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Binding of flavor compounds in tofu

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DEDICATION

This study is dedicated in the memory of my father, Edward H. Kaan. His high value of education and constant quest for knowledge have given inspiration and motivation to me. To him learning was not something that took place just in the early years, but should continue throughout one's life.

INTRODUCTION

Tofu is the name used in the Orient for soybean curd. Tofu is made much like cheese, by pressing whey from curds formed by coagulating milk, in this case, soymilk. It has long been a traditional part of the diet in many oriental countries like Japan. However, in the United States, it is rather a new item on the grocery shelf. Upon tasting tofu for the first time, many find that it has an objectionable flavor. This same sort of experience happened years ago to first-time consumers of plain yogurt. Just as the yogurt sales increased once flavored yogurts came on the market, tofu also could benefit from added flavors.

Tofu is a good source of protein and it contains no lactose. This enables it to be used in the diet of people who have a lactose intolerance and cannot drink milk or eat milk products. Also, tofu is a good source of calcium, if it is made with calcium sulfate. Another benefit is that since tofu is made from a plant source, soybeans, it contains no cholesterol. Therefore it can be safely used in cholesterol-restricted diets. For these reasons, the addition of tofu to the diet of many people can be quite beneficial.

If flavors are to be added to tofu to enhance its appeal to consumers, a number of questions arise. One,

during the preparation of tofu, when should the flavors be added to give the best distribution and retention of the flavor? Secondly, which type of flavor compounds will be retained within tofu? For example, would an alcohol bind more easily than an aldehyde? In this study, we investigated the interaction or binding of flavor compounds in the tofu food system. Flavor compounds with different functional groups, different chain lengths and different molecular shapes were studied.

LITERATURE REVIEW

Soybean production in the United States has been increasing. In 1970, 40 to 50 million metric tons of soybeans were produced. In 1980, 81.6 million metric tons were produced, resulting in $8.6 billion in sales of soybeans and soybean products. In comparison to other crops, 30% of U.S. crop land in 1980 was devoted to growing soybeans, almost equal to corn at 31.9% and wheat at 32.1% (American Soybean Association, 1981). Much of the U.S. soybean crop is used for soybean oil production (Smith et al., 1960), although a small but increasing percentage is used for soybean food products. Tofu (soybean curd), soymilk and soy products brought in $150 million in sales during 1986 (Anon, 1987a). Consumption of soy products, specifically tofu, could increase if the flavor was improved.

A comparison can be drawn to the increase in sales of yogurt products. Dietary and health concerns of Americans, and the availability of fruit flavored yogurts have greatly enhanced consumer acceptance of this food product (Anon, 1978b). By 1978, sales of yogurt were $500 million and in 1985 yogurt sales topped $1 billion (Anon, 1978a; Anon, 1987a).

Nutritional Value of Tofu

Tofu has a high nutritional value. It contains only 52.6 calories per 100 g, is high in calcium, if made with calcium sulfate, and because it is of plant origin, has no cholesterol (Marson, 1984). Tofu composition differs slightly because of varied preparation techniques and soybean varieties. Fukushima (1980) reported the typical content of tofu as 88% water, 6% protein, and 3.5% oil. According to Wang (1984) the typical oriental tofu is 85% water, 7.5% protein, and 4.3% oil. Shurtleff and Aoyagi (1975) list similar values for tofu composition. But Wang (1984) states that Japanese tofu has 87 to 90% water and Chinese tofu has 50 to 60% water. In a recent U.S. study (Schaefer, 1986), tofu had 85.8% moisture, 7.3% protein and 3.9% oil. The Soyfoods Association of America recently set standards for tofu as follows: soft tofu, 5 to 6.4% protein; regular tofu, 6.5 to 9.9% protein; firm tofu, 10 to 13.9% and extra firm tofu, 14% or more protein (Anon., 1987b). Skim milk has a protein content of 3.3%, plain yogurt 3.5%, cheddar cheese 25%, eggs 13.6%, fish (haddock) 20% and hamburger (10% fat) 26.8% protein (U.S.D.A., 1981). In comparison, tofu can have protein values between 6 and 14%. Soybean curd has also been determined to have a digestibility of 96% (Peng, 1982).

It must be noted, however, that soy protein is low in the sulfur amino acids, methionine and cysteine (Torun et al., 1981). According to Bodwell and Marable (1981) the nutritional value of soy protein was 85 to 95% that of milk or egg protein. It was noted, though, that rat assays do not provide an accurate estimate of the protein nutritive value for humans.

Unheated soybeans contain several anti-nutritional factors such as inhibitors of the enzymes trypsin and chymotrypsin, phytohemagglutinins which bind carbohydrate substances, anti-vitamins and goitrogens. All of these are easily destroyed by heat. A part of tofu preparation includes a heat treatment, approximately 10 to 20 minutes of boiling, which inactivates these anti-nutritional factors (Liener, 1981).

A few other anti-nutritional factors in soybeans are heat stable. The 2-S globulin, an allergen, can cause an allergic reaction in some people. This is the reason some infants cannot drink soymilk. Also not sensitive to heat are the flatulence factors raffinose and stachyose. Humans do not have the enzyme necessary to break these carbohydrates down into absorbable sugars (Liener, 1981).

Phytic acid present in tofu may reduce the availability of certain minerals, especially zinc, from this product. If

meat, a very good source of zinc, is replaced entirely in the diet with a food source having decreased zinc availability, an impaired zinc status could result (Schaefer, 1986).

Worldwide, 90% of calories and 70% of the protein consumed come from plants directly. With an increase in the world's population (6 to 7 billion expected by the year 2000), even more reliance may be placed on plant protein sources (Esen, 1982). Soy protein foods may become increasingly important, despite the limitations mentioned.

Off-Flavors

One limitation has been a major problem in ready acceptance of soy protein for food use. That is the problem of off-flavors in soy foods.

Honig and Rackis (1975) studied soybeans in different stages of maturity to identify the major volatiles present, many of them causing off flavors in soybeans. They found total volatiles to decrease from a maximum of 113 ppm during early maturation to 0.1 ppm at maturity. Methanol, the major constituent, accounted for 30 to 90% of the total volatile compounds. Other compounds were ethanol and ethanal plus small amounts of propanal, acetone, pentane, pentanal and hexanal. Maximum amounts of ethanal correlated

with peak lipoxygenase activity. But, surprisingly, hexanal, a major off-flavor component, exhibited little direct relation to lipoxygenase activity. When soybeans were macerated in water in the presence of air, a large increase in volatiles including hexanal was noted. Honig and Rackis (1975) postulated that the higher level of volatiles reflected an increase in autoxidation during maceration. Hsieh et al. (1981) isolated 25 compounds in defatted soybean flour and concurred that the compounds could be autoxidative decomposition products of soy lipids. 2-pentyl furan and ethyl vinyl ketone were found to be mainly responsible for beany and grassy odors of soy flour. Even though the content of hexanal was low, it was a major component of the "green" flavor, due to its low flavor threshold. Hsieh et al. (1981) also noted that hexanal represented 25% of the volatiles of soybean milk. Sessa and Rackis (1977) also found these volatiles in soy products. They stated oxidative deterioration of the free and esterified unsaturated fatty acids, specifically linoleic and linolenic acids, was primarily responsible for formation of objectionable flavors in legumes. They further suggested that soybean lipoxygenase catalyzed the formation of hydroperoxides from fatty acids. With uptake of oxygen, the hydroperoxides decomposed to volatile and non-volatile constituents.

Ames and Macleod (1984) also found linoleic and linolenic acids as the most common oxidation substrates in textured soy protein (TSP). Present in highest concentration in TSP aroma were aliphatic aldehydes, with hexanal representing 15.5% of the volatiles. It was concluded that aldehydes affect soy flour flavor to a greater extent than other volatiles.

Del Rosario et al. (1984) compared raw soybeans to heated soybeans. In raw soybean volatiles, hexanal was identified, along with 1-hexanol, 1-pentanol, and α-pinene. But acetic acid was the major volatile constituent. After heating, alcohols, esters, terpenoids, and acetic acid decreased while 2-pentyl furan increased. Hexanal exhibited a 15-fold increase upon heating. Pentanal and significant amounts of 1-octen-3-ol (mushroom-like aroma) also were found in heated soybeans.

Some non-volatile constituents of soybeans isolated by Murphy (1981) and Pratt et al. (1982) were the isoflavones genistein, daidzein and glycitein. Huang et al. (1981) stated that these isoflavones, particularly glycitein, which has a herb-like astringency and bitterness, might contribute to objectionable flavor of soy protein products.

The off-flavor compounds mentioned contribute to the characteristic green beany, grassy and somewhat bitter

flavors associated with soy products. To minimize these off-flavors various treatments have been investigated.

Methods to Eliminate Off-Flavors

Various investigators have attempted to improve flavor by grinding unsoaked soybeans with hot water at temperatures between 80 and 100°C for about 10 minutes (Wilkens et al., 1967; Escueta and Banzon, 1979), to inactivate the lipoxygenase enzyme, thought to be responsible for off-flavors. Although this treatment improved the soy products, some off-flavors were still present. Other treatments have used steam to blanch the soybeans (Cowan et al., 1973; Sessa and Rackis, 1977).

Various soaking treatments have also been investigated. One involved the use of a continuous stream of running water to soak dehulled soybeans (Del Rosario and Maldo, 1979). Salts such as NaOH, Na_2CO_3, $NaHCO_3$ plus 15% ethanol (Ashraf and Snyder, 1981); or nontoxic bromate or iodate salts (Chung, 1981) have also been added to the soaking water in an attempt to reduce off-flavors. One experimenter added sodium salts to the soymilk (Bourne et al., 1976).

Solvent extractions using hexane and alcohol were studied to determine if removing some of the fat and also deactivating the lipoxygenase enzyme would further reduce

off-flavors (Cowan et al., 1973; Honig et al., 1979). But not all the solvent could be easily removed from the soy products and both experimenters added a "toasting" process to help drive off the solvents. However a review by Warner et al. (1983) indicated that large amounts of residual solvents were left in soy products treated in this manner and that the solvents contributed to poor flavor quality.

Another treatment that has been evaluated was an NAD+/- regenerating system, consisting of aldehyde dehydrogenase and diaphorase. Because green bean flavor is caused mainly by aldehydes like hexanal, which have very low threshold levels, this treatment would oxidize the aldehydes to acids which have higher threshold levels and thus help to improve the flavor (Takahashi et al., 1980). Proteolytic enzymes such as papain, bromelin, and pepsin have been used to reduce off-flavors, with limited success (Fujimaki et al., 1968). A recent patent sought to reduce oxidative off-flavors by heating soybeans in a vacuum (Gupta et al., 1986).

All of these treatments have reduced off-flavors to some extent. A few treatments, such as the vacuum heating process by Gupta et al. (1986), have produced soy products nearly free of off-flavors.

Since soy products are relatively bland when off-flavors are not present, further gains in the acceptability of soy products might be achieved by adding desirable flavor compounds.

Flavored Tofu Products

Many research projects have studied masking off-flavors in soy foods by adding desirable flavors. The Book of Tempeh (Shurtleff and Aoyagi, 1979) mentions the simple additon of salt, to improve tofu flavor, by the Chinese. Chen et al. (1984) noted that a popular snack food in China was semi-dried, spiced soybean curd. The firmest variety of Chinese tofu is boiled in a mixture of soy sauce and seasoning (Tsai et al., 1981).

The addition of coconut cream did not seem to improve the flavor of soymilk (Escueta, 1979; Escueta et al., 1985); however, coconut milk slightly improved the flavor profile of soymilk (Del Rosario and Maldo, 1979). Peanut milk (Nakayama, 1985) and sunflower seed milk (Vijayalakshmi and Vaidehi, 1982) additions did not seem to give an improvement in tofu flavor. A simulated cheese spread has been developed from a tofu base by adding margarine, mayonnaise, flavoring and coloring (Nolan, 1983). Cheese whey, a by product of cheese production, and soymilk were combined to

create a new product "Ohio curd" (Peng, 1982). The product was coagulated with glucono δ-lactone (GDL). With water and heat, the GDL ring opens to become an acid and the drop in pH results in soymilk coagulation (Campbell, 1972). Several patents (Kenkyujo, 1982; Matsuura, 1985; Sugisawa et al., 1985) have been granted which used GDL to produce silken tofu, or tofu in which the whey is retained.

Added sweeteners have also been used in soy products. Researchers have added sucrose and vanilla essence to soymilk samples presented to sensory panelists (Bourne et al., 1976; Blesa et al., 1980). A cultured soymilk beverage, "mil-mil", sweetened with glucose and fructose and colored with carrot juice is sold in shelf-stable, aseptic, decilitre cartons in Japan (Anon., 1979). Low calorie dessert products have been developed in California in which tofu was sweetened with honey. Flavors such as strawberry, almond, or chocolate were added to the sweetened tofu (Anon., 1984).

Fermentation has long been known to improve the flavor of tofu. For example, "Natto" in Japan (Sugawara et al., 1985) and "IBU or biang" in Indonesia (Shurtleff and Aoyagi, 1979) are popular fermented tofu products. Swartz et al. (1985) used yeast fermentation to reduce beany flavors. Hashimoto et al. (1985) used Saccharomyces sake to culture a

fermented tofu. In 1980, research (Patel et al., 1980) was begun on lactic fermentation of soymilk and by 1986 many yogurt-type soy products were on the market. Some were unflavored, but many had flavors (such as strawberry, cherry, blueberry, peach, banana, spiced apple, raspberry, kiwi, orchard fruits, tropical fruits, coffee, chocolate or vanilla-almond) added. Sales of this type of product brought in $1.75 million in 1986 (Anon., 1987a).

Flavor Compounds

The task of adding flavors to foods is a complex one. Which type of flavoring compounds should be added? What level of incorporation should be used for optimum effect? The ease of release of a flavor compound, its vapor pressure, its interaction with components of the medium into which it is to be added, and its reaction to heat can change the character of the flavor compound (Schutte and Van Den Ouweland, 1979).

One must also consider consumer preferences. Natural rather than synthetic materials may be preferred by some consumers. But natural raw materials are, at times, in short supply and usually more expensive. Yet the consumer also prefers the food items which are lower in cost (Woollen, 1981). A great many of the flavor compounds used

in foods are "nature-identical", which means chemically they are identical to the ones found in foods (Dixon, 1981). They are commonly used and their use may increase in view of the limited availability of natural raw materials.

Flavor has been defined as "the sum of those characteristics of a material taken into the mouth, perceived principally by the senses of taste and smell, and also by the general pain, tactile and temperature receptors in the mouth, as received and interpreted by the brain" (Teranishi et al., 1971). Thus each food releases a mixture of volatile compounds to form a distinctive flavor. The aroma of a food is very important to flavor perception. The role of aroma components in flavor becomes apparent when one catches a cold (Heath and Reineccius, 1986).

The total amount of flavor compounds in foods is very small. The quantity of flavor material in a natural raw food ranges from 100 parts per million (ppm) to only a few ppm. For example, bananas have 12 to 18 ppm of flavor volatiles, raspberries 2 to 5 ppm, strawberries 2 to 8 ppm, tomatoes 3 to 5 ppm, beef 30 to 40 ppm and cocoa around 100 ppm. In a prepared food, the concentration of flavor compounds may be only a few parts per billion (ppb) (Emberger, 1985).

