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Identification and characterization of volatile compounds in bovine plasma

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Identification and characterization of volatile compounds in bovine plasma

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ABSTRACT

Bovine plasma proteins (BPP) are an economical source of high quality proteins that enhance gel strength and improve the emulsion capacity of food systems. However, off-flavors in the spray-dried plasma often result in finished food products with an unacceptable flavor, limiting the use of BPP. The objectives of this research were to identify the source of the volatile compounds in plasma and to test the effects of antioxidant addition, processing conditions and storage time on the formation of volatile compounds.

Heat and oxygen exposure during the spray drying process initiate lipid oxidation in BPP as there are few oxidation compounds in liquid plasma and a significant amount in the spray-dried plasma, with hexanal being the principal volatile compound identified.

Antioxidants (TBHQ, PG, BHT, rosemary) were added to BPP concentrate prior to drying in either a gas-fired or electric spray drier. The plasma dried with a gas-fired drier showed a significant ($p<0.05$) decrease in hexanal content compared with a control sample in the TBHQ, PG and BHT treatments while the rosemary treatment was not significant. Also, control samples from the electric drier had a significantly lower ($p<0.05$) total volatile count than control samples from the gas drier.

The effect of residence time (zero and 20 minutes) and storage time (zero and eight weeks) was studied on plasma samples dried in a gas-fired drier and treated with either TBHQ or a rosemary concentrate. Data showed that longer

residence and storage times both significantly ($p<0.05$) increased the amounts of hexanal produced in the spray-dried plasma.

The spray drying process initiates lipid oxidation in BPP but the amount of volatiles formed can be reduced through the addition of antioxidants, use of an electric spray drier or by minimizing residence and storage times. All of these factors will help to reduce the formation of volatile flavor compounds and improve the quality of spray-dried BPP.

1. GENERAL INTRODUCTION

Bovine plasma contains proteins with excellent functional properties that are high in nutritional value and less expensive than other protein sources such as meat and egg. Bovine plasma proteins (BPP) can be used as binders and extenders in the manufacture of sausage, as a gel-enhancer in the manufacture of surimi and as an egg white replacement in the manufacture of cakes. BPP are a complete protein fraction with no limiting essential amino acids (Duarte and others 1999) and the high lysine content makes BPP a good fortifier for cereal based foods (Satterlee 1974). BPP are a by-product of the slaughter industry and large quantities are available as over 1,000,000 liters of bovine blood is produced daily (Jobling 1986).

With all of its beneficial properties, BPP have the potential for widespread use in the food industry as an inexpensive, high quality protein source. However, the use of BPP has been limited due to the off-flavors it imparts to finished products when added at higher concentrations. Caldironi and Ockerman (1982a) reported that flavor limited the acceptability of sausages when BPP were added at levels greater than 10% of the total protein. A study of 100% egg white replacement by spray-dried BPP in high-ratio white cakes reported the majority of panelists noted an objectionable flavor in the cakes (Myhara and Kruger 1998).

A spray-dried powder is the most economical form to transport, store and use BPP, however the drying process may lead to off-flavor formation through lipid oxidation reactions. These off-flavors limit the amount of BPP that can be added to foods before they can be detected in the finished products.

Antioxidants are commonly used to reduce the formation of off-flavors due to lipid oxidation. Primary antioxidants, whether natural or synthetic, function by donating an H• radical and interrupt the propagation step of the free radical chain reaction, slowing lipid oxidation. A study of ground beef showed that off-flavors that develop after cooking the meat could be reduced if antioxidants were added to the raw, ground mixture (St. Angelo and others 1990).

This thesis addresses the possible sources of the off-flavors in bovine plasma and methods to either remove the off-flavors or prevent them from forming. Variations of the spray drying process including gas or electric driers and different residence times were compared to evaluate their effect on the formation of off-flavors. The effectiveness of liquid plasma treatments including lipid removal and antioxidant addition were also tested. If the cause of the off-flavors is identified and controlled, the addition of BPP to foods would not be limited by off-flavors in the finished food product and the functional properties of BPP could be fully utilized.

2. LITERATURE REVIEW

By-Products of Meat Animals

Domesticated animals are produced primarily for their meat, which serves as a high quality protein source in human diets. The meat however, only accounts for approximately 50% of the weight of the animal, resulting in large quantities of by-products. Approximately 125,000 head of cattle are slaughtered daily in the United States (USDA 2002) generating 68 million pounds of by-products. Due to the large amounts of by-products produced, uses for them have been devised not only for economic gain, but also to reduce pollution and public health concerns.

In the United States, there are two categories of by-products: edible and inedible. Edible by-products must be collected in a sanitary manner and pass inspection. Some examples include organ meats, edible fats, blood, gelatin, rennin and intestines. Inedible by-products are unsuitable for human consumption and include hides, skins, wool, hair, feathers, manure, bone and any of the "edible" components that did not pass inspection. Inedible by-products have a wide variety of uses such as clothing, lubricants, adhesives and fertilizer (Ockerman and Hansen 2000).

Blood collection and utilization is important in by-product processing because of the large quantities of protein that can be salvaged. Measured on an as is basis, whole bovine blood is 17.3% protein with plasma and the cellular fraction containing 5.5 and 11.8% of the protein, respectively (Howell and Laurie 1983). Approximately 6-7% of an animal's usable protein is contained in its blood, which in beef amounts to nearly one pound of protein per animal (Ockerman and Hansen 2000).

Collection and Processing of Blood for Food Applications

It is estimated that over 1,000,000 liters of bovine blood are produced in the United States daily (Jobling 1986), making it readily available as a raw material. The majority of animal blood is used as fertilizer and in animal feed (Johnson 1988), but because of its nutritional content and functional properties, blood components are also a viable choice as ingredients in human food.

Blood is collected directly from the stunned animal at the slaughterhouse. The most common and sanitary collection method is the insertion of a hollow knife into the neck of a hanging animal, which severs the carotid artery and jugular vein. Gravity carries the blood through food grade tubing connected to the knife and allows the blood to drain directly into a closed container. As the blood is collected, it is mixed with an anticoagulant such as sodium citrate or citric acid (Halliday 1973).

The collection of blood from cattle typically takes 6 min. For each animal, 12-16 liters of whole blood is obtained and from that, 2-3 kg of protein are recovered (Wismer-Pedersen 1979). Blood from healthy animals obtained in a sanitary manner also has a low microbial count, usually less than 10^4 cfu/ml (Gordon 1971).

Collected blood that is approved for food use is cooled immediately to 3-5 ^{0}C using a plate heat exchanger to reduce bacterial growth. The cooled blood is held in an insulated storage tank prior to separation by centrifugation into a plasma fraction and a cellular fraction which make up 60-70% and 30-40% of whole blood, respectively (Wismer-Pedersen 1979).

Since raw blood has a shelf-life of only 24-48 hours at refrigerated temperatures, it must either be utilized immediately as an ingredient or processed

into a frozen or dried form for extended storage (Halliday 1973). The cellular fraction is approximately 35% solids; therefore it can be efficiently frozen or dried without any further processing. The plasma fraction, however, is only 8.5% solids and must be concentrated prior to freezing or drying. Concentration is accomplished by either membrane filtration or evaporation. Once concentrated to 25-27% dry matter, plasma is either spray dried and placed in air-tight containers or frozen and stored at -25 ^{0}C (Stevenson 1979).

Benefits of Blood Utilization

Blood has traditionally been an ingredient in foods such as puddings, breads and sausages but as food production has become more industrialized, many traditional uses are less popular (Bates and others 1974). However, food manufacturers, in efforts to minimize costs while still producing a high quality product, recognize the benefits of utilizing the components in blood.

Nutritional Benefits.

The protein efficiency ratio (PER) of bovine plasma is higher than that of casein with scores of 2.15 and 1.94, respectively (Young and others 1973). Blood proteins have no limiting essential amino acids and are abundant in lysine, resulting in the high PER value (Duarte and others 1999). The high lysine content also makes blood proteins a beneficial addition to cereal-based products which are often limited nutritionally by lysine (Satterlee 1974).

Iron deficiency anemia is often associated with low-income groups who do not consume enough lean meat due to its higher cost (Jenkins 1977). Heme iron, the most available form for absorption (Layrisse and Martinez-Torres 1971), is present in the cellular fraction of blood and incorporation of blood proteins into less expensive foods such as sausage can increase iron intake. The addition of whole blood at a level of 5% to a sausage mix can increase the iron content from 8 mg/kg to 27.5 mg/kg (Wismer-Pedersen 1979).

Plasma Protein Functional Properties.

Plasma proteins are utilized for their functional properties in order to replace or extend muscle, egg white and other proteins. The functional properties provided by bovine plasma that are desired for use in food products include gelation, emulsification, foaming and protease inhibition.

Gelation occurs when a solution of protein and water is heated. The proteins unfold, exposing functional groups that form hydrogen bonds and hydrophobic interactions. The protein cross-linking forms a stable network that entraps water, forming a gel. Plasma proteins form a coagulum-type gel that is irreversible due to the hydrophobic protein-protein interactions and disulfide bonding that has taken place (Walstra 1996). Plasma gels are strongest and most stable when prepared at pH 9.0 and heated for 60 minutes at 90 ^{0}C (O'Riordan and others 1989). A comparison of a control meat batter and a meat batter containing 4% blood plasma powder demonstrates the effect of temperature on gel strength. When heated to 75 ^{0}C, both meat batters showed similar firmness but when heated to 95 ^{0}C the

batter with added plasma was 1.5 times firmer. The difference was attributed to an increase in protein cross-linking through disulfide bond formation (Harper 1978).

An emulsion is defined as a system made up of two immiscible phases, usually an oil phase and a water phase, separated by an emulsifier (Das and Kinsella 1990). Plasma proteins are used as emulsifiers in systems with an aqueous continuous phase. The emulsion is formed when fat globules are coated with the protein surfactant and trapped within the matrix that forms through protein-protein interactions. The fat globule is stabilized when the proteins unfold and align themselves so that the hydrophobic groups are in the lipid phase and the hydrophilic region is in the aqueous phase, forming a protein film. Cross-links are then formed throughout the continuous phase and a stable three-dimensional structure results (Barbut 1995). The thickness of the protein film that forms depends on the amount and quality of protein present in the system. Studies by Caldironi and Ockerman (1982 a, b) showed blood plasma to have an emulsion capacity similar to muscle proteins, thus the addition of plasma to meat emulsions increases the total protein content without compromising the quality.

