Abstract

FRIEDECK, KRISTOFER GREGG. Soy Protein Fortification of a Low Fat Dairy-Based Ice Cream. (Under the direction of Dr. MaryAnne Drake.)

Since the inception of the 1998 FDA approved health claims linking soy and health, soy protein has been investigated as a potential ingredient in many foods. Products containing soy protein have been characterized as having beany or woody off-flavors, which are unacceptable to many American consumers. Frozen dairy dessert products, in comparison, are appealing to many consumers and may potentially serve as carriers for functional ingredients such as soy protein. Understanding the flavor and sensory impact of soy protein in a dairy-based frozen dessert is important in designing soy fortified dairy products with high market acceptability. The goals of this research are to identify and characterize aroma active compounds contributing to off flavors in soy protein fortified dairy-based frozen desserts, and assess associated sensory impacts.

Descriptive sensory analysis was conducted on frozen dairy dessert mixes formulated with 0, 2, and 4% soy protein isolate (SPI). Duplicate samples (750g) containing an internal standard were distilled by high vacuum transfer followed by extraction of the distillate with diethyl ether. Extracts containing volatile compounds were separated into neutral-basic and acidic fractions and analyzed by gas chromatography-mass spectroscopy (GC-MS) and gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA). Comparison of retention indices, odor properties, and GC-MS data against reference standards was performed for identification of compounds. SPI fortified dessert mixes displayed different textural and color properties in comparison to the 0 % SPI control. Soy fortification increased mouth

viscosity and mouthcoating. Green/grassy and doughy/fatty flavors increased in intensity with added SPI. GC-O analysis revealed higher concentrations of hexanal (green), (*Z*)-4-heptanal (fishy/oily), 2-acetyl-1-pyrroline (nutty/popcorn), and (*E,E*)-2,4-decadienal (fatty/frier oil) in the SPI fortified samples compared to controls. Consumer testing revealed lower acceptability of SPI fortified ice creams in conjunction with lower intensities of pleasing ice cream flavors with added SPI. Chocolate flavored SPI fortified ice creams received higher acceptability scores than vanilla flavored SPI fortified ice creams. Consumers indicated a general knowledge of the healthfulness of low fat dairy desserts and soy foods. This information will aid in the design and optimization of an acceptable soy fortified low fat dairy ice cream.

Soy Protein Fortification of a Low Fat Dairy-based Ice Cream

by
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Biography

Kristofer Gregg Friedeck was born on January 4, 1975, in Seguin, Texas to Walter and Jan Friedeck. His family also includes his wife Melanie, and sister, Michelle.

Kristofer graduated from Southwest Texas State University in August, 2000 with a Bachelor of Science degree in Nutrition and Foods. Kristofer began work in Houston Texas at H.E.B. Grocery Company in November 2000 where he worked as a Chef. He left H.E.B. in April 2001 to pursue a Master of Science degree in Food Science at North Carolina State University.

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Introduction

Soybeans have served as a major source of dietary protein for many people throughout Asia for over 1,000 years. In other parts of the world, however, soybeans have been sought after mostly for their oil. Though the soybean's protein content is greater than 50%, soybean meal from oil processing has been used mostly as animal feed in the Western world (Hettiarachchy and Kalapathy 1997). Recent developments in processing technology and a need to meet demands of new soyfood consumers has brought on the development of a new class of soyfoods, known as "the second generation of soyfoods" (Liu, 1997a). This "second generation of soyfoods" includes among others soy sausages, soy yogurt, and soy cheeses, and soy-based dairy analogs. These foods utilize protein ingredients derived from defatted soybean meal including soy protein concentrate (SPC), soy protein isolate (SPI), and texturized soy protein (Liu 1997a).

Consumption of soyfoods has been on the rise since the establishment of the October, 1998 U.S. FDA-approved soy health claim, which links the intake of products high in soy protein with positive health benefits such as lower risk for heart disease (Henkel 2000; Federal Register 1999). Recently, the market for SPI has seen expansive growth due to the nutritional properties of SPI which make it useful in multiple food applications, such as nutritional beverages, supplements, nutraceuticals, and nutrition bars (Parle 2000). Though this market has enormous growth, there still remains a major problem in expanding the use of soy protein, its characteristic "beany", grassy, and bitter flavors (Kinsella 1979). Primary efforts made in the marketing of soy protein materials for food purposes have usually focused on nutritional value, functionality, and price, and

have underestimated the two main flavor problems associated with these products: the inherent off flavor and the lack of attractive, positive flavors such as that of meats and dairy (Schutte and Ouweland 1979).

Previous research has investigated flavor characteristics of soy protein derivatives. Compounds contributing to the "beany" odor of aqueous soy protein isolate solutions have been studied. Trans-2, 4-decadienal was found to be a major odor contributor to the oxidized, fatty off-aroma of SPI (Boatright and Lei 1999). The high proportion of unsaturated fatty acids in soybeans and an abundance of lipoxygenases are factors that can lead to the development of undesirable flavors in soybean products (Wolf and Cowan 1975). Lipoxygenases catalyze the oxidation of polyunsaturated fatty acids of soy lipids and produce hydroperoxides. The degradation of hydroperoxides leads to the formation of certain volatile compounds which are responsible for the beany, grassy taste of various soybean products, including soy protein products (Liu 1997b). Hydroperoxide degradation, as a subsequent action of lipoxygenase activity, is thought to produce aldehydes (Boatright and Crum, 1997). Aldehydes appear to be the source of beany, grassy off flavors in soy protein products (Boatright and Crum 1997; Davis and others 1987; Qvist and von Sydow 1974; Rackis and others 1979).

