UTILIZATION OF A LIQUID SMOKE FRACTION AS A REACTIONARY, CARAMEL-TYPE FLAVOR IN WHIPPED CREAM APPLICATIONS VIA MAILLARD REACTION MECHANISMS

by

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ABSTRACT

Smoke flavored foods continue to be a popular choice among consumers. In this study, a caramel-type flavor in whipped cream applications via Maillard reaction pathways was evaluated. A highly refined liquid smoke fraction was developed using a delignified pulp wood source, and a patented activated carbon filtration process. To maximize sensory and reactionary capabilities, a liquid smoke fraction with phenol and carbonyl concentrations of 0.07mg/ml and 12.9g/100ml, respectively, was developed. Heavy cream containing a 0.075% addition of the refined liquid smoke fraction was evaluated when reacted at 50, 63, and 72°C for 15 sec prior to chilling at 0°C for 12 h, and whipping for 8 min using a handheld mixer. Sensory analysis showed the addition of liquid smoke increased whipped cream sweetness and caramel flavors, while imparting minimal off-flavors. Probable Maillard pathways were predicted for the reaction taking place between the liquid smoke and the dairy proteins upon thermal processing. This technology can be used to develop other foods which are not traditionally smoke flavored.

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INTRODUCTION

The food industry is continually searching for new, innovative flavors and concepts to improve product marketing, quality, and labeling. This type of research has generated much interest in the field of reactionary flavors, also known as process or thermal processing flavors. A thermal process flavor is defined by International Organization of the Flavor Industry (IOFI) as:

> "a product prepared for its flavouring properties by heating food ingredient and/or ingredients which are permitted for use in foodstuffs or in process flavourings" (IOFI, 1989).

Many current food markets are interested in reactionary flavors, particularly in the dairy industry, where a flavor additive may be used to increase sweetness, add interesting flavors via reactions, minimize sugar usage levels, and maintain a clean product label and ingredient declaration.

The Maillard reaction is a very important component of reactionary flavor chemistry. The mechanisms of the Maillard reaction can be used to help illustrate the reactions taking place between the proteins present in dairy cream and the carbonyl components active in liquid smoke fractions, resulting in caramel-type flavors present in the final product.

Liquid smoke is a fairly common food additive used primarily in the meat industry because it functions as an antimicrobial agent, colorant, and flavor additive. Liquid smoke has been used for its browning agents in various applications, including meats, doughs, and cheese applications because they are a good source of carbonyls that contribute to Maillard browning reactions.

Whipped cream has an adequate amount of proteins available for reaction, which are known to actively participate in the Maillard reaction mechanisms upon thermal processing. This makes heavy cream an excellent medium for researching the reactivity of liquid smoke carbonyls with dairy proteins.

It is hypothesized that the liquid smoke carbonyls will react with the dairy proteins via Maillard reaction mechanisms to develop a sweet, caramel-type flavor in the final whipped cream product. Specific objectives in this experiment were (1) to create a highly processed liquid smoke fraction that is concentrated in carbonyl content, yet low in phenol content, (2) to determine the amount of active ingredients present in the liquid smoke fraction that is available for reaction, (3) to determine the reactionary impact a liquid smoke fraction addition will have on heavy cream when added prior to thermal processing in the production of whipped cream, (4) to determine temperature requirements needed for the reaction to take place and yield the most effective and economical product, (5) to determine the threshold of active chemical components that will yield the most acceptable final product while minimizing smoke, chemical, and/or off-flavor notes. Sensory studies will be utilized to determine how the liquid smoke affects the whipped cream flavor, and the temperature and concentration levels that are most likely to yield a consumer acceptable product. The main goal was to determine if liquid smoke would be an acceptable additive in dairy products that could prove affective for dairy markets and to justify this project by creating a product that is useful in these applications.

CHAPTER 1 – LITERATURE REVIEW
BROWNING REACTIONS OF FOOD SYSTEMS

Food browning may be the result of many different reactions and via a multitude of mechanisms; however, the browning of food products may be generalized into two main reaction categories: oxidative and non-oxidative. Oxidative, or enzymatic browning occurs when oxygen reacts with a phenolic substrate where polyphenol oxidase enzyme is acting as a catalyst. This method of browning occurs in fruits and vegetables, including apples and lettuce when the tissues are exposed to air, but does not directly involve carbohydrates. Enzymatic browning is often viewed as an unacceptable reaction, where non-enzymatic browning may be sometimes seen as a benefit in food applications. Non-oxidative, non-enzymatic browning is a reaction of proteins with carbohydrates, and may also include caramelization (Christen and Smith, 2000).

Browning is essential in developing food production and storage guidelines. While the fruit and vegetable industries concentrate on browning prevention, other food industries focus on the promotion of browning reactions, particularly in the meat and flavor businesses.

In addition to the Maillard reaction, there are three types of non-enzymatic browning reactions known to occur in food applications. The degradation of ascorbic acid, lipid peroxidation, and sugar-sugar caramelization reactions have all been identified as non-enzymatic browning mechanisms in the food industry (Davies and Labuza, 1997). Each of these pathways is in some way chemically related to the Maillard reaction.

The degradation of ascorbic acid is a type of non-enzymatic browning reaction that is chemically similar to that of sugars; however, amino acids are not required for

browning to occur. Ascorbic acid is highly reactive, and degrades by two pathways. Both mechanisms proceed through dicarbonyl intermediates and eventually form browning compounds (Davies and Labuza, 1997).

Another form of non-enzymatic browning, lipid peroxidation occurs by way of oxygen on fatty acids, particularly unsaturated fatty acids. Similar to the Maillard reaction, the oxidation forms ketones and aldehydes which consequently react with amino acids forming brown pigments. It is believed that peroxidation products promote the browning reaction of the Amadori products, which are major intermediates in the Maillard reaction (Davies and Labuza, 1997).

Sugar-sugar caramelization reactions are a form of non-enzymatic browning mechanisms that occur at high temperatures (> 80ºC) (Davies and Labuza, 1997). The sugar-sugar interactions are highly complex, and may result in many intermediate compounds and end products similar to those associated with the Maillard reaction.

The primary method of browning in food systems is the Maillard reaction. Non-oxidative browning, or the Maillard reaction is a very complex system of reactions leading to the formation of several end-products, affecting colors, flavors, and aromas. For Maillard browning to take place, an amino compound (protein), a reducing sugar, and water must be present. (Christen and Smith, 2000).

MAILLARD REACTION

INTRODUCTION

The Maillard reaction was first discovered in 1908 by two Englishmen, Ling and Malting, who studied color formation in beer. However, it was not until 1912 that Louis

Camille Maillard, a French chemist, described a browning reaction involving reducing sugars and amino groups. This specific reaction is now attributed to Maillard, not because he was the first to report the reaction, but for realizing its significance in diverse fields, including geology, pathology, and medicine (Davies and Labuza, 1997). While the discovery of the Maillard reaction was initially linked to a food application, there is a lack of direct research on the Maillard reaction in much of the food industry, including confectionary, meat, and beverage applications. Today, a significant portion of research is concentrated on the medical applications of the reaction.

Adaptations of Hodge's classical illustration of the Maillard reaction, as shown in Figure 1, are still used today to display the mechanism of this highly complicated reaction (Hodge, 1953). Because of its complexity, the Maillard reaction is generally divided into three stages. The initial stage consists of sugar-amine condensation and Amadori rearrangement. The reaction steps for this stage are the most well defined, but no actual browning occurs during these initial reactions. The second phase involves sugar dehydration, and amino acid degradation via the Strecker reaction. Toward the end of stage two, there is a possibility for flavor formation to occur, but this is not necessarily true for all applications. The final stage is responsible for the formation of heterocyclic nitrogen compounds and browning (Davies and Labuza, 1997; Lee and Nagy, 1983; Mauron, 1981).

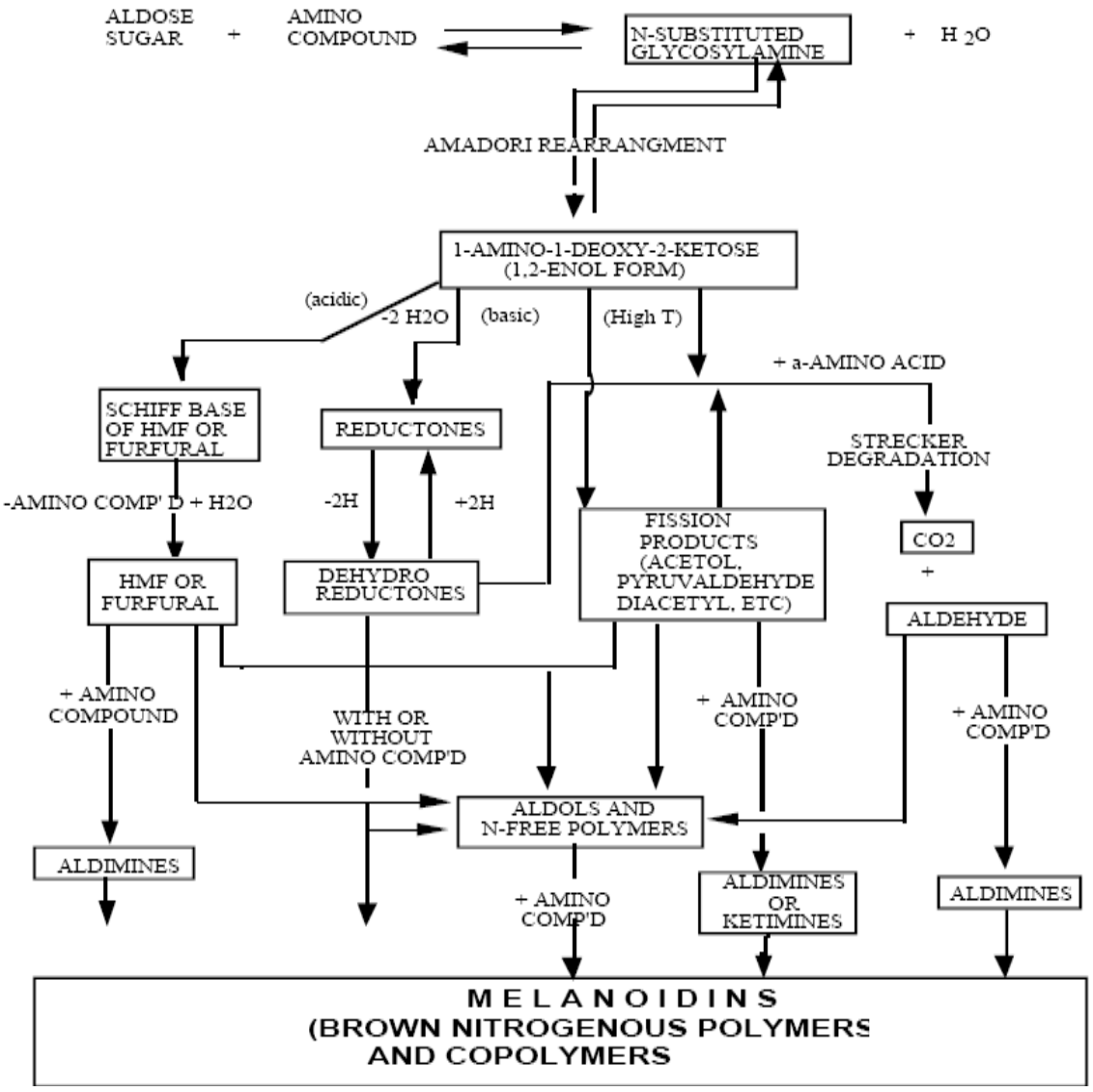

Figure 1 - Hodge diagram; Maillard browning pathways (Hodge, 1953).

