Efficacy of Odor Scavengers in Reducing Odor Compounds in Water, Milk, and Soymilk

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ABSTRACT

Odor detection thresholds of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were determined in spring water, high temperature short time (HTST) 2% fat milk, and extended shelf life soymilk. The efficacy of odor scavenger's β-cyclodextrin, D-sorbitol, and nylon 6 in removing these odors was also determined. The odor thresholds of the different odor and media combinations were as follows: hexanal in spring water, milk, and soymilk were 585, 339, and 536 ppb respectively; 2-heptenal in spring water, milk, and soymilk were 2,092, 2,322, and 3,184 ppb respectively; 2-pentanone in spring water, milk and soymilk were 24,925, 29,255 and 33,271 ppb respectively; and 2,4-nonadienal in spring water, milk, and soymilk were 164, 326, and 243 ppb respectively. These amounts reference the initial spiked concentration that was added directly to the media. Both hexanal and 2,4-nonadienal had lower thresholds than 2-heptenal and 2-pentanone in all of the media. The odor detection thresholds of 2-heptenal, 2-pentanone, and 2,4-nonadienal did show a significant difference between soymilk and water, but not for milk. The efficacy of the odor scavengers were determined by use of solid phase micro-extraction gas chromatography (SPME-GC) and sensory evaluation. Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were spiked at 1,000, 3,000, 30,000, and 300 ppb respectively in all three media. β-cyclodextrin, D-sorbitol, and nylon 6 were added at a level of 0.1% w/v and 1.0% w/v. In all of the media, β-cyclodextrin was found to significantly reduce hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal at both 0.1% w/v and 1.0% w/v. Nylon 6 was not found beneficial.

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CHAPTER 1

INTRODUCTION

Milk is an extremely complicated entity comprised of lipids, proteins, carbohydrates, and minerals. Over 400 volatile compounds in milk products have been identified (Walstra and Jenness 1984). The combination of these volatiles results in both favorable and unfavorable flavors and aromas in the end product. These volatiles can develop as a result of thermal processing, storage, autooxidation of lipids, light exposure and packaging migration (Valero and others 2001). This particular literature review will focus on changes in aroma due to different forms of oxidation in milk and soymilk.

Oxidation is detrimental to the quality of most foods. The result of oxidation is the production of secondary compounds such as aldehydes, ketones, and alcohols. Theses secondary products can produce unfavorable flavors and aromas in foods. Studies have identified packaging, processing, and storage techniques that would lower or eliminate the formation of these compounds (Azzara and Campbell 1992; Friedrich and Acree 1998; Shaw and others 1984; Wenzel and Lankmayr 2000). However, oxidation can still occur in the product. Therefore antioxidants, oxygen scavengers, and odor scavengers have been identified for efficacy in lowering or completely removing the volatiles that cause the odors in products (Feigenbaum and others 1998; Gavara and others 1997; Larson 1997; Robertson 1993).

This study was conducted in three studies. During the first study the odor thresholds of hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal were determined in spring water, 2% fat high temperature short time (HTST) milk, and extended shelf life soymilk. This information assisted in determining the level in which to spike the media in the second and third phases. SPME-GC was also used to determine recovery levels in all sample combinations.

The second study was to determine the efficacy of β-cyclodextrin, D-sorbitol, and nylon 6 as odor scavengers for the 12 combinations of odors and media in phase one. The scavenger

combinations that were found to significantly different from the original odor concentration would be used in the third study of this study.

The final study was to determine by sensory evaluation if panelists could determine a difference in odors when the scavengers β-cyclodextrin, D-sorbitol, and nylon 6 were added. Only the odor/medium/scavenger combinations that were found significantly different in phase two were used in this study. Fifteen odor triangle difference tests were conducted to determine the efficacy of the odor scavengers in spring water, milk, and soymilk.

CHAPTER II

REVIEW OF LITERATURE

Flavor and Aroma Acceptability of Milk Products

One of the most important factors in the consumer acceptance of a product is the flavor and aroma. It has been stated that there are three basic elements responsible for the flavor of milk: (1) pleasant mouth feel due to presence of macromolecules such as colloidal proteins and fat globules, (2) sweet and salty taste due to lactose and milk salts, respectively, and (3) a weak and delicate aroma due to numerous volatile compounds present at, near or below their odor threshold levels. Carbonyl compounds, alcohols, free fatty acids, and various sulfur compounds have been found to play important roles in fresh milk flavor (Cadwallader and Howard 1998).

No food or beverage product is immune to off-flavor or odor development. Milk is rather bland-tasting compared to many other foods and beverages. As a result, extremely low concentrations of contaminants can cause noticeable changes in the aroma and flavor, depending on the contaminant's chemical nature (Forss and Sugisawa 1981). Flavor and odor development in milk can result from several different factors. Thermal processing, especially ultra-high-temperature (UHT) heat processing, storage, autooxidation of lipids, light exposure, and package migration are some of the most significant causes. However this literature review will focus directly on the oxidation of lipids.

Light-induced off flavors and odors are the most common milk flavor defect (Frankel 1998). They have two distinct components. Initially, a burnt, active sunlight flavor develops and predominates for approximately two to three days. Degradation of sulfur-containing amino acids of whey proteins is likely responsible for this, called light-activated flavor.

A second light-activated flavor or odor is attributed to lipid oxidation. This off flavor is often characterized as metallic or "cardboardy", which usually develops after two days and does not

dissipate. Aldehydes, especially pentanal and hexanal, and to a lesser degree, ketones, alcohols and hydrocarbons form as a result of these lipid oxidation reactions (Akoh and Min 2002; Frankel 1998; Gunstone 1996).

Various malodorous carbonyl compounds are produced from light reacting with unsaturated fatty acids in milk fat triglycerides. Autooxidation of unsaturated fatty acids involves a free radical reaction, forming fat hydroperoxides that thermally degrade to various malodorous compounds, e.g., hexanal, the predominant lipid reaction byproduct produced from linoleic acid (Akoh and Min 2002).

Soymilk Acceptability

The use of soybeans as a source of food is increasing in the United States. The past two to three decades have seen an increase use of soy in food products in the United States. However, the present use of soy in food is only a small percent of its potential use. With the growing concern for minimizing health risks, the public is looking for alternatives to the use of animal proteins and products (Chang 1996; Chien and Snyder 1983). The nutritional climate would appear to be an opportunity for the soy industry to significantly increase utilization of soy in food (Wilkens and others 1969). However, the soy industry may not be totally prepared to meet the challenge. In the past, incorporation of soy into food products has resulted in undesirable changes in product color, flavor and texture (Chien and Snyder 1983; Emmert and Baker 1995; Kwok and others 2000).

Even with the reported health benefits of soybeans, the major factor of acceptance is the aroma and flavor. Soy products are known for the beany flavor and unpleasant odor. For this reason, the food industry is trying to find ways to improve the acceptability of soy products. Many methods for improving the flavor and aroma of soy milk start with the processing of the soybeans (Matsuura and others 1989; Wilkens and others 1967; Wilkens and others 1969).

Of the three functional attributes of soy (color, flavor, and texture), flavor has been regarded as the major roadblock to the increased use of soy in food. Hydroxy fatty acids present in soybeans

have been reported to be bitter (Chien and Snyder 1983). Storage of soy flour, concentrates, or isolates leads to increased product bitterness by an autooxidative process. Since bitterness can develop after inactivation of lipoxygenases and peroxidases during processing, ways to control the bitterness should be investigated. The main cause for the undesirable odor and flavor of soy milk is due to two types of lipid oxidation in the soy milk (Kwok and others 2000).

Lipoxygenase is found to be a key contributor to the production of off-flavors and odors in soy products. Lipoxygenase initiates the sequence of oxidative chemical reactions which lead to bitterness in soy. Inactivation of lipoxygenase, before or during crushing, would help prevent bitterness. This approach has been extensively reviewed and attempted (Forss and Sugisawa 1981; Iwuoha and Umunnakwe 1997). However with the inactivation of lipoxygenase, considerable storage protein denaturation was observed (Wilkens and others 1967). It has been shown that the only way to eliminate this problem is to develop soybean cultivars that lack the major lipoxygenase isozyme. Recent literature that looked at lipoxygenase-free cultivars noted the beans to have better flavor upon processing than the beans with normal lipoxygenase activity (Davies and others 1987; Iwuoha and Umunnakwe 1997; Kwok and others 2000; Torres-Penaranda and Reitmeier 2001). Lipoxygenase-free cultivars are being grown today, however research on the flavor or aroma of these cultivars has not been compared with the regular soybeans.

Unfortunately, lipoxygenase is not the only contribution to flavors and aromas in soybeans. Bitterness produced by autooxidation during storage cannot be eliminated by developing lipoxygenase-free cultivars since the lipid oxidation reactions involved are chemical rather than enzymatic (Iwuoha and Umunnakwe 1997). Soybeans are rich in polyunsaturated lipids such as linoleic and linolenic acids. Soybean oil contains about 55% linoleic acid and thus is susceptible to oxidation (Forss and Sugisawa 1981). Process-generated oxidation of unsaturated lipids could yield degradation products with a significant flavor impact, e.g., oxidative induced beany flavor (Kwok and others 2000).

Among the degradation products of polyunsaturated fatty acids, hexanal was reported to play the most important role in beany flavors, and hexanal comprised about 25% of the volatile

components in soymilk (Torres-Penaranda and Reitmeier 2001). According to Hashim and Chaveron (1998), hexanal had a strong grassy odor and when the content was above 250 ppb in soy milk, every panelist could detect its presence. Although hexanol possesses a harsh grassy and painty odor, its threshold is much higher than hexanal. Therefore, conversion of hexanal to hexanol is useful to decrease the intensity of off-flavors of soy products.

Lipid Oxidation

Lipids, proteins, and carbohydrates are the major structural components of food and are also the major source of flavors in foods. Lipids play a vital role in the metabolism of cells by providing a source of energy and reserve storage materials. As lipids oxidize, they form hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as aldehydes, ketones, acids and alcohols (Akoh and Min 2002; Eaton 1994). However, most volatiles that impart undesirable flavors are carbonyl compounds (Frankel 1998). There are many catalytic systems that can oxidize lipids. Among these are light, temperature, enzymes, metals, metalloproteins and microorganisms (Frankel 1998; Gunstone and others 1994).

Although there are several means for off-flavors and aromas to develop, there are two specific oxidative pathways: metal-induced and light-induced. Each of these causes of lipid oxidation results in severely different flavor and aromas that can be found as unpleasant. Metal-induced oxidation is detected by taste and, when intense, by odor. The descriptors for this off-flavor and odor include cardboard-like, metallic, painty, fishy, puckery and copper penny on tongue (rarely found) (Gunstone and Norris 1983; Land 1982). Light-induced processes in dairy products are mainly oxidations, which lead to formation of off-flavors, loss of nutrients, and formation of oxidation products, some of which are suspected to be toxic (Hashim and Chaveron 1998). Light-induced oxidation is also detected by smell and taste. The descriptors include burnt feathers, cabbagey, chemical-like odor and taste on front of tongue and, when strong, tastes like wet wood, tallowy (Azzara and Campbell 1992).

Milk is susceptible to formation of off-flavors by various mechanisms. The polyunsaturated acids oleic, linoleic, linolenic, and arachidonic are the most important precursors for the

formation of aldehyde compounds due to their prevalence in milk products (Forss and Sugisawa 1981). The unsaturated phospholipids of the membrane were reported to play a vital role in initiating oxidation of milk fat (Azzara and Campbell 1992). The action of lipid oxidation is due to the exposure of the lipids in the milk system to different variables such as light, metals, etc. Here listed are seven descriptors of off-flavors in milk based on causes: heated, lipolyzed, microbial, transmitted (from weed or feed), light-induced, oxidized, and miscellaneous (Shahidi 1997). The mechanism of lipid oxidation occurs in three steps: initiation, propagation, and termination.

Light induced off-flavor has two distinct components. Initially a burnt, activated sunlight flavor develops and predominates for about two or three days. Degradation of sulfur-containing amino acids of the whey proteins has been blamed for this reaction. The second component has been contributed to lipid oxidation (Marsili and Miller 1998). Light-induced off-flavors in milk can also be initiated by protein oxidation (Emmert and Baker 1995). Exposure of milk to light results in the development of off-flavors and causes the destruction of several key nutrients such as riboflavin, ascorbic acid and the essential amino acid methionine. Factors affecting the extent of off-flavor formation or nutrient loss include wavelength and intensity of light, duration of exposure, type of packaging materials, and the product temperature (Cadwallader and Howard 1998).

It has been reported that C6 to C10 alk-2-enals, butanone, pentanone, acetaldehyde, n-pentanal and n-hexanal are all formed in milk upon exposure to fluorescent light or sunlight (Cadwallader and Howard 1998). Some differences have been found between the volatile carbonyl compounds in milk due to either light-induced oxidation or autooxidation (metal-induced oxidation). Milk samples exposed to light show the presence of alkanals and 2-enals. These compounds are not present in autooxidized milk samples. Similarly, 2,4-dienals are present in autooxidation milk samples, but are not present in milk samples exposed to light. Based on the above evidence, several researchers have postulated that photoxidation involves the mono-ene fatty acids of the triglycerides while autooxidation involves the poly-ene of the phospholipids (Shipe 1980; Wishner 1964). However, hydroperoxides are formed by both the autooxidation or photo-

induced oxidation of fatty acids and are the principle source of off-flavors developed by lipid oxidation.

Oxidation of lipids is primarily dependent on the degree of unsaturation of their fatty acid constituents, but is also affected by other components present in the food matrix as well as conditions under which the product is stored. Hydroperoxides are the primary products of lipid oxidation, but hydroperoxides, despite their deleterious effects decompose readily to form a myriad of products such as aldehydes, ketones, alcohols, and hydrocarbons, among others. Some of these secondary oxidation products have threshold values in the parts per billion range and thus have a major impact on flavor deterioration of foods in which they are present (Eaton 1994; Shipe 1980). The rate of autooxidation increases with the degree of unsaturation. Linoleate is oxidized 10 times faster than oleate; linolenate 20 – 30 times faster. Hydroperoxides of unsaturated fatty acids formed by autooxidation are very unstable and break down into a wide variety of volatile and non-volatile flavor compounds.

Aldehydes are the most significant volatile flavor compounds produced during the breakdown of the alkoxy radicals (Shahidi 1997; Shipe 1980). Aldehydes can be produced by the scission of the lipid molecules on either side of the radical. The products formed by these scission reactions depend on the fatty acids present, the hydroperoxide isomers formed, and the stability of the decomposition products (Nawar 1996). Hexanal has long been used as an index of oxidative deterioration in foods. Hexanal is the primary oxidation product of linoleic acid. Some aldehydes, particularly the unsaturated aldehydes, are very potent flavor compounds.

Autooxidation is the direct reaction of molecular oxygen with organic compounds under mild conditions. Oxygen has a special nature in behaving as a biradical by having two unpaired electron (*O-O*) in the ground state and is said to be in a triplet state (Akoh and Min 2002). The oxidation of lipids proceeds like that of many other organic compounds by a free chain mechanism, which can be described in terms of initiation, propagation, and termination processes. These processes often consist of a complex series of reactions the first step in lipid oxidation. In this step a radical is formed due to the exposure to various variables.

Aliphatic aldehydes are the most important breakdown products of hydroperoxides because they are major contributors of unpleasant odors and flavors in food products (Frankel 1998). The polyunsaturated acids: oleic, linoleic, linolenic, and arachidonic are the most important precursors for the formation of aldehyde compounds due to their prevalence in milk products.

Sensory Evaluation for Odor Detection

Sensory analysis methods can be divided into four categories: sensitivity, quantitative, qualitative and affective (Bi and Ennis 1998). Sensory threshold is a measure of human sensitivity to a given stimulus. Determination of sensory threshold is an essential element in sensory analysis and is important today for a variety of purposes including the selection of panelists and the study of ingredient variation limits in products.

A sensory threshold can be defined generally as a stimulus intensity that produces a response in half of the trials (Bi and Ennis 1998). Thresholds are the limits of sensory capacities. There are several different thresholds that can be determined such as the absolute threshold, the recognition threshold, the difference threshold, and the terminal threshold (Meilgaard and others 1999). However, in this project the absolute threshold (detection threshold) will be used. The absolute detection threshold is the lowest stimulus capable of producing a sensation.

By finding the detection thresholds, scientists can determine at what level a chemical can be in a food without being detected. Detection thresholds can be used for level detection of odors in foods, such as secondary products developed from lipid oxidation in dairy products. Research has been conducted on the flavor detection threshold hold of acetaldehyde in milk with different fat levels (van Aardt and others 2001). The project looked at the effect of shelf life and light exposure on acetaldehyde concentrations when the milk was packaged in HDPE and PETE bottles (van Aardt and others 2001).

Considerable control has to be taken during threshold testing to result in accurate thresholds. Experience shows that with practice and training (Lawless and Heymann 1998) it is possible to

obtain reproducibility levels of ± 20% for a given panel and ± 50% between one large panel (>25) and another. The important factors, in addition to repeated training with the actual substance under test, is that subjects will pride themselves and hope to please the experimenter by finding the lowest threshold, and this must be counteracted by meticulous attention to the details of sample preparation and sample presentation so as not to leave clues to their identity (Bi and Ennis 1998).

