase the lycopene level in certain body tissues, and acts as an antioxidant that may trap the highly reactive oxygen molecules. Lycopene would also increase the overall anti-oxidant capacities, which further reduce the oxidative damage to lipid i.e. lipoprotein, membrane lipids; and also proteins such as significant enzymes, and DNA or other genetic materials, thereby lowering the oxidative stress. This reduced oxidative stress may lead to the reduced risk of cancer and cardiovascular disease. In addition, the increased lycopene levels in our body may also regulate the gene functions, improve intercell communications, modulate hormone and immune response, or regulate metabolism (Agarwal 2000). These functional characteristics help lower the risk for chronic disease

In general, the 'active' form of lycopene is the *cis*-conformation (15-*cis*, 13-*cis*, 9-*cis*, and 5-*cis* lycopene). The term 'active lycopene' is equivalent to the term of 'bioavailability', which is the measure of the uptake of an ingested substance by the body as assessed by its concentration in the blood or the quantifiable biologic or functional effects of that nutrient (Stahl and Sies 1996). Although about 90% of the dietary lycopene is found in stable linear all-*trans* conformations, after food processing and cooking, these *trans*-isomers are transformed to *cis*-isomers to some extent, <10% (isomerization). Human

tissues, however, contain only *cis*-conformations. The *cis*-isomers of lycopene are better absorbed than the all-*trans* form due to their non-linear (bend configuration), greater solubility in human micelles, as well as their lower tendency to aggregate; thus, they are more bioavailable to further function as antioxidants (Boileau 2002).

Due to lycopene's health benefits and attractive color, lycopene has become a valuable natural colorant in food industry. Recently, consumers have a growing interest in the use of "natural" ingredients in food products, such as lycopene itself, that are perceived as safer and healthier than the synthetic counterpart (Feder 2009). Current applications of lycopene mostly include juice, nutritional beverages and bars, smoothies, snack foods, cheese and yogurt.

Lycopene is unsaturated with eleven conjugated double bonds (covalent bonds), which makes lycopene considerably reactive with light, heat, oxygen, acid, and metal ions which can cause problems during processing (Bruno and others 2007). Chang and others (2006) reported the use of freeze-drying as a mean to produce shelf-stable lycopene from tomato. Freeze-drying is better than other commonly used drying methods, such as hot-air-drying. As the matter of fact, freeze-drying is considered the best method to dry and preserve lycopene sensitive pigments and its antioxidant capacities. Freeze-dried lycopene-rich fruit, such as the autumnberry, can be regarded as a source of food additives for fortification and natural colorant. Additionally, from the economic viewpoint, the extract lycopene from freeze-dried autumnberry could be further developed as food additives useful in other food applications such as instant food products.

2.3. Background of Autumnberry

Autumnberries are the red berry-like fruit mottled with silvery brown dots of the autumn olive plant (*Elaeagnus umbellate* Thund.). Autumnberry is also commonly known as autumn olive berry and Japanese silverberry. The autumn olive plant is a large shrub or a small tree that has fragrant, ivory-yellow flowers; silvery-green leaves with waxy margins but not toothed; silvery-scaly twigs; and brown-dotted stems with a few sharp thorns hidden among the leaves (Pyle and Willis 2002).

Autumn olive and Russian olive (*Elaeagnus angustifolia* L.) are the two major species of *Elaeagnus* in the Unites States, although the latter is found mainly in New England and is less frequently seen in other regions. The autumn olive itself has four known cultivars: 'Cardinal,' 'Ellagood,' 'Elsberry,' and 'Redwing' (Kartesz 2002; Pyle and Willis 2002). Originally from southern Europe, and western and central Asia (China, Korea, and Japan), autumn olive was first introduced to the US around 1830 as an ornamental plant (Dirr 1983). Autumn olive grows at a rapid rate during spring to summer throughout the eastern US, from Maine to Alabama and west to Wisconsin. The fruit is produced and ready for harvest in the summer to fall (Strax 2006).

A mature autumn olive tree (20 years old) can reach a maximum height of 4.5 m and generally fruit production is abundant. This species can tolerate a wide range of environmental conditions; for example, it is easily adapted to various soil types, does not need much water and nutrients, and is hardy to -31°C (Kartezs 2002; Black 2005). Autumn olive is valued because of it serves

different functions: 1) it attracts wildlife, mainly birds and foxes that also help spread the seeds; 2) it prevents erosion; 3) it carries out nitrogen fixation due to its nitrogen-fixing root nodules thus allow it to thrive in infertile habitats; and 4) it enhances certain types of agro-forestry, for example as a "nurse" tree, which prepares the ground for black walnut trees (Fordham and others 2001). Feral populations of autumn olive have invaded throughout the eastern US due to their persistent nature, seed distribution by wild animals, and ability to survive in inferior soil and environment conditions by fixing nitrogen. Autumn olive is on the United States Department of Agriculture (USDA) Natural Resources Conservation Service's invasive species list, meaning that it should not be cultivated where it is not already established. However, some other important crop species and agro-forestry plants are also similarly listed for example water chestnut, Japanese honeysuckle, garlic mustard, and Japanese barberry (Kartezs 2002; Strax 2006).

In 2001, USDA Fruits and Phytonutrients Laboratory researchers published the facts that autumnberries have a high carotenoid content; especially lycopene (30-70 mg/100 g), which is approximately 17 times more abundant in autumnberries than in fresh tomato. Lycopene, a potent antioxidant, is suitable for nutraceutical use as well as a natural red colorant in food products (Fordham and others 2001). Wang and others (2007) studied the antioxidant capacity and anti-cancer properties of six genotypes of autumnberry. Although some genotypes have higher antioxidant capacity than others, the results indicated that the extracts from all autumnberry genotypes successfully inhibited proliferation of

human leukemia HL-60 cancer cells and human lung epithelial cancer A549 cells, and also induced apoptosis (programmed cell death) of HL-60 cells. These results suggest that consuming autumnberry may have positive health effects for human; although further studies are needed for confirmation (Wang 2007).

Despite autumnberry's palatability to human and its high lycopene content, only few references are available to human consumption of autumnberry in the United States. These berries are also high in acidity, similar to blueberries and blackberries, but somewhat astringent, with slightly sweet notes. Autumnberry, however, is normally consumed in Asia (Tanaka 1976; Parmar and Kaushal 1982). The annual productivity of autumnberry ranged from 0.5 to 15 kg per tree with approximately 8-10% of the total berry weight is in the seed (Black 2005). This sweet-tart fruits was utilized into jams, jellies, and fruit leather. It could also be used for juice, flavoring, and other food products. Incorporating this fruit into a baked product, however, has never been done.

2.4. Common Drying Methods of Fruits and Vegetables

Drying is the oldest universal method used to preserve a wide range of fruits and vegetables, and other food products. Drying involves heat that evaporates water and a mechanism that removes the moisture from foods to the level where the growth of microorganisms and chemical reactions are slowed down mainly to prevent spoilage. At the same time, drying also reduces the weight and volume of foodstuffs and prolongs their shelf life, thereby, minimizing the cost as well as the difficulties of packaging, storage, and transportation cost.

When drying fruits and vegetables, other valuable characteristics, such as nutritive value, flavor, and color are important to retain. Common drying methods for fruits and fruit-based products that yield fruits powder include spray-drying, drum-drying, and freeze-drying.

Mechanisms of spray-drying involve the transformation of liquid food products (i.e., in a solution, suspension, or paste) into dried particulate end products (i.e., powders, agglomerates, or granules) where the liquid feed is atomized or sprayed into a hot dry medium that evaporates the moisture. Only limited varieties of fruit and vegetable have been spray-dried. Fruit juices, pulps, and pastes can not be effectively spray-dried without incorporating additive such as maltodextrin to prevent caking. The temperature used in spray-drying fruits and vegetables is usually quiet high, for example, tomato paste is spray-dried at inlet temperature ranging from 138-150°C and at 75-90°C of the outlet temperature, which can degrade some of nutrients in the food product to some extent. Specific care of the final products must also be taken especially as they are both hygroscopic and thermoplastic (Mujumdar 2006).

In the drum-drying process, the initial product has to be in liquid, slurry, or pureed form where it is applied as a thin layer on the outer surface of a slowly revolving and heated hollow stainless steel drum. It is one of the simplest and economical drying methods. Typical drum-drying food products are usually in powders and flakes forms, which include milk and milk products, soup mixes, instant cereals, and potato flakes, which can be quickly rehydrated. The applications in fruits, however, are not widely used especially for fruits that are

high in sugar and low in fiber such as in berries. The addition of fiber such as low methoxyl pectin to these fruits pureeds is also needed for better final drum-dried outputs (Barbosa-Canovas 2005; Mujumdar 2006). The raw materials have to be able to withstand a high temperature (>140°C) for a short time. In fruit pureeds, this can caramelized or molten the sugar as well as degrading the heat sensitive compounds such as enzymes, vitamins, and protein (Desobry 1997; Abonyi 2001).

2.5. Lycopene

2.5.1. Structure of Lycopene

Lycopene is responsible for the natural orange-to-red pigment in most fruits and vegetables with higher concentrations in tomato, guava, rosehip, watermelon, and pink grapefruit (Bruno and others 2007). Lycopene is the most common subclass of carotenoids in the human diet. In the carotenoids family, overall 600 different subclasses have been extracted from plants, and more than 20 of these are from tomato alone. Lycopene along with α-, β-, γ-, and ζ-carotenes are classified as the hydrocarbon carotenes, while another major class of carotenoids, oxygenated xanthophylls, includes β-cryptoxanthin, lutein, and zeaxanthin. Lycopene is a lipohilic (oil-soluble) pigment/phytochemical, and naturally exists in the all-*trans* form. On the other hand, xanthophylls are more polar than carotenes due to the oxygenation, and they impart the yellow-to-brown color in plants (Shi and others 2002).

Lycopene structure is characterized as a polyene hydrocarbon with a symmetrical and acyclic structure containing 13 double bonds of which 11 are conjugated double bonds arranged in a linear array and having molecular formula of $C_{40}H_{56}$ (Figure 2.5.1.1.). In addition, the isomerization of lycopene from the naturally predominant thermodynamically stable *trans*-form to less stable *cis*-geometric forms happens as the result of exposure to heat, light, oxygen, acid, or the present of metallic ions such as Cu^{2+} and Fe^{3+}.

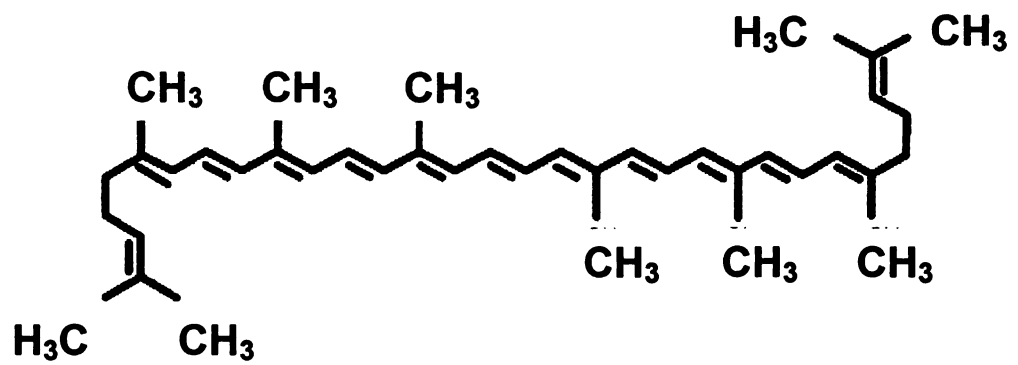


Figure 2.5.1.1. *Trans*-lycopene (molecular weight = 536.89 g/mol).

Different isomers have different stabilities due to their molecular energy as follows, highest stability: 5-cis ≥ all-trans ≥ 9-cis ≥ 13-cis > 15-cis > 7-cis > 11-cis: lowest (Agarwal 2000).

2.5.2. Lycopene Degradation during Processing

The major causes of lycopene degradation in food processing are isomerization and oxidation. In general, lycopene undergoes isomerization during thermal processing which converts lycopene from more stable (*trans*) to less stable state (*cis*). This transformation results in the changes of the ratio of

trans and *cis* isomers present in the food products, which also affects its biological activities (Bruno and others 2007). Other physical and chemical factors such as elevated temperature, light exposure, oxygen, extreme pH, and the involvement of metal ions (Cu^{2+}, Fe^{3+}), degrade lycopene in food products (Shi and others 2007).

2.5.2.1. Impact of Temperature on Lycopene Stability

In most cases, the duration of thermal treatment has less effect on the degradation of lycopene if the heating temperature is less than 100°C. The application of higher temperatures will result in more significant lycopene loss especially when the heating time is long. In addition, lycopene in general undergoes isomerization with the application of thermal processing. When the temperature is increased above 100°C, for instance to 180°C, in general, both the *trans* and *cis* isomers of lycopene will degrade. The level of conversion of *trans* isomers to *cis* isomers increases with the increase in treatment temperature up to 100°C; however, it drops significantly at 180°C. The temperature increase from 100°C to 180°C causes approximately 76% decrease in total lycopene content. In general, the increasing temperature (100°C to 180°C) or increasing heating time increases the degradation of *trans* and *cis* isomer of lycopene (Shi and others 2007).

In the processing of tomato paste, aseptic technique is widely used at temperatures below 100°C for 4 to 5 s. During this thermal processing, the transformation of all-*trans* isomers to the *cis*-form of lycopene occurs. The *cis*-

form has been proven to be more bioavailable (easily absorbed by human tissues) than the *trans*-form due to its non-linear bend structure, greater solubility of in human micelles, and/or the lower tendency to aggregate. Interestingly, even though the thermal treatment above certain temperature and time can degrade the total lycopene, it can also be concluded that heating increases the bioavalability of lycopene of tomato (Gartner and others 1997; Boileau and others 2002). Additionally, the total antioxidant activity considerably increases with heat processing although other components, such as vitamin C, are reduced by heat processing (Bruno and others 2007).

2.5.2.2. Impact of Light on Lycopene Stability

In general, light exposure causes total lycopene degradation. A study suggested that after 6 d of light exposure at room temperature, approximately 94% of *trans*-lycopene degraded, and at the same time the percentage of the *cis*-form increased inconsistently until day 2 when the *cis*-lycopene decreased. Overall, during the light exposure, the isomerization and the lycopene degradation occur simultaneously (Lee and Chen 2002).

Other study suggested that the exposure of lycopene to light caused no significant change to total and all-*trans* lycopene, although significant loss of cis-isomer lycopene was observed. In addition, the light irradiation caused the decrease in total lycopene, *trans*, and *cis* isomers meaning that light induces lycopene oxidation which leads to total lycopene degradation (Shi and others 2007).

2.5.2.3. Impact of Oxygen on Lycopene Stability

The oxidation of lycopene is irreversible and will lead to fragmentation of the molecule, producing acetone, methylheptenone, laevulinic aldehyde and probably glyoxal, which cause apparent color loss and typically hay or glass-like odors evolve. Generally, lycopene undergoes destabilization about three times higher in the presence of oxygen than in the absence of oxygen (Shi and others 2007). Nitrogen or argon flushing can be used to replace the atmospheric oxygen in the headspace of the storage containers; or by leaving headspace as minimum as possible in the containers. Furthermore, *cis*-isomers of lycopene are more susceptible to autoxidation than the *trans*-form (Anguelova and Warthesen 2000).

2.6. Freeze-drying

In food industry, freeze-drying, or lyophilization, is used to prepare dehydrated food powders from their original liquid state. Freeze-drying has gained in popularity in recent years and is considered the most attractive drying method in extending the shelf-life of foodstuffs. During freeze-drying, the moisture in the product is withdrawn in the form of water vapor via sublimation from its frozen state facilitated by vacuum suction. Freeze-dried products have been identified of having superior qualities in term of taste, aroma, color/appearance, texture/structure, and nutritional value retention.

The apparent advantage of this freeze dehydration process is that moisture removal from the foodstuffs can be achieved without exposing them to

high temperature. Additionally, during freeze-drying, the product structure is maintained in a more tolerable state, resulting in maximum nutrient and flavor volatiles retention; minimum destruction to the products' structure and texture; minimized shrinkage and movement of the soluble solid due to the solid ice structure in the products and the porous structure of the product assist in rapid and complete rehydration of the product (Welti-Chanes 2007; Mujumdar 2006). Therefore, freeze-drying is an ideal method for drying fruits and vegetables that are generally high in nutritional/volatile compounds, heat sensitive, and delicate in shape, structure, and texture. The final freeze-dried fruits are generally dry, light, and porous, retaining their original shape and structure which makes it convenient for packing and shipping. These products can be stored for more than one year with minimum losses on their physical, chemical, microbiological, and organoleptic properties if properly packaged (Oetjen and Haseley 2004; Barbosa-Canovas 2005).

2.6.1. Principles of Freeze-drying

The main components of freeze-dryer are an evaporator and a condenser that are located inside of a vacuum chamber, a refrigeration system, and a vacuum pump. The evaporator generates heat as the source of energy for drying, and the condenser gathers the vapors produced from the products. The steam ejector or vacuum pump facilitates the vacuum and low-pressure conditions in the chamber. Figure 2.6.1.1. shows the simplified schematic of research-scale freeze-dryer.

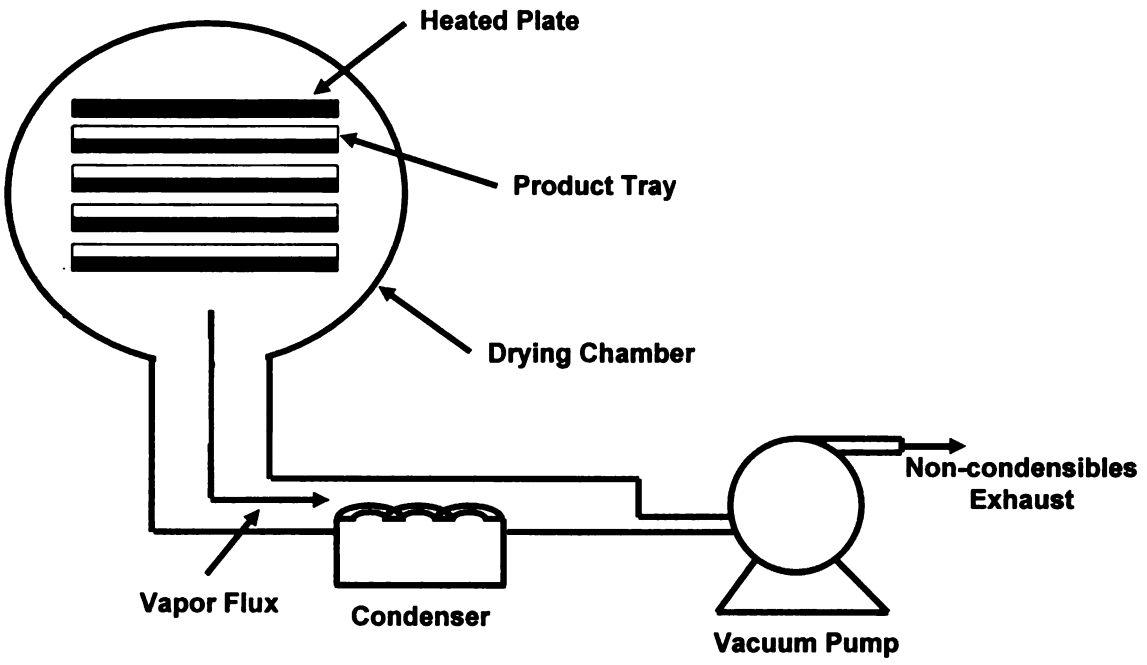


Figure 2.6.1.1. Freeze-dryer simplified schematic (source: Barbosa-Canovas and others 2005).

Two steps of freeze-drying process involve the freezing of the product with the aid of dry ice to approximately –20 to –40°C or below, and the application of heat to the product to directly sublime the ice in the product to water vapors under vacuum and low pressure condition. This sublimation can only be accomplished below the triple point of the water (at <627 Pa, 0°C) shown in Figure 2.6.1.2..

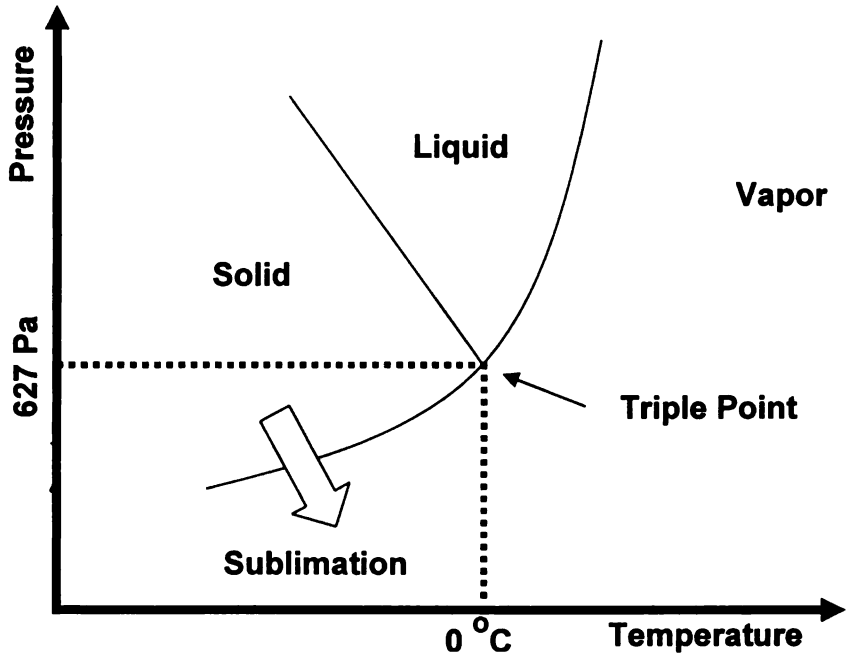


Figure 2.6.1.2. Phase diagram of water (source: Barbosa-Canovas and others 2005).

Freeze-dryer, as it is indicated previously, is designed to remove maximum amount of aqueous solution or other solvents in a food product under controlled conditions without degrading other components. In the initial stage, the refrigerator system of the freeze-dryer and the dry ice cooled the product rapidly to below its eutectic point (the point where water goes 'directly' from solid to liquid without partially melting to a solid-liquid combination) to support the sublimation process. The vacuum system discharges all non-condensable gasses from the chamber, which basically facilitates the water vapor migration from the product to the condenser as well as creating the vapor pressure differential necessary to enhance sublimation process. This vacuum condition also helps prevent oxidation of the food sample by removing the air (Barbosa-

Canovas and others 2005).

In the vacuum chamber, controlled heat was applied to the frozen sample from the heating plate. The temperature in the condenser must be about –40°C. In the last stage of freeze-drying cycle, a higher heat setting again is desired to discharge any of the remaining vapors. The applied heat to the frozen sample results in the constant vapor migration from the product to the condenser, which supplies sufficient energy to drive off the vapors to ensure continued sublimation. The water vapor molecules leave the product and migrated toward the low-pressure areas in the vacuum system surrounding the condenser. Once the vapors got into contact with the condenser, the vapors emitted the energy and turned into ice. The refrigeration system automatically seeks the lowest possible temperature in proportion with the product load. At the end of the process, a desirable moisture content of the final freeze-dried product is 1% - 4% (Oetjen and Haseley 2004).

2.6.2. Issues and Concerns during Vacuum Evaporation

Despite its great extent of benefits for drying fruits and vegetables, freeze-drying has some drawbacks. Freeze-drying is high in processing, energy, and capital costs due to the slow drying rate, the use of vacuum and heat, and the needs of specific packaging materials.

Resistance to heat and mass transfer causes the slow and long drying time. This happens because it is difficult to obtain a homogenous ice crystal distribution in the frozen food products to speed up the freeze-drying process. The energy costs are expensive because the materials have to be completely

frozen first and the use of vacuum at low pressure and the heat supply to sublime the ice as well as the bound water (a water portion of a tissue that does not form ice crystals until temperature lower than -20°C). Although it is light and convenient, the hygroscopic freeze-dried food product needs a special packaging material to control oxidation as well as to prevent the moisture absorption from surroundings (Welti-Chanes 2007; Mujumdar 2006).