The gelation and emulsifying properties of plasma contribute to increased water-holding capacity (WHC) of the food systems to which they are added. WHC refers to the ability of a protein matrix to retain water through chemical bonds or physical entrapment against the force of gravity (Damodaran 1996). Meat products with increased WHC are more tender and juicy, have increased cook yields and reduced purge when packaged under vacuum. The addition of 2% plasma to sausage products increased yields 4-5% because of decreased drip loss (Dill 1976).

Caldironi and Ockerman (1982 a) also found the plasma to be an effective water binder when added to sausage products at levels ranging from 5-12%.

Another functional property of blood plasma protein is foaming capacity. A foam is similar to an emulsion except that the discontinuous phase is air instead of oil (Walstra 1996). Plasma and egg albumin have equivalent foaming capacities, but plasma has a lower stability. After beating for two minutes, egg albumin and pH 9.0 bovine plasma had equal initial foam volumes. After ten minutes the volumes had decreased to 94 and 74% of the initial volume for egg albumin and plasma, respectively (Tybor and others 1975).

In the manufacture of baked products, bovine plasma can be used as a replacement for egg white. In studies of egg white replacement in cakes, Johnson and others (1979), Lee and others (1991) and Myhara and Kruger (1998) concluded that bovine plasma exhibits similar gel formation ability and foaming capacity to that of egg white because there were no significant differences in cake volumes between the egg white treatments and the 100% plasma replacement treatments.

Another functional property of plasma proteins, although not fully understood, is protease inhibition. In the manufacture of surimi, proteolysis is a common problem that results in decreased gel strength due to the destruction of high molecular weight proteins. The addition of 1% bovine plasma protein to surimi significantly reduced proteolysis and resulted in a 70% stronger gel than surimi with added egg white or potato extract (Morrissey and others 1993). Seymour and others (1997) also demonstrated that a 1% addition of bovine plasma protein significantly increased the gel strength of Pacific whiting surimi as compared to surimi with no

autolysis controlling additives. The increased gel strength was attributed to an increased amount of protein cross-links which are resistant to protease hydrolysis.

Off-Flavors in Bovine Plasma

Despite the numerous benefits of incorporating blood proteins into food systems, the amount of protein that can be added is often limited by off-flavors in the finished products. In high-ratio white cakes, a significant number of panelists preferred the flavor of the control cake made with egg white as compared to the cake made with spray-dried bovine plasma as a 100% replacement for egg white. Sixty-four percent of those panelists commented that the plasma replacement cakes had an undesirable flavor (Myhara and Kruger 1998). In sausage products where plasma was added to enhance emulsion capacity, the amount of plasma that could be added was limited by off-flavors in the finished sausage (Satterlee and others 1973; Caldironi and Ockerman 1982). Panelists noted the sausage had an objectionable flavor when plasma was added at concentrations higher than five percent. The use of frozen plasma is preferred because spray-dried plasma contributes to off-flavors in the finished product (Wismer-Pedersen 1979).

Lipid Oxidation

Lipid oxidation is the reaction of lipids with oxygen. This deteriorative process forms aldehydes, ketones, acids and alcohols within the food matrix resulting in off-flavors and odors. Autoxidation follows a free-radical chain reaction mechanism that occurs in three steps: initiation, propagation and termination.

The initiation step in the autoxidation of lipids is the formation of a free radical through exposure of an unsaturated fatty acid to oxygen. This reaction has an activation energy of approximately 146 kJ/mol and requires a catalyst for the initial free radicals to be produced (Bateman and others 1953). A transition metal such as iron, copper or nickel, is often the catalyst but the presence of radiation, heat or light can also initiate the reaction. The propagation step occurs as free radicals from the initiation step react with oxygen and form peroxy radicals. The peroxy radicals then abstract an allelic hydrogen from another unsaturated fatty acid forming a hydroperoxide and a new radical fatty acid. This reaction sequence continues until all radical species react with one another to form stable, nonradical compounds (Farmer and others 1942).

The hydroperoxides formed in the propagation step are very unstable and readily decompose to form hydrocarbons, alcohols, aldehydes and ketones, the major compounds responsible for off-flavors in foods. Decomposition of hydroperoxides begins with the scission of the bond between the two oxygen molecules leaving hydroxy and alkoxy radicals. The next step involves carbon-carbon bond cleavage of the alkoxy radical, with the location of the bond breaking determining the final compound produced (Simic and others 1992). Other factors that determine the carbonyl compound generated are the chain length and degree of unsaturation of the fatty acid as well as the location of the hydrogen atom which was abstracted (Frankel and others 1983).

Many factors influence the rate of lipid oxidation reactions. Oxygen concentration, temperature, water activity, degree of unsaturation and disposition of

fatty acids, pH and the presence of pro-oxidants such as iron can affect lipid oxidation reactions. By controlling these factors, lipid oxidation can be minimized.

The amount of oxygen a food is exposed to either in the processing environment or within the packaging material directly affects the rate and amount of lipid oxidation that occurs. When oxygen is present at a concentration greater than 4% at room temperature, the rate of oxidation is not limited by available oxygen molecules (Karel 1992). When oxygen is present at lower concentrations, the rate of oxidation is dependent on the amount available. Rapeseed oil stored at 50 ^{0}C showed increased oxygen consumption from the headspace as the oxygen concentration increased. The oxygen concentrations and rates of consumption were 0.03, 0.3, 1.0, 1.8% and 0.01, 0.03, 0.04, 0.05 mL/day, respectively (Andersson and Lingnert 1999). The amount of oxygen to which foods are exposed can be controlled by packaging. In spray-dried whole egg, vacuum packaging showed a significant decrease in the loss of polyunsaturated fatty acids compared to product that was only sealed in a polypropylene bag (Guardiola and others 1997). Another method of controlling oxygen exposure to foods is through the use of oxygen absorbers or scavengers. While studying cholesterol oxidation products (COPS) in spray- dried whole milk, Chan and others (1993) showed after six months of storage at 40 ^{0}C, the packages containing oxygen absorbers had only 0.07% (μg/g lipid) COPS while the packages with no oxygen absorbers had a level of 5.9% COPS.

The degree of unsaturation of fatty acids also affects the rate at which oxidation occurs because as the number of double bonds increases, so does the number of potential oxygen addition sites. Relative rates of oxidation are 1:10:20:40

for oleic, linoleic, linolenic and arachidonic acids, respectively at 37 ^{0}C (Holman and Elmer 1947). The rates of oxidation in polyunsaturated lipids are considerably higher than monounsaturated lipids due to activated methylene groups along the carbon chain. These rates were disputed by Cosgrove and others (1987) saying that the earlier studies did not account for different rates of initiation. In experiments with controlled initiation rates, Cosgrove and others (1987) calculated the relative rates of oxidation to be 10:20:30 for linoleic, linolenic and arachidonic acids, respectively at 37 ^{0}C. In addition to the number of double bonds, fatty acid geometry influences oxidation rates in that *cis* isomers generally oxidize faster than *trans* isomers. Relative oxidation rates of linoleoyl glycerols, from slowest to fastest are: free fatty acids, monoacylglycerols, diacylglycerols, triacylglycerols, phospholipids (Cosgrove and others 1987). The high rate of reactivity of phospholipids is attributed to their high levels of unsaturation and increased oxygen exposure due to their close proximity to the surface of the lipid molecule (Hornstein and others 1961).

Temperature has a direct effect on all chemical reactions including lipid oxidation of foods, which makes temperature control critical during processing and storage. Several studies have researched this subject and show that increased temperatures result in increased lipid oxidation. Han and others (1995) researched the effect of heating on the oxidation of ground beef by comparing samples with five different temperature treatments: no heat, 55, 70, 85 and 100 ^{0}C. Using thiobarbituric acid reactive substances values, it was determined that all levels of heat treatment produced significantly more ($p<0.05$) oxidation products than did the previous temperature level, with the exception of 100 ^{0}C showing no significant

difference from 85 ^{0}C. Processing temperatures can also affect the extent of lipid oxidation. In a study of oxysterol (OS) formation in spray dried eggs, two different sets of spray drying temperatures were compared. Drying the eggs with inlet and outlet temperatures of 225 and 140 ^{0}C, respectively produced significantly ($p<0.01$) more OS than eggs processed at inlet and outlet temperatures of 170 and 117 ^{0}C (Guardiola and others 1997).

Storage temperatures also impact the degree of lipid oxidation foods experience. During the storage of spray dried whole milk, cholesterol oxidation products were found in significantly higher concentrations ($p<0.05$) in samples stored at 40 ^{0}C for six months than in corresponding samples stored at 20 ^{0}C (Chan and others 1993). In rapeseed oil stored at either 35 or 50 ^{0}C, the rate of volatile formation measured in ng headspace volatiles/L/day, was higher in samples stored at 50 ^{0}C (Andersson and Lingnert 1999). In general, the rate of oxidation of polyunsaturated fatty acids is decreased by a factor of 2-3 for every 10 ^{0}C decrease in temperature (Hardy and Smith 1976).

The water activity of foods also affects the rate of lipid oxidation. At water activities less than 0.1 and between 0.6 and 0.8, the rate of lipid oxidation is fastest, with water activities between 0.2 and 0.3 having the slowest rate (Labuza 1984). The high rate of reaction at water activities less than 0.1 is due to the availability of the lipid to react with oxygen because there is little water to form a protective layer. As the water activity increases to 0.2 - 0.3, the small amounts of water slow lipid oxidation by hydrating metal catalysts, binding free radicals and protecting the lipid from oxygen. When levels of 0.6 are reached, oxidation is again at a high rate due

to the mobilization of reactants by the water (Gopala and Prabhakar 1992). Roozen and Linssen (1992) compared the development of lipid oxidation products in spray-dried milk stored at 37 ^{0}C for 10 weeks at water activities of 0.11, 0.24 and 0.34. The spray-dried milk stored at 0.24 water activity had developed fewer lipid oxidation products than the treatments at 0.11 and 0.34, both of which had similar development of oxidation products.

Transition metals act as pro-oxidants in lipid oxidation reactions because of their ability to catalyze the initiation and propagation steps. Metals such as iron, nickel or copper function by activating a molecule of oxygen to form singlet oxygen and a peroxy radical. Metals can also react directly with the fatty acids to form an alkyl radical. In the propagation step, metals accelerate the decomposition of hydroperoxides, resulting in the generation of off-flavors and odors (Nawar 1996). Iron-catalyzed lipid oxidation is a problem often encountered when dealing with muscle foods due to the presence of myoglobin and hemoglobin. Han and others (1995) studied the effects of the addition of iron in various forms on lipid oxidation of ground beef. All treatments with added iron produced significantly ($p<0.05$) more lipid oxidation compounds than did the control beef with no additional iron.

