ABSTRACT

SPICER, VALERIE DENISE. The Effects of Protein Concentration and Temperature on Flavor Delivery and Volatility of 2,4-Dimethylbenzaldehyde and Ethyl Butyrate in Whey Protein Isolate Solutions. (Under the direction of Dr. E. A. Foegeding and Dr. Duane K. Larick.)

The development of reduced fat foods is a continual challenge in the food industry. Among the many attributes fat contributes to food products is flavor. Fat replacers are used to regain some of these lost attributes as fat is removed from foods. These fat substitutes affect the rate and concentration at which flavor molecules are released during mastication. Whey is a source of protein for protein-based fat replacers. The effects this ingredient has on flavor intensity was studied utilizing 2,4-Dimethylbenzaldehyde (DMB) and Ethyl Butyrate (EB). The objective of this project was to develop a headspace gas chromatography method to measure the changes in volatility of these two flavor compounds in various concentrations of WPI solutions and to compare these results to sensory findings. There was a significant decrease in the volatility of DMB with increases of protein concentration from 0 to 2%. Aroma and taste intensity also decreased with increasing protein concentration. The volatility of EB and taste intensity showed no significant differences with successive increases in protein concentration. However, aroma shows significant decreases in intensity with increases in protein concentration at the 0 to 2% and 4 and 6% levels. There were significant positive correlations between volatility and sensory results.

The Effects of Protein Concentration and Temperature on Flavor Delivery and Volatility of 2,4-Dimethylbenzaldehyde and Ethyl Butyrate in Whey Protein Isolate Solutions

by
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Biography

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Chapter 1

Introduction

The development of low-fat and reduced-fat foods remains a challenge in the food industry as fat contributes many different attributes to food products, such as texture and flavor. As fat is removed from a formulation, the other ingredients such as proteins, carbohydrates, and minerals increase automatically and proportionately, and interact differently with flavor than fat (Hatchwell 1994). The dilemma of the food scientist is that the consumer wants to enjoy the taste and texture of high-fat foods without the unnecessary calories. Hence, the flavor system must be reformulated to reflect the differences that occur in the flavor/fat versus flavor/fat-substitute interactions (Schirle-Keller and others 1992).

It has been demonstrated that replacement of these lost attributes cannot be accomplished by simply removing fat and replacing it 1:1 with another ingredient (Clark 1994). Typically, a combination of ingredients is used to jointly provide the many functional properties of fat (Clark 1994). Fat replacers composed of proteins interact differently with aroma compounds than fat and are utilized in products such as frozen desserts, dairy products, salad dressings, and margarine. Whey protein products are a source of protein for these replacers and the effect whey protein has on flavor intensity will be studied in this project using both a sensory and analytical method.

Sensory evaluation is used extensively by the food industry and academia, and allows companies to use a sample of the population to give them helpful information about issues such as consumer preference, and how the reformulation of a product will be perceived or noticed by the consumer. The class of sensory test chosen in this investigation was descriptive analysis. Panelists were trained and interval scaling was used to measure relative differences in intensity.

The objective of this project was to develop a headspace gas chromatography method to analyze the changes in volatility (intensity) of two separate flavor compounds in various concentrations of whey protein isolate solutions and to compare these results to the sensory findings.

Review of Literature

Roles of Fat in Flavor

The roles of fat in flavor development are very important when attempting to reformulate foods at a reduced fat level. Fat provides a medium for flavor delivery, adds a creamy texture and mouthfeel to foods, serves as a flavor precursor, and adds gloss and sheen to the appearance of food (Lucca and Tepper 1994). Removing fats from foods results in a loss of flavor medium and the flavor becomes unbalanced. The fat-soluble flavor volatiles, normally in the lipid phase now stand out in the flavor profile.

Successfully reformulating food requires the utilization of other mediums such as protein-, carbohydrate-, or lipid-based fat replacers. These fat mimetics cannot become a solvent for flavor volatiles, as fat does, but can impart some of the characteristics lost when reducing fat in foods.

Flavor

According to Jasinski and Kilara (1985) and Taylor and Linforth (1996), the flavor of a food is the overall impression in the nose and on the tongue caused by aroma (volatile) and taste (non-volatile) substances. Two types of aroma, as well as flavor, are integrated to give the overall flavor profile of foods. Orthonasal aroma is perceived through direct sniffing through the nasal passages and retronasal aroma is experienced

during mastication as the food volatiles are released into the back of the throat via the pharynx to the nasal cavities (O'Neill 1996).

Many products, such as bananas, oranges, or lemons can be recognized solely on the basis of their aroma. Odor is considered to be 70 – 80 percent of flavor (Reineccius 1994). Typically, methods used for analyzing flavor focus on the volatile components because of their importance in overall flavor and because they are more amenable to instrumental analysis (Taylor and Linforth 1996).

Flavor Release

During mastication, the breakdown of the food matrix and the interaction with saliva causes the release of the volatiles into the mouth. The physicochemical environment changes significantly with the addition of saliva, the increased surface area of the broken down food particles, and changes in temperature that may occur. These changes affect flavor release, the rate of release and concentration of flavor released into the headspace from food or other medium. Static headspace gas chromatography samples those volatiles released (aroma) and contained in the air above the food system in a sealed system at equilibrium. The composition of the headspace depends on the partitioning of volatiles between the air phase and the different phases present in the food.

Factors that affect partitioning of the volatiles between air and food (medium) include dispersion medium, temperature, degree of solubility in the medium, and the concentration of volatiles in the liquid phase (Nawar

1971; Franzen and Kinsella 1974; Ebeler and others 1988; Schirle-Keller and others 1992, 1994).

Full-fat foods have less volatiles than reduced-fat foods. In full-fat foods, the rate of release and concentration of flavor volatiles in the headspace is much slower and lower, respectively. When fat is removed, the vapor pressure or volatility is much higher and the overall flavor perception is more intense and harsh and has less texture or creaminess (Hatchwell 1994). This means a change in the flavor profile that requires reformulation in an effort to regain lost attributes and rate of release.

Flavor Compound Analysis

Food volatiles are usually found in trace amounts and constitute an integral part of the flavors perceived by consumers. Typically, because of their trace concentrations in food, they must be isolated and concentrated in some manner prior to instrumental analysis (Girard and Nakai 1991). Numerous techniques are employed to study flavor constituents released from foods based on volatility. They include dynamic headspace, direct injection, and static headspace (Reineccius 1994).

Dynamic headspace methods are based on the concept of purging volatiles from the sample and concentrating them on an adsorbant trap. According to Reineccius (1994), this approach favors the isolation of constituents with the highest vapor pressure and is suitable for liquid samples. A disadvantage of using dynamic headspace is that the method is not favorable for liquid samples containing protein because it causes

foaming, which can interfere with the entire process. The flavor system studied in this research utilized a whey protein matrix.

The direct injection technique involves injecting the food sample into the apparatus itself (gas chromatography) and heating to volatilize aroma constituents. This method is limited to liquid samples that usually require some chemical modification before sampling.

Equilibrium or static headspace analysis involves a sample, liquid or solid, that is enclosed in a sealed vessel and allowed to equilibrate for a period of time to allow flavor volatiles to partition between the sample and the headspace. The advantage of this method is that samples, liquid or solid, containing volatiles can be analyzed. Above ambient incubation temperatures are sometimes used to increase the volatilization of flavor components prior to analysis. The limitations include the size of the sampling device as well as the required use of dilute concentrations of the volatiles. The sampling technique utilized for this research was static headspace gas chromatography.

The vapor of the volatiles released from the sample are analyzed when utilizing static headspace, and proceed to follow the usual schematic of gas chromatography. The sample is injected by a semi-automated headspace sampler and swept into the column by an inert carrier gas (Helium) and passes through the column containing the stationary phase. This column separates the volatile components from one another based on their physicochemical properties such as polarity or boiling point (Harris

1982). The aroma compounds pass through a detector, which sends a signal to a recorder as the volatiles emerge from the column. The recorder represents each compound as a peak and the area under the peak is integrated as a numerical value of peak area. A large concentration of a compound should correspond to a comparatively large peak area, and vice versa.

The volatility or headspace concentration of a compound is affected by factors such as vapor pressure and temperature, type of medium, degree of solubility, concentration, miscibility with other organic compounds, and presence of salts or sugars (Franzen and Kinsella 1974). An increase in the volatility of a compound would result in an increase in release of the volatile from the matrix.