Sensory characteristics of flavor compounds

Aldehydes, alcohols, ketones and esters have been identified as important volatile flavor compounds in fruits. Hexanal, for example, is a large contributor to banana flavor (Eskin, 1979). According to Heath and Reineccius (1986), each of these types of flavor compounds have varying sensory characters. Lower molecular weight aldehydes have an unpleasant odor. Higher ones have a pleasing fruity character. Dilution also plays a role in determining the flavor sensation imparted by a chemical. Aldehydes with 8 to 10 carbons,though bitter at high concentrations, become floral upon dilution. Alcohols are among the most important of flavoring materials and are extensively found in nature. Lower molecular weight alcohols have a sweet odor, while ones with higher molecular weights are unpleasant. Higher molecular weight ketones, starting with C-7, are widely used in imitation flavorings. As the carbon number increases, the fruity odor changes to a floral note. Esters vary in their characters. Each one must be considered individually; however, overall they have a fruity note. Most important of all in using flavors in foods is the realization that a flavor compound may change not only with dilution or concentration, but also when in combination with other compounds and when incorporated into a matrix such as tofu.

Levels of incorporation

Heath and Reineccius (1986) suggest levels of flavors that should be used in sensory evaluation. Natural flavors should be tested at 0.5 to 1% concentration, liquid artificial flavors at 10 to 50 ppm and dried artificial flavors at 50 to 100 ppm.

Also important in choosing a proper concentration level is knowing the threshold level of each compound, or the level at which it can be detected by 50% of the population. Fazzalari (1978) has compiled listings of odor and taste threshold data for the American Society of Testing and Materials. For the flavor compounds used in this study, the following values were given. Benzaldehyde has a taste threshold in water of approximately 2 ppm. Pentanal has a taste threshold in water of 7×10^{-1} ppm and in milk 1.3×10^{-1} ppm; hexanal 2.5×10^{-1} ppm in water and 5×10^{-2} ppm in milk; heptanal 1.2×10^{-1} ppm in milk. Taste thresholds were not listed for hexanol and 3-hexanone.

Another consideration is the air-water partition coefficients of flavor compounds (Buttery et al., 1969). This value is proportional to the volatility of a flavor compound, and indicates how readily it is released into the air surrounding a food. Esters have the greatest air-water partition coefficients followed by aldehydes, ketones and

finally alcohols which have the lowest coefficient values. Buttery et al. (1969) also noted that as the carbon chain length increased, the air-water partition coefficient values rose.

Methods of incorporation

Heath and Reineccius (1986) mention several ways to incorporate flavorings into a food. Flavors may be blended into the product during preparation or bulk mixed after preparation of the food has been completed. Another method is to allow the consumer to mix in the flavorings, for example stirring the fruit flavorings on the bottom of the container into yogurt, or mixing a packaged spice blend into the product before heating.

Tofu Preparation

History of tofu preparation

The preparation of tofu, or soybean curd, was first recorded more than 2,000 years ago by Liu An (179 to 122 BC), a Chinese king of the Hsi-Han dynasty. It was introduced into Japan in 1183 and then into other Asian countries such as Taiwan (Tsai et al., 1981; Wang and Hesseltine, 1982). Between summer and autumn, soybeans were washed, soaked and ground in water, then boiled and filtered to produce soymilk. Bittern (nigari in Japanese), a bitter liquor that remains after salt is crystallized from seawater, was used to coagulate the soymilk. The resulting soft curds were ladled into press boxes where most of the whey was removed. The longer the soy curds were pressed, the harder the tofu became (Yung-Shung, 1981; Wang and Hesseltine, 1982). In general, the Chinese preferred a harder tofu which had more of the whey pressed out and the Japanese a softer tofu (Wang, 1984).

Today tofu is prepared in much the same way with only slight variations in this method to help standardize results. Commercially, $CaSO_4$, or gypsum, is usually used to coagulate soymilk (Wang and Hesseltine, 1982). To produce silken tofu, some manufacturers use glucono δ-lactone (GDL) which coagulates soymilk when it is heated, by forming an

acid (Fukushima, 1981). For home preparation any acid such as lemon juice or vinegar can be used to coagulate soymilk (Shurtleff and Aoyagi, 1979).

Effects of soybean varieties on tofu

When Japanese and U.S. soybean varieties were compared, a higher protein content was found in the Japanese varieties by Smith et al. (1960). However when varieties were grown in the same environmental conditions, differences were not attributable to country of origin. It was found that if a soybean variety was high in protein, then the resultant tofu would be higher in protein and lower in oil content. Varieties with a light hilum and high protein content were preferred for making tofu (Wang et al., 1983). Johnson (1984) also found that different varieties of soybeans produced tofus with different compositions.

7S and 11S proteins

The major protein components of soybeans are 2S, 7S, 11S and 15S globulins. 2S and 15S globulins represent a smaller portion of the protein content, 15% and 9.1% respectively, while 7S and 11S globulins represent the larger portion of the protein content, 34% and 41.9% respectively (Fukushima, 1980).

When gels from 7S and 11S components were studied, it was determined that 11S formed a harder gel than 7S (Saio, 1981). Whereas 7S gels involve mostly hydrogen bonding, 11S globulin gels involve electrostatic interactions and, most importantly, strong disulphide bonds (Saio et al., 1971; Utsumi and Kinsella, 1985). Microscopic pictures of tofu show a network of protein granules in a honeycomb-like structure with coalesced oil droplets (Saio, 1981). When viewed microscopically, gels made from the 11S protein fraction showed that protein was aggregated into lumps and in 7S gels the protein was more dispersed. This finding indicates that a tighter bonding system is present in 11S protein gels (Saio et al., 1969). Further, it was found that tofu from soybean varieties higher in the 11S protein component was somewhat harder. Wang et al. (1983) and Johnson (1984), however, indicated that tofu processing conditions could affect tofu quality more than varietal differences.

Soaking the soybeans

Lo et al. (1968a) found soaking to decrease the protein content of the resulting soymilk produced. Soaking 8 hours, however, facilitated grinding of the beans, giving a better suspension of bean solids during extraction (Lo et al., 1968b). Park et al. (1985) found curd yield increased from

45% to 55.4% when soaking time was increased from 5 to 24 hours. Wang (1984) found that a soak time of 16 to 18 hours at 20 to 22°C was suitable to hydrate the soybeans. Also 20 to 40% of the oligosaccharides, which are the undesirable gas forming factors, leached out into the soak water.

Boiling treatment

As mentioned previously, a boiling time of 10 to 20 minutes is sufficient to inactivate anti-nutritional factors in soymilk. Okada et al. (1980) also found heating for 15 minutes helped to reduce the beany flavor, but longer periods resulted in degradation of free amino acids and development of off-flavors.

As the ground soybeans are heated, the proteins become denatured, which is important to obtain proper curd formation. Heating longer than 20 minutes, however, will reduce the solids recovery and thus the total tofu yield (Wang, 1984).

Factors in coagulation

Uniform tofu products made from the same lot or variety of soybeans can be produced by utilizing a selected set of conditions (Wang and Hesseltine, 1982).

Temperature Schaefer (1986) studied coagulation temperatures for soymilk of 70, 80 and 90°C. As the

temperature increased, the amount of moisture retained in the tofu decreased and the hardness increased. Yet the amount of protein in the whey did not differ significantly. Saio (1981) stated that at higher temperatures the 11S proteins were more involved in network bonding, thus resulting in an increased hardness of the tofu.

A variety of coagulation temperatures have been used in research studies. Wang and Hesseltine (1982) used 70°C; Yung-Shung (1981) recommended 75 to 85°C; Fukushima (1981) used 75°C and Saio (1979) used 90°C. The desired hardness of the tofu must be considered when choosing a coagulation temperature.

Stirring Once the soymilk and coagulant have been poured into the same container, some mixing may be desirable, but it has been noted that this is a critical step in determining the hardness of the tofu produced. As mixing is increased, the volume of tofu and moisture retention decreases and the hardness of tofu increases (Saio, 1979; Wang, 1984).

Coagulant Wang and Hesseltine (1982) reviewed four different kinds of coagulants: $CaSO_4$, $CaCl_2$, $MgSO_4$ and $MgCl_2$. They indicated that of all the coagulants $CaSO_4$ resulted in the greatest weight of tofu, due to higher moisture and solids content. Concentrations of 0.02 to 0.04

M gave the highest nitrogen recovery for all coagulants, with $CaSO_4$ giving the highest values and 0.02 M the highest nitrogen recovery for $CaSO_4$. The effect on yield and nitrogen recovery may be the reason $CaSO_4$ is preferred in commercial production.

Coagulation Unlike gelation, which results in an ordered continuous network of molecules, coagulation involves a random aggregation of denatured molecules (Hermansson, 1979). Heating unfolds the soybean protein molecules exposing their disulfide and hydrophobic amino acid side chains. When calcium sulfate is added to soymilk, coagulation of the protein occurs. Coagulation is due to the decreased negative charge on the protein as a result of the formation of calcium ion bridges between negatively charged acidic amino acid residues of the protein molecules. After calcium addition, the unfolded molecules aggregate owing to the decrease of electrostatic repulsion, then form an irreversible coagulate. Acids such as gluconic acid, formed by heating glucono δ-lactone, protonate the acidic acid residues and thus reduce the electrostatic repulsion, thereby forming a coagulate (Fukushima, 1980).

Salts It has been noted that added sodium ions can interfere with the coagulation process and possibly increase the amount of coagulant needed (Nakashima and Murakami,

1980). Salts may have a stabilizing effect on the 11S globulin and interfere with calcium ion bond formations with the disulfide amino acid side chains. Salts do not seem to interfere with bonding in 7S gels (Hermansson, 1979; Utsumi and Kinsella, 1985).

Microbiological Concerns

Often a few soybeans in a batch may be purple specked. This discoloration is caused by a fungus, Cerocospora kikuchii, an organism which commonly grows on the seed coat of soybeans. Use of discolored beans may give a slightly red tint to soymilk (Taira et al., 1980).

A potentially more serious problem is microbial growth in commercial tofu. However, if sanitary practices are maintained during tofu production and tofu is properly refrigerated during transport and retail display, it can be relatively free of microorganisms. It must be remembered that tofu is just as good a medium for microbial growth as is cow's milk or cheese (Kovats et al., 1984; Kooij and Boer, 1985; Rehberger et al., 1984).

Headspace Analysis

When we perceive an odor from a flower, baked bread, fresh peaches, or wine, we are smelling the volatile

molecules in the gaseous atmosphere around those items, or their headspace. The nose is known to be more sensitive than the most sensitive instrument available (Teranishi, 1981). For experimental purposes, we can place a food item in a closed system and after a time at a chosen temperature, an equilibrium will be established between the volatile molecules in the vapor phase above the sample and the volatile molecules within the sample itself. Low-boiling volatile compounds, having higher vapor pressures, will be present in larger quantities than high-boiling compounds with lower vapor pressures (Jennings, 1978; Lamparsky, 1985). The concentration of the volatile compounds and the interaction of the volatile compounds with other substances such as protein and lipids present in the sample is also important (Franzen and Kinsella, 1975; Ter Heide, 1985).

Headspace analysis consists of direct sampling of the gaseous mixture surrounding a sample within a closed system, which is in equilibrium, with a gas-tight syringe. The headspace gas sample is immediately injected into a gas chromatograph for separation and quantification of the compounds present (De Pooter et al., 1985; Kolb, 1985).

The advantages of headspace analysis are that it does not disturb the sample, which can be further analyzed, and it is a simple method in which little preparation is needed.

Also accurate quantitative analysis of volatile components is possible. The major limitation is that compounds must have some degree of volatility in order to be detected by the gas chromatograph. Many important volatile compounds are present in only trace amounts in the headspace of a sample (Teranishi et al., 1971; Heath, 1981; Bassette, 1984; Ter Heide, 1985).

Experimental conditions for headspace analysis

Heath (1981) describes the general conditions necessary for headspace analysis recommended by the International Organization of the Flavour Industry (IOFI). A constant temperature heating bath, headspace flasks with inert septa and seals, gas-tight syringes and a gas chromatograph with flame ionization or other suitable detector should be used.

To prepare a headspace gas sample, a headspace flask is filled to 1/3 of its volume with a prepared sample. The flask is closed with an air-tight septum and seal. Then the flask is immersed in a constant-temperature water bath up to the neck of the flask. After equilibration, the needle of the glass syringe is inserted into the flask through its septum and the piston is moved slowly up and down a few times. The syringe is slowly filled with the gas phase above the sample, then the needle is removed from the vial and the syringe is adjusted to a specified volume. The

sample is immediately injected into a gas chromatograph which has been previously brought to specified conditions.

To quantify a particular compound from the resulting gas chromatographic peak area value, either an internal or external standard can be used. If an external standard is used, calibration curves must be run under identical conditions, including using a similar matrix for the calibration standard. It is best if both sample and calibration standard can be run at the same time (Heath, 1981; Bassette, 1984).

A relatively new method of quantification of a compound is called multiple headspace extraction. It is thought to eliminate matrix effects. Multiple injections are made until all volatiles in the headspace flask are gone. The sum of the areas for a particular volatile compound is proportional to the total amount present in the flask in both phases, the gas phase and the sample itself. It must be noted that the compound studied must have a high volatility (Hiltunen et al., 1985; Kolb, 1985).

With a sample in which the compounds present are unknown, Kovats values can be helpful in identification (Jennings and Shibamoto, 1980). Another very useful tool in flavor compound identification has been the combined gas chromatograph-mass spectrometer. NMR (nuclear magnetic

resonance spectrometry) and IR (infrared spectrometry) are also used in compound identification (Teranishi et al., 1971; Heath, 1981; De Pooter et al. 1985).

Problems

Glass syringes When glass syringes are used, some limitations must be noted. Buttery et al. (1969) found a small amount of adsorption of compounds to the glass surfaces. For propanal and hexanal, they found the degree of adsorption was negligible, less than 2%. However they calculated that for higher boiling point organic compounds a higher degree of adsorption might occur. Nonanal was calculated to have 80% adsorption. Wyllie et al. (1978) felt by filling and emptying the needle while taking a headspace gas sample, adsorptive demands would be satisfied. Franzen and Kinsella (1975) suggested adsorption could be eliminated by coating syringes and containers with teflon, silane or other inert materials.

Kolb (1985) felt in addition to adsorption problems, that a small undefined amount of headspace sample might be lost by expansion of the gas through the needle. Pressurizing the vial with an inert gas to a constant pressure was suggested as a solution to this problem.

Memory peaks After one injection of a sample, the following injection of a pure sample may show some compound

present where none actually exists in the sample. This is due to a slightly delayed retention somewhere in the gas chromatographic system. If this is a problem, it may be necessary to run blank samples between actual headspace samples (Bassette, 1984).

Fog problem If a sample is too hot, vapors may condense in the sample flask, on the septum or the syringe, changing the concentration of the volatile components in the gas. Therefore it is important to have the sample only as warm as is necessary for proper equilibrium conditions. Also the syringe temperature should be the same as the flask temperature when the headspace gas sample is taken (Heath, 1981).

Equilibration Before actual samples are run, it is necessary to determine the proper time, at the chosen temperature, necessary to bring the samples to equilibrium. Unless this is done the gas phase sample will not be representative of the true concentration of a compound. To determine equilibrium at the chosen temperature, the time before a gas sample is taken should be increased until the peak size remains constant (Bassette, 1984; Kolb, 1985).

C.V.s Coefficient of variability, or % sample error, is often used to determine proper sampling conditions. Coefficient of variability is the sample

standard deviation expressed as a percentage of the sample mean (Steel and Torrie, 1980). If the C.V. values are low, this is a good indication of very high sampling precision (Thissen, 1982; Hiltunen et al., 1985). If they are high, either the technique or equipment may be a problem and should be solved before proceeding.