The pH of a food system also seems to affect the rate of lipid oxidation although the mechanisms are not fully understood. In a study of beef heart surimi-like material, Srinivasan and others (1996) demonstrated a significant decrease in lipid oxidation products as the pH of the material increased from 5.5 to 6.0 to 7.0. Similar results were obtained by Richards and Hultin (2002) when the oxidation of cod muscle was studied at pH 6.0, 7.2 and 7.6. They found that as the pH increased

so did the lag time prior to off-odor formation. At pH 6.0, a sensory panel detected off-odors after 24 hours but at pH 7.6, off-odors were not detected until 14 days.

Antioxidants

To combat the deteriorative effects of lipid oxidation, antioxidants are often added to food systems. Antioxidants are substances that delay the onset or slow the rate of lipid oxidation by reducing free radicals to nonradical chemical species. The subsequent antioxidant radical is very stable and does not react further with lipid compounds. Antioxidants can also function by binding to prooxidative substances and preventing them from initiating the oxidation chain reaction (Jadhav and others 1996). Selection of the appropriate antioxidant is based on its effectiveness in the type of fat in the product, solubility in the oil or water phase of the food, the ability to be dispersed evenly throughout the food system, approval of federal regulations and the ability to survive processing so that the antioxidant can inhibit oxidation in the finished product (Buck 1984).

Antioxidants are classified as either primary (chain-breaking) or secondary according to their mode of action. Primary antioxidants are phenolic compounds that disrupt the lipid oxidation chain reaction by donating hydroxyl hydrogens to lipid and peroxy radicals, converting them into more stable products. Secondary antioxidants inhibit the initiation of lipid oxidation by scavenging oxygen, chelating metal ions, absorbing ultraviolet light or deactivating singlet oxygen (Gordon 1990).

Antioxidants are also classified as either natural or synthetic based upon their source. Natural antioxidants can be isolated from oilseeds, fruits, vegetables, spices

and other sources and are readily accepted by consumers because they are generally regarded as being safe. Examples of common, naturally occurring antioxidants include carnosine, citric acid, ascorbic acid and tocopherols. Disadvantages of natural antioxidants include being more expensive and imparting off-flavors or colors to the products they are added. Another disadvantage of natural antioxidants is variability. Different preparations of the same antioxidant compound often contain different amounts of active ingredients and care must be taken so that the proper amount of the antioxidant compound is added.

Synthetic antioxidants are phenolic compounds that have been developed to overcome the negative aspects of natural antioxidants. Synthetics are usually less expensive, more efficient and do not add unwanted flavors or colors to the finished product. Some common synthetic food antioxidants are butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT), and propyl, octyl and dodecyl gallates. To their detriment, synthetic antioxidants have strict limitations on usage and lower consumer confidence due to questions of safety. The total amount of synthetic antioxidants that can be added to a food product alone or in combination is limited to 200ppm based on the fat content (Pokorny 1991).

BHA and BHT are insoluble in water and highly soluble in fat. They also have high steam volatility, making them undesirable in spray drying processes. BHA and BHT are often added to food packaging materials and limit oxidation by migrating into the food during storage. TBHQ is often added to frying oils to protect them from oxidation and to provide carry through protection to the fried foods. Propyl gallate (PG) has been shown to be effective as an antioxidant in oils and fresh sausages.

PG is unsuitable for frying operations since it loses effectiveness at high temperatures due to its low melting point of 148 ^{0}C (Dziezak 1986).

St. Angelo and others (1990) compared the ability of different antioxidants to reduce lipid oxidation in cooked ground beef patties. The results showed that after two days of storage at 4 ^{0}C PG, TBHQ and a combination of BHA and BHT all significantly reduced the amount of hexanal formed in the meat when compared to a control with no antioxidants added. Different concentrations (125, 250, 375, 500 ppm) of water-based rosemary extract were also added to the ground beef in the same study. The treatments were able to reduce the amounts of hexanal formed, with increasing concentrations generally providing more protection against oxidation, but the results were not significant.

Antioxidants have also been utilized in spray drying applications. Huber and others (1995) added antioxidant preparations of BHA, tocopherols or ascorbyl palmitate to liquid egg yolk prior to spray drying in an electric-powered drier to test the inhibition of COPS formation as compared to a control with no antioxidant. They found that all antioxidant treatments reduced the amounts of COPS formed, with the tocopherols being most effective. In a similar study, Guardiola and others (1997) compared the addition of propyl gallate or alpha-tocopherol to liquid egg. They found that propyl gallate was able to inhibit oxysterol formation in the finished spray-dried egg.

Analysis of Volatile Flavor Compounds

Solid-phase microextraction (SPME) is a technique for a rapid, solventless extraction of volatile compounds such as those formed from lipid oxidation. It was originally developed for chemical analysis of wastewater by Berlardi and Pawliszyn in 1989 but has since been applied to food analysis. Benefits include minimal sample preparation, no chemical usage and the prevention of artifact formation (Harmon 1997).

SPME is performed using a fused silica fiber coated with a thin film of stationary phase. During collection of volatiles, the film acts as an organic solvent to which the compounds adsorb. Choice of fiber coating depends on analyte characteristics such as polarity. After sample volatiles have adsorbed onto the fiber, they can be thermally desorbed directly onto the injection port of a gas chromatograph for identification and quantification of compounds (Harmon 1997).

SPME has been compared to more traditional headspace analysis techniques to determine its relative sensitivity, accuracy and reproducibility. Jelen and others (1998), while analyzing esters and alcohols present in beer, found no significant differences in the levels of compounds detected in a comparison of SPME and static headspace analysis by automatic injection. The results yielded a correlation coefficient of r=0.9993. A study evaluating samples of cola compared SPME with dynamic headspace concentration onto a Tenax trap. Both methods were found to be equally reproducible (Elmore and others 1997). Each of the previous studies concluded than SPME is easier and less expensive than other headspace analysis

methods and the compounds detected by SPME are dependent on the polarity of the fiber used.

Steenson and others (2002) used SPME to analyze corn and soybean oils and identified the major volatile compound as 2,4-decadienal and hexanal, both known products of lipid oxidation. They found SPME to be a simple, reproducible and sensitive method for the measurement of volatile compounds.

Hexanal is a breakdown product of linoleic acid (Labuza 1971) and has been shown to be an accurate indicator of lipid oxidation. Analysis of volatiles generated by lipid oxidation often results in hundreds of compounds. Hexanal content has shown a high correlation with sensory panels and objective tests when studying the extent of lipid oxidation. Fritsch and Gale (1977) studied hexanal formation in low fat foods and concluded that hexanal is an accurate indicator of lipid oxidation. Their comparison of sensory panel flavor scores and hexanal content measured by gas chromatography resulted in a correlation of 0.99. A study of cooked beef yielded similar results. The scores from a sensory panel trained to recognize warmed-over flavor were compared to hexanal values of reheated beef obtained using gas chromatography, gave a correlation coefficient of 0.80 (St. Angelo and others 1987).

The 2-thiobarbituric acid (TBA) test is another method commonly used to determine the extent of lipid oxidation in foods. An advantage of this test is that it can be performed directly on food products without having to first extract the lipid fraction. The TBA test measures the amount of malonaldehyde, a breakdown product of lipid peroxides, present in the sample and correlates well with sensory data (Shahidi 1994).

3. MATERIALS AND METHODS

Standard Plasma Processing

Liquid plasma from the slaughter plant is shipped to the processing plant, stored at 2 ^{0}C in holding tanks and processed within 72 hours. The first step in plasma processing is microfiltration (MF) to reduce the bacterial load followed by reverse osmosis concentration (ROC) to increase the solids content from 8 to 24%. The concentrated plasma is then spray-dried and bagged for shipping (Nnanna 2001). Finished product plasma from this basic process will be referred to as 'regular' plasma throughout the thesis. Another product, which has lactic acid added at the ROC step to give a desired whiter color, will be referred to as '600N'.

Plasma Characterization

Sample Collection

Samples of MF, ROC and spray-dried bovine plasma were collected to represent each processing stage from the Proliant plasma processing facility in Fremont, NE. One set each of regular and 600N plasma were collected on separate processing dates. All samples collected were packed on ice for transport to Ames, IA then stored at -10 ^{0}C prior to analysis.

Lipid Analysis

Lipids in the plasma were extracted with chloroform and methanol based on the Bligh and Dyer (1959) method. Ten grams of plasma powder were suspended in 20 g of distilled deionized water and homogenized with 30 mL methanol and 15 mL

chloroform using a PowerGen 125 homogenizer (Fisher Scientific, Chicago, IL) for 2 min. An additional 15 mL of chloroform were added to the mixture and it was blended for another 2 min. The mixture was centrifuged at 500 x g for 10 min (Beckman J2-21, Palo Alto, CA). The upper layer consisting of methanol and water was removed by aspiration. The bottom layer, chloroform and plasma, was filtered with vacuum through a Buchner funnel. The plasma residue and filter paper were blended with 30 mL chloroform for 2 min and again filtered using the Buchner funnel. The filtrate was washed twice with 10 mL chloroform. The collected chloroform-lipid extract was passed through anhydrous sodium sulfate and the sodium sulfate was then rinsed with 30 mL of chloroform. The filtrate was concentrated to 10 mL using a rotary evaporator. Lipid content was determined by drying 1 mL extract under nitrogen.

Thin layer chromatography was used to separate and identify the types of lipid present in the extract. One microliter of extract from each sample was spotted onto a 20 x 20 cm PET polyester plate with silica gel as the stationary phase (Supelco, Bellefonte, PA). The plate was developed in an eluting solvent system of hexane, diethyl ether and acetic acid (85:15:2, v/v) until the solvent front was within 2 cm of the top of the plate. The plate was developed in an iodine chamber for 5 min to visualize the components. The lipids were identified by comparison to accepted literature values (Christie 1989).

Fatty acid methyl esters (FAME) were prepared by heating the lipid extract with boron trifluoride-methanol reagent (Supelco) according to the procedure described by Morrison and Smith (1964). Approximately 2 mL aliquots of lipid

extract were dried under nitrogen prior to the addition of 1 mL boron triflouride-methanol reagent. The mixture was capped with a teflon septum, vortexed, then heated to 100 ^{0}C for 30 min in a 20 mm Boekel digital dry bath incubator (Feasterville, PA). Once cooled to room temperature, the FAME samples were transferred to centrifuge tubes with 2 mL hexane and 1 mL deionized water. The mixture was vortexed for 30 s then centrifuged at 500 x g (Sorvall GLC-1, Newtown, CT) for 10 min. After centrifugation, the upper hexane layer was transferred to a vial for analysis.