Methods for Controlling Oxidation

Antioxidants. Antioxidants are substances which delay the onset or slow the rate of oxidation of oils thus preventing the formation of oxidation breakdown products. The disadvantage of antioxidants is that they merely slow oxidative rancidity not prevent it. Antioxidants can be divided into two categories: primary antioxidants and synergists (Larson 1997).

Primary antioxidants such as a phenolic type antioxidant, function as free radical acceptors forming stable resonance hybrid compounds that will not propagate further oxidation of the glyceride. Effectiveness of an antioxidant depends on its activation energy, rate constants, oxidation-reduction potential, ease of antioxidant loss or destruction and solubility (Larson 1997). Synergists such as citric, phosphoric, thiodipropionic, ascorbic and tartaric acids and lecithin, promote the action of the antioxidants but have little effect if present alone (Land 1982).

Packaging Interactions. Packaging plays a major role in the quality of foods. By using different materials for the packaging, the food is protected from exposure that could cause changes in the food. Plastic polymers, such as HDPE and PETE have been used in contact with food products for many years. Milk is currently packaged in HDPE containers and PETE is extensively used for carbonated soft drinks, mineral waters, beers, wines and other food products. There have been recent studies on the effects of different packaging materials on the flavor of milk products (van Aardt and others 2001).

Light-induced oxidation can be slowed with the use of colored or opaque packaging. By keeping light, oxygen, and odors out of the food product, the packaging can help to keep the product

fresh. However, consumers prefer milk containers that are translucent. Therefore the use of colored or opaque packaging may not be accepted by consumers. Trying to keep the packaging clear has lead to the development of active packaging. Active packaging usually consists of several layers. Different polymers are incorporated into the layers for specific purposes such as oxygen barriers, odor barriers, and ethylene barriers.

Odor Scavengers. The use of active packaging systems to selectively remove off-flavors and odor compounds and improve the flavor quality of foods is an area only recently being explored (Gavara and others 1997; Hernandez-Munoz and others 2001; Li and Paik 1996). However, now some researchers are looking into adding specific potential binding agents into the packaging system (that will interact with the odorous compounds within the milk system and headspace of the container). These odor scavengers would remove or decrease the volatiles by adsorption or absorption.

β–cyclodextrin has been shown to be a desirable food additive to remove bitter compounds and also to protect flavors, vitamins, and natural colors (Hedges 1998; Rekharsky and Inoue 1998). β–cyclodextrin has been used to selectively extract components from complex mixtures such as orange and grapefruit juice (Crini and others 1998; Mizobuchi and others 1981; Reinhardt and others 1995; Shaw 1990; Shaw and Wilson III 1983). Complexes produced using β–cyclodextrin resist oxidation, evaporation, degradation by UV and visible light, chemical attack and inter- or intra-molecular reactions. Many of these complexes are stable in solution as well as in the crystalline state.

Cyclodextrin polymers have potential for wide application in food processing because they separate molecules according to molecular size and shape. These polymers have excellent physical properties for use with aqueous solutions since they are easily wettable; they are stable when treated with dilute aqueous alkaline solutions that are commonly used in the food processing industry to clean equipment (Cserhati and others 2000). They are approved for food use because the polymers are made from naturally occurring carbohydrates that have very low toxicity (Cserhati and others 2000) .

Several factors can affect the selectivity of the polymer to form inclusion complexes with specific organic molecules. The choice of α-, β-, or γ-cyclodextrin for polymerization offers some selectivity because the size of the cavity is different for each of these forms of cyclodextrin. Cost is an important factor to be considered if large-scale studies are planned, because β-cyclodextrin is by far the least expensive of the three forms, α-cyclodextrin is second in cost, and γ-cyclodextrin is very expensive ($60 per gram for research quantities) (Shaw 1990). Thus, polymers from β-cyclodextrin seem to have the most potential for general commercial consideration.

β-cyclodextrin has been used for the debittering of orange juice and grapefruit juice samples. A pilot-plant study showed that a fluidized bed of β-cyclodextrin in a 7.6cm column could reduce the concentration of two bitter components (limonin and naringin) in grapefruit juice to an acceptable level (Shaw and others 1984). Both limonin and naringin were reduced enough in these juices to make them significantly preferred over the original juice (Shaw and others 1984; Shaw 1990; Shaw and Wilson III 1983).

In a study conducted by (Cserhati and others 2000) the binding characteristics of a water-soluble β-cyclodextrin polymer was determined by charge-transfer reverse phase thin layer chromatography. Also (Mizobuchi and others 1981) conducted a study on the sorption behavior of low molecular weight organic vapors on β-cyclodextrin resins. He found that β-cyclodextrin would be suitable as sorbents to collect polar volatile organic compounds.

Besides the mentioned studies, many patents have been found that have used cyclodextrins for the removal or odors in different products. The Proctor & Gamble Company has proposed several patents for the main purpose of odor removal with cyclodextrins. Such products include diapers, panty-liners, barrier films, trash bags, and other odor absorbing products (Bobo Jr. 1993; Brunner and others 1998; Sahar 2000; Sivik and others 2000; Trinh and Phan 1998; Wood and Beaverson 1997).

The scavenger ability of β–cyclodextrin is due to the shape of the molecule. This configuration gives a torus shaped molecule with the cavity being hydrophobic and the outer surface being

relatively hydrophilic. In the presence of water both the inner and outer surfaces hydrate. The hydrogen bonding of the water inside the cyclodextrin and the molecule itself causes distortion. The hydrated cyclodextrin molecule therefore is in a high energy state, and reduction of that energy by replacement of the water in the cavity is the secret of this product.

Sorbitol is another compound of interest to help reduce or eliminate odors in foods and the packaging that contains the foods. Sorbitol and other alcohols react with aldehydes and ketones in a reversible nucleophilic addition reaction. An acid catalyst protonates the carbonyl oxygen and subsequently eliminates water from the hemiacetal intermediate to produce an acetal. The acetal formed from sorbitol and other higher molecular weight alcohols is less volatile then the aldehydes and ketone reactant.

Sorbitol was used to develop an edible film for the coating of such products as gelatin capsules, sausage casings, or chocolate coatings (McNeely and Woodward 1993). Edible films made up of sorbitol and candelilla were found to be able to enhance the food quality by acting as moisture, gas, aroma, and lipid barriers and providing protection to the food product after the primary package is opened. Polyols such as sorbitol, have also been used in thermoplastic moldings in an effort to reduce the level of acetaldehyde resulting after processing of the thermoplastics (Sahar 2000; Schaper 1989).

Nylon 6 has been used as an addition to a polymer complex to help reduce the off-flavors and odors in primary packaging for food products. Patents have been shown to use polyamide active agents to act as a scavenger or flavor barrier (Bell and others 1998; Mills and Stafford 1993; Mills and Stafford 1994). In one patent, a polyester/polyamide blend consisted of a PET or PEN polyester component with a concentration of 99.5-98.0% w/w and a polyamide component with a concentration of 0.05-2% w/w (Mills and Stafford 1993). The polyamide component can be selected from: low molecular weight partially aromatic polyamides having an average molecular weight less than 15,000, low molecular weight aliphatic polyamides having an average molecular weight of less than 7,000, or wholly aromatic polyamides and mixtures thereof. The mechanisms by which the polyamide removes acetaldehyde and other byproducts generated by

ozonated liquids is believed to be by the nucleophilic addition of the free amino group on the polyamide to aldehydes or ketones to form imines (Mills and Stafford 1993).

Methods of Analysis

Methods that measure primary changes of lipids may be classified as those that quantify loss of reactants (unsaturated fatty acids), addition of oxygen or changes in iodine value or formation of primary lipid oxidation products which forms hydroperoxides. Since the quality of milk is a concern in the food industry, methods have been developed for flavor determination of dairy products. Flavor analysis of milk products has included mostly sensory evaluations and analytical analysis. Even though milk has been evaluated by its flavor, test methods for the detection of odors in milk are less common.

Analysis of the aroma of dairy products is a complex problem due to the heterogeneous nature of milk. Significant levels of lipids, proteins and carbohydrates in milk make it difficult to separate flavor-active chemicals based on general properties like polarity or volatility. Several analytical methods have been used to study the aroma of dairy products: gas chromatography-mass spectrometry (GC/MS) and gas chromatography-olfactometry (GC/O) are most commonly used (Marsili 1999; Marsili and Miller 1998; Morales and others 1998; Ney 1989). Identification of these compounds is carried out using extraction, distillation, concentration and chromatography.

Another method to isolate flavor compounds is headspace analysis. Static headspace analysis involved sampling air equilibrated above a food sample and injection into a gas chromatography for identification and quantification. This analysis can be restricted by the level at which the volatile compounds are present.

A relatively new variation of adsorption technique called solid phase micro-extraction (SPME) has been developed for the extraction of volatile and semi-volatile compounds from waste water samples (Yang and Peppard 1994). The SPME method is based on adsorption of analytes on a suitable fiber coated with a sorbent. This fiber can be immersed in the liquid sample or exposed

to the headspace above the sample. The adsorbed compounds can then be thermally desorbed in a GC injection port (Arthur and Pawliszyn 1990).

Although SPME was originally developed for the analysis of pollutants in water samples, it has been more recently applied for the analysis of volatile flavor compounds in foods and beverages. Caffeine in beverages was successfully determined by using SPME with an uncoated fused silica fiber (Hawthorne and others 1992). SPME-GC has also been used for the analysis of light induced lipid oxidation products in milk (Marsili 1999; van Aardt and others 2001). SPME provides many advantages over conventional sample preparation techniques. It is very simple, rapid, uses no solvent for extraction, are relatively low cost, and does not result in dilution of the volatiles (Marsili 1999; Yang and Peppard 1994).

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Chapter III

Odor Detection Thresholds for Hexanal, 2-Heptenal, 2-Pentanone, and 2,4-Nonadienal in Water, Milk, and, Soymilk

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A. ABSTRACT

Odor detection thresholds of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were determined in spring water, high temperature short time (HTST) 2% fat milk, and extended shelf life soymilk. These odors in food systems, particularly dairy products, are of great importance since they can cause a negative effect in the foods. Hexanal, 2-heptenal, 2-pentanone, and 2,4 – nonadienal have been found to result from lipid oxidation in foods with various fat contents, and can cause concern with the flavor and aroma of the food. Sensory odor detection threshold testing of all odor and medium combinations was duplicated using a panel of 12 untrained volunteers.

The odor detection thresholds of the different odor and media combinations were as follows: hexanal in spring water, milk, and soymilk were 585, 339, and 536 ppb respectively; 2-heptenal in spring water, milk, and soymilk were 2,092, 2,322, and 3,184 ppb respectively; 2-pentanone in spring water, milk and soymilk were 24,925, 29,255 and 33,271 ppb respectively; and 2,4-nonadienal in spring water, milk, and soymilk were 164, 326, and 243 ppb respectively. These values correspond directly to the spiked concentration of odor that was directly added to the media.

The odor detection thresholds of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal did show a significant difference when comparing the three media. There was a noticeable lower odor detection threshold when water was used as the medium. The odor detection thresholds increased slightly for milk and then again for soymilk. Solid phase micro-extraction (SPME) was verified as an effective method for recovery of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal in all media.

Key words: Lipid Oxidation, Milk, Odor, Solid Phase Micro-Extraction, Soymilk, Threshold

B. INTRODUCTION

The odor and flavor of dairy products greatly influence the acceptability of the product by consumers. An important factor concerning odor of dairy products is oxidation which occurs during processing or storage. Lipid oxidation has been recognized as a major factor in the quality of dairy products (Frankel 1998; Gunstone 1996). Lipid oxidation results in the formation of secondary aldehydes, ketones, and alcohols that contribute to odors in dairy products. Some of these volatiles include: C6 to C10 alk-2-enals, butanone, pentanone, acetaldehyde, n-pentanal and n-hexanal (Eaton 1994; Friedrich and Acree 1998; Mistry and Min 1992; Shipe 1980).

The level at which these secondary lipid oxidation products form are important for the quality of dairy products. Human thresholds are used to determine the acceptable level of secondary products that can be in the model system without being detected or cause a negative effect on the product. Currently flavor thresholds have been found for several aldehydes, ketones, and alcohols in various dairy products. The thresholds also can change according to different factors such as temperature, time, testing surroundings, and the range of characteristics of the panelists. Detection threshold can be defined as an energy level below which no sensation would be produced by a stimulus and above which a sensation would reach consciousness (Bi and Ennis 1998; Lawless and Heymann 1998).

The objectives of this study were to determine: 1) the odor threshold for hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal in high temperature short time (HTST) 2% fat milk, extended shelf life soymilk, and spring water; and 2) if the method of solid phase micro-extraction for hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal recovery was effective.

C. MATERIALS AND METHODS

High-temperature short-time (HTST) pasteurized milk (2% fat), extended shelf life soymilk, and natural spring water were used as the media for odor detection. An untrained panel of 12 people and a three-sample alternate forced choice test series was used for sensory analysis of all media. Quantification of hexanal, 2-heptenal, 2-pentanone, and 2,4 - nonadienal was completed on all media using solid phase micro-extraction method coupled with gas chromatography (SPME-GC).

(i) Media used

HTST freshly pasteurized milk with 2% fat was obtained from Valley Rich Dairy in Salem, VA. Extended shelf life Westsoy ™ plain soymilk was obtained from Kroger Co. in Blacksburg, VA. Kroger ™ spring water was obtained from Kroger Co. in Blacksburg, VA. The milk, soymilk, and water were bought on the day of sample preparation and stored in one liter amber-glass bottles until time of use.

(ii) Preparation of hexanal, 2-heptenal, 2-pentanone, and 2,4 - nonadienal spiked samples

Hexanal was obtained from Sigma (St. Louis, MO). Trans-2-heptenal (97%), 2-pentanone (99.5%), and trans, trans-2,4-nonadienal (90%) were obtained from Aldrich (Milwaukee, WI). HTST milk, soymilk, and water were used as testing media for odor threshold testing for all of the volatiles the day the media was purchased. All samples were spiked volumetrically, in geometric progression of concentration, with 10 concentrations of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal separately in each medium. The concentrations were different among volatiles due to the varying odor potency of each chemical. Hexanal concentrations for milk/soymilk/water were: 0; 50; 100; 200; 400; 800; 1,600; 3,200; 6,400; 12,800 ppb. 2-Heptenal concentration steps for milk/soymilk/water were: 0; 500; 1,000; 2,000; 4,000; 8,000; 16,000; 32,000; 64,000; 128,000 ppb. Spiked concentrations for 2-pentanone in

milk/soymilk/water were: 0; 10,000; 20,000; 40,000; 80,000; 160,000; 320,000; 640,000; 1,280,000; 2,560,000 ppb. 2,4-Nonadienal concentrations for milk/soymilk/water were: 0; 62.5; 125; 250; 500; 1,000; 2,000; 4,000; 8,000; 16,000 ppb. The samples were thoroughly mixed and stored at 4ºC in sealed amber glass containers with aluminum foil between the lid and the container until sensory testing.

(iii) Quantification of hexanal, 2-heptenal, 2-pentanone, and 2,4 –nonadienal in milk, soymilk, and water

Concentrations of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal spiked in the three media were determined. Six milliliters of samples were placed in 20 ml amber vials (Supelco, Inc Bellefonte, PA). A magnetic stir bar was placed in the amber vials, then the vials were capped with a phenolic cap that contained a PTFE/silicone septa (Supelco, Inc Bellefonte, PA). Samples were held at 4ºC until the next day when they were analyzed using headspace SPME-GC.

Before SPME-GC analysis, 2g of sodium chloride was added to the milk and soy milk samples to increase the partitioning of the volatiles from media containing fat (Page and Lacroix 1993; Zhang and others 1994). The samples were placed one at a time in a modular heating block that was positioned on top of a stirring hot plate. The SPME unit was clamped into position above the sample vial. A 75 µm Carboxen-PDMS (Poly-dimethylsiloxane) SPME fiber (Supelco, Inc Bellefonte, PA) was exposed to the static headspace about 1 cm above the surface of the samples for 20 minutes at 45ºC (± 1ºC). Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were adsorbed onto the exposed SPME fiber and then withdrawn from the septum and inserted into the injection port of the gas chromatograph. The injector temperature was 250ºC. The fiber was left exposed in the injection port for 15 minutes before removing to minimize the possibility of carryover.

Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were thermally desorbed in the injection port of a Hewlett Packard gas chromatograph (Model 5890A, Hewlett Packard, Avondale, PA) equipped with a HP 5895A ChemStation and a flame ionization detector. Separation was

completed on a HP-5 capillary column (25 m x 0.32 mm, 1.05 μm) (Supelco, Inc. Bellefonte, PA) with helium as the gas flow at 1.0 ml/min. The temperature program began at 50ºC for 0.5 min, then raised at 15ºC/min. to a temperature of 180ºC for 0.5 min. The temperature then was raised 20ºC/min. to a final temperature of 240ºC. All injections were made in the splitless mode. Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal identification and quantification were based on retention time and peak area results for the standard solutions using the method of an external standard (McNair and Miller 1998).