Solvent Extraction

Most extractions of compounds are complex processes which include some sort of distillation. However a simpler, quicker method involves shaking an aqueous food sample and solvent in a bottle. After the sample is centrifuged, the solvent extract is sampled by syringe for direct injection into a gas chromatograph. It is also noted that multiple extractions with small amounts of solvent each time are more efficient than one extraction with a large amount of solvent (Damodaran and Kinsella, 1981b; Heath and Reineccius, 1986).

Selection of the proper solvent is important to completely extract a flavor compound. An important consideration is the polarity of the solvent. A solvent with polarity similar to the compounds studied should be chosen. Also the solvent chosen should have a boiling point which is lower than the compounds being extracted (Sugisawa, 1981).

It is important that introduction of contaminants be avoided, since contaminant peaks might obscure the desired peaks. When using highly sensitive gas chromatographs, it is thus very important to use very pure solvents. Also, if food samples contain lipids, the lipids are extracted along with flavor compounds. These lipids can build up and contaminate the injection port and column, of the gas chromatograph. (Teranishi et al., 1971; Heath and Reineccius, 1986).

Binding

The foods we eat are a very complex system made of many components: water - up to 95%, protein - 1 to 25%, lipid - 1 to 40%, carbohydrate - 1 to 80%, minerals - 1 to 5%, vitamins - ppm and flavor compounds - ppb to ppm (Teranishi et al., 1971). Also complex is the interaction of these various components. When studying flavor compounds one must take into account the effect the other components have on them.

Solms et al. (1973) have studied how fat, or lipid, carbohydrate and protein each affect volatile flavor compounds. With lipids, the interaction was simple. As the amount of fat increased, the amount of a flavor compound necessary to get the same flavor intensity also needed to be

increased. With carbohydrates no specific trends were observed. Some flavor compounds did bind to carbohydrates, while others did not. Also, different carbohydrates did not behave same way. For example, methyl cellulose did not show much binding, while semicrystaline cellulose did bind volatile compounds. Starch interacted with ligands only when they were present in appreciable amounts. Protein did, however, exhibit some specific reaction patterns with flavor compounds. As a protein became denatured, the amount of bound ligand increased and was found to be resistant to vacuum distillation. The resistance to removal may be indicating irreversible binding. When the protein was hydrolyzed, the flavor compound was released and could be removed by vacuum distillation (Arai et al., 1970). Solms et al. (1973) commented that if a ligand was soluble in a food system then it would be available to interact with the hydrophobic zones of a protein molecule and unfold it, exposing more nonpolar residues, making them available for further binding. Thus, the interaction of flavor compounds was more complex than could be explained by a simple partitioning of the molecules between the gas phase and the food sample.

In tofu, the proteins have been heat denatured and are aggregated, or coagulated, owing to a decrease of

electrostatic repulsion (Fukushima, 1980). Being unfolded, portions of the protein molecule may be readily available for binding to flavor compounds.

Franzen and Kinsella (1975) found different food elements to compete somewhat for binding of flavor compounds. When hexanal or 2-hexanone was added to soy concentrate, quar gum or emulsifier, binding was exhibited. However when the three food ingredients were combined the total binding shown, although greater than the individual components, was not as large as the sum of binding of the three food constituents.

Nawar (1966) did a study on headspace volatiles in which the medium affected the amount of volatiles in the headspace. If the flavor compound was very miscible with the medium, for example ethanol in water, then almost no volatiles were present in the headspace, a strong indication of binding. Only a slight increase of binding with increasing ethanol concentration was noted. When the flavor compound was not miscible in the medium, for example heptane in water, then headspace values for the flavor compound alone and in water did not significantly differ. He also examined some combinations of flavor compounds and mediums. When a flavor compound exhibited some binding, indicated by a lowered volatile headspace concentration, and another

similar medium was added, a competition for binding occured. For example, 2-heptanone had a loose affinity for ethanol but when water was added it replaced some 2-heptanone molecules. A resulting increase of 2-heptanone in the headspace was noted. This behavior was more noticeable at lower concentrations than at higher concentrations, as would be expected due to saturation effects. Dumont and Land (1986) felt it was also important to keep the flavor compound concentration low in studies because flavor compounds are found in low concentrations in foods. This way the data can also easily be integrated with sensory evaluation studies.

In simple water solutions, esters and aldehydes have high volatility, and high air-water partition coefficients, followed by ketones. Alcohols have the lowest volatility. Also up to nine carbons, the higher molecular weight homologs of each series were more volatile than lower molecular weight homologs (Buttery et al., 1969).

Several trends in binding have been observed in research studies dealing with binding. For one, as the alkyl chain length increases up to about C-9, binding also increases (Aspelund and Wilson, 1983; Cornell et al., 1971; Crowther et al., 1980; Damodaran and Kinsella, 1981b). Also as the concentration of a volatile flavor compound

increases, so does the amount of binding exhibited (Cornell et al., 1971). This follows the simple air-water partition trends mentioned previously.

In aqueous systems, aldehydes bind to soy protein very strongly, ketones bind less strongly and alcohols exhibit little if any binding (Beyeler and Solms, 1974; Gremli, 1974). However in dry systems, alcohols exhibit the strongest binding to soy protein, followed by aldehydes, ketones and methyl esters binding less strongly. Finally, hydrocarbons were found to have the weakest affinity for soy proteins (Crowther et al., 1980; Aspelund and Wilson, 1983). Obviously water seems to affect the binding affinity of alcohols for soy protein.

Aspelund and Wilson (1983) hypothesized that van der Waals forces and hydrogen bonds were involved in the binding of flavor compounds to soy protein. Hydrocarbons had just a van der Waals attraction, but ketones had one hydrogen bond and binding by alcohols involved two hydrogen bonds.

Gremli (1974) studied the reversibility of binding to soy protein. Ketones were found to bind completely reversibly, while aldehydes exhibited irreversible binding. Hexanal and heptanal had minimal irreversible binding, but higher molecular weight aldehydes showed an increasing ability to bind irreversibly to soy protein. Gremli felt

that if little or no binding was exhibited by a flavor compound, then its flavor intensity would not be affected. However, if a flavor compound exhibited irreversible binding, then the flavor impact would be suppressed and more of the flavor compound would be needed to achieve the desired flavor perception level.

Damodaran and Kinsella (1981a) found the 11S soy protein fraction to exhibit no binding with 2-nonanone, whereas the 7S soy protein fraction did. In fact, 7S had the same binding value as the whole soy protein. It may be that 7S is more available to hydrogen bonding than 11S, with its disulfide residues.

In an experiment using pea proteins, Dumont and Land (1986) found the amount of binding to increase proportionally as the amounts of pea protein present increased. Cornell et al. (1971) studied binding of esters to milk proteins. They found trends similar to those with soy proteins. Binding increased with increasing alkyl chain length and increasing flavor compound concentration. Chain branching decreased binding, indicating non-polar rather than polar protein-ligand interactions were involved. It was also found that calcium-free milk proteins bound less of a flavor compound than did natural milk proteins, possibly due to reduction in the non-polar environment available for

interaction. In this study we investigated the interaction of flavor compounds in the tofu food system, which includes proteins, fats, carbohydrates and water.

MATERIALS AND METHODS

Preliminary Sensory Panels

To begin this study, preliminary 'bench-top' sensory panels were conducted with a variety of flavors to determine: 1) whether it would be possible to flavor tofu by adding flavoring materials to the soymilk before coagulation and pressing; and 2) what flavors might be compatible with tofu. A small group of judges (6) were asked to evaluate flavored tofus by using a line-scale preference test described by Larmond (1977). A sample evaluation form is shown in Figure 1.

For each sample, flavoring was added to the heated soymilk at approximately 75°C just prior to the coagulation step, in which 80°C soymilk was poured into a bowl containing food-grade calcium sulfate. Flavored tofu blocks were sealed in plastic bags and refrigerated overnight. The next day samples were cut into one inch cubes and allowed to come to room temperature for one hour before the sensory panel began. Individual booths and normal lighting conditions were used. Tap water was provided for tasters to rinse between samples. Random numbers were assigned to each sample (Larmond, 1977).

Name _____
Date _____

We would like your opinion of these samples of _____ and _____ flavored tofu.

Please taste the samples in the following order:

Make a vertical line on the horizontal line to indicate your opinion of these samples of _____ and _____ flavored tofu.

dislike it very much ——————————————— like it very much

Other comments:

FIGURE 1. Sensory evaluation form

Tofu Preparation

A single variety of soybeans was used in these flavor binding studies to reduce varability due to soybean variety, location grown and weather conditions. They were Vinton 81 soybeans, grown in 1983, obtained from Strayer Seed Farms, Hudson, Iowa. The soybeans were stored in plastic bags at refrigerator temperatures (0-5°C).

Soymilk preparation

For each experimental unit, the soybeans were weighed, inspected for diseased or damaged soybeans, washed and then soaked overnight in room-temperature water. The next day, the beans were rinsed again. Small portions of the soybeans were ground with equal amounts of water (approximately 1 cup water per 1 cup beans) for 2 minutes in a Waring blender. Additional water was used to rinse the blender. A total of 3800 ml of water was used per 454 g (1 lb) of soybeans. The resulting soybean slurry was heated to boiling and simmered for 15 minutes. Due to the formation of foam, which needed to be occasionally stirred down, only 1 lb or less of soybeans was cooked at one time.

Next the mixture was strained through 4 layers of cheesecloth and the cloth was squeezed until the residue was almost dry. The resulting liquid is called soymilk and the

solid material retained by the cheesecloth is called okara. For this study, the okara was discarded.

Percent solids determination

Percent solids in soymilk samples were determined by using a method described by Johnson and Wilson (1984) and modified by Schaefer (1986). To provide soymilk with a uniform 6% solids content, each sample needed to be tested for percent solids content and then adjusted by dilution. The percent solids of soymilk was obtained by correlating absorbance values to percent solids derived from moisture tests on soymilk prepared from the Vinton soybeans used in this study.

Soymilk was diluted and transmittance readings were read, at 400 nm, on a Bausch & Lomb spectrophotometer, model 340. Concentrations of 100%, 80%, 60%, 50% and 25% soymilk were prepared. One gram of each sample was weighed into a 250-ml volumetric flask and water was added to the 250 ml mark. Then a transmittance reading was taken on an aliquot of this diluted sample. This allowed readings to be taken on very dense soymilk samples. The procedure was repeated and a mean transmittance value was obtained which was then converted to absorbance. Thus, for each percent dilution concentration two samples were taken and an absorbance value was obtained.

Moisture content was determined for each diluted sample, according to the AOAC (1980) method 14.084. Three samples of each concentration were weighed, frozen, freeze-dried and then placed in a vacuum oven to achieve complete dryness. The dried samples were cooled and weighed to give a percent solids value.

This entire procedure was done on three different batches of soymilk made from the Vinton 81 soybeans used for this study. The mean values obtained are shown in Figure 2. These values were used during the study to determine percent solids of soymilk from the Vinton 81 soybeans. All soymilk was then adjusted to give a uniform 6% solids as suggested by Schaefer (1986). For example, for soymilk with 7.8% solids the calculation would be as follows:

$$\frac{(7.8\%\ \text{Solids} - 6\%\ \text{Solids})\ (800\ \text{ml})}{7.8\%\ \text{Solids}} = 185\ \text{ml water to add}$$

$$185\ \text{ml water} + 615\ \text{ml } 7.8\%\ \text{soymilk} = 800\ \text{ml } 6\%\ \text{soymilk}$$

Coagulation and pressing

To prepare one tofu block, 800 ml of 6% solids soymilk was heated to 80°C and then poured into a glass bowl containing $CaSO_4 \cdot 2H_2O$ dispersed in 30 ml boiling water. The 30 ml was subtracted from the amount of water added to give 6% solids soymilk. The amount of $CaSO_4 \cdot 2H_2O$ was calculated

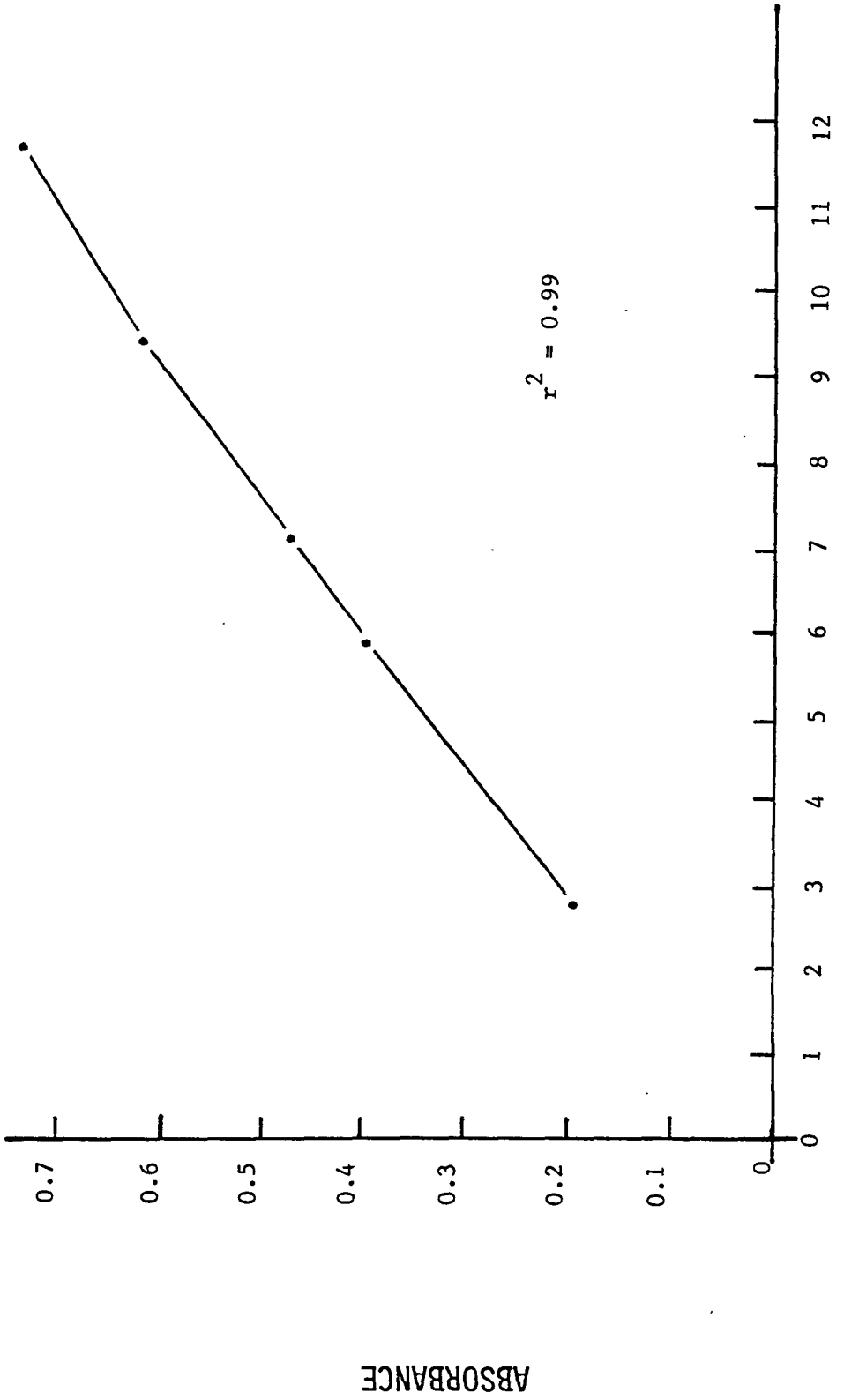

FIGURE 2. Percent solids for 1981 Vinton Soybeans

to give 0.02 M in the soymilk. For example, 800 ml of soymilk would require approximately 2.75 g $CaSO_4 \cdot 2H_2O$ to be 0.02 M in calcium sulfate.