The FAME were separated using gas chromatography. A 1 μL aliquot was injected into a 220 ^{0}C gas chromatograph (GC) (Hewlett Packard 6890 Series, Wilmington, DE) injection port with a 20:1 split. FAME were separated using a cyanopropyl CP-Sil-88 capillary column (100 m x 250 μm x 0.20 μm Chrompak, Middelburg, The Netherlands). The column had a constant pressure of 138.0 kPa with helium as the carrier gas. Column flow rates to the flame ionization detector were air, 400 mL/min; nitrogen, 25 mL/min and hydrogen, 30 mL/min. The initial GC oven temperature of 90 ^{0}C was held for 5 min before increasing 10 ^{0}C/min to 120 ^{0}C then finally a 3 ^{0}C/min increase to 220 ^{0}C. Fatty acids were identified using an external standard (Supelco).

Total Solids / Moisture Content

Total solids in the MF and ROC plasma were determined by oven drying. Nine milliliter aliquots were measured into pre-weighed aluminum weighing dishes (#08-732, Fisher Scientific, Chicago, IL) and placed into a 100 ^{0}C oven (Model OV-

18A, Blue M Electric, Watertown, WI) for 24 hours. The dry samples were allowed to cool in a dessicator prior to weighing. Total solids in the liquid plasma were calculated and expressed as a percentage using the following formula:

$$\frac{\text{Dry Sample wt.}}{\text{Wet Sample wt.}} \times 100 = \% \text{ Solids in Plasma}$$

Moisture content of spray dried plasma was also determined using the oven drying method. Three grams of plasma powder were measured into a pre-weighed aluminum weighing dish, dried and re-weighed as described above. Moisture content of the dry plasma samples was calculated and expressed as a percentage using the following formula:

$$1 - \frac{\text{Dry Sample wt.}}{\text{Wet Sample wt.}} \times 100 = \% \text{ Moisture in Powder}$$

pH

pH measurements of the plasma samples were obtained using a digital pH meter (Model 420A, Orion Research, Inc., Beverly, MA) calibrated with buffers at pH 7.0 and 10.0 (Fisher # SB107-500 and SB115-500, respectively). Fifty milliliter samples of MF, ROC and 12% spray dried plasma suspensions of were measured at 20 ^{0}C.

Volatile Analysis

Spray-dried plasma was reconstituted to a 12% wt/vol concentration with deionized water by stirring the mixture at room temperature until all solids were suspended. Plasma samples from the MF and ROC processing steps were

analyzed without modification. Fifty milliliters of the suspended, MF or ROC plasma were transferred to 125 mL glass bottles containing stir bars and crimp sealed with PTFE/Silicone septa (Supelco).

Gels were prepared by heating 100 mL of the 12% plasma suspension in a 90 ^{0}C water bath for one hour. The gel was removed from the water bath and allowed to cool at room temperature for 30 min before being transferred to a Whirlpak^{TM} bag. In the bag, the gel was crushed by hand in order to maximize surface area and volatile release. The bag was then closed tightly leaving 3 cm of headspace.

All prepared samples were placed in a 40 ^{0}C water bath and allowed to temperature equilibrate for 10 min. Liquid samples were stirred using a Thermolyne stir/hot plate (Model #SPA1025B, Dubuque, IA) while the gels were analyzed without stirring.

Volatiles were collected by inserting a solid-phase microextraction (SPME) 2 cm-50/30 μm divinylbenzene/ carboxen/polydimethylsiloxane stable flex fiber assembly (Supelco, Bellefonte, PA) into the headspace of each sample for 30 min. The volatiles were thermally desorbed from the fiber into a 220 ^{0}C splitless injection port of a GC (Hewlett Packard 6890 Series) for 3 min. Volatiles were separated using a 5% diphenyl / 95% dimethylsiloxane fused-silica capillary column (SPB-5, 30 m x 0.25 mm x 0.25 μm film thickness, Supelco). The oven temperature was held at 33 ^{0}C for 3 min and increased at the following rates: 5 ^{0}C/min from 33-80 ^{0}C, 4 ^{0}C/min from 80-95 ^{0}C, 5 ^{0}C/min from 95-115 ^{0}C and 10 ^{0}C/min from 115-200 ^{0}C. Column pressure was held constant at 124.0 kPa with helium gas as the carrier.

Flow rates to the flame ionization detector were: air, 400 mL/min; nitrogen, 25 mL/min and hydrogen, 30 mL/min. Peaks were identified with standards (Aldrich, Milwaukee, WI and Sigma, St. Louis, MO) by comparing retention times and confirmed using GC/MS.

A gas chromatograph-mass spectrometer (Trio 1000, Fisions Instruments, Danvers, MA) with a quadrupole mass analyzer was used for the confirmation of the identity of the volatile compounds. The conditions for the mass spectrometer were as follows: source electron energy, 70 eV: source electron current, 150 μA; ion source temperature, 220 ^{0}C; interface temperature, 220 ^{0}C; source ion repeller, 3.4 V; electron multiplier voltage, 600 V; and scan range, 41-250 m/z. Mass spectra of the volatile flavor compounds were compared to the NBS Library for identification.

Experimental Processing Treatments to Improve Flavor Quality

Plasma Modification

Modifications to the liquid plasma were developed by Proliant (Ames, IA) in order to evaluate their effects on the formation of lipid oxidation compounds during spray drying. Plasma with fibrinogen removed was prepared by heating liquid plasma to 50 ^{0}C for one hour. The fibrinogen precipitate was collected and removed prior to spray drying. MF and ROC plasma were treated with silica at a 2% concentration to remove lipids in the plasma. The plasma was centrifuged to remove the silica and any adsorbed lipids prior to spray drying.

The treatments and a 600N control, all from the same processing date, were spray dried in the Proliant pilot plant (Ames, IA). Samples of the spray dried plasma

were analyzed as suspensions for volatile content as described above. Only one sample per treatment was analyzed due to limited amounts of plasma available.

Antioxidant Addition

TBHQ and ethylenediaminetetraacetic acid (EDTA) (Ashland Distribution Co, Columbus, OH) were added at 200 and 100 ppm, respectively to MF and ROC plasma to determine their effectiveness at reducing volatile compound formation in the spray dried product. The treatments and a 600N control, all from the same processing date were dried at the ISU pilot plant (Ames, IA) using an electric powered spray drier (Anhydro Compact Spray Dryer, 480V; APV Crepaco, Attleboro Falls, MA). A second control from the same processing date was dried at the Proliant processing facility (Fremont, NE) in a gas-fired spray drier (Blaw-Knox 750 Box drier, Mora, MN). Samples of the spray-dried plasma were prepared and analyzed as suspensions and gels for volatile content as described above.

This experiment was repeated using only TBHQ, added at 200 ppm to MFP and ROC plasma. Treatments of regular and 600N were prepared and spray-dried at the ISU pilot plant (Ames, IA). Control samples of regular and 600N plasma from the same processing date as the treatments were dried at the Proliant processing facility (Fremont, NE). Samples of the spray-dried plasma were prepared and analyzed as suspensions and gels for volatile content as described above. Again, only one sample per treatment was analyzed due to the limited amount of plasma.

Experiment 1. Antioxidant Addition and Spray Drier Comparison

After preliminary research was completed, two experiments were devised to study factors that affect lipid oxidation. The first study focused on the addition of antioxidants to the plasma prior to spray drying and the type of spray drier, either electric or propane gas-fired.

Four antioxidants, TBHQ, PG, BHT and rosemary, were used in this study. TBHQ, PG and BHT (Eastman Chemical Co., Kingsport, TN) were added to regular plasma concentrate at a level of 200 ppm based on lipid content of the ROC plasma. Rosemary (Duralox MANC-213) (Kalsec, Kalamazoo, MI) was added at 2500 ppm.

Antioxidant treated and regular bovine plasma were dried in either a gas-fired drier at the Proliant pilot plant (Ames, IA) or an electric drier in the ISU pilot plant (Ames, IA). Both driers were set with an inlet temperature of 204 ^{0}C and an outlet temperature 95 ^{0}C. All samples were stored in airtight containers and frozen prior to analysis. Proliant Inc. (Ames, IA) processed all samples.

Spray-dried plasma samples were prepared and analyzed for volatile compounds using gas chromatography as described in the 'Plasma Characterization, Volatile Analysis' section above.

The experiment was designed as a two-way factorial with antioxidant treatment and spray drier type as the main factors. The experiment was replicated three times using samples from three different processing dates. Individual analyses were conducted in duplicate. Statistical analysis of peak areas was done using analysis of variance and mean separation methods to determine significance of main factors and interactions using the SYSTAT software program.

Experiment 2: Spray Drier Residence Time and Powder Storage Study

This study was designed to show the effects of spray drier residence time and plasma powder storage time on the formation of lipid oxidation compounds. This experiment utilized regular bovine plasma and the Proliant pilot plant (Ames, IA) propane-fired spray drier.

To determine the effect of spray drier residence time on volatile compound formation, dried plasma was collected either directly from the outlet (regular) or by brushing down the powder that adhered to the walls in the drying chamber after twenty minutes (BD). Storage effects were determined by analyzing plasma powders immediately after processing (0 weeks) and after an eight-week storage period. Samples were stored in sealed plastic bags at room temperature with minimal light exposure. Proliant, Inc. (Ames, IA) dried and stored all samples.

Spray-dried plasma samples were prepared and analyzed for volatile compounds using gas chromatography as described in the 'Plasma Characterization, Volatile Analysis' section above.

A three-way factorial experiment with antioxidant treatment (control, 200 ppm TBHQ, 1000 ppm rosemary concentrate (RC) (Pinus TKI, Race, Slovenia)), spray drier residence time (0 and 20 min) and plasma powder storage time (0 and 8 weeks) as the main factors was replicated twice using samples from different processing dates. Individual analyses were conducted in duplicate. Statistical analysis of peak areas was done using analysis of variance and mean separation methods to determine significance of main factors and any interactions using the SYSTAT software program.

4. RESULTS AND DISCUSSION

Preliminary Research

Plasma Characterization

Lipids were extracted from spray-dried regular and 600N plasmas. The total lipid content of the samples was 3.77 and 3.99%, respectively. Differences in lipid content are due to raw material variation between processing dates. Thin layer chromatography analysis of the lipid fraction showed the presence of phospholipids, free fatty acids, triacylglycerols and cholesterol esters. Each of these compounds is susceptible to oxidation, with the phospholipids having the greatest potential due to their high degree of unsaturation and close proximity to the surface of the membrane resulting in increased exposure to oxygen and metal catalysts (Lea 1957). A study by Larick and others (1989) concluded that the phospholipid content of bovine meat directly affects the amount of off-flavors formed during cooking.