STAGE 1

The Maillard browning reaction begins with a condensation reaction between the carbonyl group of a reducing sugar, and the free amino group of an amino acid yielding an N-substituted aldosylamine. This is a nucleophilic reaction, where the amino acid NH_2 group attacks the electrophilic carbonyl groups of the sugar compounds. It is essentially an amine-assisted dehydration reaction of sugar. As the condensation product forms, it quickly loses water and converts into a Schiff base. This portion of the reaction is acid-base catalyzed and may be reversed. The Schiff base then cycles into the aldosylamine. This is followed by the Amadori rearrangement forming a ketosamine (Davies and Labuza, 1997). When a ketose, such as fructose is reacted with an amine, an aminoaldose is formed via the Heyns reaction. Imines are intermediates to the Heyns reaction. The resulting aminoaldose is unstable and reacts readily to form Amadori compounds (Ledl and Schleicher, 1990). An example of these initial steps is depicted in Figure 2 (Christen and Smith, 2000). Glycosylamine is formed through the loss of water and a ring closure. Then the resulting glycosylamine undergoes Amadori rearrangement to yield 1-amino-2-keto sugar (Christen and Smith, 2000).

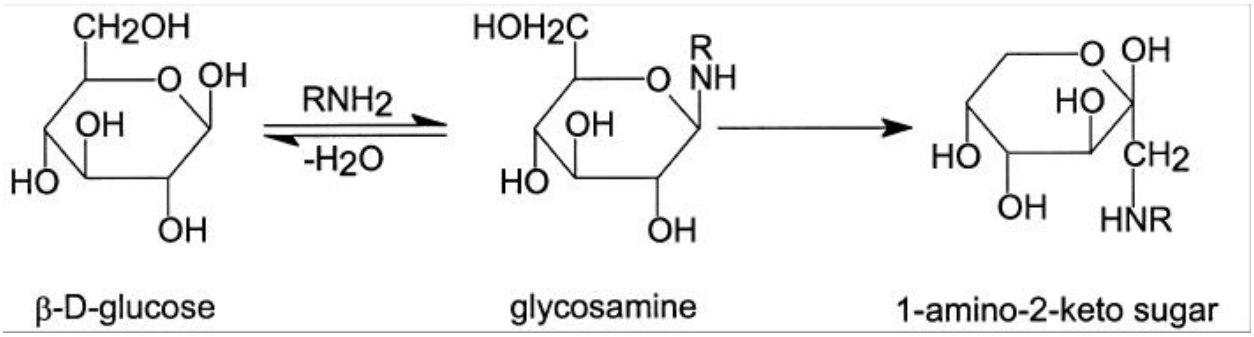

Figure 2 - Early stage Maillard Reactions: formation of glycosylamine and the Amadori rearrangement of a glycosylamine to produce a 1-amino-2-keto sugar (Christen and Smith, 2000).

STAGE 2

The second stage of the Maillard reaction involves the degradation of the Amadori product. This occurs via one of three main pathways, depending on the specific situation and environment.

1. The free hydrogen of the amino group of the Amadori product (ketosamine) may react with a second aldose molecule forming a diketosamine. The resulting compound is less stable than the monoketosamine and readily converts to yield a nitrogen-free carbonyl compound and a monofructosamine (Davies and Labuza, 1997).
2. In acidic media, enolization of the Amadori product can occur via two distinct pathways, as seen in Figure 3 (Christen and Smith, 2000). One pathway gives a 1,2-eneaminol after enolization and proceeds through a 3-deoxyosone, while the other yields a 1-amino-2,3-enediol after enolization and advances through methyl α-dicarbonyl compounds (Christen and Smith, 2000). Both pathways result in the production of Maillard reaction end products (melanoidin pigments) that contain pyrazine and imidazole rings, as well as lower molecular weight compounds, including hydroxymethyl furfural (HMF) and reductones (Christen and Smith, 2000). The 2,3-enolization mechanism is favored in neutral and weakly alkaline conditions (Feather, 1981).
3. Another possible pathway is the Strecker degradation of amino acids. This involves the oxidative degradation of amino acids via carbonyl compounds, which develop from the degradation of ketosamines. Amino acids react to form Schiff bases, and then undergo acid-catalyzed decarboxylation in this

degradation reaction. The newly formed Schiff base is easily hydrolyzed to yield an amine and an aldehyde (Davies and Labuza, 1997). Strecker degradation is characterized by the formation of CO_2. The final result is a transamination reaction which is believed to be needed for the incorporation of nitrogen into melanoidins (Mauron, 1981).

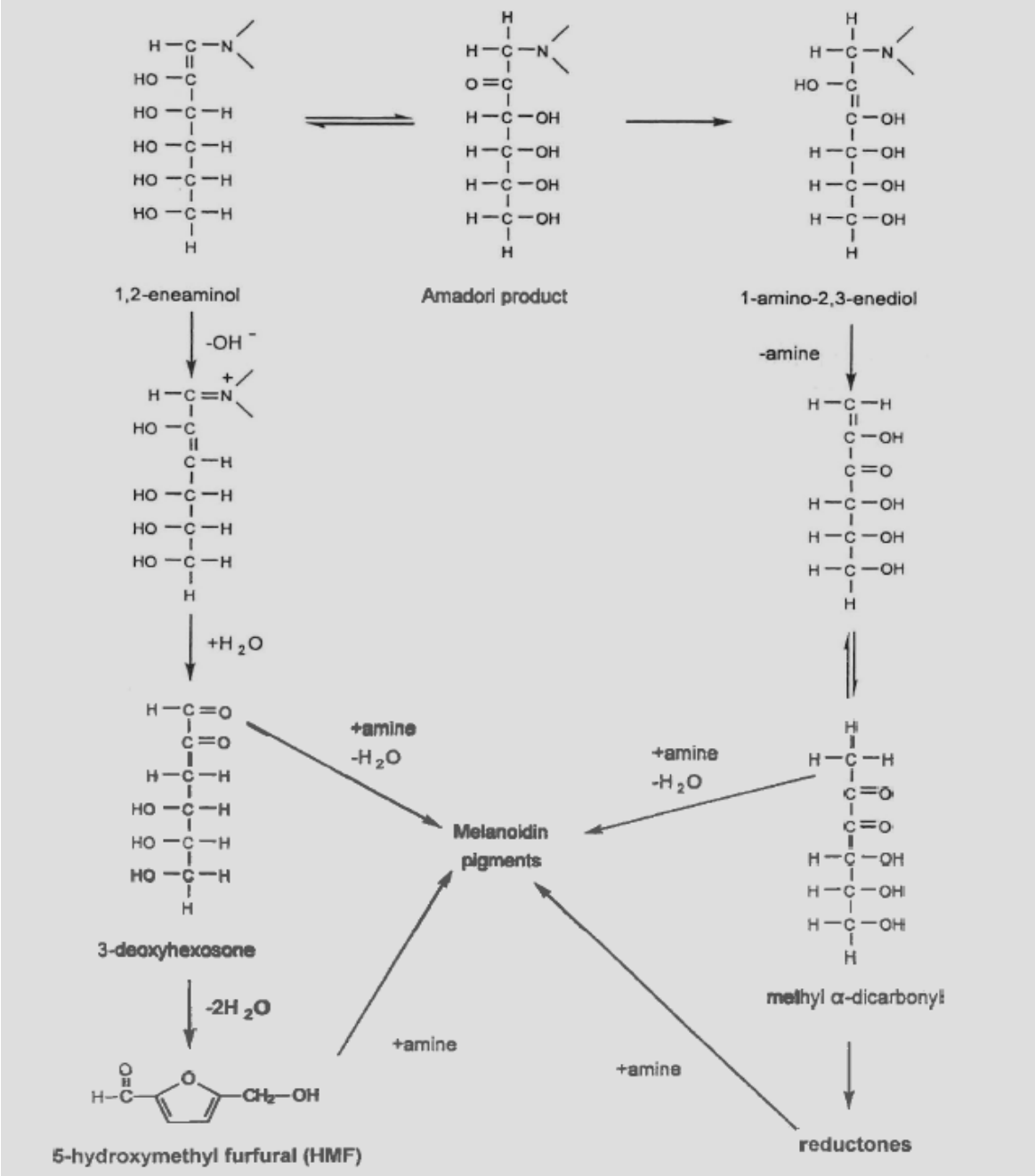

Figure 3 - Possible degradation pathways of the Amadori product to produce melanoidin pigments (Christen and Smith, 2000).

STAGE 3

The third stage of the Maillard reaction is characterized by the formation of melanoidins, brown-pigmented compounds, as well as roasted and toasted aromas and flavors. The formation of melanoidins is the result of highly reactive intermediates that are formed during the Maillard reaction (Mauron, 1981). The chemistry of those intermediates is not completely understood and their formation mechanisms remain unclear. As browning reactions proceed, the molecular weight of the resulting compounds increases until they become insoluble (Davies and Labuza, 1997).

LIQUID SMOKE FLAVORINGS

INTRODUCTION

Food smoking is one of the oldest and most primitive food technologies. Initially, food was hung over fires as a means of protection against competing predators. In addition to physical protection, smoking also provided a special taste, aroma, and color to foods, while enhancing preservation via smoke's natural dehydrating, bactericidal, antimicrobial, and antioxidant properties (Maga, 1988). Although more modern methods such as pasteurization, cooling, and deep freezing have essentially replaced smoking as a preservation method, consumers have become accustomed to the reactionary functions of liquid smoke, including color and flavor. Therefore, the main purpose of smoking food today is the ability to achieve a desired product flavor and an appealing product color.