2.7. Analyses of Antioxidant Capacity

Lycopene, one of the most prominent phytochemicals along with other dietary antioxidants such as phenolic compounds, vitamin C and E, has relatively high protective effects against oxidative stress and reduces the risk of developing certain types of cancer, inflammation, cardiovascular diseases, and age-related disorders. Therefore, more and more researchers are studying the measurement of antioxidant capacity of food stuffs in daily human consumption. However, this study has been an ongoing challenge to separate each antioxidant compound independently for analysis because of the interactions of these antioxidants with other components in the food system, as well as the possible synergistic effects amongst the antioxidant compounds in the matrix (Motchnik and others 1994; Cao and Prior 2001; Koracevic and others 2001).

2.7.1. Total Phenolic Assay

The total phenolic assay is also known as the Folin-Ciocalteau (FC) colorimetry assay. Folin-Ciocalteu assay was initially developed in 1927 for the

analysis of proteins (tyrosine). Since then, this method has been standardized for determining the antioxidant capacity and phenolics in variety of foods products including wine and dietary supplements in general (Singleton and Rossi 1965; Prior and others 2005).

The basic mechanism of this assay involves oxidation and reduction reactions, which is the measurement of oxidation of phenols by the reagent containing a mixture of tungsten and molybdenum oxide. The outcome of this metal oxide reduction during the assay is a blue colored solution that exhibits a broad light absorption with a range of 745 – 750 ηm in general, and a maximum at 765 ηm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. This color development is normally slow; however, proper elevated temperature can be applied to speed up the reaction. Excessive heating can cause rapid subsequent color loss and timing the assay measurement becomes an issue. Regardless of its ease of use and high precision, any compounds in the sample of interest containing phenolic groups such as reducing sugars, ascorbic acid, amino acids, enediols, and reductones will be detected, thus, limits the efficacy of this method for determining specific flavanols or flavonoids in food samples. In addition, some other substances, for example nonphenolic organic substances such as adenine, adenosine, benzaldehyde, glycine react with the Folin-Ciocalteau reagent. Some inorganic substances, such as sodium phosphate, react with the reagent and interfere with the final measurement of elevated phenolic content (Singleton and Rossi 1965; Prior and others 2005; Wrolstad and others 2005).

2.7.2. Oxygen Radical Absorbance Capacity ($ORAC_{FL}$)

$ORAC_{FL}$ is Oxygen-Radical Absorbance Capacity assay utilizing fluorescein (FL) (3',6'-dihydroxyspiro[isobenzofuran-1[3*H*],9'[9*H*]-xanthen]-3-one) as the fluorescent probe and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), as a peroxyl radical generator. This assay measures the ability of a compound or group of compounds to quench (the term "absorb") oxygen radicals, which indicates the antioxidant potential of foods.

Principal mechanism of $ORAC_{FL}$ involves the measurement of antioxidant inhibition of peroxyl radical induced oxidations by AAPH and thus demonstrates the classical chain breaking antioxidant activity by hydrogen atom transfer mechanism. Peroxyl radical reacts with a fluorescent probe to yield a non-fluorescent product, which can be easily monitored by measuring fluorescence in the function of time at incubation temperature at 37°C (Prior and others 2005). The proposed fluorescein oxidation pathway in the presence of AAPH according to Ou and others (2001) is presented in Figure 2.7.2.1..

The ORAC reactants consists of fluorescein as the fluorescent probe, AAPH as the radical generator, and food sample containing antioxidants at proper series of dilutions or Trolox dilutions (a cell-permeable, water-soluble derivative of vitamin E with known potent antioxidant properties) as the control, which are dissolved in sodium phosphate buffer solution (Davalos and others 2004). The incubation temperature for the reaction mixture is 37°C and the fluorescence is measured every minute until the fluorescence is completely lost. Technically, the higher the antioxidant capacity of a product, the longer this

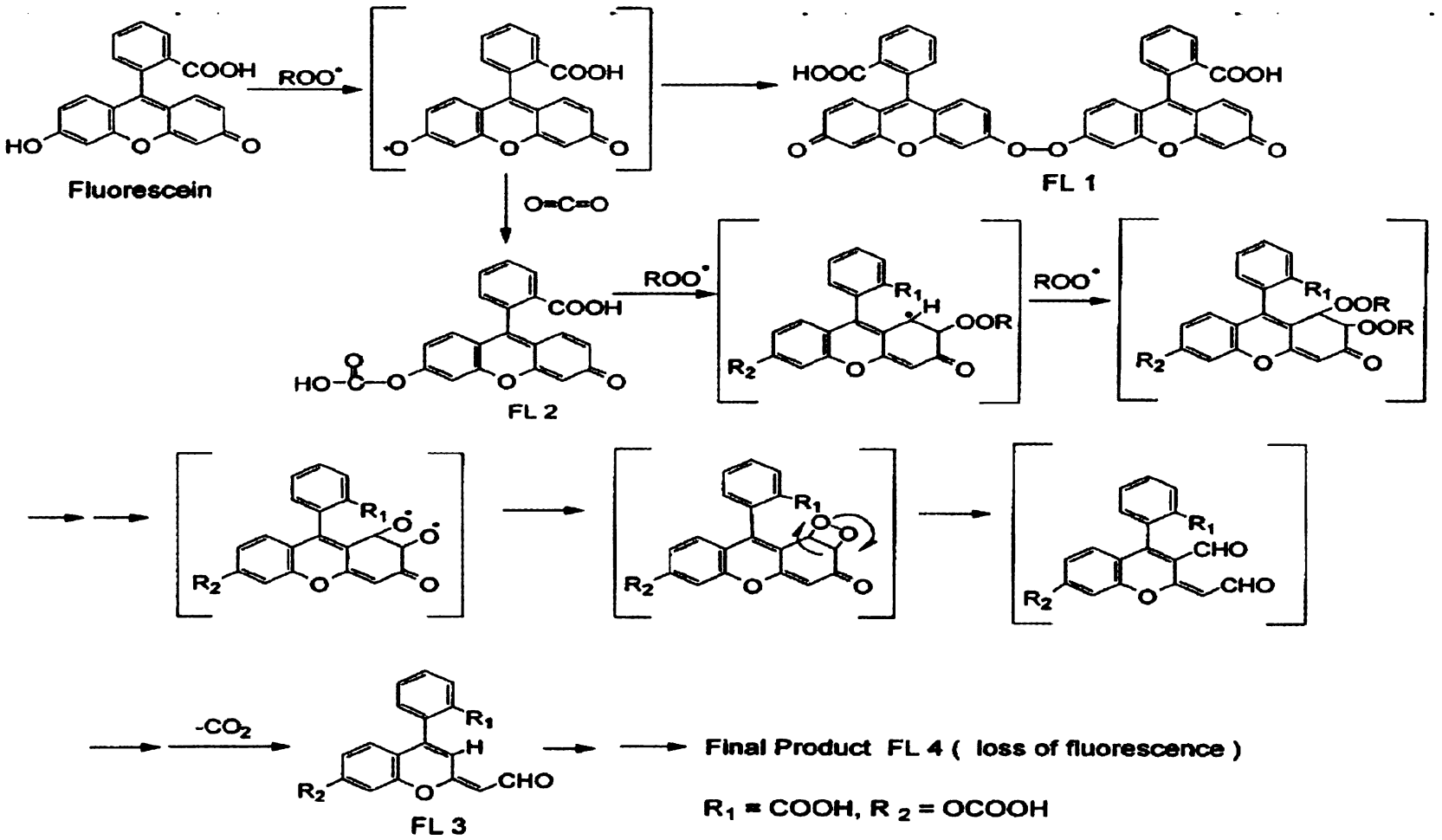


Figure 2.7.2.1. Proposed Fluorescin (FL) oxidation pathway in the presence of AAPH (source: Ou and others 2001).

reaction goes to completion. Data analysis of the results of ORAC assay is obtained by calculating the area under the kinetic curve (AUC) and net AUC (AUC_{sample} - AUC_{blank}); while the standard curve is obtained by plotting the concentrations of Trolox in the blank sample against AUC of the sample containing antioxidants (AUC_{sample} - AUC_{blank}) (Figure 2.7.2.2.). The calculated data are expressed as Trolox Equivalents (TE) as micro mol of TE per gram or liter of sample (μmol of TE/g OR μmol of TE/L).

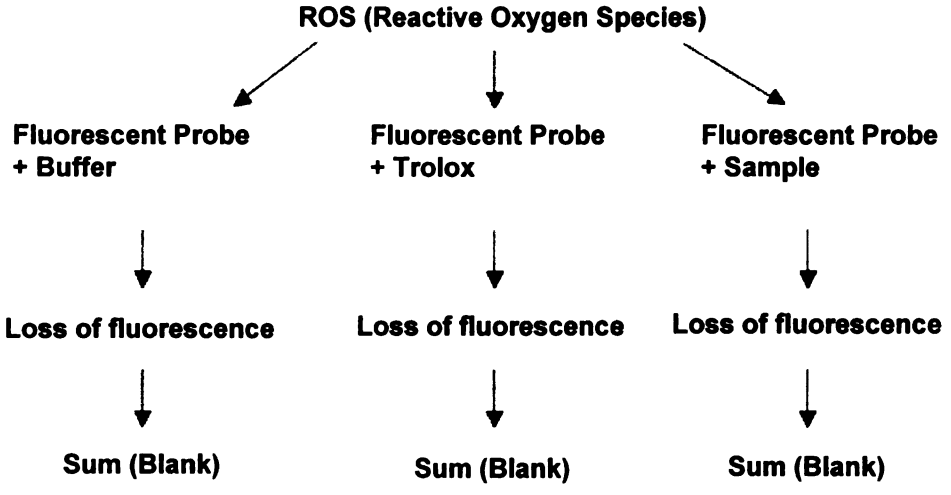


Figure 2.7.2.2. Total antioxidant properties calculations using $ORAC_{FL}$ (Source: Davalos and others 2004).

2.7.3. Spectrophotometery for Lycopene Determination

Spectrophotometry is a rapid, practical, and economical technique for lycopene content determination in food products that is widely used in the current research. Other possible methods for lycopene analysis include High

Performance Liquid Chromatography (HPLC) and color evaluation (Schoefs 2002; Anthon and Barrett 2007; Sandei and others 2009). In general, spectrophotometer consists of a spectrometer for producing light of any specific wavelength (color), and a photometer for measuring the light intensity. The cuvette filled with sample liquid containing compound of interest is placed between the spectrometer beam and the photometer for analysis.

The absorbance (A_λ) is defined as follows:

$$A_\lambda = -\log_{10}(I / I_o)$$

where I is the intensity of light at a selected wavelength (λ) that has passed (transmitted) through a sample, which intensity is measured by the photometer. The photometer delivers a voltage signal to a galvanometer. This signal changes as the amount of light absorbed by the sample changes. While I_o is the light intensity before it enters the sample or so called incident light intensity If the color development is associated to the concentration of a compound of interest present in a solution, then that concentration can be measured by determining the extent of light absorption at the appropriate wavelength (Gore 2000).

This device has been used for lycopene content determination since 1980s when this technique was initially introduced due to its short-time analysis, good accuracy and reproducibility (Barrie and Soderstrom 1989). The identity of carotenoids can be confirmed by their UV-vis absorption spectra, which usually

carried out by UV-visible (λ) spectrophotometric detector at around 450 ηm for general subclasses of carotenoids generally, and at 475 ηm specifically for lycopene as shown in Figure 2.7.3.1. (Minguez-Mosquera and others 2002).

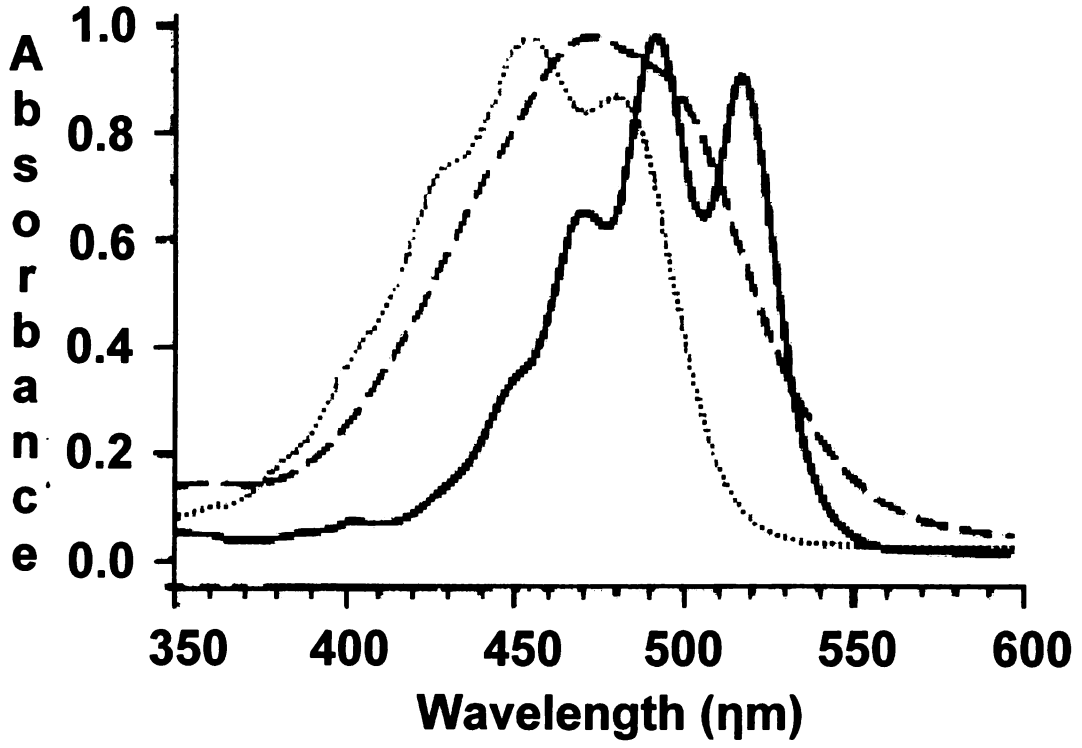


Figure 2.7.3.1. UV-vis light absorption spectrum of selected carotenoids (...) β-carotene, (---) capsanthin, and (—) lycopene (source: Minguez-Mosquera and others 2002).

CHAPTER 3: MATERIALS AND METHODS

3.1. Plant Material

3.1.1. Autumnberry Collection

Three batches of whole autumnberries were manually harvested during the same harvest time (October 2007) from different locations of a farm owned by Paul Siers in Mount Pleasant, Michigan. The berries were separated from leaves and twigs using a mechanical harvester (BEI, Inc., South Haven, MI). They were then spray-washed with water. The weights of the three batches of autumnberries after sorting were 15.92 kg, 2.93 kg, and 1.70 kg. The first batch was used for bread-making as well as for physicochemical analyses. The second and third batches were only used for physicochemical analyses.

3.1.2. Autumnberry Puree

Washed whole berries were mechanically mashed into pureed using a fruit-pureeding machine (Sterling Electric, Inc., Indianapolis, Indiana), which removed the seeds. The sorting and pureeing were done on-site immediately after the harvesting. The final weight of pureed for batches 1, 2, and 3 were 14.51 kg, 2.67 kg, and 1.55 kg, respectively. Citric acid solution 0.2% (w/w) was added into the puree to retard the post-harvest enzymatic degradation (Hui, 2006). The puree was transferred to individual 500-mL capacity wide-mouth French clear square glass bottles. The bottles were loosely closed with vinyl-lined screw caps, and wrapped in parafilm, and kept inside a closed cardboard

box to minimize exposure to light. These pureed samples were stored in a freezer at –20°C until further processing.

3.2. Freeze Drying

3.2.1. Sample Preparation and Freezing

The glass bottles containing frozen pureed autumnberry were thawed in a refrigerator at 4°C for 2 to 3 d. The thawed pureed was transferred to square plastic molds to form 20 x 20 cm slabs, which were returned to the freezer for 2 d. Prior to the freeze-drying process, the frozen slabs were further frozen in a styrofoam box filled with dry ice to approximately –20 to –40°C.

3.2.2. Operation

The freeze-dryer used in this experiment was a research-scale Freeze Mobile 12 with Unitop 600SL chamber consisting of three heating plates (The Virtis Company, Gardiner, NY).

Refrigeration

Mesh trays (51 x 25 cm), made out of 316-stainless steel, were put on each heating plates inside of the chamber. Pieces of dry ice were placed on top of the trays to completely cool down the chamber for about 30 to 45 min. The remaining pieces of dry ice were taken out of the chamber. The frozen autumnberry slabs were placed on the mesh trays (one slab per tray).

Sublimation

The vacuum vents were closed prior to the beginning of sublimation process. The mechanical oil in the vacuum pump was changed prior to each run, and the oil level was also checked. Fisherbrand 19 mechanical pump oil (Fisher Scientific, Pittsburgh, PA), with specifications of ultimate pressure at 25°C, 1.13 x 10^{-2} Pa; pour point of –15°C; and flash point of 212.78°C, was used for the continuous high vacuum pump throughout the freeze-drying process. Boekel hyvac flushing oil (Boekel Ind. Inc., Philadelphia, PA) was used to wash out any sediments and contaminants from the vacuum pump for 1 h between runs. Once the chamber was completely sealed, the pressure of the unit decreased gradually until around 4 to 5 Pa. Each freeze-drying run took approximately 3 to 4 d to obtain samples at the desired moisture content of 2% to 3% (wet basis).

Storage

The freeze-dried slabs were transferred to zip-lock bags, placed in a desiccator with drierite, and transported to a dark-dry room where these slabs were crushed manually using mortar and pestle into powder. The samples were repeatedly crushed until the particles passed a USA Standard Mesh Sieve ASTM Specifications Number 50 (300 µm) (Central Scientific Co., Chicago, IL). The freeze-dried powder was transferred to dark-brown narrow-necked 500 mL glass bottle, leaving a minimum headspace to prevent oxidation, and then sealed with parafilm. The samples were stored in a dark freezer at –20°C until further analysis.

3.3. Moisture Content Analysis

Moisture content of the pureed, freeze-dried, and the bread samples were determined using the vacuum oven drying method (Nielsen, 2003). Each batch of freeze-dried autumnberry powder was measured for its moisture content in triplicates. Approximately 2 to 2.5 g of freeze-dried sample was added to the oven-dried aluminum-weighing dish. The weight was recorded to the nearest 0.0001 g. The samples were dried at 70°C for 5 h in a bench-top vacuum dryer model 1430 (VWR Scientific, San Dimas, CA) equipped with a vacuum pump (Gast Manufacturing Corporation, Benton Harbor, MI, USA). After drying, the samples were cooled down in a desiccator and weighed to the nearest 0.0001 g. The weight loss was determined to calculate the moisture content of the samples. Moisture content (MC) was expressed in percentage of wet basis:

[(weight of initial sample – weight of dry sample)/weight of initial sample] x 100

3.4. Water Activity (A_w)

Water activity (A_w) of the pureed, freeze-dried samples, and the bread samples at all levels were measured using Aqua Lab Water Activity Meter (Decagon Devices, Pullman, WA) in triplicate readings.

3.5. Sugar Content Analysis

Sucrose content and total sugar content of both autumnberry pureed and freeze-dried autumnberry were estimated using the adaptation of Lane-Eynon titration method (AOAC Method 923.09, 920.183b).

3.5.1. Standard Solution Preparation

A stock solution was prepared in 1-L volumetric flask by dissolving 9.5 g sucrose crystal (Mallinckrodt Baker, Inc., Phillipsburg NJ) with 100 mL distilled water. Five milliliters of concentrated hydrochloric acid (HCl) (EMD Chemicals Inc., Gibbstown, NJ) was added to the solution, which was equilibrated at room temperature for 3 d. The volume of the solution was then brought up to 1 L with distilled water.

The invert sugar standard solution was made by neutralizing 50 mL of the stock solution with sodium hydroxide (NaOH) solution 0.1 to 1 N to pH 7.0. The volume of this pH-adjusted solution was brought up to 250 mL with distilled water. The standard solution contained 2 mg of invert sugar/mL solution.

3.5.2. Determining the Titration Factor

In 150-mL Erlenmeyer flasks, 5 mL each of Fehling's reagent A (cupric sulfate standard) and Fehling's reagent B (potassium sodium tartrate solution alkaline) (Fluka Chemika/Sigma-Aldrich, St. Louis, MO), 25 mL of distilled water, 15 mL of the standard solution, and glass beads were added. The solution was kept boiling throughout the titration. Two minutes after boiling, 2 to 3 drops of methylene blue solution ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) were added as a color indicator. The blue solution was titrated against the standard solution in a burette until the solution turned a clear maroon color. The amount of standard solution used for titration was recorded. The titration factor, F, was determined using the following formula:

$$F\ (mg) = V_1\ (mL) \times 2\ mg/L$$

where, V_1 = 15 mL + volume of standard solution used in titration.

3.5.3. Sample Preparation for Invert Sugar Determination

Approximately 25 g of the sample was dissolved with 225 mL of distilled water, stirred constantly for 30 to 45 min, and equilibrated at room temperature for 15 min. The clear upper part of the solution was used in titration.

3.5.4. Sample Preparation for Total Sugar Determination

Fifty milliliters of the dissolved sample solution (1:9) was mixed with 5 mL of concentrated HCl, and then put in a water bath at 65-57°C for 5 min. After cooling, the pH of the solution was adjusted to 7.0 using 0.1 to 1 N of NaOH and the volume was brought to 100 mL.

3.5.5. Determining the Invert Sugar and Total Sugar Content

The procedure mentioned in section 3.5.2 was followed in estimating the invert sugar and total sugar content of the samples with some changes: 15 mL standard solution was added; and instead of filling the burette with the standard solution, the sample solution was filled into it. The invert sugar and total sugar was calculated using the following formulas:

$$\textit{Invert sugar (g/100 mL of diluted sample)} = \frac{\textit{F (g) x dilution factor x 100}}{V_2\ (mL)}$$

where V_2 = volume of sample solution used in titration. The sucrose content can also be calculated:

$$Sucrose = (Total\ Sugar - Invert\ Sugar) \times 0.95$$

3.6. Oxygen Radical Absorbance Capacity ($ORAC_{FL}$)

3.6.1. Sample Preparation

Approximately 1 g each of autumnberry pureed, freeze-dried autumnberry, and autumnberry bread were analyzed for their antioxidant capacity.

3.6.2. Reagent and Standard Preparation

Fluorescein sodium salt, 2,2'-Azobis (2-amidionopropane) dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO). Black polystyrene, round bottom, assay plates with 8 x 12 wells (part# 3792) were obtained from Corning Incorporated (Corning, NY).

The ORAC assay was performed as described by Huang and others (2002) where 0.414 g AAPH was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) to obtain a final concentration of 153 mM. The AAPH was prepared fresh during the experiment. The fluorescein stock solution of 4×10^{-3} mM was prepared in 75 mM buffer (pH 7.4) and wrapped in foil and placed in refrigerator. The fluorescein stock solution was prepared every three months. Prior to analysis, a fluorescein working solution was made daily by diluting the fluorescein stock solution 1:1000 with 75 mM phospahate buffer (pH 7.4). The

Trolox standards were prepared by dissolving 0.25 g trolox to 500 mL of the 75 mM phosphate buffer (pH 7.4) to give a 1.89 x 10-3 M stock solution. The stock solution was diluted prior to each analysis with the same phosphate buffer to 6.25,12.5, 25, 50, and 100 µM working solutions.

3.6.3. Experimental Setup for $ORAC_{FL}$

The outer wells of the plates were filled with 300 µL of water, while the interior wells were used for experimental analyses. Into all experimental walls, 150 µL of working sodium fluorescein solution was added; to the blank wells, 25 µL of 75 mM phosphate buffer (pH 7.4) was added; to the standard wells, 25 µL of trolox dilutions were added; and to the sample wells, 25 µL of appropriate dilution were added. The plate was incubated for 30 min at 37°C in the FLx800 Multi-Detection Microplate Reader (Biotek Instruments, Winooski, VT) after which reactions were initiated by the addition of 25 µL of AAPH solution that was freshly prepared. The microplate reader was controlled by the Biotek Gen5 software where it was programmed to shake the microplate automatically for 10 s prior to each reading. Detection parameters were set at 485 ηm, 20 ηm bandpass, excitation filter and a 528 ηm, 20 ηm bandpass, emission filter. The fluorescence was monitored over time and recorded every 90 s.