Soy-based dairy analogs such as soymilk, soy yogurt, and soy ice cream are available; however there are few soy-fortified dairy-based food products that might appeal to a more traditional dairy consumer (Berry 2002). Drake and others (2000; 2001) evaluated soy protein fortification of dairy yogurts. Although physical and sensory properties were altered, consumer studies indicated an interest and a potential market for soy fortified dairy yogurts and other foods (Drake and Gerard 2003). Frozen foods

represent another opportunity for soy protein addition. The frozen treats market has been and continues to be a leading, high gross food market, representing nearly $ 7 billion or 26% of the total frozen food sales in grocery stores (Anonymous 2002a). Ice cream represents greater than two-thirds of consumer dollars spent on frozen treats (Anonymous 2002b). Currently, there is no literature encompassing the soy protein isolate fortification of a low fat dairy based ice cream for the purpose of meeting any soy protein regulatory labeling requirements. The purpose of this research was to investigate the chemical and sensory effects of soy protein isolate fortification on a low fat dairy-based frozen dessert.

Literature Review

Soybean History

The soybean is widely believed to have originated 4000-5000 years ago in the north and central regions of China (Liu 1997a). Historically, soybeans have played an important part in Asian culture, both as a food and as a medicine (Messina 1995). Soybeans have been consumed throughout Asia for more than 1000 years in a variety of traditional soyfood products. Asian countries still utilize soybeans largely for traditional soyfood production. In 1999, Asian countries accounted for nearly half of the 23 million tons of soybeans exported by the U.S. (Soya Bluebook 2001). Traditional soyfoods, those foods prepared from whole soybeans (Scott and Aldrich 1983), are typically divided into two categories: nonfermented and fermented.

Traditional nonfermented soyfoods include fresh green soybeans, whole dry soybeans, soy nuts, soy sprouts, whole-fat soy flour, soymilk, tofu, okara, and yuba (Goblitz 1995). Fresh green soybeans and whole dried soybeans are prepared and consumed in much the same fashion as Western bean dishes, eaten plainly or serving as additions to soups and stews. In addition to the drying of soybeans to create whole dried varieties, dry roasting may also be employed to create a crunchier product known as a soy nut. Soy nuts can be eaten whole as a snack food, or used in food applications similar to dry roasted peanuts. Soy sprouts, prepared by the soaking, washing, and sprouting of soybeans, are consumed as a vegetable throughout the year in many Asian countries, and are used in soups, salads, and side dishes. Whole-fat soy flour, prepared from the grinding of whole dried soybeans, is used in bakery applications in the place of milk

powder applications or as a substitute for whole-wheat flour. Preparations of soymilk, tofu, okara, and yuba begin with the soaking of whole soybeans, followed by rinsing, grinding, and filtering. The insoluble residue at this point is called okara, it can be used in the making of a dish, salted as a pickle, or fermented in the production of tempeh. Further processing of the filtered soybean liquid includes cooking to yield soymilk. Tofu is made from the further processing of soymilk in which a coagulant is added to precipitate the protein from the soymilk. The precipitate is pressed into a solid, which can then be dried, frozen, or fried. Soymilk is also used in Asian cultures to produce yuba, a creamy yellowish protein-lipid film formed on the top of boiling soymilk. Yuba comes in the form of sheets, sticks, or flakes, and is served as a delicacy, cooked with meat or vegetables, or in soups (Liu 1997c).

Traditional fermented soyfoods include tempeh, miso, soy sauces, natto, and fermented tofu (sufu) and soymilk products (Golbitz 1995). Tempeh is a product produced from the fermentation of dehulled, boiled soybeans by *Rhizopus oligosporus*. This fermentation yields a cakelike product with a clean yeasty odor, covered and completely penetrated by mycelium. Tempeh is usually served as a main dish or meat substitute that, when sliced and deep-fried, has a nutty flavor, pleasant aroma, and crunchy texture (Liu 1997c). Miso is the Japanese word for bean paste and refers to fermented pastelike products, which are used as bases for soups and flavoring agents. Varieties of miso are categorized by the substrate used for making the starter culture, and include rice miso, barley miso, and soybean miso. Miso production begins with making a starter culture, or koji, which consists of a cereal (rice, barley, or soybeans) which, is soaked, cooked, cooled, and inoculated with a mixture of strains of *Aspergillus orzae and*

Aspergillus soyae. The koji is then mixed into previously soaked, steamed soybeans along with salt, and a yeast/bacteria inoculum, which is allowed to ferment and ripen prior to blending and mashing to form the miso (Liu 1997d). Soy sauce is a salty, sharp tasting, dark-brown liquid extracted from fermented mixture of soybeans and wheat. Soy sauce is widely used as an all purpose seasoning in Asia, and is also the widest-accepted fermented soyfood product in western countries (Liu 1997d). Natto is a sweet, aromatic product made from the fermentation whole soybeans with *Bacillus natto*. It is a flavoring agent that is eaten with rice or as a sauce at breakfast or dinner. A number of traditional fermented soyfood products also exist that are made from the fermentation of tofu and soymilk. Sufu, or Chinese cheese, is produced by the fermentation of fresh tofu by fungi, such as Mucor hiemalis or Actinomucor elegans. Sufu is a cubed product with a firm texture, salty taste, and specifically characteristic flavor that is consumed mainly as an appetizer or relish. Soymilk is used to produce soybean yogurt, which is similar to western dairy yogurt, and is basically a mixture of soymilk, whey, and sucrose that has been cooked and cooled and inoculated with *Lactcoccus acidophilus* (Liu 1997d).