The invention of liquid smoke is credited to E.H. Wright, a late 19th century Kansas pharmacist. The product was initially used in a domestic setting for the curing of bacon and hams, as well as for the flavoring of products such as stews and baked beans.

Smoke flavors have been produced on a large scale since the 1970's, and are now progressively replacing traditional smoking methods. Liquid smoke methods have gained popularity over traditional methods due to several advantages: ease and speed of application, uniformity of product, final product reproducibility, functionality and reactionary properties, and cleanliness of application. Since its introduction into the food industry, liquid smoke has taken on several functionalities independent of smoking meats. Via US Patent 5637339, liquid smoke may be fractionated, de-phenoled (de-flavored), and further refined to yield a product applicable as a functional ingredient in a variety of food applications, including dairy, vegetable, coatings, and pastry industries (Moeller, 1997).

PRODUCTION METHOD

Before the chemical composition of liquid smoke can be understood, one should have a basic comprehension of the production methods of liquid smoke flavorings. Smoke flavorings are produced as aqueous solutions, as well as oil based solutions and powders. The smoke process begins with a drying stage in which sawdust is dried to a moisture content of approximately 2-3% and fed into a hopper, as detailed in US Patent 4,298,435 (Ledford, 1981). Next, the furnace stage consists of the pyrolysis of the sawdust, with the resulting smoke being absorbed into a water medium via a scrubbing operation. The liquid smoke is fed to settling tanks where the polymerization of tar components is completed. The tar is removed from the tanks, and the final product can then be sold, concentrated via evaporation, further refined, or blended to achieve the various product specifications.

The liquid smoke production process begins at the time the dried sawdust is place in the hopper. From the hopper, the sawdust is continuously fed to the indirect-heat calciner furnace via a screw conveyor. The screw feeder extends into the retort where baffle extensions are spaced in order to convey the sawdust through the furnace. According to US Patent 4,298,435, the retort is heated to approximately 900°F (Ledford, 1981). The smoke vapor produced during the pyrolysis of the sawdust is drawn into a scrubber column filled with ceramic saddles. As the smoke rises in the column, a counter-current stream of water absorbs the smoke components. The smoke absorbed in the water exits the bottom of the scrubber column and enters a water fed makeup tank. From the makeup tank, the aqueous smoke solution is pumped through a plate and frame cooler and then sent back to the top of the scrubber column. The temperature of the cooling water to the plate and frame exchanger is controlled to maintain the re-circulating smoke solution within a 140-150°F range. The liquid smoke is then pumped to a holding tank where it is allowed to settle and precipitate tars. After the tars and polymerized polyaromatic hydrocarbons are removed, the liquid smoke is ready for distribution or further processing.

CHEMICAL COMPOSITION

The composition of wood smoke is directly related to the type of wood source. Generally, trees are composed of approximately 45% cellulose, 20-30% lignin (polyphenol), and 25-35% hemicellulose. All wood sources yield smoke that is a very complex mixture of over 400 different compounds including alcohols, carbonyls, esters, furans, lactones, phenols, and others. Fortunately for chemists, the identification of compounds present in wood smoke is possible through the use of gas

chromatography/mass spectrometry methods (Guillen and Ibargoitia, 1999). Some of the over 400 volatiles identified in liquid smoke are summarized in Table 1 (Maga, 1988). This list includes only a few of the 48 acids, 22 alcohols, 131 carbonyls, 22 esters, 46 furans, 16 lactones, 75 phenols, and 50 miscellaneous compounds known to exist in liquid smoke (Maga, 1988).

Table 1. Compounds identified in wood smoke (Maga, 1988).

Acids	Alcohols	Carbonyls	Esters
Formic	Methyl	Methanal	Methyl Formate
Acetic	Ethyl	Propanal	Methyl Acetate
Glycolic	Propyl	Acetone	Methyl Propionate
Propionic	Isopropyl	Acetol	Methyl Butyrate
Isobutyric	Isobutyl	Diacetyl	Methyl Crotonate
Benzoic	Propan-2-on-ol	Hydroxyacetaldehyde	Ethyl Benzoate
Sorbic	Cyclohexanol	Pentanone	Methyl Valerate
Isovaleric	Benzylalcohol	Cyclopentanone	Methyl Isobutyrate
3-Butenoic	Butan-2-on-1-ol	Benzaldehyde	Cresyl Acetate
Valeric	Amyl	Hexanal	Methyl Palmitate
Furans	**Lactones**	**Phenols**	**Miscellaneous**
Furfuryl Alcohol	Butyrolactone	2,6-Xylenol	Pyrazine
Furans	Butenolide	Cresol	Pyrrole
2-Methylfuran	Angelica Lactone	Diethylphenol	Pyridine
3-Acetylfuran	Hydroxyvalerolactone	4-Butylphenol	Maltol
Propylfuran	2-Methyl-2-Butenolide	4-Propylphenol	Ethanediol
Amylfuran	Methylvinyl-2-Butenolide	4-Vinylphenol	Toluene
Benzofuran	2,3-Dimethyl-2-Butenolide	3-Methoxyphenol	Styrene
2-Furoic Acid	2,3,4-Trimethyl-2-Butenolide	Guaiacol	Benzene
2-Furfural	Crotonolactone	Pyrocatechol	Indene
5-Methylfurfural	4-Ethyl-2-Methyl-2-Butenolide	Isoeugenol	Naphthalene

From a logical perspective, it could be assumed that smoked food color results from the deposition of smoke particles directly on a food surface, rather than via a chemical reaction. Because deposition of smoke particles on an organic surface yields a darker color than the same particles deposited on an inert surface, it is concluded that there is more involved than simple physical absorption of particulate matter. This indicates that a chemical reaction has occurred (Maga, 1988). This chemical reaction

involves the interaction of carbonyls in the smoke vapor phase with amino groups, derived from proteins in the food substrate, representing a series of non-enzymatic browning reactions similar to the Maillard reaction. Although, based on temperature conditions, some of the present carbonyls may be more influential than others in the Maillard reaction browning process. Of the compounds listed in Table 2 (Maga, 1988), the carbonyls known to be most reactive are glycoaldehyde, methylglyoxal, and glyoxal (Riha and Wendorff, 1993).

Table 2 - Carbonyls associated with the formation of color in smoked foods (Maga, 1988).

Formaldehyde
Glycoaldehyde
Glyoxal
Acetone
Hydroxyacetone
Methylglyoxal
Diacetyl
Furfural

The idea that protein amino groups are involved in the browning reactions of liquid smoke has been clearly demonstrated by the use of chemically modified collagen. The modified collagen's amino groups can be converted to hydroxyl groups where no color will develop during liquid smoke application, whereas with normal collagen, extensive color formation will result (Maga, 1988).

Specific phenols associated with the vapor phase of smoke are also believed to in color formation of smoked foods. However, these phenols must be relatively high in molecular weight in order to possess an adequate number of hydroxyl groups to cross-link proteins at multiple sites through hydrogen bonding mechanisms (Maga, 1988).

However, in most instances the concentration of high molecular weight polyphenols is rather low, particularly compared to the amount of carbonyls available for reaction. The phenolic compounds are widely known to supply the traditional strong smoked flavors to the food substrate. This information led to the knowledge that phenol removal would both minimize smoke flavor and concentrate the amount of reactive carbonyls present. Thus, a low phenol, high carbonyl liquid smoke fraction would present the most effective product for Maillard testing where smoke flavor is an undesirable attribute.

In order to develop a liquid smoke fraction suitable for reaction in whipped cream applications, processing and fractionation beyond basic liquid smoke production is required. Base liquid smoke is naturally acidic, and therefore must be buffered with sodium bicarbonate in order to achieve an acceptable flavor profile. Also, from a sensory standpoint it is necessary to remove a majority of the phenolic compounds present in the base liquid smoke product. The phenolic compounds are removed via filtration using activated carbon as indicated in US Patent 5637339 (Moeller, 1997). Base liquid smoke has an approximate phenolic content of 17mg/ml. Through activated carbon filtration, a liquid smoke fraction of less than 0.08mg/ml phenol content was developed for this study. In removing a significant portion of the phenolic compounds, multiple benefits are realized; a desirable flavor is achieved and the reactionary, carbonyl compounds are concentrated.

SMOKE BROWNING REACTIONS

Since carbonyl-amine reactions may yield numerous compounds, some research has been done to identify resulting pigmented compounds via simple model systems. Some of the model systems and resulting compounds known to contribute to color in

smoked foods are listed in Table 3 (Maga, 1988). Several of the identified end products have the ability to be formed from multiple starting materials; therefore, the amount of final end product is theoretically increased (Maga, 1988).

Table 3 - Model system reactants in smoke that contribute to color (Maga, 1988).

Glycolic aldehyde + aminoethanol → 1-hydroxyethyl-3-hydroxymethyl-2-pyrrolaldehyde
Glycolic aldehyde + aminoethanol + formaldehyde → 3-unsubstituted-1-hydroxymethyl-2-pyrrolaldehyde
Xylose + aminoethanol + formaldehyde → 3-unsubstituted-1-hydroxymethyl-2-pyrrolaldehyde
Methylglyoxal + methylamine → 1,5-dimethyl-4-hydroxy-2-pyrrolealdehyde
Dihydroxyacetone + methylamine → 1,5-dimethyl-4-hydroxy-2-pyrrolealdehyde

In comparing these end products with those obtained between aldose-amine reactions, as typical of the Maillard reaction, the basic mechanism is the same for the formation of color compounds in protein present systems: 1) the formation of conjugated unsaturated compounds: 2) the attachment of these compounds to hydroxyl groups: and 3) the resulting products condensing into brown pigmented compounds (Maga, 1988). The most notable variance between the Maillard reaction and the smoke non-enzymatic browning reaction is that in the formation of smoke color, the initial carbohydrate degradation occurs in the smoke production process. Therefore, the resulting reactive compounds may be brought into direct contact with reactive amino groups at the food surface without further rearrangement, while in the Maillard reaction a significant amount of rearrangement occurs before the final reaction takes place (Maga, 1988).

DAIRY PROTEINS

> "In 1877 O. Hammarsten distinguished three proteins in milk: casein, lactalbumin and lactoglobulin. He also outlined a procedure for their separation: skim milk is diluted then acidified with acetic acid. Casein flocculates, while the whey proteins stay in solution. This established a specific property of casein: it is insoluble in weakly acidic media. It was later revealed that the milk protein system is much more complex" (Belitz et al., 2004).