Flavor Interactions

The volatility of a flavor compound is closely related to the vapor pressure of the compound. Vapor pressure is the pressure of the accumulated volatile(s) above a liquid at a given temperature in a sealed vessel at equilibrium. An increase in vapor pressure would be signified by an increase in the concentration of the volatile in the vapor phase. This can be achieved by increasing temperature, adding salts, reducing oil or fat level, and various other ways and is compound and matrix dependent.

Ebeler and others (1988) showed a significantly reduced vapor pressure of menthone and isoamyl acetate in a nonpolar medium (soybean oil) that was not exhibited in the relatively polar medium, water.

They also showed that increased protein concentrations, in water, greatly decreased the headspace concentration of menthone. The headspace concentration (HC) of isoamyl acetate, however, showed only minor changes (Ebeler 1988). These minor changes were explained by the differences in solubility of the two flavorants. Menthone is relatively less polar than isoamyl acetate, and may have interacted with the hydrophobic regions of the protein to a greater extent.

Bohnenstengel and others (1993) conducted three experiments to determine the factors influencing headspace gas chromatography. Two of the studies focused on sampling device volume and interactions of compounds with the sample matrix. The model solution contained equal amounts of 16 flavor compounds dissolved in diethyl ether, which were chosen because they differed in chemical structure, volatility, and polarity. Increasing sampling device volume increased peak areas for the higher boiling point or lower volatility compounds while only slightly decreasing the lower boiling point or higher volatility compounds. All test matrices (water, glycerol, paraffin or gelatine) had an influence on headspace composition. Adding the flavor solution to each matrix generally caused a decrease in the peak areas of the flavor compounds (Bohnenstengel and others 1993). Parrafin had the greatest effect, and the explanation offered focused on a better solubility of the test compounds in this matrix. The more polar compounds were less influenced by paraffin.

Schirle-Keller and others (1992, 1994) illustrated that the functional group of a flavor compound is a factor affecting the partitioning between air and food (or medium). The protein-based fat replacers utilized in these experiments show strong interactions with aldehydes (particularly unsaturated aldehydes) via relative vapor pressure, with the exception of acetaldehyde (polar compound).

Girard and Nakai (1991) used static headspace chromatography to study volatiles in canned salmon. Vials of fish flakes were incubated at temperatures ranging from 35°C to 125°C for one hour. As incubation temperatures increased, overall peak intensities and the number of peaks increased. Two observations were also noted. Samples tempered at 110°C for more than an hour experienced bulging septums from increased internal pressure, resulting in weaker crimped seals causing leaks. Also, when varying incubation time from 15 minutes to 5.5 hours at a constant incubation temperature of 75°C, after 3.5 to 4 hours, the total area reached a plateau. Initially, the total chromatographic area followed a linear relationship with incubation time.

The advantages of using static headspace analysis are that the method does not require any isolation and concentration processes for the sample, which are time-consuming, reduces the possibility of artifact formation, and gives reproducible ratio changes of the volatile components in the vapor (Kepner and others 1964). These factors result in

reproducible and accurate analysis of volatile components in food systems.

One of the problems faced with static headspace chromatography is “carry over”. The syringe used to sample the vapor for analysis can adsorb a significant amount of the volatile being analyzed. This problem was addressed in the work of Buttery and others (1969) and O’Keefe and others (1991). Buttery and others’ (1969) work noted that there was negligible adsorption on the syringe for hexanal (boiling point 126 °C); however, with nonanal (boiling point 190°C), the syringe adsorbed about 80% of the vapor concentration. They summarized that the degree of adsorption by the syringe is greater the higher the boiling point of the volatile. “Carryover” was present during the analysis of one of the flavorants in this research project, and if the system was not completely cleared out, the result would be will give misleading data that was not reproducible.

Buttery and others (1969) explained that although adsorption by syringes can be misleading, “sampling with a single component can be adequate for relative quantitative measurements of the concentrations in a vapor.” Buttery and others (1969) went on to reason that adsorption by the syringe for the compound should be approximately proportional to its concentration. Hence, the amount remaining for injection would be proportional to the original concentration. This problem was present in

this research, and the syringe had to be “cleaned” by running water blanks after each sample.

Static headspace analysis can be utilized in many food applications including the analysis of fermentation products such as ethanol production (Girard and Nakai 1991), an objective tool in testing milk flavor quality (Christensen and Reineccius 1992), as well as in many quality assurance/quality control protocols in the food industry (Reineccius 1994). This review focuses on static headspace chromatography and its use in the analytical measurement of flavor or volatile release in whey protein isolate systems.

Whey Protein

One method used to manufacture whey protein isolate (WPI) is cation exchange, in which the whey stream is reduced to an acidic pH and proteins are positively charged (Huffman 1996). The protein is pumped into a tank that contains negatively charged resin beads. As the whey proteins attach to the resin beads, the lactose, lipids, and minerals flow through and are removed. The pH of the tank is then brought up to alkaline pH, which releases the protein and the process of ultrafiltration and spray drying continues, producing a highly concentrated whey protein product that is low in lactose, fat, and minerals.

An alternative process to manufacture WPI is by combining two additional steps, microfiltration (to remove fat) and lactose hydrolysis (to

remove lactose) before the ultrafiltration step. The WPI used in this research project was produced utilizing the cation exchange method.

Functionality of Whey Protein Isolate

Whey proteins may be used in a wide range of food applications because of their various functional properties. These functional properties include solubility, viscosity, emulsification, foaming and whipping, gelation, and nutrient source (Fenemma 1985; Huffman 1996).

Native whey proteins demonstrate excellent solubility and low viscosity over a wide pH range; however, heat-denatured proteins (heating to temperatures above 70°C) can aggregate and precipitate at their isoelectric point. The isoelectric point is the pH value at which the protein is electrically neutral (Fenemma 1985). The isoelectric point of whey proteins range from 4.5 – 5.3 (Huffman 1996).

Heat causes whey protein to unfold, exposing additional hydrophobic sites that are unavailable in the native unheated protein (Huffman 1996). As the protein loses solubility and forms protein aggregates, the viscosity of the protein solution increases. This functional property is used to thicken foods, such as soup and gravy.

Whey proteins can also function as emulsifiers because they contain both hydrophilic (water-loving) and hydrophobic (lipid-loving) regions. The protein encapsulates oil, allowing the water and oil to stay in close proximity and prevent the partitioning of oil (lipid) and water. This function allows whey protein ingredient to be used in salad dressings.

Another functional property of whey protein is foaming and whipping. When air is incorporated, the protein forms interfacial membranes around the air and is desirable for use in whipped toppings, ice cream, and meringues (Fenema 1985).

Whey proteins in aqueous solution are also able to form gels upon denaturation at concentrations as low as 4% (Morr and Foegeding 1990). The gelation properties can be used to change liquid products into solids and to modify texture characteristics in food such as cakes and seafood.

Finally, whey proteins contain essential amino acids that can be added to foods that lack adequate amounts. These proteins add nutritional value to infant formulas, sport drinks, and other health foods.

Although whey proteins have numerous functional properties, the ability of proteins to bind flavors, to serve as a flavor carrier, and affect flavor perception is the focus of this project. References will be made to WPI and whey protein concentrate (WPC). The main difference in the two products is that WPI contains a higher % of protein (usually >90%) and less fat, lactose (sugar), and minerals. This fact allows minimal interactions with these components, and focuses more on the protein interactions with flavor.

Fat Replacers

The development of low-fat and reduce-fat foods remains a challenge in the food industry as fat contributes many different attributes to food products, such as texture and flavor. In an effort to regain these

lost attributes, it has been found that this cannot usually be accomplished by simply removing fat and replacing 1:1 with another ingredient, but by using combinations of ingredients to jointly provide the many functional properties of fat (Clark 1994). These ingredients are fat replacers or fat substitutes and fall under three categories: protein-based, carbohydrate-based, and lipid-based. Fat replacers composed of proteins and carbohydrates interact differently with aroma compounds than fat and altering the type of fat or total fat content of food affects the rate and concentration at which food flavor molecules are volatized during consumption (Hatchwell 1994). Fat substitutes are utilized in frozen desserts, dairy products, salad dressings, and margarine. Lipid-based fat replacers are either emulsifiers or fat analogs that have been modified or synthesized so that they are no longer metabolized completely, providing fewer calories (Glueck and others 1994). Currently, lipid-based fat replacers are used in bakery goods, frozen desserts, and as a replacement for oil and shortening in baking and deep fat frying. This section will focus on protein-based fat replacers, some of their functional properties, and their interactions with aroma compounds.