Plasma and beef muscle contain similar amounts of unsaturated fatty acids, (Table 1), but plasma contains more polyunsaturates making it more susceptible to oxidation. The primary unsaturated fatty acid in beef muscle is monounsaturated (oleic acid, 39.99%, Andrae and others 2001) while plasma is predominately polyunsaturated linoleic acid (28.25%). Holman and Elmer (1947) showed that linoleic acid undergoes oxidation reactions ten times faster than oleic acid.

Andrae and others (2001) reported the linoleic acid content in beef muscle to be 3.78% while analysis of the plasma showed an average content of 28.25% (Table 1). With high levels of linoleic acid, plasma is likely to undergo oxidation when

exposed to the heat of the spray drying process and form undesirable oxidation products such as hexanal.

Lipid oxidation in cooked beef has been the subject of many studies (St. Angelo and others 1990; Drumm and Spanier 1991; Han and others 1995; Boylston and others 1996). It is accepted that heat exposure during cooking initiates oxidation reactions in unsaturated lipids resulting in the undesirable off-flavors in beef. Hexanal, a breakdown product of linoleic acid, contributes to the off-flavors with its grassy and painty flavor characteristics (St. Angelo and others 1987).

Table 1. Percent fatty acid composition of 600N plasma, regular plasma and beef muscle.

Fatty Acid	600N Plasma	Regular Plasma	Beef Muscle[a]
14:0	0.40	0.31	2.85
16:0	13.33	13.72	26.94
18:0	23.87	21.08	16.90
Saturated Fatty Acids	37.60	35.11	46.69
18:1	13.41	13.13	39.99
18:2	28.22	28.29	3.78
18:3	0.60	0.74	0.08
20:4	3.30	2.90	0.83
Unsaturated Fatty Acids	45.53	45.06	44.68

[a]Andrae and others 2001

Total solids in MF and ROC plasma were 8.76 and 25.21%, respectively. These values fall within the expected ranges of solids content for processed bovine

plasma as reported in the literature (Stevenson 1979) and the plasma should not have required excess heat or extended time to be adequately spray-dried.

Volatile analysis of regular and 600N plasma showed few compounds to be present in the ROC, but increased amounts of volatiles were present in spray-dried plasma samples of suspensions and heat-set gels (Tables 2 and 3) despite the fact the ROC plasma contained twice as many solids. Total volatile contents as measured by peak area for regular plasma were 66.42, 178.93, 277.56 and 600N, 104.60, 433.14, 730.49 for ROC, 12% suspensions and 12% gels, respectively. The lack of volatiles in the liquid plasma and subsequent development of volatiles after spray drying supports the hypothesis that the heat and oxygen exposure the plasma receives during spray drying initiates lipid oxidation reactions resulting in volatile compound formation. The removal of water results in the molecules having greater accessibility to atmospheric oxygen making them more likely to oxidize (Jadhav and others 1996). Studies by Chan and others (1993) and Guardiola and others (1997) of whole milk and egg, respectively, also demonstrate that spray drying promotes oxidation. Tsai and Hudson (1984), while studying cholesterol oxidation products (COPS) in egg yolk, found no oxidation compounds in raw or freeze-dried egg yolk, but significant amounts of COPS were present in spray-dried samples. The further heat exposure of the plasma during gel formation, 90 ^{0}C for one hour, also seemed to increase lipid oxidation as greater amounts of volatiles were present in the gels as compared to the suspensions. This is expected as heat is a known catalyst for lipid oxidation reactions.

Table 2. Volatile compound content[a] of regular plasma.

Compound	ROC Plasma	12% Suspension	12% Gel
Pentanal	ND[b]	2.25 ± 1.01	3.74± 1.77
Hexanal	ND	59.86 ± 0.88	63.49 ± 22.98
Heptanal	ND	1.80 ± 0.27	2.59 ± 0.59
1-Octen-3-ol	ND	5.68 ± 0.64	6.73 ± 0.32
2-Octanone	ND	4.69 ± 0.33	10.38 ± 0.06
Octanal	ND	ND	2.01± 0.03
Nonanal	ND	ND	4.51 ± 0.76

[a]Peak areas. Values are an average of two replications ± one standard deviation.
[b]None Detected.

Table 3. Volatile compound content[a] of 600N plasma.

Compound	ROC Plasma	12% Suspension	12% Gel
Pentanal	ND[b]	9.16 ± 3.37	28.17 ± 1.97
Hexanal	ND	237.84± 13.54	497.12 ± 12.06
Heptanal	ND	7.74 ± 0.50	17.78 ± 0.64
1-Octen-3-ol	1.21± 0.86	13.53 ± 0.93	16.52 ± 2.06
2-Octanone	1.28 ± 0.01	6.41 ± 1.18	19.98 ± 0.16
Octanal	ND	1.35 ± 0.13	4.77 ± 1.00
1-Octanol	ND	1.91 ± 0.37	1.84 ± 0.31
Nonanal	ND	3.10 ± 0.49	3.99 ± 0.95

[a]Peak areas. Values are an average of two replications ± one standard deviation.
[b]None Detected.

Volatile compounds identified in the plasma were aldehydes, ketones and alcohols, all of which are typical products of lipid oxidation reactions involving the unsaturated fatty acids linoleic, oleic and arachidonic (Ladikos and Lougovois 1990). The content of hexanal, a known indicator of lipid oxidation (Fritsch and Gale 1977; St. Angelo and others 1987), correlates to the amount of heat to which the plasma had been exposed. No hexanal was present in ROC samples of either regular or 600N plasma. After spray drying and heat treatment to form a gel, hexanal content, as measured by peak area, for regular plasma was 60.86 and 98.91, and for 600N, 237.84 and 497.12 for suspensions and gels, respectively.

The lower pH of the 600N plasma seemed to have a catalytic effect on the oxidation reactions as nearly twice as many total volatiles were detected than in the regular plasma samples. This could be attributed to the fact that lower pH values cause iron to be released from heme molecules and reduce the iron from Fe^{3+} to Fe^{2+} resulting in a catalyst for lipid oxidation (Jacobsen and others 2001). Heme may be present in plasma if platelets are not completely removed during centrifugation of the whole blood. A study of ground poultry at pH values of 3, 5, 6, 7 and 9.5 showed decreasing oxidation with increasing pH as measured by thiobarbituric acid (TBA) values after a 14 day storage period. At pH 6 and 9.5, TBA values of 5 and 0.4 (mg malonaldehyde/kg ground turkey) were recorded, respectively (Chen and Waimaleongora 1981). Chang and others (1961) found that off odors and flavors are usually undetectable at TBA values less than 0.5.

Experimental Processing Treatments to Improve Flavor Quality

Plasma Modification

Analysis of fibrinogen removal (FR), lipid removal at the microfiltration step (LR-MF) and lipid removal at the reverse osmosis concentration step (LR-ROC) plasma samples showed a decrease in lipid oxidation compounds for each treatment as compared to the control plasma (Figure 1). It is known that proteins have the ability to bind flavor compounds (Damodaran 1996) and the FR treatment was to reduce volatile compounds by removing any compounds bound to the fibrinogen (Nnanna 2001). This was somewhat successful in that the total volatiles and hexanal were reduced by 20 and 28%, respectively, but the remaining serum proteins are also capable of binding volatile compounds. Damodaran and Kinsella (1980) showed that carbonyls have a high affinity for serum proteins, making the heat shock treatment an incomplete solution.

Lipid removal treatments were to reduce lipid oxidation by removing the lipid from the liquid plasma prior to spray drying. LR – MF resulted in a greater decrease of hexanal and total volatiles than the other plasma modification treatments (Figure 1). By reducing the amount of lipid present in the plasma, the amount of potential oxidation reactants is also reduced and ultimately fewer volatile compounds will be formed. The LR – ROC samples showed minimal change but this is most likely due to the fact that the same amount of silica was added to both the MF and ROC treatments. The LR – ROC plasma was concentrated and therefore needed three times more silica to get the same results.

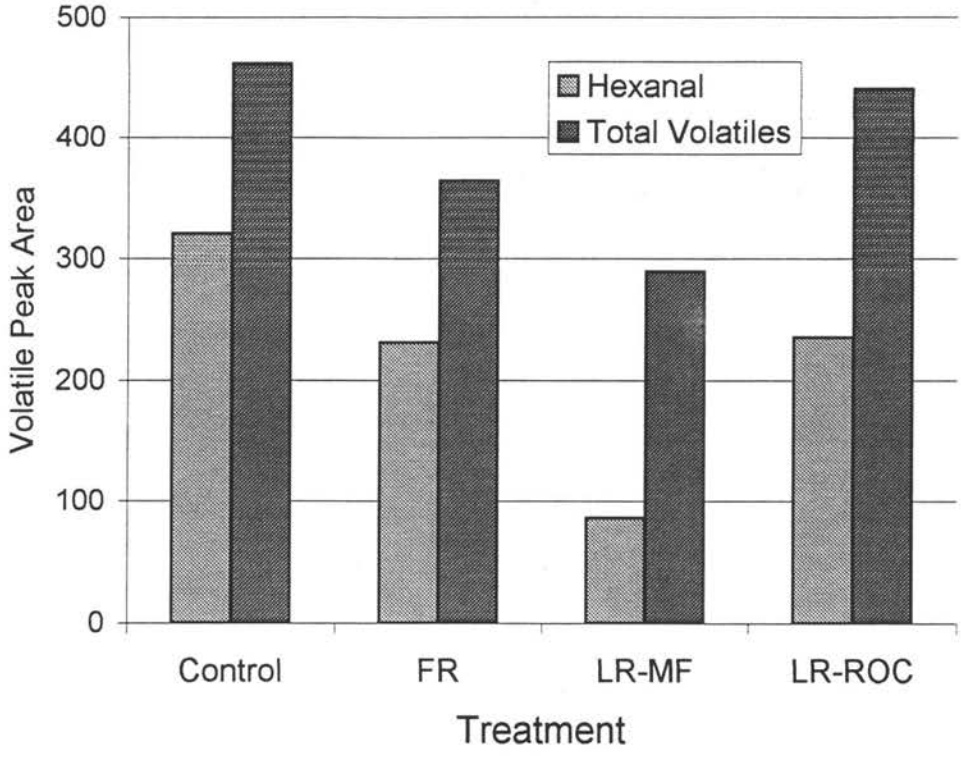

Figure 1. Volatile content of plasma treatment suspensions.
FR Fibrinogen Removal
LR-MF Lipid Removal - Microfiltration Step
LR-ROC Lipid Removal -Reverse Osmosis Concentration Step

Antioxidant Addition

The preliminary research provided evidence that the off-flavors in the spray-dried bovine plasma could be attributed to lipid oxidation. Tables 2 and 3 show that few volatiles were present in the liquid ROC plasma and increased amounts of oxidation products were present in samples made from spray-dried powders. Figure 1 shows the effectiveness of lipid removal prior to spray drying to prevent volatile compound formation in the LR – MF treatment. Although the silica treatment to remove lipids before spray drying proved successful, it would require considerable capital investments to implement on a commercial production scale so another alternative was sought.