3.6.4. Data Analysis of $ORAC_{FL}$

ORAC values were computed according to Cao and Prior (1999). The net area under the curve (AUC) of the standards and samples were calculated using the trapezoidal rule as shown below:

$$AUC = \left[\frac{R1}{2} + R2 + R3 + \ldots + R_{n-1} + \frac{Rn}{2} \right] \Delta t$$

Where R1 is the fluorescence reading at the initial time of the reaction and Rn is the final measurement of fluorescence. While Δt *is the time difference between each reading.*

The net AUC is determined by AUC_{sample} - AUC_{blank}.The standard curve was obtained by plotting the trolox concentrations against the net AUC of different trolox concentrations. The ORAC values of the samples could then be calculated automatically using the Biotek Gen5 software by interpolating the sample's net AUC against the trolox standard curve, with the dilution factor taken into account. Results are generally expressed as trolox equivalents (TE) as micromol of TE per gram or per liter of sample (μmol of TE/g OR μmol of TE/L).

3.7. Total Phenolics by Folin-Ciocalteau Colorimetry

The total phenolics assay used in this study followed the protocol written in Handbook of Food Analytical Chemistry by Wrolstad and others (2005).

3.7.1. Preparation of saturated sodium carbonate solution

Anhydrous sodium carbonate, 200 g, was dissolved in 800 mL distilled water and then boiled. After cooling, a few crystals of sodium carbonate were

added into the solution, and equilibrated for 24 h at room temperature. The solution was passed through Whatman no. 1 filter paper, brought up to 1 L using distilled water, and stored at room temperature until further use.

3.7.2. Preparation of Gallic acid standard solution

Gallic acid, 0.5 g, was dissolved in 10 mL ethanol and diluted to 100 mL with distilled water to make a 5-g/L-concentration solution. Standard solutions with concentrations of 12.5, 25, 50, 75, 100, 125, 250, and 500 mg/L, were made by diluting 0.25, 0.5, 1, 1.5, 2, 2.5, 5, and 10 mL to 100 mL with distilled water, respectively. These solutions could be kept for further use approximately for two weeks at 4°C with 98% potency retention.

3.7.3. Sample Preparation and Analysis of Total Phenolics

Approximately 0.5 g of the sample in the form of filtered pureed or freeze-dried solid was extracted using 50 mL of 80% methanol. The samples were put in an ultrasonic bath for 20 min, and then centrifuged (Sorvall RD-5B Refrigerated Superspeed Centrifuge by DuPont Instruments for 15 min at 7826*g*. One mL of sample aliquot or the standard solution was added to 25 mL volumetric flask containing 9 mL distilled water, and then one mL of Folin-Ciocalteau's phenol reagent 2 N was added into all mixture, then shaken, and equilibrated at room temperature for 5 min. Saturated sodium carbonate, 10 mL, was added to the mixture, diluted to 25 mL with distilled water, and incubated for 90 min at room temperature. After the incubation, the blue color developed.

The absorbance readings were measured using UV-visible spectrophotometer model Spectronic 21D (Milton Roy, Ivyland, PA) at 750 ηm wavelength. The standard curve was constructed using the absorbance data of the gallic acid standards versus gallic acid concentration. The linear regression equation form the standard curve was then used to calculate the concentration of total phenolics in the samples with taking into account the dilution factor used. The results were expressed as gallic acid equivalents (GAE) mg / mL sample.

3.8. Color Analysis

Color analysis on pureed and freeze-dried autumnberry, as well as on the fortified bread at all levels (control, 3%, 6%, 9% flour basis) was done using LabScan XE colorimeter which includes the EasyMatch QC software an electronic recordkeeping version that is 21 CFR 11 compliant (Hunter Lab, Reston, VA). The type of color test chosen was reflectance using Hunter Lab and CIE D65/10 (day light at 10^{o} angle). The pureed and freeze-dried samples were analyzed using 1.75" glass sample cup with 1.75" port opening, while the bread crust and crumb samples were directly put on top of the 1.75" post.

3.9. Titratable Acidity

AOAC Official Method 942.15 (2000) in fruit products was used to measure the titratable acidity of the pureed and freeze-dried autumnberry with some adjustments. Ten mL of the juice from pureed or the extracted juice from 10 g of freeze-dried sample was combined with 190 mL distilled water. The aliquot of 50

mL was titrated with 0.1 N NaOH to the end point of pH of 8.1 – 8.2 using phenolphthalein indicator. The acid content was expressed as percentage (w/w, wet weight).

3.10. Spectrophotometric Method for Lycopene Measurement

The low volume hexane extraction method (LVHEM) was used to measure the lycopene content of pureed and freeze-dried autumberry samples, and bread samples. Fish (2002) method on LVHEM was performed. Approximately 1 g (determined to the nearest 0.05 g) triplicate samples were weighed into the 50 mL - polypropylene tubes that contained 5 mL of 0.05% (v/v) butylated hydroxytoluene (BHT) in acetone, 5 mL of 95% HPLC grade ethanol, and 10 mL of hexane. The tubes were put in the sonicator, Bransonic 2510, (Branson Ultrasonic Corporation, Danbury, CT) for 20 minutes for homogenization. Samples were extracted on a gyratory water bath orbital shaker G76 (New Brunswick Scientific, Edison, NJ) at 180 rpm for 15 min on ice. After shaking, 3 mL of distilled water were added into each tube, and shaken for additional 5 min. The tubes were left on a stable surface at room temperature for 5 min to allow the phase separation. The upper hexane layer was transferred to the spectrophotometer cuvettes. The absorbance readings were measured using UV-visible spectrophotometer model Spectronic 21D (Milton Roy, Ivyland, PA) at 503 ηm wavelength blanked with hexane. The lycopene standard curve was obtained using pure lycopene from Wako Chemicals USA, Inc. (Richmond, VA).

3.11. Bread-making

A fifty-pound bag of Bread flour (Seal of Minnesota-Bakers Flour AD) containing bleached wheat flour, malted barley flour, and potassium bromate was obtained from ConAgra, Omaha, NE. The flour composition information provided by the manufacturer includes 14.83% protein, 82.37% carbohydrates, and 2.81% fat. Method 10-10B: optimized straight-dough bread-making method of American Association of Cereal Chemists International (AACCI) was used in making the autumnberry-fortified bread at different levels: control, 3%, 6%, and 9% fortification (flour basis).

Farinograph

A farinograph (C. W. Brabender Instrument, Inc., South Hackensack, NJ) was used to estimate the water absorption of the flour and measure dough characteristics of flour, i.e., development time, dough stability and softening. This information is important in optimizing the bread-making process.

The moisture content of the bread flour was determined using IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). The amount of flour used for the farinograph was determined using the following formula (for flour with 14% of moisture content or 86% of dry matter, 50 g of flour is needed for farinograph readings):

$$\text{Weight of flour for farinograph readings} = \frac{50 \times 86}{100 - \text{MC of flour}}$$

The amount of the freeze-dried autumnberry incorporated with the flour at 3%, 6%, and 9% levels were also adjusted to 14% moisture content using the following formula:

$$\text{Weight of freeze-dried sample incorporated} = \frac{\%\ \text{level of fortification} \times 86}{100 - \text{MC of freeze-dried sample}}$$

The weight of flour used for bread-baking (100 g flour basis) is two times the weight of flour used for farinograph. Based on the farinogram, two important pieces of information were obtained: (1) the water absorption of the flour (water needed for optimum dough forming) was estimated plus 2 mL of water, and (2) the mixing time of the dough to reach optimal stability. Distilled water was used throughout the process.

Baking Process

Method 10-10B: optimized straight-dough bread-making method of American Association of Cereal Chemists International (AACCI) was used to make the bread samples, with some modification. The formulation for the bread includes salt-sugar solution, ascorbic acid solution, and yeast suspension, while other ingredients listed in the method were not used in this particular experiment. The baking time was adjusted to 20 minutes for 400 – 425°F.

3.11.1. Bread Texture

The bread firmness was measured according to AACC Method 74-09 (AACC 2000) using a TA-XT2i Texture Analyser that includes Texture Expert-

Stable Micro Systems version 1.22 software (Texture Technologies Corp, Scarsdale, NY). A 50 mm diameter cylindrical probes, for 25% of compression; at a test speed of 1.0 mm/s was used in this firmness test. At least triplicates slices of bread (25 mm thickness each) cut from the center of the bread loaf were tested.

3.11.2. Bread Volume and Density

The volume and density of each bread samples were estimated using a volumeter filled with rape seeds. The density (ρ) was then calculated as mass over volume (*m/v*).

3.12. Statistical Analysis for the Physicochemical Properties

The physicochemical properties of autumnberry in two presentations (pureed and freeze-dried) were measured in three batches of product. The differences in physicochemical properties between the two presentations of autumnberry were the point of interest. Each batch of each presentation was analyzed repeatedly three times. For each physicochemical property of interest, a general linear mixed model was fitted using the MIXED procedure of SAS (SAS Institute Inc, Cary, NC). The statistical model included the fixed effect of presentation (pureed versus freeze-dried) and the random effect of batch. In addition, a random interaction between presentation and batch was fitted to the model to account for sub-sampling (technical replication) in the experimental design. Least square mean estimates (with standard errors) at each

presentation were also shown. Pertinent pairwise comparisons were performed using Tukey's adjustment to avoid inflation of Type I error rate. A likelihood ratio test (also to determine homogenous or heterogeneous variances applied) was estimated for each treatment to improve the model fit. Model assumptions were evaluated using residual plots and assumptions were considered to be appropriately met.

3.13. Sensory Tests and Evaluations

A preliminary consumer acceptability testing (n=52) using a 9-point hedonic scale (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, 9-like extremely) was conducted. The samples presented were bread containing 10% and 20% autumnberry pureed. This test was done to acquire consumer perception and acceptance on the attributes of autumnberry-containing bread such as aroma, color, appearance, flavor, body/texture, and overall acceptance. Some general survey-typed questions: "How do you like the idea of bread containing antioxidant lycopene?" "Would you purchase bread with health benefits over regular white bread?" "Please rank the three samples in order of preference for each attributes" were also asked during the test. The resulting data was analyzed using an ANOVA and Tukey's test.

A trained panel (n=12) was further conducted to evaluate the final samples of fortified bread (using freeze-dried autumnberry) at different levels (control, 3%, 6%, and 9%). Sensory characteristics of interest were crust color, crumb color,

firmness, crumb cell structure uniformity, intensity of yeasty flavor, and intensity of autumnberry flavor. The duration of the training session was eight weeks with additional three weeks for conducting triplicates descriptive tests using randomized samples, unstructured line scaling method (0-15, less to more). The trained panelists were asked to assess the samples' sensory characteristics, as trained. This test was repeated three times. Sensory scores were recorded for each treatment assessed by each panelist at each run, utilizing SIMS 2000 sensory software (Sensory Computer System, Morristown, NJ). The resulting data was further studied utilizing SAS statistical software (SAS Institute Inc, Cary, NC).

The trained panel utilized commercial breads as the references since there are no universal standard for bread characteristics. For each sensory attribute of interest, the references were set up in a 5-point increment. For the firmness attribute, the panelists were trained to bite the bread (excluding the crust) using their front teeth as their first judgment and then bring it back to the wisdom teeth for further evaluation. The yeasty flavor references were prepared using a bread mix with increased active dry yeast content: 0%, 5%, 10%, 15%, and 20% (w/w). Lastly, the references for autumnberry flavor were prepared using the autumnberry pureed and water mixture at 0%, 3%, 6%, and 9% (v/v).

3.14. Statistical Analysis for Sensory

An ANOVA and Tukey's test were applied to the preliminary consumer acceptability, or so called discrimination/acceptance test results. This type of test is simple, requiring untrained panelists, and commonly used method with results proven to be reliable. ANOVA was used to analyze interaction effects between variables, therefore, to test more complex hypotheses. Furthermore, this test was conducted to examine the significant difference between the sensory attributes of each sample. Some additional questions were also asked to the panelists to gain consumers' perception on the product idea, their preference among samples, as well as their purchasing habit.

Furthermore, for each sensory characteristic of interest, a general linear mixed model was fitted using the MIXED procedure of SAS. The model integrated the fixed effect of treatment at four levels: 0%, 3%, 6%, and 9% fortification, and the random effect of panelist. In addition, a random interaction between panelist and treatment was fitted to the model to account for sub-sampling in the experimental design. A likelihood ratio test (either homogenous or heterogeneous variances) was estimated for each treatment to improving the model fit. Residual plot and model assumptions for each characteristics of interest were evaluated and considered appropriately met. Least square mean estimates for levels of the fixed effects are provided. Pertinent pairwise comparisons were performed using Tukey's adjustment to avoid inflation of Type I error rate.

Additionally, Principal Component Analysis (PCA) on the trained panel sensory results was performed using XLStat (XLStat, New York, NY). PCA basically transforms a certain number of data points (variables) into a smaller number of principal components. PCA reveals the internal structure of the data in a way which best explains the relationship between data points with sensory attributes.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1. Statistical Analysis of the Physicochemical Properties of Pureed and Freeze-dried Autumnberry

The mixed model of SAS was considered to be valid if the residual plots for each physicochemical characteristic of interest were well distributed (cloud of points) without depicting a certain trend or shape, such as a fan-shape. In this case, the statistical analyses of all physicochemical attribute of interest were valid. In addition, there was no significant difference among the three batches of pureed autumnberry in term of all physicochemical properties of interest. There was also no significant difference among the three batches of freeze-dried autumnberry examined.

4.1.1. CIE L*a*b* Color

The CIE L*a*b* is 3-dimensional color space specified by the International Commission on Illumination (*Commission Internationale d'Eclairage*–CIE). CIE L*a*b* characterizes all color visible to the human eye and was created to serve as a tool independent model to be used as a reference. It is the color scale used as a universal standard and uniform color scale so that the color values or measurements could be easily compared. L* = 100 represents a perfect reflecting diffuser, thus, the color appears to be white, while L* = 0 represents black. The a* and b* axes have no precise numerical limits whether the value is negative or positive. The negative a* and positive a*correspond to green and

red, respectively, while the negative b* is blue and positive b* is yellow (HunterLab 2008).

A significant effect of presentation of autumnberry was identified on color L* ($P<0.0001$), such that color L* was greater in freeze-dried samples compared to pureed. This means the color of the freeze-dried samples was perceived to be lighter than that of the pureed ones. On the CIE color a* attribute, a significant effect of presentation of autumnberry was identified on color a* ($P<0.0001$), such that color a* was greater in pureed sample compared to freeze-dried. In this case, the pureed samples had more red color perception than the freeze-dried counterpart. A significant effect of presentation of autumnberry was identified on color b* ($P<0.0001$), such that color b* was greater in pureed compared to freeze-dried. This means that the pureed impacts more yellow color perception than the freeze-dried sample (Figure 4.1.1.1.).

Although freeze-drying is considered to be the best drying method to preserve natural color of fruits, the significant loss of moisture in the freeze-dried sample changes the surface characteristics of the fruit (smooth and porous) and alters its light reflectivity, hence, it is perceived to be lighter in color. During storage, the open porous texture of the freeze-dried sample also allows oxygen to enter and cause oxidative deterioration of lipids, which overtime can gradually diminish the color of freeze-dried fruits (Fellows 2000). In addition, based on the observation, sugar in autumnberry tended to crystallize after freeze-drying, forming a thin white layer on the surface of the autumnberry slab. This also

contributed to the perceived lighter color of the freeze-dried autumnberry compared to the pureed sample.

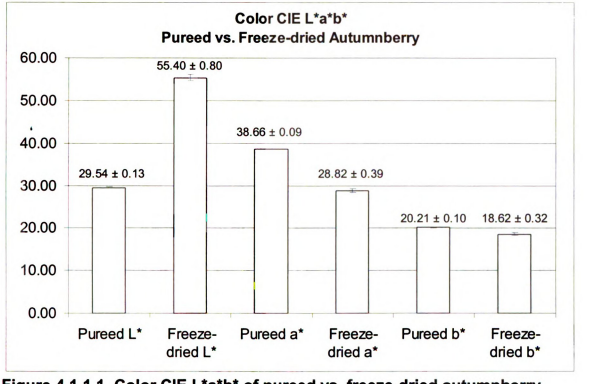


Figure 4.1.1.1. Color CIE L*a*b* of pureed vs. freeze-dried autumnberry.

4.1.2. Acid Content

The contents of four major acids commonly found in berries: citric, lactic, acetic, and malic acids were analyzed in both pureed and freeze-dried autumnberry. A significant effect of presentation of autumnberry was identified for the citric, lactic, and acetic acid content ($P<0.0003$), such that pureed samples had greater concentration of citric acid than freeze-dried counterpart. A significant effect of presentation of autumnberry was also identified ($P<0.0001$)

such that the pureed autumnberry had greater concentration of malic acid than freeze-dried autumnberry (Figure 4.1.2.1.).

The predominant organic acids in berries such as citric and malic acid, with the compliment of phenolic acids, are responsible for the titratable acidity of fruits. Titratable acidity is considered as a better overall indicator of fruit quality, whereas the pH is often a poor marker of fruit quality characteristics (Nielsen 2003). In case of autumnberry, its acidity is comparable to blueberries and blackberries, but somewhat more astringent, with slightly sweet notes (Tanaka 1976; Parmar and Kaushal 1982). The titratable acidity for citric acid (the most predominant organic acid in berry fruits) of fresh blueberries comparing to the experimental pureed autumnberries from this study is ranging from 0.54 to 1.13 and 0.41 to 0.44, respectively (Zhao 2007). The concentration of organic acids present in fruits is also crucial for fruits preservation, for example, maintaining a low pH in processed fruits such as in jams and jellies. Additionally, different acids own various levels of effectiveness in lowering heat resistant of microorganisms: lactic acid > citric acid > acetic acid (Ranganna 1986).

The air containing free-radicals could easily penetrate the smooth and porous texture of freeze-dried autumnberry during the grinding process from slab into powder. The porous structure and the larger surface area of freeze-dried autumnberry powder further facilitated oxidation during experiment. When the oxidation took place, these organic acids acted as free-radical quenchers, thus they degraded overtime. Organic acid degradation might also have occurred

during storage especially due to storage temperature fluctuation and/or improper packaging materials (Fellows 2000). These factors may have caused the significant differences in organic acid contents between the pureed and freeze-dried autumnberry with freeze-dried autumnberry having lower organic acids compared to the pureed counterpart.

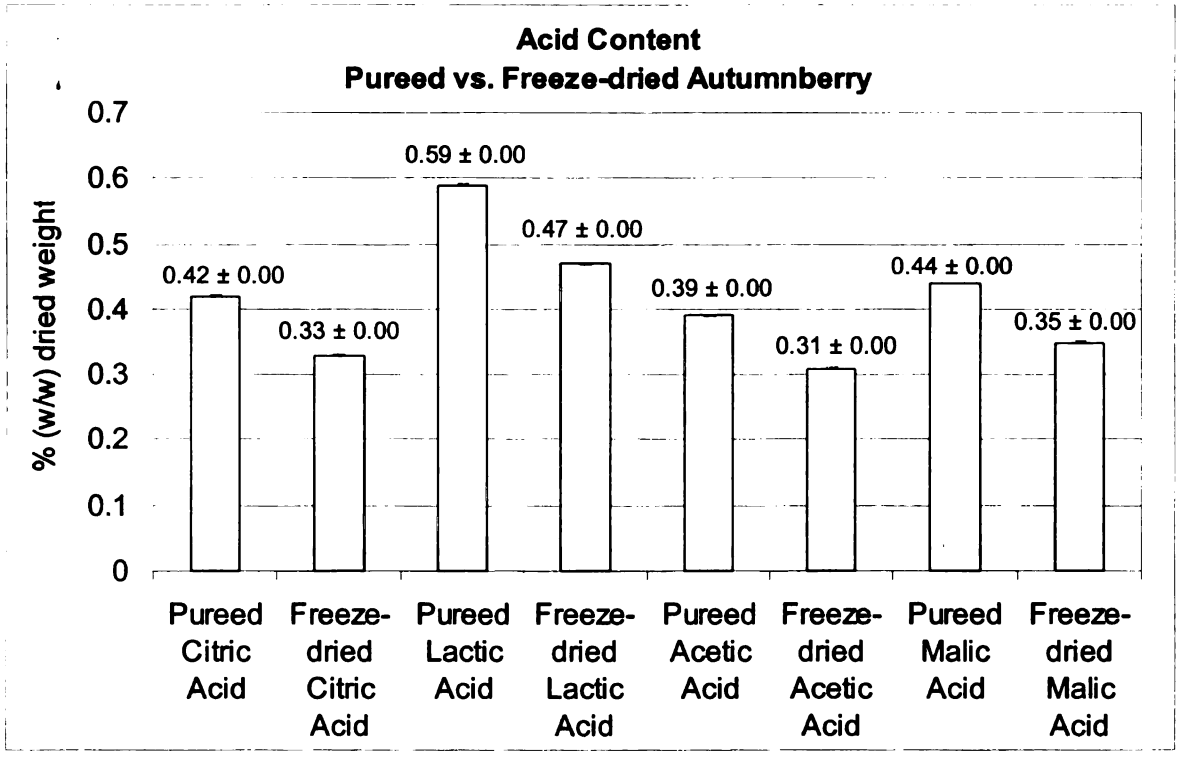


Figure 4.1.2.1. Acid contents of pureed vs. freeze-dried autumnberry in dried weight basis.

4.1.3. Sugar Content

High sugars and high acids are essential for desirable berry flavor. The acidity from predominant organic acids in berry fruits is counterbalanced by the sugar content (Kader 1991). Invert sugar, total sugar, and sucrose contents of pureed and freeze-dried autumnberry were analyzed. Invert sugar is a mixture of

equal parts of glucose and fructose resulting from the hydrolysis of sucrose, which is achieved through the action of invertase or a concentrated acid. It is found naturally in fruits and honey. Invert sugar is also produced artificially for use in the food industry because it is sweeter than sucrose and it also has lower tendency to crystallize (Damodaran and others 2008).

According to sugar content analysis on autumnberry in this study, there were no significant differences between the pureed and freeze-dried autumnberry in term of invert sugar ($P = 0.36$), total sugar ($P = 0.64$), and sucrose ($P = 0.13$) (Figure 4.1.3.1.). Thus, the freeze-drying process did not change the sugar content of autumnberry. The sugar content of autumnberry is higher but still comparable to that of the other berries claimed to have similar sweetness/sourness. The sucrose and total sugar contents of autumnberry, blueberry, and blackberry (g/100g of wet weight) were as follows: 1 and 15, 0.11 and 9.96, and 0.07 and 4.88 (USDA National Nutrient Database for Standard Reference 2006).

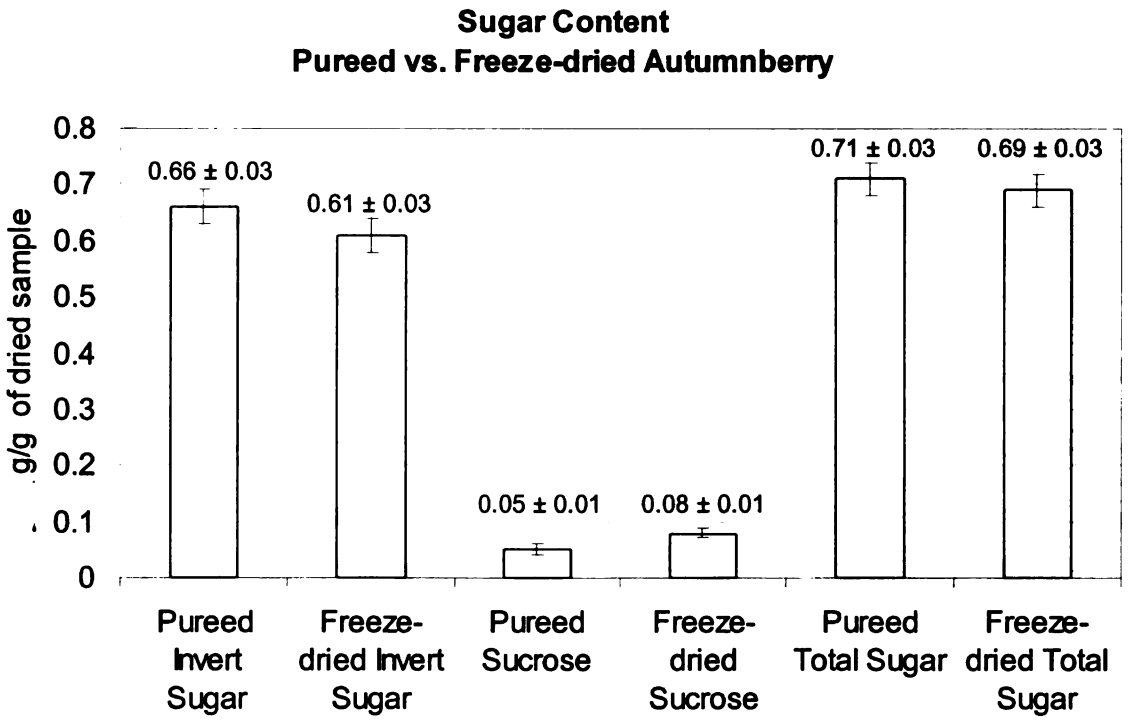


Figure 4.1.3.1. Sugar contents of pureed vs. freeze-dried autumnberry in dried weight basis.