Although soybeans were introduced to Europe in the 1700s, little interest developed until the early 1900s, primarily because of the plants inferior flavor quality compared to the native oil and meal products. Soybeans were introduced to the Eastern United States (U.S.) in the late 1800's with production spreading to the Midwest by 1920. Through the early 1930s, soybeans were grown primarily as a pasture and forage crop. However, by 1947, 85% of the crop was harvested for seed processing in the production of oil (Orthoefer 1978). As demand increased, markets developed for soybean oil and later for the high-quality soybean meal used as a protein source for animal feeds.

The U.S. is the leading producer of soybeans worldwide. U.S. production in the harvest year 1999-2000 was 71.9 billion metric tons, 46.2% of the total world production. In 1999, the U.S. exported 32% of its total soybean production at a total value of $4.5 billion (Soya Bluebook 2001). Initially, growth of the U.S. soybean industry was influenced more by the shortage of oil and its relatively high price than the need for dietary protein (Mounts and others 1987). Soybeans dominate production of the seven major oilseeds traded on the international market which include coconut, peanut, cottonseed, rapeseed, sunflower seed and palm kernel. Since 1970, soybean production has been at least double that of any other oilseed, increasing in world oilseed production share from 32% in 1965 to over 50% in the 1980s and 1990s (Smith and Huyser 1987; Soya Bluebook 2001).

Soybeans contain about 40 % fat (Messina 1997) and oil from soybeans is the world's leading vegetable oil and accounts for well over half the fats and oils going into food products in the U.S. The major uses domestically are cooking and salad oils, shortening, and margarine (Scott and Aldrich 1983). The bulk of the soybean oil produced is consumed as salad oil. Industrial applications for soybean oil also exist and include soap manufacture, paints, resins, and drying oil products (Orthoefer 1978).

Soybean Oil Extraction

Nearly all of the soybeans produced in the U.S. are processed in oil extraction plants (Ferrier 1974). The general functions of oilseed extraction are oilseed preparation, solvent extraction, solvent and oil recovery, meal desolventizing and finishing (Becker 1978). With hexane being the accepted solvent for oil extraction of soybeans and oilseeds throughout the world, modern soybean processing generally involves oil extraction by the

use of hexane to produce at first crude soybean oil and defatted meal (Becker 1978; Orthoefer 1978). Solvent extraction of soybeans is a diffusion process in which the solvent (hexane) selectively dissolves miscible oil components (Proctor 1997). Solvent extraction primarily encompasses first, the recovery of lipids from a seed structure which has been prepared to facilitate its penetration by a solvent, and second, the diffusion of the lipid-solvent mixture or miscella to the surface of the solid (Milligan 1976).

The processing begins with the cleaning, cracking and dehulling of dried soybeans. The cracked beans are then conditioned in a steam-jacketed cooker, a vertical stack type dryer or a rotary steam-tube dryer type (Mounts and others 1987). Next conditioned, cracked beans are flaked by being passed between horizontal, pressurized, smooth rollers producing flakes approximately 0.01-.0015 in. thick (Proctor 1997). Properly prepared flakes are essential to a consistent and high-quality extraction (Becker 1978). Flaking is important prior to solvent extraction as solvent can much more readily flow through a bed of flakes than through a bed of soy meats or fine particles (Proctor 1997).

After flaking, the flakes are transported to the extractor by enclosed mass-flow type enclosed conveyors designed to minimize flake breakage (Mounts and others 1987). The extractor is the heart of the process and must convey large volumes of solids, contact these solids with equally large volumes of circulating liquids, and efficiently separate liquids and solids in such a way as to minimize stage-to-stage carryover of liquid on solids (Milligan 1978). Most commercial extraction is by continuous, countercurrent methods (Proctor 1997). Several types of solvent extractors are available and utilize both immersion and percolation technology (Becker 1978; Proctor 1997).

Proceeding through extraction, the miscella becomes richer in oil, resulting in the oil/hexane mixture obtained by flake extraction consisting of 70-75% oil and 25-30% hexane (Mounts and others 1987; Proctor 1997). After extraction, the miscella is filtered to remove the suspended fines, and the solvent is stripped from the oil by a combination of thin film evaporators to ensure complete removal of solvent (Ortheofer 1978). The oil, essentially free of solvent, is cooled to ambient temperature and pumped to storage (Mounts and others 1987).

Soybean Meal from Oil Extraction

In addition to being a valuable source of edible oil, the soybean is also recognized as an excellent source of protein for feeding both animals and man (Liener 1993). Soybeans are approximately 37 % protein (Messina 1997). The major tonnage of soy protein products is derived from dehulled, solvent-extracted intermediates known as defatted or "white" flakes (Lusas and Riaz 1995).

Extracted flakes contain about 50% protein and are mainly used as protein for animal feed (Ferrier 1974). The treatment of the flakes after they leave the extractor determines the properties of the final products and their final use. Flakes coming from the extractor contain about 30% hexane, and the removal of solvent is concerned with controlling three variables that influence protein denaturation and protein solubility: time, temperature, and moisture content (Orthoefer 1978; Wolf and Cowan 1971). In the process of solvent extraction, defatted "white" flakes intended for food use are processed by flash solvent-removing systems which incorporate heat and vacuum to remove residual solvent (Lusas and Riaz 1995; Soy Protein Council 1987). When a plant

operation is for producing oil and meal for animal feed, the desolventizing is usually carried out in one unit referred to as a desolventizing toaster (Johnson 1974).