The functional properties of protein-based fat replacers include those of the original protein, and are dependent on the source and the processing steps. For example, microparticulated proteins (MPPs) are subjected to high heat (causing heat coagulation) and high shear, which produces particles of less than 0.3μm in diameter. At this diameter, the

tongue cannot distinguish the individual particles, thus giving the perception of creaminess (Lucca and Tepper 1994). These MPPs, when used as a fat replacer improve mouthfeel, ice-crystal control, and foam stabilization in semi-solid food products, such as ice cream, and provide increased tenderness and crumb quality in baked goods (Hatchwell 1994).

Schirle-Keller and others (1992), conducted an experiment that utilized five fat replacers, including a whey-based microparticulated protein, Simplesse 100 (S-100), and compared their interactions with flavor compounds to flavor interactions in oil. Using static headspace gas chromatography, relative vapor pressure (rvp) (peak area of flavorant in system / peak area of flavorant in water) was determined for each fat replacer and compared to rvp in oil. While the rvp values were substantially greater than the oil-based system, S-100 was found to interact with flavor compounds more like oil than any of the other fat replacers (Schirle- Keller and others 1992; 1994).

Nonpolar compounds, such as limonene, exhibited rvp values of 0.6 - 0.8, which indicates an interaction or reduction in headspace concentration (Schirle-Keller and others 1992). Most water-soluble flavor compounds, such as acetaldehyde, showed little interaction; their vapor pressure was not reduced due to oil or fat replacer (Schirle-Keller and others 1992; 1994).

One explanation offered for the oil-like behavior of Simplesse 100 was that the replacer contained a small amount of residual fat (1.72%)and

at the fat replacer usage level (10%), there would be approximately 0.17% fat in the S-100 model system studied (Schirle-Keller and others 1992). The nonpolar compounds may have interacted with the small amount of fat, resulting in a reduced vapor pressure.

Flavor Binding

The flavor binding property of proteins allows them to be utilized as a flavor carrier in fabricated foods. In order to function as a good flavor carrier, the protein should bind flavors tightly, retain them during processing, and release them during mastication of food in the mouth (Fennema 1996). Flavor-protein interactions depend on the protein source, the flavor compound, the presence of water, and the presence of lipids (Franzen and Kinsella 1974). Three methods used to study flavor binding to proteins include equilibrium dialysis, headspace analysis, and relative vapor pressure.

Equilibrium dialysis involves a twin- chambered cell separated by a semi-permeable membrane. One side contains a soluble protein and the other side contains the flavor compound of interest. At equilibrium, the concentration of the flavor not bound by protein is equal on both sides of the membrane. Flavor bound by the protein is calculated by the difference in the concentration of flavor extracted from the two cells. The concentration of flavor on the non-protein side of the cell represents the free flavor concentration. Equilibrium dialysis can be used to determine

binding affinities to flavor compounds and number of binding sites of a protein.

Several proteins, β-lactoglobulin (50%), α-lactalbumin (12%), bovine serum albumin (5%), immunoglobulin (13%), and proteose-peptone (23%) combine to make whey protein (Jansinski and Kilara 1985). Other articles, such as Morr and Foegeding (1990), quote different composition % for the individual proteins that make up WPC.

Jansinski and Kilara (1985) incorporated two flavor compounds into whey protein concentrate, β-lactoglobulin, α-lactalbumin, and bovine serum albumin to determine flavor binding. The two flavorants were 2-nonanone (ketone) and nonanal (aldehyde). Using equilibrium dialysis, the scientists determined that WPC (88.3% protein) bound the largest number of molecules and had the highest binding affinity when compared to the individual protein fractions.

The binding affinity of a ligand to a protein depends on the structural state of the protein (Damodaran and Kinsella 1981) (Jansinski and Kilara 1985). Kinsella and Damodaran (1980) observed in 1% soy protein solution that the binding constants increased in the case of partially denatured soy protein (1240 M^{-1}) compared to native soy (930 M^{-1}); however, it did not change the number of binding sites. They suggest that the quarternary structure may undergo reorganization of the subunits that may enhance the hydrophobicity of the hydrophobic sites, thus increasing the binding affinity for the ligand.

Kinsella and Damodaran (1980) also reported that there is a difference in binding constants for flavor ligands from protein to protein. For example, the intrinsic binding constant for 2-nonanone to bovine serum albumin is approximately 1800 M^{-1}, whereas with soy protein it is only 930 M^{-1}. This indicates that 2-nonanone has a stronger binding affinity for BSA than soy protein, which they suggest may be because the hydrophobicity of the binding sites in the BSA may be more than that of soy protein. In aqueous solutions, nonpolar groups tend to aggregate so that the area of direct contact with water is minimized (Fennema 1996). This interaction with nonpolar groups is known as hydrophobic interactions.

Flavor release is the concentration of flavor compound that is not bound by protein and is available for flavor perception. Static headspace gas chromatography focuses on the flavor released from the protein matrix and can establish, by difference, the amount of flavor bound by the protein. This method can evaluate the partitioning behavior of the flavor compound between the vapor phase and the liquid phase. Vapor/liquid partition coefficients are calculated using the formula:

$$K = y/x$$

where "y" and "x" are the molar fraction of the aroma compound in the vapor and liquid phases, respectively (Draux and Voilley 1997). When K=1, the concentration of the aroma compound is found to be equal in the vapor phase and the liquid phase. As K increases, so does the relative

volatility of the compound. The K value can be used to compare volatilities between compounds.

Hydrophilic compounds interact independently in nonpolar matrices; however, hydrophobic compounds would significantly decrease in their headspace concentrations (Brauss and others 1999). This fact would be reflected in a decrease in the K value for hydrophobic (nonpolar) compounds, which would indicate a difference in partitioning of the compound.

Andriot and others (2000) calculated the partition coefficient for a homologous series of ketones and observed the partition coefficient changed decreased as protein was added. They observed an increase in the vapor-liquid partition coefficient for compounds with increasing hydrophobicity.

Relative vapor pressure is used to compare interactions of matrices to water to see if there is an interaction and can be described by the following formula:

$$rvp = \frac{\text{Matrix x day A volatility 1 + Matrix x day A volatility 2 + ...}}{\text{Water day A volatility 1 + Water day A volatility 2 + ...}}$$

in which the matrix volatilities are divided by repetitions of water volatilities for the same day. The water reference system is used when comparing interactions of the volatile with interactions in other matrices. An rvp value of ≈ 1 is indicative of no interaction with the flavor and matrix. As rvp decreases, the value indicates an interaction. For example, Schirle-Keller and others (1992) found that Simplesse 100 (S-100) exhibited rvp values

of 0.6 - 0.8, indicating an association between the protein-based fat replacer and nonpolar flavor compounds. The rvp values of the other fat replacers interacting with nonpolar flavor compounds were close to 1. Nonpolar compounds are soluble in oil and have limited solubility in water. The polarity of the volatile affects the solubility of the volatile in different matrices and helps determine the amount of interaction between the matrix and volatile. S-100 was relatively less polar than the other fat replacers, but not oil. As a result, the interactions of S-100 with nonpolar compounds, determined by rvp, were closer to oil (rvp close to 0) than the others. Polar compounds, such as acetaldehyde and diacetyl had rvp values close to 1, even for oil, indicating little to no interaction between the matrices.

Sensory Evaluation

Before the consumer tastes a food product, the first sensation is aroma via the nasal cavities. As the food is consumed (mastication), further aroma responses are experienced as food volatiles are released into the back of the throat via the pharynx to the nasal cavities (O'Neill 1996). These two aroma sensations are orthonasal and retronasal, respectively, and integrate with the flavor sensations involved in taste in the mouth to produce the overall taste of the product. Modifications of food products can change their flavor release profile, such as in a reduced-fat food, in which the flavor tends to be released from the food matrix at a quicker rate and greater intensity (Lucca and Tepper 1994).