4.1.4. Moisture Content

On the moisture content attribute, a significant effect of presentation of autumnberry was identified on moisture content ($P<0.0001$), such that pureed samples (80.30%) had greater moisture content than freeze-dried samples (2.32%). Freeze-drying decreased a great deal of water content in autumnbery puree which is a favorable condition to inhibit any potential action(s) of microorganisms and enzyme that would spoil or degrade the fruit faster (Welti-Chanes and Hui 2007).

4.1.5. Water Activity (Aw)

Water activity of the pureed and freeze-dried autumnberry samples from each batch was also measured using Water Activity Meter: AquaLab Series 3 (Decagon, Pullman, WA). There was a statistical significant difference between the pureed autumnberry (0.97 ± 0.00) and freeze-dried autumnberry (0.13 ± 0.01). The water activity of the pureed presentation ranged from 0.964 – 0.991 ± 0.009, which showed that the pureed form, provided an environment for bacteria and certain types of yeast to grow. Freeze-drying was able to lower the water activity to Aw ranging from 0.088 – 0.164 ± 0.033 (Table 4.1.5.1) (Damodaran and others 2008).

Table 4.1.5.1. Water activity of autumnberry pureed and freeze-dried

Presentation	Water Activity (Aw)	Average	Standard Deviation
Pureed Batch 1	0.983	0.975	0.009
Pureed Batch 1	0.975		
Pureed Batch 1	0.971		
Pureed Batch 2	0.965		
Pureed Batch 2	0.966		
Pureed Batch 2	0.964		
Pureed Batch 3	0.991		
Pureed Batch 3	0.981		
Pureed Batch 3	0.975		
Freeze-dried Batch 1	0.091	0.133	0.033
Freeze-dried Batch 1	0.089		
Freeze-dried Batch 1	0.088		
Freeze-dried Batch 2	0.142		
Freeze-dried Batch 2	0.163		
Freeze-dried Batch 2	0.145		
Freeze-dried Batch 3	0.156		
Freeze-dried Batch 3	0.157		
Freeze-dried Batch 3	0.164		

4.1.6. Total Phenolics

On the total phenolics content, no significant difference was detected between the freeze-dried samples (6.73 ± 0.34 mg GAE/g of dried sample) and the pureed (7.32 ± 0.34 mg GAE/g of dried sample) counterpart on the total phenolics attribute (P = 0.31). Total phenolic acids can impart bitter or astringent flavors in most of berry fruits. Together with other predominant organic acids, phenolic acids contribute to the basic taste components of most of berries (Zhao 2007). The total phenolics content of blueberry and blackberry were 5.31 and 6.60 mg GAE/g of wet weight, respectively (Zheng and Wang 2003; Wu and others 2004). The average total phenolics content of the experimental autumnberry in this study was found to be 1.44 mg GAE/g of wet weight, which was less compared to that of blueberry and blackberry.

4.1.7. ORAC (Total Antioxidant)

For total antioxidant (ORAC) content, a significant effect of presentation of autumnberry was identified on total antioxidant content (P<0.0001), such that the freeze-dried sample (102.36 ± 3.27 μmol TE/g dried sample) had lower ORAC values than those of pureed sample (144.14 ± 4.86 μmol TE/g dried sample). In this experiment, the freeze-drying process notably degraded the total antioxidant in autumnberry.

Literature reported the ORAC value of blueberry, blackberry, cranberry, cherry, raspberry, and strawberry as follows: 61.84, 52.45, 92.56, 33.44, 47.65,

and 35.41 μmol TE/g wet weight, respectively (Zheng and Wang 2003; Wu and others 2003). While the average ORAC value of the experimental autumnberry was 28.43 μmol TE/g wet weight. According to this data, autumnberry is relatively comparable to cherry and strawberry in term of total antioxidant capacity.

Antioxidants in general are sensitive to heat and oxidation. Although most berries contain only small amounts of lipids, the oxidation (incorporation of air into the porous structure of freeze-dried fruits) of unsaturated fatty acids in the fruits produces hydroperoxides, which react further by oxidation to produce aldehydes, ketones, and acids, and eventually cause rancidity and bad odor. Antioxidants in the berries undergo auto-oxidation to slow down this process (Fellows 2000). The highly unsaturated structure makes it easier for antioxidant lycopene to be oxidized. This explains why the total antioxidant capacity after freeze-drying (including storage time) was lower than that of the original pureed autumnberry.

4.1.8. Lycopene Content

A standard curve of lycopene was constructed as a reference for determining the lycopene content in the pureed and freeze-dried autumnberry, and bread samples (Figure 4.1.8.1.). Lycopene has UV-visible light absorption spectrum characteristics due to the presence of conjugated double bonds of its hydrocarbon chain (polyene). This means the positions of the bands of maximum light absorption (λ_{max}) area are a function of the number of conjugated

double bonds in the molecule. The lipophilic lycopene has UV-vis absorption maxima ranging from 446 to 503 ηm in low polarity solvents such as hexane. Thus, for lycopene content determination, the samples absorbance readings were taken at 503 ηm UV-vis (Minguez-Mosquera 2002).

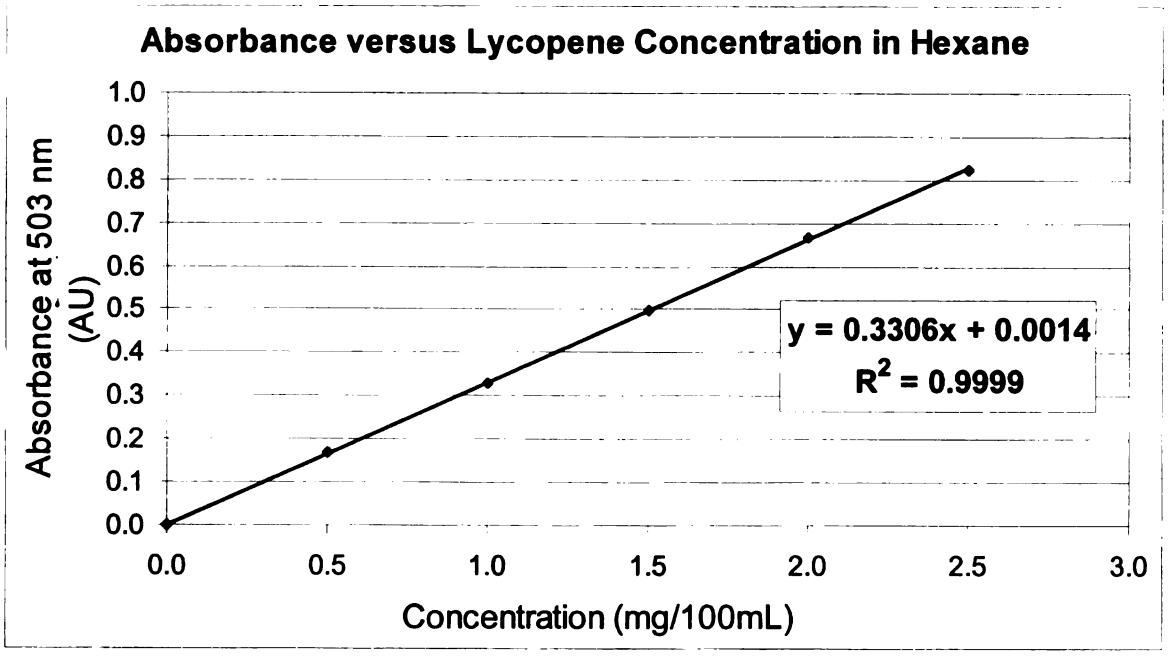


Figure 4.1.8.1. Lycopene standard curve using the spectrophotometric method.

For lycopene content, a significant effect of presentation of autumnberry was identified on lycopene content ($P<0.0001$), such that pureed sample (2.90 ± 0.04 mg/g dried sample) contained higher lycopene than those of freeze-dried (0.70 ± 0.00 mg/g dried sample) (Table 4.1.8.1.). The average degradation of lycopene after freeze-drying in this study was 75.86%, which was considerably high compared to the degradation of lycopene from tomato after freeze-drying: 20-40%. In addition, the tomato's lycopene degradation was higher in freeze-dried tomato samples compared with oven-dried samples between 25 and 75°C.

This loss also increased with the exposure of tomato solids to air, light, and high temperature during storage (Sharma and Maguer 1996; Nguyen and Schwartz 1998). According to this experiment, the lycopene in autumnberry is less stable compared to lycopene in tomato after freeze-drying.

Table 4.1.8.1. Summary of lycopene content of pureed and freeze-dried autumnberry *(P = pureed, FD = Freeze-dried, number in the sample ID represents the batch number)*

Sample ID	Run	Absorbance at 503 nm (AU)	Lycopene Content (mg/g wet weight)	Lycopene Content (mg/g of dried sample)	Average	Standard Deviation
P1	1	1.710	0.5168	2.627	2.90	0.1287
P2	1	1.888	0.5707	2.901		
P3	1	1.900	0.5743	2.890		
P1	2	1.889	0.5710	2.897		
P2	2	1.998	0.6039	3.072		
P3	2	1.885	0.5698	2.876		
P1	3	1.880	0.5682	2.857		
P2	3	1.880	0.5682	2.889		
P3	3	1.980	0.5985	3.061		
FD1	1	2.301	0.6956	0.711	0.70	0.0133
FD2	1	2.222	0.6716	0.687		
FD3	1	2.301	0.6956	0.713		
FD1	2	2.222	0.6717	0.688		
FD2	2	2.301	0.6956	0.711		
FD3	2	2.301	0.6956	0.713		
FD1	3	2.301	0.6956	0.713		
FD2	3	2.222	0.6717	0.688		
FD3	3	2.222	0.6717	0.687		

There are insufficient and inconsistent (no clear trend) data on the effect of freeze-drying on carotenoids, including lycopene. Some of the examples: ethanol extracts of tomato skins contained more lycopene than freeze-dried skins (Inakuma and others 1998); frozen or boiled soybeans had a higher lutein and

beta-carotene content than freeze-dried beans (Simonne and others 2000); freeze-drying preserved more carotenoids from daylily (*Hemerocallis disticha*) flowers than air-drying (Tai and Chen 2000); freeze-drying also preserved more carotenoids in eight Malaysian medicinal plants than oven-drying at 50°C for 9 h or at 70°C for 1 h. No solid conclusions can be drawn from these varied studies. It seems that freeze-drying can have a negative effect on carotenoid preservation (Jones 1979). In the case for autumnberry in this study, freeze-drying retained only 24.14% of lycopene from its original pureed form.

The comparisons of average lycopene content of autumnberry vs. other fruits and tomato products are shown in Figure 4.1.8.2. (Bramley 2000 and Boileau 2002). The average lycopene content of the experimental autumnberry was 57.13 ± 2.45 mg/100g wet weight. The literature based lycopene content of autumnberry ranged from 30 to 70 mg/100g wet weight. Compared to the lycopene content of other selected fruits and tomato products, experimental autumnberry had the highest lycopene content. The spectrophotometric method, however, is not useful to differentiate the *trans* and *cis* lycopene, thus, the lycopene content estimated in this study was the overall lycopene including both *trans* and *cis* isomers.

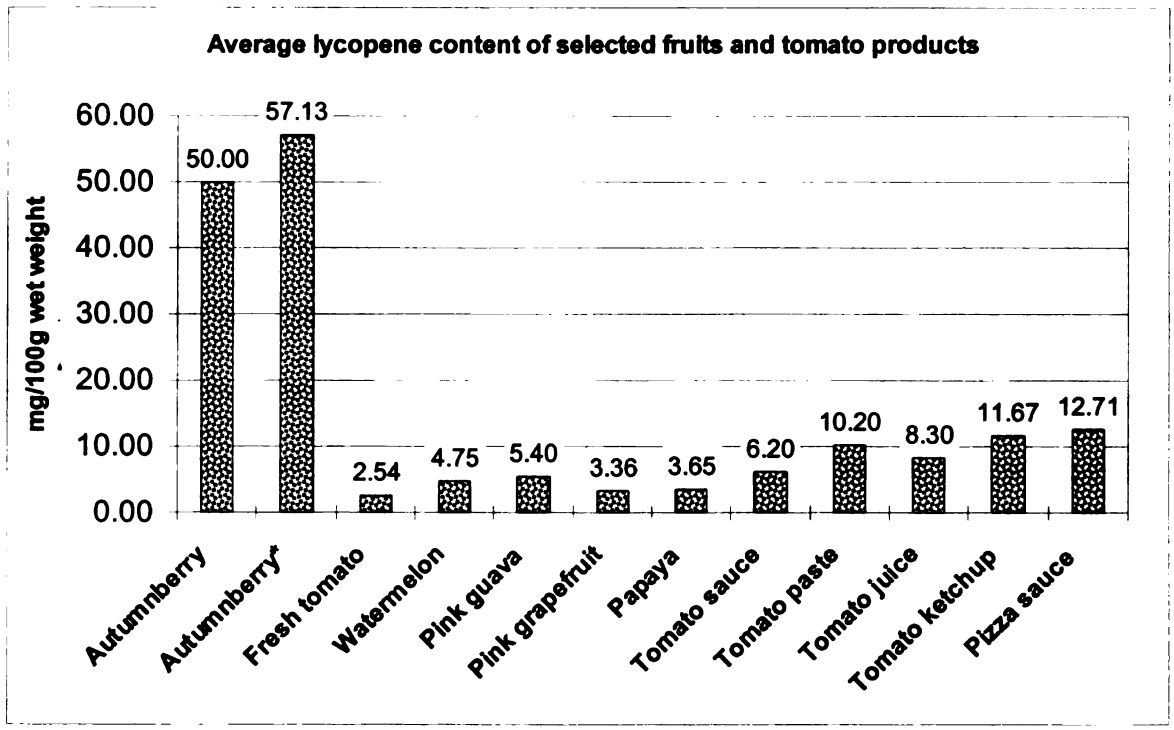


Figure 4.1.8.2. Average lycopene content of selected fruits and tomato products (Bramley 2000 and Boileau 2002).
- Autumnberry: average lycopene content – literature based.
- Autumnberry*: average lycopene content – experimental based.

4.1.9 Conclusions

In term of color, the freeze-dried format had lighter color, less intense red color, and about the same blue and green color perception compared to pureed format. The pureed autumnberry had more citric, lactic, acetic, and malic acid content compared to freeze-dried counterpart. In term of sugar content, both pureed and freeze-dried autumnberry contained the same level of invert sugar, total sugar, and sucrose as compared to the pureed format. The freeze-drying brought down the high moisture content in the pureed to a level where the water activity was very low. Thus, freeze drying preserved the autumnberry from enzymatic activity as well as from mold, yeast, and bacteria growth, and also

made the sample more dry, concentrated, and convenient for storage and future applications.

Total phenolics content for both pureed and freeze-dried presentations were not significantly different, meaning that the freeze-drying process had no or a little effect on total phenolics of autumnberry. On the other hand, total antioxidant capacity and lycopene content of the pureed autumnberry were higher than the freeze-dried counterpart. According to the statistical analyses, there were no significant differences between batches of pureed and freeze-dried samples for all physicochemical properties examined. Thus, the freeze-dried sample from all batches could be used for bread-making. The summary table of the physicochemical properties raw data is provided in Table A.2..

4.2. Bread Analyses

4.2.1. Bread Physical Analysis

For the bread physical analysis, the samples were analyzed for their weight/loaf, loaf volume, density, and firmness. Table 4.2.1.1. provides the results summary of the bread physical analysis. Figure 4.2.1.1., 4.2.1.2., 4.2.1.3., and 4.2.1.4. represent the typical results of the bread firmness (texture analysis) for the bread control, 3% fortified bread, 6% fortified bread, and 9% fortified bread samples, respectively.

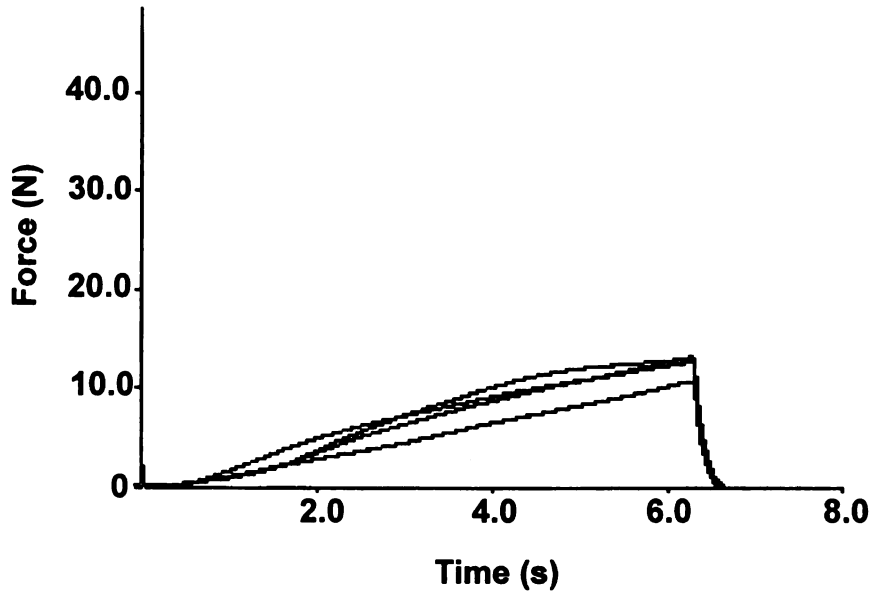


Figure 4.2.1.1. Typical results of the control bread texture analysis.

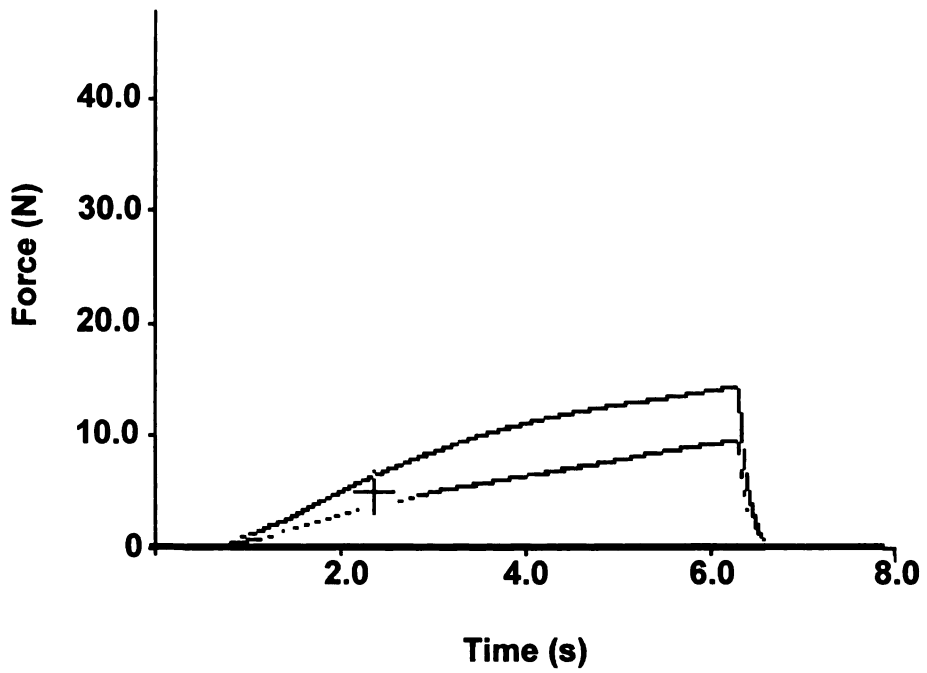


Figure 4.2.1.2. Typical results of the 3% fortified-bread texture analysis.

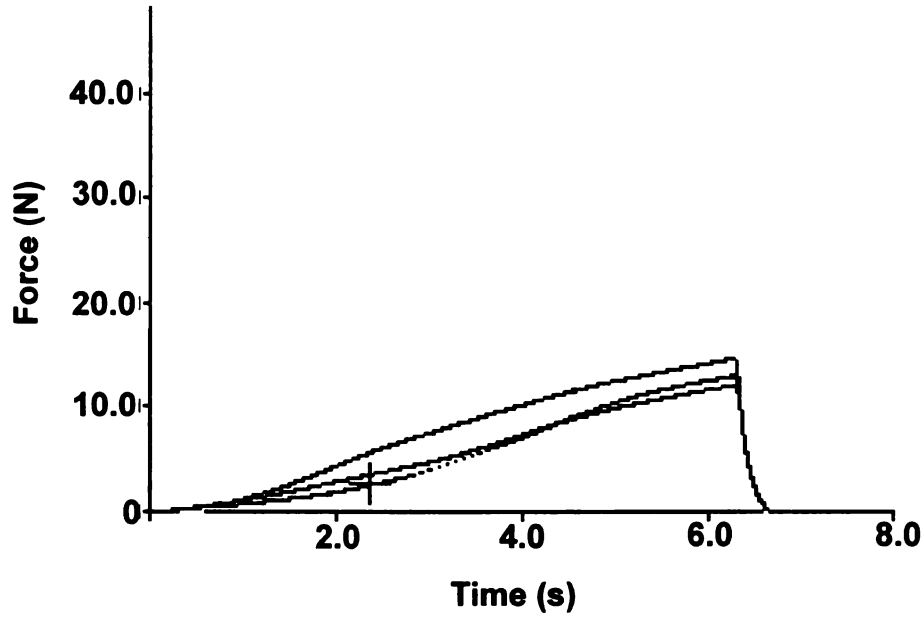


Figure 4.2.1.3. Typical results of the 6% fortified-bread texture analysis.

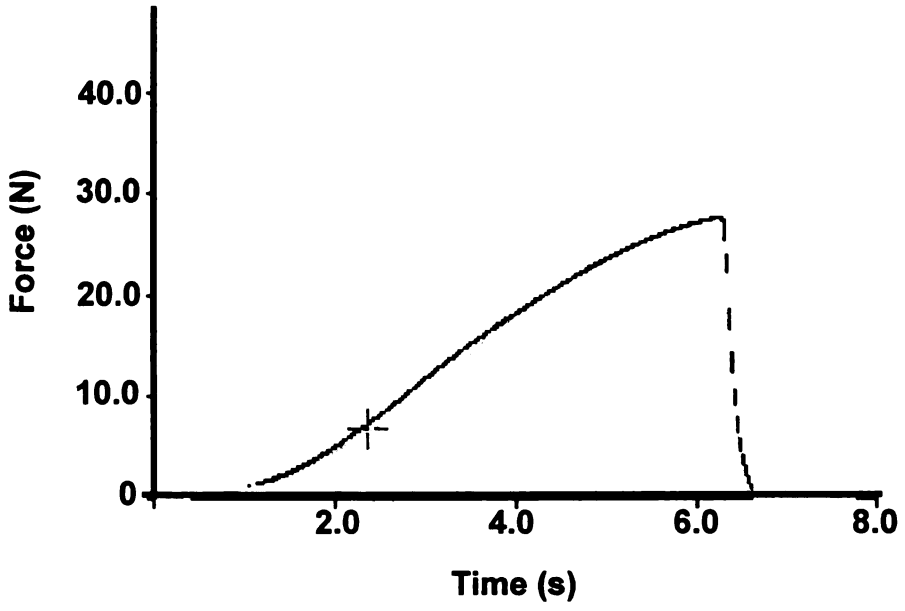


Figure 4.2.1.4. Typical results of the 9% fortified-bread texture analysis.