(iv) Sensory Aroma Threshold Testing

Sensory testing was completed on the three media and volatile compounds (hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal) for a total of 12 odor detection threshold tests. Each odor detection threshold test consisted of a three-sample alternate forced choice test series that was used with a panel of 12 people at a sample temperature of 4ºC. The study was repeated twice to verify that the thresholds were within 20% of each other (Lawless and Heymann 1998).

A panel of 12 people was randomly selected for each of the 12 aroma detection threshold tests. Panelists were seated in individual sensory booths. Panelists were presented with a warm up sample that was spiked at supra-threshold level of the odor in question (hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal). This was done to familiarize the panelists with the expected odor discrimination. The panelists were asked to fill out a human subjects consent form while waiting for the first tray of samples.

The panelists were presented with 10 three sample sets of triangle tests. Triangle sets were presented on three trays, with three, three, and four sample sets respectively. The samples were presented in rows of three with ascending concentrations starting at zero in the first row. The samples were randomized within rows and each numbered sample with randomly selected three-digit numbers. Each three-sample set included either two samples of un-spiked media and one spiked sample of one of the odors (hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal) or one un-spiked sample and two spiked samples, depending on the randomized order that was selected

on the Sample Preparation Guide. The panelists were informed to smell the samples and choose the sample that smelled "different" within each row of three samples.

The panelists were instructed to remove the lid and swirl the sample, then place the bottle under the nose and smell. The panelists were also instructed to rest their nose for a couple of minutes when getting fatigued. Panelists were not informed of the ascending concentration characteristics of the samples, although they might have acquired the knowledge by participating in several sensory panels (Lawless and Heymann 1998). However since 12 different combinations of different mediums and volatiles were used, the ascending order characteristics would be difficult to follow.

(v) Data evaluation

The aroma detection thresholds were interpreted in two ways, geometric mean threshold and logistic regression. The thresholds of the individual panelists were determined by taking the last incorrect concentration and the first correct concentrations (when at least three subsequent choices were correct) and calculating the geometric mean (Lawless and Heymann 1998). The geometric mean is the antilog of the last incorrect concentration and the first correct concentration's mean. The group threshold was calculated by taking the log of the mean of all the individual geometric means combined. Each of the sensory panels was duplicated. The group aroma detection thresholds were then compared to ensure that they were within 20% of each other as specified by Lawless and Heymann (1998). The panel responses for the determination of the 12 aroma detection thresholds were recorded as <-> which indicates the lack of detection in the spiked sample by the panelists, and <+> which indicates the detection of the given volatile in the spiked sample.

Logistic regression is a technique for predicting the probability of "success" as a function of some predictor variable. In this aroma detection threshold determination the concentration of the given volatile (hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal) in the specific medium (milk, soy milk, spring water) is the predictor variable and a correct identification of a spiked sample is a success.

Let x = the concentration of the volatile in the medium and let p(x) = the probability that a panelist correctly identified a sample that contained one of the given volatiles. The logistic regression model is:

$$P(x) = 1 / [1 + \exp(-\alpha-\beta x)]$$

where α and β are parameters that are estimated from the data. Data was analyzed using SAS (2002).

D. RESULTS AND DISCUSSION

Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal odor thresholds were measured in two different ways. By using logistic regression, the probability of "success" – the probability that the volatile-spiked samples will be identified correctly – as a function of volatile concentration in the medium can be predicted. By using the geometric mean approach, the concentration of volatile, below which the subject lack the sensitivity to detect the volatile in a sample, can be determined.

(i) Geometric Mean Approach

Individual aroma thresholds were calculated in each sensory test and, from this, the group threshold was estimated. Table 1 reports the group threshold of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal in the different media. The sensitivity of individual panelists varied shown by the minimum and maximum thresholds found in each group of samples. For example the minimum and maximum thresholds found for hexanal in water were 75 and 4,800 ppb respectively. This is considered normal due to the different sensitivities of panelists. There are many factors that can affect the detection of an odor. Age, sex, medications, and many other factors have been noted (Lawless and Klein 1991; Meilgaard and others 1999).

There was a significant difference in the odor thresholds in different media when comparing water with milk and soymilk. In general the panelists could detect the odor in water at a lower level than in milk or soymilk. For example, the group odor threshold for 2-pentanone in water was 24,925 ppb while in milk and soymilk it was 29,255 ppb and 33,271 ppb respectively. This same trend was found for 2-heptenal and 2,4-nonadienal with group thresholds for 2-heptenal being 2,092, 2,322, and 3,184 ppb and group thresholds for 2,4-nonadienal being 164, 326, and 243 ppb in water, milk, and soymilk respectively. This difference in odor thresholds can be due to the lack of fats, proteins and other components in water compared to milk and soymilk. Water by itself has a very bland aroma which would allow panelists to detect the odor faster. With other aromas in competition with the odor in question in the media milk and soymilk, the panelist would have a more difficult time identifying that particular odor. The only odor that did not follow this trend was hexanal. Panelists found a lower odor threshold of hexanal in milk (339 ppb) than in water (585 ppb). Even though the threshold testing was conducted in a controlled environment, sensory thresholds can vary due to testing circumstances.

The odor detection thresholds of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal in soymilk was found slightly higher. This is due to the overall aroma of the soymilk. Soymilk is becoming a popular alternative to regular dairy milk, however soymilk is not highly favored for its flavor or aroma (Torres-Penaranda and Reitmeier 2001; Wilkens and others 1967; Wilkens and others 1969). With all of the aroma compounds found in soymilk, the panelists were not able to pinpoint the odor in question until it was spiked at a much higher level than in both milk and spring water.

Both 2,4-nonadienal and hexanal had lower thresholds than 2-pentanone and 2-heptenal. This indicates that the general public would be more sensitive to lower concentrations of 2,4-nonadienal or hexanal in foods. This is particular important since hexanal is found to be a main source of oxidation odor and flavor in many fat containing products. It is noted by a grassy smell and taste.

2-Pentanone was found to have the highest odor detection thresholds. In Table 1, the odor detection thresholds of 2-pentanone were 24,925, 29,255 and 33,271 ppb in water, milk and

soymilk respectively. 2-pentanone has an alcohol-like smell that is not very overpowering in a medium as noted by the group thresholds. Therefore the concern of odors caused by 2-pentanone, which is a product of lipid oxidation, is not as high as the concern for hexanal which was detected at a much lower concentration by the panelists.

Table 1. Odor detection thresholds for hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal in water, milk, and soymilk as determined by the geometric mean approach

Medium/Volatile Combination	Group Threshold[1] (ppb)	Min/Max Individual Thresholds	% Variation between Replications
Hexanal in Water	585	75 / 4800	15.5
Hexanal in 2% Milk	339	25 / 4800	10.5
Hexanal in Soy Milk	536	75 / 2400	12.1
2-Pentanone in Water	24925	5000 / 120000	17.9
2-Pentanone in 2% Milk	29255	15000 / 480000	15.8
2-Pentanone in Soy Milk	33271	5000 / 240000	17.9
2-Heptenal in Water	2092	500 / 24000	13.9
2-Heptenal in 2% Milk	2322	750 / 48000	15.9
2-Heptenal in Soy Milk	3184	1500 / 12000	12.2
2,4-Nonadienal in Water	164	31 / 188	18.6
2,4-Nonadienal in Milk	326	94 / 3000	18.7
2,4-Nonadienal in Soy Milk	243	94 / 3000	5.6

[1]12 different panelists for each of two replications

Table 1 also shows variability in individual thresholds of panelists. Lawless and Heymann (1998) confirm that individuals have very different abilities to detect flavor compounds. Some panelist may not even be sensitive enough to detect the odor compounds at all. It is important, however, to still include these individuals in a group threshold test, since they are part of the general public and will also consume the product.

Since individual thresholds vary substantially, it can influence a group threshold a great deal. Therefore, valid threshold measurements require group threshold values with <20% variability between two replications (Lawless and Heymann 1998). The variability between the replicated odor detection threshold tests in this study can be found in Table 1. There was a noticeable

variability among the replicates in each of the threshold tests but all were less than 20%. This illustrates as Lawless and Heymann (1998) noted that individuals have very different abilities to detect odor compounds. All of the replications had varibilites below 20% so the odor detection thresholds were within the normal range of variability. Even though water had the lowest odor thresholds out of the three media, it did not exhibit a lower variability. This shows that panelists will have variability no matter what the odor or media may be.

(ii) Logistic Regression

Logistic regression and the geometric mean approach are both measuring detection threshold in two different ways. The geometric mean approach looks directly at the thresholds of each individual and uses those specific numbers to calculate the group thresholds. Logistic regression, however, predicts where a certain percentage of the panelists will correctly identify the four odors in each medium.

Figures 1 and 2 show the probability of correct identification of each odor (hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal) in all media. Lawless and Heymann (1998) suggests an arbitrary level of 50% above chance guessing for determining threshold when an alternative approach, such as logistic regression is used. This level is calculated by making use of Abbott's formula (Finney 1971):

$$\text{Adjusted proportion correct} = (\text{observed proportion} - \text{chance}) / (1 - \text{chance})$$

$$0.5 = (0.667 - 0.33) / (1 - 0.33)$$

The 50% above chance guessing for the triangle test thus requires 66.7% correct identification. For example, in soy mik, the logistic regression predicts that at a concentration of 1,021 ppb of hexanal, 66.7% of the panelists should be able to identify the soymilk that is spiked with hexanal.

The probability of correct identification of odor-spiked samples in all media at the threshold concentrations found when using the geometric mean approach is shown in Table 2. This means

that at the threshold level for hexanal in soymilk (536 ppb) 53.4% of the panelists used would be able to correctly identify a hexanal-spiked sample. Thresholds calculated using logistic regression were found to be higher than the geometric mean calculation for all the odors in question.

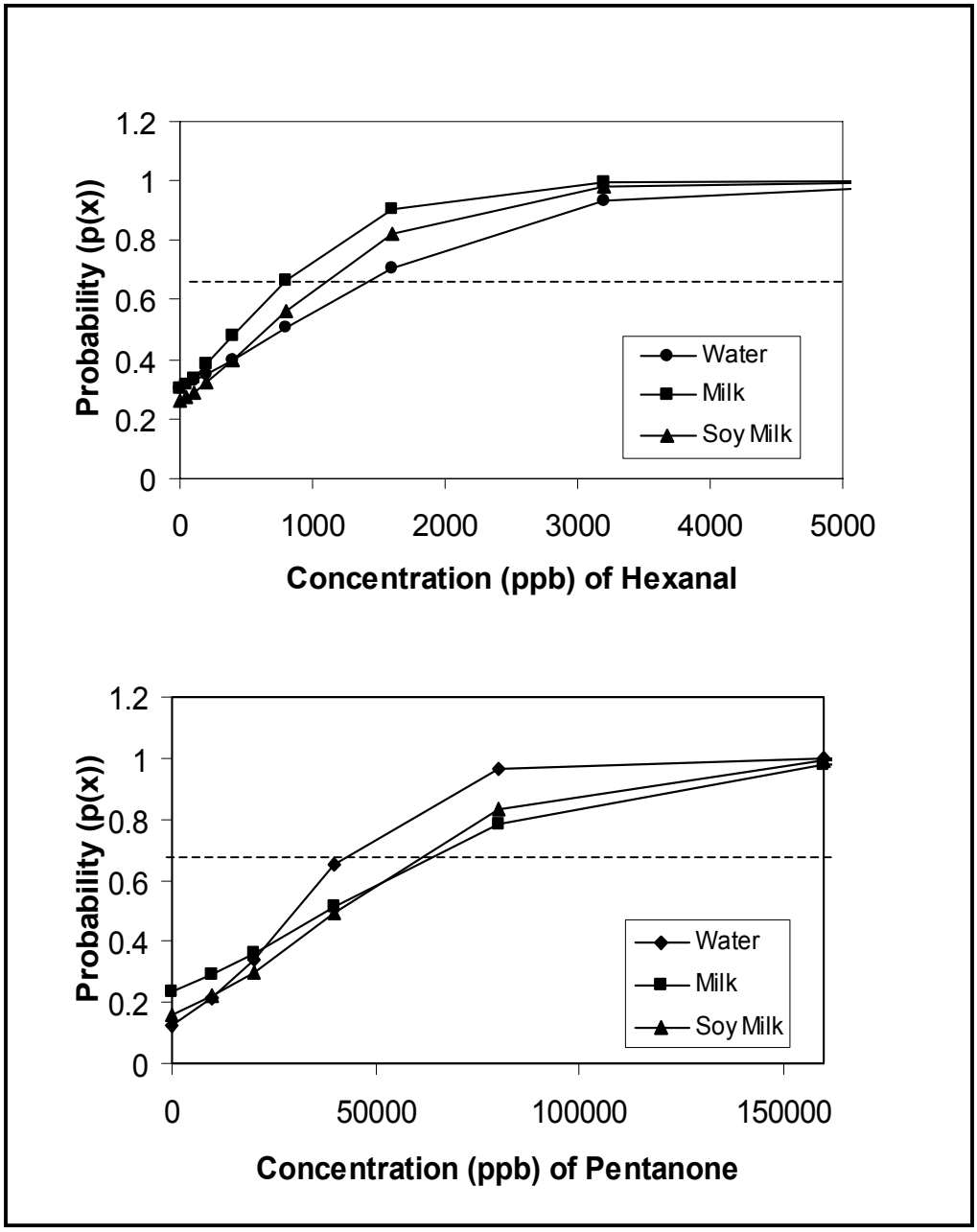

Figure 1. Probability of correct identification of hexanal and 2-pentanone at increasing concentrations using logistic regression analysis when $p(x) = 1 / [1 + \exp(-\alpha - \beta x)]$

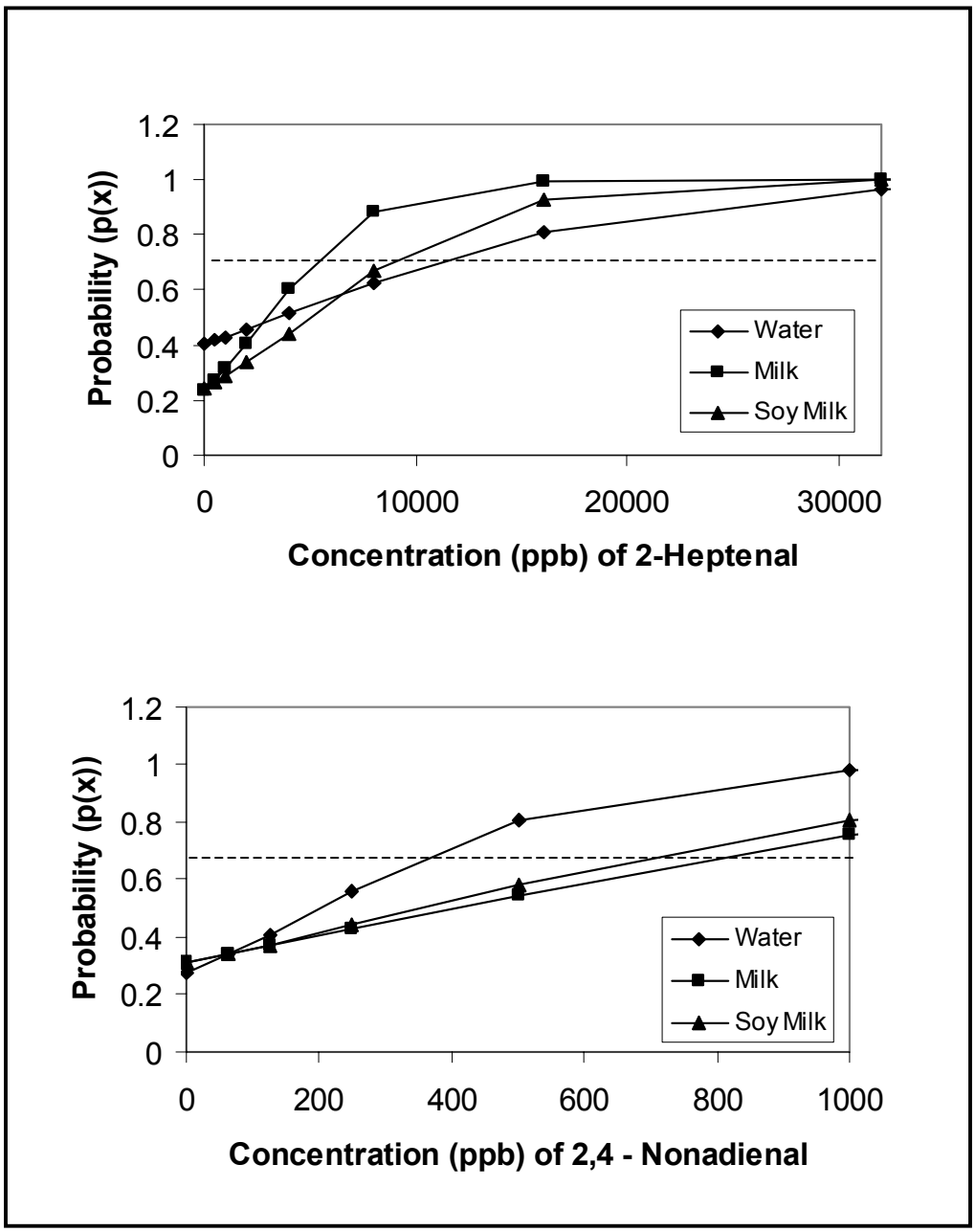

Figure 2. Probability of correct identification of 2-heptenal and 2,4-nonadienal at increasing concentrations using logistic regression analysis when $p(x) = 1 / [1 + \exp(-\alpha - \beta x)]$

Table 2. Predicted odor concentration of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal at probability of 66.7% using logistic regression as compared to group thresholds using geometric means

Odor	Medium	Predicted Thresholds[1] At p(x) = 0.667 (ppb)	Group Geometric Mean Threshold [2] (ppb)	Probability (%)[3] for Group Thresholds (ppb)
Hexanal	Spring water	1180	535	42.8
	Milk (2% fat)	856	321	40.1
	Soy milk	1021	620	53.4
Pentanone	Spring water	39,620	24,925	39.6
	Milk (2% fat)	51,853	29,255	43.2
	Soy milk	52,954	33,271	45.1
2-Heptenal	Spring water	4,174	1,752	43.9
	Milk (2% fat)	4,723	2,184	41.5
	Soy milk	3,953	3,184	43.6
2,4 – Nonadienal	Spring water	364	164	45.7
	Milk (2% fat)	642	274	43.3
	Soy milk	631	241	42.5

[1] Calculated at p(x) = .667 from logistic regression with $p(x) = 1 / [1 + \exp((-\alpha - \beta x)]$

[2] Calculated using geometric mean

[3] Probabilities calculated with $p(x) = 1 / [1 + \exp((-\alpha - \beta x)]$ when using group thresholds obtained from geometric mean approach

(iii) Solid phase micro-extraction

Solid phase micro-extraction coupled with gas chromatography (SPME – GC) is a solventless extraction technique that is simple, relatively cheap, and effective for isolating and detecting low levels of aroma compounds in foods and beverages (Marsili 1999). SPME has been used to isolate various aldehydes in nonfat and low fat milk (Marsili and Miller 1998; van Aardt and others 2001).