0.8 l (0.02 moles/l x 172.1 g/mole) = 2.75 g

MW $CaSO_4 \cdot 2H_2O$ = 172.1 g

After standing for 12 minutes, the coagulum was spooned into a cheesecloth-lined press box and pressed for 20 minutes. Three layers of cheesecloth were used to line the press box and cover the soymilk curds.

The press box was constructed of stainless steel mesh of 1 mm thickness with 6 mm diameter holes, which allowed the whey to flow out. The outer dimensions of the pressbox measured 5" x 4" x 3". Three weights totaling 4 kg were used to press whey from the soy curds. The first weight, of 1 kg, was flat and covered the top of the box and slid downward as the tofu was compressed. Two more circular weights of 1 and 2 kg respectively, fitted onto the top of the first weight (Figure 3).

After being pressed, each tofu block was weighed, sealed in a plastic bag and refrigerated overnight before samples were taken.

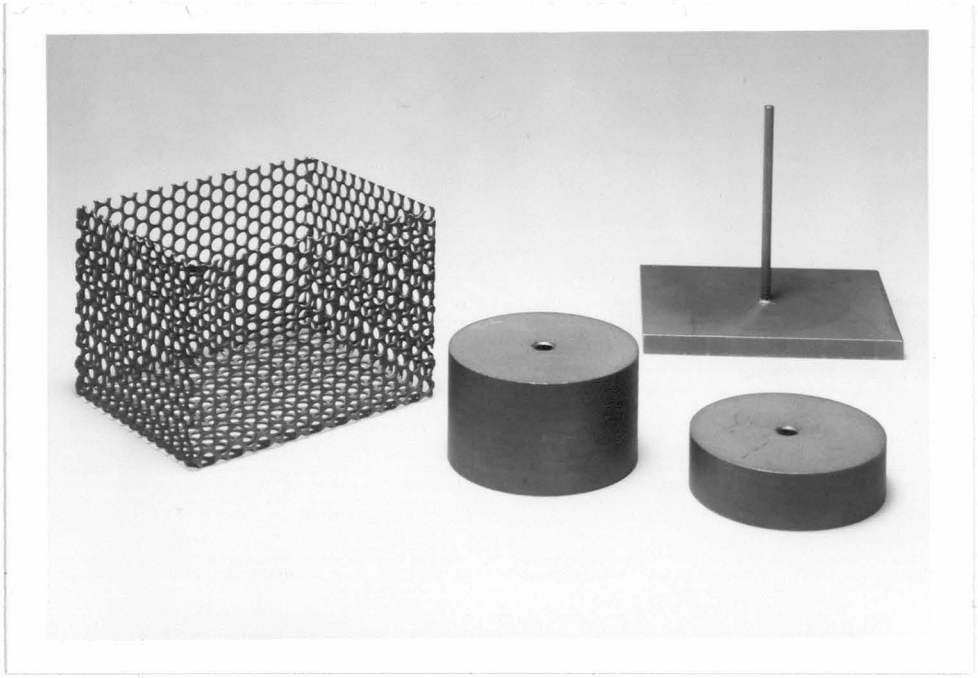

FIGURE 3. Press Box

Tofu Blend Procedure

This part of the study was conducted to determine the binding profile of tofu, after it was prepared, for six compounds in a closed system. For these experiments, an unflavored tofu block made from 1200 ml of soymilk was blended with water to give a blend of 55% tofu and 45% water. A Tekmar SDT Tissumizer fitted with a SDT182EN shaft, using a speed control setting of 40 to 50, was used to blend the tofu in a glass container. The tofu was blended to facilitate mixing tofu with the flavor compounds added.

The tofu blend was then divided into 50-gram samples contained in 150-ml glass headspace vials. Specified quantities of the flavor compounds were pipetted into the vials, which were capped with a teflon coated rubber septum and an aluminum seal which was crimped, giving an airtight seal. Each vial was inverted twice and then mixed on a Burrell wrist action shaker for 10 minutes.

The vials were then heated in a water bath at 30°C for 2 hours. Headspace samples of 1 ml were taken with a Hamilton gas-tight 2.5-ml syringe and injected into a gas chromatograph.

Samples of the tofu blend were taken for moisture determinations. The dried tofu was subsequently analyzed for protein and lipid content.

Four concentrations (90, 130, 170 and 210 ppm) of six compounds were studied. The flavor compounds were three aldehydes (pentanal, hexanal, and heptanal), a ketone (3-hexanone), an alcohol (hexanol), and an aromatic compound (benzaldehyde). The concentrations of the compounds were chosen to be in the range in which flavor compounds would be found in foods.

This entire procedure was repeated two more times, for a total of 3 trials. Each vial was tested once during each trial.

Flavored Tofu Blocks

In this section of the study, the binding profile of tofu for two of the six flavor compounds, hexanal and benzaldehyde, was investigated. Flavor compounds were added to the soymilk just before coagulation, rather than mixing them into tofu after it was prepared.

Flavored tofu blocks were prepared by adding hexanal and benzaldehyde to the soymilk. Concentrations in the soymilk were those used in previous studies. Because flavor compounds were added to the soymilk, which was then coagulated and pressed, the concentrations in the tofu blocks could differ from that in the soymilk. Therefore, it was necessary to determine the concentration for each block of tofu.

Each tofu block was cut into very small pieces and mixed by hand, wearing disposable plastic gloves. This was done to insure representative sampling. Then two samples of each tofu block, for headspace analysis, were taken as well as moisture samples, which would be later used for protein and lipid determinations. Samples from each tofu block were also taken for a solvent extraction procedure, to determine the flavor compound concentration within each tofu block. The solvent procedure may not absolutely remove all traces of the flavor compound from the tofu samples. Therefore the concentrations determined were only close approximations.

After analysis, tofu headspace values for each tofu block were then plotted against the determined concentrations of the flavor compounds.

Solvent extraction procedure

Through repeated trials, the following method was found to give nearly complete extraction of flavor compounds from tofu. Three one-gram samples were taken from each tofu block. Each sample was placed in a 10-ml glass tube which was fitted with a teflon-coated screw cap. Then 3 ml of tap water were immediately pipetted on top of the sample. Tubes were then capped and refrigerated.

The next day, each sample was homogenized in the tube by using a Tekmar SDT Tissumizer fitted with a Tissumizer

Microprobe, type SDT080EN. A speed control setting of 30 to 40 was used. Next 3 ml of HPLC-grade hexane was pipetted into the tube. Capped tubes were vortex-mixed for approximately one minute at a setting of 5 to 6 to obtain a thorough blending of the water-mixed tofu sample with hexane. The tube was then centrifuged, by using an International Centrifuge, for 10 minutes at a 40 rheostat setting equivalent to 1600 rpm or 600 xg (relative centrifugal force). The supernatant was removed with a glass disposable-pipette and placed into a small glass vial with an aluminum-foil lined cap to prevent leakage.

A second hexane washing was performed for samples containing the flavor compound hexanal and a third hexane washing was necessary to remove benzaldehyde from tofu.

All samples were frozen until analyzed on a gas chromatograph.

Hexane calibration curves for flavor compounds

Calibration curves for hexanal and benzaldehyde were prepared by the following procedure. Four serial dilutions in HPLC-grade hexane (30 to 70 ppm) were made for each compound. A 0.50 µl sample was injected into the gas chromatograph, with a Hamilton liquid syringe.

This procedure was replicated three times. It was done at the beginning, middle and end of the gas chromatographic

analysis of flavor compounds extracted from tofu samples. This was done to insure uniformity of conditions for 'standards' and extracted tofu samples.

Plots of concentration versus peak area were then used to determine the concentration of hexanal or benzaldehyde in the solvent extracts of tofu.

Headspace Analysis

Headspace analysis of the tofu samples was performed according to the I.O.F.I. (International Organization of the Flavor Industry) recommended methods as outlined in Heath (1981). Glass headspace vials of 150-ml volume were filled to approximately one third of their volume with 50 g of tofu or tofu blend. They were then capped with teflon-coated rubber septa and aluminum outer seals, which were crimped on. This created an air-tight atmosphere for each tofu sample.

For samples taken from tofu blocks, vials were refrigerated for one day and then allowed to come to room temperature. Each vial was then heated in a constant temperature water bath for exactly 2 hours at 30°C (see time-temperature study). This treatment allowed the volatile flavor compounds in each sample to be brought into equilibrium with the air, or headspace, above the sample using identical conditions for each sample.

A cleaned, gas-tight syringe was warmed in a needle cleaner (Hamilton Syringe Cleaner fitted to a high vacuum hose), to maintain a similar temperature with the interior of the vial. The needle of the syringe (Hamilton 1002 RN Gastight Syringe 2.5-ml capacity) was pushed through the septum of the vial and the piston was moved up and down once before the syringe was slowly filled to 2.5-ml. The volume was then adjusted to 1.0 ml and the headspace sample was immediately injected into the gas chromatograph.

Due to the low volatility of high boiling flavor compounds and therefore minute amounts present in the headspace, some flavor compounds such as vanillin (bp 285°C), could not be used in this study. Therefore all the flavor compounds used have low boiling points and thus high vapor pressures.

Time - Temperature study

A time-temperature study was done to determine the optimum conditions to bring a sample to equilibrium before injection of headspace. Three trials were conducted. In each trial three temperatures (25°C, 30°C and 37°C) and three time lengths (1 hour, 2 hours and 3 hours) were studied. One volatile compound from the tofu, with a retention time of approximately 3.2 minutes was chosen as the indicator. This compound was not identified. Total

area units under the indicator peak, as calculated by the integrator, were used for comparison. A thermostatically temperature-controlled water bath was used for this study.

Water headspace calibration curves

Glass serum-type vials (as used for all tofu samples) with 150-ml capacity were filled one-third full with 50 ml of water. The flavor compounds were pipetted into each vial to give dilutions of 90, 130, 170 and 210 ppm. Vials were then capped as mentioned before.

Each vial was placed in a water bath and held at 30°C for 2 hours. A 1-ml headspace sample was injected into the gas chromatograph. Area values for each sample were obtained from the printout of the attached integrator.

Gas Chromatographic Analysis

For analysis of the flavor compounds pentanal, hexanal, heptanal, hexanol, 3-hexanone and benzaldehyde, a fused silica DB-5 capillary column (30M x 0.254 mm) was used, which was obtained from J & W Scientific, Inc. DB-5 (a durabond liquid phase, non-extractable, cross linked and surface bonded) is composed of 95% dimethyl siloxane and 5% phenyl. The film thickness is 1.0 micron. Also the DB-5 column is non-polar and able to separate compounds at or below their boiling points (Hayes, 1987). All compounds in this study eluted below their boiling points (Table 1).

TABLE 1. Flavor compounds, boiling points and GC elution temperatures[a]

Compound	BP(°C)	GC Elution Temp.(°C)
Pentanal	103	95
Hexanal	128	109
Heptanal	155	127
Benzaldehyde	179	140
Hexanol	158	120
3-Hexanone	123	107

[a]Observed on the DB-5 column used in this study.

This capillary column was installed on a Varian Gas Chromatograph, model 3700, equipped with an inlet splitter and a flame ionization detector. For the flame operation, air flow was 300 ml/min and hydrogen was 30 ml/min. The carrier gas used was nitrogen. Column flow rate was 0.97 ml/min at 25°C (room temperature) and 0.87 ml/min at 80°C (operating temperature). Make-up gas was used to achieve a 30 ml/min nitrogen gas flow to the detector.

The inlet splitter was set at 20:1 for all injections. The split was checked each day before injections began to insure uniformity. Also 2 μl of methane were injected to insure the gas chromatograph was working properly. A needle sharp peak of the non-retained gas would indicate the gas

chromatograph was in good-operating condition (Jennings, 1980).

Gas chromatograph parameters were as follows:

Injector temperature	150°C
Detector temperature	250°C
Column temperature	80°C hold 3 min, rise 6°C/min to 150°C
Sensitivity	1×10^{-12}

A Hewlett Packard integrator, model 3390A was used in conjunction with the gas chromatograph. The following parameters were used for the integrator:

Attenuation	4
Chart speed	1
Peak width	0.04
Threshold	1
Area rejection	700

All headspace samples were taken with a gas-tight Hamilton syringe. After each injection the needle was cleaned with a Hamilton Syringe Cleaner for 4 minutes.

For all liquid samples 0.50 μl was injected onto the gas chromatograph by using the same parameters as for headspace samples, including a split of 20:1. A 7101 N

Hamilton liquid needle was used for all liquid samples and was thoroughly cleaned, between samples, with H.P.L.C.-grade hexane.

It was important to insure that the column was clean and free from extraneous compounds. Each day after the methane injection, a control sample was injected before the experimental samples. For liquid samples this was HPLC-grade hexane, and for headspace samples this was a sample of headspace above tofu without any added flavor compounds.

Protein, Moisture and Lipid Analysis Procedures

Protein determination

To determine nitrogen, a macro-Kjeldahl procedure was used, according to a modification of the AOAC (1980) method 14.086. Dried tofu was weighed onto glassine powder paper and placed into digestion tubes with 15 ml of concentrated H_2SO_4 and 2 Pro-Pac tablets (MT-37, Alfie Packers Inc., Omaha, NE). The next day, samples were digested and distilled by using the Tecator digestion block and distillation apparatus. Distillate was mixed with a methyl red-bromcresol green indicator and titrated with approximately 0.1 N HCl. Percent nitrogen was then calculated and converted to percent protein by using the 6.25 conversion factor.

Moisture determination

After overnight refrigeration in sealed plastic bags, tofu blocks were weighed and samples for various analyses were taken. Moisture analysis was performed using a modification of the AOAC (1980) method 14.084. For the tofu-blend samples, moisture samples were taken after the tofu was blended with water. Each tofu sample was placed in pre-weighed aluminum 'weighing boats' and weighed. The samples were then covered and frozen till solid, about 18 hours. They were then freeze dried for 24 hours at a vacuum of 12 MT (Millitorr), by using shelf heat of approximately 80°C. Finally samples were transferred in dessicators to a vacuum oven and dried at 100°C with a vacuum setting of 1 x 10^5 MT. After samples were completely dry, they were cooled in a dessicator and their dry weights were recorded. Percent moisture was then calculated.

Lipid determination

Crude lipid analysis was performed, using a modification of the AOAC (1980) method 14.089, on dried tofu samples by using a Goldfisch fat extractor. Hexane (60 to 70°C boiling range) was used as the solvent. Approximately 1-g tofu samples were weighed onto Whatman filter paper, (Qualitative 4, filter speed - fast) and placed in extraction thimbles. They were then placed in the extractor

for approximately six hours. All residual solvent was evaporated from the glass retaining beakers under a hood and finally in a drying oven set at 80°C for 30 minutes. The beakers were then cooled in desiccators and weighed. This weight was subtracted from the weight of the retaining beakers when they were clean and desiccated before analysis began. Percent crude lipid of each sample was then calculated.

Experimental Design and Statistical Analysis

In the tofu blend study, six trials (three trials divided in half by concentration levels) were conducted. For each trial, all compounds and half of the concentrations were mixed with tofu blend. In addition, there were control samples which contained no added chemicals. One headspace sample was taken from each vial. Compounds were run alternately on the gas chromatograph to avoid memory peaks. The same procedure was followed for water 'standards', except all concentrations were run at each trial. The control vial for 'standards' was a vial containing water.

For the tofu block study, two trials were conducted. (A third trial was not considered in the statistical analysis, due to malfunction of the gas-tight syringe.) For each trial, tofu blocks containing each concentration of all

chemicals were prepared once, plus one control, or unflavored tofu block. Enough tofu was available for two samples per block for headspace analysis. One headspace sample was taken per vial. An additional water 'standard' of all compounds and concentrations was run concurrently with the tofu block headspace samples, to insure uniformity of conditions for standards and tofu samples.