Sensory evaluation is a scientific method used to “evoke, measure, analyze and interpret responses to food products as perceived through the five senses: sight, smell, touch, taste and hearing” (Stone and Sidell 1993). This segment of science measures human responses to foods, while minimizing potentially biasing effects such as brand identity and other information that influences consumer perception (Lawless and Heymann 1998). Sensory methods are used by companies, especially in product development, to study the effects of reformulation, packaging, processing, storage, etc., in order to make better decisions about the success of a new or improved product quality. For example, sensory evaluation could be used to determine if substituting a cheaper ingredient in a product formulation would significantly change the product (human perception) and/or decrease consumer acceptance.

Choosing the appropriate test method that will answer the questions asked about the product is the most important to the sensory scientist, and leads to decisions about panel selection and a method of measurement or scaling. Sensory test methods are grouped into three major classes that include difference testing, descriptive analysis, and hedonic testing (Lawless and Heymann 1998).

The goal of difference testing is to determine if any difference exists between food samples, and this type of sensory test includes the triangle procedure, duo-trio test, and paired comparison test (Lawless and Heymann 1998). In the triangle procedure, two products are the same,

while a third product is different. Judges are asked to pick the odd sample from among the three. The duo-trio test contains a reference, a test sample that matches the reference, and a test sample that is different. The objective is to correctly match the test sample that matches the reference. Finally, the paired comparison test allows panelists to choose which of two products is more intense in a specific attribute.

Descriptive analysis, according to O'Mahoney (1995), is probably the most commonly used technique in the industry and is a very informative sensory evaluation tool. This type of sensory test can answer questions about changes in intensity of sensory attributes, and are often compared with instrumental analysis data. Panelists assess a food and give responses for a range of sensory attributes, generally requiring training to ensure accurate communication e.g., same vocabulary of descriptors in order to categorize information more easily (O'Mahoney, 1995). Armbrister and Setser (1994) used a descriptive analysis panel to compare the sensory properties of chocolate chip cookies made with vegetable shortening (control) and cookies that substituted fat replacers at the 50% and 75% levels. The protein, carbohydrate, and lipid-based fat replacers could not achieve the texture, appearance, and flavor that the control exhibited. Although some fat replacers could simulate some of the characteristics of the full-fat cookie, no individual fat replacer could attain all of the characteristics. Hansen and Heinis (1992) studied how whey proteins affected the flavor perception of three flavor compounds using

quantitative descriptive analysis. The 12 member trained panel was utilized to determine changes in flavor intensity of benzaldehyde, citral, and d-limonene. Benzaldehyde and d-limonene flavor intensity declined with an increased protein concentration, but citral did not exhibit a significant drop with increased protein concentration. Reasons for the decrease of intensity of the two flavor compounds included hydrophobic interactions.

Hedonic testing is the third major class of sensory tests and attempts to measure the products that consumers prefer and the degree of liking or disliking. For example, panelists may evaluate a product with several levels of sucrose (sugar) and asked to rate how much they like the products. The 9-point hedonic scale is utilized, which rates “dislike very much” = 1 and “like very much” = 9. A neutral response is at the center of the scale, which is = 5. The response of the panelists would give the sensory scientist information on the best concentration of the substance, a hedonic optimum.

Descriptive analysis, which was the sensory test method chosen for this project, is often compared to some form of analytic instrumental analyses. Selecting panelists and the appropriate objectives for this test is very important and some form of training is required.

As panelists are trained to study changes in a specific sensory attribute, the elimination or reduction of cross-sensory interference is required (O’Mahoney, 1995). For example, if panelists are asked to

distinguish products by smell, it would be important that they could not see, or taste them. This keeps the brain from integrating other sensory input, and potentially biasing the sensory results.

To quantify differences in intensities among specific sensory attributes, descriptive analysis assigns numbers as a method of measurement. Four popular ways of scaling these measurements are nominal scaling, ordinal scaling, interval scaling, and ratio scaling. Interval scaling assigns numbers at equal intervals, representing equal subjective responses that can represent actual degrees of difference and is utilized in this flavor project (Lawless and Heymann 1998). As a result of introducing actual degrees of measurement, this scaling method can introduce powerful statistical methods (Lawless and Heymann 1998).

Summary

Considerable flavor research has been done on whey protein concentrate and soy protein. Through such techniques as headspace chromatography, equilibrium dialysis, and sensory evaluation, it has been established that proteins bind flavor compounds and affect flavor perception (Jasinski and Kilara 1985; O'Keefe and others 1991; Hansen and Heinis 1992; Schirle-Keller 1992, 1994; Brauss and others 1999).

Research on whey protein isolate, a purer form of whey protein, has included studies on its superior functionality compared to whey protein concentrate. The protein's flavor binding capacity, however, needs to be explored. This research project uses static headspace chromatography to

study this flavor binding function and supports it with sensory evaluation in order to gain a better understanding of how the protein affects flavor perception.

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Chapter 2

The Effects of Protein Concentration and Temperature on Flavor Delivery and Volatility of 2,4-Dimethylbenzaldehyde and Ethyl Butyrate In Whey Protein Isolate Solutions

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Abstract

The effects whey protein isolate has on flavor intensity was studied utilizing 2,4-Dimethylbenzaldehyde (DMB) and Ethyl Butyrate (EB). A headspace gas chromatography method was developed to measure the changes in volatility of these two flavor compounds in 0, 2, 4, and 6% WPI solutions at 45, 55, 65, and 75C and compared to sensory findings. Statistical analyses utilized the SAS program. There was a significant decrease in volatility of DMB with increases of protein concentration from 0 to 2% w/v. Sensory aroma and taste intensity also decreased with increasing protein concentration. The volatility and taste intensity of EB showed no significant differences with successive increases in protein concentration. However, aroma showed significant decreases in intensity with increases in protein concentration at the 0 to 2% and 4 to 6% levels. There were significant positive correlations between volatility and sensory results.

Introduction

Today's consumer wants to enjoy the taste and texture of high-fat foods without the unnecessary calories. The development of low-fat and reduced-fat foods, however, remains a challenge in the food industry as fat contributes many attributes to food products, such as texture and flavor. The differences that occur in the flavor/fat versus flavor/fat substitute interactions lead to a necessary reformulation of the food system (Schirle-Keller and others 1992). Typically, a combination of ingredients are used to jointly provide the many functional properties of fat (Clark 1994).

Fat replacers composed of proteins interact differently with aroma compounds than fat and altering the type of fat or total fat content of food affects the rate and concentration at which food flavor molecules are volatized during consumption (Hatchwell 1996). The functional properties of protein-based fat replacers include those of the original protein and are dependent on the source and processing steps.

The flavor binding property of proteins can be utilized as a flavor carrier in fabricated foods. Flavor-protein interactions depend on the protein source, the flavor compound, the presence of water, and the presence of lipids (Franzen and Kinsella 1974). One method used to study flavor binding to proteins is static headspace gas chromatography, which measures the amount of flavorant in the vapor state in a sealed system at equilibrium. This measure of flavor analysis focuses on the

flavor released from the food matrix and can establish by difference, the amount of flavor bound by the protein. This method can evaluate the partitioning behavior of the flavor compound between the vapor phase and the liquid phase.

Descriptive sensory analysis can be used to measure changes in intensity of flavor compounds. As flavor is a combination of taste and aroma, the project evaluated changes in aroma and taste due to protein concentration. The objectives of this project were to develop a static headspace gas chromatography method to analyze and measure the changes in volatility of 2,4-Dimethylbenzaldehyde and Ethyl butyrate in various concentrations of whey protein isolate solutions and to compare these results with sensory findings.

Materials and Methods

Materials

The flavorants utilized were 2,4-Dimethylbenzaldehyde and Ethyl butyrate (Aldrich, Milwaukee, WI). The whey protein isolate, BiPro, was obtained from Davisco Foods International (Le Sueur, MN), and contained 92.2% protein, according to Kjedahl analysis results.

Sample Preparation

The flavor solution was prepared by weighing out approximately 0.03 g of the flavorant into a tared boat funnel, and rinsing with deionized water into a 1000 mL volumetric flask, which was then filled to volume. A 1 mL aliquot from the 1000 mL was transferred to a 100 mL volumetric flask, which was then filled to volume.