In this study SPME was used to verify the concentration of the four odor compounds from the headspace of water, milk, and soymilk. Calibration curves for hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal were based on peak area results for the standard solutions using the method of external standards (McNair and Miller 1998). The correlation coeffiecients from the calibration curves can be found in Table 3. The calibration curve of hexanal in spring water is in Figure 4.

In our study, solid phase micro-extraction method was an effective method for concentration identification of hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal from the headspace of water, milk, and soymilk. All correlation coefficients of the odors were above 0.924. There was no significant differences between the spiked concentrations and the recovery concentrations. Hexanal spiked in spring water at a concentration of 100 ppb was recovered at a concentration of 96 ppb. The retention times of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were 5.33, 7.67, 3.9, and 11.39 seconds respectively. Figure 3 show 2-pentanone in milk. As the concentration was increased, the peak area also increased.

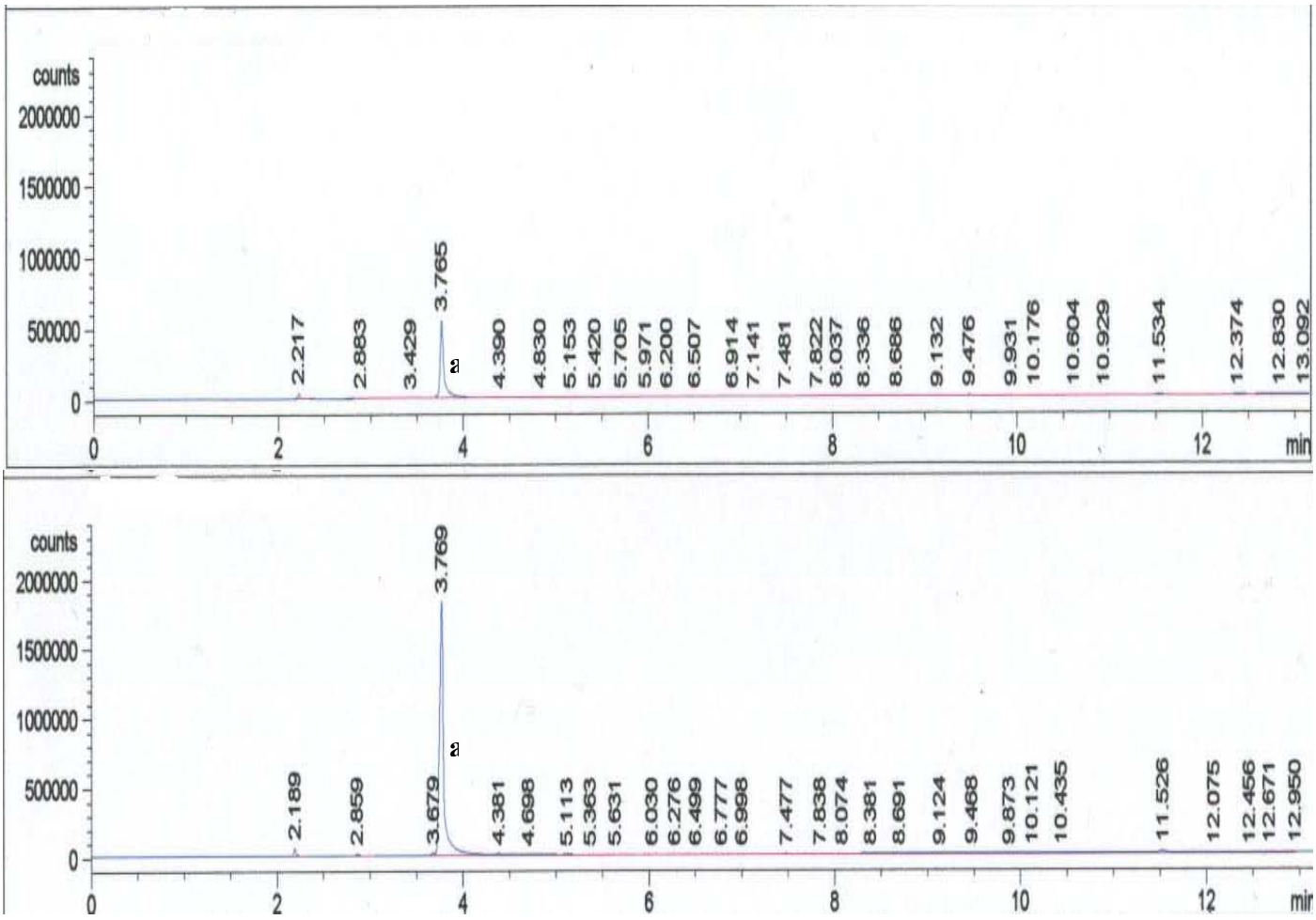

Figure 3. 2-Pentanone (a) in 2% fat milk at a concentration of 10,000 and 80,000 ppb respectively

Table 3. Hexanal recovery from spring water spiked with hexanal using solid phase micro-extraction concentration technique

Spiked Concentration (ppb)	Recovered Concentration (ppb ± sd)[1]
100	96 ± 5.5
200	211 ± 16.0
400	440 ± 18.2
800	834 ± 38.8
1600	1703 ± 21.0
3200	3184 ± 81.1
6400	6523 ± 196.5

[1] mean of 3 reps ± standard deviation

Table 4. Hexanal recovery from HTST (2% fat) milk spiked with hexanal using solid phase micro-extraction concentration technique

Spiked Concentration (ppb)	Recovered Concentration (ppb ± sd)[1]
200	192 ± 16.3
400	360 ± 26.5
800	831 ± 34.0
1600	1543 ± 22.4
3200	3095 ± 42.5
6400	6284 ± 109.2
9600	9765 ± 176.5

[1] mean of 3 reps ± standard deviation

E. CONCLUSION

Hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal had different odor threshold levels. Although soymilk had the highest threshold values for all of the odors, these were not significantly different from milk, but was significantly different from spring water. This could be due to the complex aromas in soymilk that resulted in the panelists having a harder time detecting the given odor. Spring water had overall lower threshold values for all of the odors. Water is usually very bland and normally does not exhibit a particular taste or odor. Therefore the panelist could more easily identify the odor at lower concentrations.

Both hexanal and 2,4-nonadienal had lower thresholds than 2-heptenal and 2-pentanone in all of the media. This would indicate that at lower concentrations, hexanal and 2,4-nonadienal could alter the aroma of water, milk or soymilk. 2-Pentanone had a very high group odor threshold in all three media. At a concentration of 29,255 ppb, 2-pentanone was detected in milk by panelists. However, this concentration is very high, and would probably not be found in water, milk, or soymilk. Therefore the reduction of 2-pentanone is not as much of a concern as the other odors.

Solid phase micro-extraction is an effective method for detecting low levels of odor compounds. It was an effective method for the recovery of hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal in spring water, milk, and soymilk in this study. The recovery of all odors was not significantly different from the known concentration.

F. ACKNOWLEDGEMENT

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Chapter IV

Efficacy of Potential Odor Scavengers in Reducing the Concentration of Hexanal, 2-Heptenal, 2-Pentanone, and 2,4 –Nonadienal in Water, Milk, and Soymilk

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A. ABSTRACT

Lipid oxidation is a concerning factor in the quality of foods. It results in the production of secondary volatiles that can cause off-flavors or odors in the food. Odor scavengers have recently been used to reduce or remove odors caused by lipid oxidation. One way to introduce these potential scavengers is to incorporate them into the packaging system. In this study, hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were the odors used as lipid oxidation products in spring water, milk, and soymilk. The odor scavengers used were β-cyclodextrin, D-sorbitol, and nylon 6. These potential odor scavengers are packaging compatible and would ultimately be added to a multilayered primary packaging system that would potentially scalp off-flavors and odors out of the foods and into the package.

The efficacy of the odor scavengers were determined by use of solid phase micro-extraction gas chromatography (SPME-GC) and sensory evaluation. Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were spiked at 1,000, 3,000, 30,000, and 300 ppb respectively in all three media. β-cyclodextrin, D-sorbitol, and nylon 6 were added to each of the combinations at a level of 0.1% and 1.0% w/v. In all media β-cyclodextrin was found to significantly reduce hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal at both 0.1% w/v and 1.0% w/v. In some cases, such as with 2-heptenal and 2,4-nonadienal in spring water, the concentrations were decreased by more than 50%.

The odor scavenger reduced the odor compounds in spring water more than in both milk and soymilk. This is due to the lack of low molecular weight compounds in water. Milk and soymilk have more flavor compounds that could also form a bond complex with β-cyclodextrin. With these other compounds bonding with β-cyclodextrin, β-cyclodextrin would not be so readily available to scavenge hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal . In water, these odors were free to complex with the β-cyclodextrin. However, β-cyclodextrin was still significant in reducing the odor compounds in all three media.

Key Words: Active Packaging, Hexanal, Lipid Oxidation, Milk, Odor, Scavengers, Solid Phase Micro-Extraction, Soymilk

B. INTRODUCTION

The odor and flavor of dairy products greatly influence the acceptability by consumers. An important factor concerning odor of dairy products is oxidation which occurs during processing or storage. Lipid oxidation has been recognized as a major factor in the quality of dairy products (Frankel 1998; Gunstone 1996). Lipid oxidation results in the formation of secondary aldehydes, ketones, and alcohols that contribute to odors in dairy products (Eaton 1994; Friedrich and Acree 1998; Mistry and Min 1992; Shipe 1980).

The use of odor scavengers in packaging has recently been developed for use in the food industry. Such compounds as β-cyclodextrin, D-sorbitol, and nylon 6 have been used as scavengers in juices and household items. β-Cyclodextrin has been used for the debittering of both orange juice and grapefruit juice in recent studies (Shaw and others 1984; Shaw 1990; Shaw and Wilson III 1983; Wagner and others 1988). D-sorbitol and nylon 6 have been used in the packaging industry as odor scavengers in items such as trash bags, panty-liners, plastic containers, etc. (McNeely and Woodward 1993; Quezada-Gallo and others 1999; Sahar 2000). With the scavenging abilities of these odor scavengers, they show potential in efficiently lowering the concentrations of low molecular weight compounds due to lipid oxidation in milk and soymilk.

The objectives of this study were to determine: (1) The efficacy of β-cyclodextrin, D-sorbitol, and nylon 6 as acting odor scavengers in significantly reducing the level of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal in spring water, milk, and soymilk by SPME-GC. (2) To determine if a sensory panel to detect the overall difference in samples with and without odor scavengers by means of triangle difference tests.

C. MATERIALS AND METHODS

High temperature short time (HTST) 2% fat pasteurized milk, extended shelf-life soy milk, and spring water were used as the media in this project. Efficacy of potential odor scavengers β-cyclodextrin, D-sorbitol, and nylon 6 was looked at to lower volatile compounds associated with lipid oxidation in dairy products (hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal).

(i) Media used

HTST milk with 2% fat was obtained from Valley Rich Dairy in Salem, VA. Extended shelf life Westsoy ™ plain soymilk was obtained from Kroger Co. in Blacksburg, VA. Kroger ™ spring water was obtained from Kroger Co. in Blacksburg, VA. The milk, soymilk, and water was bought on the same day of sample preparation and stored in one liter amber-glass bottles until time of use.

(ii) Preparation of hexanal, 2-heptenal, 2-pentanone, and 2,4 - nonadienal spiked samples

Hexanal was obtained from Sigma (St. Louis, MO). Trans-2-heptenal (97%), 2-pentanone (99.5%), and trans, trans-2,4-nonadienal (90%) were obtained from Aldrich (Milwaukee, WI). HTST milk, soymilk, and water samples were used as the media the same day purchased. All samples were spiked volumetrically at or above the group thresholds that were found in the previous study. Spiking the samples at or above threshold made sure that in this study, the odor in each combination would be detected by the panelists for the sensory overall difference triangle tests. Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were spiked at 1,000, 3,000, 30,000, and 300 ppb respectively. Spiked samples were stored at 4ºC in amber glass bottles until analysis.

(iii) Addition of odor scavengers (β-cyclodextrin, D-sorbitol, and nylon 6)

The potential odor scavengers β-cyclodextrin, D-sorbitol, and nylon 6 and were purchased from Aldrich (Milwaukee, WI). The odor scavengers were added individually to the spiked samples at levels of 0.1% and 1.0% w/v. The spiked samples were then stirred with a magnetic stir bar until dissolved. All samples were stored at 4ºC while being slowly shaken until the next day of analysis.

(iv) Quantification of hexanal, 2-heptenal, 2-pentanone, and 2,4 –nonadienal in milk, soymilk, and water with the addition of odor scavengers

Concentrations of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal spiked in HTST milk, soymilk, and spring water were determined in the presence of odor scavengers β-cyclodextrin, D-sorbitol, and nylon 6. A control (without the scavenger) was also prepared for comparison. Six milliliters of sample were placed in 20 ml amber vials (Supelco, Inc Bellefonte, PA). A magnetic stir bar was placed in the amber vials, then the vials were capped with a phenolic cap that contained a PTFE/silicone septa (Supelco, Inc Bellefonte, PA). Samples were held at 4ºC while being slightly shakened until the next day when it was analyzed using headspace SPME-GC.

Before SPME-GC analysis, 2g of sodium chloride was added to the milk and soy milk samples to increase the partitioning of the volatiles from mediums containing fat (Yang and Peppard 1994; Zhang and others 1994). The samples were placed one at a time in a modular heating block that was positioned on top of a stirring hot plate. The SPME unit was clamped into position above the sample vial. A 75 μm Carboxen-PDMS (Poly-dimethylsiloxane) SPME fiber (Supelco, Inc Bellefonte, PA) was exposed to the static headspace about 1 cm above the surface of the samples for 20 minutes at 45ºC (± 1ºC). Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were adsorbed onto the exposed SPME fiber and then withdrawn from the sample's septum and inserted into the injection port of the gas chromatograph. The injector temperature

was 250ºC. The fiber was left exposed in the injection port for 15 minutes before removing to minimize the possibility of carryover.

Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal were thermally desorbed in the injection port of a Hewlett Packard gas chromatograph (Model 5890A, Hewlett Packard, Avondale, PA) equipped with a HP 5895A ChemStation and a flame ionization detector. Separation was completed on a HP-5 capillary column (25 m x 0.32 mm, 1.05 μm) (Supelco, Inc. Bellefonte, PA) with helium as the gas flow at 1.0 ml/min. The temperature program began at 50ºC for 0.5 min, then raised at 15ºC/min. to a temperature of 180ºC for 0.5 min. The temperature was then raised 20ºC/min. to a final temperature of 240ºC. All injections were made in the splitless mode. Hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal identification and quantification were based on retention time and peak area results for the standard solutions using the method of an external standard (McNair and Miller 1998).

(v) Sensory Evaluation

A triangle test was done on all medium/odor/scavenger combinations at a temperature of 4ºC that were found significantly different by means of SPME-GC. This was to eliminate the work of sensory testing when no noticeable differences would be able to be detected by the panelists. Each sensory test consisted of a three-sample alternate forced choice test series that was used with a panel of 36 people.