Table 4.2.1.1. Summary table of the physical analyses of bread samples

Bread Sample	Weight/loaf (g)	Average	Standard Deviation	Volume (cc)	Average	Standard Deviation	Density (g/cc)	Average	Standard Deviation	Peak Force (N)	Average	Standard Deviation
0%	138.90	138.40	0.50	760.00	771.70	10.41	0.18	0.18	0.00	9.76	10.64	0.78
0%	137.90			780.00			0.18			10.92		
0%	138.30			775.00			0.18			11.25		
3%	139.90	139.20	0.95	775.00	745.00	32.79	0.18	0.19	0.00	12.57	12.37	0.84
3%	138.10			750.00			0.18			13.10		
3%	139.50			710.00			0.20			11.46		
6%	141.90	141.90	0.75	625.00	631.70	7.638	0.23	0.22	0.00	13.39	14.29	0.79
6%	141.20			630.00			0.22			14.81		
6%	142.70			640.00			0.22			14.68		
9%	145.20	145.20	0.65	550.00	545.00	5.000	0.26	0.27	0.00	27.86	27.22	1.18
9%	144.60			540.00			0.27			27.94		
9%	145.90			545.00			0.27			25.85		

The average weight of a loaf of control bread, 3%-fortified, 6%-fortified, 9%-fortified was 138.4g, 139.2g, 141.9g, and 145.2g respectively. The weight of the bread increased as the bread was fortified with higher level of freeze-dried autumnberry. The same trend applied to the bread density (from 0.1793 g/cc to 0.2667 g/cc). The peak force reflected the firmness of the bread samples. The larger the peak force meant that the more force was needed to compress the bread, thus, the firmer texture. In this case, the higher the level of autumnberry fortification, the firmer the bread texture (10.64 to 27.22 N). The volume of the bread loaf, however, was decreasing at higher level of fortification from 771.7 cc (control) to 545.0 cc (9%-fortified). The 9%-fortified bread had the heaviest weight, smaller volume, higher density, hence, firmest texture. The 3%-fortified bread was the closest to the control in term of its physical attributes.

The bread dough fermentation was facilitated by active dry yeast. The pH of the bread dough went down due to the addition of acidic freeze-dried autumnberry. This elevated acidity disturbed the protein matrix in the flour, which lessened the ability of the protein for trapping the CO_2 produced by the yeast fermentation that responsible for leavening the bread. In addition, berries also contain high in fiber which also changed the protein network/structure, and as a result decreased the CO_2 entrapment by the protein to leaven the bread (Zhao 2007). The additional sugar from the freeze-dried autumnberry retarded the fermentation process. In ideal condition, low levels of sugar act as food for yeast and sugar normally speeds up the fermentation. However, at the elevated sugar levels, the osmotic pressure exerted by the sugar slows down the yeast

fermentation. (Hui, Corke, and others 2006). Thus, less CO_2 was produced to leaven the bread, which resulted in lower bread volume in higher fortification of freeze-dried autumnberry. This also explained the big pocket holes in the bread control (less dense) and smaller pocket holes in the 9% fortified bread (denser).

4.2.2. Bread Physicochemical Analyses

4.2.2.1. Bread Moisture Content and Water Activity

Table 4.2.2.1.1. Summary Table of Bread Moisture Content and Water Activity

Bread Sample	Water Activity (Aw)	Average	Standard Deviation	Moisture Content (%)	Average	Standard Deviation
0%	0.94	0.92	0.02	4.13	4.10	0.09
0%	0.91			4.17		
0%	0.91			4.00		
3%	0.95	0.94	0.01	4.08	4.12	0.05
3%	0.94			4.17		
3%	0.93			4.11		
6%	0.95	0.93	0.01	3.97	4.04	0.08
6%	0.93			4.12		
6%	0.93			4.01		
9%	0.95	0.95	0.00	4.02	4.04	0.05
9%	0.95			4.11		
9%	0.95			4.01		

The water activity of the bread with freeze-dried autumnberry fortification did not change significantly compared to the bread control. The fortified bread had slightly elevated water activity. The moisture content of the fortified bread samples, however, was slightly lower than the moisture content compared to the control. In conclusion, the water activity and the moisture content of the fortified bread were comparable to the control bread.

4.2.2.2. Bread ORAC-value (Total Antioxidant Capacity)

Table 4.2.2.2.1. Summary of ORAC-values of the bread samples in dried weight basis (after baking)

Bread Sample ID	Run	ORAC-value in dry matter (μmol TE/g)	Average	Standard Deviation
Control	1	0.16	0.19	0.03
Control	2	0.21		
Control	3	0.19		
3%	1	1.43	1.36	0.14
3%	2	1.46		
3%	3	1.19		
6%	1	2.17	2.13	0.14
6%	2	1.97		
6%	3	2.24		
9%	1	2.62	3.45	0.74
9%	2	4.05		
9%	3	3.68		

On average, the bread samples (average of 140g/loaf) contained increasing total antioxidant capacity (in dried weight) of 190.40 μmol TE, 298.20 μmql TE, and 483.00 μmol TE of for 3%, 6%, and 9% fortified bread samples, respectively. The bread control contained very low amount of total antioxidant of 26.6 μmol TE/140g of bread (Table 4.2.2.2.1.).

Initially, a loaf of 3%, 6%, and 9% fortified-bread (average of 140g/loaf) consisted of 2.64 g, 5.28 g, and 7.92 g of freeze-dried autumnberry, respectively. Since on average, the freeze-dried autumnberry contained 102.36 μmol TE/g of freeze-dried autumnberry in dried basis (Section 4.1.7.), thus, before the baking process, these loaves of bread had approximately 270.23, 540.46, and 810.69

μmol TE of antioxidant capacity for 3%, 6%, and 9% fortified bread. Comparing the total antioxidant content before and after baking process, the fortified bread samples 3%, 6%, and 9% only retained 70.46%, 55.18%, and 59.58% of total antioxidant capacity.

4.2.2.3. Bread Lycopene Content

Table 4.2.2.3.1. Summary of lycopene content of the bread samples

Sample ID	Run	Lycopene Content (mg/g of dried sample)	Average	Standard Deviation
B Control	1	0.000	0.00000	0.0000
B Control	2	0.000		
B Control	3	0.000		
B 3%	1	0.001451	0.0013	0.0002
B 3%	2	0.001452		
B 3%	3	0.001136		
B 6%	1	0.002079	0.0022	0.0002
B 6%	2	0.002082		
B 6%	3	0.002395		
B 9%	1	0.003025	0.0030	0.0003
B 9%	2	0.002713		
B 9%	3	0.003340		

The average weight of a loaf of bread was 140 g. The bread samples contained 0.18 mg lycopene/140g bread, 0.31 mg lycopene/140g bread, and 0.42 mg lycopene/140g bread for 3%, 6%, and 9% fortified bread samples respectively (Table 4.2.2.3.1.). The bread control, however, did not contain any lycopene.

A loaf of 3%, 6%, and 9% fortified-bread (average of 140g/loaf) consisted of 2.64 g, 5.28 g, and 7.92 g of freeze-dried autumnberry, respectively, before

baking. Since in average, the freeze-dried autumnberry contained 0.7 mg of lycopene/g of freeze-dried autumnberry in dried basis, thus, before the baking process, these loaves of bread had approximately 1.85 mg, 3.70 mg, and 5.54 mg of lycopene. Comparing the lycopene content before and after baking process, the fortified bread samples 3%, 6%, and 9% fortified bread only retained 9.73%, 8.38%, and 7.58% of lycopene.

4.2.3. Conclusions

The fortified bread samples were comparable to the control in term of water activity and moisture content attributes. The total antioxidant and the lycopene content of bread sample with higher level of fortification were higher, thus, had higher nutraceutical value. There was also a similar pattern that the lower the degree of fortification, the better the retention of both total antioxidant capacity and lycopene.

4.3. Sensory Results and Analyses

4.3.1. Consumer Acceptability Panel

The raw data of the consumer acceptability test was attached in the Table A.1.. Refer to Table A.1.1 to Table A.1.6. for the ANOVA test summary of each sensory attribute. The Tukey's test summary is presented in table 4.3.1.1 below.

Table 4.3.1.1. Tukey's test summary for consumer acceptability test

	Aroma	Color	Appearance	Body Texture	Flavor	Overall Acceptance
Standard Error of Mean (SEM)	0.12	0.20	0.16	0.15	0.17	0.15
Least Significant Difference (LSD)	0.35	0.55	0.46	0.41	0.47	0.43
Mean Differences						
Control vs. 10%	0.23	0.52	0.79	0.12	0.19	0.25
Control vs. 20%	0.70	1.33	1.19	0.21	0.27	0.79
10% vs. 20%	0.47	0.81	0.40	0.10	0.46	0.54

Significant differences were perceived between control and 20% samples, and also between 10 and 20% samples in term of aroma, color, and overall acceptance, while no significant differences were detected between control and 10% samples. Significant differences in term of aroma, color, and overall acceptance were detected in 10 and 20% fortification samples. On the other hand, no significant differences were detected in body/texture and flavor among all three treatments. In terms of appearance, the control appeared to be significantly different compared to the other two samples, while 10 and 20% samples were not significantly different from each other (Figure 4.3.1.1.).

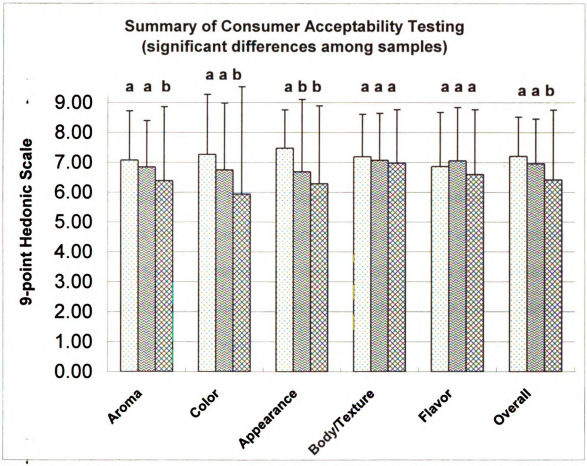


Figure 4.3.1.1. Summary of consumer acceptability test for control, 10%, and 20% fortification (flour basis) using autumnberry pureed for each sensory attribute.

As of the results of this preliminary sensory study show, panelists had higher acceptance toward the 10% over the 20% bread sample. The aroma for 20% bread was significantly low compared to the 10% and control samples. The 10% bread sample was the most preferable sample in terms of flavor compared to other samples. Ninety percent of the panelists liked the idea of bread containing antioxidants/functional ingredient. Seventy-seven percent of them would buy breads with health benefits versus regular white bread if they were available in the market. They liked the appearance, body texture, and overall acceptance of the autumnberry bread compared to the control. In conclusion,

panelists liked the idea of autumnberry-fortified bread (high lycopene bread). They also liked the bread sample that had subtle, moderate levels of autumnberry fortification, which they thought had closer sensory profile to regular white bread that they are used to consume on a regular basis.

4.3.2. Statistical Analysis for Trained Panel / Descriptive Analysis

Residual plots for each sensory characteristics of interest were examined for the outliers and shape. The respond values distribution on the residual plot should be well distributed (cloud of points) without depicting a certain trend or shape, i.e. a fan-shaped, to be valid.

4.3.2.1. Crust Color

On the crust color attribute, heterogeneous variances were estimated for each treatment. A significant effect of freeze-dried autumnberry fortification on the perception of crust color of bread samples was identified ($P<0.0001$), such that as the levels of freeze-dried autumnberry fortification increased from 0% to 6%, the greater the crust color scorings were recorded (Figure 4.3.2.1.). This means that as the level of fortification increased, the darker the crust color of the bread samples were (light golden brown to dark brown). However, no difference in crust color was identified between 6% and 9% bread samples fortification. The effect of supplementation on crust color scoring was additive and linear ($P<0.0001$).

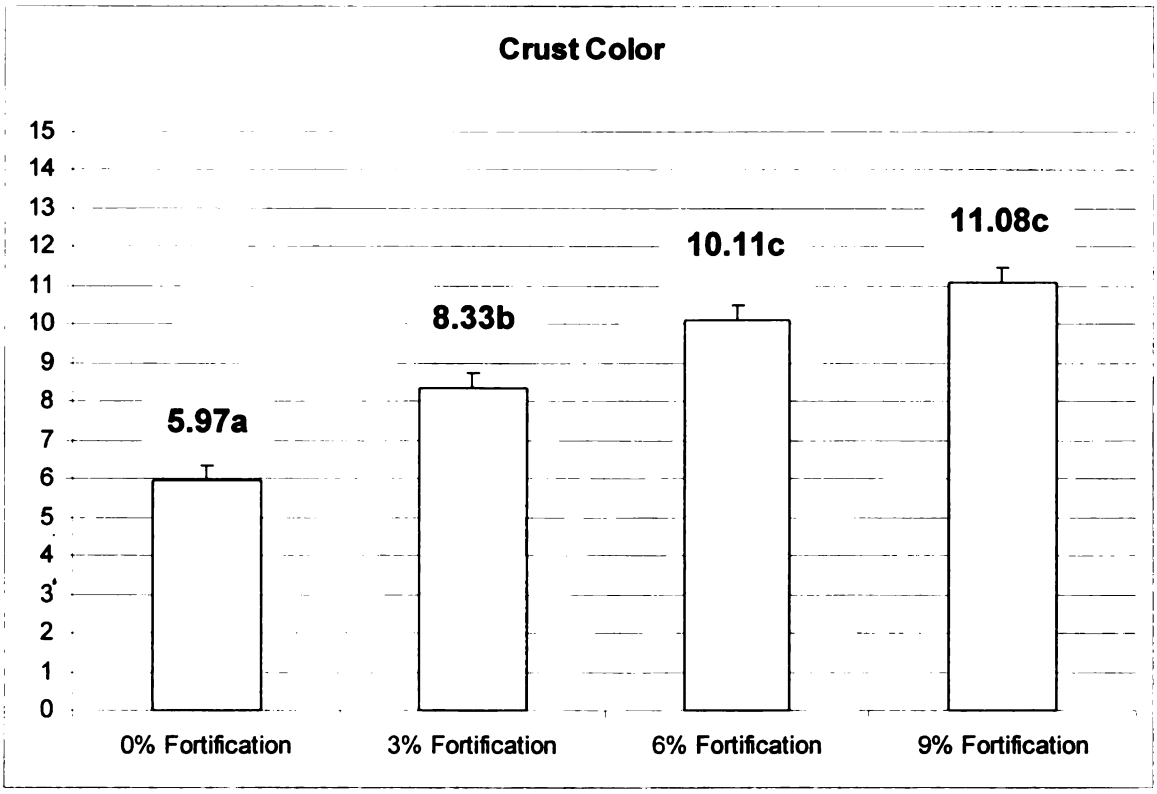


Figure 4.3.2.1. Trained panel crust color perception scores (increasing score indicates perceived darker color).

4.3.2.2. Crumb Color

For the crumb color, homogenous variances were estimated for each treatment. A significant effect of autumnberry fortification on crumb color perception of bread samples was identified ($P<0.0001$), such that greater levels of autumnberry fortification yielded greater crust color scoring at all levels. Each 3% increase in autumnberry fortification yielded a significant increment on crumb color perception. The greater the crust coloring recorded, the color perception was more off from the white color. In addition, a cubic trend ($P = 0.05$) was identified between autumnberry fortification and crumb color perception.

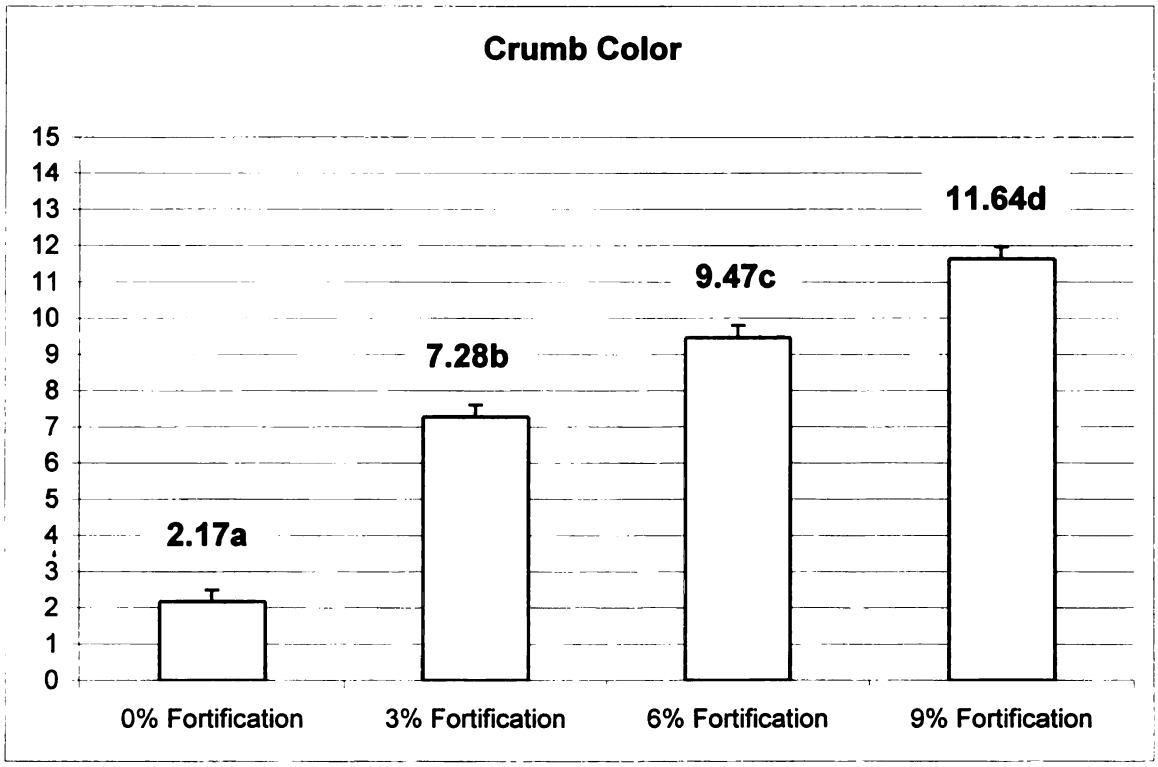


Figure 4.3.2.2. Trained panel crumb color perception (increasing score indicates perceived darker color).

4.3.2.3 Crumb Cell Uniformity

On the crumb cell uniformity, homogenous variances were estimated for each treatment. A significant effect of autumnberry fortification on crumb cell uniformity perception was identified ($P<0.0001$) as crumb cell uniformity scoring for 9% fortification was greater than that for 0% bread sample (Figure 4.3.2.3.). This means that the crumb cell was more uniform on the 9% fortification sample than the 0% sample. No significant differences were identified between any of the other levels of fortification. Additionally, the relationship between fortification level and crumb cell uniformity scoring was linear ($P=0.0002$).

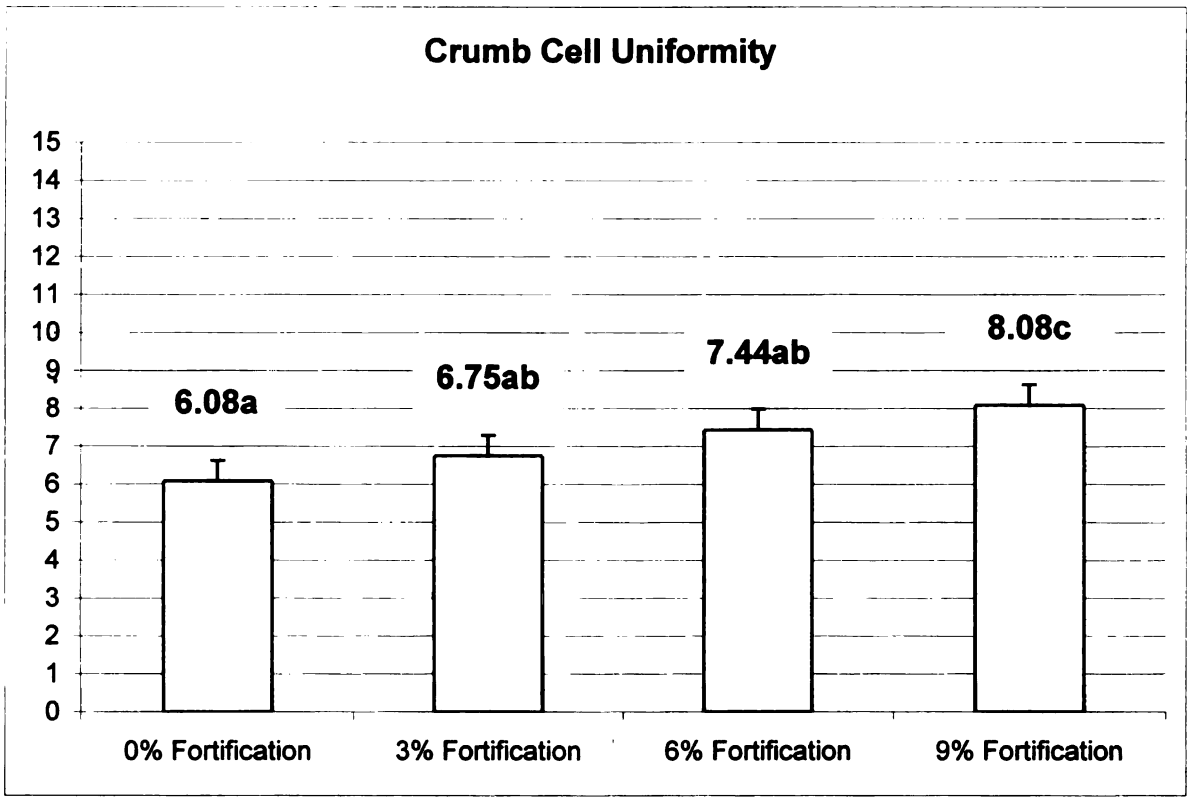


Figure 4.3.2.3. Trained panel crumb cell uniformity perception (increasing score indicates more uniform crumb cells).

4.3.2.4. Firmness

On the firmness attribute, homogenous variances were estimated for each treatment. At $P<0.0001$, a significant effect of autumnberry fortification on firmness perception of the bread samples was identified. Fortification at 6% and 9% levels resulted in greater firmness than 0% fortification (Figure 4.3.2.4.). Also, 9%, but not 6%, fortification yielded greater firmness than 3%. On the other hand, no significant difference was identified between 6% and 9% fortification samples and between 0% and 3%. Additionally, the relationship between fortification level and firmness scoring was linear ($P<0.0001$).