In 1939, Mellander used electrophoresis to determine the three phases of casein: α–, β–, and γ-casein, which make up the main portion of critical milk proteins (Belitz et al., 2004). Whey proteins, β-lactoglobulin A and B, and α-lactalbumin, can be differentiated genetically using various methods such as high-performance liquid chromatography and capillary electrophoresis (Bobe et al., 1998; Fairise and Cayot, 1998). The milk proteins exist in a range of naturally occurring genetic variants that differ from each other by a few amino acid substitutions, and may be differentiated by identifying and separating the proteins by those variants (Bikker et al., 2000). Clear separation of major whey proteins, β-lactoglobulin A and B, and α-lactalbumin, may be achieved by capillary electrophoresis (Fairise and Cayot, 1998). The amino acid composition of casein and whey proteins of milk is presented in Table 4 (Belitz et al., 2004). These proteins, or at least a portion of them, are critical in initiating and promoting the Maillard reaction. Specifically, glycine is known to readily react with liquid smoke carbonyls to produce Maillard end-products (Lappin and Clark, 1951).

Table 4 - Amino acid composition (g AA/100g protein) of casein and whey protein of bovine milk (Belitz et al., 2004).

Amino Acid	Whey	Casein
alanine	5.5	3.1
arginine	3.2	4.1
aspartic acid	11.0	7.0
cystine	3.0	0.3
glutamic acid	15.5	23.4
glycine	3.5	2.1
histidine	2.4	3.0
isoleucine	7.0	5.7
leucine	11.8	10.5
lysine	9.6	8.2
methionine	2.4	3.0
phenylalanine	4.2	5.1
proline	4.4	12.0
serine	5.5	5.5
threonine	8.5	4.4
tryptophan	2.1	1.5
tyrosine	4.2	6.1
valine	7.5	7.0

WHIPPED CREAM

Heavy dairy cream is an emulsion with a fat content of 35-40%. When a sample of heavy cream is whipped, the air bubbles that are created during agitation cause fat globules to begin to partially coalesce in chains around the air bubbles (Goff, 1995).

As seen in Figure 4, when the fat partially coalesces, it causes the fat-stabilized air bubbles to link together (Goff, 1995). As the process progresses, chains of fat-stabilized air bubbles are formed. Water, lactose, and proteins are bound in the open areas around the fat-stabilized air bubbles. The crystalline fat content is essential for the fat globules

to partially coalesce into 3-dimensional structures, giving the whipped cream its stiff, smooth texture. If the fat globules were allowed to fully coalesce into larger globules, structure-building would not be feasible. Crystals, within the globules, cause the globules to stick together in chains and clusters, while allowing them to retain their individual identities and structures. If whipped cream is whipped excessively, the fat will churn and butter will form (Goff, 1995).

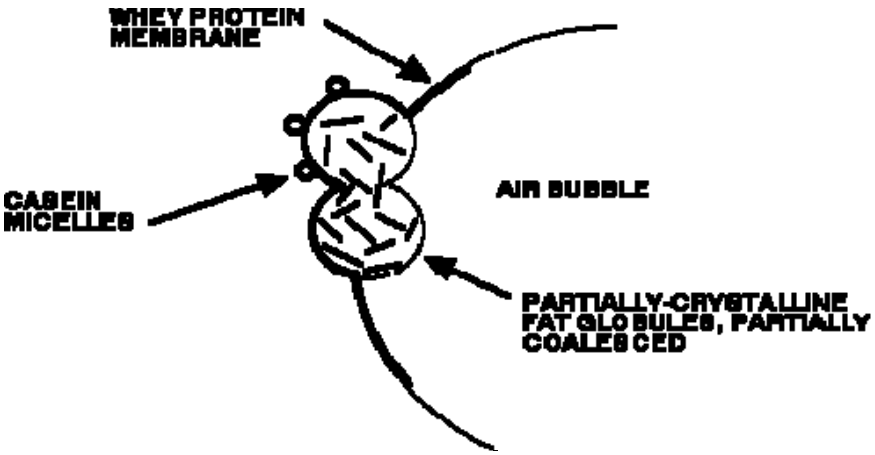

Figure 4 - Structure during whipped cream formation (Goff, 1995).

CHAPTER 2

UTILIZATION OF A LIQUID SMOKE FRACTION AS A REACTIONARY, CARAMEL-TYPE FLAVOR IN WHIPPED CREAM

INTRODUCTION

Research shows liquid smoke and whipped cream to be very complex solo ingredients, comprised of a variety of reactive chemicals. The blend of these two ingredients in the presence of thermal processing creates a complicated chemical environment that is believed to promote Maillard reactions. Laboratory testing is required to create a more controlled environment for product development, and drawing conclusions regarding this application.

Two separate developments are required for this project: (1) development of a sensory acceptable, chemically reactive liquid smoke fraction (AM-10) and (2) development of a finished whipped cream/AM-10 product that develops a sweet flavor via thermal processing. There are several laboratory tests and evaluations needed to support the project objectives, including: (1) the carbonyl and phenolic content of the liquid smoke fraction (AM-10) must be controlled in order to optimize product development, (2) sensory testing is necessary to determine the level of liquid smoke flavor that may be added to the whipped cream to optimize the sweet, caramel-type notes while minimizing off-flavors, (3) sensory testing is needed to characterize and quantify the flavor differences between the control sample of whipped cream and the liquid smoke treated sample when thermally processed at different temperatures, (4) laboratory and research findings are needed to predict possible Maillard reaction pathways for this application, and (5) laboratory results are required to successfully design a focus group

session that will be useful in determining additional product development strategies, go-to-market ideas, and further required research.

MATERIALS AND METHODS

AM-10 (liquid smoke fraction) product development

Introduction

In developing a liquid smoke fraction suitable for this application, many factors were taken into consideration: (1) wood source, (2) concentration of carbonyls, (3) amount of phenolic compounds present, and (4) physical color of the product.

Several wood sources are available for processing into liquid smoke flavors, including, but not limited to, hardwood, hickory, mesquite, alder, and beech. However, due to the sensitive flavor requirements of this project, a special wood source was determined as the most efficient. According to US Patent 6214395, delignified wood pulp may be used as a source for generating liquid smoke with high browning capabilities (Moeller and Ramakrishnan, 2001). This source was chosen because the delignified pulp wood has an acceptable phenol to carbonyl ratio (low initial phenol content when compared to other wood sources). This source is the most conducive to creating a low flavor, high carbonyl liquid smoke upon further refinement. The carbonyl and phenol evaluations are done via wet laboratory analysis. Following US Patents 6214395 and 5637339, the liquid smoke fraction will be highly processed via filtration to achieve the most acceptable carbonyl and phenol levels possible (Moeller, 1997; Moeller and Ramakrishnan, 2001).

Carbonyl concentration in the liquid smoke fraction is a critical parameter. High carbonyl content is required to ensure an adequate amount of components are available for reaction with the amino acids present in the whipped cream. Particular care was taken in the liquid smoke refinement processes to ensure the highest carbonyl content was maintained.

Phenolic, or flavor content and physical color of the liquid smoke fraction are linked attributes. The higher molecular weight phenolic compounds are responsible for the majority of the brown color associated with typical liquid smoke products. Multiple carbon filtration steps were utilized to remove the phenolic compounds (color producing components) from the liquid smoke.

Carbonyl content

A wet laboratory analysis method was utilized to determine the amount of carbonyl components present in the liquid smoke fraction that are available to participate in the Maillard reaction. Detailed below is the method for analyzing carbonyl content of the liquid smoke fractions used in this study. This is the standard industry method for determining carbonyl content as described in US Patents 4,594,251 and 4,876,108 (Nicholson, 1986; Underwood and Graham 1989).

Chemicals:

Chemicals needed for this wet laboratory analysis were carbonyl-free methanol (OmniSolv), 2,4-dinitrophenylhydrazine (97%, Aldrich, St. Louis, MO), potassium hydroxide (KOH, 45%(w/w aqueous solution), Ricca Chemical Company, Arlington, TX), distilled water, 2-butanone (Aldrich, Milwaukee, WI), concentrated hydrochloric acid (HCl, 37% (w/w), Mallinckrodt, Paris, KY).

Reagents:

The 2,4-dinitrophenylhydrazine solution was prepared by mixing 5g of 2,4-dinitrophenylhydrazine and 250ml of carbonyl-free methanol to a 500ml beaker. The beaker was placed in a 50°C oven (VWR, model 1330FM-2; 220 volt) for 1.5 h, and stirred every 30 min with a stir rod.

The potassium hydroxide solution was prepared by dissolving 10g of KOH into 20ml of distilled water in a 100ml volumetric flask. The flask was then diluted to volume with carbonyl-free methanol.

The 2-butanone standard was prepared by weighing 1.700g of 2-butanone into a 10ml glass beaker. This was then funneled into a 1000ml volumetric flask. Any residual 2-butanone was rinsed from the 10ml beaker using carbonyl-free methanol while filling the 1000ml volumetric flask with the carbonyl-free methanol.

Procedure:

A standard curve was prepared by adding 10, 15, and 20ml (referred to as A, B, and C, respectively) of the 2-butanone standard solution to three separate 100ml volumetric flasks and diluting to volume with carbonyl-free methanol. The test samples were prepared by placing 50µl of the liquid smoke sample in a 50ml volumetric flask and filling to volume with carbonyl-free methanol. A 1.0ml addition of the 2,4-dinitrophenylhydrazine reagent was added to seven separate graduated test tubes. For the sample tubes (three tubes, as three separate lots were tested), a 1.0ml sample of the liquid smoke solution was added. For the standard curve graduated test tubes, a 1.0ml sample of each of the 2-butanone standards was added. A reagent blank graduated test tube was prepared by adding 1.0ml of carbonyl-free methanol. An addition of 0.05ml of

concentrated HCl was added to all test tubes. The tubes were mixed thoroughly using a vortex mixer, covered first with aluminum foil, and then covered additionally with parafilm. All samples were placed in a 50˚C oven (VWR, model 1330FM-2; 220 volt) for 30 min. The samples were removed from the oven and allowed to cool at room temperature for 2 min. Five ml of KOH was added to each tube. All graduated test tubes were diluted to 25ml with carbonyl-free methanol and mixed thoroughly using a vortex mixer. Each sample was allowed to rest and react for 15 min. The absorbance of each sample was read, using a 1 cm cuvette, at 480nm using a spectrophotometer (Spectronic, Genesis) after zeroing with the reagent blank sample.