Antioxidants are compounds that inhibit lipid oxidation by preventing or interrupting the free-radical chain reaction. The subsequent antioxidant radical formed is very stable and does not react further with lipid compounds (Jadhav and others 1996). Antioxidant treatments could be implemented in commercial production by simply adding antioxidants to the plasma prior to spray drying and would not require any additional equipment.

Initial antioxidant trials compared the effectiveness of EDTA and TBHQ. The TBHQ treatment was effective and fewer volatile compounds were formed than in the control sample with no antioxidant, however the addition of EDTA did not reduce volatile compound formation (Table 4). EDTA functions as an antioxidant by chelating metal ions and preventing them from catalyzing oxidation reactions. EDTA is best utilized in combination with a primary antioxidant or ascorbic acid (Madhavi and others 1996). In this application the heat from the spray dryer catalyzed the

oxidation reaction making the EDTA addition ineffective. TBHQ was more effective because it is a primary antioxidant and it was able to eliminate the free radicals formed by the heat of the spray drier.

Antioxidant addition to either MF or ROC plasma was also compared resulting in antioxidant addition to MF plasma being more effective at reducing volatile compound formation than the addition to ROC (Table 4). After a discussion of the exact procedure used it was found that the same amount of antioxidant was added in both treatments, therefore the ROC treatment contained three times less antioxidant per mg lipid than did the MF treatment. The experiment was repeated with the correct proportions of antioxidants and there was little difference between the two treatments (Table 5). It was decided to add the antioxidants to the ROC plasma to prevent any interference with the concentration process.

This experiment also brought attention to the effect different types of spray driers have on volatile compound formation. Two different control samples with no added antioxidants were prepared from plasma produced on the same processing date, but dried with different types of spray driers. A gas-fired drier at the Fremont, NE processing facility was used for one control sample and an electric-powered drier at the ISU pilot plant (Ames, IA) was used for the other. Table 4 shows that approximately five times more hexanal and twice as many total volatiles were produced in the sample from the gas-fired drier compared to the electric drier. The Fremont, NE sample was also processed under milder heating conditions with inlet and outlet temperatures of 175 and 80 ^{0}C, respectively while the ISU pilot plant sample was exposed to temperatures of 204 and 95 ^{0}C. Lower temperatures in the

Table 4. Volatile compound content[a] of suspensions of spray-dried plasma[b] with EDTA[c] or TBHQ[d] added at either the MF[e] or ROC[f] processing step.

	MF		ROC	
Treatment[g]	Hexanal	Total Volatiles	Hexanal	Total Volatiles
ISU Control	52.82	284.65	173.90	451.78
EDTA	76.65	242.93	195.25	422.70
TBHQ	29.98	217.71	144.21	433.47
Fremont Control	266.04	512.61	266.04	512.61

[a] Peak area. One replication per treatment.
[b] Plasma from one processing date, ISU control, EDTA and TBHQ treatments spray-dried at the ISU pilot plant. Fremont Control spray-dried at Proliant processing facility, Fremont, NE.
[c] Ethylenediaminetetraacetic acid.
[d] Tertiary butyl hydroquinone.
[e] Microfiltrated.
[f] Reverse Osmosis Concentrate.

Table 5. Volatile compound content[a] of suspensions of spray-dried plasma[b] with TBHQ[c] added at either the MF[d] or ROC[e] processing step.

Treatment	Hexanal	Total Volatiles
TBHQ at MF[f]	61.89	102.51
TBHQ at ROC – Regular	39.78 ± 3.05	124.06 ± 5.40
Control – Regular	223.64 ± 18.29	787.57 ± 26.34
TBHQ at ROC – 600N	162.39 ± 8.70	261.69 ± 13.58
Control – 600N	487.14 ± 22.67	751.09 ± 32.16

[a] Peak area. Values are averages of two replications ± one standard deviation.
[b] Plasma from one processing date. TBHQ treatments spray-dried at the ISU pilot plant. Control treatments spray-dried at Proliant processing facility, Fremont, NE.
[c] Tertiary butyl hydroquinone
[d] Microfiltrated.
[e] Reverse Osmosis Concentrate
[f] Only one replication for this treatment

Fremont drier should have resulted in fewer volatile compounds produced, which indicates a gas-fired drier will produce more oxidation compounds. Chan and others (1993) found that a gas-fired drier produced significantly more oxidation compounds in whole milk than did the electric drier. This was attributed to the formation of nitrogen oxides, which are known catalysts of lipid oxidation, in the gas drier.

Experiment 1: Effects of Antioxidants and Spray Drier Type

Moisture, lipid content and pH were measured for each of the control plasma samples (Table 6). Moisture content ranged from 4.25 to 5.97% with an average of 5.12% and lipids ranged from 2.49 to 3.96% with an average of 3.11%. The pH values averaged 8.88 with a standard deviation of 0.1. Variations in the plasma lipid content are due to the nature of the raw materials. The cattle from which the plasma is collected will have different concentrations of lipid in their blood due to variations in diet or the amount of time between the last meal and slaughter. The range of moisture contents could be due to differences in moisture content of concentrated plasma, however, in samples from code dates 1151 and 1155 the electric-dried samples had lower moisture contents even though they were made from the same plasma. This indicates the electric drier may be more efficient as both driers were set to the same temperatures.

There were no significant correlations between moisture or fat content with the amount of volatiles formed. This indicates that plasma composition is not the only factor determining the amount of volatiles formed, but processing conditions also impact the oxidation reactions.

Table 6. Moisture, lipid and pH values of spray-dried plasma.

Code Date	Spray Drier	Moisture Content (%)	Lipid Content (%)	pH
1108	Electric	5.97	3.05	8.81
1124	Gas	5.05	3.25	8.89
1151	Electric	4.25	3.96	8.86
1151	Gas	5.24	3.17	8.75
1155	Electric	4.27	2.49	8.97
1155	Gas	6.35	2.62	9.02

The control suspensions prepared with powder from the electric drier had significantly lower ($p<0.05$) amounts of lipid oxidation products and fewer total volatiles than the control suspensions from the gas drier (Figures 2 and 3). This indicates that gas driers increase the amount of volatiles produced through lipid oxidation. In a study of cholesterol oxidation in spray-dried whole milk, Chan and others (1993) showed that the nitrogen oxides produced in gas-fired spray driers increase the amount of oxidation compounds formed in the finished product. They found whole milk dried in an electric-powered drier had significantly fewer oxidation products than the milk dried in a gas-fired drier.

In the suspensions made from plasma dried in a gas-fired drier, a significant decrease ($p<0.05$) in hexanal content, as compared with the control, was observed in the TBHQ and PG treatments. This finding agrees with Jadhav and others (1995) who found TBHQ and gallate esters to be the most stable and effective antioxidants for a spray-drying process while BHT is steam volatile and less effective. BHT is also water insoluble and may not have been distributed as well as TBHQ and PG in the hydrophilic plasma (Sims and Fioriti 1980).

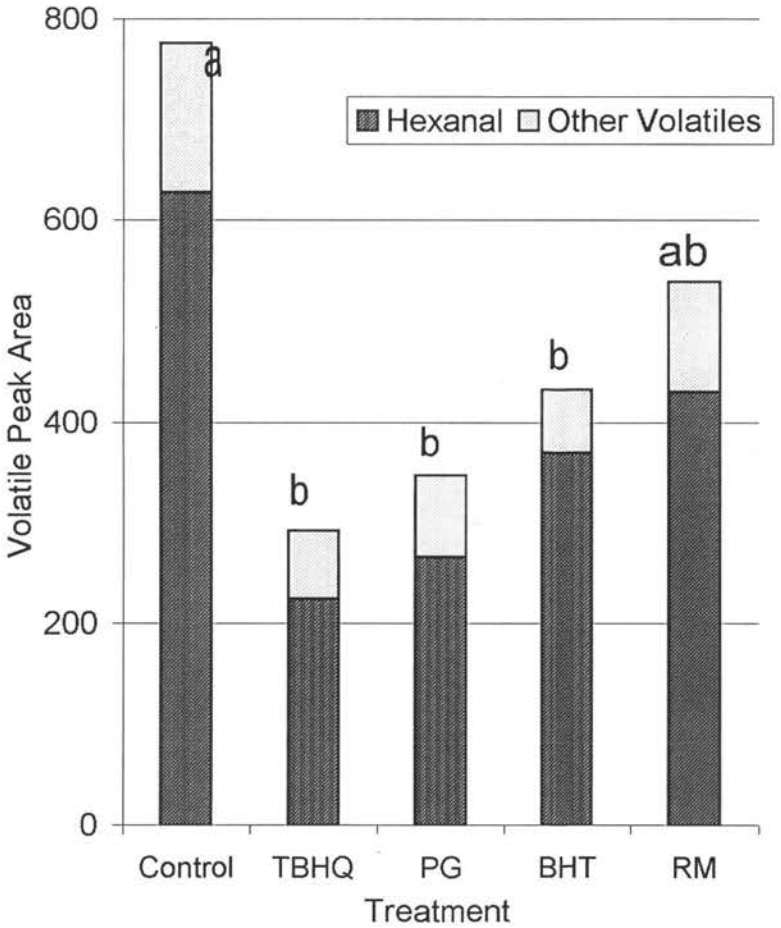

Figure 2. Volatile content of suspensions of gas-dried plasma. Treatments with different letters are significantly different ($p<0.05$)