An appropriate amount of whey protein isolate was hydrated in deionized water (25ºC) for 30 minutes utilizing a magnetic stir bar. The hydrated protein was poured into a 100-mL flask to obtain 2, 4, and 6% whey protein isolate solutions. The flavor-protein solution was obtained by adding 2mL of the diluted flavored solution to the 100 mL volumetric flask containing the whey protein solution. The solution was mixed and filled to volume with deionized water.

A Varian model 3700 gas chromatograph (Varian Corp., Palo Alto, CA), equipped with a DB-5 capillary column (30m x 0.32μm film thickness) (J & W Scientific, Folsom, CA) and HS-6 semi-automated sampler (Perkin-Elmer, Foster City, CA) was utilized to analyze the volatile release of the

synthetic flavorants. The head pressure was 15 psi with a column flow of 2.3 mL/min at 50°C and a split ratio of 5 to 1. The injector and detector were maintained at 230°C.

Samples (2 mL) were placed in a headspace vial with a crimp cap. Vials were placed in the semi-automatic headspace sampler for a predetermined equilibration time and temperature. Sample vials were pressurized for 4 min prior to semi-automated sampling for 1 min and injection on the DB-5 capillary column. The oven was programmed from 50°C to 220°C at 6°C/min. Eluted compounds were detected with a Flame Ionization Detector (FID). The peak area was integrated using Maxima 820 Chromatography Workstation (Millipore, Waters Chromatography Division, Millford, MA). To standardize peak areas for differences in sample weight and flavor concentration due to weighing, a "volatility" factor was calculated using the equation listed below:

$$\text{Volatility} = \frac{\text{Peak Area}}{(\text{Concentration})*(\text{Weight of sample})}$$

Methods Development

Concentration Study

Water samples with 2,4-Dimethylbenzaldehyde added at concentrations of 400, 500, 600, 700, and 800 ng/2mL were analyzed using a 30 min equilibration time at 45° and 75° C.

Equilibration Time Study

Water samples containing 2,4-Dimethylbenzaldehyde at a concentration of 600 ng/2mL were analyzed at 15, 30, 45, and 60 min and at 45° and 75° C.

Temperature Study

Water samples containing 2,4-Dimethylbenzaldehyde at a concentration of 600 ng/2mL were analyzed using 30 min equilibration time and at 45°, 55°, 65°, and 75° C to evaluate the impact of temperature on volatility.

Protein-Flavor Time Study

Whey protein solutions containing 2, 6, and 10% protein containing 600 ng/2mL 2, 4-Dimethylbenzaldehyde were analyzed at 75° C for 0, 2, 4, 6, 8, 12, 24, and 48 hr. This study was done to see if volatility was influenced by contact time with the whey protein.

Binding/Release Study

Ethyl butyrate and 2,4-Dimethylbenzaldehyde, at a concentration of 600 ng/2mL, were held for 30 min equilibration time at temperatures 45°, 55°, 65° and 75° C in 0, 2, 4, and 6% WPI.

Sensory Analysis

Samples

The food-grade flavorants utilized were 2, 4-Dimethylbenzaldehyde and Ethyl butyrate (Aldrich, Milwaukee, WI). Whey protein solutions and flavor solutions were prepared using distilled

water by Culligan (Goldsboro, NC). The samples were presented in disposable 7-mL scintillation vials and new 50-mL Erlenmeyer flasks with stoppers (Kimax/Fisher, Atlanta, GA).

A series of whey protein isolate solutions were prepared containing 0, 2, 4, and 6% protein by mixing for 30 min in approximately 400 mL of distilled water. The protein solution was transferred to a 500-mL volumetric flask and mixed, without bringing the solution up to volume. The mixed solution was divided equally, using a graduated cylinder, and poured into two separate 250-mL volumetric flasks. One flask was to be used for the morning analysis and the other refrigerated for use in the afternoon analysis.

The flavored stock solution was prepared in distilled water at a concentration of 600 ppm (0.6 g/L), and the appropriate volume was added via glass pipette to each protein concentration that were then filled to volume prior to analysis. The concentration of each of the protein solutions was 20 ppm of the individual flavorant.

Training

Panelists were introduced to the flavorants in two phases. Phase one introduced the panelists to the aroma and taste of 1) water and 2) 6% protein, with and without flavorant. These samples were introduced to give panelists an idea of the range of intensities they would encounter and provided anchoring for the ten-point scale utilized in evaluating the flavorants (Fig. 1). The second phase consisted of re-establishing the

anchors for the flavorant using the references and introducing four unknown samples. Two samples were analyzed for flavor and aroma and the entire group was calibrated based on the central tendency of the overall scores. This calibration was introduced in an effort to reduce the number of outliers caused by differences in taste and aroma sensitivity of the panelists. The two phases consisted of two days during the original training and was condensed to one day prior to evaluating the second flavorant.

Flavor

Three reference samples, consisting of water with flavor, water without flavor, and protein without flavor were presented to panelists at each testing. During training, references were given preset intensity ratings of 9, 0, and 0, respectively, and were used to anchor the 10-point scale (0 to 9) used for the descriptive panel analysis. At each session, panelists received five samples to score, one of each of the four protein concentrations (0, 2, 4, and 6%) and a blind sample, which duplicated one of the other four samples. The eight samples, including the three references were pipetted into disposable 7-mL scintillation vials and covered with aluminum foil. Panelists were instructed to evaluate the samples listed on the score sheet. After swishing for 3 – 5 sec in the mouth, the flavor intensity was scored from 0 to 9, indicated by circling the appropriate number. The tasters took a bite of unsalted cracker and a sip of water before evaluating the next sample.

Aroma

References and samples for the aroma analysis were prepared from the same flavored protein solutions as described above and presented in random order using random 3-digit numbers. The 20-mL samples were measured in a graduated cylinder and poured into 50-mL Erlenmeyer flasks and capped. These flasks were also covered with aluminum foil to eliminate the opportunity for visual evaluation. At each session, the panelist was presented with 8 samples. The three reference samples that were used for taste, the 0, 2, 4, and 6% protein-flavor solutions, and a blind sample, which was a random duplicate of one of the protein-flavor solutions.

Panelists were asked to swirl the flask 5 times, take 2 - 3 short sniffs, cap the flask, and score the sample. Some options used if needed to clear the nostrils were to smell the reference of distilled water without flavor or to sniff the back of their unperfumed hand. Re-evaluation of the aroma samples was allowed after a waiting period of 2 min.

Experimental Design

Statistical analysis of the data utilized the SAS program (Cary, NC) and included analytical and sensory data. The analytical data presented a full factorial model and involved two experimental factors, temperature and protein concentration. There were four levels of temperature (45°, 55°, 65°, and 75°C) and four levels of protein concentration (0, 2, 4, and 6%). The sensory data involved a complete randomized block design, utilizing

protein concentration (0, 2, 4, and 6%) as an experimental factor and 10 judges as a block, averaging over 3 days.

Results and Discussion

The goal of the methods development section of the research was to develop a method that would establish parameters for 2,4-Dimethylbenzaldehyde (DMB) that resulted in optimum reproducibility of volatility.

Table 1 illustrates the carry over problem with volatility of DMB at 45, 55, 65, and 75°C. The boiling point of DMB is 212°C and adsorption of the compound on the syringe needle was expected. Running water blanks between samples, the cleaning procedure, greatly improved reproducibility. O'Keefe and others (1991) reported a carryover of 10% of the initial peak area after a blank injection of air followed nonanone (b.p. 190°C). The DMB (b.p. 212°C) flavor experienced a carryover of 32% to 63%, decreasing with an increase in temperature.

Concentration Study

The next variable that was analyzed for reproducibility was the concentration of DMB at 45°C and 75°C (Fig. 2). The concentration of 600 ng per 2 mL of DMB was chosen as the concentration to be used in all future tests because the volatility showed the greatest repeatability at both 45º and 75ºC.

Equilibration Time Study

A study was done evaluating the influence of equilibration time (Fig. 3) on optimum reproducibility of volatility for DMB. The equilibration time is the time the flavor ligand is allowed to interact with the medium in a

sealed vessel at a given temperature. The flavor partitions itself between the medium and air phases and remains constant at equilibrium. Fig. 3 shows the volatility of duplicate samples of DMB at 45°C and 75°C for equilibration times of 15, 30, 45, and 60 min. Optimum reproducibility was achieved at 30 min equilibration time. A search of the literature finds similar equilibration times used for some headspace gas chromatography research. Such research includes Schirle-Keller and others (1994) who analyzed aldehydes, ketones, and sulfur compounds after equilibrating at 60°C for 40 min. Andriot and others (2000), established equilibrium at 60°C for 15 min for methyl ketones.