Phenol content

In developing a liquid smoke fraction that would be acceptable from a sensory prospective, the concentration of phenolic compounds was evaluated. Based on the analysis results, the liquid smoke will be further processed until an acceptable level is achieved. The procedure for the determination of the amount of phenol content is a modified JAOAC method for detecting the amount of phenols as 2,6-dimethoxyphenol (Tucker, 1942).

Chemicals:

Chemicals needed for this wet laboratory analysis were distilled water, 2,6-dimethoxyphenol (98%, Fluka, Steinheim, Germany), 2,6-dichloroquinone-4-chloroimide (95%, Aldrich, St. Louis, MO), sodium hydroxide pellets (NaOH, J.T. Baker, Phillipsburg, NJ), ethanol (anhydrous, denatured reagent grade, AAPER, Shelbyville, KY), granular potassium chloride (KCl, Reagents Inc., Charlotte, NC), granular boric

acid (Reagents Inc, Charlotte, NC), and 0.10N sodium hydroxide (NaOH, Mallinckrodt, Paris, KY).

Reagents:

The 8.3 pH buffer solution was prepared by adding 0.32g of granular NaOH, 3.728g of granular KCl, and 3.20g of boric acid to a 1000ml volumetric flask and diluting to volume with distilled water. The solution was allowed to mix thoroughly for 10 min using a stir bar and stir plate. The pH of the solution was then tested. If the pH was below 8.2, it was adjusted with NaOH. If the pH was above 8.4, it was adjusted with boric acid. Increments of 0.1g or less were used for adjustments until the desired 8.3 pH was achieved.

The color reagent solution was prepared by adding 0.25g of 2,6-dichloroquinone-4-chloroimide to a 250ml glass beaker in a fume hood, and adding 30ml of denatured reagent grade ethanol. Using a stir rod, the solution was thoroughly mixed. This solution was stored in a refrigerator until time of use.

The 2,6-dimethoxyphenol solution was prepared by adding 1.0g of 2,6-dimethoxyphenol to a 1000ml volumetric flask and diluted to volume with distilled water. Using a stir bar and stir plate, the solution was thoroughly mixed for 20 min.

Procedure:

A standard curve was prepared by adding 0.05, 0.10, and 0.15ml (A, B, and C, respectively) of the 2,6-dimethoxyphenol standard solution to three separate 100ml volumetric flasks and diluting to volume with distilled water. The flasks were capped and inverted several times to mix thoroughly. The liquid smoke test samples were

prepared by adding 1.0ml of liquid smoke to a 100ml volumetric flask and diluting to volume with distilled water. The flask was capped and inverted several times to mix thoroughly. Then 2.0ml of the diluted liquid smoke solution was added to a 50ml volumetric flask and diluted to volume with distilled water. The flask was capped and inverted several times to mix thoroughly. To 25ml test tubes, 5.0ml of the 8.3 pH buffer was added. Seven test tubes were prepared; three for the liquid smoke test sample (as three separate lots were tested), three for the standard curve samples, and one for the reagent blank sample. For the reagent blank sample, 5.0ml of distilled water was added to the 25ml test tube. For the standard curve samples, 5.0ml of each of the 2,6-dimethoxyphenol standard solutions was added to 3 separate 25ml test tubes. For the liquid smoke test samples, 5.0ml of the diluted liquid smoke solutions was added to three separate 25ml test tubes. Each test tube was adjusted to a 9.8 pH by adding 1.0ml of 0.10N NaOH to each sample. A dilution of the 2,6-dichloroquinone-4-chloroimide color reagent was prepared by adding 2.0ml of the color reagent to 30ml of distilled water in a beaker. One ml of the diluted 2,6-dichloroquinone-4-chloroimide solution was added to each test tube. All test tubes were mixed thoroughly using a vortex mixer. The samples were allowed to rest and react for 25 min. All samples were read for absorbance using a 1cm cuvette at 580nm using a spectrophotometer (Spectronic, Genesis) after zeroing with the reagent blank sample.

Whipped cream/AM-10 product development

Preliminary testing: threshold and temperature evaluation

The level of AM-10 to be added to the cream and the thermal processing temperatures were determined in a round table evaluation. During this evaluation, panelists were familiarized with the product and agreed on attributes to be evaluated. Then, the panelists individually evaluated the intensities of each attributes. Overall flavor was evaluated, along with four positive attributes and three negative attributes. The positive attributes were creamy dairy, sweet, cooked, and caramelized; the negative attributes were chemical, cooked, and metallic. The lexicon found in Table 11 illustrates how each attribute was defined by the panel.

The initial screening consisted of samples with 0.5, 1.0, and 1.5% AM-10 which were heated to 50, 75, and 90°C for 15 s. All samples were found to be high in smoky/chemical notes. In the second screening, samples with 0.1% and 0.05% AM-10 heated to 50°C were evaluated. No significant difference was noted between the Control and the 0.05% samples while the 0.1% AM-10 samples still had noticeable smoky/chemical notes. In the next evaluation, cream with 0.075% AM-10 heated to 50, 75, and 90°C for 15 s were evaluated. Slight differences were observed between the control and 50°C samples, but 75°C and 90°C samples were high in smoky/chemical notes. Temperatures were, therefore, decreased to 63°C and 72°C for 15 s which are more representative of pasteurization temperatures used in the dairy industry.

Sensory evaluation

A sensory test panel, consisting of eight trained panelists, was assembled to determine the impact of the selected liquid smoke fraction as reacted with heavy cream prior to whipping. Each panelist was trained by completing a minimum of 50 h in the sensory evaluation of foods, with at least 20 h focused on dairy products, and has extensive experience (3-7 years) in descriptive analysis.

The sensory test objectives were to determine the effects of AM-10 added to whipped cream prior to thermal processing and whipping. Also, sensory panels were used to determine what level of AM-10 added to whipped cream would result in sweet caramelized notes, rather than off-flavors. This testing was designed to characterize and quantify sensory differences between control whipped cream and whipped cream treated with the AM-10 smoke fraction when heated to different temperatures.

Eight trained panelists evaluated individual product attributes using difference from 5, where the Control = 5, and the minimum and maximum deviations from the control are 1 and 9, respectively (Table 9). Also, the aroma, appearance, flavor, aftertaste, and overall degree of difference from the control sample was represented by the Deviation from Reference (DFR) where the Control = 10, and the maximum deviation from the Control = 0 (Table 10).

The following samples were prepared for evaluation: Unheated cream (Great Value Heavy Whipping Cream, Expiration Date: Sept 24, 2008) chilled at 0°C for 12 h, and whipped using a handheld mixer on medium setting (Hamilton Beach Hand Held Mixer Model 62676) for 8 min; Cream (Great Value Heavy Whipping Cream, Expiration Date: Sept 24, 2008) heated to 50, 63, and 72°C, chilled at 0°C for 12 h, and whipped

using a handheld mixer on medium setting (Hamilton Beach Hand Held Mixer Model 62676) for 8 min; and Cream (Great Value Heavy Whipping Cream, Expiration Date: Sept 24, 2008) with a 0.075% AM-10 addition, heated to 50, 63, and 72°C, chilled at 0°C for 12 h, and whipped using a handheld mixer on medium setting (Hamilton Beach Hand Held Mixer Model 62676) for 8 min. All samples were prepared in 500g batches, in 800ml beakers. Each sample was heated after the addition of AM-10 (where applicable) to the desired temperature for 15 s using a hot plate (Pyro Multi Magnestir Lab-Line) and magnetic stir bar on medium setting. Temperature endpoints were determined using a digital thermometer (Acurite), sampling at the center of the fluid paying close attention to avoid touching the thermometer to the bottom of the beaker. Approximately one ounce samples were served to the panelists in two ounce plastic soufflé cups with matching lids. The products were blind coded with three-digit numbers, and evaluated at approximately 40°F under white lighting. Each panelist was given a ballot to complete (Figure 9.), and the panel results were averaged to yield final results.

Possible Maillard reaction pathways

Heating milk in a classical sterilization process results in Maillard reactions between lactose and amino groups, resulting in the formation of hydroxymethyl furfural (HMF) (Belitz et al., 2004). With the addition of the liquid smoke fraction, and carbonyls available for reaction, the heated cream may take many Maillard reaction pathways yielding melanoidins.

By definition, the Maillard reaction is a complex mechanism, or set of mechanisms that result in the formation of melanoidins. The pathway or pathways taken

by the cream/smoke fraction solution is dependent on many variables: pH, moisture content, temperature, and concentration of active components. Due to the complexity of the reaction and the amount of variables, it is difficult to predict the specific mechanisms. It is also probable that multiple pathways could be followed in this application that would yield the same or similar Maillard end products. Based on research of liquid smoke, dairy proteins, whipped cream, and the Maillard reaction, it is possible to predict favorable Maillard reaction pathways that may be followed in this application.

Focus group

A focus group of 15 food people was assembled to determine possible market usages for this technology, as well as go-to-market strategies. The 12 food scientists participating represented specialties in the following industries: dairy, meat, coatings, reactionary flavors, sweet flavors, fruit flavors, ready to eat sauces, vegetables, sensory, liquid smoke flavors, food emulsifiers, and beverages. Also, three people outside the food industry participated to voice the opinions and ideas of an average consumer.

Each focus group participant tasted the control whipped cream, the heated control whipped cream (50°C), and the 0.075% AM-10 whipped cream test sample (50°C). The 0.075% AM-10 sample thermally processed to 50°C was giving to the focus group because that sample was deemed most acceptable by sensory evaluation. The task of the focus group was to identify any potential customers or markets for this application.

RESULTS AND DISCUSSION

AM-10 (liquid smoke fraction) product development

The following manufacturing steps were taken to develop the most effective liquid smoke fraction: (1) initial smoke manufacturing utilizing a delignified pulp wood source, (2) buffering of the liquid smoke using sodium bicarbonate to reach an acidity of less than 2%, and (3) multiple filtration steps utilizing activated carbon to achieve a wet laboratory result of less than 0.08mg/ml phenol content. The wet laboratory results of the liquid smoke fraction (AM-10) at each manufacturing step are indicated in Table 5.

Table 5 - Wet laboratory results of AM-10 at each manufacturing step.

	AM-10 prior to buffering	AM-10 after buffering	AM-10 final product
Phenol (mg/ml)	2.1	1.9	0.07
Carbonyl (g/100ml)	13.9	12.5	12.9

Carbonyl content

The final AM-10 carbonyl results are reported as mg/100ml of 2-butanone (Table 6). All absorbance values and 2-butanone concentrations were graphed in Figure 5.