Soy Protein Preparations

Since the 1950s, U.S. production of soy products for human consumption has grown enormously (Soy Protein Council 1987). Still, only a minimal portion (5% in 1986) of annual worldwide soybean production is processed into traditional soyfoods. Most of the remaining portion is crushed for oil production and defatted meal. Although the oil is mainly used for human consumption, about 2 % of the protein-rich meal is further processed into protein products for food ingredients, with the remaining larger portion, approximately 54% to animal feed based on 1986 figures (Berk 2001; Liu 1997).

For human consumption, the basic edible compounds made from defatted soybean flakes and meal fall into three categories: soy flours and grits, soy protein concentrate (SPC), and soy protein isolate (SPI) (Scott and Aldrich 1983). These three groups are classified based on protein content, with soy flour ranging from 56-59%, SPC from 65-72%, and SPI from 90-92% on a moisture-free basis (Soy Protein Council 1987). Traditional soyfoods are not classified with these products because, as mentioned earlier, they are prepared from whole soybeans using Oriental processes and will not be a main focus of this review.

Over 90% of soybeans consumed by humans *in the U.S.*, excluding soybeans used for soy oil, are in the form of soy-protein products (Soyatech 1990). Edible soybean proteins are used primarily as secondary ingredients in a variety of processed foods (Mounts and others 1987). A noteworthy quality of soybean protein is that, through process manipulation, it can be prepared to have different functional properties that can

be useful in a variety of food systems and applications (Bressani 1981). Functionality of soy protein is related to its surface-active properties, gelling abilities, and fat and water absorption (Orthoefer 1978). Since the 1960's, soy protein used for nutritional and functional food applications has been used in every consumer food category (Soy Protein Council 1987). Until the development of textured soybean proteins in the early 1970s, the major reason for adding soy proteins to foods in the U.S. was for their functional properties rather than as a source of dietary protein (Wolf and Cowan 1975).

Traditionally, in the U.S., food applications of soy protein have included meat extenders and analogs, ingredients in bakery products, and dairy analogs. Additionally, soy protein has been added to other various products to serve certain functions, such as protein in cereals and weight loss beverages, emulsifiers, and as carriers for artificial spices (Hettiarachychy and Kalapathy 1997). Table 1 shows important food uses for soy protein.

Soy flour and grits are the least refined forms of soy protein used for human consumption (Soy Protein Council 1987). Soy flour and grits are obtained by grinding and screening defatted flakes to various sizes (Kalapathy and Hettiarachychy 1997). Per 100 gram sample, defatted soy flour contains 51.46 grams of protein, 33.93 grams of carbohydrate, 17.5 of which is dietary fiber, and 1.22 grams of fat (USDA 2001). Soy flours are available in enzyme-active forms, which are non-heat treated. This form of flour contains enzymes in their active form, which facilitates the natural bleaching of the flour for use bakery applications. Enzyme-active forms of soy flour utilize the ability of soybean lipoxidase to naturally bleach the carotenoid pigments present in the flour. Soybean flours are also available in various degrees of water solubility, expressed as

Protein Dispersibility Index or Nitrogen Solubility Index (Lusas and Rhee 1995). These parameters indicate the extent of protein denaturation and the intensity of heat treatment which has been applied to the starting material. The specification of a specific value of NSI reflects a compromise between the need to maintain the functional properties of the soy proteins or some enzyme activity, and the desire to inactivate anti-nutritional factors and eliminate the beany taste, all in function of the end use (Berk 1992). Uses of soy flour and grits include milk replacers, baked goods, pasta products, infant formulas, additives to coarsely ground meat products, candies, confections, and desserts (Rakosky 1974; Soy Protein Council 1987).

Soy protein concentrates contain a minimum of at least 65% protein on a moisture-free basis, and essentially are flours from which the water- or alcohol-soluble components, including flatulence–promoting sugars and strong flavor compounds, have been leached before drying (Lusas and Rhee 1995). Rakosky (1974) noted three commercial means to immobilize protein in the making of soy protein concentrates: heat, isoelectric field, and alcohol washing (Fig 1). Soy protein concentrates, compared to soy flours, can be used in the same foods as flours but in greater quantities due to their improved flavor, color, and higher protein content (Kinsella 1979). Soy protein concentrates and isolates may also be textured by thermoplastic extrusion and steam texturization for use as meat extenders (Johnson and others 1992). In addition to meat extenders textured soybean proteins can be combined with starches and other powdered proteins to produce textured products that can be used to simulate ground meat, chunks, and strips (Klahorst, 2001).

Of the products made from defatted soybean meal, soy protein isolates are the most highly refined (Soy Protein Council 1987). Traditional isolate production processes include first solubilizing the protein in mild alkali and removing the unsolubilzed fiber by centrifugation. Next, reconcentration of the protein at its isoelectric point (pH 4.2-4.5) and mechanical decanting takes place followed by washing the precipitate with water and again reconcentration by decanting. Neutralization to pH 6.8 occurs next, with the final step being spray-drying (Lusas and Rhee 1995; Hettiarachychy and Kalapathy 1997).

Soy protein isolates were historically developed for making spun fibers for use in meat analogs and sometimes, restructured meats (Campbell 1981; Riaz 1999). Isolated soy proteins are available in a variety of physical forms including dry powder, dry granules, and frozen fiber (Richert and Kolar 1987). As shown in Table I, isolates can be used in a wide range of foods. The form of isolate used in a specific food application varies according to its characteristics such as solubility, gelation, emulsification, dispersibility, viscosity, and retort (Orthoefer 1978; Richert and Kolar 1987; Soy Protein Council 1987). Enzyme-modified isolates are also commercially available in such products as whipping proteins for use in aerated foods (Riaz 1999).