A panel of 36 people was randomly selected for each of the 19 sensory tests. Panelists were seated in individual sensory booths. Panelists were asked to fill out a human consent form while waiting for the first tray of samples. The panelists were presented with a total of nine samples in one session. Five different sessions were completed. Triangle sets were presented on three trays, with one triangle set on each tray. The samples were randomized within rows and numbered with randomized three-digit numbers. Each three-sample set included either two samples without scavengers and one sample with a scavenger, or one sample without scavenger and two samples with a scavenger present, depending on the randomized order that was selected

on the sample preparation guide. The panelists were informed to smell the samples from left to right and choose the sample that smelled "different" within each row of three samples.

The panelists were instructed to remove the lid and swirl the sample, then place the bottle under the nose and smell. The panelists were also instructed to "rest" their nose in between testing of sample sets for a couple of minutes when getting fatigued. Panelists were not informed of the scavenger or odor in question, although they might have acquired the knowledge by participating in several sensory panels. However since 19 combinations of different mediums/volatiles/scavengers were used, the pattern of order would be difficult to follow.

(vi) Data Evaluation

The efficacy of the potential odor scavengers was determined in two ways: quantitatively by SPME-GC and by sensory evaluation. The data obtained from SPME-GC were analyzed by both a 3-way factorial design and a complete randomized design in SAS (2002). The Tukey-Kramer Honestly Significant Difference Test was used as a multiple comparison to find significant differences in the levels of odors with and without the addition of odor scavengers at an $\alpha \leq 0.05$.

Sensory evaluation was completed on all the sample combinations that were determined to be significantly different from the controls by SPME-GC. Thirty-six panelists were used in this project. The results were determined by the number of panelists that correctly identified the odd sample in the triangle test. Using Table T8 "Critical Number of Correct Responses in a Triangle Test" at a pre-selected $\alpha \leq 0.05$, the minimum number of correctly identified samples for a panel of 36 is 18 (Meilgaard and others 1999). Thus, for all sensory panels in which 18 or greater correct responses were found, the samples are considered to be significantly different from the control.

D. RESULTS AND DISCUSSION

Odor scavengers β-cyclodextrin, D-sorbitol, and nylon 6 were evaluated to effectively reduce the amounts of hexanal, 2-pentanone, 2-heptenal, and 2,4–nonadienal in spring water, milk, and soymilk. By using SPME-GC and sensory evaluation, the odor scavengers that significantly reduced the amount of volatiles in the three media were detected. Both methods of analysis (SPME-GC and sensory evaluation) found a significant decrease of hexanal, 2-pentanone, 2-heptenal, and 2,4–nonadienal in spring water, milk, and soymilk with the addition of the potential odor scavengers β-cyclodextrin, D-sorbitol, and nylon 6. However, SPME-GC was found to be more sensitive to the separation of treatments in order to show a reduction in odors than the human sensory panel.

(i) SPME-GC Evaluation

The use of odor scavengers was found to be effective for the reduction of hexanal, 2-pentanone, 2-heptenal, and 2,4–nonadienal in spring water, milk, and soymilk by evaluating the data from SPME-GC. Figures 1, 2, and 3 show the differences in concentration recovery in all combinations of samples.

The odor scavengers reduced the odor compounds in spring water more than in both milk and soymilk. This is due to the lack of low molecular weight compounds in water. Milk and soymilk have more flavor compounds that would also form a bond complex with β-cyclodextrin. With these other compounds bonding with β-cyclodextrin, the concentration of hexanal, 2-pentanone, 2-heptenal, and 2,4–nonadienal, that were spiked into the samples, would decrease in a less amount because of competition. In water, the spiked odors were free to complex with the β-cyclodextrin without competition. However, this odor scavenger was still significant in reducing the odor compounds in all three mediums.

In all media β-cyclodextrin was found to significantly reduce hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal at both 0.1% and 1.0% w/v. In some cases, such as with 2-heptenal and 2,4-nonadienal in spring water, the concentrations were decreased by more than 50%. The level of β-cyclodextrin did make a difference in the reduction of odor compounds. At a level of 1.0%

w/v the effectiveness of the scavenger was greater than at 0.1% w/v. By looking at the results it could be concluded that by adding even more β-cyclodextrin the concentration of the odors would keep decreasing. However, since β-cyclodextrin would ultimately be added into packaging system, the level of β-cyclodextrin would be determined by the surface area of interaction inbetween the food and the packaging system and also the packaging capacity for added β-cyclodextrin.

D-sorbitol was found to significantly reduce 2,4-nonadienal at 1.0% w/v in spring water, 2-heptenal and 2,4-nonadienal at both 0.1% and 1.0% w/v in milk, and 2,4-nonadienal at both 0.1% and 1.0% w/v in soymilk. D-sorbitol was not as effective as an odor scavenger as β-cyclodextrin in this study. The addition of either 0.1% or 1.0% w/v did not increase the effectiveness of the scavenger.

Nylon 6 only had a significant difference at a level of 1.0% w/v on hexanal in milk. Nylon 6 is a non-soluble polymer that has been used in polymer complexes to remove or absorb odors in industry (Brunner and others 1998; Hatzidimitriu and others 1987). Nylon 6 did show noticeable reduction in the odors of all combinations, but these were not found to be significant.

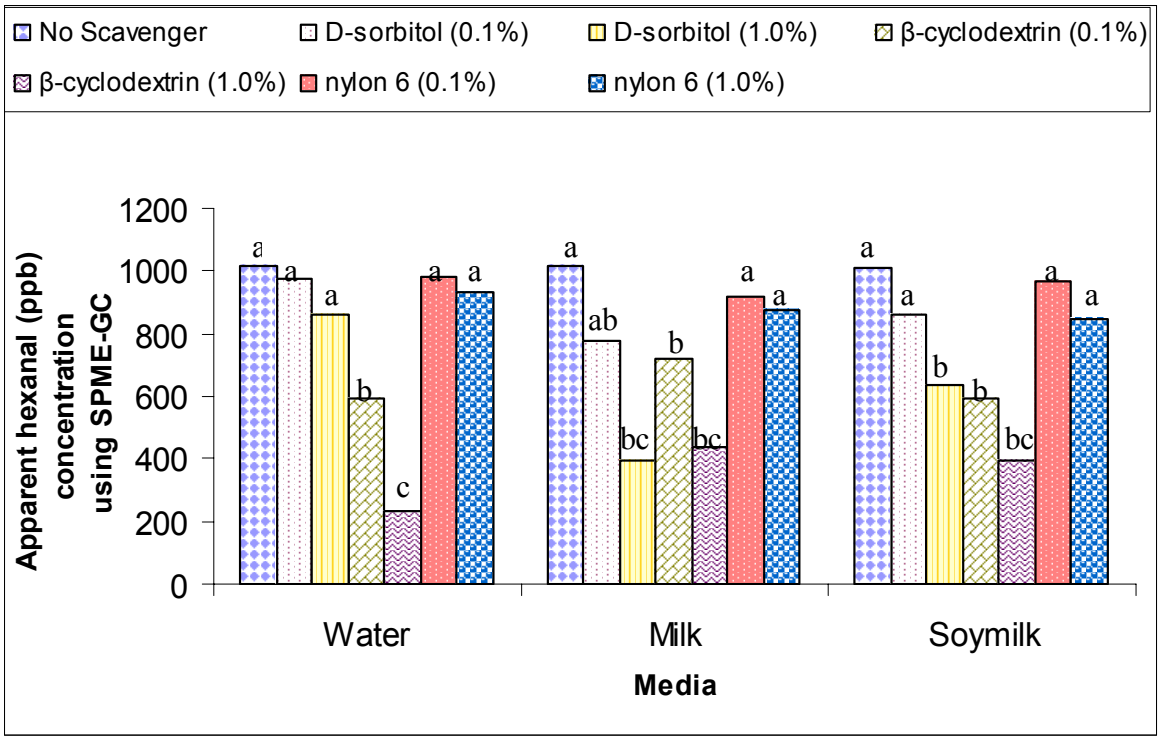

Figure 1. Apparent hexanal concentration (ppb) in the headspace of water, milk, and soymilk (originally spiked concentration of 1,000 ppb) in the presence of odor scavengers (0.1% and 1.0% w/v) $\alpha \geq 0.05$ as indicated by lower case letters.

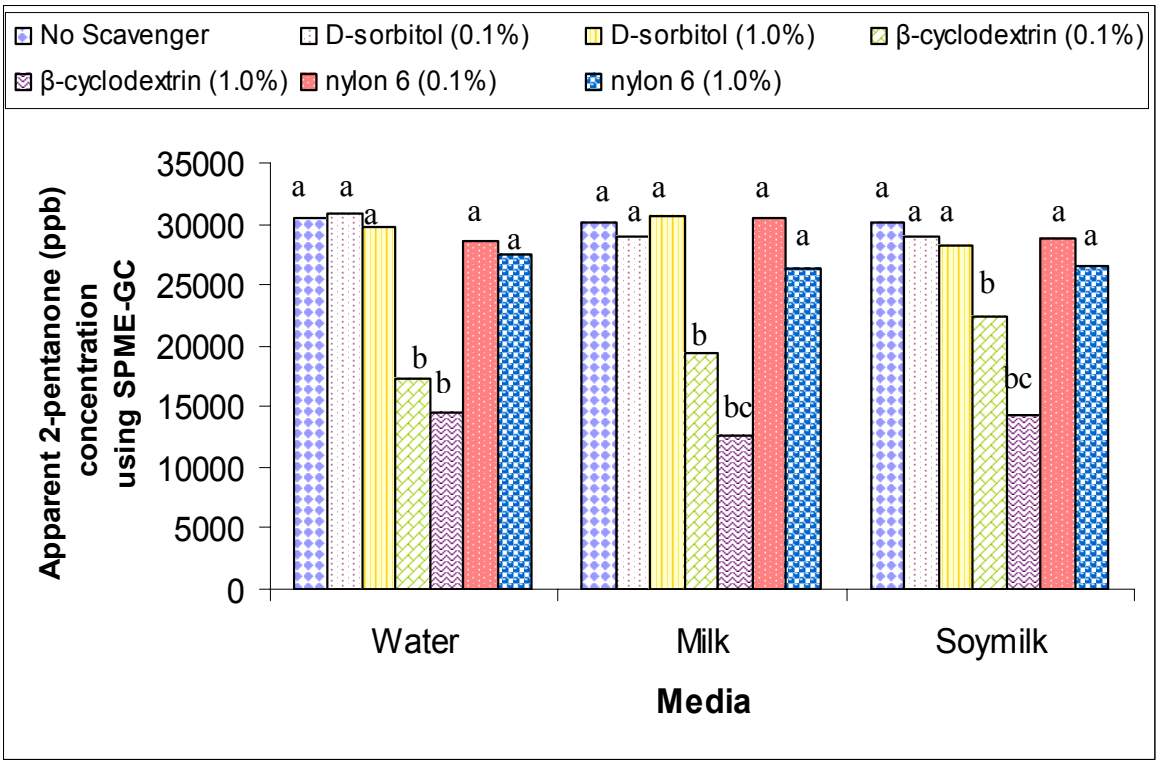

Figure 2. Apparent 2-Pentanone (ppb) concentration in the headspace of water, milk, and soymilk (originally spiked at 30,000 ppb) in the presence of odor scavengers (0.1% and 1.0% w/v) at $\alpha \geq 0.05$ as indicated by lower case letters.

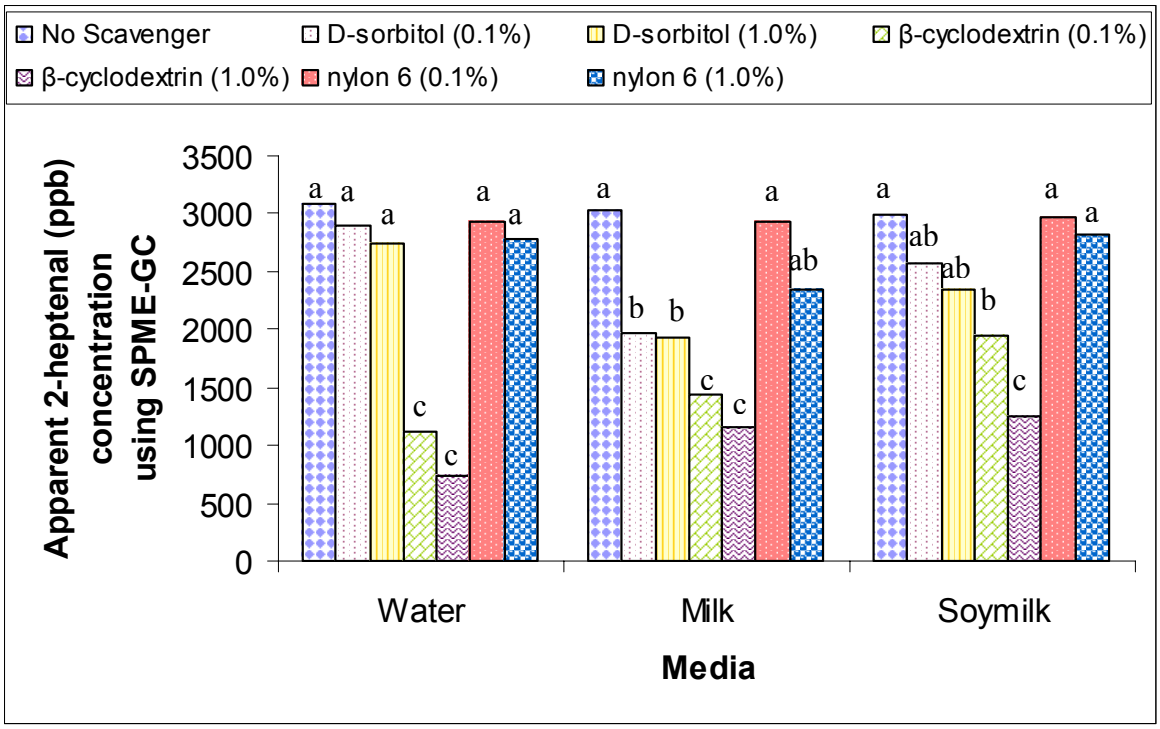

Figure 3. Apparent 2-Heptenal (ppb) concentration in the headspace of water, milk, and soymilk (originally spiked at 3,000 ppb) in the presence of odor scavengers (0.1% and 1.0% w/v) at $\alpha \geq 0.05$ as indicated by lower case letters.

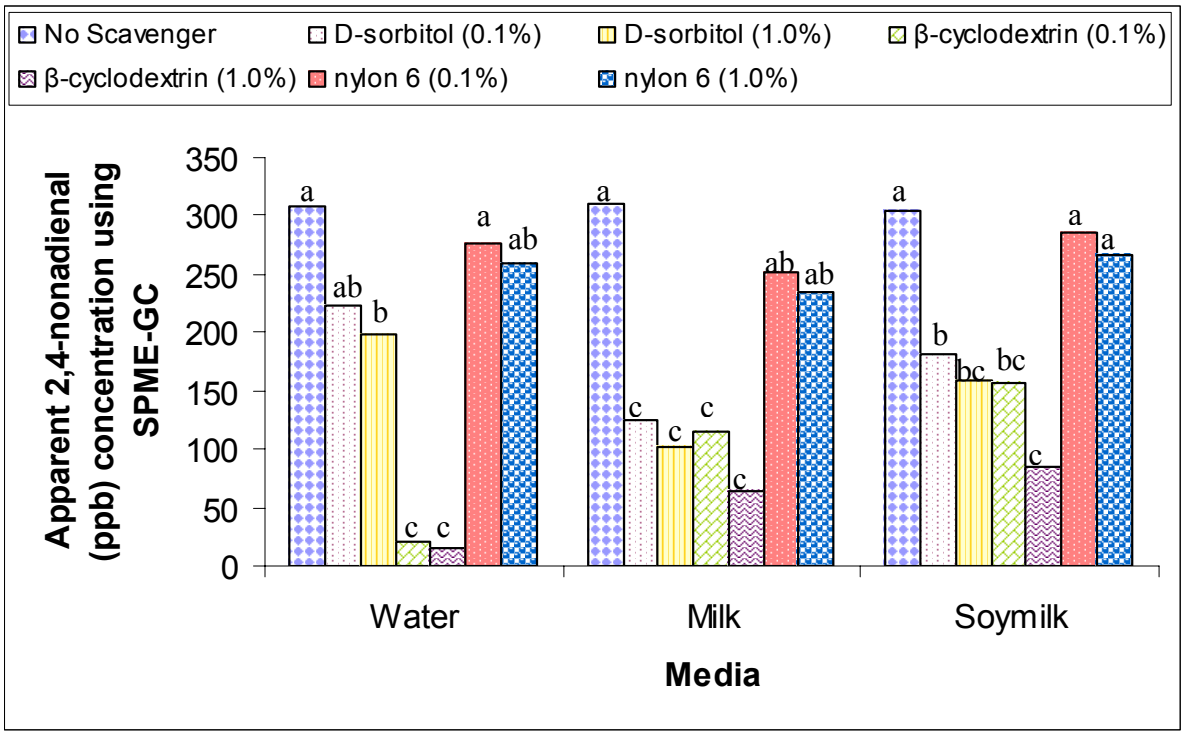

Figure 4. Apparent 2,4-Nonadienal (ppb) concentration in the headspace of water, milk, and soymilk (originally spiked at 300 ppb) in the presence of odor scavengers (0.1% and 1.0% w/v) at $\alpha \geq 0.05$ as indicated by lower case letters.