Table 6 - AM-10 carbonyl results for three separate lots (* mathematically determined values).

Sample	2-butanone mg/100ml	Absorbance (480nm)
AM-10 (Lot 1)	13.0*	0.788
AM-10 (Lot 2)	12.8*	0.776
AM-10 (Lot 3)	12.9*	0.782

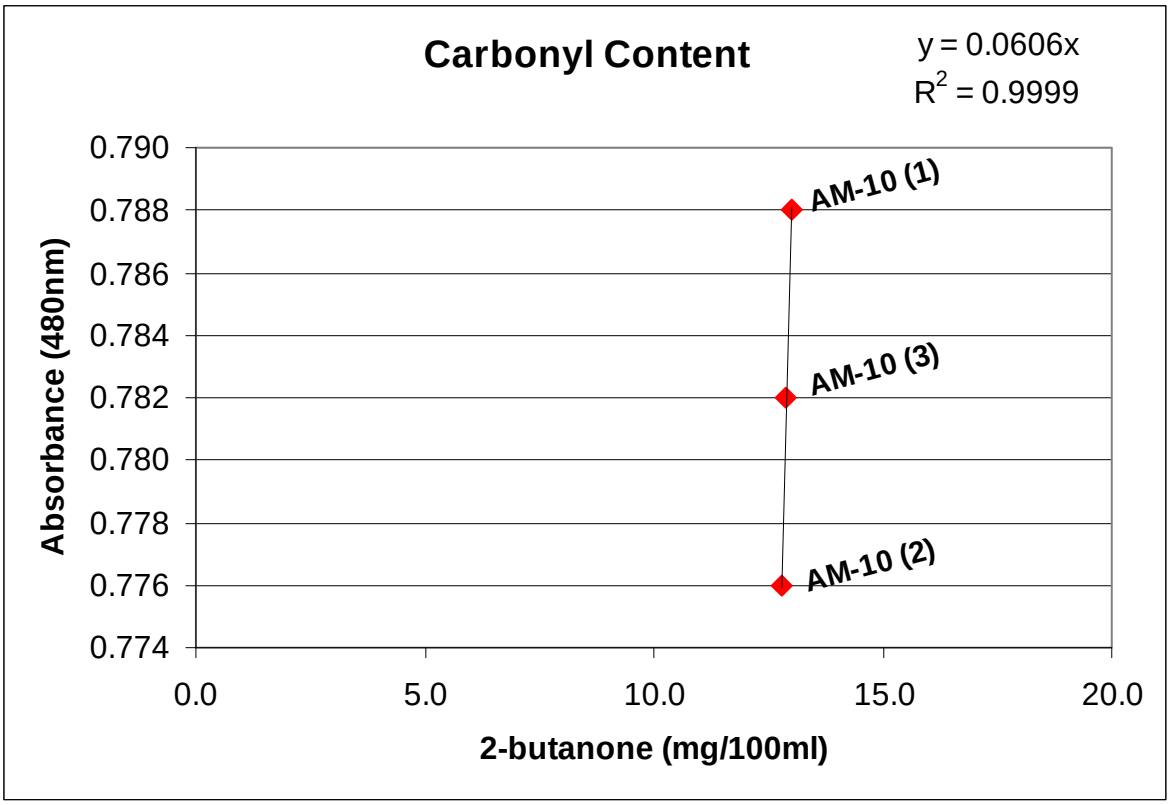

Figure 5 - Carbonyl content results graph of AM-10 liquid smoke fraction; carbonyl content of three separate lots in mg/100ml (represented as 2-butanone versus absorbance at 480nm).

The three AM-10 carbonyl values were averaged to get the reportable value of 12.9mg/100ml of 2-butanone. The 12.9mg/100ml carbonyl content allowed for an acceptable liquid smoke product, with high carbonyl content available for reaction to be utilized in this application. A table of the carbonyl values at each processing step is presented in Table 5. The effects of filtration and refinement processes on the AM-10 carbonyl content are also represented in Table 5.

Phenol content

The final AM-10 phenol results are reported as mg/ml of 2,6-dimethoxyphenol (Table 7). All absorbance values and 2,6-dimethoxyphenol concentrations are graphed in Figure 6.

Table 7 - AM-10 phenol results for three separate lots (* mathematically determined values).

Sample	2,6-dimethoxyphenol (mg/ml)	Absorbance (580nm)
AM-10 (Lot 1)	0.072*	0.026
AM-10 (Lot 2)	0.067*	0.024
AM-10 (Lot 3)	0.075*	0.027

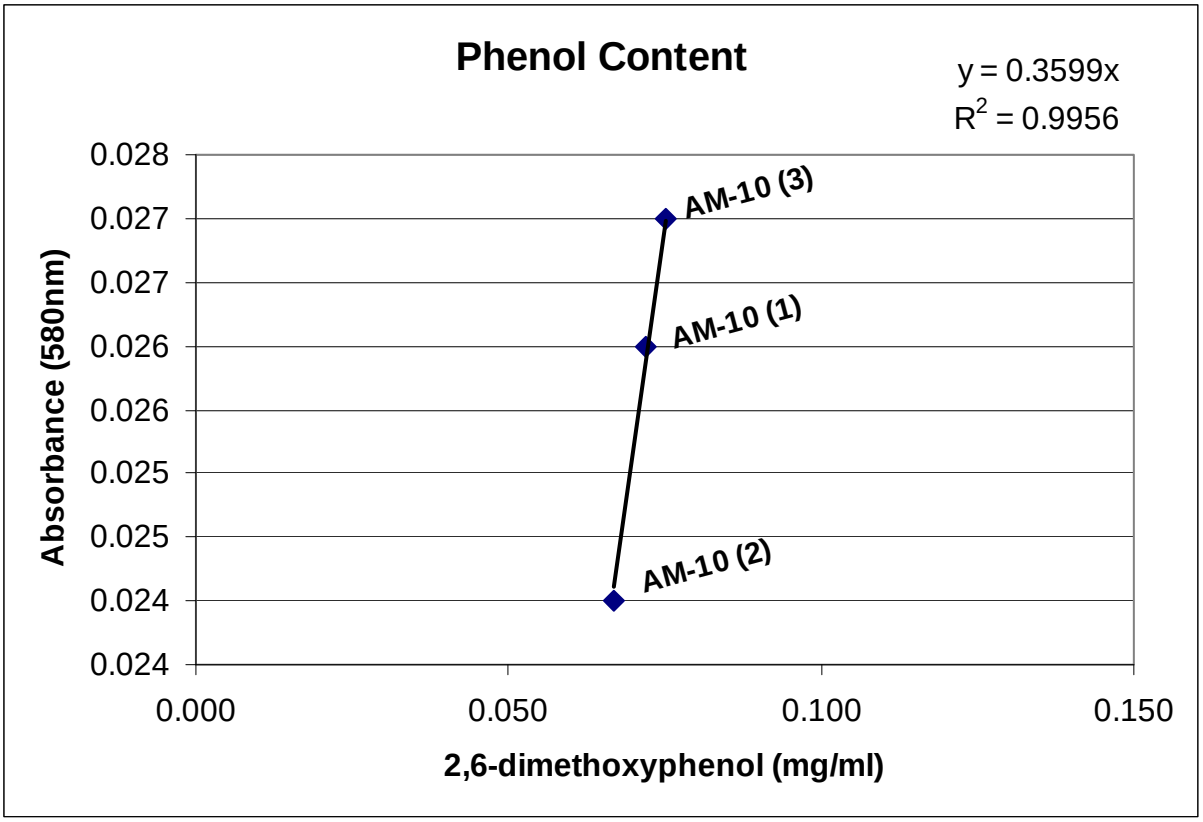

Figure 6 - Phenol content results graph of AM-10 liquid smoke fraction; phenol concentration of three separate lots in mg/ml (represented as 2,6-dimethoxyphenol versus absorbance at 580nm).

The three AM-10 phenol values were averaged to get the reportable value of 0.07mg/ml of 2,6-dimethoxyphenol. A phenol content of 0.07mg/ml allowed for a product with a very low smoke flavor profile to be utilized in this process. Liquid smokes used in conventional meat applications have a phenol range of approximately 6-20mg/ml; therefore, a phenol value of 0.07mg/ml is extremely low by liquid smoke industry standards. A chart of the phenol values at each processing step is presented in Table 5. The effects of filtration and refinement processes on the AM-10 phenol content are also represented in Table 5.

Whipped cream/AM-10 product development

Sensory testing

Eight trained sensory panelists found the addition of AM-10 to slightly enhance the sweetness of the whipped cream. An increase in caramelized notes was also observed, but panelists associated this with cooked dairy notes and not necessarily browned sugar/caramel notes. Both the control and 0.075% AM-10 cream solution heated to 72°C resulted in increased chemical and metallic notes, indicating heating alone causes the development of off-flavors. Differences were noted in appearance and texture; however, additional work with a standardized method of whipping is needed to determine if there is any correlation to temperature and/or the addition of AM-10.

The results of the attribute analysis are as follows: Heating the control to 50°C resulted in a cooked flavor. With the addition of AM-10, the sample had a similar

cooked flavor, but also showed slightly increased overall flavor, and additional creamy/dairy, sweet, and caramelized notes. Heating the control to 63°C resulted in increased cooked flavor. With the addition of AM-10, the sample was found to have increased overall aroma, overall flavor, creamy dairy, caramelized, and cooked notes. Heating the control to 72°C resulted in increased overall and cooked aroma and flavor. With the addition of AM-10, the sample showed increased overall and cooked aroma and flavor, along with increased sweet and caramelized flavor notes. Also, color differences were observed between the control and the test samples; however, there was no clear correlation to the addition of AM-10 or temperature differences. Controls heated to 50°C and 72°C were noted as slightly more yellow than the unheated control. Very slight yellow color was also noted in all other samples except the 0.075% AM-10 treated sample that was heated to 72°C. Texture differences were also observed. In general, the heated samples did not whip as well as the unheated control. The 0.075% AM-10 sample heated to 72°C was the closest to the control in texture. Controls heated to 50°C and 63°C were noted as being grainy. All results are reported as averages of the panel results (Table 8 and Figure 7). The standard deviations are represented in Table 12.

Table 8 - Qualification and quantification of sensory descriptors.