Health Benefits of Dietary Soy Protein

Among the oilseeds the soybean assumes a most prominent position; not only is its protein content high (30-46%), but this protein, when properly processed, is of good nutritional quality (Liener 1978). However it is not the amount of protein in soybeans that is notable, but also the amino acid pattern of soybean protein (Messina 1995). Protein from soybean provides all nine essential amino acids and a form of protein acceptable in almost all diets (Riaz 2000). Unlike other legumes, the limiting amino acids

in soy protein, methione and cysteine, are at high enough levels to meet the protein needs of humans when consumed at the recommended level of protein intake (Messina 1997; Young 1991).

Though the high value of nutrition associated with the amino acid composition of soy protein has been determined, its full nutritional potential is reached only after a certain amount of heat treatment (Liener 1981). Liener (1994) lists the heat labile antinutritional factors in untreated soybeans as protease inhibitors, lectins, and antivitamins. Protease inhibitors, such as the Kunitz trypsin inhibitor and the Bowman-Birkman inhibitor, along with lectins are assumed to be responsible for growth depression in animal studies because of their ability to reduce the digestibility of proteins (Liu 1997b; Liener 1994). Lectins, also known as hemagluttinins, have the ability to agglutinate red blood cells from various species of animals and have a high binding affinity for carbohydrates and a number of cell surfaces which may, in the intestines, be responsible for the poor nutritive value of raw soybeans in the (Liener 1981; Liener 1994; Damodaran 1996). Liener (1981) states that heat treatment is an effective means of inactivating antivitamins D, E, and B_{12} and the effects of these compounds may also be counteracted by vitamin and mineral supplementation in the diet.

Other antinutritional factors associated with soybeans are heat stable and are listed by Liener (1994) as saponins, tannins, estrogens, flatulence factors, allergens, and phytates. Saponins are structurally related compounds in plants that have an adverse effect on animal growth (Liener 1981). It appears though, that the saponins from soybeans are innocuous to chicks, rats, and mice, even when fed at levels three times greater than that in levels found in soybeans (Liener 1994). Tannins are phenolic

compounds that posses the ability to combine with proteins and other polymers and precipitate proteins. Tannins may also bind zinc and inhibit its absorption (von Elbe and Schwartz 1996; Groff and Gropper 1999). For this reason tannins are of nutritional concern, though the levels of tannins in soybeans are low compared to other legumes which have left soybeans virtually ignored in terms of the possible nutritional significance of its tannin content (Liener 1994). Estrogens in soybeans, chemically characterized as isoflavones, can be demonstrated to exhibit estrogenic activity when fed at sufficiently high levels, levels that could only be reached if the isoflavone containing plant material was the sole constituent of the diet (Liener 1994). Certain soybean products, mainly soy flours, containing some percentage of the carbohydrates stachyose and raffinose have the ability to cause flatulence in humans (Liener, 1994). This flatulence is the result of the intact, indigestible carbohydrates being metabolized by human microflora in the large intestine resulting in the production of gas (Liener 1991; Groff and Gropper 1999). Allergens associated with soybeans are of concern as soy products become more widely used, although allergens will display their effects only in those individuals possessing hypersensitivity to the allergens and are generally of harmless when consumed by most others regardless of the amount (Liener 1994). It is known that the phytate content of soybeans used in the diet increases the requirement for certain metallic minerals (Liener 1994). Phytate has been shown to decrease absorption of calcium and zinc in humans. Though phytate in soybeans is partially eliminated by heat treatment, other techniques including enzymatic hydrolysis, ion exchange chromatography, and pH control during soy isolate production have been explored (Groff and Gropper 1999; Liener 1991).

In addition to soybeans supplying adequate protein to the diet, studies have shown that protein from soybeans may be beneficial to human health in other ways. Aside form soy protein being low in saturated fat and cholesterol free; there may be many more advantages to consumption of soy in the diet (Messina 1995). It has been recognized for some time now that consumption of plant proteins often results in significant lowering of low-density lipoproteins (LDL) and total cholesterol levels, which are associated risk factors for cardiovascular disease. (Friedman and Brandon 2001; Krummel 1996).

A recent ecological study found a statistically significant inverse correlation between heart disease mortality rate and total amount of soy products and soy protein intake in women (Nagata 2000). In a clinical study Balmir and others (1996), while studying the effects of an ethanol extract of isolated soy protein on plasma lipid and thyroid hormone concentrations, decreases in serum total and LDL cholesterol in rats and hamsters fed soy protein were observed, in comparison with others fed casein. Without knowing the component of the extract responsible for the reduction in blood cholesterol, it was apparent that consumption of soy protein in and of itself lowers cholesterol (Balmir 1996). A 2-month study carried out by Damasceno and others (2001) assessed the influences of casein and soy protein isolate on lipoprotein oxidation and atherosclerosis progression in cholesterol fed rabbits. Atherosclerosis is the result of atherogenesis, a degenerative systematic process involving arteries. The process of degeneration appears to be affected partly by lipids and lipoproteins, most importantly LDL, which has been oxidized or otherwise modified (Groff and Gropper 1999). The study demonstrated that, compared to casein, soy protein isolate promoted a decrease of lipid peroxides, which had

beneficial effects over atherosclerosis progression in the cholesterol-fed rabbits (Damasceno 2001).