Temperature Study

Fig. 4 illustrates how changes in temperature affected volatility of DMB. There was an expected increase in volatility with increase in temperature from 45°C to 75°C for the samples analyzed in triplicate. The main concern, however, was the increase in variability at temperatures 65°C and 75°C. One possible source of error could be leaks around the crimped seals of the vials that might occur as pressure increases inside the vial at higher temperatures. As the temperature increased (45, 55, 65, and 75°C) repeatability decreased. The two lower temperatures were optimal for reproducibility.

Protein-Flavor Time Study

Whey protein isolate solutions (2, 6, 10%) with DMB were evaluated for any changes in volatility due to the time the flavor ligand was

in contact with the protein. Over a 48-hr period there was little variation in volatility, and this is illustrated in Figure 5. As anticipated, there was a decreased in volatility, from approximately 5 to less than 2, as protein increased from 2 to 10 percent.

The results of these preliminary tests were evaluated to establish parameters for flavor concentration, equilibration time, and temperature for any flavor compounds studied. The compound DMB was utilized for the methods development stage because of its high boiling point (low volatility). By establishing parameters with a low volatility compound, it was assumed that the parameters would be sufficient to analyze relatively higher volatility compounds such as Ethyl butyrate (EB).

The standard concentration that was utilized in the following analyses was 600 ng per 2 mL sample and the standard equilibration time was 30 min. The temperatures were optimal for analysis at 45°C and 55°C; however, all temperatures (45°C, 55°C, 65°C, 75°C) were used in order to determine if any trends that developed changed as temperature increased. These temperatures represent stages of protein denaturation.

Binding/Release Study

The measurement of volatility of DMB using headspace gas chromatography revealed that temperature, protein concentration, and the temperature*protein concentration interaction (Fig. 6) were all highly significant ($p<0.0001$). Mean volatility increased with increasing temperature and decreased with increasing protein concentration.

Using Tukey's procedure, multiple comparisons were evaluated. An increase in protein concentration from 0 to 2% resulted in a significant decrease in volatility at 45 and 55°C; however, further increases in protein concentration had no influence on volatility. At 65°C, increases from 0 to 2% and 4 to 6% showed significant decreases in volatility. There were significant decreases in volatility at each level of increased protein concentration at 75°C.

The analysis of the sensory data of DMB answered two main questions. They were, if protein concentration had a significant effect on taste and aroma, and if this association corresponded with the trends revealed in the analytical part of the experiment.

The protein concentration effect was highly significant ($p>0.0001$) when analyzing taste intensities of DMB, but there was also substantial variability among the judges. The individual and average intensity scores are shown in Table 2. Subsequent pairwise comparisons among the four protein means were statistically significant except for the one involving the two highest levels of protein.

The analysis of aroma intensities (data shown in Table 2) revealed that protein concentration effect was highly significant ($p<0.0001$), and the variability among judges was not ($p>0.05$) statistically different. The mean aroma measurement at 0% protein was significantly different from 2% protein, but further increases in protein concentration were not significantly different.

Temperature was taken into account when comparing sensory and analytical findings. Aroma intensities were observed at ~ 25°C (room temperature), taste intensities were observed at ~37°C (body temperature), and the analytical samples were run at 45, 55, 65, and 75°C. The analytical samples at 45°C were compared to the taste and aroma samples.

The 45°C samples of protein showed a significant decrease in volatility of protein was increased from 0 to 2% protein. The aroma intensities were also found to decrease as protein concentrations were increased from 0 to 2% protein. Statistically significant decreases in taste intensity were seen as protein level increased from 0 to 2% and 2 to 4%. The aroma scores and the headspace concentrations were more in agreement than taste and headspace. The taste samples seemed more sensitive (more statistical differences); however, there was a statistical difference among the judges that was not found in aroma. This may be due to difficulty in ignoring whey flavor increase as protein increased from 0 to 6%.

Analysis for the results of Ethyl butyrate using headspace gas chromatography used log-transformed data to accomplish homogeneity of variance that was not present using the normal data. This was not necessary for the DMB data. Analysis of the data showed that volatility was temperature ($p<0.0001$) and protein concentration ($p<0.01$) dependent (Fig. 7). There was no significant temperature*protein

concentration interaction. Subsequent comparisons revealed that all temperature means differed significantly and there was an incomplete separation among the four % protein means where 0,2,and 4% protein were not significantly different and 4 and 6% were not significantly different.

The protein concentration effect was significant ($p=0.015$) when analyzing taste intensities of Ethyl butyrate and the variability among judges was not statistically different (Table 3). The pairwise comparisons among the four protein means revealed that while increasing protein concentration, taste did not differ significantly ($p>0.05$), with the exception of the comparison of 0 to 6% protein, where a significant difference was seen.

The aroma intensity data (Table 3) showed that volatility was influenced by protein concentration ($p<0.0001$) and the variability among judges was not. All of the comparisons of mean aroma by protein level were significant except when comparing 2 and 4%.

Volatility, aroma intensities, and taste intensities were compared for Ethyl butyrate (EB). Comparisons of successive increases from 0 to 6% showed no significant ($p>0.05$) differences in volatility for 0, 2, and 4% protein and between 4 and 6% protein. Aroma intensity samples showed no significant ($p>0.05$) differences in volatility as protein was increased from 2 to 4%. Panelists were able to distinguish the upper and lower limits of the aroma samples. Taste intensity samples showed no significant

($p>0.05$) differences for successive increases from 2 to 6%. Panelists were not able to distinguish differences as protein concentration increased, but could distinguish 0% from 6%. Taste intensity agreed with volatility results.

The analytical and sensory findings of the two flavorants offer differences in results. DMB volatility most closely correlated with aroma sensory findings and EB volatility most closely correlated with taste sensory findings.

The two flavorants were found to react differently analytically (Fig. 6 and Fig. 7). DMB showed a decrease in volatility with an increase in protein concentration that was significant. EB, although statistically found to be different due to protein, had a small mean square error value (0.006) which resulted in small changes in volatility being statistically different. Figure 6 illustrates how small these changes were when comparing an increase in protein concentration at the same temperature.

Table 4 shows the relative vapor pressure values of the two flavorants with increasing protein concentration at each temperature. The flavorant DMB shows a strong interaction with whey protein; however, EB does not, with rvp values remaining close to 1 even at 6% protein. One explanation offered in the literature is that the hydrophobic binding sites in the whey protein have little or no affinity for the ester (Kinsella and Damodaran, 1980). The volatility of EB is the same with or without protein.

A comparison of the physical differences between DMB and EB may offer an explanation to these findings. EB is an ester that is slightly soluble in water and has a boiling point of 120°C (Reineccius, 1994). The flavorant DMB is an aromatic aldehyde that is insoluble in water and has a boiling point range of 212 - 215°C (Reineccius, 1994). The results (Fig. 6 and Fig. 7) showed that the volatility of both compounds increased with temperature, and that EB was more volatile than DMB.

The difference in boiling point predicts that EB should be relatively more volatile than DMB. The difference in solubility suggests that DMB is relatively less polar than EB. The matrices and temperatures that the flavorants were subject to were the same. Kinsella and Damodaran (1980) reported that the binding of a flavor ligand is due mostly to hydrophobic interactions.

The results show that the volatility of both compounds increases with temperature, and that EB is more volatile than DMB. The two flavorants also interacted with protein differently. EB showed small changes in volatility due to increasing protein concentration. The flavorant DMB, however, showed definite decreases in volatility.

The increase in protein concentration causes the matrix to become relatively less polar than water. This fact would increase the solubility of the flavorants because they are both relatively nonpolar, and should cause the flavorants to decrease in volatility. This was not so with EB.

The increase in temperature would cause the proteins to unfold, exposing all hydrophobic regions and possible binding sites. This should also cause a decrease in volatility as protein concentration is increased. The expectation was that as the protein became more concentrated there would be more binding sites available.