For the solvent extraction study, three samples were taken per tofu block. Two injections were made per extraction vial. For hexanal, mean peak areas of 2 extractions were summed to get a total peak area for each tofu sample taken. For benzaldehyde, mean peak areas of 3 extractions were summed to get a total peak area for each tofu sample taken. Hexane 'standards' of all compounds and concentrations were run once per 'standard' trial. Four hexane 'standard' trials were completed concurrently with the solvent extraction trials, to insure uniformity of sampling conditions.

For moisture determinations, enough sample was taken to insure a sufficient amount of solids for protein and lipid determinations. For the tofu block study, 3 samples for moisture analysis were taken per tofu block. For the tofu blend study, 12 samples were taken per tofu blend, to insure sufficient solids for protein and lipid analyses. Tofu

block moisture determinations were done in 3 trials. Tofu blend moisture determinations were completed in one trial.

For lipid determinations, three trials were run on each tofu blend. The accuracy of lipid determinations was found to be very precise, therefore only two trials were run for each tofu block. For protein determinations three trials were run for the tofu blend and tofu block samples.

For moisture, lipid, protein and solvent extraction procedures, coefficient of variability (C.V.) was used to determine procedure accuracy. For protein determinations, percent recovery of a nitrogen standard was also calculated.

To analyze the amounts of compounds bound, the Iowa State University computer system, Statistical Analysis System (SAS) package was used (Statistical Analysis System, 1982).

RESULTS AND DISCUSSION

Bench-top Sensory Panels

A variety of flavorings was added to tofu, to determine which of them might be compatible with tofu. A second objective of the preliminary studies was to determine whether flavors added to soymilk prior to coagulation and pressing of tofu were retained in the tofu.

The flavorings evaluated are listed in Table 2.

TABLE 2. Flavorings studied for addition to tofu

Fruit Flavors	Dairy and Meat Flavors	Others
Banana	American Cheese	Mushroom
Strawberry	Natural Cheddar Cheese	Onion
Pineapple	English Cheddar Cheese	
	Beef	
	Seafood	
	Bacon	
	Butter	

When the bacon and beef flavorings were added to soymilk, curdling of the soymilk occurred and the resulting tofu had an unacceptable grainy texture. This phenomenon could be due to ions present. Bourne et al. (1976) observed some curdling of soymilk, at the alkaline pH of 7.5, with

$NaHCO_3$ added. Salt, or more specifically sodium ions, may have affected the texture of tofu, with several other flavorings used in our studies. With the addition of onion, butter and seafood flavorings to the soymilk, a slight softening of the tofu texture was observed. Several researchers have noted softening of protein gels with the presence of NaCl. This is possibly due to stabilization of the 11S protein fraction by salt, and interference with 11S calcium ion bond formations (Hermansson, 1979; Nakashima and Murakami, 1980; Babajimopoulos et al., 1983; Utsumi and Kinsella, 1985). Nakashima and Murakami (1980) stated that with sodium salts present in soymilk, more calcium ions were needed for coagulation of the soymilk.

It was observed during preparation of these flavored tofu blocks that some of the flavorings were lost in the whey as the soy curds were being pressed. There could also have been volatile loss at the temperature used and physical loss on the equipment used. This loss would require an increase in the amount of flavor needed to achieve a desired flavor intensity. Also if a flavor compound was expensive, its loss during tofu preparation may not be acceptable. This loss must be considered in making a decision to add flavorings at the soymilk stage of tofu preparation.

The flavorings of strawberry, American cheese and seafood blended uniformly within the tofu block and were found to be acceptable additions to tofu by the sensory panelists. Seafood-flavored tofu had the highest hedonic score of the flavors tested, 4 on a 5-inch line scale. It must be noted that salt within the seafood flavoring softened the texture of the tofu.

Time - Temperature Study

Prior to conducting headspace studies, conditions necessary to bring enclosed tofu samples to equilibrium needed to be determined. Once equilibrium was reached, the gas phase above the tofu sample was representative of the concentration of the compounds present in tofu (Bassette, 1984; Kolb, 1985). In this study, temperatures of 25°C (room temperature), 30°C and 37°C (body or mouth temperature) and time lengths of 1, 2 and 3 hours were investigated in three trials. The resulting mean peak areas are shown in Table 3.

Although slightly higher peak areas were found at 37°C, the tofu samples changed in color, during equilibration. The heat could change the flavor compounds, or break them down into fragments, or artifacts (Bassette, 1984). In addition, fogging or condensation was observed in the

TABLE 3. Time - Temperature study, mean peak areas (n=3)

	Mean peak area		
Temperature	1 Hour	2 Hours	3 Hours
25°C	4742	8060	12595
30°C	16007	26957	26435
37°C	30508	27855	28148

bottles held at 37°C, which could change the concentration of volatile components within the gas phase (Heath, 1981). At 30°C fogging or heat changes were not observed.

After two hours at 30°C, the peak size remained relatively constant. Therefore two hours and 30°C were chosen as the conditions necessary to bring the gas phase into equilibrium with the tofu sample, in an airtight glass vial.

Headspace Sampling

Direct headspace analysis involves the removal by a gas-tight syringe of small portions of the gas phase above the sample. Once a gas 'headspace' sample is removed, the equilibrium conditions established within the glass vial could be changed, subsequently changing the concentrations of the compounds in the gas phase. Thus, only the initial

gas sample is absolutely representative of the concentration of a flavor compound in the tofu sample. However, it has been noted that second gas samples, which differ only 10% or less from initial headspace samples, indicate good reproducibility of peak areas and sampling conditions (Thissen, 1982). For this reason, a short study was conducted to determine if the technique used in these studies had good reproducibility.

Two compounds, benzaldehyde and hexanal were added to tofu at the soymilk stage. Flavored tofu samples from each block were then placed in two separate headspace vials. One vial was brought to equilibrium and two gas phase samples were taken from the single vial. The second gas phase sample was taken just 20 minutes after the first. The glass vial was held in the water bath during the 20 minutes to maintain equilibrium conditions as much as possible. The following day, the duplicate vials were sampled under identical conditions. Resulting peak areas are shown in Table 4.

The mean coefficient of variability (C.V.) was 4.3%. All but 2 duplicate injections were under 10% sample error, or C.V. This indicated a good reproducibility between duplicate gas samples taken from the same vial. The low C.V.s also indicated adequate control of experimental

TABLE 4. Duplicate headspace samples from the same vial

Compound	Vial	Conc. (ppm)	1st Injection[b]	2nd Injection[b]	C.V.(%)[a]
Benzaldehyde	1	110	218,850	222,300	1.1
"	2	110	219,280	246,820	8.4
"	1	170	292,080	293,860	0.4
"	2	170	279,630	342,000	14.2
"	1	230	424,410	501,110	11.7
"	2	230	360,750	357,860	0.6
"	1	330	538,430	568,030	3.8
"	2	330	464,340	520,250	8.0
Hexanal	1	30	339,800	340,880	0.2
"	2	30	296,880	294,610	0.5
"	1	100	1,094,600	1,083,500	0.7
"	2	100	1,131,400	1,123,500	0.5
"	1	130	1,379,900	1,270,700	5.8
"	2	130	1,408,900	1,254,200	8.2
"	1	220	-	-	-[c]
"	2	220	2,190,400	2,223,200	1.1

[a]Mean C.V. = 4.3%.

[b]G.C. peak area values.

[c]Sample lost due to syringe plunger malfunction.

conditions. In the other studies to be discussed, only one gas phase sample was taken per vial.

Tofu Blend Study

For this study six flavor compounds were used: pentanal, hexanal, heptanal, benzaldehyde, hexanol and 3-hexanone. Each compound chosen had a low boiling point (see Table 1), which made it suitable for headspace analysis. Higher boiling compounds have low vapor pressures. Due to this fact, they are often not present in the headspace at levels which can be detected by gas chromatographs (Lamparsky, 1985).

Concentrations of 90, 130, 170 and 210 ppm were chosen to be close to the levels of 50 to 100 ppm suggested for sensory evaluation of artificial flavors (Heath and Reineccius, 1986). Although tofu samples containing these compounds were not tested by sensory panels, the data from these studies could be related to future studies which utilized sensory evaluation, because of the concentration range chosen. It must be noted that threshold concentrations for these compounds are even lower than the levels employed in these studies. Some threshold values for compounds used in this study are listed in Table 5.

Fazzalari (1978) defined detection threshold as "the minimum physical intensity detection by a subject where he (or she) is not required to identify the stimulus, but just detect the existence of the stimulus".

TABLE 5. Threshold values[a]

Compound	in water	in milk
Pentanal	0.7 ppm	0.13 ppm
Hexanal	0.3 ppm	0.05 ppm
Heptanal	-	0.12 ppm
Benzaldehyde	2.0 ppm	-
Hexanol	none listed	
3-Hexanone	none listed	

[a]American Society of Testing and Materials data (Fazzalari, 1978).

Specified concentrations of the flavor compounds were pipetted into headspace vials containing 50 g of tofu blend (55% tofu, 45% water). The vials were capped and then mixed. Next, each vial was brought to equilibrium and a headspace sample of 1 ml was taken and injected into a gas chromatograph for analysis. The resulting headspace values are listed in Table 6.

Moisture analysis and subsequent lipid and protein determinations were performed on tofu blends prepared in this study (Table 7).

Each C.V. listed in Table 6 was based on 3 G.C. peak areas, from separate sample vials, with the same compound and concentration. Most C.V.'s are at or below 10%, but some headspace samples, for example heptanal in the higher

TABLE 6. Headspace mean peak areas for compounds added to tofu blend

Compound	Conc.(ppm)	Mean peak area[a]	C.V.(%)[b]
Pentanal	90	2,085,700	8.8
"	130	3,015,533	1.0
"	170	3,691,333	1.6
"	210	4,891,367	10.0
Hexanal	90	1,288,267	7.4
"	130	1,578,900	10.6
"	170	2,356,667	8.6
"	210	2,669,900[c]	3.2
Heptanal	90	1,037,857	12.6
"	130	1,682,233	9.8
"	170	2,135,367	24.7
"	210	2,356,500	24.3
Benzaldehyde	90	220,217	6.5
"	130	258,450	14.0
"	170	383,315[c]	6.4
"	210	440,607	12.2
Hexanol	90	169,930[c]	1.0
"	130	259,243	11.5
"	170	302,833	5.9
"	210	347,127	6.2
3-Hexanone	90	1,771,700	10.8
"	130	2,402,767	6.7
"	170	3,202,933	13.3
"	210	3,755,833	3.3

[a] n = 3 trials.

[b] Coefficient of variability = (Standard deviation/Sample mean) 100.

[c] n = 2 trials, due to syringe malfunction.

TABLE 7. Mean values of moisture[a], lipid[b] and protein[b] determinations for tofu blends[c].

Trial	Moisture (%)	C.V. (%)	Lipid (%)	C.V. (%)	Protein (%)	C.V. (%)
1	92.17	0.9	2.04	2.7	4.43	0.4
2	92.15	1.2	2.07	3.7	4.48	0.2
3	91.85	1.0	2.15	0.5	4.61	0.6
4	92.05	1.3	2.08	1.2	4.49	0.5
5	92.08	1.0	2.09	0.5	4.50	0.4
6	92.26	0.7	2.04	1.1	4.52	7.4

[a] n=12 trials.

[b] n=3 trials.

[c] 55% tofu, 45% water.

concentration ranges, varied more than 10%. Determinations for moisture, lipid and protein generally had low C.V.s (Table 7), indicating precise experimental conditions.

Headspace trials for each compound and concentration in water were run concurrently. These 'standards' were then used in determinations of the amount of each compound bound within the tofu blend. Water 'standards' are listed in Table 8.

The difference between the peak area obtained from the water 'standard' headspace sample and the peak area obtained

TABLE 8. Headspace standards for flavor compounds in water

Compound	Conc.(ppm)	Mean GC Peak Area[a]	C.V.(%)
Pentanal	90	2,896.033	6.5
"	130	3,820,700	6.5
"	170	5,600,767	1.6
"	210	6,766,733	4.0
Hexanal	90	2,969,800	9.4
	130	3,594,833	7.0
"	170	4,978,167	7.1
"	210	6,122,233	5.9
Heptanal	90	6,606,667	6.9
"	130	10,216,233	10.0
"	170	11,699,667	5.6
"	210	15,361,667	9.6
Benzaldehyde	90	397,200	9.4
"	130	624,717	5.2
"	170	753,690	7.4
"	210	1,090,123	16.6
Hexanol	90	286,297	11.4
"	130	342,310	3.2
"	170	451,513	5.3
"	210	545,627	8.6
3-Hexanone	90	2,895,100	10.7
"	130	4,093,333	6.3
"	170	5,052,767	4.1
"	210	6,049,433	6.1

[a]n=3 trials.

from the flavored tofu headspace sample is an indication of binding of the flavor compound by the tofu blend. Similar to the formulas used by Dumont and Land (1986) and Thissen (1982), the equation used in these studies to determine the amount of binding was as follows:

$$C_{Bd} = \frac{\bar{X}\ \text{Std.} - \text{Tofu}}{\bar{X}\ \text{Std.}} \quad C_I$$

or C_{Bd} = Amount Bound x initial conc.(g)

C_{Bd} = concentration (g) of the compound bound

C_I = initial concentration (g) of the flavor compound

$\bar{X}$ Std.= mean peak area of the water 'standard' headspace

Tofu = peak area of the flavor compound in the headspace above tofu

Using pentanal at 90 ppm as an example:

$$C_{Bd} = \frac{2{,}896{,}033 - 2{,}085{,}700}{2{,}896{,}033} \times (9 \times 10^{-5}\ g)$$

$C_{Bd} = 2.52 \times 10^{-5}$ g or 25.2 ppm

To determine the amount bound on a solids basis:

C_{Bd} / mean amount of solids per g tofu

For example, pentanal at 90 ppm -

C_{Bd} per g solids = 2.52×10^{-5} g/0.0791 g

C_{Bd} per g solids = 3.19×10^{-4} g or 319 ppm

To determine the amount bound on a protein basis:

C_{Bd} / mean amount of protein per g tofu

For example, pentanal at 90 ppm -

C_{Bd} per g protein = 2.52×10^{-5} g/0.0451 g

C_{Bd} per g protein = 5.59×10^{-5} g or 55.9 ppm

Shown in Figure 4, Figure 5 and Figure 6 are graphical presentations of the amounts of the compounds bound at the four concentrations used in these studies. Means are shown, but individual data were used for statistical analysis.