(ii) Recovery of Odors

With the three media used in this study, there were no significant differences with the three media used for recovery of odors. Figure 1 shows the apparent concentration (ppb) of hexanal in spring water, 2% fat HTST milk, and soymilk in the presence of the odor scavengers. The concentration of hexanal in the controls (no scavenger present) was 1029 ppb in water, 1009 ppb in milk, and 1012 ppb in soymilk. Salt was added to both milk and soymilk to help the partitioning of the odors into the headspace of the bottles. This addition of salt could have lead to no significant differences found in between water, milk, and soymilk.

The concentration of hexanal spiked in 2% fat HTST milk at 1,000 ppb in the presence of 1.0% w/v odor scavengers can be found in Figure 5. In this combination, β-cyclodextrin was the most effective scavenger for hexanal. In water the apparent hexanal concentration was reduced from 1029 ppb to 594 ppb with 0.1% w/v β-cyclodextrin and 263 ppb with 1.0% w/v β-cyclodextrin. This can be noted by the large reduction in the peak of hexanal in the figure. D-sorbitol also showed a decrease in the concentration of hexanal, however the difference is harder to see in the chromatograph shown in Figure 5.

The effects of odor scavengers can also be seen in Figures 2, 3, and 4. Figure 6 shows the apparent concentrations of 2-pentanone in 2% fat HTST milk in the presence of 1.0% w/v odor scavengers. The odor scavengers had similar effects as seen in both Figures 5 and 6. β-cyclodextrin again, was found have a significant difference on the concentration of 2-pentanone in milk.

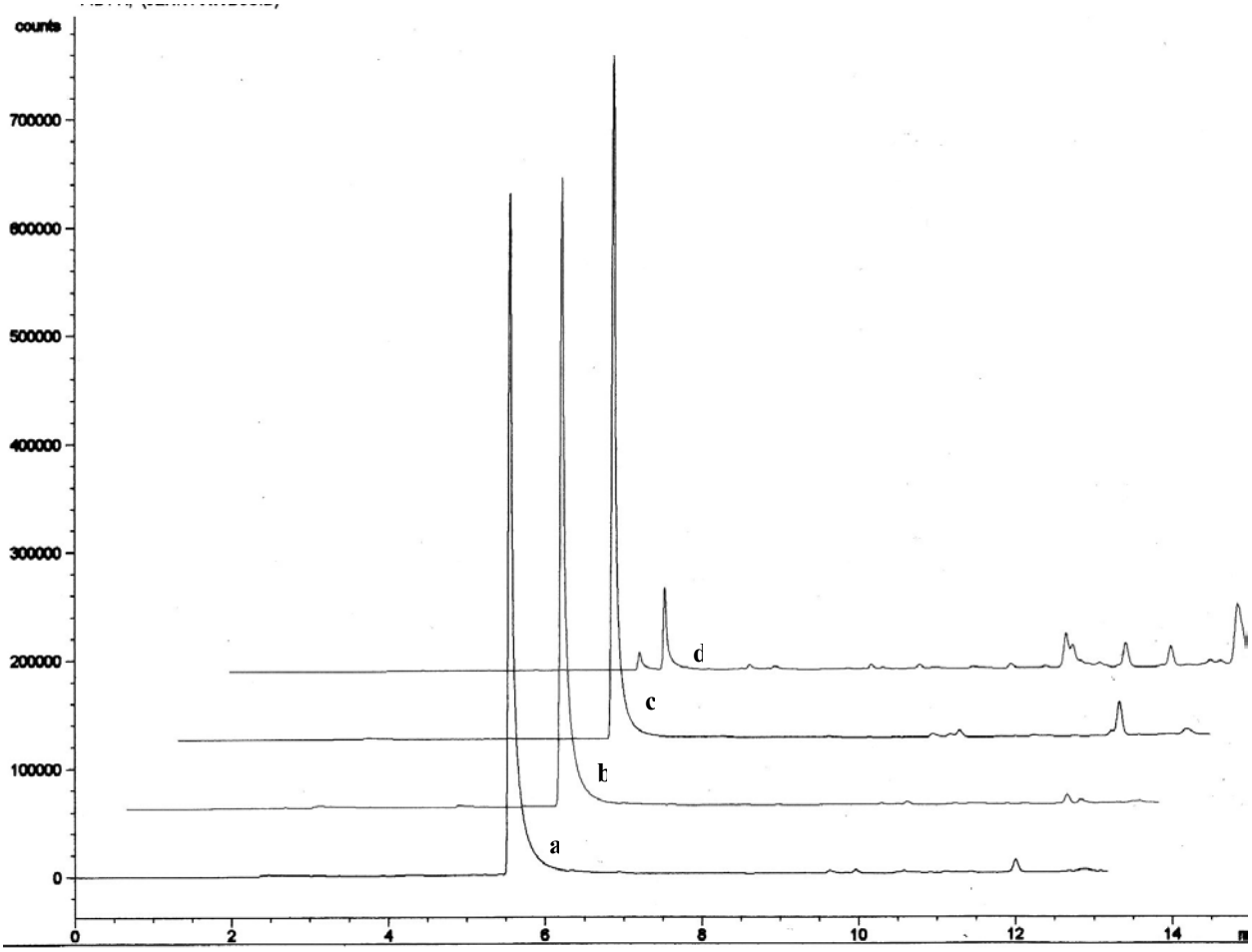

Figure 5. Hexanal spiked in HTST 2% fat milk at 1000 ppb ((a) no scavenger, (b) Nylon 6 added at 1.0% w/v, (c) D-sorbitol added at 1.0% w/v, and (d) β-cyclodextrin added at 1.0% w/v). Each peak is offset by twenty seconds for clarity.

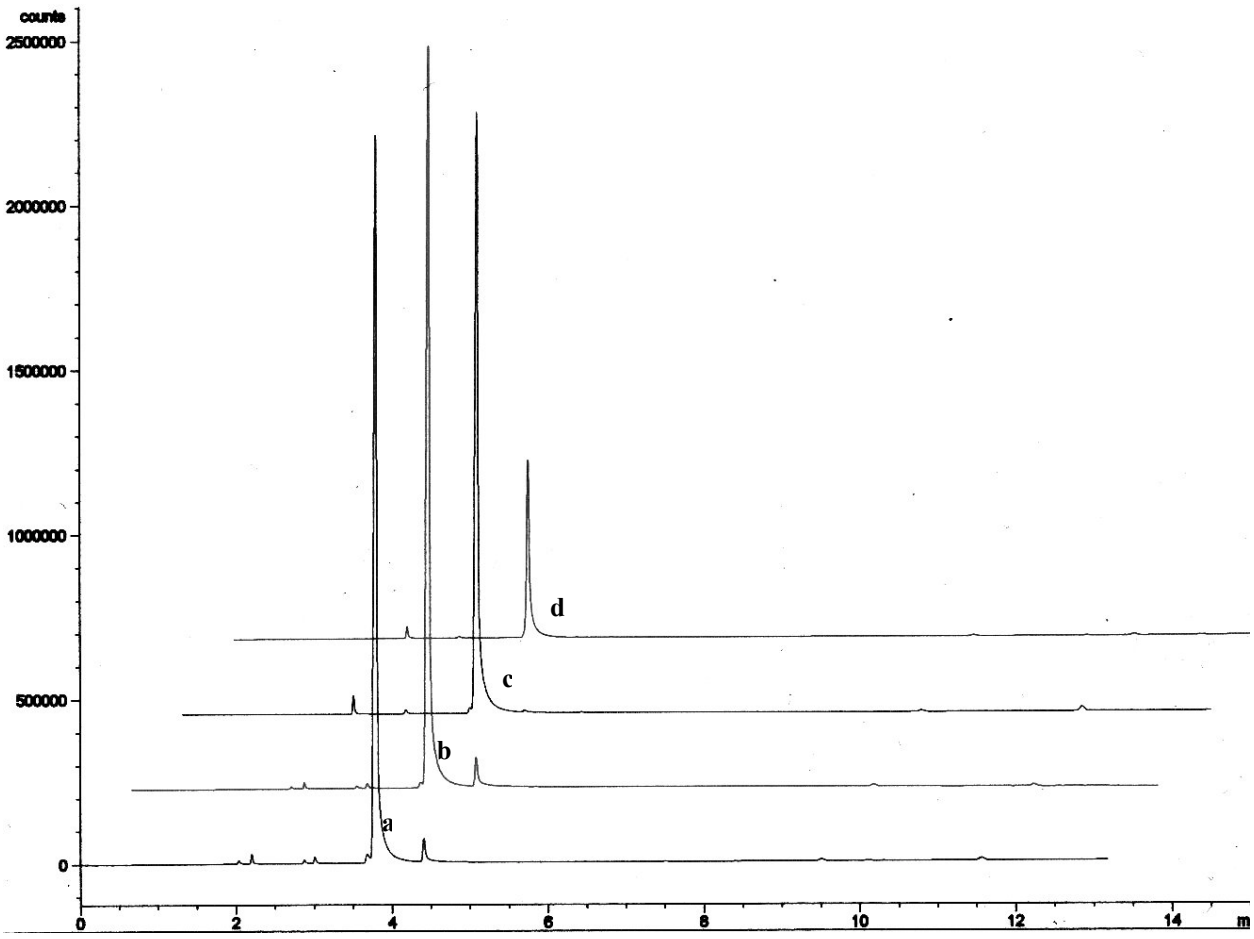

Figure 6. 2-pentanone spiked in HTST (2% fat) milk at 10,000 ppb ((a) no scavenger, (b) Nylon 6 added at 1.0% w/v, (c) D-sorbitol added at 1.0% w/v, and (d) β-cyclodextrin added at 1.0% w/v). Each peak is offset by twenty seconds for clarity.

(iii) Sensory Evaluation

Overall difference triangle tests were conducted on all the combinations of odor/medium/scavenger that were found to be significantly different by means of SMPE-GC. Nineteen triangle tests were completed and the results can be found in Table 1.

β-cyclodextrin was found significantly different in 10 out of the 19 combinations. In spring water, milk, and soymilk, β-cyclodextrin significantly reduced the concentration of hexanal, 2-pentanone, and 2-heptenal to an extent that was detectably different from the control. A small reduction of 2,4-nonadienal in all of these media was found, however it was not significant.

D-sorbitol was not as effective as β-cyclodextrin, however some significant differences were found. D-sorbitol was found to significantly reduce the concentration of hexanal, 2-pentanone, and 2-heptenal in milk. 2,4-nonadienal was reduced significantly in both spring water and soymilk.

There were noticeable differences in a few of the combinations involving nylon 6 as the scavenger. However nylon 6 did not significantly reduce any of the odors in spring water, milk, or soymilk. Due to this reason, nylon 6 was not used in the sensory evaluation of the scavenger efficacy.

The people that participated in the panel consisted mainly of members that participated in the threshold study of hexanal, 2-pentanone, 2-heptenal, and 2,4-nonadienal. Therefore they were familiar with the odors in this overall difference test. The panelists showed that they did not have the same sensitivity as SPME-GC when detecting differences in samples. Out of the 19 sensory triangle tests, five were found to be significantly different by the panelists. Panelists found that the addition of β-cyclodextrin in both hexanal spiked spring water and milk made a significant difference in the odor. They also found a significant difference in 2-pentanone spiked milk with the addition of both β-cyclodextrin and D-sorbitol. The final difference was found in 2-heptenal spiked soymilk with the addition of D-sorbitol. The use of a trained sensory panel could have found more significant differences between samples with scavengers. However,

since the overall goal of this study was to determine if the difference would be evident to the general public, a general panel was used.

Table 1. Odor/medium/scavenger combinations that were found to be significantly different at an $\alpha \leq 0.05$ from the control by SPME-GC

	Odor				Media		
Scavengers	hexanal	2-pentanone	2-heptenal	2,4-nonadienal	Spring water	milk	soymilk
β-cyclodextrin	X				X	X	X
β-cyclodextrin		X			X	X	X
β-cyclodextrin			X		X	X	X
β-cyclodextrin				X	X	X	X
D-sorbitol	X					X	X
D-sorbitol		X				X	
D-sorbitol			X			X	
D-sorbitol				X	X	X	X

Table 2. Sensory triangle overall difference test results of scavenger efficacy for odor removal

Sample Combination (odor/medium/scavenger)	Number of Sensory Panelists	Number of Correct Responses in the Triangle Sensory Test
hexanal/water/β-cyclodextrin	36	21*
hexanal/milk/β-cyclodextrin	36	18*
2-pentanone/milk/β-cyclodextrin	36	18*
2-pentanone/milk/d-sorbitol	36	18*
2-heptenal/milk/d-sorbitol	36	18*

***** denotes panelists found a significant difference between samples with (1.0% w/v) and without a scavenger present (18 correct responses needed)

E. CONCLUSIONS

Using SPME-GC a significant difference in the concentration of hexanal in both spring water and 2% fat HTST milk was found in the presence of β-cyclodextrin. A significant decrease in 2-pentanone in 2% fat HTST milk with the addition of both β-cyclodextrin and D-sorbitol was also found. The final significant decrease was found in 2-heptenal in soymilk with the addition of D-sorbitol. All of these combinations were also found to have an overall difference in odor between the samples with and without the odor scavengers by a sensory panel. This shows that β-cyclodextrin would be a good additive to the packaging system that contains milk or soymilk to decrease the odors caused by lipid oxidation. However, since β-cyclodextrin is very reactive with low molecular weight compounds, there is a possibility that desirable aromas could also be scavenged by β-cyclodextrin. This reactivity could lead to further research involving β-cyclodextrin as a pontential scavenger in food packaging.

In general, nylon 6 was not effective as an odor scavenger in this study. Nylon 6 did show a slight decrease in odors, however was not found significant enough for use. Even though nylon 6 was not found effective in this study, it is still currently being used for its scavenger abilities of other compounds in packaging systems and should not be left out of the potential scavengers for odor removal.

β-cyclodextrin and D-sorbitol were found to be effective odor scavengers in this study by both SMPE-GC and sensory evaluation. There were 19 total odor/scavenger/media combinations that resulted in significantly lower concentrations of the odor than the controls (without scavengers) when interpreting the data from SPME-GC. However, only 5 of these combinations proved to be detected as a significant difference by a sensory panel that represented the general public. It is possible that if the sensory panel was trained in identifying these odors, the overall differences found by the sensory panel could have been larger.

F. ACKNOWLEDGEMENTS

I would like to thank the Center for Advanced Processing and Packaging Studies for the funding of this project.

I would also like to thank Kim Waterman for her help with the sensory evaluation and all the students, faculty, and staff that participated in the sensory panels. Without them I would not have been able to complete my research.

Finally I would like to thank my committee members Dr. J. E. Marcy, Dr. S. E. Duncan, and Dr. S. F. O'Keefe for their help and encouragement in this project.

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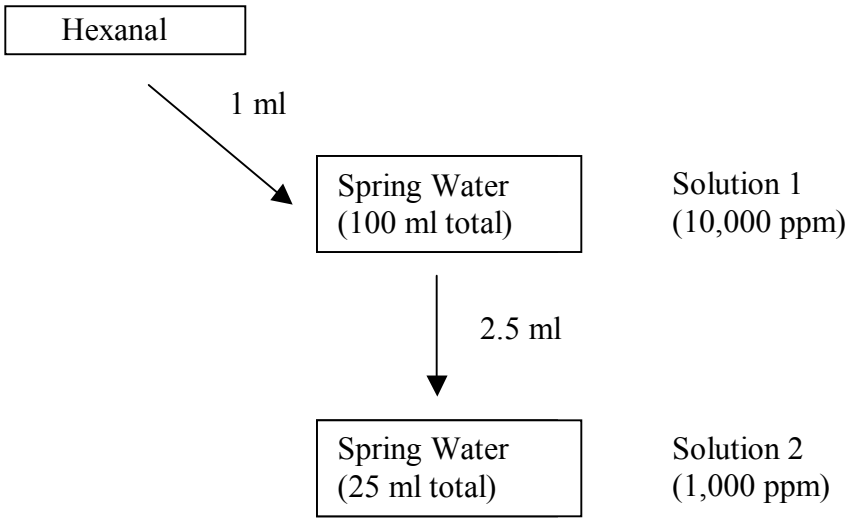
APPENDIX A: Dilution Chart for Spiking

Example: Hexanal Spiking



Concentration (ppb)	Volume of solution (ml)	Solution #	Total volume of medium (L)
50	0.05	1	1
100	0.1	1	1
200	0.2	1	1
400	0.4	1	1
800	0.8	1	1
1,600	0.16	2	1
3,200	0.32	2	1
6,400	0.64	2	1
12,800	1.28	2	1

APPENDIX B: Human Subjects Consent Form

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: *Efficacy of Potential Aroma Scavengers to Reduce or Remove Aromas Caused by Lipid Oxidation in Milk and Soy Milk Medias*

Principal Investigator: Jenny Norton, Masters Candidate in Food Science

I. THE PURPOSE OF THIS PROJECT

The purpose of this project is to:

(i) Establish the detection threshold of hexanal, 2-heptenal, 2-pentanone, and 2,4-nondienal in water, milk, and soy milk, and.