Ebeler and others (1988) studied the influence of protein concentration on headspace concentration and aroma intensity of isoamyl acetate and menthone. Protein decreased both intensity and headspace concentration. In 1992, Schirle-Keller and others showed that nonpolar compounds, including aldehydes and esters, exhibited rvp values of 0.6 - 0.8 in whey protein based fat replacer systems, indicating interactions. These interactions were most similar to the oil system than any of the other fat replacers analyzed.

Hansen and Heinis (1992) found a decrease in flavor intensity for benzaldehyde with an increase in whey protein concentrate. Schirle-Keller and others (1994) found aldehydes to be reactive with protein-based fat replacers, and had very similar vapor phase concentrations as fat-containing systems, suggesting possible similar flavor profiles.

Conclusions

An analytical method, suitable for evaluation of volatility of flavorants in whey protein isolate was developed. This method was utilized to evaluate the influence of protein concentration and temperature on volatility and these data were compared to sensory taste and aroma. DMB analytical samples of volatility were highly correlated with the aroma sensory samples. EB analytical samples of volatility were highly correlated with taste sensory samples.

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Table 1. Comparison of reproducibility of volatility between initial and cleaning method with duplicate samples of 2,4-Dimethylbenzaldehyde.

Temp. (C)	Initial Volatility	Cleaning Method Volatility
45	0.77	1.28
45a	2.26	1.36
55	1.76	2.73
55a	3.77	2.38
65	4.48	3.64
65a	6.14	3.67
75	4.89	6.33
75a	8.08	6.81

[a] **'a' at the end of temperature represents the duplicate sample at at the same temperature.**

Table 2. Sensory data for 2,4-Dimethylbenzaldehyde.[a]

Taste

Judge	0%	2%	4%	6%
1	6	5	2.3	4.3
2	2.3	3.3	0.7	3
3	7.7	6	5.3	4
4	7	4.7	3.7	4.3
5	7.3	6	5	4.7
6	7.7	6.3	3.3	1.7
7	8	6.7	1.3	3
8	6.3	2	1.3	3
9	8	4.3	4.3	0.3
10	6.7	4	1.7	4
Average	6.7	4.8	2.9	3.2

Aroma

Judge	0%	2%	4%	6%
1	7.3	4	4	3.3
2	8	3	4	3.3
3	8.8	4.7	3	1.3
4	5.7	4.3	2	4.3
5	4.7	3	2	1.3
6	7	3.3	3.3	1
7	5.7	7	3.3	5.7
8	7	4	3.7	6
9	6.7	4.7	4	4
10	9	3.3	6	2
Average	7	4.1	3.5	3.2

[a] **Each number represents an average of three intensity scores ranging from 0 to 9.**

Table 3. Sensory data for Ethyl butyrate.[a]

	Judge	0%	2%	4%	6%
Taste	1	7.3	4.7	3	2.3
	2	7.7	5	6.3	4.3
	3	7.7	4.3	3.7	3.7
	4	7	5.7	5	2.7
	5	2.7	4.3	6	5.3
	6	8	6.7	3.7	2
	7	6.3	6.3	5	2.7
	8	6.3	4.3	4	2.7
	9	1.7	4.3	4	5.7
	10	6	4.3	2.3	5
Average		6.1	5	4.3	3.6

	Judge	0%	2%	4%	6%
Aroma	1	8	5.3	6.3	2.3
	2	7.3	5.7	4.3	3.7
	3	7.3	5.7	4.3	3.3
	4	6.3	4.3	3.3	3.3
	5	6.3	6.3	4	2.7
	6	7.7	3.7	4	2.7
	7	6	4	6	2.7
	8	5.3	2.7	4	3
	9	8	5	5	3
	10	6.7	5	3.3	1.7
Average		6.9	4.8	4.5	2.8

[a] Each number represents an average of 3 intensity scores ranging from 0 to 9.

Table 4.
Relative Vapor Pressure of Ethyl butyrate and 2,4-Dimethylbenzaldehyde.

Ethyl Butyrate

%Protein	45C	55C	65C	75C
0	1	1	1	1
2	0.9	0.97	1	1
4	0.86	0.88	0.97	1
6	0.8	0.88	0.91	1

2,4-Dimethylbenzaldehyde

%Protein	45C	55C	65C	75C
0	1	1	1	1
2	0.33	0.22	0.35	0.6
4	0.2	0.13	0.23	0.36
6	0.18	0.07	0.1	0.25

Fig. 1. Ten point scale used for aroma/taste evaluation.

Sensory Evaluation Score Sheet

Name ________________

Taste Aroma

Sample#

										Max Intensity
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9
______	0	1	2	3	4	5	6	7	8	9

Fig. 2 The Effects of Concentration Variation on Volatility of 2,4-Dimethylbenzaldehyde