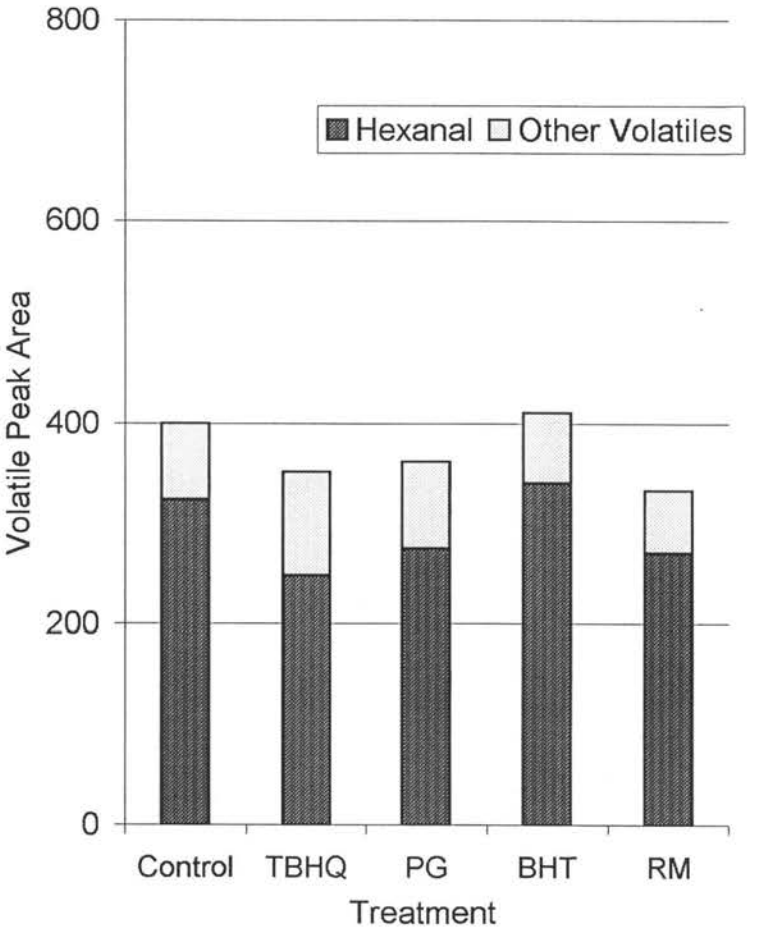

Figure 3. Volatile content of suspensions of electric-dried plasma. No significant difference between treatments ($p<0.05$)

TBHQ (*tert*-butyl hydroquinone), PG (propyl gallate), BHT (butylated hydroxytoluene), RM(rosemary)

The results of this experiment also showed the gas-dried TBHQ, PG and BHT treatments all had significantly fewer ($p<0.05$) total volatiles than the gas-dried control, demonstrating the effectiveness of antioxidant addition. However, antioxidant addition in the electric-dried samples had no significant effect ($p<0.05$) on volatile content when compared with the control. This suggests antioxidants are best utilized in a gas-fired system where high amounts of volatile compounds are generated from lipid oxidation reactions. Guardiola and others (1995), in a study of antioxidant effects on polyunsaturated fatty acid losses in spray-dried foods, concluded that antioxidants are most effective when processing conditions promote oxidation. The conditions in the gas-fired drier would tend to promote oxidation more than the electric drier, thus the antioxidants showed significant effects in the gas-dried samples.

While each of the synthetic antioxidants tested decreased the amounts of volatile compounds produced, the rosemary treatment had no significant effects. The rosemary preparation used was Duralox MANC-213 (Kalsec, Kalamazoo, MI) and according to the product specifications, only 20-27% of the 2500 ppm added to the plasma possessed antioxidant activity. The rosemary preparation also contained between 5 and 9% canola oil, which would increase the total amount of unsaturated fatty acids and the potential for lipid oxidation. St. Angelo and others (1990) in a study of antioxidant-treated, cooked ground beef showed that the addition of rosemary could reduce the amount of volatile oxidation compounds. Hexanal content was reduced from 82 (peak area x 0.001) to 19 in control and 500 ppm rosemary samples, respectively. However, beef treated with PG, BHA/BHT or

TBHQ (all at 125ppm), prepared under the same conditions as the rosemary treatment, each had hexanal counts of 0.1, demonstrating the effectiveness of synthetic antioxidants.

In the analysis of the gels (Figures 4 and 5), no significant results ($p<0.05$) were found for antioxidant effects, drier effects or interactions between the two factors. This was unexpected because the main difference between the plasma suspensions and plasma gels was a 90^0C heat treatment for one hour. Antioxidant activity should have still remained at this level of heat and shown results similar to the suspensions. Hamama and Nawar (1991) demonstrated that phenolic antioxidants were still active after heating at 185^0C for one hour. The results may have been affected by the fact the solid gels, in contrast to the suspensions, were analyzed in a static state and most likely reduced recovery of volatiles. Also, binding of the volatile compounds by the proteins in the gels may have decreased volatility of the lipid oxidation products (Kinsella 1978).

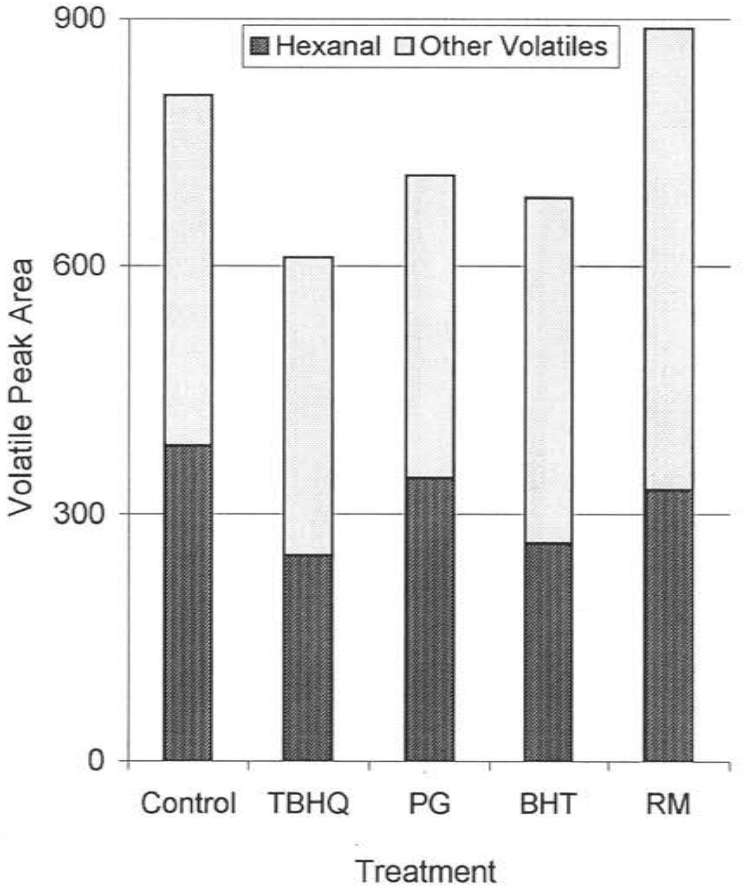

Figure 4. Volatile content of gels of gas-dried plasma. No significant difference between treatments ($p<0.05$).

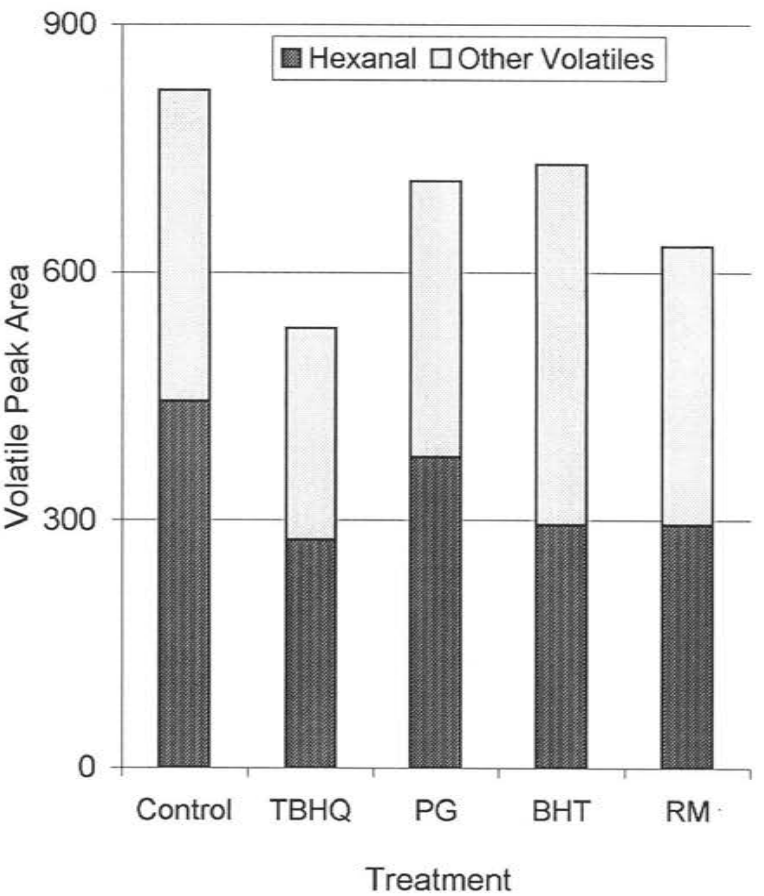

Figure 5. Volatile content of gels of electric-dried plasma. No significant difference between treatments ($p<0.05$).

TBHQ (*tert*-butyl hydroquinone), PG (propyl gallate), BHT (butylated hydroxytoluene), RM(rosemary)

Experiment 2: Spray Drier Residence Time and Powder Storage Study

A second experiment was designed to study the effects of spray drier residence time and powder storage on the development of lipid oxidation compounds in bovine plasma. Residence time treatments were regular and brushdown (BD) with regular modeling normal processing conditions and BD simulating excess heat exposure. The BD samples were produced by allowing the spray drier to run for twenty minutes and then collecting the powder that adhered to the sides of the drier. Three plasma treatments were compared, a control with no antioxidant; TBHQ, chosen based on previous results showing it to be capable of reducing lipid oxidation; and a rosemary concentrate (RC), selected to identify a natural antioxidant that is comparable in effectiveness to synthetic antioxidants.

The results showing volatile compound formation in suspensions are listed in Tables 7 and 8. For hexanal and octanal content, the RC BD treatment formed significantly higher amounts than the corresponding treatment with a normal residence time. This shows that excess heat promotes lipid oxidation and limits the effectiveness of antioxidants.

RC was not effective as an antioxidant for plasma powder stored for eight weeks. Both the BD and regular treatments showed significant increases in hexanal, 1-octene-3-ol, octanal and t-2-nonenal (Table 8) after eight weeks of storage. Hall and Cuppett (1993) reported that rosemary lost its antioxidant effects after 36 hours of storage when added to soybean oil. Subsequently, large amounts of oxidation products formed during the remainder of their experiment.

Table 7. Volatile compound formation in suspensions from Experiment 2 as influenced by residence time and antioxidant treatment or storage time.