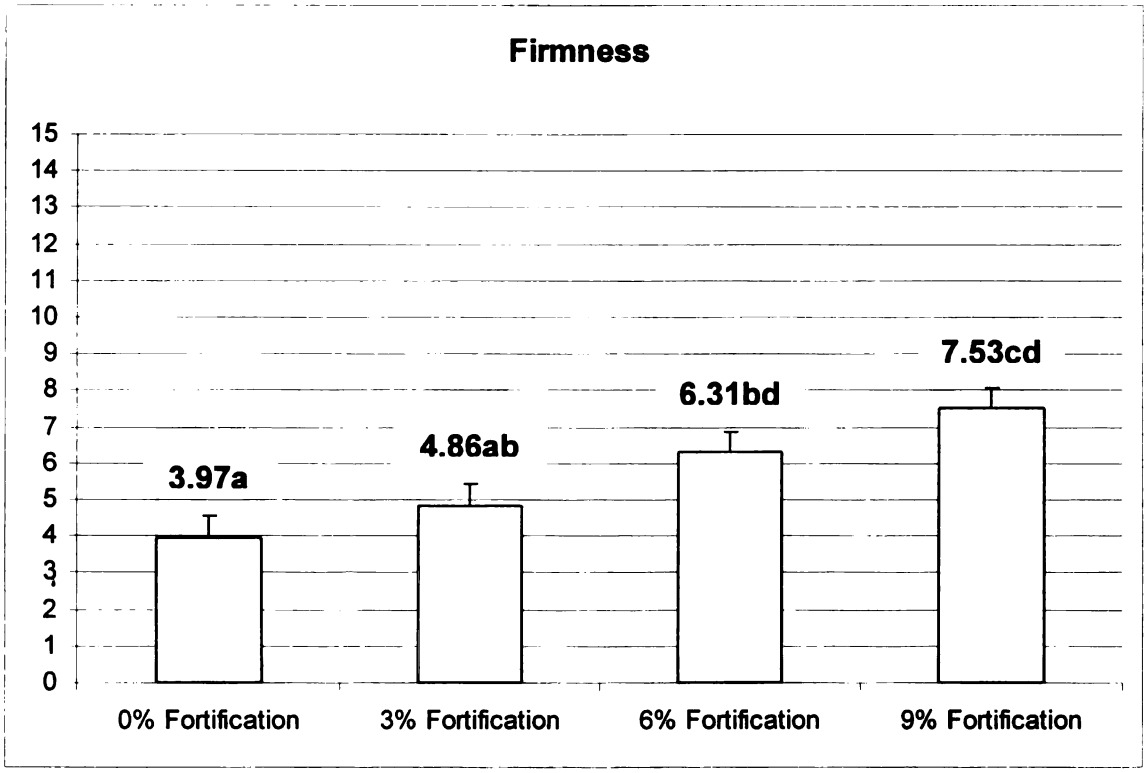


Figure 4.3.2.4. Trained panel firmness perception (increasing score indicates more firmer crumb).

4.3.2.5. Yeasty Flavor

For the yeasty flavor attribute, homogenous variances were estimated for each treatment. A linear effect was identified between level of autumnberry fortification and yeasty flavor of bread samples (P=0.0142), such that yeasty flavor scoring of the bread tended to decrease linearly with greater fortification levels. No pairwise comparison between fortification levels turned out to be significant (P=0.0870) (Figure 4.3.2.5.).

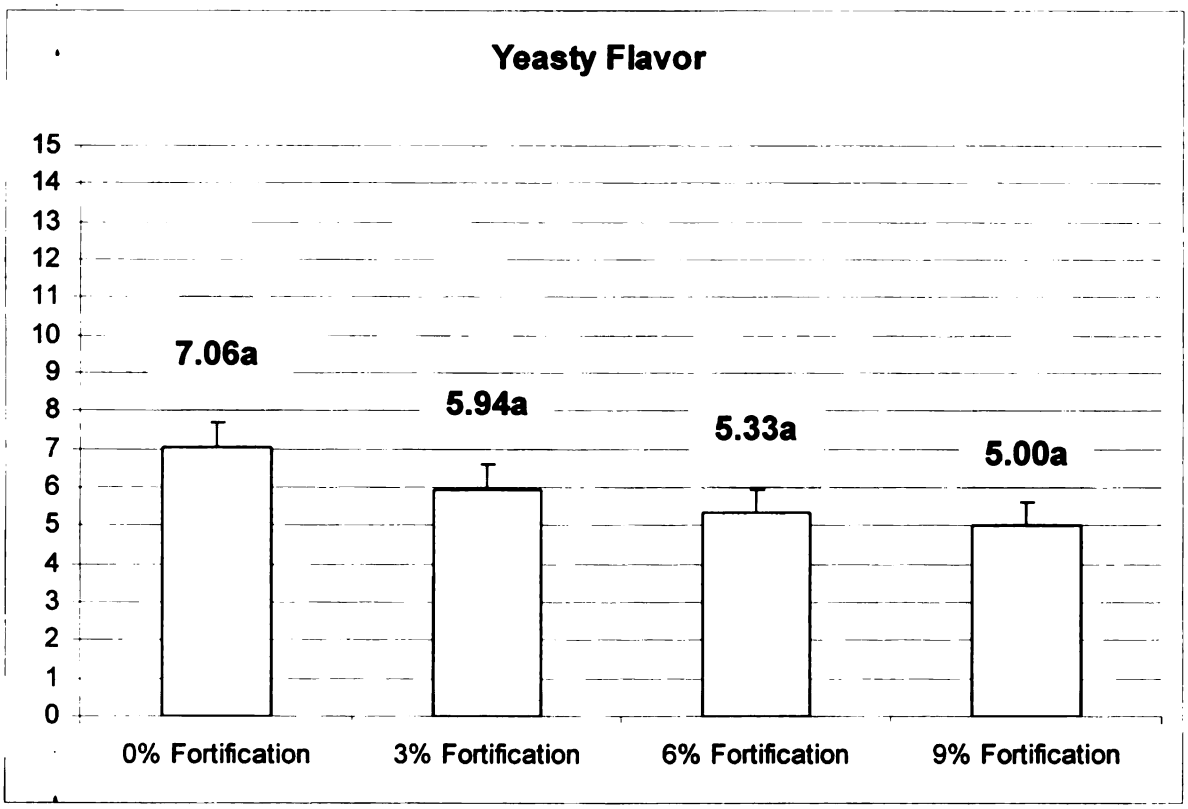


Figure 4.3.2.5. Trained panel yeasty flavor perception (increasing score indicates more yeasty flavor).

4.3.2.6 Autumnberry Flavor

The significant effect of autumnberry fortification on the autumnberry flavor scoring was identified ($P<0.0001$). The intensity of autumnberry flavor increased linearly with increased level of fortification ($P<0.0001$). The 9% fortification samples yielded the greatest intensity of autumnberry flavor scoring whereas 0% fortification yielded the least autumnberry flavor scoring (Figure 4.3.2.6). Estimates of residual variation varied with supplementation treatment and appeared to increase with level of supplementation. There are significant differences in the autumnberry flavor among all treatments.

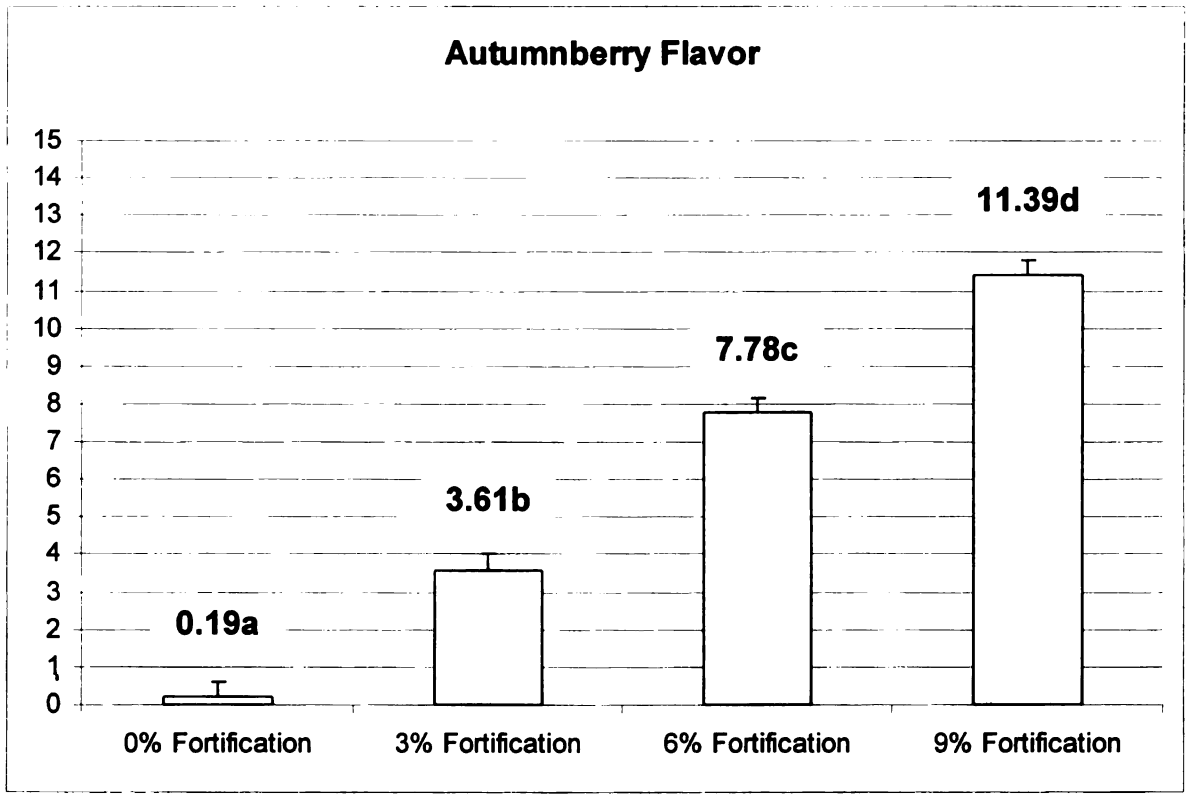


Figure 4.3.2.6. Trained panel autumnberry flavor perception (increasing score indicates more yeasty flavor).

4.3.3. Principal Component Analysis (PCA)

According to the PCA results (Figure 4.5.3.1.), the yeasty flavor was a distinct characteristic to the control bread sample. While the crumb cell uniformity was closely related to 9% bread samples. The 6% bread samples were closely related to crust color, crumb color, and autumnberry flavor attributes, so was the firmness. In this case, the 3% bread samples were not closely related to any of the attributes. It is also important to note that the relationship between yeasty flavor and crumb cell uniformity were opposite to each other.

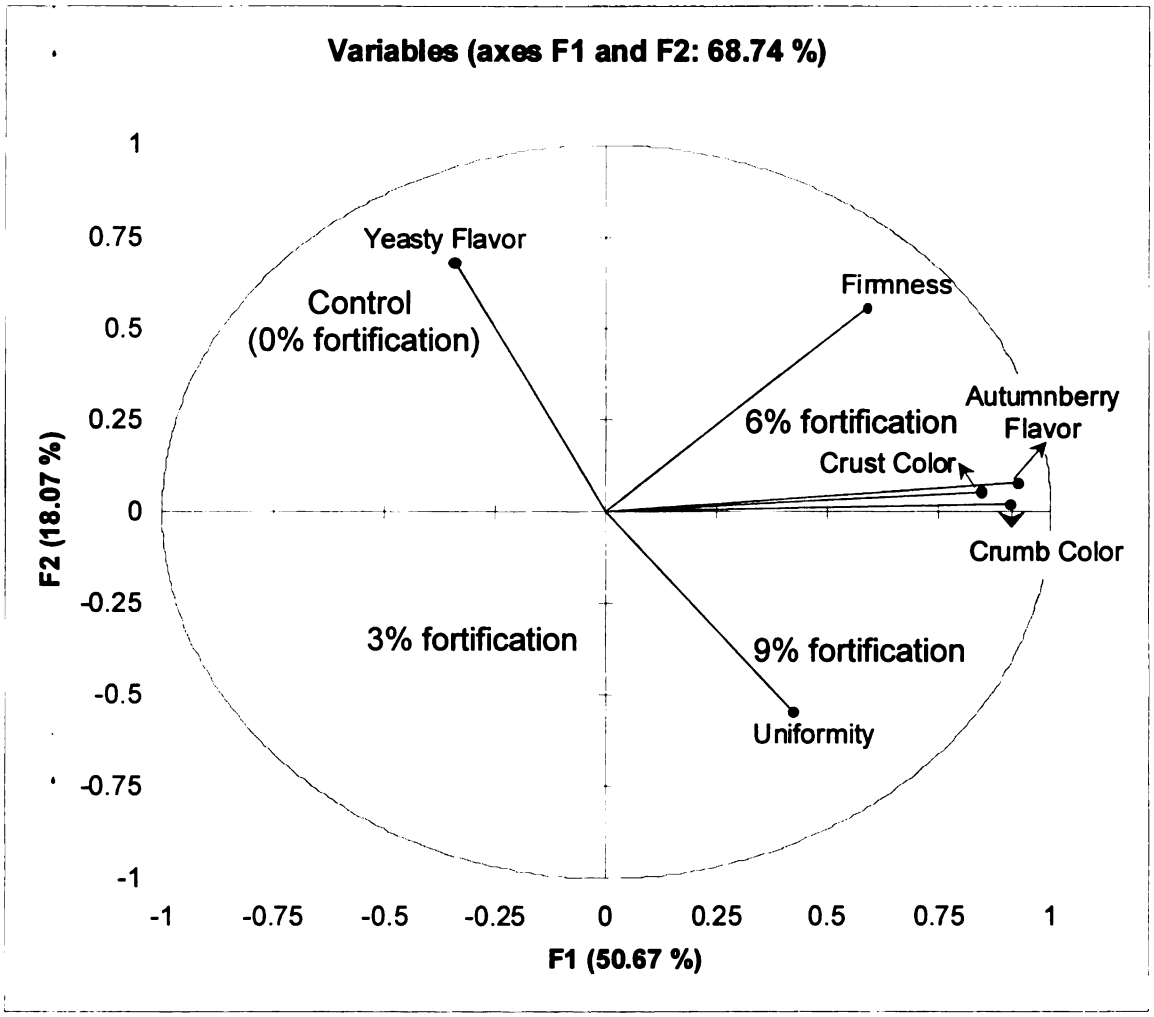


Figure 4.3.3.1. Principal Component Analysis (PCA) graph for the sensory trained panel.

4.3.4. Conclusions

Autumnberry is a low-cost underutilized fruit with potential for highly valuable antioxidants or nutraceuticals applications. Overall, autumnberry fortified bread had high consumers acceptability. The bread fortified with lower level of autumnberry was preferred compared to the higher level of fortification. The flavor of the fortified bread was comparable to that of sourdough bread.

FUTURE STUDIES AND RECOMMENDATIONS

The following topics are recommended for future research:

1) To study the bioavailability of lycopene extracted from autumnberry.
2) To study different applications of autumnberry in food products.
3) To investigate different drying methods that may be more efficient and cheaper than freeze-drying.

APPENDICES

Table A.1. Raw Data of Consumer Acceptability Panel

Panelist	Sample	AROMA	COLOR	APPEARANCE	BODY TEXTURE	FLAVOR	OVERALL ACCEPTANCE
ANONYMOUS001	Control	7	8	8	7	7	7
ANONYMOUS002	Control	7	5	7	6	6	6
ANONYMOUS003	Control	8	8	8	9	9	8
ANONYMOUS004	Control	9	9	9	9	9	9
ANONYMOUS005	Control	5	4	5	6	6	6
ANONYMOUS006	Control	8	8	8	8	7	8
ANONYMOUS007	Control	8	6	7	7	7	7
ANONYMOUS008	Control	8	8	8	6	7	7
ANONYMOUS009	Control	7	8	8	7	7	7
ANONYMOUS010	Control	8	8	8	8	8	8
ANONYMOUS011	Control	8	8	8	8	7	8
ANONYMOUS012	Control	5	7	8	3	4	5
ANONYMOUS013	Control	8	9	8	8	7	8
ANONYMOUS014	Control	8	8	8	8	8	8
ANONYMOUS015	Control	8	7	7	7	7	7
ANONYMOUS016	Control	7	8	8	7	7	8
ANONYMOUS017	Control	8	8	8	7	7	8
ANONYMOUS018	Control	7	8	8	7	8	7
ANONYMOUS019	Control	7	4	4	4	5	4
ANONYMOUS020	Control	7	6	7	7	7	7
ANONYMOUS021	Control	7	5	6	7	6	7
ANONYMOUS022	Control	9	9	9	9	9	9
ANONYMOUS023	Control	7	8	8	7	7	7
ANONYMOUS024	Control	9	9	9	8	8	8
ANONYMOUS025	Control	7	8	7	8	7	8
ANONYMOUS026	Control	9	8	8	8	8	8
ANONYMOUS027	Control	5	8	8	8	8	8
ANONYMOUS028	Control	6	6	4	7	5	6
ANONYMOUS029	Control	7	7	7	7	7	7
ANONYMOUS030	Control	8	9	9	9	8	8
ANONYMOUS031	Control	4	5	6	7	4	5
ANONYMOUS032	Control	7	8	8	6	6	7
ANONYMOUS033	Control	7	7	7	7	7	7
ANONYMOUS034	Control	7	8	8	8	7	8
ANONYMOUS035	Control	9	9	8	6	5	8
ANONYMOUS036	Control	4	5	5	6	5	5
ANONYMOUS037	Control	8	8	8	8	9	9
ANONYMOUS038	Control	6	7	7	7	7	7
ANONYMOUS039	Control	7	8	8	7	6	7
ANONYMOUS040	Control	7	8	9	7	7	8

Table A.1. Continued

ANONYMOUS041	Control	8	8	7	8	8	7
ANONYMOUS042	Control	6	4	7	6	3	4
ANONYMOUS043	Control	5	6	6	8	5	6
ANONYMOUS044	Control	6	8	8	5	6	7
ANONYMOUS045	Control	7	8	8	8	8	8
ANONYMOUS046	Control	4	5	7	7	8	7
ANONYMOUS047	Control	6	5	7	6	5	6
ANONYMOUS048	Control	7	8	8	7	7	8
ANONYMOUS049	Control	8	8	8	8	8	8
ANONYMOUS050	Control	8	8	8	9	8	8
ANONYMOUS051	Control	8	8	8	8	7	8
ANONYMOUS052	Control	7	7	8	8	8	8
ANONYMOUS001	10%	7	6	6	8	8	8
ANONYMOUS002	10%	7	7	8	8	7	7
ANONYMOUS003	10%	8	8	8	8	9	8
ANONYMOUS004	10%	7	4	3	4	7	7
ANONYMOUS005	10%	7	7	8	8	8	8
ANONYMOUS006	10%	8	8	8	7	6	7
ANONYMOUS007	10%	8	8	8	8	8	8
ANONYMOUS008	10%	6	6	5	5	6	6
ANONYMOUS009	10%	8	6	7	6	6	6
ANONYMOUS010	10%	8	6	7	8	8	8
ANONYMOUS011	10%	4	5	4	6	7	6
ANONYMOUS012	10%	5	6	5	5	7	6
ANONYMOUS013	10%	8	8	8	8	8	8
ANONYMOUS014	10%	7	8	8	8	8	8
ANONYMOUS015	10%	7	7	7	8	8	8
ANONYMOUS016	10%	6	4	4	4	3	3
ANONYMOUS017	10%	7	6	6	7	7	6
ANONYMOUS018	10%	7	8	7	8	8	8
ANONYMOUS019	10%	6	5	4	6	6	6
ANONYMOUS020	10%	8	7	7	7	8	8
ANONYMOUS021	10%	7	8	6	7	7	7
ANONYMOUS022	10%	9	9	9	9	9	9
ANONYMOUS023	10%	7	8	8	7	8	8
ANONYMOUS024	10%	8	8	8	9	7	7
ANONYMOUS025	10%	7	8	7	7	7	7
ANONYMOUS026	10%	8	8	7	8	9	8
ANONYMOUS027	10%	8	8	8	8	8	8
ANONYMOUS028	10%	7	6	7	7	8	6
ANONYMOUS029	10%	8	8	8	7	7	7
ANONYMOUS030	10%	8	9	9	9	8	8
ANONYMOUS031	10%	7	7	8	7	6	7
ANONYMOUS032	10%	7	8	8	7	7	7
ANONYMOUS033	10%	7	4	3	4	4	4
ANONYMOUS034	10%	7	6	7	7	8	7
ANONYMOUS035	10%	8	6	7	8	8	8

Table A.1. Continued

ANONYMOUS036	10%	4	6	6	4	4	4
ANONYMOUS037	10%	8	7	7	8	9	8
ANONYMOUS038	10%	7	7	7	7	7	7
ANONYMOUS039	10%	6	4	4	7	5	5
ANONYMOUS040	10%	6	6	7	7	7	7
ANONYMOUS041	10%	7	9	8	8	7	8
ANONYMOUS042	10%	5	7	7	7	7	7
ANONYMOUS043	10%	4	3	3	6	5	5
ANONYMOUS044	10%	6	6	7	7	7	7
ANONYMOUS045	10%	6	8	7	6	4	6
ANONYMOUS046	10%	7	9	8	8	8	8
ANONYMOUS047	10%	4	7	7	7	7	7
ANONYMOUS048	10%	5	4	5	7	6	6
ANONYMOUS049	10%	8	8	8	8	8	8
ANONYMOUS050	10%	8	7	7	8	8	8
ANONYMOUS051	10%	8	7	7	8	7	7
ANONYMOUS052	10%	5	5	5	7	7	6
ANONYMOUS001	20%	3	3	3	4	4	4
ANONYMOUS002	20%	3	8	8	9	8	9
ANONYMOUS003	20%	3	7	7	7	7	7
ANONYMOUS004	20%	3	3	3	7	6	6
ANONYMOUS005	20%	3	8	8	8	8	8
ANONYMOUS006	20%	3	6	6	7	6	6
ANONYMOUS007	20%	3	7	8	7	7	7
ANONYMOUS008	20%	3	5	5	7	6	5
ANONYMOUS009	20%	3	6	7	7	7	7
ANONYMOUS010	20%	3	6	7	8	8	8
ANONYMOUS011	20%	3	8	7	7	6	6
ANONYMOUS012	20%	3	7	7	7	7	8
ANONYMOUS013	20%	3	4	5	8	4	4
ANONYMOUS014	20%	3	8	8	7	7	7
ANONYMOUS015	20%	3	6	6	8	7	7
ANONYMOUS016	20%	3	2	4	5	4	3
ANONYMOUS017	20%	3	7	7	6	8	8
ANONYMOUS018	20%	3	8	8	8	8	8
ANONYMOUS019	20%	3	4	4	7	6	6
ANONYMOUS020	20%	3	8	8	8	9	8
ANONYMOUS021	20%	3	8	8	8	8	8
ANONYMOUS022	20%	3	9	9	9	8	8
ANONYMOUS023	20%	3	6	7	8	8	7
ANONYMOUS024	20%	3	6	7	8	7	7
ANONYMOUS025	20%	3	5	6	5	6	6
ANONYMOUS026	20%	3	7	7	7	4	5
ANONYMOUS027	20%	3	7	7	8	4	5
ANONYMOUS028	20%	3	3	6	2	8	6
ANONYMOUS029	20%	3	8	8	7	7	7
ANONYMOUS030	20%	3	8	8	8	8	7

Table A.1. Continued

ANONYMOUS031	20%	3	7	6	7	4	5
ANONYMOUS032	20%	3	7	7	7	7	7
ANONYMOUS033	20%	3	3	3	7	4	3
ANONYMOUS034	20%	3	4	5	8	7	7
ANONYMOUS035	20%	3	4	6	6	9	7
ANONYMOUS036	20%	3	4	5	4	6	4
ANONYMOUS037	20%	3	7	7	8	6	7
ANONYMOUS038	20%	3	8	8	8	8	8
ANONYMOUS039	20%	3	6	6	6	8	7
ANONYMOUS040	20%	3	6	6	6	6	7
ANONYMOUS041	20%	3	7	8	8	7	8
ANONYMOUS042	20%	3	6	6	7	7	7
ANONYMOUS043	20%	3	2	2	6	6	5
ANONYMOUS044	20%	3	3	4	7	7	4
ANONYMOUS045	20%	3	6	7	7	4	4
ANONYMOUS046	20%	3	9	8	9	9	9
ANONYMOUS047	20%	3	6	6	6	6	6
ANONYMOUS048	20%	3	3	4	5	6	4
ANONYMOUS049	20%	3	7	7	8	8	8
ANONYMOUS050	20%	3	7	7	8	7	7
ANONYMOUS051	20%	3	4	5	7	6	7
ANONYMOUS052	20%	3	5	5	6	4	5

Table A.1.1. Summary and ANOVA table for aroma

SUMMARY for AROMA						
Groups/Treatments	**Count**	**Sum**	**Average**	**Variance**		
Control	52	368	7.08	1.64		
10%	52	356	6.85	1.54		
20%	52	332	6.38	2.48		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Treatments	12.92	2	6.46	3.42	0.04	3.06
Panelists	288.77	153	1.89			
Total	301.69	155				

Table A.1.2. Summary and ANOVA table for color

SUMMARY for COLOR						
Groups/Treatments	**Count**	**Sum**	**Average**	**Variance**		
Control	52	378	7.27	2.00		
10%	52	351	6.75	2.23		
20%	52	309	5.94	3.58		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Treatments	46.5	2	23.25	8.92	0.001	3.06
Panelists	398.81	153	2.61			
Total	445.31	155				