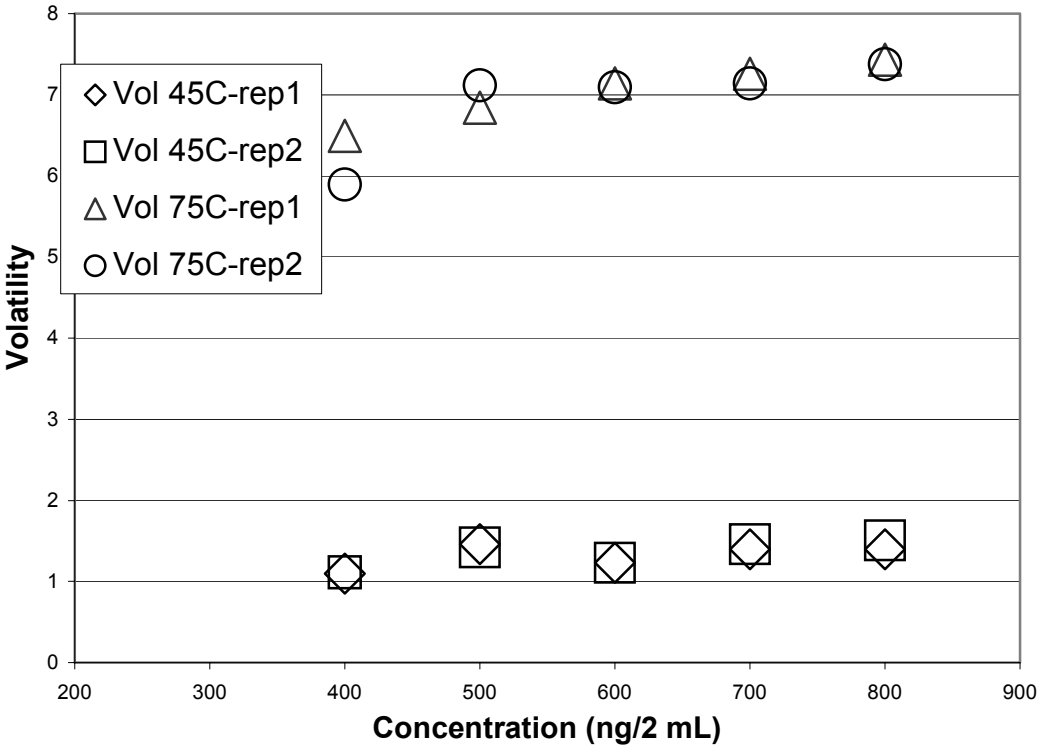

Fig. 3 Equilibration Time Study on Volatility of 2,4-Dimethylbenzaldehyde

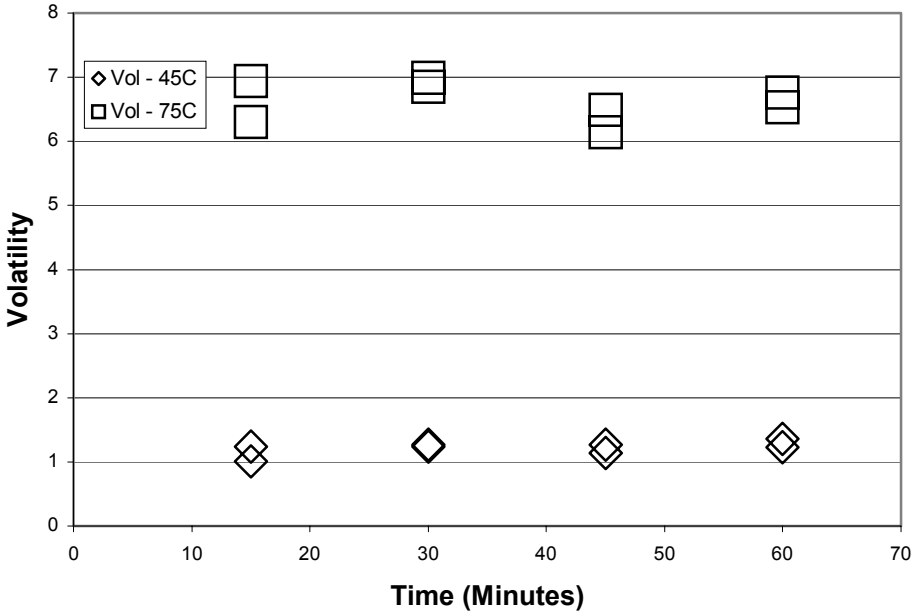

Fig. 4 Variation of Temperature on Volatility of 2,4-Dimethylbenzaldehyde

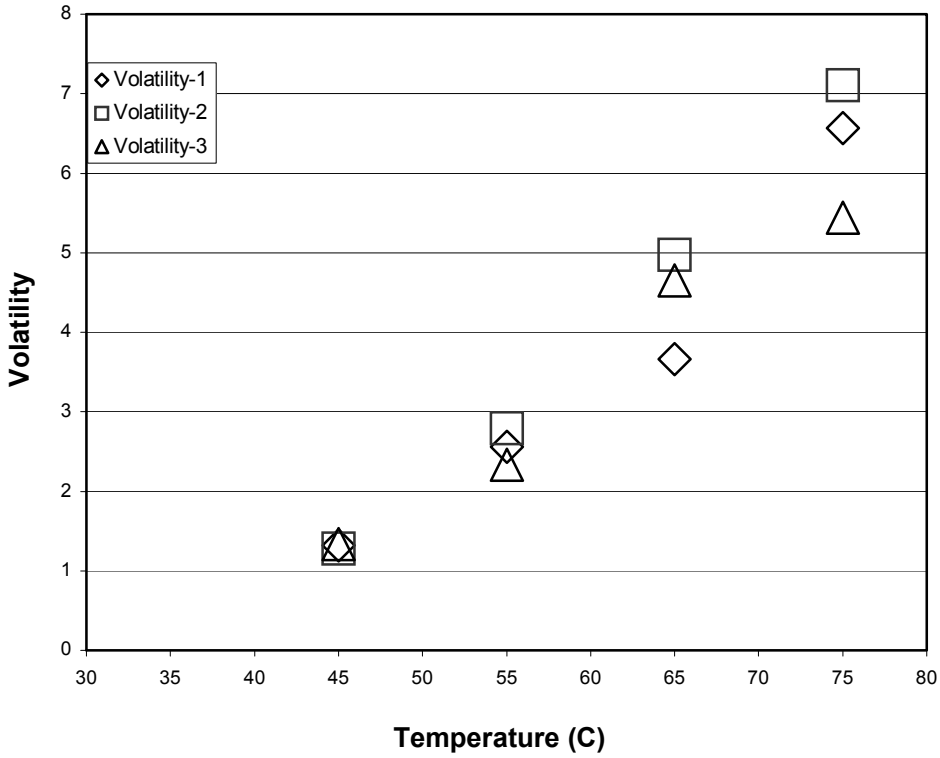

Fig. 5 Time Effect Study of Volatility on 2,4-Dimethylbenzaldehyde in Whey Protein Solution

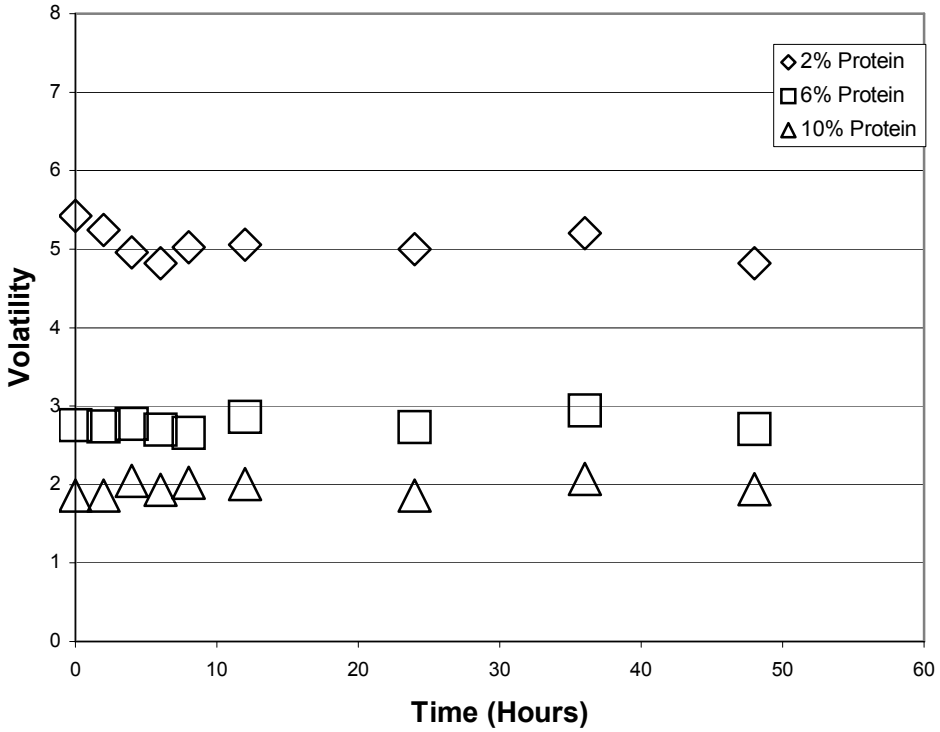

Fig. 6 Volatility Study of 2,4-Dimethylbenzaldehyde

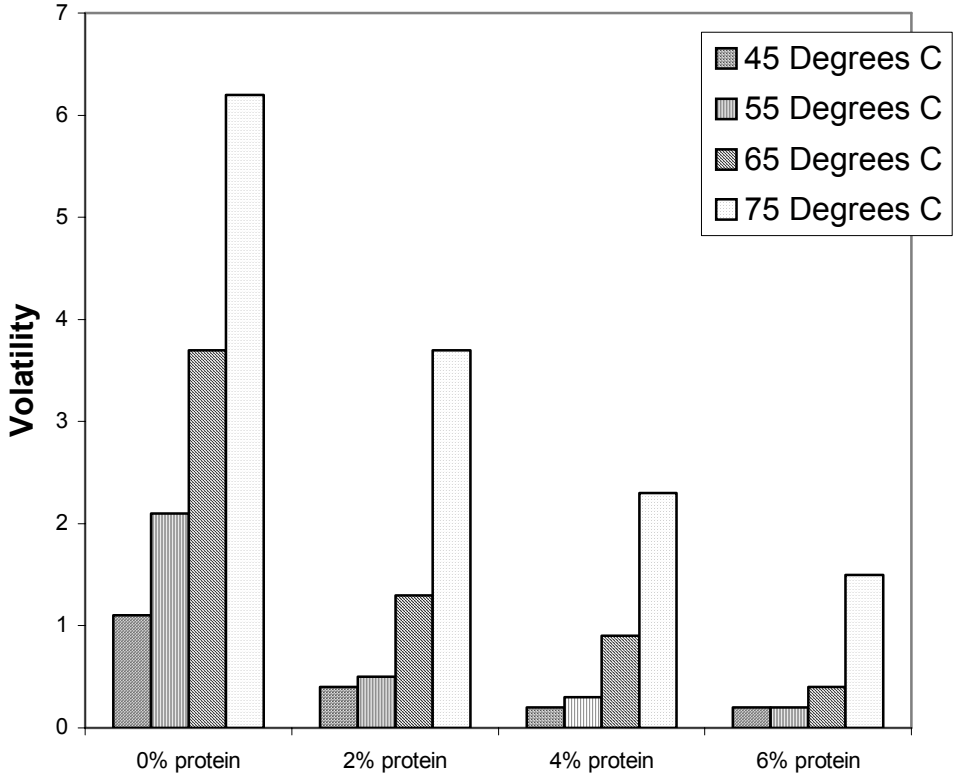

Fig.7 Volatility Study of Ethyl Butyrate