II. PROCEDURES

There will be __1__ sessions over a period of __1__ day involving about __15__ minutes at each session. You will be presented with approximately __30__ samples at each session. As a panelist, it is critical to the project that you attend the session.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

_____

_____

_____

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide information that may be helpful to the determination of threshold values in water, milk, and soy milk. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

IV. EXTENT OF AN0NYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION

For participation in the project, you will receive a piece of candy as a token of our appreciation for your time and support of this project.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require tasting six (6) yogurt samples.

_____

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address _____

Phone _____

IX. SUBJECT'S PERMISSION (provide tear off for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require: (list sessions to be attended or other requirements.)

_____

Signature

Should I have any questions about this research or its conduct, I should contact:

Jenny Norton (540) 231-8675
Investigator/Phone

Dr. Susan E. Duncan (540)231-8675
Investigator/Phone

Food Science & Technology (540)231-6806
Faculty/Phone

Appendix C: Sample Preparation

MILK

Table 1. Sample Setup and Presentation

Triangle Presentation	Sample – Code	Sample – Code	Sample – Code	Order
1	A- 356	R- 814	R-634	R,R,A
2	B- 941	R-498	R-781	B,R,R
3	C- 622	R- 675	R-563	R,C,R
4	D- 822	R- 581	R-857	R,R,D
5	E- 168	R- 137	R-245	R,E,R
6	F- 471	R- 226	R-196	F,R,R
7	G- 375	R- 349	R-918	R,G,R
8	H- 657	R- 752	R-479	R,R,H
9	I- 291	R- 963	R-322	I,R,R
10	J- 879	R- 737	R-952	R,R,J

R – Indicates the reference or un-spiked sample
A to J – Represents the samples spiked with increasing concentrations of aroma compounds

SOY MILK

Table 2. Sample Setup and Presentation

Triangle Presentation	Sample – Code	Sample – Code	Sample – Code	Order
1	A- 452	R- 221	R-823	R,R,A
2	B- 512	R-433	R-397	B,R,R
3	C- 647	R- 484	R-987	R,C,R
4	D- 832	R- 853	R-209	R,R,D
5	E- 139	R- 481	R-732	R,E,R
6	F- 177	R- 249	R-816	F,R,R
7	G- 215	R- 932	R-726	R,G,R
8	H- 712	R- 913	R-642	R,R,H
9	I- 193	R- 219	R-388	I,R,R
10	J- 528	R- 245	R-610	R,R,J

R – Indicates the reference or un-spiked sample A to J – Represents the samples spiked with increasing concentrations of aroma compounds.

APPENDIX D: Scorecard for Sensory Evaluation of Odor Thresholds

Thresholds of Lipid Oxidative Products in Water, Milk, and Soy Milk (Aroma Only)#1

Name:_____
Number:_____
Date:_____

Instructions**: DO NOT CONSUME SAMPLES**. **This is an aroma study only.** You will be provided with a warm-up example to familiarize you with the expected aroma of discrimination. Please complete the human subjects consent form while resting from the warm-up sample. You will then receive ten sets of samples, each consisting of three samples. Two of the three samples in a set are the same and one is different. **Smell samples by removing cap and waving the bottle under your nose**. Smell the samples from left to right. Once you smell a sample DO NOT go back. The initial aroma will dissipate. Circle the number of the sample in each set of three that smell "different". Rest between each set of samples.

Circle the different sample in each row

Tray 1

Sample Row 1:	814	634	356
Sample Row 2:	941	498	781
Sample Row 3:	675	622	563

Send tray through and wait for tray 2

Circle the different sample in each row

Tray 2

Sample Row 1:	581	857	822
Sample Row 2:	137	168	245
Sample Row 3:	471	226	196

Send tray through and wait for tray 3

(continue on to other side of page)

Circle the different sample in each row

Tray 3

Sample Row 1:	349	375	918
Sample Row 2:	752	479	657
Sample Row 3:	291	963	322
Sample Row 4:	737	952	879

Send tray and score sheet back through the sensory door. Thank you so much for your help!

APPENDIX E:

Human Threshold of hexanal in spring water

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**50**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**	**12800**	**Threshold**
1	-	-	+	+	+	+	-	+	+	+	2400
2	-	-	-	+	+	+	+	+	+	+	150
3	-	-	+	+	+	-	-	+	+	+	2400
4	-	+	+	-	-	-	+	+	+	+	1200
5	-	-	+	-	-	-	-	+	+	+	2400
6	-	+	-	-	-	+	+	+	+	+	600
7	-	-	+	-	-	-	-	+	+	+	2400
8	-	-	-	+	-	-	+	+	+	+	1200
9	-	-	-	-	+	+	+	+	+	+	300
10	-	-	-	+	+	+	+	+	+	+	150
11	-	+	-	-	+	+	+	+	+	+	300
12	-	-	+	+	+	+	+	+	+	+	75

- = incorrect response
+ = correct response

Group Threshold **635**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**50**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**	**12800**	**Threshold**
1	-	+	-	-	-	+	+	+	+	+	600
2	-	-	-	-	-	+	+	+	+	+	600
3	-	-	+	+	-	-	+	+	+	+	1200
4	-	-	+	-	-	-	-	+	+	+	2400
5	-	-	-	+	+	-	-	-	+	+	4800
6	-	-	+	+	+	+	+	+	+	+	75
7	-	-	+	+	+	+	+	+	+	+	75
8	-	-	-	-	+	-	+	+	+	+	1200
9	-	-	-	-	-	+	+	+	+	+	600
10	-	-	+	-	-	-	-	+	+	+	2400
11	-	-	-	+	+	+	+	+	+	+	150
12	-	-	+	+	+	+	+	+	+	+	75

- = incorrect response
+ = correct response

Group Threshold: **534**

Average between duplication: **585**

Human Threshold of Hexanal in HTST Milk (2% milkfat)

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**50**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**	**12800**	**Threshold**
1	-	+	+	-	-	-	-	+	+	+	2400
2	-	+	+	+	+	+	+	+	+	+	25
3	-	-	+	-	-	+	+	+	+	+	600
4	-	-	-	-	+	+	+	+	+	+	300
5	-	+	-	-	-	+	+	+	+	+	600
6	-	+	-	+	-	-	+	+	+	+	1200
7	-	+	+	+	+	+	+	+	+	+	25
8	-	+	-	-	-	-	+	+	+	+	1200
9	-	+	+	+	+	+	+	+	+	+	25
10	-	+	-	+	-	+	+	+	+	+	600
11	-	-	-	-	+	+	+	+	+	+	300
12	-	-	+	+	-	-	+	+	+	+	1200

- = incorrect response

+ = correct response

Group Threshold **322**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**50**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**	**12800**	**Threshold**
1	-	-	+	-	-	+	+	+	+	+	600
2	-	-	-	-	-	+	+	+	+	+	600
3	-	-	-	-	+	+	-	+	+	+	300
4	-	-	+	+	+	+	+	+	+	+	75
5	-	+	-	-	+	+	+	+	+	+	2400
6	-	-	-	+	+	+	+	+	+	+	150
7	-	-	-	-	+	+	+	+	+	+	300
8	-	-	-	+	+	+	+	+	+	+	150
9	-	-	-	+	+	+	+	+	+	+	150
10	-	-	-	+	-	-	-	-	+	+	4800
11	-	-	-	-	+	+	+	+	+	+	300
12	-	-	-	+	+	+	+	+	+	+	150

- = incorrect response

+ = correct response

Group Threshold: **356**

Average between duplication: **339**

Human Threshold of Hexanal in Soymilk

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**50**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**	**12800**	**Threshold**
1	-	-	-	-	+	+	+	+	+	+	300
2	-	-	+	-	-	-	+	+	+	+	1200
3	-	+	-	-	+	-	-	+	+	+	2400
4	-	-	+	+	+	+	+	+	+	+	75
5	-	-	+	-	-	-	+	+	+	+	1200
6	-	-	+	+	-	-	-	+	+	+	2400
7	-	-	+	+	+	+	+	+	+	+	75
8	-	+	-	-	-	-	+	+	+	+	1200
9	-	-	-	+	-	-	-	+	+	+	2400
10	-	-	+	+	+	+	+	+	+	+	75
11	-	-	+	+	+	+	+	+	+	+	75
12	-	+	+	-	-	-	+	+	+	+	1200

- = incorrect response
+ = correct response

Group Threshold **505**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**50**	**100**	**200**	**400**	**800**	**1600**	**3200**	**6400**	**12800**	**Threshold**
1	-	-	+	-	-	-	-	+	+	+	2400
2	-	-	-	+	+	+	+	+	+	+	150
3	-	-	-	+	+	+	+	+	+	+	150
4	-	-	+	+	+	+	+	+	+	+	75
5	-	+	-	-	-	-	+	+	+	+	1200
6	-	+	+	-	-	-	+	+	+	+	1200
7	-	-	-	-	+	+	+	+	+	+	300
8	-	+	-	-	-	+	+	+	+	+	600
9	-	-	-	-	-	+	+	+	+	+	600
10	-	-	+	-	-	-	-	+	+	+	2400
11	-	-	+	-	-	+	+	+	+	+	600
12	-	-	-	+	-	-	+	+	+	+	1200

- = incorrect response
+ = correct response

Group Threshold: **566**

Average between duplication: **536**

Human Threshold of pentanone in spring water

Replicate 1

Panelists	0	10000	Concentration (ppb) 20000	40000	80000	160000	320000	640000	1280000	2560000	Individual Threshold
1	-	+	+	+	+	+	+	+	+	+	5000
2	-	-	+	+	+	+	+	+	+	+	15000
3	-	-	-	+	+	+	+	+	+	+	30000
4	-	-	-	-	+	+	+	+	+	+	60000
5	-	-	+	+	+	+	+	+	+	+	15000
6	-	-	+	+	+	+	+	+	+	+	15000
7	-	-	-	+	+	+	+	+	+	+	30000
8	-	-	+	+	+	+	+	+	+	+	15000
9	-	+	-	-	+	+	+	+	+	+	60000
10	-	+	-	-	+	+	+	+	+	+	60000
11	-	-	-	+	+	+	+	+	+	+	30000
12	-	-	-	-	-	+	+	+	+	+	120000

- = incorrect response
+ = correct response

Group Threshold **27375**

Replicate 2

Panelists	0	10000	Concentration (ppb) 20000	40000	80000	160000	320000	640000	1280000	2560000	Individual Threshold
1	-	-	-	+	+	+	+	+	+	+	30000
2	-	-	+	+	+	-	+	+	+	+	15000
3	-	-	-	+	+	+	+	+	+	+	30000
4	-	-	-	-	+	+	+	+	+	+	60000
5	-	-	+	+	+	+	+	+	+	+	15000
6	-	-	-	+	+	+	+	+	+	+	30000
7	-	-	-	+	+	+	+	+	+	+	30000
8	-	-	+	+	+	+	+	+	+	+	15000
9	-	-	-	+	+	+	+	+	+	+	30000
10	-	-	+	+	+	+	+	+	+	+	15000
11	-	-	+	+	+	+	+	+	+	+	15000
12	-	-	+	+	+	+	+	+	+	+	15000

- = incorrect response
+ = correct response

Group Threshold: **22474**

Average between duplication: **24925**

Human Threshold of pentanone in milk

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**10000**	**20000**	**40000**	**80000**	**160000**	**320000**	**640000**	**1280000**	**2560000**	**Threshold**
1	-	-	+	+	+	+	+	+	+	+	15000
2	-	-	-	+	+	+	+	+	+	+	30000
3	-	-	+	+	+	+	+	+	+	+	15000
4	-	-	-	+	+	+	+	+	+	+	30000
5	-	-	+	+	+	+	+	+	+	+	15000
6	-	-	+	+	+	+	+	+	+	+	15000
7	-	-	+	-	-	-	-	+	+	+	480000
8	-	-	+	+	+	+	+	+	+	+	15000
9	-	-	+	+	+	+	+	+	+	+	15000
10	-	+	-	-	-	-	+	+	+	+	240000
11	-	-	-	+	+	+	+	+	+	+	30000
12	-	-	-	+	+	+	+	+	+	+	30000

- = incorrect response

+ = correct response

Group Threshold **317834**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**10000**	**20000**	**40000**	**80000**	**160000**	**320000**	**640000**	**1280000**	**2560000**	**Threshold**
1	-	-	+	+	+	+	+	+	+	+	15000
2	-	-	-	+	+	+	+	+	+	+	30000
3	-	-	-	+	+	+	+	+	+	+	30000
4	-	-	-	+	+	+	+	+	+	+	30000
5	-	-	-	+	+	+	+	+	+	+	30000
6	-	-	+	+	+	+	+	+	+	+	15000
7	-	-	+	+	+	+	+	+	+	+	15000
8	-	-	-	+	+	+	+	+	+	+	30000
9	-	-	-	+	+	+	+	+	+	+	30000
10	-	-	-	-	+	+	+	+	+	+	60000
11	-	-	+	+	+	+	+	+	+	+	15000
12	-	+	-	-	+	+	+	+	+	+	60000

- = incorrect response

+ = correct response

Group Threshold: **26727**

Average between duplication: **29255**

Human Threshold for pentanone in soymilk

Replicate 1

			Concentration (ppb)								Individual
Panelists	**0**	**10000**	**20000**	**40000**	**80000**	**160000**	**320000**	**640000**	**1280000**	**2560000**	**Threshold**
1	+	-	-	+	+	+	+	+	+	+	30000
2	-	-	+	+	+	+	+	+	+	+	15000
3	-	-	+	-	-	-	+	+	+	+	240000
4	-	-	-	+	+	+	+	+	+	+	30000
5	-	-	-	-	+	+	+	+	+	+	60000
6	-	-	-	+	+	+	+	+	+	+	30000
7	-	+	+	+	+	+	+	+	+	+	5000
8	-	-	-	+	+	+	+	+	+	+	30000
9	-	+	-	-	-	-	+	+	+	+	240000
10	-	-	+	+	+	+	+	+	+	+	15000
11	-	-	-	-	+	+	+	+	+	+	60000
12	-	-	-	+	+	+	+	+	+	+	30000

- = incorrect response

+ = correct response

Group Threshold **36542**

Replicate 2

			Concentration (ppb)								Individual
Panelists	**0**	**10000**	**20000**	**40000**	**80000**	**160000**	**320000**	**640000**	**1280000**	**2560000**	**Threshold**
1	-	-	-	+	+	+	+	+	+	+	30000
2	-	-	+	+	+	+	+	+	+	+	15000
3	-	-	-	+	+	+	+	+	+	+	30000
4	-	-	+	+	+	+	+	+	+	+	15000
5	-	-	-	+	+	+	+	+	+	+	30000
6	-	-	+	+	+	+	+	+	+	+	15000
7	-	+	-	-	+	+	+	+	+	+	60000
8	-	-	-	+	+	+	+	+	+	+	30000
9	-	-	-	+	+	+	+	+	+	+	30000
10	-	+	-	-	-	+	+	+	+	+	120000
11	-	-	+	+	+	+	+	+	+	+	15000
12	-	-	-	-	+	+	+	+	+	+	60000

- = incorrect response

+ = correct response

Group Threshold: **30000**

Average between duplication: **33271**

Human Threshold of 2-heptenal in spring water

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**32000**	**64000**	**128000**	**Threshold**
1	-	-	+	+	+	+	+	+	+	+	750
2	-	-	-	-	+	+	+	+	+	+	3000
3	-	-	-	+	+	+	+	+	+	+	1500
4	-	-	-	+	+	+	+	+	+	+	1500
5	-	-	-	-	+	+	+	+	+	+	3000
6	-	-	-	+	+	+	+	+	+	+	1500
7	-	-	-	+	-	+	+	+	+	+	6000
8	-	+	-	-	-	-	+	+	+	+	12000
9	-	+	-	-	-	+	+	+	+	+	6000
10	-	-	+	+	+	+	+	+	+	+	750
11	-	-	-	-	+	+	+	+	+	+	3000
12	-	-	-	+	+	+	+	+	+	+	1500

- = incorrect response
+ = correct response

Group Threshold **2247**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**32000**	**64000**	**128000**	**Threshold**
1	-	-	+	+	+	+	+	+	-	+	750
2	-	-	-	+	+	+	+	+	+	+	1500
3	-	-	-	+	+	+	+	+	+	+	1500
4	-	-	-	+	+	+	+	+	+	+	1500
5	-	-	-	-	+	+	+	+	+	+	3000
6	-	-	-	+	+	+	-	+	+	+	24000
7	-	-	-	+	+	+	+	+	+	+	1500
8	-	-	-	+	+	+	+	+	+	+	1500
9	-	-	-	+	+	+	+	+	+	+	1500
10	-	-	-	+	-	-	-	+	+	+	24000
11	-	+	+	+	+	+	+	+	+	+	250
12	-	-	+	+	+	+	+	+	+	+	750

- = incorrect response
+ = correct response

Group Threshold: **1936**

Average between duplication: **2092**

Human Threshold of 2-heptenal in milk

Replicate 1

			Concentration (ppb)								Individual
Panelists	**0**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**32000**	**64000**	**128000**	**Threshold**
1	-	-	+	+	+	+	+	+	+	+	750
2	-	-	-	+	+	+	+	+	+	+	1500
3	-	-	+	+	+	+	+	+	+	+	750
4	-	+	-	-	-	+	+	+	+	+	6000
5	-	+	-	-	-	+	+	+	+	+	6000
6	-	-	+	+	+	+	+	+	+	+	750
7	-	-	+	+	+	+	+	+	+	+	750
8	-	-	-	+	+	+	+	+	+	+	1500
9	-	-	+	-	-	-	+	+	+	+	12000
10	-	-	+	+	+	+	+	+	+	+	750
11	-	-	-	-	+	-	-	+	+	+	24000
12	-	-	+	-	-	-	+	+	+	+	12000