When analyzed statistically, the data plotted in Figure 4 were found to be linear, indicating that an increase in binding was directly proportional to an increase in compound concentration. Studies by Cornell et al. (1971) had similar results. The difference in binding between the aldehydes, pentanal, hexanal and heptanal, in an analysis of variance (Anova) test was found to be highly significant ($p<0.0001$). In an additional T-test, these compounds were significantly different from each other at the 0.05 level. Thus, as the carbon chain length increased, the amount of binding also increased. This concurs with previous studies (Cornell et al., 1971; Crowther et al., 1980; Damodaran and Kinsella, 1981b; Aspelund and Wilson, 1983). The amount of binding of heptanal increased more with increased concentration than did hexanal; and hexanal more than pentanal. The amount of

FIGURE 4. Binding profile of flavor compounds in tofu blend (means n=3)

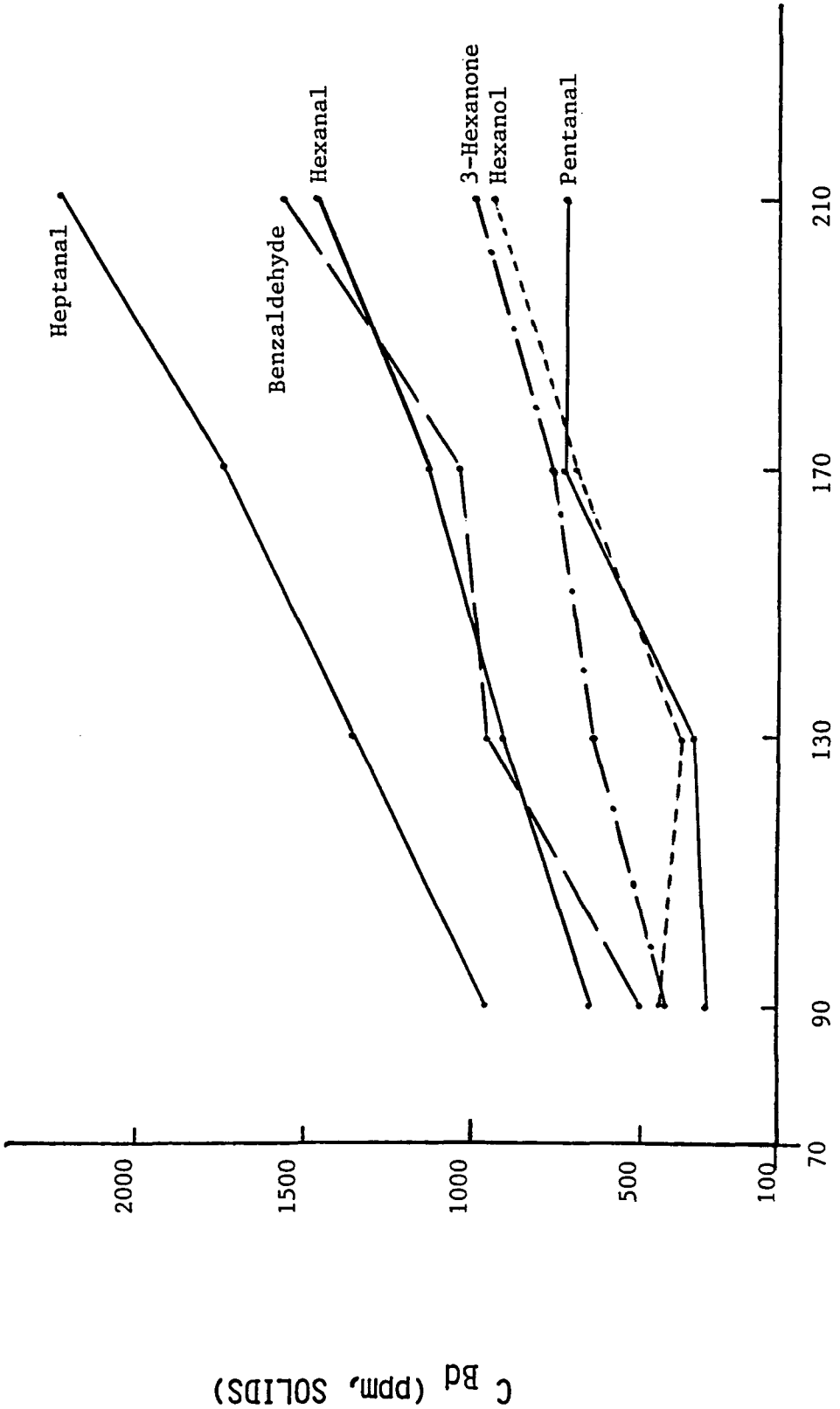

FIGURE 5. Binding profile of flavor compounds in tofu blend, on a solids basis (means n=3)

FIGURE 6. Binding profile of flavor compounds in tofu blend, on a protein basis (means n=3)

binding of hexanal, a straight-chain aldehyde, and benzaldehyde did not differ significantly in an Anova test ($p<0.6466$).

When comparing compounds with different functional groups, some differences were noted. Hexanal, an aldehyde, exhibited greater binding than did 3-hexanone, a ketone, or hexanol, an alcohol. This was similar to results from other studies in aqueous systems (Beyeler and Solms, 1974; Gremli, 1974). Aspelund and Wilson (1983) found in dry systems, however, that alcohols exhibited more binding than aldehydes. It seems as though water competes with alcohol for binding to soy protein. Nawar (1966) noted that different components within a medium could compete for binding. In the current study, a T-test showed that the aldehyde was significantly different from the ketone and the alcohol at the 0.05 level. It was noted at 130 ppm that the ketone and the alcohol were different at the 0.05 level (T-test). But overall, the ketone and the alcohol were not significantly different, at the 0.05 significance level (T-test).

Flavored Tofu Block Study

In this study, two of the six flavor compounds, hexanal and benzaldehyde, were investigated. As mentioned previously, the flavor compounds were added to the soymilk during tofu preparation. Samples from each flavored tofu block were taken for headspace analysis, for moisture, lipid and protein determinations (Table 9) and for the solvent extraction procedure, to try to determine the concentration of the flavor compound within the tofu block. Two trials for each compound and concentration were completed.

TABLE 9. Values from moisture, lipid and protein determinations for tofu blocks

Determination	Mean(%)	C.V.(%)
Moisture	86.50[a]	1.1
Lipid	3.21[b]	0.8
Protein	7.64[a]	0.9

[a] n=81; 27 blocks (2 compounds x 4 concentrations, plus an unflavored tofu block; 3 trials per variable) x 3 trials per block.

[b] n=54; 27 blocks x 2 trials per block.

TABLE 10. Standards for flavor compounds in hexane

Compound	Conc.(ppm)	Mean Peak Area[a]
Benzaldehyde[b]	30	76,129
"	43	103,398
"	57	123,048
"	70	156,246
Hexanal[c]	30	53,585
"	43	89,827
"	57	108,244
"	70	126,610

[a] n=4 trials.

[b] Correlation coefficient (r) = 0.9727.

[c] Correlation coefficient (r) = 0.9177.

After the solvent extraction procedure, 'standards' of the compounds in hexane (Table 10) were used to calculate the concentrations represented by peak areas resulting from the extracted tofu samples (Table 11). The concentrations determined per gram of tofu were then used as the initial concentrations in the formula to calculate amount of binding represented by the headspace peak areas (Table 12) of flavored tofu block samples.

Also, additional 'standards' for hexanal and benzaldehyde in water were run concurrently with the flavored tofu-block samples (Table 13).

TABLE 11. Solvent extraction sample concentration determinations

Cmpd. a	Mean pk. area[b] of sample	Determined conc. (g per ml)[e]	C per g tofu (g)[c]	Wt. tofu block (g)	Amt. per block (g)	C added (g)[d]
6-al	39,761	4.6×10^{-10}	5.5×10^{-5}	331.44	1.8×10^{-2}	7.2×10^{-2}
"	23,774	2.3×10^{-10}	2.8×10^{-5}	282.06	7.8×10^{-3}	"
"	67,608	8.6×10^{-10}	1.0×10^{-4}	336.43	3.5×10^{-2}	1.04×10^{-1}
"	66,845	8.5×10^{-10}	1.0×10^{-4}	305.09	3.1×10^{-2}	"
"	82,899	1.1×10^{-9}	1.3×10^{-4}	345.60	4.5×10^{-2}	1.36×10^{-1}
"	85,011	1.1×10^{-9}	1.3×10^{-4}	293.57	3.9×10^{-2}	"
"	105,181	1.4×10^{-9}	1.7×10^{-4}	358.64	6.0×10^{-2}	1.68×10^{-1}
"	134,722	1.8×10^{-9}	2.2×10^{-4}	295.40	6.5×10^{-2}	"
B-al	84,631	8.7×10^{-10}	1.0×10^{-4}	336.22	3.5×10^{-2}	7.2×10^{-2}
"	85,380	8.8×10^{-10}	1.1×10^{-4}	286.12	3.0×10^{-2}	"
"	125,366	1.4×10^{-9}	1.7×10^{-4}	335.41	5.6×10^{-2}	1.04×10^{-1}
"	130,063	1.5×10^{-9}	1.7×10^{-4}	299.40	5.2×10^{-2}	"
"	167,244	1.9×10^{-9}	2.3×10^{-4}	342.42	7.9×10^{-2}	1.36×10^{-1}
"	166,408	1.9×10^{-9}	2.3×10^{-4}	303.67	7.0×10^{-2}	"
"	272,458	3.3×10^{-9}	3.9×10^{-4}	344.37	1.4×10^{-1}	1.68×10^{-1}
"	231,112	2.7×10^{-9}	3.3×10^{-4}	298.63	9.8×10^{-2}	"

[a]6-al = hexanal; B-al = benzaldehyde.

[b]n=3 trials.

[c]x (g per ml) / 2.5 x 10^{-5} ml (injection vol) x 3 ml (dilution vol per 1 g tofu sample) = compound (g) per g tofu sample.

[d]Compound conc. added to soymilk during preparation of tofu block.

[e]y (sample peak area) - b (intercept) / m (slope) = x (determined conc.); benzaldehyde hexane 'standard': b = 1.70 x 10^{4}, m = 7.8 x 10^{13}; hexanal hexane 'standard': b = 7.8 x 10^{3}, m = 7.0 x 10^{13}.

TABLE 12. Headspace mean peak areas for compounds within tofu blocks

Compound	Conc.(ppm)	Mean Peak Area[a]	C.V.(%)
Hexanal	55	649,970	-[b]
"	28	317,945	12.3
"	100	1,153,150	6.9
"	100	1,320,600	10.4
"	130	1,544,200	1.3
"	130	1,752,200	13.2
"	170	1,724,700	5.8
"	220	2,332,400	5.0
Benzaldehyde	100	175,020	-[b]
"	110	246,345	7.8
"	170	298,290	5.2
"	170	332,185	12.4
"	230	375,930	2.1
"	230	430,265	9.4
"	390	461,230	6.9
"	330	546,960	7.9

[a] n=2 sample vials per tofu block.

[b] n=1 sample vial peak area due to syringe malfunction with second vial.

From the water 'standards', values were calculated for the concentration found within the tofu blocks. The same formula was used to calculate the amount of each compound bound:

$$C_{Bd} = \frac{\text{Calculated Std - Tofu}}{\text{Calculated Std}} \quad C_I$$

TABLE 13. Headspace standards for flavor compounds in water

Compound	Conc.(ppm)	Mean Peak Area[a]	C.V.(%)
Hexanal[b]	90	2,850,700	12
"	130	3,707,700	8
"	170	4,940,975	6
"	210	6,151,625	5
Benzaldehyde[c]	90	407,608	9
"	130	650,178	9
"	170	776,803	8
"	210	1,066,480	15

[a] n=4 trials.

[b] Correlation = 0.9748; intercept = 2.367×10^5; slope = 2.784×10^8.

[c] Correlation = 0.9398; intercept = -63449.1; slope = 5.2581×10^7.

Concentrations bound per g of solids and per g of protein also were calculated. Graphical presentations of the binding profiles are shown in Figure 7 for tofu, in Figure 8 for solids and in Figure 9 for protein.

Analysis of variance of amounts bound for hexanal and benzaldehyde, when the compounds were added at the soymilk stage in tofu preparation, showed no statistical difference in binding ($p<0.7389$). This result agrees with results from the tofu blend study. Results shown in Table 11 indicate that although similar amounts of hexanal and benzaldehyde