Initiation of atherogenesis may begin in response to some form of endothelial cell injury possibly resulting from mechanical stress or high levels of oxidized LDL, which is known to be toxic to endothelial cells (Groff and Gropper 1999). Large molecular size LDL has also been shown to be injurious to arterial vessels (Kanazawa and others 1997). In vitro and in vivo human and rabbit experiments by Kanazawa and others (1997) reported reduced plasma cholesterol LDL levels, decreased accumulation of cholesterol on arterial vessel walls, suppression of LDL in the production of peroxidized LDL, and suppression in the enlargement of LDL molecules upon incorporating soy protein into the diet. Bakhit and others (1994) displayed that 25 grams of soy protein added to a low fat, low cholesterol diet significantly lowered total cholesterol in men with elevated blood lipids. In 1995 a clinical study by Wong and others resulted in similar findings. The study included hypercholesterolemic subjects which were fed diets conforming to the national Cholesterol Education Program step 1 diet for lowering plasma cholesterol levels in humans, containing either animal protein or soy protein as >75% of total protein intake. The study resulted in greater percent reduction in plasma total cholesterol level for subjects who were fed the soy protein diet rather than the animal protein diet (Wong and others 1995). In a meta-analysis of 38 controlled clinical trials, Anderson and others (1995) examined the relation between soy protein and serum lipid concentrations in humans. The analysis indicated that the consumption of soy protein is associated with significant decreases in serum cholesterol, LDL cholesterol, and triglyceride concentrations and is strongly related to the subjects' initial serum cholesterol

concentrations (Anderson and others 1995). In reviewing clinical studies, Sirtoriet and others (1995) found that generally only subjects with elevated cholesterol showed a clear-cut response to dietary protein change.

Human and non-human clinical studies have focused on soy protein products and their effects on plasma cholesterol levels since the early 1940s (Carroll 1991). Still, despite years of research, the bioactive component of soy protein responsible for changes in blood lipids still remains unknown (Potter 1998). In a review of animal and human studies, Carroll (1995) cited many attempts that have been made to explain the mechanism of action by which dietary proteins and amino acids alter serum total and LDL cholesterol levels. Attempts included hormonal mechanisms involving insulin, glucagon, or thyroid hormones, as well as suggestions involving effects on intestinal absorption of cholesterol and on fecal bile acid excretion (Carroll and Kurowska 1995; Sirtori and others 1995).

Recent research has suggested that soy isoflavones may have an effect resulting in the reduction of LDL cholesterol (Ho and others 1999). Isoflavones are a class of phytoestrogens which are estrogenic compounds in plants (Kurzer and Xu 1997). The only significant dietary sources of isoflavones in the diet are soybeans (Yang and others 2001). Kirk and others (1998) designed a study to determine if isoflavones in soy protein isolate confer protection from atherosclerosis, reduce total plasma cholesterol levels and protect against lipoprotein oxidation in atherosclerosis-susceptible mice. The study utilized female C57BL/6J and female LDL receptor-deficient mice (LDLr-null). The results of the study showed that isoflavone consumption did not alter atherosclerosis, plasma cholesterol levels or oxidative susceptibility in LDLr-null mice, but did lead to a

30% decrease in plasma cholesterol levels in C57BL/6 mice. Without excluding other biologically active compounds that may have been co-extracted with the isoflavones, the researchers concluded that the results were attributable to soy isoflavones (Kirk and others 1998). Conversely, Hodgson and others (1998) found that serum lipid concentrations of humans were not affected by dietary supplementation of isoflavones.

In addition to a reduced risk for heart disease, evidence also supports the hypothesis that adequate isoflavone intake from a product like soy protein may reduce risks for cancer, osteoporosis, and symptoms of menopause (Kurzer and Xu 1997). In a review of animal cancer studies, Hawrylewicz and others (1995) noted that it has been established that several components in soybeans, including isoflavones, inhibit a variety of tumors in various disease tisues in the animal model.

Research done by Donghua and others (1999) demonstrated that isoflavones reduced experimental metastasis of melanoma cells in mice while also inhibiting growth of metastatic tumors that had developed in the lungs. Estrogen treatment of human prostate cancer in mice resulted in inhibition of cancer growth in a study by Adlercreutz and others (2000). Possible mechanisms of estrogen involved in this inhibition have also been demonstrated for dietary phytoestrogens and researchers conclude that in regards to prostate disease, phytoestrogens such as soy isoflavones are strong candidates as well (Adlercreutz 1995; Adlercreutz 2000; Evans 1995). Animal models have often been used to investigate the chemoprotective effects of both synthetic and naturally occurring micronutrients in the diet, however isoflavones have also displayed an inhibitory effect on the growth of many types of cell lines in vitro (Barnes 1995; Molteni and others 1995).

Despite these few findings, clinical data supporting the potential value of phytoestrogens in cancer prevention is still lacking (Setchell and Cassidy 1999).

Isoflavones from soybeans have also been studied in attempts to assess their potential role as an effective treatment for menopausal symptoms and osteoporosis in women. Burke and others (2000) attempted to compare the effects of soy isoflavones and traditional hormone replacement therapy (HRT) in treatment of menopausal symptoms. This study resulted in the findings that, although both HRT and soy isoflavones exhibited beneficial effects on cardiovascular disease, soy isoflavones resulted in less significant relief from menopausal symptoms and osteoporosis than did HRT. Along with these findings, researchers at this time do not view soy isoflavones as a viable alternative to HRT (Burke and others 2000).