Table A.1.3. Summary and ANOVA table for appearance

SUMMARY for APPEARANCE						
Groups/Treatments	**Count**	**Sum**	**Average**	**Variance**		
Control	52	389	7.48	1.27		
10%	52	348	6.69	2.41		
20%	52	327	6.29	2.60		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Treatments	38.24	2	19.12	9.12	0.001	3.06
Panelists	320.73	153	2.10			
Total	358.97	155				

Table A.1.4. Summary and ANOVA table for body/texture

SUMMARY for BODY/TEXTURE						
Groups/Treatments	**Count**	**Sum**	**Average**	**Variance**		
Control	52	374	7.19	1.41		
10%	52	368	7.08	1.56		
20%	52	363	6.98	1.78		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Treatments	1.17	2	0.58	0.37	0.69	3.06
Panelists	242.75	153	1.59			
Total	243.92	155				

Table A.1.5. Summary and ANOVA table for flavor

SUMMARY for FLAVOR						
Groups/Treatments	**Count**	**Sum**	**Average**	**Variance**		
Control	52	357	6.87	1.81		
10%	52	367	7.06	1.78		
20%	52	343	6.60	2.17		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Treatments	5.59	2	2.79	1.46	0.24	3.06
Panelists	293.40	153	1.92			
Total	298.99	155				

Table A.1.6. Summary and ANOVA table for overall acceptability

SUMMARY for OVERALL ACCEPTANCE						
Groups/Treatments	**Count**	**Sum**	**Average**	**Variance**		
Control	52	375	7.21	1.31		
10%	52	362	6.96	1.49		
20%	52	334	6.42	2.33		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Treatments	16.88	2	8.44	4.94	0.01	3.06
Panelists	261.29	153	1.71			
Total	278.17	155				

Table A.2. Raw Data of Physic ochemical Properties of Autumnberry Pureed and Freeze-dried

Sample ID	Color L*	Color a*	Color b*	% Citric Acid (w/w)	% Lactic Acid (w/w)	% Acetic Acid (w/w)	% Malic Acid (w/w)	Invert Sugar (g/g of dried sample)	Total Sugar (g/g of dried sample)	Sucrose (g/g of dried sample)	MC (wb)	Aw	Total Phenolics (mg GAE/g of dried sample)	ORAC value (umol TE/gof dried sample)	Lycopene (mg/g of dried sample)
Puree Batch 1	29.52	38.50	20.00	0.42	0.59	0.39	0.44	0.75	0.77	0.03	80.33	0.98	6.34	139.78	2.63
Puree Batch 2	29.39	38.75	20.25	0.42	0.59	0.39	0.44	0.72	0.77	0.05	80.56	0.98	7.64	162.35	2.90
Puree Batch 3	29.45	38.66	20.22	0.42	0.59	0.39	0.44	0.72	0.77	0.05	80.13	0.97	7.83	154.42	2.89
FD Batch 1	53.68	29.49	19.03	0.34	0.47	0.31	0.35	0.75	0.77	0.02	2.23	0.09	6.66	89.19	0.71
FD Batch 2	55.89	29.20	18.88	0.33	0.47	0.31	0.35	0.75	0.77	0.02	2.20	0.09	6.55	94.57	0.69
FD Batch 3	55.44	29.02	18.67	0.34	0.47	0.31	0.35	0.74	0.77	0.03	2.48	0.09	7.05	101.11	0.71
Puree Batch 1	29.35	38.59	20.19	0.42	0.59	0.40	0.44	0.72	0.76	0.05	80.29	0.99	6.54	164.16	2.90
Puree Batch 2	29.51	38.70	20.35	0.41	0.58	0.39	0.43	0.72	0.77	0.05	80.34	0.98	7.57	141.79	3.07
Puree Batch 3	29.55	38.65	20.21	0.41	0.58	0.39	0.44	0.73	0.78	0.05	80.19	0.98	7.86	124.73	2.88
FD Batch 1	54.94	28.33	18.06	0.33	0.47	0.31	0.35	0.52	0.67	0.14	2.33	0.14	6.65	102.08	0.69
FD Batch 2	55.78	28.31	18.22	0.33	0.47	0.31	0.35	0.52	0.67	0.14	2.21	0.16	6.75	111.08	0.71
FD Batch 3	56.33	28.59	18.43	0.33	0.47	0.31	0.35	0.52	0.67	0.14	2.49	0.15	6.59	95.16	0.71
Puree Batch 1	29.72	38.79	20.30	0.42	0.59	0.39	0.44	0.52	0.58	0.06	80.11	0.97	6.30	135.04	2.86
Puree Batch 2	29.65	38.62	20.20	0.42	0.59	0.39	0.43	0.52	0.58	0.06	80.33	0.97	7.62	125.49	2.89
Puree Batch 3	29.69	38.69	20.17	0.41	0.58	0.39	0.43	0.52	0.58	0.06	80.45	0.96	8.18	149.49	3.06
FD Batch 1	56.02	28.69	18.91	0.34	0.47	0.31	0.35	0.56	0.64	0.07	2.44	0.16	6.67	118.85	0.71
FD Batch 2	55.67	28.74	18.67	0.33	0.47	0.31	0.35	0.56	0.64	0.07	2.33	0.16	6.77	112.47	0.69
FD Batch 3	54.88	28.99	18.70	0.33	0.47	0.31	0.35	0.56	0.62	0.07	2.19	0.16	6.85	96.73	0.69

Appendix 3. SAS Output for Physicochemical Properties

A. 3.1. CIE Color L*

Covariance Parameter Estimates

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	**0.6436**	0.3218	2.00	0.0228
Residual	Presentation Pure	**0.01687**	0.008437	2.00	0.0227

Fit Statistics

-2 Res Log Likelihood	13.6
AIC (smaller is better)	17.6
AICC (smaller is better)	18.5
BIC (smaller is better)	15.8

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	8.42	9117.66	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	**55.4033**	0.2674	8	207.18	<.0001
Presentation	Pure	**29.5367**	0.04330	8	682.12	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	Pure	25.8667	0.2709	8.42	95.49	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Adjustment	Adj P
Presentation	FD	Pure	Tukey-Kramer	**<.0001**

A.3.2. Color a*

Covariance Parameter Estimates

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	**0.1548**	0.07740	2.00	0.0227
Residual	Presentation Pure	**0.007461**	0.003731	2.00	0.0228

Fit Statistics

-2 Res Log Likelihood	-4.3
AIC (smaller is better)	-0.3
AICC (smaller is better)	0.6
BIC (smaller is better)	-2.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	8.77	5373.98	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	**28.8178**	0.1312	8	219.73	<.0001
Presentation	Pure	**38.6611**	0.02879	8	1342.75	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	Pure	-9.8433	0.1343	8.77	-73.31	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Adjustment	Adj P
Presentation	FD	Pure	Tukey-Kramer	**<.0001**

A.3.3. Color b*

Covariance Parameter Estimates

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	**0.1051**	0.05256	2.00	0.0228
Residual	Presentation Pure	**0.009450**	0.004725	2.00	0.0228

Fit Statistics

-2 Res Log Likelihood	-5.5
AIC (smaller is better)	-1.5
AICC (smaller is better)	-0.6
BIC (smaller is better)	-3.3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	9.43	198.89	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	**18.6189**	0.1081	8	172.29	<.0001
Presentation	Pure	**20.2100**	0.03240	8	623.69	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	Pure	-1.5911	0.1128	9.43	-14.10	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Adjustment	Adj P
Presentation	FD	Pure	Tukey-Kramer	**<.0001**

A.3.4. Citric Acid

Covariance Parameter Estimates

Cov Parm	Standard Estimate	Z Error	Value	Pr Z
Batch	1.306E-6	3.146E-6	0.42	0.3390
Presentation*Batch	1.185E-6	3.033E-6	0.39	0.3480
Residual	5.286E-6	2.158E-6	2.45	0.0072

Fit Statistics

-2 Res Log Likelihood	-141.3
AIC (smaller is better)	-135.3
AICC (smaller is better)	-133.3
BIC (smaller is better)	-138.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	2	3444.16	0.0003

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	0.3346	0.001191	3.66	281.00	<.0001
Presentation	Pure	0.4168	0.001191	3.66	350.08	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	-0.08226	0.001402	2	-58.69

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.0003	Tukey-Kramer	0.0003

A.3.5. Lactic Acid

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Standard Estimate	Z Error	Value	Pr Z
Batch	2.585E-6	6.225E-6	0.42	0.3390
Presentation*Batch	2.344E-6	6.002E-6	0.39	0.3480
Residual	0.000010	4.27E-6	2.45	0.0072

Fit Statistics

-2 Res Log Likelihood	-130.4
AIC (smaller is better)	-124.4
AICC (smaller is better)	-122.4
BIC (smaller is better)	-127.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	2	3444.16	0.0003

Least Squares Means

Effect	Presentation	Standard Estimate	Error	DF	t Value	Pr > |t|
Presentation	FD	0.4706	0.001675	3.66	281.00	<.0001
Presentation	Pure	0.5863	0.001675	3.66	350.08	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Standard Estimate	Error	DF	t Value
Presentation	FD	Pure	-0.1157	0.001972	2	-58.69

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.0003	Tukey-Kramer	0.0003

A.3.6. Acetic Acid

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Standard Estimate	Z Error	Value	Pr Z
Batch	1.149E-6	2.766E-6	0.42	0.3390
Presentation*Batch	1.042E-6	2.667E-6	0.39	0.3480
Residual	4.648E-6	1.897E-6	2.45	0.0072

Fit Statistics

-2 Res Log Likelihood	-143.3
AIC (smaller is better)	-137.3
AICC (smaller is better)	-135.3
BIC (smaller is better)	-140.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	2	3444.16	0.0003

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	0.3137	0.001116	3.66	281.00	<.0001
Presentation	Pure	0.3909	0.001116	3.66	350.08	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	-0.07713	0.001314	2	-58.69

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.0003	Tukey-Kramer	0.0003

A.3.7. Malic Acid

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	7.991E-7	0	.	.
Residual	Presentation Pure	0.000015	7.443E-6	2.00	0.0228

Fit Statistics

-2 Res Log Likelihood	-151.4
AIC (smaller is better)	-147.4
AICC (smaller is better)	-146.5
BIC (smaller is better)	-149.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	8.86	4255.88	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	0.3503	0.000298	8	1175.60	<.0001
Presentation	Pure	0.4364	0.001286	8	339.34	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	-0.08612	0.001320	8.86	-65.24

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	<.0001	Tukey-Kramer	<.0001

A.3.8. Invert Sugar

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Standard Estimate	Error	Z Value	Pr Z
Batch	2.28E-22	.	.	.
Presentation*Batch	0	.	.	.
Residual	0.01085	0.003838	2.83	0.0023

Fit Statistics

-2 Res Log Likelihood	-22.6
AIC (smaller is better)	-20.6
AICC (smaller is better)	-20.3
BIC (smaller is better)	-21.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	16	0.89	0.3600

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	0.6103	0.03473	16	17.57	<.0001
Presentation	Pure	0.6566	0.03473	16	18.91	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	-0.04628	0.04911	16	-0.94

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.3600	Tukey	0.3600

A.3.9. Total Sugar

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Standard Estimate	Z Error	Value	Pr Z
Batch	0	.	.	.
Presentation*Batch	0	.	.	.
Residual	0.006336	0.002240	2.83	0.0023

Fit Statistics

-2 Res Log Likelihood	-31.2
AIC (smaller is better)	-29.2
AICC (smaller is better)	-28.9
BIC (smaller is better)	-30.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	16	0.23	0.6395

Least Squares Means

Effect	Presentation	Standard Estimate	Error	DF	t Value	Pr > |t|
Presentation	FD	0.6909	0.02653	16	26.04	<.0001
Presentation	Pure	0.7088	0.02653	16	26.71	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Standard Estimate	Error	DF	t Value
Presentation	FD	Pure	-0.01791	0.03752	16	-0.48

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.6395	Tukey	0.6395

A.3.10. Sucrose

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Standard Estimate	Z Error	Value	Pr Z
Batch	0	.	.	.
Presentation*Batch	0	.	.	.
Residual	0.001404	0.000497	2.83	0.0023

Fit Statistics

-2 Res Log Likelihood	-55.3
AIC (smaller is better)	-53.3
AICC (smaller is better)	-53.0
BIC (smaller is better)	-54.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	16	2.62	0.1250

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	0.07812	0.01249	16	6.25	<.0001
Presentation	Pure	0.04952	0.01249	16	3.96	0.0011

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	0.02860	0.01767	16	1.62

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.1250	Tukey	0.1250

A.3.11. Moisture Content

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
Batch	0	.	.	.
Presentation*Batch	0.000959	0.005355	0.18	0.4289
Residual	0.01748	0.007135	2.45	0.0072

Fit Statistics

-2 Res Log Likelihood	-14.3
AIC (smaller is better)	-10.3
AICC (smaller is better)	-9.4
BIC (smaller is better)	-12.1

Solution for Fixed Effects

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		80.3033	0.04756	4	1688.55	<.0001
Presentation	FD	-77.9811	0.06726	4	-1159.5	<.0001
Presentation	Pure	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	4	1344343	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	2.3222	0.04756	4	48.83	<.0001
Presentation	Pure	80.3033	0.04756	4	1688.55	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	Pure	-77.9811	0.06726	4	-1159.5	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Adjustment	Adj P
Presentation	FD	Pure	Tukey	<.0001

A.3.12. Water Activity (Aw)

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Group	Standard Estimate	Z Error	Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	0.001114	0.000557	2.00	0.0228
Residual	Presentation Pure	0.000084	0.000042	2.00	0.0227

Fit Statistics

-2 Res Log Likelihood	-79.7
AIC (smaller is better)	-75.7
AICC (smaller is better)	-74.7
BIC (smaller is better)	-77.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	9.2	5321.27	<.0001

Least Squares Means

Effect	Presentation	Standard Estimate	Error	DF	t Value	Pr > |t|
Presentation	FD	0.1328	0.01113	8	11.93	<.0001
Presentation	Pure	0.9746	0.003055	8	318.97	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Standard Estimate	Error	DF	t Value
Presentation	FD	Pure	-0.8418	0.01154	9.2	-72.95

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	<.0001	Tukey-Kramer	<.0001

A.3.13. Total Phenolics

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Group	Standard Estimate	Error	Z Value	Pr Z
Batch		0.05790	0.2455	0.24	0.4068
Presentation*Batch		0.2771	0.2839	0.98	0.1646
Residual	Presentation FD	0.02194	0.01258	1.74	0.0406
Residual	Presentation Pure	0.01887	0.01096	1.72	0.0426

Fit Statistics

-2 Res Log Likelihood	3.1
AIC (smaller is better)	11.1
AICC (smaller is better)	14.7
BIC (smaller is better)	7.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	2	1.85	0.3065

Least Squares Means

Effect	Presentation	Standard Estimate	Error	DF	t Value	Pr > |t|
Presentation	FD	6.7275	0.3378	3.91	19.92	<.0001
Presentation	Pure	7.3197	0.3373	3.89	21.70	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Standard Estimate	Error	DF	t Value
Presentation	FD	Pure	-0.5922	0.4350	2	-1.36

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	0.3065	Tukey-Kramer	0.3065

A.3.14. ORAC value

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	96.1967	48.0983	2.00	0.0228
Residual	Presentation Pure	212.32	106.16	2.00	0.0228

Fit Statistics

-2 Res Log Likelihood	129.2
AIC (smaller is better)	133.2
AICC (smaller is better)	134.1
BIC (smaller is better)	131.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	14	50.92	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	102.36	3.2693	8	31.31	<.0001
Presentation	Pure	144.14	4.8571	8	29.68	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	-41.7796	5.8549	14	-7.14

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	<.0001	Tukey-Kramer	<.0001

A.3.15. Lycopene

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Group	Estimate	Standard Error	Z Value	Pr Z
Batch		0	.	.	.
Presentation*Batch		0	.	.	.
Residual	Presentation FD	0.000178	0.000089	2.00	0.0228
Residual	Presentation Pure	0.01657	0.008284	2.00	0.0228

Fit Statistics

-2 Res Log Likelihood	-52.1
AIC (smaller is better)	-48.1
AICC (smaller is better)	-47.2
BIC (smaller is better)	-49.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Presentation	1	8.17	2590.61	<.0001

Least Squares Means

Effect	Presentation	Estimate	Standard Error	DF	t Value	Pr > |t|
Presentation	FD	0.7013	0.004443	8	157.83	<.0001
Presentation	Pure	2.8967	0.04290	8	67.51	<.0001

Differences of Least Squares Means

Effect	Presentation	Presentation	Estimate	Standard Error	DF	t Value
Presentation	FD	Pure	-2.1954	0.04313	8.17	-50.90

Differences of Least Squares Means

Effect	Presentation	Presentation	Pr > |t|	Adjustment	Adj P
Presentation	FD	Pure	<.0001	Tukey-Kramer	<.0001

Appendix 4. Consumer Testing Consent Form

Consent Form - Consumer Panel Acceptance Test
Evaluation of Bread containing Autumnberry

Dear Participant:

Michigan State University graduate student is investigating consumer perceptions of autumn berry-containing bread. We would like you to take about 15 minutes (including the time you spend reading this letter) to help us evaluate autumnberry bread samples. We are asking for volunteers, 18 years or older, who consume bread in regular basis. *If you have a known food allergy* to the ingredients of the bread: wheat flour, autumn olive berry pureed, sugar, yeast, partially hydrogenated shortening, and salt, *please do not volunteer for this study.*

If you meet the above requirements, we would like you to look at and taste the bread samples. You will be given 5 samples to look at, smell, taste, and answer questions related to the product quality. If you agree to taste these and provide your evaluation based on the survey questionnaire, please sign the consent form below. You will be given an ice cream cup for your evaluation and completion of the survey.

If you believe there is a potential of an allergic reaction upon sniffing and tasting, notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have.

Your responses are collected anonymously. We have no way to connect you, as an individual, to the completed survey form. You are free to not answer any question you choose, but please try to answer every question. We are not able to use incomplete responses nor are we able to provide the incentive for incomplete responses.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator. Feel free to contact Dr. Janice

Appendix 4. Continued

Harte, the principle investigator, via phone at 517 355 8474 x105 or harteja@msu.edu for any inquiry you might have due to your participation in our study.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN OUR STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON YOUR REQUEST.

SIGNED_____ DATE_____

Appendix 5. Trained Sensory Evaluation Consent Forms

Trained Panel Consent Form
Evaluations of Freeze-dried Autumnberry Fortified Bread
Samples: Yeast-raised bread fortified with freeze-dried autumnberry
(AACC Method 10-10)

Before you decide to sign this consent form and continue to participate in our study, please read carefully and thoroughly the reverse side of this form for the sample ingredients and preparation information, purpose and procedure of this study, potential risks and benefits from your participation, our assurance of your privacy, your rights as a human subject in our study, etc.

We are asking that panelists participate in the physicochemical and sensory properties evaluation of autumnberry bread that will be conducted for 8-10 weeks period. Training will consist of approximately 8-10 sessions of 30 - 45 minutes. After training, evaluations will be scheduled for 3 times over a 2-week period. Evaluations should last about 15-30 minutes. It is important for this research that we have the same panelists participate for each evaluation when ever possible. We will make every effort to accommodate your schedule and time needs. However, if you cannot attend any evaluation please inform the researchers when contacted each month. Your signing this consent form will indicate your agreeing to participate when possible.

If you have any question during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator. Feel free to contact Dr. Janice Harte, the principle investigator of this study, via phone at 517-355-8474, ext. 105 or write her at 114 Trout Food Science and Human Nutrition Building, Michigan State University, East Lansing, MI 48823. You also can reach us via email at harteja@msu.edu for any inquiry you might have due to your participation in our study.

If you have read all the information we offer to you in this consent form and decide to participate in our study and give us your valuable response to our questionnaire, you can go ahead and sign this form now. Otherwise, you can stop here and feel free to discontinue participation in our study without any penalty.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN OUR STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE

Appendix 5. Continued

HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON YOUR REQUEST.

SIGNED: _____ DATE: _____

TRAINED PANEL CONSENT FORM
Participant Copy
Evaluations of Yeast-raised Bread Fortified with Freeze-dried Autumnberry
(AACC Method 10-10 for bread-making)

Invitation to participate: You are invited to participate in the study that assesses some physicochemical and sensory properties of yeast-raised bread fortified with freeze-dried autumnberry.

Purpose of the study: We are investigating the effect of the fortification of freeze-dried autumnberry at different levels on in yeast-raised bread in terms of crust and crumb color, firmness, cell uniformity, and the intensity of the yeast and/or autumnberry flavor.

Procedure of the study: Each panelist would be served different variations of slice of bread at room temperature condition. Each sample will be coded with a random 3-digit code. We are asking that panelists participate in this study at which last for 8-10 weeks period. Training will consists of approximately 8-10 sessions of 30-45 minutes. Instructions to the test would be provided on a given sheet. Participants will be asked to rate the samples based on Universal Scale in which consists of 15 points spectrum scale on color and texture attributes.

Samples preparation: Breads were prepared in MSU Baking Lab/Cereal Lab 124 G of Trout FSHN Building using AACC approved baking equipment.

Potential risks: The breads are consisted of bread flour (wheat gluten), freeze-dried autumnberry, salt, sugar, vegetable shortening, bread machine yeast, ascorbic acid, water. If you have any known allergic reaction(s) to these listed ingredients, please do not participate in this trained panel. These cookies pose no adverse health risk. Though none is anticipated, if you have a problem **upon consuming these samples,** please notify the on-site sensory evaluation

Appendix 5. Continued

coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have. Your response is confidential and we will protect your confidentiality to the full extent of the law.

Expected benefits: This study will enable the researchers to establish the relationship between sensory evaluation and experimental (mechanical) data on physicochemical properties of autumnberry fortified breads.

Assurance of confidentiality: Any information obtained in connection with this study that could be identified with you will be kept confidential by ensuring that all consent forms and response sheets are securely stored. All data collected and analyzed will be reported in an aggregate format that will not permit associating subjects with specifics response or findings. **Your privacy will be protected to the maximum extent allowable by law.**

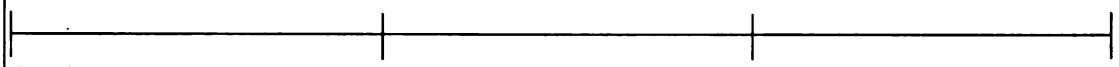
Withdrawal from the study: Participation in this study is in voluntary basis. You may refuse to grade any of the cookies without penalty, and your decision to refuse participation or discontinue participation during this study will be fulfilled promptly and unconditionally.