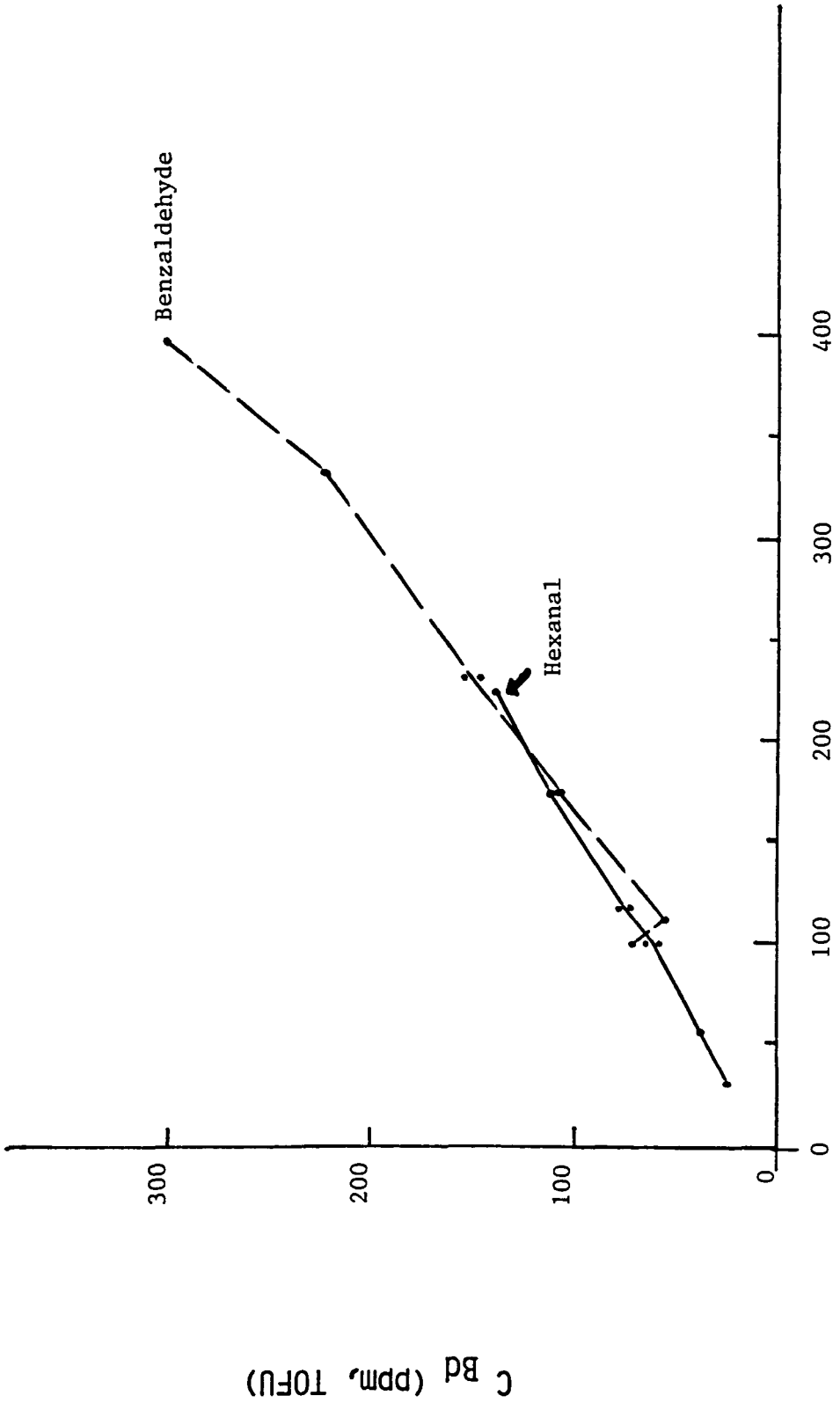

FIGURE 7. Binding profile of flavor compounds in tofu blocks

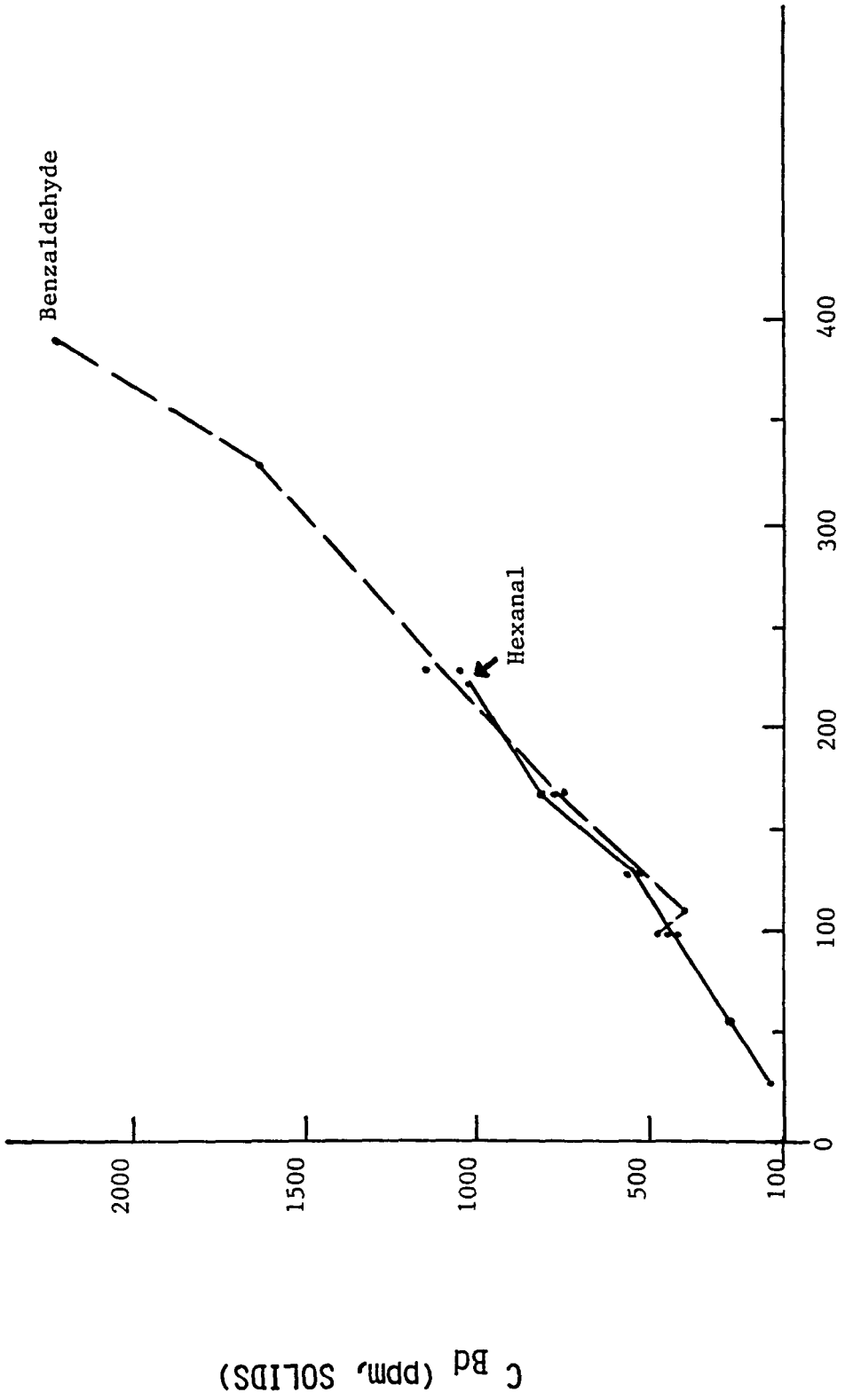

FIGURE 8. Binding profile of flavor compounds in tofu blocks, on a solids basis

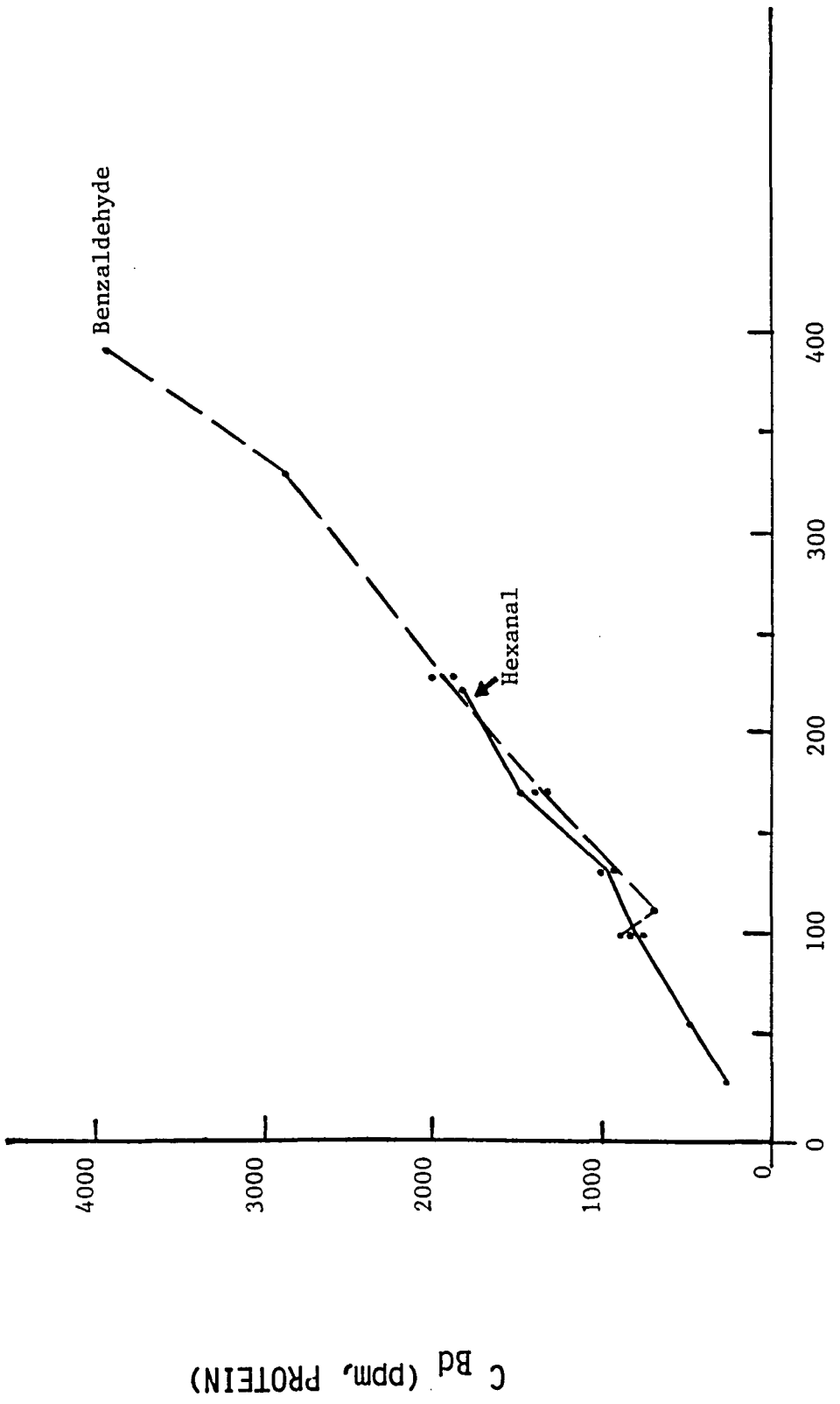

FIGURE 9. Binding profile of flavor compounds in tofu blocks, on a protein basis

were added to the soymilk, more hexanal was lost during preparation, while benzaldehyde was retained to a greater extent within the tofu block. The structure may be affecting retention, the ring-shaped aldehyde being retained more than the straight-chained aldehyde.

When the concentrations bound for hexanal and benzaldehyde were analyzed in the tofu blend, versus those added to tofu at the soymilk stage, they were found to be significantly different at the 0.05 level (T-test) (Figure 10). They were also different ($p<0.05$), when compared on a solids basis (Figure 11) and a protein basis (Figure 12).

It was noted that, on a solids and protein basis, the compounds bound at a greater level in the tofu blend than within the tofu block. When comparing tofu blend and tofu block on a wet-weight basis, (Figure 10) the tofu block binds compounds more than the tofu blend. The tofu blend has 7.9% solids, 92.1% moisture and the tofu block has 13.5% solids, 86.5% moisture. In the tofu blend, the solids have been broken up and dispersed in water, whereas, in the tofu block, some of the water is trapped by the coagulated soy proteins. As shown in Figure 11, although fewer solids are present, greater binding is exhibited by the tofu-blend soy solids, which includes protein. Perhaps the more dispersed state of the denatured proteins allows them to bind greater