Since late 1999, U.S. patents have been issued to companies for methods for isolating and concentrating isoflavones from plant materials for use an an addition to ordinary soy products (Pszczola 2000). Technology has been applied to making isoflavone rich protein materials by extraction to be used in addition to other ingredients in making products such as ready to drink soy protein beverages, powdered soy protein beverages, food bars, and soy yogurt (U.S. Patent 5,994,508). This process of extraction, described by U.S. patent 5,994,508, utilizes pH adjustment to precipitate protein and concentrate isoflavones, then cool temperature extraction is used to separate the specific isoflavone rich proteins. The use of ion-exchange technology has also been applied recently to isolate phytochemicals (U.S. Patent 6,020,471). The method of patent 6,020,471 involves isolating isoflavones using an anion exchange resin with an alcohol or organic solvent as a release agent. Other methods of isolating and concentrating

isoflavones include their recovery from carbohydrate by products, such as soy molasses (U.S. Patent 6,083,553). Patent 6,083,553 involves condensing soy molasses and separating isoflavone-enriched material that is then treated in a way as to convert isoflavone conjugates to isoflavone glucosides. The glucosides are then recovered as enriched aglucone isoflavones.

While researchers continue to assess the many possible health benefits of soy, the food industry and consumers in the market for healthy foods have recognized the cholesterol lowering abilities of soy proteins. On October 26, 1999, the U.S. Food and Drug Administration (FDA) authorized the use, on food labels and in food labeling, of health claims for the association between soy protein and reduced risk of coronary heart disease (CHD). The qualifying criterion for a food to bear the claim is 6.25 grams of soy protein per reference amount customarily consumed. Additionally, the food shall meet the nutrient requirements for "low fat", "low cholesterol" foods. Sources of soy protein identified in the soy protein ruling include foods composed of or derived from whole soybeans and foods that contain processed soy protein ingredients (Federal Register 1999). Model health claims may be used in accordance with FDA regulations regarding food labels and food labeling:

> (1) 25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies ____ grams of soy protein (Federal Register 1999).
>
> (2) Diets low in saturated fat and cholesterol that includes 25 grams of soy protein a day may reduce the risk of heart disease. One serving of [name of food] provides ____ grams of soy protein (Federal Register 1999).

Soy and Functional Foods

Recently, the food industry has seen expansive growth in what is known as "functional food". Recent growth of functional foods far outpace that of conventional foods and supplements, and has attracted the interests of both consumers and food producers (van Poppel 1998) (Locklear 1999). van Poppel (1998) defined functional foods as food that exerts a beneficial health effect beyond the recognized traditional nutritional value of such a food. Within the grouping of functional foods are two categories; 1) potential functional foods (those with the potential for use in human nutrition), and 2) established functional foods (those with proven benefits in human nutrition)

(Riaz 2000). Riaz (2000) categorized soybeans and soy products as established functional foods.

As previously mentioned, soyfoods have been consumed for centuries among Asian populations. It is not until recently that the Western world has seen an increased popularity in soyfoods due to a unique and favorable cultural context (Levington 1987). Levington (1987) observed eight factors that have helped bring acceptance and retail success for soyfoods and a foundation for continued market existence. The first observation was the fact that the aging Baby Boom population has welcomed and propelled the natural foods industry as the supportive context for soyfoods. The next observation was the growth of the natural foods industry itself and the emergence of pioneered "nutrition centers" within supermarkets. The third observation by Levington was the entrance of natural foods into mainstream markets as "light", low-calorie, cholesterol-free has caught the interest of today's dieting and health conscious consumers.

Fourth, the growing Asian population has influenced eating trends and founded an ethnic wave in prepared foods and restaurant fare. A fifth factor in the recent growth in the soyfoods market is a medical awareness and realization of the link between diet and disease. Sixth, the new found interest in gourmet cooking, exotic foods and new "chic" foods has given rise to consumers searching for and uncovering new food like soyfoods. Rising costs of living are seen as yet another factor in the rise in soyfoods as people discover the comparatively inexpensive high quality protein soyfoods offer. Although some soy products, especially analogs of meat, may not be cost comparative. Levingtons' eighth contributing factor was a summarization of the previous seven, and an observance of the fact that soyfoods today are associated with being "natural", "organic", "wholesome", and "simple" (Levington 1987).

Functional foods include the subgroup nutraceuticals, which is defined as a substance considered a food or part of a food that provides medical or health benefits, including the prevention and treatment of disease (American Dietetic Association 1995). From 1995-1999 sales of nutraceuticals, had increased 28.5%, from $12.3 billion to $15.8 billion (Rogers 2001). Many foods made from soy, or those foods containing soy protein in various forms, carry the FDA soy protein health claim and fall into the category of nutraceuticals.

Since the FDA approval of health claims associating soy protein with reduced risks of CHD, there has been much exploration into the development of new ways to include soy into established product formulations as well as new foods with soy (Berry 2000). In 2000, sales of soy products topped $161 million, with a majority of sales coming from mainstream retail grocery stores rather than natural foods outlets

(Thompson 2000). Soy represents a considerable market with high margins and growth rates, educated customers, premium pricing, and a healthy food platform (Tischler 2002).

While there are many acceptable soy products for health conscious consumers such as soy milk and soy ice cream, these products have not been accepted by other consumer groups, such as those that consume traditional dairy products (Berry, 2002). Additionally there exists consumer objection to soy products due to their characteristic "beany" taste. An approach to overcoming this hurdle while still reaching customers with products containing soy is to use soy protein as an ingredient for fortification rather than a base for functional and nutraceutical food products (Berry 2000).