- = incorrect response
+ = correct response

Group Threshold **2523**

Replicate 2

			Concentration (ppb)								Individual
Panelists	**0**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**32000**	**64000**	**128000**	**Threshold**
1	-	-	-	+	+	+	+	+	+	+	1500
2	-	+	-	-	-	+	+	+	+	+	6000
3	-	-	-	+	+	+	+	+	+	+	1500
4	-	-	-	+	+	+	+	+	+	+	1500
5	-	-	-	-	+	+	+	+	+	+	3000
6	-	-	-	+	+	+	+	+	+	+	1500
7	-	-	-	-	+	+	+	+	+	+	3000
8	-	+	-	-	-	-	+	+	+	+	12000
9	-	-	-	+	+	+	+	+	+	+	1500
10	-	-	+	+	+	+	+	+	+	+	750
11	-	-	+	+	+	+	+	+	+	+	750
12	-	+	-	-	+	+	+	+	+	+	3000

- = incorrect response
+ = correct response

Group Threshold: **2121**

Average between duplication: **2322**

Human Threshold of 2-heptenal in soymilk

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**32000**	**64000**	**128000**	**Threshold**
1	-	-	-	+	-	+	+	+	+	+	6000
2	-	-	-	+	+	+	+	+	+	+	1500
3	-	-	-	-	+	+	+	+	+	+	3000
4	-	-	-	+	+	+	+	+	+	+	1500
5	-	-	-	+	+	+	+	+	+	+	1500
6	-	-	-	-	+	+	+	+	+	+	3000
7	-	-	-	-	+	+	+	+	+	+	3000
8	-	-	-	-	+	+	+	+	+	+	3000
9	-	+	-	-	-	-	+	+	+	+	12000
10	-	-	-	+	+	+	+	+	+	+	1500
11	-	+	-	-	-	+	+	+	+	+	6000
12	-	-	-	-	+	+	+	+	+	+	3000

- = incorrect response
+ = correct response

Group Threshold **3000**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**32000**	**64000**	**128000**	**Threshold**
1	-	+	-	-	-	+	+	+	+	+	6000
2	-	-	-	-	+	+	+	+	+	+	3000
3	-	-	-	+	+	+	+	+	+	+	1500
4	-	-	-	-	-	-	+	+	+	+	12000
5	-	-	-	+	+	+	+	+	-	+	1500
6	-	-	-	-	+	+	+	+	+	+	3000
7	-	+	-	-	-	+	+	+	+	+	6000
8	-	-	-	-	+	+	+	+	+	+	3000
9	-	-	-	-	+	+	+	+	+	+	3000
10	-	+	-	-	-	+	+	+	+	+	6000
11	-	-	-	-	+	+	+	+	+	+	3000
12	-	-	-	+	+	+	+	+	+	+	1500

- = incorrect response
+ = correct response

Group Threshold: **3367**

Average between duplication: **3184**

Human Threshold of 2,4 – nonadienal in spring water

Replicate 1

			Concentration (ppb)							Individual
Panelists	**0**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**Threshold**
1	-	+	-	-	+	+	+	+	+	+	375
2	-	-	+	+	+	+	+	+	+	+	93.75
3	-	-	+	+	+	+	+	+	+	+	93.75
4	-	-	+	+	+	-	+	+	+	+	1500
5	-	-	-	+	+	+	+	+	+	+	187.5
6	-	-	+	+	+	+	+	+	+	+	93.75
7	-	-	+	+	+	+	+	+	+	+	93.75
8	-	-	-	+	+	+	+	+	+	+	187.5
9	-	-	-	+	+	+	+	+	+	+	187.5
10	-	+	-	-	-	+	+	+	+	+	750
11	-	-	+	+	+	+	+	+	+	+	93.75
12	-	+	-	+	+	+	+	+	+	+	187.5
									Group Threshold		**199**

- = incorrect response

+ = correct response

Replicate 2

			Concentration (ppb)							Individual
Panelists	**0**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**Threshold**
1	-	-	+	+	+	+	+	+	+	+	93.75
2	-	+	-	+	+	+	+	+	+	+	187.5
3	-	-	+	+	+	+	+	+	+	+	93.75
4	-	-	-	+	+	+	+	+	+	+	187.5
5	-	+	-	-	-	+	+	+	+	+	750
6	-	-	-	+	+	+	+	+	+	+	187.5
7	-	-	-	+	+	+	+	+	+	+	187.5
8	-	-	+	+	+	+	+	+	+	+	93.75
9	-	+	-	-	-	+	+	+	+	+	750
10	-	-	+	+	+	+	+	+	+	+	93.75
11	-	-	-	+	+	+	+	+	+	+	187.5
12	-	+	+	+	+	+	+	+	+	+	31.25
									Group Threshold:		**161**

- = incorrect response

+ = correct response

Average between duplication: **164**

Human Threshold of 2,4 – nonadienal in milk

Replicate 1

	Concentration (ppb)									Individual
Panelists	**0**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**Threshold**
1	-	-	-	-	+	+	+	+	+	+	375
2	-	+	-	-	-	+	+	+	+	+	750
3	-	-	+	-	+	+	+	+	+	+	375
4	-	-	-	+	+	+	+	+	+	+	187.5
5	-	+	-	-	-	-	+	+	+	+	1500
6	-	-	+	+	+	+	+	+	+	+	93.75
7	-	-	+	+	+	+	+	+	+	+	93.75
8	-	+	-	-	+	+	+	+	+	+	375
9	-	-	+	-	-	-	-	+	+	+	3000
10	-	-	+	+	+	+	+	+	+	+	93.75
11	-	-	+	+	+	+	+	+	+	+	93.75
12	-	-	-	+	+	+	+	+	+	+	187.5

- = incorrect response

+ = correct response

Group Threshold **298**

Replicate 2

	Concentration (ppb)									Individual
Panelists	**0**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**Threshold**
1	-	-	-	+	+	+	+	+	+	+	187.5
2	-	-	-	+	+	+	+	+	+	+	187.5
3	-	+	-	-	+	+	+	+	+	+	375
4	-	+	-	-	-	-	-	+	+	+	3000
5	-	-	+	+	+	+	+	+	+	+	93.75
6	-	-	-	+	+	+	+	+	+	+	187.5
7	-	-	-	+	+	+	+	+	+	+	187.5
8	-	-	+	+	+	+	+	-	+	+	6000
9	-	-	-	+	+	+	+	+	+	+	187.5
10	-	-	-	+	+	+	+	+	+	+	187.5
11	-	-	-	+	+	+	+	+	+	+	187.5
12	-	+	-	-	-	+	+	+	+	+	750

- = incorrect response

+ = correct response

Group Threshold: **354**

Average between duplication: **326**

Human Threshold of 2,4 – nonadienal in soymilk

Replicate 1

	Concentration (ppb)										Individual
Panelists	**0**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**Threshold**
1	-	-	-	-	+	+	+	+	+	+	375
2	-	-	+	+	+	+	+	+	+	+	93.75
3	-	-	+	+	+	+	+	+	+	+	93.75
4	+	-	-	+	+	+	+	+	+	+	187.5
5	-	-	+	-	-	+	+	+	+	+	750
6	-	-	+	+	+	+	+	+	+	+	93.75
7	+	-	+	+	+	+	+	+	+	+	93.75
8	-	-	-	+	-	-	+	+	+	+	1500
9	+	-	-	+	+	+	+	+	+	+	187.5
10	-	+	-	+	+	+	+	+	+	+	187.5
11	+	-	-	-	-	+	+	+	+	+	750
12	+	+	-	-	+	+	+	+	+	+	375

- = incorrect response

+ = correct response

Group Threshold **250**

Replicate 2

	Concentration (ppb)										Individual
Panelists	**0**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	**4000**	**8000**	**16000**	**Threshold**
1	-	-	-	+	+	+	+	+	+	+	187.5
2	-	-	-	+	+	+	+	+	+	+	187.5
3	+	-	-	+	+	+	+	+	+	+	187.5
4	-	-	+	+	+	+	+	+	+	+	93.75
5	-	-	-	+	+	+	+	+	+	+	187.5
6	-	-	-	+	+	+	+	+	+	+	187.5
7	-	-	-	+	+	+	+	+	+	+	187.5
8	-	+	-	-	+	+	+	+	+	+	375
9	-	-	-	-	+	+	+	+	+	+	375
10	-	+	+	-	-	-	-	+	+	+	3000
11	-	-	-	+	+	+	+	+	+	+	187.5
12	-	-	+	+	+	+	+	+	+	+	93.75

- = incorrect response

+ = correct response

Group Threshold: **236**

Average between duplication: **243**

APPENDIX F: Human Consent Forms to Triangle Test for Scavenger Efficiency

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: *Efficacy of Aroma Scavengers in Reducing or Eliminating Odor Compounds Caused by Lipid Oxidation in Milk and Soy Milk*

Principal Investigator: Jenny Norton, Masters Candidate in Food Science

I. THE PURPOSE OF THIS PROJECT

The purpose of this project is to:

(ii) Determine the efficacy of D-sorbitol, β-cyclodextrin, and nylon 6 as aroma scavengers in reducing the amount of hexanal, 2-heptenal, 2-pentanone, and 2,4-nonadienal in milk and soy milk samples.

II. PROCEDURES

There will be_4_ sessions over a period of _4_ day involving about __10__ minutes at each session. You will be presented with approximately _9__ samples at each session. As a panelist, it is critical to the project that you attend the session.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

_____
_____
_____

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide information that will show whether the aroma scavengers listed above are affective in reducing the odor caused by lipid oxidation in milk and soy milk. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy. However, this is an aroma test so risks are minimal.

IV. EXTENT OF AN0NYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION

For participation in the project, you will receive a piece of candy as a token of our appreciation for your time and support of this project.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in soon as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require tasting six (6) yogurt samples.

_____

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address _____

Phone _____

----------(tear off)----------

IX. SUBJECT'S PERMISSION (provide tear off for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require: (list sessions to be attended or other requirements.)

_____

Signature

Should I have any questions about this research or its conduct, I should contact:

Jenny Norton (540) 231-8675
Investigator/Phone

Dr. Susan E. Duncan (540)231-8675
Investigator/Phone

Food Science & Technology (540)231-6806
Faculty/Phone

APPENDIX G: Worksheet for triangle test set-up

DATE _____ WORKSHEET TEST CODE _____

THIS SHEET IS FOR ORDER PRESENTATION

SAMPLE IDENTIFICATION	**CODE**
SAMPLE WITH OUT SCAVENGER	N
SAMPLE WITH SCAVENGER	S

CODE SERVING CONTAINERS AS FOLLOWS:

TRAY ONE (FIRST SAMPLE SET)

PANELIST #	**ORDER OF PRESENTATION**
1, 7, 13, 19, 25, 31	N – S – S (239, 692, 193)
2, 8, 14, 20, 26, 32	S – N – S (692, 541, 193)
3, 9, 15, 21, 27, 33	S – S – N (193, 692, 239)
4, 10, 16, 22, 28, 34	S – N – N (692, 239, 541)
5, 11, 17, 23, 29, 35	N – S – N (541, 193, 239)
6, 12, 18, 24, 30, 36	N – N – S (239, 541, 692)

TRAY TWO (SECOND SAMPLE SET)

PANELIST #	**ORDER OF PRESENTATION**
1, 7, 13, 19, 25, 31	N – S – S (168, 421, 510)
2, 8, 14, 20, 26, 32	S – N – S (421, 392, 510)
3, 9, 15, 21, 27, 33	S – S – N (510, 421, 168)
4, 10, 16, 22, 28, 34	S – N – N (421, 392, 168)
5, 11, 17, 23, 29, 35	N – S – N (392, 510, 168)
6, 12, 18, 24, 30, 36	N – N – S (168, 392, 421)

TRAY TWO (SECOND SAMPLE SET)

PANELIST #	**ORDER OF PRESENTATION**
1, 7, 13, 19, 25, 31	N – S – S (521, 103 , 384)
2, 8, 14, 20, 26, 32	S – N – S (384, 932, 103)
3, 9, 15, 21, 27, 33	S – S – N (103, 384, 521)
4, 10, 16, 22, 28, 34	S – N – N (384, 521, 932)
5, 11, 17, 23, 29, 35	N – S – N (932, 103, 521)
6, 12, 18, 24, 30, 36	N – N – S (521, 932, 103)

TRAY ONE
N - 239 541
S - 692 193

TRAY TWO
N - 168 392
S - 421 510

TRAY THREE
N - 521 932
S - 103 384

APPENDIX H: Scorecard for Scavenger Efficiency Triangle Test

TRIANGLE TEST

PANELIST NO.:_____ NAME:_____ DATE:_____

TYPE OF SAMPLE:_____

INSTRUCTIONS

DO NOT CONSUME SAMPLES!!! THIS IS AN AROMA TEST ONLY.

REMOVE THE LIDS ONE AT A TIME AND SMELL THE SAMPLES FROM LEFT TO RIGHT. TWO SAMPLES ARE IDENTICAL; ONE IS DIFFERENT. SELECT THE ODD/DIFFERENT SAMPLE AND INDICATE BY PLACING AN X NEXT TO THE CODE OF THE ODD SAMPLE.

SAMPLES ON TRAY	INDICATE ODD SAMPLE	REMARKS
_____	☐	_____
_____	☐	_____
_____	☐	_____

IF YOU WISH TO COMMENT ON THE REASONS FOR YOUR CHOICE OR IF YOU WISH TO COMMENT ON THE PRODUCT CHARACTERISTS, YOU MAY DO SO UNDER REMARKS.

PLEASE SEND TRAY BACK THROUGH AND WAIT FOR SECOND TRAY

TRIANGLE TEST

PANELIST NO.:_____ NAME:_____ DATE:_____

TYPE OF SAMPLE:_____

INSTRUCTIONS

DO NOT CONSUME SAMPLES!!! THIS IS AN AROMA TEST ONLY.

REMOVE THE LIDS ONE AT A TIME AND SMELL THE SAMPLES FROM LEFT TO RIGHT. TWO SAMPLES ARE IDENTICAL; ONE IS DIFFERENT. SELECT THE ODD/DIFFERENT SAMPLE AND INDICATE BY PLACING AN "X" NEXT TO THE CODE OF THE ODD SAMPLE.

SAMPLES ON TRAY	INDICATE ODD SAMPLE	REMARKS
_____	☐	_____
_____	☐	_____
_____	☐	_____

IF YOU WISH TO COMMENT ON THE REASONS FOR YOUR CHOICE OR IF YOU WISH TO COMMENT ON THE PRODUCT CHARACTERISTICS, YOU MAY DO SO UNDER REMARKS.

PLEASE SEND TRAY BACK THROUGH AND WAIT FOR TRAY TWO.

TRIANGLE TEST

PANELIST NO.:_____ NAME:_____ DATE:_____

TYPE OF SAMPLE:_____

INSTRUCTIONS

DO NOT CONSUME SAMPLES!!! THIS IS AN AROMA TEST ONLY.

REMOVE THE LIDS ONE AT A TIME AND SMELL THE SAMPLES FROM LEFT TO RIGHT. TWO SAMPLES ARE IDENTICAL; ONE IS DIFFERENT. SELECT THE ODD/DIFFERENT SAMPLE AND INDICATE BY PLACING AN "X" NEXT TO THE CODE OF THE ODD SAMPLE.

SAMPLES ON TRAY	INDICATE ODD SAMPLE	REMARKS
_____	☐	_____
_____	☐	_____
_____	☐	_____

IF YOU WISH TO COMMENT ON THE REASONS FOR YOUR CHOICE OR IF YOU WISH TO COMMENT ON THE PRODUCT CHARACTERISTICS, YOU MAY DO SO UNDER REMARKS.

PLEASE SEND TRAY BACK THROUGH. THANK YOU VERY MUCH!!!

Vita

Jenny was born on December 20, 1977 in Bentonville, Arkansas to Randy and Susan Norton. She was raised in Centerton, Arkansas along with her brother, Jake, and her sister, Amber. Jenny attended public school in Bentonville, Arkansas. After high school, she decided to pursue her undergraduate degree at the University of Arkansas in Fayetteville in Food Science. While at the U of A, Jenny received several local and national scholarships to help pay her way through her undergraduate degree. She also spent a summer in Scotland for an internship. Jenny received her Bacholers of Science Degree in Food Science in December 2000. She then moved to Blacksburg, Virginia where she was offered a full assistantship in the Food Science and Technology Department at Virginia Tech to pursue her Masters of Science Degree. While at Virginia Tech, Jenny worked on aroma and packaging issues associated with food products and also received the Graduate Student of the Year Award in 2002. Jenny completed her Masters of Science Degree in Food Science and Technology in September 2003. She accepted a full assistantship at Virginia Tech and is currently pursuing a Doctoral Degree in Food Science and Technology.