	Residence Time	
Compound	Regular	Brushdown
Pentanal	7.30[b]	23.68[a]
Hexanal	176.53	349.64
Control	203.65[by]	259.7[by]
TBHQ[1]	111.57[by]	220.63[by]
RC[2]	214.38[by]	568.59[ax]
2-Heptanone	23.29[a]	21.27[a]
1-Octen-3-ol	67.65[a]	78.58[a]
2-Octanone	44.93	72.99
Zero Weeks	31.09[bx]	39.05[bx]
Eight Weeks	58.77[by]	106.93[ay]
Octanal	17.43	25.95
Control	22.97[by]	9.12[by]
TBHQ	14.73[by]	13.57[by]
RC	14.57[by]	55.15[ax]
Nonanal	12.16[a]	10.01[a]
t-2-Nonenal	36.01[a]	37.48[a]

[a, b]Residence time effects. Means are pooled unless interactions between storage time or antioxidant treatment are significant ($p<0.05$). Means within the same row with the same superscript are not significantly different ($p<0.05$).
[x,y] Antioxidant or storage effects. Means for each compound in the same column with the same superscript are not significantly different ($p<0.05$).
[1] Tertiary butyl hydroquinone 200 ppm.
[2] Rosemary concentrate 1000 ppm.

Table 8. Volatile compound formation in suspensions from Experiment 2 as influenced by storage time and antioxidant treatment.

Compound	Storage Time	
	Zero Weeks	Eight Weeks
Pentanal	12.57[a]	18.40[a]
Hexanal	202.22	323.96
Control	183.10[ax]	280.25[ay]
TBHQ[1]	202.07[ax]	130.13[ay]
RC[2]	221.48[bx]	561.46[ax]
2-Heptanone	0.00[b]	41.56[a]
1-Octen-3-ol	27.55	118.88
Control	26.04[bx]	98.76[ay]
TBHQ	24.79[bx]	91.01[ay]
RC	31.20[bx]	166.87[ax]
2-Octanone	35.07[b]	82.85[a]
Octanal	9.20	34.18
Control	10.06[ax]	22.02[ay]
TBHQ	8.14[ax]	20.17[ay]
RC	9.38[bx]	60.34[ax]
Nonanal	0.00[b]	19.17[a]
t-2-Nonenal	11.28	62.20
Control	16.53[ax]	39.39[ay]
TBHQ	12.53[ax]	41.02[ay]
RC	4.80[bx]	106.19[ax]

[a, b]Storage effects. Means are pooled unless interactions with antioxidant treatment are significant ($p<0.05$). Means within the same row with the same superscript are not significantly different ($p<0.05$).

[x,y] Antioxidant effects. Means within the same column for the same compound with the same superscript are not significantly different ($p<0.05$).

[1] Tertiary butyl hydroquinone 200 ppm

[2] Rosemary concentrate 1000 ppm

The gel results for this experiment are shown in Figures 6 and 7, but as discussed in the previous experiment, their accuracy is in question. For hexanal content, antioxidant treatments showed no significant effects, but spray drier residence time and storage time did affect volatile compound formation. As noted in the suspensions, BD samples showed significant increases in content of lipid oxidation products. Not only were the BD samples higher than the regular samples for both zero and eight weeks, but the zero week BD samples contained more hexanal than did the eight week regular samples. This demonstrates that excess heat exposure increases the rate of oxidation, resulting in more volatile compounds to be formed in these samples than in samples processed under milder conditions and stored for eight weeks. For total volatiles, BD samples again were significantly higher than samples with a regular residence time.

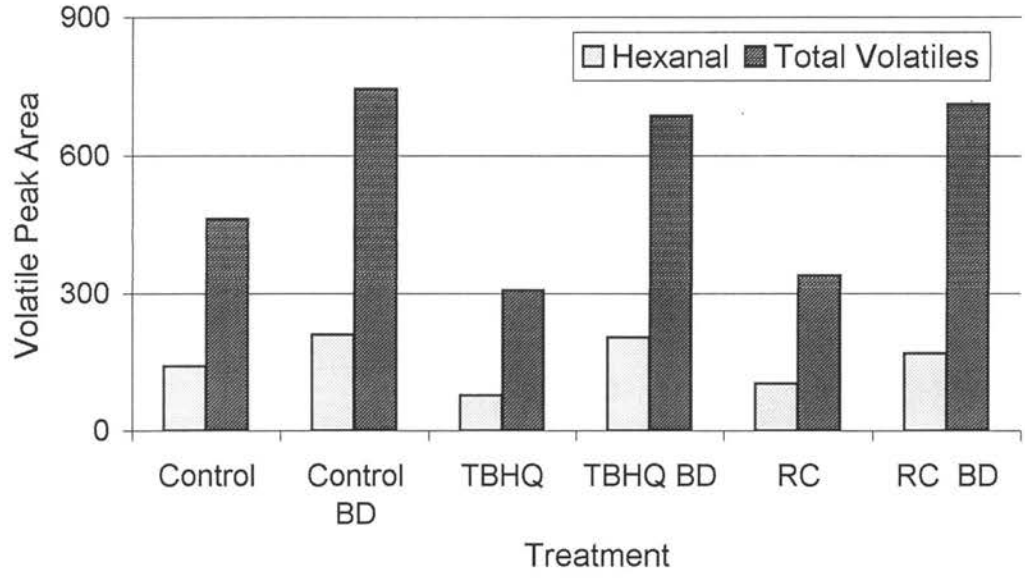

Figure 8. Spray-dried plasma gels after zero weeks of storage. BD ("Brushdown"), 20 min residence time. TBHQ (tert-butyl hydroquinone) RC (Rosemary concentrate).

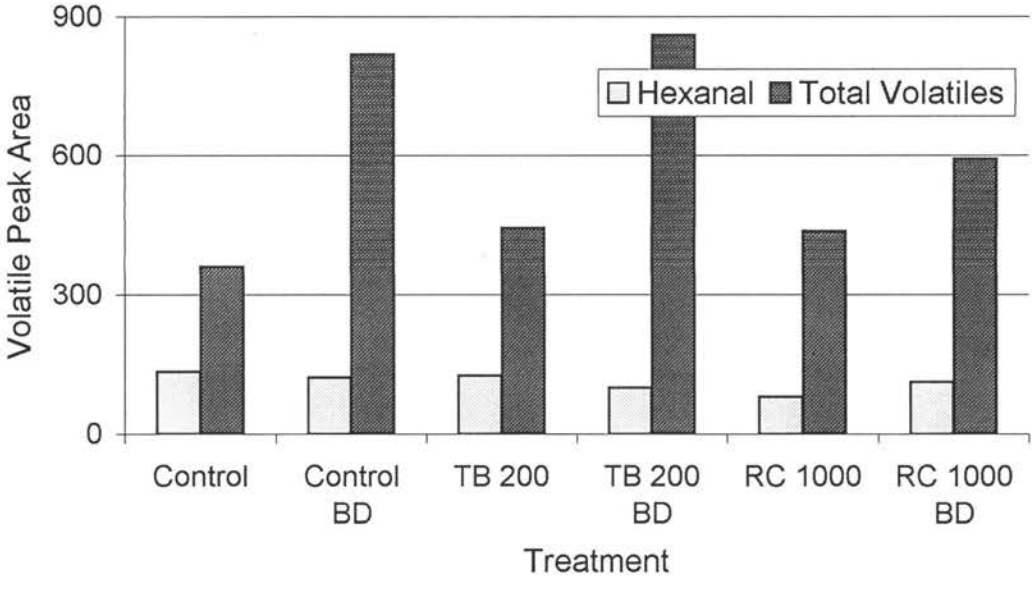

Figure 9. Spray-dried plasma gels after eight weeks of storage. BD ("Brushdown"), 20 min residence time. TBHQ (tert-butyl hydroquinone) RC (Rosemary concentrate).

5. CONCLUSIONS

The spray drying process initiated lipid oxidation as liquid bovine plasma that had not received any heat treatment contained few oxidation products, but after spray drying contained significant amounts. Gas-fired spray driers, due to their production of nitrogen oxides, were found to produce more volatile oxidation compounds in spray-dried plasma than samples from an electric-fired drier. A longer residence time during spray drying and increased storage time each significantly increased the amount of volatiles present in the plasma as well.

The addition of synthetic antioxidants, TBHQ, propyl gallate or BHT, to liquid plasma prior to spray drying successfully decreased the amount of lipid oxidation compounds formed. The rosemary preparations chosen for this study were unable to significantly decrease the amount of volatiles present. The removal of lipids prior to spray drying also reduced the amount of oxidation compounds, but this method would be very expensive to implement.

Lipid oxidation, initiated by the spray drying process, is the primary cause of the volatile flavor compounds present in spray-dried bovine plasma. By controlling processing factors such as temperature, residence time and type of spray drier, the amount of oxidation compounds formed can be minimized. Also, the addition of synthetic antioxidants to liquid plasma prior to spray drying can reduce the amount of volatile flavor compounds present in the finished product.

Future research in this area should include an analysis of the water activity of spray-dried plasma and its effects on oxidation rates. Also, the effectiveness of other natural antioxidants, such as tocopherols should be investigated.

APPENDIX

Table 1a. Volatile compounds in suspensions from gas-dried plasma.

Compound	Control	TBHQ[1]	PG[2]	BHT[3]	Rosemary
Hexanal	626.43[a]	224.17[b]	265.29[b]	369.59[b]	430.16[ab]
2-Heptanone	20.06	9.69	9.93	8.10	11.87
Heptanal	15.45[a]	0.00[b]	0.00[b]	0.00[b]	0.00[b]
1-Octen-3-ol	77.87	34.23	38.62	38.17	54.65
2-Octanone	25.18	17.45	20.07	8.50	24.72
Octanal	10.55	6.67	13.42	8.37	18.28
Total Volatiles	775.55[a]	292.22[b]	347.33[b]	432.74[ab]	539.67[ab]

[1]Tertiary butyl hydroquinone. [2] Propyl gallate. [3]Butylated hydroxytoluene.
[a] Means with the same superscript within a row are not significantly different ($p<0.05$).

Table 2a. Volatile compounds in suspensions from electric-dried plasma.

Compound	Control	TBHQ[1]	PG[2]	BHT[3]	Rosemary
Hexanal	322.94	247.73	274.54	339.83	270.04
2-Heptanone	7.61	4.21	8.84	4.79	0.00
Heptanal	7.04	8.63	8.49	3.26	3.85
1-Octen-3-ol	31.48	34.80	36.16	36.85	24.51
2-Octanone	17.91	24.05	22.80	15.10	16.61
Octanal	12.79	31.68	10.60	10.76	17.83
Total Volatiles	399.77	351.10	361.42	410.59	332.85

[1]Tertiary butyl hydroquinone. [2] Propyl gallate. [3]Butylated hydroxytoluene
No significant differences between treatments for volatile compounds present ($p<0.05$).

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