Appendix 6. Descriptive Analysis Questionnaire

Descriptive Analysis of Autumnberry Bread
Trained Panel Test Session # _____

Name: _____ Date: _____

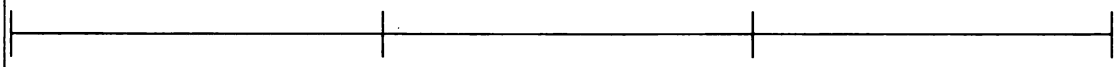
1) Crust Color:



0 5 10 15
light dark

Comments: _____
_____

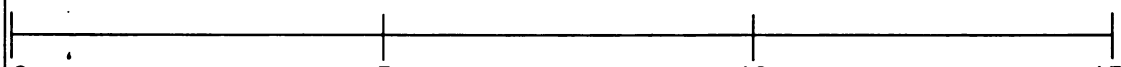
2) Crumb Color:



0 5 10 15
light dark

Comments: _____
_____

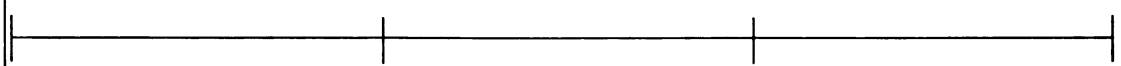
3) Firmness:



0 5 10 15
soft hard

Comments: _____
_____

4) Crumb Structure Uniformity:



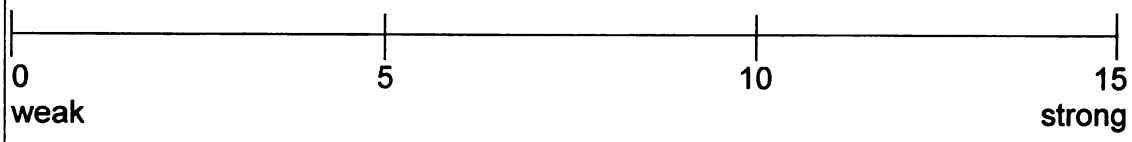
0 5 10 15
less uniform more uniform

Appendix 6. Continued

Comments: _____

_____

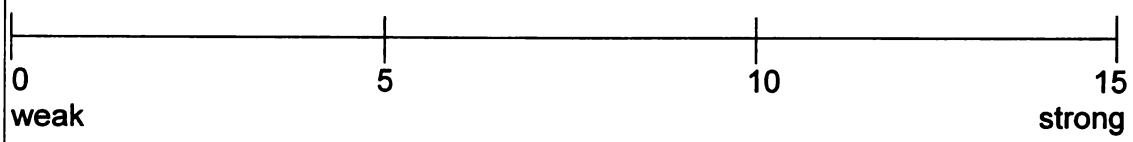
5) Yeasty Flavor:



Comments: _____

_____

6) Autumnberry Flavor:



Comments: _____

_____

Table A.7. Bread Attributes References for Sensory Trained Panel

Bread Characteristics	Food Products	Score
Firmness	Sunbeam whole grain white bread	1
	Stone-ground 100% whole wheat Pepperidge Farm bread	8
	Mini Bagel pre-sliced Pepperidge Farm	15
Yeast Flavor	Great Value bread mix without added yeast suspension	1
	Great Value bread mix with added yeast (doubled)	8
	Great Value bread mix with added yeast (tripled)	15
Autumnberry Flavor	Distilled water	0
	Autumnberry juice 5%	5
	Autumnberry juice 10%	10
	Autumnberry juice 15%	15

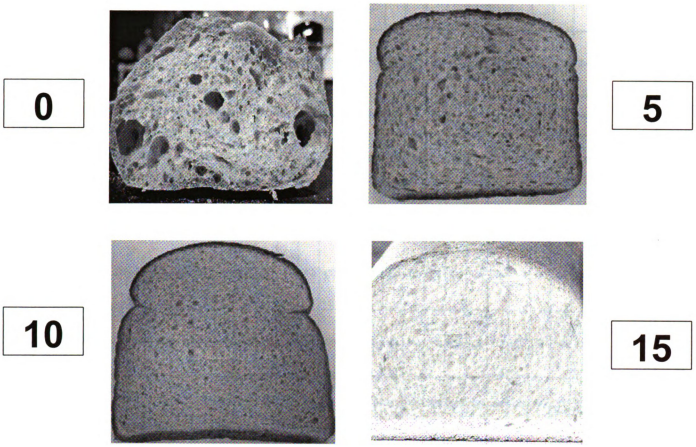


Figure A.7.1. References for Bread Crumb Cell Uniformity.

Table A.8. Raw Data for Descriptive Analysis Testing

Treatment	Panelist	Run	Crust Color	Crumb Color	Crumb Cell Uniformity	Crumb Firmness	Yeasty Flavor	Autumnberry Flavor
0%	1	1	4	1	5	10	6	0
0%	2	1	7	1	5	3	10	0
0%	3	1	7	0	12	1	5	0
0%	4	1	8	5	4	5	10	0
0%	5	1	6	1	8	7	10	0
0%	6	1	2	2	6	1	7	1
0%	7	1	7	1	7	0	9	0
0%	8	1	2	9	4	2	3	0
0%	9	1	6	0	3	3	6	0
0%	10	1	8	3	9	3	5	0
0%	11	1	2	2	11	7	6	0
0%	12	1	7	3	9	7	4	0
0%	1	2	5	2	3	4	9	0
0%	2	2	8	1	5	3	13	0
0%	3	2	8	0	5	1	5	0
0%	4	2	7	7	6	6	4	0
0%	5	2	5	2	2	10	10	0
0%	6	2	7	3	4	6	2	0
0%	7	2	8	1	10	6	9	0
0%	8	2	7	3	7	3	7	0
0%	9	2	5	1	5	4	9	1
0%	10	2	8	3	6	2	9	0
0%	11	2	3	3	6	7	5	4
0%	12	2	7	3	5	3	4	0
0%	1	3	6	1	3	4	11	0
0%	2	3	4	1	6	3	11	0
0%	3	3	8	0	9	1	5	0
0%	4	3	7	5	7	3	2	0
0%	5	3	7	1	3	10	12	0
0%	6	3	7	2	3	1	7	0
0%	7	3	7	2	7	4	12	0
0%	8	3	5	2	9	2	9	0
0%	9	3	5	1	4	2	4	0
0%	10	3	7	1	6	2	7	0
0%	11	3	2	3	10	5	3	1

Table A.8. Continued

0%	12	3	6	2	5	2	4	0
3%	1	1	6	8	5	7	2	3
3%	2	1	9	8	4	3	6	5
3%	3	1	9	7	9	5	4	4
3%	4	1	5	5	4	4	6	4
3%	5	1	5	5	4	4	6	4
3%	6	1	8	8	5	5	5	5
3%	7	1	9	7	9	4	4	5
3%	8	1	2	9	4	2	3	0
3%	9	1	7	8	12	2	3	4
3%	10	1	8	8	5	4	5	4
3%	11	1	8	10	8	6	9	10
3%	12	1	9	8	5	6	4	5
3%	1	2	5	7	3	7	4	1
3%	2	2	9	6	10	5	9	5
3%	3	2	9	5	5	5	3	4
3%	4	2	8	7	9	7	6	4
3%	5	2	10	5	6	10	10	3
3%	6	2	9	8	6	5	4	1
3%	7	2	8	9	8	5	6	3
3%	8	2	9	7	11	1	7	2
3%	9	2	10	6	11	1	1	3
3%	10	2	8	7	5	3	7	2
3%	11	2	9	7	8	3	6	6
3%	12	2	10	9	8	5	4	2
3%	1	3	8	7	5	7	3	5
3%	2	3	8	7	5	3	9	4
3%	3	3	9	7	8	6	5	4
3%	4	3	8	5	6	6	9	1
3%	5	3	13	7	4	12	9	5
3%	6	3	10	9	8	2	6	4
3%	7	3	10	8	5	4	7	2
3%	8	3	9	7	6	4	11	3
3%	9	3	10	8	6	2	10	3
3%	10	3	8	7	10	7	8	2
3%	11	3	9	7	8	8	6	5
3%	12	3	9	9	8	5	7	3
6%	1	1	9	9	5	8	2	10
6%	2	1	10	10	6	7	3	10
6%	3	1	9	10	10	6	5	6

Table A.8. Continued

6%	4	1	9	10	9	5	7	10
6%	5	1	7	7	5	5	5	6
6%	6	1	12	12	7	5	5	5
6%	7	1	13	11	12	3	3	8
6%	8	1	10	9	10	8	5	6
6%	9	1	12	10	4	6	5	9
6%	10	1	8	10	6	5	7	5
6%	11	1	13	10	12	10	9	11
6%	12	1	10	9	8	8	7	8
6%	1	2	8	9	5	8	2	4
6%	2	2	9	9	8	5	5	9
6%	3	2	9	9	10	7	3	7
6%	4	2	9	10	5	5	10	12
6%	5	2	12	11	7	12	10	11
6%	6	2	11	9	10	5	5	7
6%	7	2	10	10	3	4	2	7
6%	8	2	9	8	7	3	5	5
6%	9	2	14	9	14	3	2	12
6%	10	2	9	10	7	5	5	4
6%	11	2	10	9	5	9	7	8
6%	12	2	11	10	6	10	6	8
6%	1	3	10	8	5	5	1	10
6%	2	3	10	9	6	7	6	9
6%	3	3	9	10	7	7	4	7
6%	4	3	8	11	6	8	9	9
6%	5	3	10	6	5	9	10	10
6%	6	3	9	9	7	3	7	7
6%	7	3	11	10	4	4	4	6
6%	8	3	10	9	5	6	6	8
6%	9	3	13	9	14	5	5	7
6%	10	3	9	10	10	6	6	7
6%	11	3	10	10	12	5	5	7
6%	12	3	12	10	6	10	4	5
9%	1	1	10	10	5	5	1	13
9%	2	1	11	12	8	9	1	14
9%	3	1	10	12	10	8	6	10
9%	4	1	9	11	5	3	9	9
9%	5	1	11	10	5	6	2	10
9%	6	1	12	12	9	10	8	10
9%	7	1	14	13	5	7	0	11

Table A.8. Continued								
9%	8	1	11	11	8	4	5	8
9%	9	1	13	14	9	10	8	13
9%	10	1	10	10	7	6	5	10
9%	11	1	13	13	12	5	6	11
9%	12	1	12	11	8	9	8	13
9%	1	2	10	10	5	5	1	13
9%	2	2	10	10	10	6	4	13
9%	3	2	10	10	15	8	3	12
9%	4	2	10	12	7	5	8	13
9%	5	2	14	13	3	13	9	13
9%	6	2	10	12	7	8	5	8
9%	7	2	11	13	10	5	5	11
9%	8	2	11	9	9	5	9	8
9%	9	2	14	13	8	2	5	14
9%	10	2	10	11	8	7	5	7
9%	11	2	12	12	8	7	6	11
9%	12	2	13	12	5	11	3	13
9%	1	3	13	11	9	7	0	14
9%	2	3	11	11	14	10	1	15
9%	3	3	9	12	11	9	4	10
9%	4	3	8	12	6	8	8	11
9%	5	3	11	12	5	13	5	14
9%	6	3	8	11	4	8	7	10
9%	7	3	11	13	13	9	1	12
9%	8	3	11	11	6	9	5	11
9%	9	3	14	14	12	6	8	13
9%	10	3	11	11	5	4	6	9
9%	11	3	10	13	11	10	11	10
9%	12	3	11	12	9	14	2	13

Appendix 9. SAS Output for Sensory Analysis

A.9.1. Crust Color

Class Level Information

Class	Levels	Values
Run	3	1 2 3
Panelist	12	1 2 3 4 5 6 7 8 9 10 11 12
Trt	4	0 0.03 0.06 0.09

Number of Observations

Number of Observations Read	144
Number of Observations Used	144
Number of Observations Not Used	0

Covariance Parameter Estimates

Cov Parm	Group	Estimate
Panelist		0.2570
Panelist*Trt		0.8463
Residual	Trt 0	2.2190
Residual	Trt 0.03	3.2221
Residual	Trt 0.06	1.5183
Residual	Trt 0.09	1.5348

Fit Statistics

-2 Res Log Likelihood	552.1
AIC (smaller is better)	564.1
AICC (smaller is better)	564.7
BIC (smaller is better)	567.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	27.9	40.47	<.0001

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	0	5.9722	0.3919	40.3	15.24	<.0001
Trt	0.03	8.3333	0.4260	39.2	19.56	<.0001
Trt	0.06	10.1111	0.3662	30.6	27.61	<.0001
Trt	0.09	11.0833	0.3668	30.5	30.21	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Trt	0	0.03	-2.3611	0.5405	36.5	-4.37	<.0001	Tukey-Kramer	0.0009
Trt	0	0.06	-4.1389	0.4948	29.9	-8.36	<.0001	Tukey-Kramer	<.0001
Trt	0	0.09	-5.1111	0.4953	29.9	-10.32	<.0001	Tukey-Kramer	<.0001

A.9.1. Continued

Trt	0.03	0.06	-1.7778	0.5222	27.2	-3.40	0.0021	Tukey-Kramer	0.0103
Trt	0.03	0.09	-2.7500	0.5227	27.3	-5.26	<.0001	Tukey-Kramer	<.0001
Trt	0.06	0.09	-0.9722	0.4752	21.4	-2.05	0.0533	Tukey-Kramer	0.1959

Coefficients for
Linear on Trt

Effect	Trt	Row1
Intercept		
Trt	0	-3
Trt	0.03	-1
Trt	0.06	1
Trt	0.09	3

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Linear on Trt	1	30.4	118.03	<.0001
Quadratic on Trt	1	30.3	3.72	0.0631
Cubic on Trt	1	28	0.02	0.8934

A.9.2. Crumb Color

Class Level Information

Class	Levels	Values
Run	3	1 2 3
Panelist	12	1 2 3 4 5 6 7 8 9 10 11 12
Trt	4	0 0.03 0.06 0.09

Dimensions

Covariance Parameters	3
Columns in X	5
Columns in Z	60
Subjects	1
Max Obs Per Subject	144

Number of Observations

Number of Observations Read	144
Number of Observations Used	144
Number of Observations Not Used	0

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	508.42724685	
1	1	482.24237824	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Panelist	0.06075
Panelist*Trt	0.8709
Residual	1.1181

Fit Statistics

-2 Res Log Likelihood	482.2
AIC (smaller is better)	488.2
AICC (smaller is better)	488.4
BIC (smaller is better)	489.7

Solution for Fixed Effects

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		11.6389	0.3297	43.7	35.30	<.0001
Trt	0	-9.4722	0.4553	33	-20.81	<.0001
Trt	0.03	-4.3611	0.4553	33	-9.58	<.0001

A.9.2. Continued

Trt	0.06	-2.1667	0.4553	33	-4.76	<.0001
Trt	0.09	0	.	.	.	.

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Linear on Trt	1	33	452.11	<.0001
Quadratic on Trt	1	33	20.92	<.0001
Cubic on Trt	1	33	4.03	0.0530

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	33	159.02	<.0001

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	0	2.1667	0.3297	43.7	6.57	<.0001
Trt	0.03	7.2778	0.3297	43.7	22.08	<.0001
Trt	0.06	9.4722	0.3297	43.7	28.73	<.0001
Trt	0.09	11.6389	0.3297	43.7	35.30	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Trt	0	0.03	-5.1111	0.4553	33	-11.23	<.0001	Tukey-Kramer	<.0001
Trt	0	0.06	-7.3056	0.4553	33	-16.05	<.0001	Tukey-Kramer	<.0001
Trt	0	0.09	-9.4722	0.4553	33	-20.81	<.0001	Tukey-Kramer	<.0001
Trt	0.03	0.06	-2.1944	0.4553	33	-4.82	<.0001	Tukey-Kramer	0.0002
Trt	0.03	0.09	-4.3611	0.4553	33	-9.58	<.0001	Tukey-Kramer	<.0001
Trt	0.06	0.09	-2.1667	0.4553	33	-4.76	<.0001	Tukey-Kramer	0.0002

A.9.3. Crumb Cell Uniformity

Class Level Information

Class	Levels	Values
Run	3	1 2 3
Panelist	12	1 2 3 4 5 6 7 8 9 10 11 12
Trt	4	0 0.03 0.06 0.09

Dimensions

Covariance Parameters	3
Columns in X	5
Columns in Z	60
Subjects	1
Max Obs Per Subject	144

Number of Observations

Number of Observations Read	144
Number of Observations Used	144
Number of Observations Not Used	0

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	687.61026712	
1	2	669.09489588	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Panelist	1.7038
Panelist*Trt	0
Residual	5.5729

Fit Statistics

-2 Res Log Likelihood	669.1
AIC (smaller is better)	673.1
AICC (smaller is better)	673.2
BIC (smaller is better)	674.1

Solution for Fixed Effects

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		8.0833	0.5448	28.7	14.84	<.0001
Trt	0	-2.0000	0.5564	129	-3.59	0.0005
Trt	0.03	-1.3333	0.5564	129	-2.40	0.0180
Trt	0.06	-0.6389	0.5564	129	-1.15	0.2530
Trt	0.09	0	.	.	.	.

A.9.3. Continued

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	129	4.83	0.0032

Coefficients for Cubic on Trt

Effect	Trt	Row1
Trt	0.09	1

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Linear on Trt	1	129	14.48	0.0002
Quadratic on Trt	1	129	0.00	0.9719
Cubic on Trt	1	129	0.00	0.9623

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	0	6.0833	0.5448	28.7	11.17	<.0001
Trt	0.03	6.7500	0.5448	28.7	12.39	<.0001
Trt	0.06	7.4444	0.5448	28.7	13.67	<.0001
Trt	0.09	8.0833	0.5448	28.7	14.84	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Trt	0	0.03	-0.6667	0.5564	129	-1.20	0.2331	Tukey-Kramer	0.6291
Trt	0	0.06	-1.3611	0.5564	129	-2.45	0.0158	Tukey-Kramer	0.0736
Trt	0	0.09	-2.0000	0.5564	129	-3.59	0.0005	Tukey-Kramer	0.0026
Trt	0.03	0.06	-0.6944	0.5564	129	-1.25	0.2143	Tukey-Kramer	0.5976
Trt	0.03	0.09	-1.3333	0.5564	129	-2.40	0.0180	Tukey-Kramer	0.0829
Trt	0.06	0.09	-0.6389	0.5564	129	-1.15	0.2530	Tukey-Kramer	0.6604

A.9.4. Firmness

Class Level Information

Class	Levels	Values
Run	3	1 2 3
Panelist	12	1 2 3 4 5 6 7 8 9 10 11 12
Trt	4	0 0.03 0.06 0.09

Dimensions

Covariance Parameters	3
Columns in X	5
Columns in Z	60
Subjects	1
Max Obs Per Subject	144

Number of Observations

Number of Observations Read	144
Number of Observations Used	144
Number of Observations Not Used	0

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	672.12490773	
1	1	639.72197978	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Panelist	2.0272
Panelist*Trt	0.4961
Residual	4.0486

Fit Statistics

-2 Res Log Likelihood	639.7
AIC (smaller is better)	645.7
AICC (smaller is better)	645.9
BIC (smaller is better)	647.2

Solution for Fixed Effects

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		7.5278	0.5681	24.1	13.25	<.0001
Trt	0	-3.5556	0.5546	33	-6.41	<.0001
Trt	0.03	-2.6667	0.5546	33	-4.81	<.0001\

A.9.4. Continued

Trt	0.06	-1.2222	0.5546	33	-2.20	0.0346
Trt	0.09	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	33	16.02	<.0001

Coefficients for Cubic on Trt

Effect	Trt	Row1
Trt	0.09	1

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Linear on Trt	1	33	47.68	<.0001
Quadratic on Trt	1	33	0.18	0.6736
Cubic on Trt	1	33	0.20	0.6603

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	0	3.9722	0.5681	24.1	6.99	<.0001
Trt	0.03	4.8611	0.5681	24.1	8.56	<.0001
Trt	0.06	6.3056	0.5681	24.1	11.10	<.0001
Trt	0.09	7.5278	0.5681	24.1	13.25	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Trt	0	0.03	-0.8889	0.5546	33	-1.60	0.1185	Tukey-Kramer	0.3911
Trt	0	0.06	-2.3333	0.5546	33	-4.21	0.0002	Tukey-Kramer	0.0010
Trt	0	0.09	-3.5556	0.5546	33	-6.41	<.0001	Tukey-Kramer	<.0001
Trt	0.03	0.06	-1.4444	0.5546	33	-2.60	0.0137	Tukey-Kramer	0.0626
Trt	0.03	0.09	-2.6667	0.5546	33	-4.81	<.0001	Tukey-Kramer	0.0002
Trt	0.06	0.09	-1.2222	0.5546	33	-2.20	0.0346	Tukey-Kramer	0.1433

A.9.5. Yeasty Flavor

Class Level Information

Class	Levels	Values
Run	3	1 2 3
Panelist	12	1 2 3 4 5 6 7 8 9 10 11 12
Trt	4	0 0.03 0.06 0.09

Dimensions

Covariance Parameters	3
Columns in X	5
Columns in Z	60
Subjects	1
Max Obs Per Subject	144

Number of Observations

Number of Observations Read	144
Number of Observations Used	144
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	692.35364706	
1	1	667.13419336	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Panelist	0.6538
Panelist*Trt	2.6863
Residual	4.2778

Fit Statistics

-2 Res Log Likelihood	667.1
AIC (smaller is better)	673.1
AICC (smaller is better)	673.3
BIC (smaller is better)	674.6

Solution for Fixed Effects

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		5.0000	0.6302	41.6	7.93	<.0001
Trt	0	2.0556	0.8279	33	2.48	0.0183
Trt	0.03	0.9444	0.8279	33	1.14	0.2622
Trt	0.06	0.3333	0.8279	33	0.40	0.6898
Trt	0.09	0	.	.	.	.

A.9.5. Continued

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	33	2.38	0.0870

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Linear on Trt	1	33	6.70	0.0142
Quadratic on Trt	1	33	0.44	0.5111
Cubic on Trt	1	33	0.01	0.9329

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	0	7.0556	0.6302	41.6	11.20	<.0001
Trt	0.03	5.9444	0.6302	41.6	9.43	<.0001
Trt	0.06	5.3333	0.6302	41.6	8.46	<.0001
Trt	0.09	5.0000	0.6302	41.6	7.93	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Trt	0	0.03	1.1111	0.8279	33	1.34	0.1887	Tukey-Kramer	0.5435
Trt	0	0.06	1.7222	0.8279	33	2.08	0.0453	Tukey-Kramer	0.1806
Trt	0	0.09	2.0556	0.8279	33	2.48	0.0183	Tukey-Kramer	0.0814
Trt	0.03	0.06	0.6111	0.8279	33	0.74	0.4656	Tukey-Kramer	0.8810
Trt	0.03	0.09	0.9444	0.8279	33	1.14	0.2622	Tukey-Kramer	0.6674
Trt	0.06	0.09	0.3333	0.8279	33	0.40	0.6898	Tukey-Kramer	0.9776

A.9.6. Autumnberry Flavor

Class Level Information

Class	Levels	Values
Run	3	1 2 3
Panelist	12	1 2 3 4 5 6 7 8 9 10 11 12
Trt	4	0 0.03 0.06 0.09

Dimensions

Covariance Parameters	3
Columns in X	5
Columns in Z	60
Subjects	1
Max Obs Per Subject	144

Number of Observations

Number of Observations Read	144
Number of Observations Used	144
Number of Observations Not Used	0

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	572.26438983	
1	1	548.24935278	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
Panelist	0.6094
Panelist*Trt	0.6395
Residual	1.9722

Fit Statistics

-2 Res Log Likelihood	548.2
AIC (smaller is better)	554.2
AICC (smaller is better)	554.4
BIC (smaller is better)	555.7

Solution for Fixed Effects

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		11.3889	0.3986	33.7	28.57	<.0001
Trt	0	-11.1944	0.4649	33	-24.08	<.0001

A.9.6. Continued

Trt	0.03	-7.7778	0.4649	33	-16.73	<.0001
Trt	0.06	-3.6111	0.4649	33	-7.77	<.0001
Trt	0.09	0	.	.	.	.

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	3	33	220.05	<.0001

The Mixed Procedure

Coefficients for Cubic on Trt

Effect	Trt	Row1
Trt	0.09	1

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Linear on Trt	1	33	659.28	<.0001
Quadratic on Trt	1	33	0.09	0.7693
Cubic on Trt	1	33	0.79	0.3810

Least Squares Means

Effect	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|
Trt	0	0.1944	0.3986	33.7	0.49	0.6288
Trt	0.03	3.6111	0.3986	33.7	9.06	<.0001
Trt	0.06	7.7778	0.3986	33.7	19.51	<.0001
Trt	0.09	11.3889	0.3986	33.7	28.57	<.0001

Differences of Least Squares Means

Effect	Trt	Trt	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Trt	0	0.03	-3.4167	0.4649	33	-7.35	<.0001	Tukey-Kramer	<.0001
Trt	0	0.06	-7.5833	0.4649	33	-16.31	<.0001	Tukey-Kramer	<.0001
Trt	0	0.09	-11.1944	0.4649	33	-24.08	<.0001	Tukey-Kramer	<.0001
Trt	0.03	0.06	-4.1667	0.4649	33	-8.96	<.0001	Tukey-Kramer	<.0001
Trt	0.03	0.09	-7.7778	0.4649	33	-16.73	<.0001	Tukey-Kramer	<.0001
Trt	0.06	0.09	-3.6111	0.4649	33	-7.77	<.0001	Tukey-Kramer	<.00

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