Sensory Attribute	Reference	Control 50˚C	0.075% AM10 50˚C	Control 63˚C	0.075% AM10 63˚C	Control 72˚C	0.075% AM10 72˚C
AROMA							
Overall Aroma	5.0	5.3	5.4	5.3	5.6	5.6	5.6
Creamy Dairy Aroma*	5.0	5.1	5.1	5.1	5.1	5.1	5.3
Cooked Aroma*	5.0	5.0	5.3	5.3	5.3	5.5	5.5
Chemical Aroma	5.0	5.1	5.0	5.0	5.3	5.4	5.3
FLAVOR							
Overall Flavor	5.0	5.4	5.8	5.0	5.9	5.9	5.6
Creamy Dairy*	5.0	4.8	5.7	5.4	5.6	5.4	5.4
Sweet*	5.0	5.4	5.7	5.0	5.4	5.2	5.7
Caramelized*	5.0	5.2	5.6	5.3	5.6	5.3	5.5
Cooked*	5.0	5.8	6.1	5.8	6.3	6.0	6.3
Metallic	5.0	5.3	5.2	5.0	5.2	5.3	5.3
Chemical	5.0	5.0	5.1	4.9	5.2	5.4	5.3
Smoky	5.0	5.1	5.2	5.0	5.2	5.2	5.3
DFR'S							
DFR Aroma	10.0	7.4	7.9	7.6	7.2	7.1	7.7
DFR Appearance	10.0	6.5	7.5	7.3	7.1	6.8	7.6
DFR Flavor	10.0	7.0	7.1	7.0	7.0	6.9	7.0
DFR Aftertaste	10.0	7.3	7.2	7.0	6.9	6.9	7.0
DFR Overall	10.0	6.9	7.2	7.0	6.9	6.9	7.1

(DFR) Deviation from reference
*Denotes positive attributes
Aroma and Flavor descriptors are represented as deviation from reference where reference = 5 (Scale 1-9; where 1-4 is less intense than control with 1 having the least possible intensity, and 6-9 is more intense than control with 9 having the greatest possible intensity)
DFR's represented as deviation from reference where reference = 10 (Scale 10-0; with 10 being same as reference and 0 differing completely from reference)

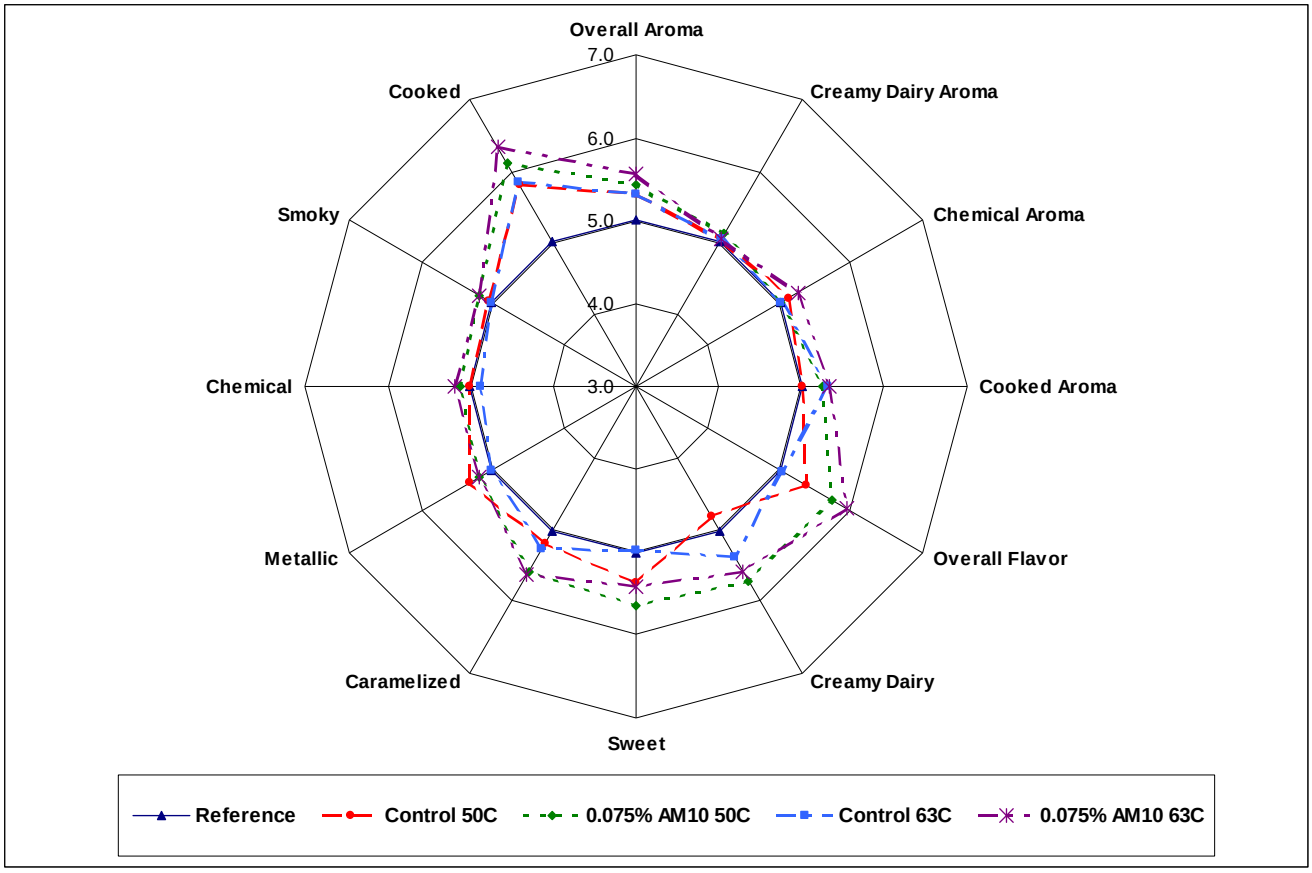

Figure 7 - Qualification and quantification of sensory descriptors of whipped cream. The reference sample is heavy cream that was chilled at 0˚C for 12 h and whipped for 8 min using a handheld kitchen mixer. Control samples were heated to 50˚C, or 63˚C for 15 sec and then chilled (0˚C for 12 h) and whipped (8 min using a handheld kitchen mixer). The 0.075% AM10 samples were prepared by adding a 0.075% addition (by weight) of a liquid smoke fraction (AM10) to the heavy cream. These samples were thermally processed (50˚C, or 63˚C for 15 sec), chilled (0˚C for 12 h) and whipped (8 min using a handheld kitchen mixer). All samples were evaluated by an eight member, trained sensory panel; and the results are reported as averages.

Possible Maillard reaction pathways

Based on the Hodge diagram represented in Figure 1, and the knowledge that heated milk yields hydroxymethyl furfural, two pathways may be viewed as the most probable in this application (Hodge, 1953). The most likely pathways are illustrated in Figure 8 (Hodge, 1953).

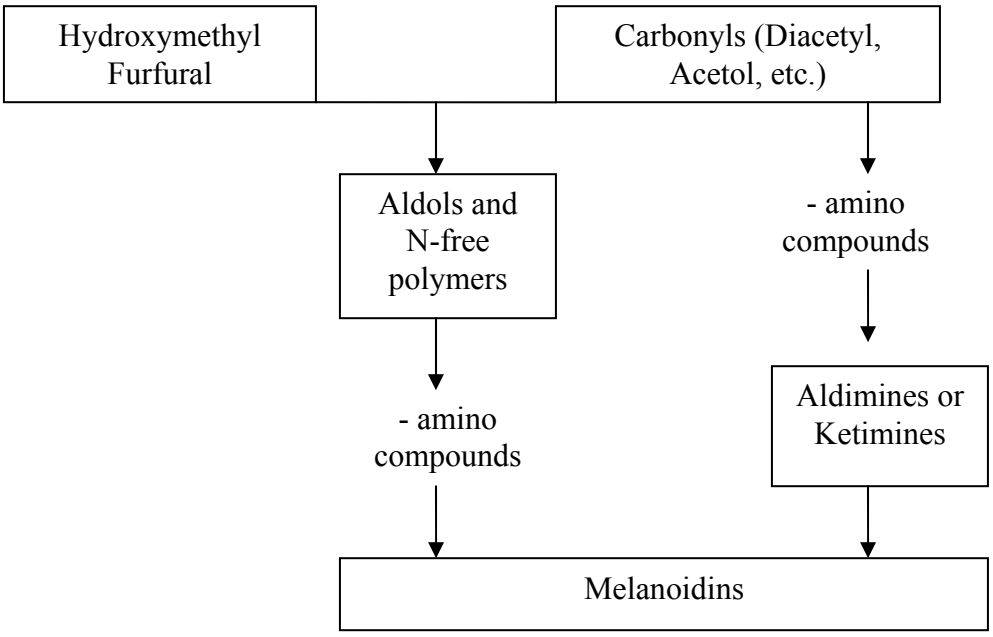

Figure 8 - Possible Maillard pathways for the heated cream and 0.075% AM-10 liquid smoke fraction solution (Hodge, 1953).

Focus group

The focus group determined that there are many possible applications for this technology, particularly in the reactionary flavor, dairy, and pastry markets. The most evident application – requiring the least research and development time at this point – was determined to be a whipped cream topping for coffee-type beverages, desserts, and as a retail product. Another proposed usage for AM-10 was as an ingredient for the reactionary flavor industry; such as, roasted, toasted, beef, bacon, and grill-type flavors. AM-10 was indicated as a potentially useful product in pastry browning applications, with particular interest noted to the idea of using it to apply grill-type markings. Also

proposed, and potentially the most promising concept was the utilization of AM-10 as a sweetener for a cream cheese filling to be used in a danish project.

Go-to-market concepts were discussed and many ideas presented. The most feasible strategies are listed in order of predicted probability of success:

1. In whipped cream as a coffee-type beverage topping
2. As a cream cheese filling sweetener for danish application
3. Retail sale of a reduced-sugar whipped cream
4. Pastry browning
5. Grill marks on pre-packaged panini and pocket sandwiches
6. As an ingredient in reactionary flavors

CONCLUSIONS

A sensory acceptable, liquid smoke fraction was successfully developed. Sweet and caramel-type flavors were noted in the final whipped cream product, and it was established (due to the lack of these flavors in the control samples) that the flavors were the result of a chemical reaction. Although other chemical reactions may be taking place, research indicates that the Maillard reaction is probably a significant contributor to the sweet, caramel-type flavors. The Maillard reaction is widely known as a complex reaction. This reaction is not completely understood, and its pathways are not easily predicted. Therefore, additional research to verify specific mechanisms may be necessary for this application; also, further laboratory and sensory testing may be beneficial in determining the most efficient reaction temperatures and AM-10 addition levels.