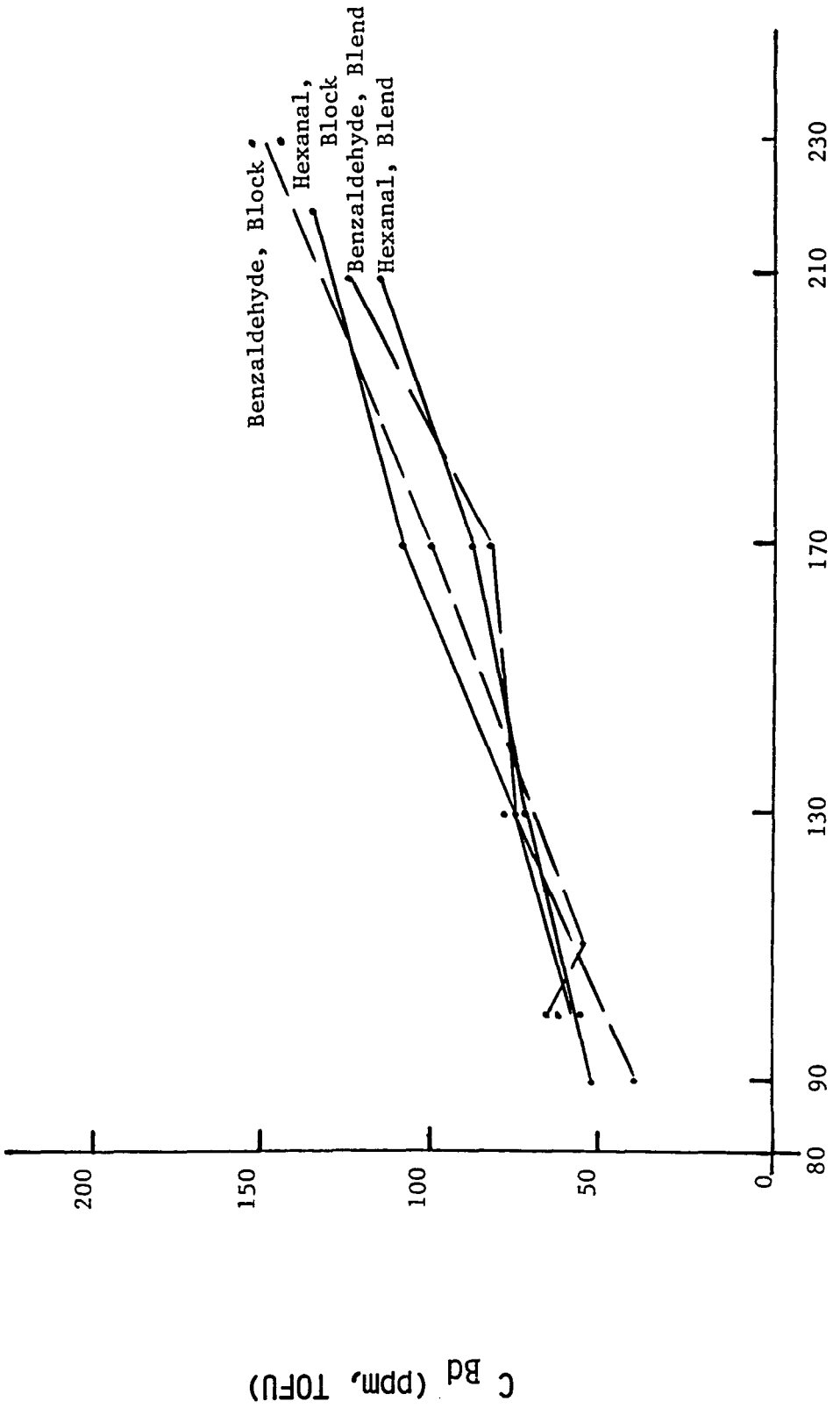

FIGURE 10. Binding profile, tofu blend vs. tofu block

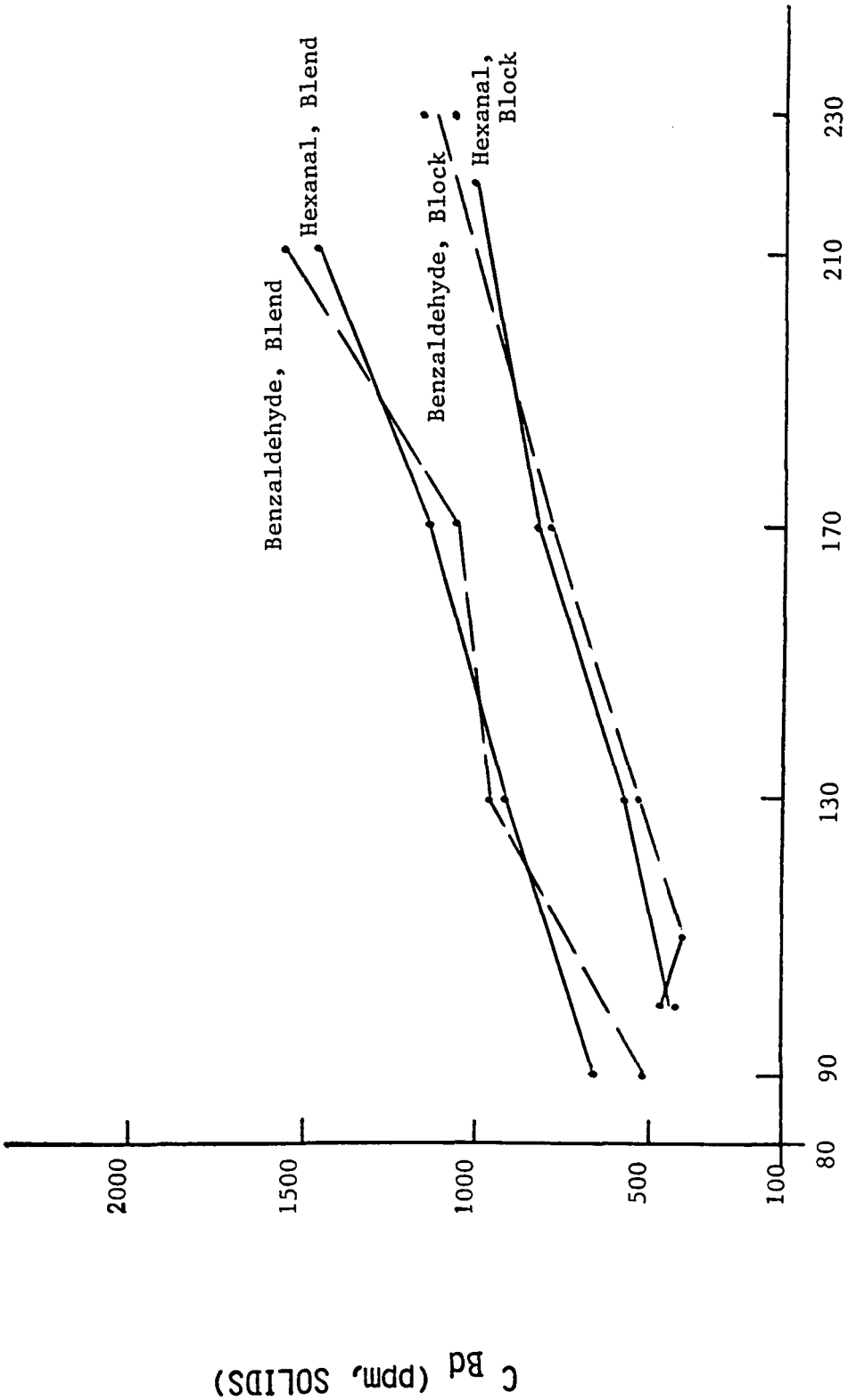

FIGURE 11. Binding profile, tofu blend vs. tofu block, on a solids basis

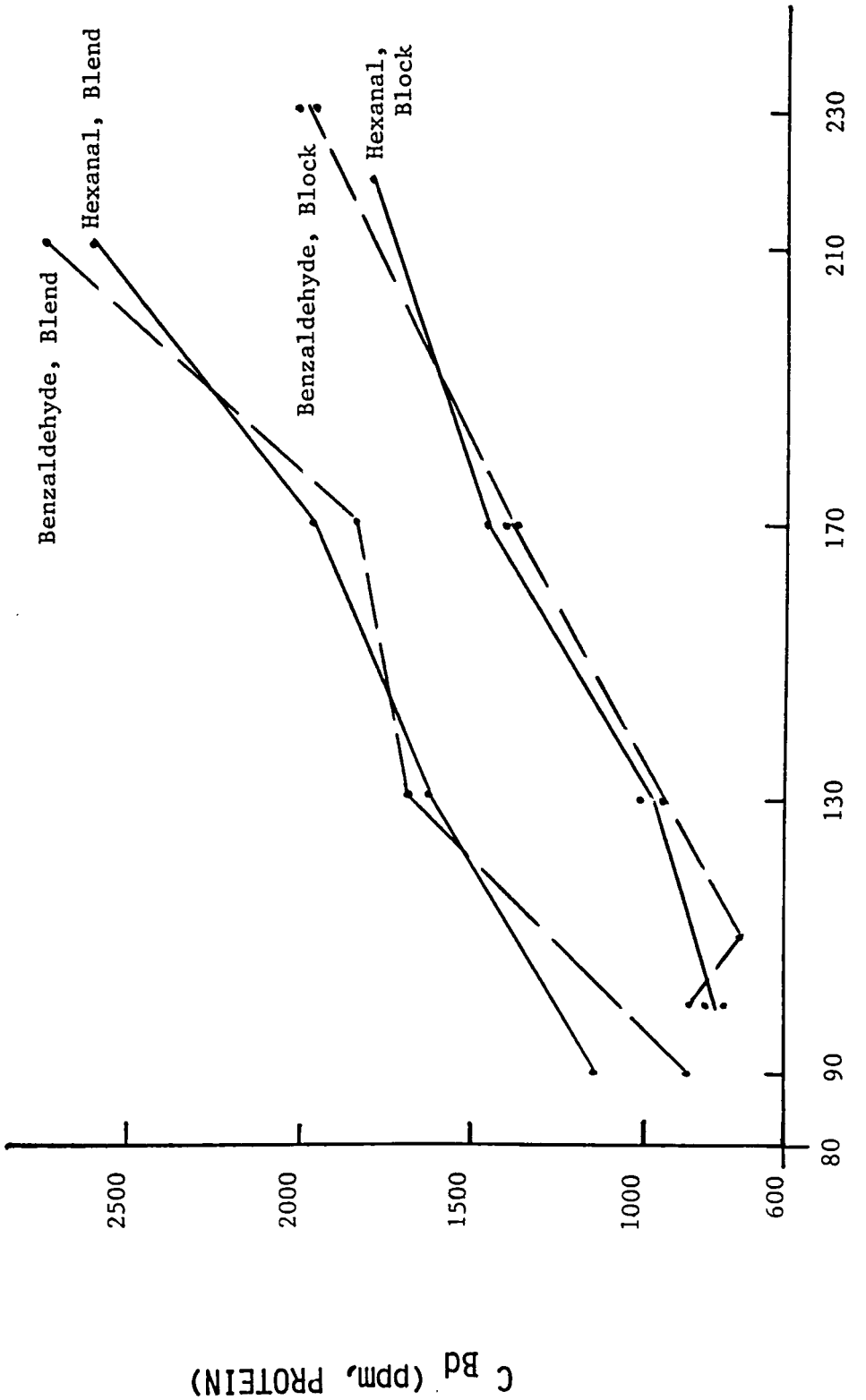

FIGURE 12. Binding profile, tofu blend vs. tofu block, on a protein basis

quantities of added flavor compounds than do the proteins confined in the coagulated tofu mass. Solms et al. (1973) stated that a soluble ligand could interact with the hydrophobic zones of the protein molecule. Arai et al. (1970) and Crowther et al. (1980) found that an increase in denaturation increased the amount of binding. In this study, it seems that, in addition, dispersion of the protein molecule seems to further enhance binding of flavor compounds. Another possibility could be that the blending, physically, further denatured the proteins. The increased denaturation could account for the increased binding.

When binding is compared on a wet-weight basis, the tofu block seemed able to bind more flavor compounds than the tofu blend system (Figure 10). An explanation could be that, during coagulation, the flavor compounds were trapped, or contained, within the tofu block. In the tofu blend, flavors were added after coagulation, thereby eliminating the possibility of entrapment.

CONCLUSIONS AND RECOMMENDATIONS

In these studies, it was found that binding increased as the concentration of the flavor compounds increased, in a linear manner. Also, as the carbon chain length of aldehydes was increased, the amount of binding increased.

At similar concentration levels, hexanal, a straight-chain aldehyde and benzaldehyde, an aromatic aldehyde, did not differ significantly in binding, either in the tofu blend or the tofu block. It was noted, however, that, when adding the compound to soymilk prior to tofu preparation, benzaldehyde was retained better than hexanal within the tofu block.

On a wet-weight basis, the tofu block was able to bind, or trap, the flavor compounds better than the tofu blend. On a solids basis, however, the tofu blend bound more of the compounds. Perhaps dispersion of the soy proteins, or physical denaturation of proteins by blending, may explain the increased amount of binding of the flavor compounds, by the solids in the tofu blend.

In the tofu blend study, it was found that hexanal, an aldehyde, was bound significantly more than 3-hexanone, a ketone, or hexanol, an alcohol. It was noted that binding of the ketone and alcohol were not significantly different. Because more of the aldehyde was bound, its flavor intensity

may be reduced. The flavor intensity of the alcohol and the ketone, because of lower amounts of binding, would not be as greatly affected.

In the bench-top sensory evaluation study, strawberry flavored tofu, which had some sugar and red food coloring added, was found acceptable by the sensory panelists. American cheese flavoring was also acceptable. The flavoring which received the highest hedonic ratings was a seafood flavoring, which contained some salt. It was noted that the salt softened the tofu texture. Future studies could also be done to select flavors to add to tofu, and consumer sensory evaluations of the flavored tofus should be conducted.

In both the preliminary bench-top study and the tofu block study, flavor compound loss was observed. Loss of flavor compounds must be considered if one should choose to add them during tofu preparation. If a flavor compound is relatively expensive, adding it to the soymilk may not be the best time of flavor compound addition. Flavor compounds and glucono δ-lactone could be added to soymilk, before it is packaged and heated, to produce a flavored silken tofu. Another possibility would be to put flavor compounds in a separate flavor packet to be added by the consumer when heating tofu. An additional alternative would be to blend

in flavor compounds, after tofu had been prepared, similar to the procedure used in the tofu blend study.

Minimal tofu preparation was needed for the tofu blend study, and also flavor compound losses were not a problem. For these reasons, future studies of binding of flavor compounds to tofu could be quickly and efficiently done with the tofu blend method. It must be noted that frequent changing of the teflon plunger tip, of the gas-tight syringe, should be done to prevent losses of the gas samples taken.

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APPENDIX

TABLE 14. Preliminary sensory panels - flavored tofu

Flavor	Conc.[a]	Mean Score[b]	Std. dev.	Lab notes:
Cheese	4 T	3.8	0.9	Flavor acceptable
Beef	2 T	2.8	1.0	Grainy, mixes poorly
Seafood	1 T	4.3	0.6	Good flavor, texture soft
Mushroom	1 T	3.6	0.5	Bitter aftertaste
Banana + sugar (1 T)	3/8 t	2.2	1.0	Not acceptable (NA) flavor
Butter + salt (1/4 t)	3/4 t	3.5	1.1	NA flavor, texture soft
Bacon	1/4 t	1.7	1.8	Soymilk curdled, strong flavor
Onion	1/4 t	3.6	0.9	Too bland, soft texture
Strawberry + sugar (1 T) + food coloring (FC)	1 t 2 drops	3.0	1.0	Flavor acceptable, sweeter ?
Pineapple + sugar (1 T) + FC (2 drops)	1/4 t	2.1	1.3	NA pineapple flavor
Seafood	3/4 t	3.6	1.3	Need stronger
Mushroom	3/4 t	2.7	1.2	Bitter aftertaste
Seafood	2 t	4.1	0.7	Slightly soft, very good flavor

Bacon	1/8 t	1.7	1.0	Texture good, NA flavor
Seafood	1 1/2 t	3.5	0.7	
"	2 t	3.2	0.8	
"	2 1/2 t	3.8	1.3	
Strawberry + sugar (2 T) + FC (2 drops)	1 t	3.1	1.1	Need higher flavor level
Nat. Cheddar Cheese	2 t	2.0	0.6	NA cheese flavor
Eng. Cheddar Cheese	2 t	1.6	1.4	" " "
Seafood	2 t	4.0	1.1	One trial
Cheese + FC	4 T	2.0	1.1	" "
Strawberry + FC + sugar (2 T)	2 t	2.3	1.5	" "
Unflavored tofu		2.3	0.8	" " ; seafood most accepted flavor

[a]Concentration added per 500 ml soymilk.

[b]5-inch line scale.

TABLE 15. Flavorings added to tofu

Flavor	Company
Artificial Banana 15280	Food Materials Corporation[a]
Artificial Bacon 16484	" " "
Natural Concentrated Onion Juice 51034	" " "
Natural Butter 16578	" " "
American Cheese (Chez-Tone 101)	Beatrice Foods Company[b]
Beef (Beatreme 2707-B)	" " "
Seafood (Seafood Base-85)	The Nestle Company, Inc.[c]
Mushroom (Artificial Mushroom-8758)	" " "
Pineapple (Imitation Pineapple)	Durkee Famous Foods[d]
Strawberry (Imitation Strawberry extract)	" " "
Natural Cheddar Cheese	Haarmann & Reimer Corporation[e]
Artificial English Cheddar Cheese	" " "

[a]Food Materials Corporation, Chicago, Illinois.

[b]Beatrice Foods Company, Chicago, Illinois.

[c]"Maggi Seasoning", The Nestle Company, Inc., White Plains, New York.

[d]Durkee Famous Foods, Cleveland, Ohio.

[e]Haarmann & Reimer Corporation, Springfield, New Jersey.

TABLE 16. Calculation of amount bound within tofu blend

Compound	ppm	C_I (g)[a]	Water std. mean pk. area[c]	Tofu mean pk. area	C_{Bd}/g tofu[b]
Pentanal	90	9×10^{-5}	2,896,033	2,085,700	2.52×10^{-5}
"	130	1.3×10^{-4}	3,820,700	3,015,533	2.74×10^{-5}
"	170	1.7×10^{-4}	5,600,767	3,691,333	5.80×10^{-5}
"	210	2.1×10^{-4}	6,766,733	4,891,367	5.82×10^{-5}
Hexanal	90	9×10^{-5}	2,969,800	1,288,267	5.10×10^{-5}
"	130	1.3×10^{-4}	3,594,833	1,578,900	7.29×10^{-5}
"	170	1.7×10^{-4}	4,978,167	2,356,667	8.95×10^{-5}
"	210	2.1×10^{-4}	6,122,233	2,669,900	1.18×10^{-4}
Heptanal	90	9×10^{-5}	6,606,667	1,037,857	7.59×10^{-5}
"	130	1.3×10^{-4}	10,216,233	1,682,233	1.09×10^{-4}
"	170	1.7×10^{-4}	11,699,667	2,135,367	1.39×10^{-4}
"	210	2.1×10^{-4}	15,361,667	2,356,500	1.78×10^{-4}
Benzaldehyde	90	9×10^{-5}	397,200	220,217	4.01×10^{-5}
"	130	1.3×10^{-4}	624,717	258,450	7.62×10^{-5}
"	170	1.7×10^{-4}	753,690	383,315	8.35×10^{-5}
"	210	2.1×10^{-4}	1,090,123	444,607	1.25×10^{-4}

Hexanol	90	9×10^{-5}	286,297	169,930	3.66×10^{-5}
"	130	1.3×10^{-4}	342,310	259,243	3.15×10^{-5}
"	170	1.7×10^{-4}	451,513	302,833	5.60×10^{-5}
"	210	2.1×10^{-4}	545,627	347,127	7.64×10^{-5}
3-Hexanone	90	9×10^{-5}	2,895,100	1,771,700	3.49×10^{-5}
"	130	1.3×10^{-4}	4,093,333	2,402,767	5.37×10^{-5}
"	170	1.7×10^{-4}	5,052,767	3,202,933	6.22×10^{-5}
"	210	2.1×10^{-4}	6,049,433	3,755,833	7.96×10^{-5}

[a]Initial compound concentration.

[b]C_{Bd} = (water standard mean peak area - tofu mean peak area / water standard mean peak area) C_I.

[c]n=3 trials.

TABLE 17. Calculation of amount bound within tofu blocks

Cmpd.[b]	ppm	C_I (g)/ g tofu[c]	Calc. water area[d]	Tofu mean pk. area	C_{Bd}/g tofu[a]
6-al	55	5.50×10^{-5}	1,767,938	649,970	3.48×10^{-5}
"	28	2.75×10^{-5}	1,016,255	317,945	1.89×10^{-5}
"	100	1.03×10^{-4}	3,020,744	1,153,150	6.37×10^{-5}
"	100	1.02×10^{-4}	3,020,744	1,320,600	5.74×10^{-5}
"	130	1.29×10^{-4}	3,855,948	1,544,200	7.73×10^{-5}
"	130	1.33×10^{-4}	3,855,948	1,752,200	7.26×10^{-5}
"	170	1.68×10^{-4}	4,969,533	1,724,700	1.10×10^{-4}
"	220	2.18×10^{-4}	6,361,559	2,332,400	1.38×10^{-4}
B-al	100	1.04×10^{-4}	462,362	175,020	6.46×10^{-5}
"	110	1.05×10^{-4}	514,943	246,345	5.48×10^{-5}
"	170	1.67×10^{-4}	830,429	298,290	1.07×10^{-4}
"	170	1.74×10^{-4}	830,429	332,185	1.04×10^{-4}
"	230	2.31×10^{-4}	1,145,915	375,930	1.55×10^{-4}
"	230	2.30×10^{-4}	1,145,915	430,265	1.44×10^{-4}
"	390	3.93×10^{-4}	1,987,212	461,230	3.02×10^{-4}
"	330	3.29×10^{-4}	1,671,726	546,960	2.21×10^{-4}

[a] C_{Bd} = (calculated water standard peak area - tofu peak area / calculated water standard peak area) C_I.

[b] 6-al = hexanal; B-al = benzaldehyde.

[c] Determined amount per g of tofu.

[d] Calculated amount of water 'standard' at concentration level.

TABLE 18. Flavor compounds

Compound	Purity	Company
Pentanal (n-valeraldehyde)	99	Sigma[a]
Hexanal	99	Aldrich[b]
Heptanal (heptaldehyde)	95	"
Hexanol (hexyl alcohol)	98	"
3-Hexanone	98	"
Benzaldehyde	98	"

[a]Sigma Chemical Company, St. Louis, Missouri.

[b]Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.

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