It is recommended that a standardized method for whipping be developed so that the AM-10 effect on the whipped cream color and texture may be more accurately qualified and quantified. Also, further research and sensory testing may be utilized to determine if developing a concentrated AM-10 and heavy cream solution may be useful as an add-back flavor. This would allow for the majority of the heavy cream to be exempt from the heating process which could possibly minimize off-flavors.

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APPENDIX

Table 9 - Sensory difference from 5 point scale; where reference = 5 and scale range is 1-9.

LESS INTENSE THAN STANDARD/CONTROL				**Control**	*MORE INTENSE THAN STANDARD/CONTROL*			
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
EXTREME	LARGE	MODERATE	SLIGHT	**TARGET**	SLIGHT	MODERATE	LARGE	EXTREME

Table 10 – Sensory deviation from reference point scale; where control = 10, and scale range is 1-10.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Completely Different	Extremely Different	Very Different	Moderately to Very Different	Moderately Different	Slightly to Moderately Different	Slightly Different	Very Slightly Different	Almost the Same as Control	**Same as Control**

Table 11 – Sensory lexicon.

Overall	**Combined strength of all flavor aromatics and basic tastes perceived in the sample**
Positive Attributes	
Creamy Dairy	**A sweet, dairy note associated with cream or other high fat dairy products**
Cooked	**A slightly brown, caramelized aromatic associated with heated milk**
Sweet	**Taste on the tongue stimulated by sugars and high potency sweeteners.**
Caramelized	**Sweet aromatic, characteristic of browned sugars and other carbohydrates**
Negative Attributes	
Metallic	**Aromatic associated with metals, tin, or iron**
Smoke	**Perception of any type of smoke flavor, whether it be hickory, apple, cherry, mesquite, or artificial; may be phenolic or tar-like**
Chemical	**A general term associated with many different compounds, such as solvents, cleaning compounds, and hydrocarbons**

Sensory Ballot

Name: _____

Whipped Cream

Attribute	Reference	279	334	420	845	157	618
Aroma							
Overall	5						
Creamy Dairy	5						
Chemical	5						
Cooked	5						
Flavor							
Overall	5						
Creamy Dairy	5						
Sweet	5						
Caramelized	5						
Metallic	5						
Chemical	5						
Smoky	5						
DFR Aroma:	10						
DFR Appearance	10						
DFR Flavor:	10						
DFR Aftertaste:	10						
DFR Overall:	10						

Figure 9 - Sensory ballot. Samples were blind coded using three digit numbers.

Table 12 - Sensory panel standard deviations.

Standard Deviations						
Sensory Attribute	**Control 50C**	**0.075% AM10 50C**	**Control 63C**	**0.075% AM10 63C**	**Control 72C**	**0.075% AM10 72C**
Overall Aroma	0.5	0.4	0.8	0.7	0.5	0.6
Creamy Dairy Aroma*	0.7	0.6	0.3	0.4	0.6	0.7
Cooked Aroma*	0.9	0.4	0.5	0.4	0.5	0.6
Chemical Aroma	0.4	0.0	0.0	0.7	0.5	0.6
Overall Flavor	0.8	0.7	0.5	0.8	0.7	0.7
Creamy Dairy*	0.9	0.6	0.7	0.5	1.0	0.7
Sweet*	0.8	0.8	0.5	0.6	1.0	0.4
Caramelized*	0.4	0.5	0.4	0.6	0.8	0.5
Cooked*	0.8	0.7	0.6	0.7	0.7	0.5
Metallic	0.6	0.3	0.5	0.4	0.7	0.6
Chemical	0.0	0.2	0.4	0.4	0.7	0.6
Smoky	0.2	0.4	0.5	0.4	0.4	0.7
DFR Aroma	0.6	0.7	1.1	0.8	0.7	1.6
DFR Appearance	0.8	0.8	0.9	0.2	0.6	0.7
DFR Flavor	0.6	0.7	0.5	0.5	0.5	0.8
DFR Aftertaste	0.9	0.8	0.7	0.6	0.3	0.9
DFR Overall	0.6	0.7	0.6	0.7	0.4	0.8

***denotes positive attributes.**

CARBONYL STANDARD CURVE AND LINEAR REGRESSION

An analysis for carbonyl content was conducted for the AM-10 product. The standard curve absorbance values were read at 480nm, and the results were recorded. A graph of the absorbance values versus the known standard curve 2-butanone concentrations was plotted. A trendline was created using these values. The linear regression was performed, yielding a correlation factor of $R^2 = 0.9996$ and $y = 0.36x$ line formula. An acceptable correlation factor was achieved (0.998 or better), so the line formula was used to calculate the 2-butanone concentration present in the liquid smoke.

Table 13 - Carbonyl Standard Curve Results Table.

Sample	2-butanone mg/100ml	Absorbance (480nm)
Blank	0.0	0.000
A	10.0	0.591
B	15.0	0.918
C	20.0	1.212

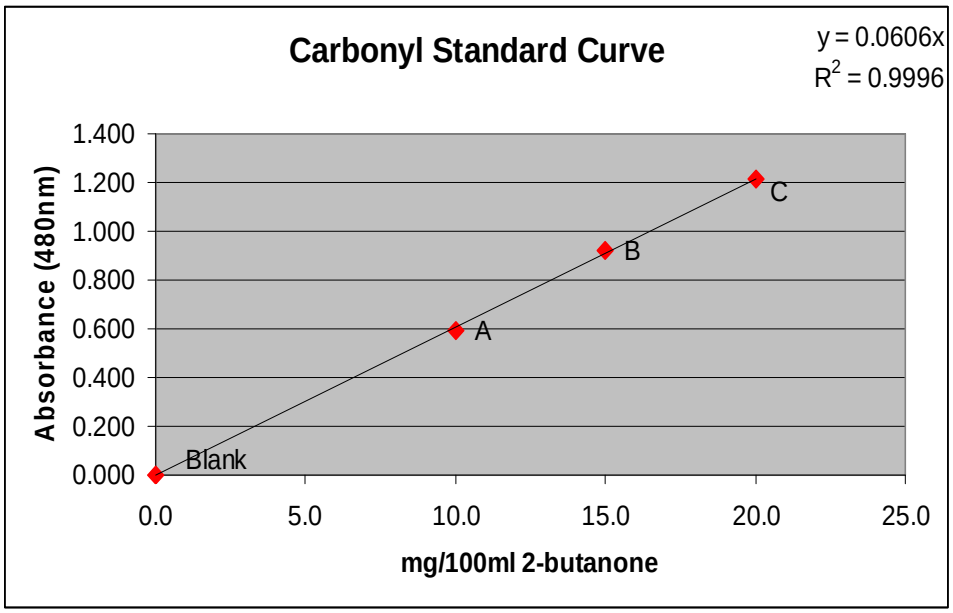

Figure 10 - Carbonyl Standard Curve Results Graph.

The AM-10 samples were tested for carbonyl content using three separate lots [AM-10 (1), AM-10 (2), and AM-10 (3)]. The absorbance values were read at 480nm and recorded. Using the y=0.0606x line formula derived from the standard curve analysis, the AM-10 absorbance values were used to mathematically calculate the 2-butanone concentration.

PHENOL STANDARD CURVE AND LINEAR REGRESSION

An analysis for phenol content was conducted for the AM-10 product. The standard curve absorbance values were read at 580nm, and the results were recorded. A graph of the absorbance values versus the known standard curve 2,6-dimethoxyphenol concentrations was plotted. A trendline was created using these values. The linear regression was performed, yielding a correlation factor of $R^2 = 1$ and $y = 0.36x$ line formula. An acceptable correlation factor was achieved (0.998 or better), so the line formula was used to calculate the 2,6-dimethoxyphenol concentration present in the liquid smoke.

Table 14 - Phenol Standard Curve Results Table.

Sample	2,6-dimethoxyphenol (mg/ml)	Absorbance (580nm)
Blank	0.000	0.000
A	0.050	0.018
B	0.100	0.036
C	0.150	0.054

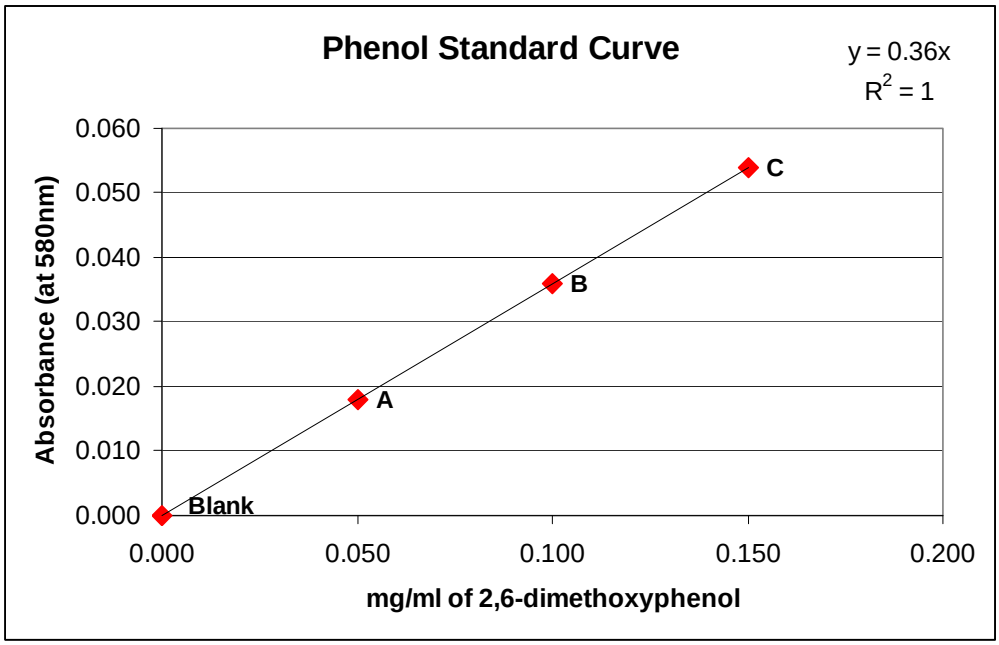

Figure 11 - Phenol Standard Curve Results Graph.

The AM-10 samples were tested for phenol content using three separate lots [AM-10 (1), AM-10 (2), and AM-10 (3)]. The absorbance values were read at 580nm and recorded. Using the y = 0.36x line formula derived from the standard curve analysis, the AM-10 absorbance values were used to mathematically calculate the 2,6-dimethoxyphenol concentration.