Though few nutraceutical food producers offer dairy products fortified with soy protein, the idea is not new. In fact, a model category of nutraceutical foods that has seen tremendous growth is fortified beverages (Theodore 2001). Fortified, value-added beverages are thought to have the potential to be $2 billion market category (Bello 2000). While new products continue to enter the market, many functional and nutraceutical products have existed on the market for years, though not recognized for their health benefits until recently (Hilliam 1998). An example of this is milk, which is arguably the first functional/nutraceutical beverage, being one of the most commonly fortified foods over the years (Anonymous 2000; Berry 2000). With constant media reminders aligning soy protein intake with good health, dairy food consumers are in search of ways to add soy protein into their diets. Dairy foods represent ideal carriers for soy protein (Berry 2002).

Analysis of flavor in foods

Along with descriptive analysis there are a number of methods used in the isolation and identification of specific flavor compounds in food. Odorants, that contribute to flavor in food, in the simplest sense, can be described as small molecules that, due to their sufficient vapor pressure, predominate in the volatile fractions of foods and beverages (Ericksson 1987). Instrumental flavor analysis of food routinely involves isolation of the volatile food components, followed by concentration and subsequent identification and quantification. Methods employed in the isolation of food flavors include headspace methods, distillation, and solvent extraction (Reineccius 1999).

Headspace techniques consist of static headspace sampling and dynamic headspace or purge and trap. Static headspace involves the placement of a sample in a sealed vial, which is warmed to increase volatile vaporization, and then allowed to stand to establish equilibrium of volatiles in the headspace. Once volatile equilibrium is reached, a small amount of headspace gas is withdrawn from the vial with a syringe and injected directly into a gas chromatograph (GC) injection port. Dynamic headspace employs the use of inert gas to move the analytes away from the sample in the headspace phase. The volatiles are swept away by a flow of carrier gas to a collection trap, which retains organic compounds and allows for the carrier gas to flow through. The trapped volatiles are usually transferred in-line to a GC for analysis (Wampler 1997).

Distillation is used in the isolation of flavor components and is especially useful in the separation of aroma components from other major food constituents. Both steam distillation and high vacuum stripping (molecular distillation), also known as high vacuum transfer (HVT), involve the condensation of volatiles in traps following their release from the food. Steam distillation involves bubbling steam, which is used as a

carrier medium, through an aqueous food to release flavor compounds and then condensing, which results in the collection of isolated volatiles. Due to the use of steam, this method is not effective for the isolation of heat sensitive compounds or those with high-boiling points. HVT, which is generally used for lipid-based foods, is a method of flavor isolation that involves the direct transfer of volatile from food to a condenser. The distance a molecule must travel, from food to condenser, must be small in comparison to the mean free path of molecule. It is for this reason HVT requires very short distances and the use of high- vacuum systems ($<10^{-3}$ Torr). HVT, by eliminating the need for high temperatures, decreases the development of artifacts and results in good recovery of volatiles compared to other methods (Reineccius 1999).

Solvent extraction techniques are used primarily on fat-free foods to obtain flavor isolates. Basis for a particular method of solvent extraction are determined from the physical state of the food, quantity of food, and extracting solvent. Nonpolar solvents, such as hexane, tricholofluormethane, and pentane, are used in alcoholic beverages. Alternatively, the low boiling point and increased polarity of diethyl ether is well suited for most other food flavor compounds (Reineccius 1999).

Distillation and extraction methods used in the isolation of flavor components from foods typically result in the production of a dilute solution of volatiles that must be concentrated prior to GC analysis. Methods used for concentrating solutions include evaporation, freeze concentration, and adsorption. Evaporation exploits the differences in boiling points of flavor compounds and solvents and is typically used to concentrate flavor isolates in organic solvents which are low in boiling point, inert, and thermally stable. Freeze concentration, used to concentrate flavor compounds in aqueous systems

(e.g., steam distillate), is based on the property that water will freeze out of solution as a pure crystal, leaving behind concentrates in the unfrozen fraction. Adsorption is used for concentrating flavor isolates in aqueous solutions. Adsorption techniques involve aqueous distillate being passed through a column of adsorbent material, followed by rinsing the eluting with an organic solvent. This process allows for both concentration and transfer of flavor compounds to a nonpolar solvent for analysis (Reineccius 1999).

The large number of compounds and their diversity within a concentrated isolate poses a problem in terms of gas chromatographic separation and measurement (Feeney and Jennings 1989). For this reason it is desirable to fractionate a flavor isolate prior to GC analysis. Common methods of fractionation for flavor analysis are acid/base and fractionation via silicic acid. Acid/base separation of flavors in an organic solvent is accomplished by first extracting with an aqueous mineral acid, then collecting the aqueous phase for reextraction with organic solvent after adjustment of pH with alkali, which serves as a basic organic phase. The acid phase is extracted from the remaining organic phase following acid extraction with aqueous alkali, then subsequent extracting after adjustment of pH with a minimal acid to produce and acid organic phase. Silicic-acid fractionation involves the flavor concentrate being passed through a silicic acid column followed by elution with a solvent gradient. Through this method, fractionation of the flavor isolate is effectively accomplished by compound polarity. Although this method is simple, there exists a major problem with the production of artifacts (Reineccius 1999).

GC Analysis in Detection and Identification of Flavor Compounds

GC analysis is used extensively in food flavor and aroma analysis and has, since the 1950s, been emergent as a key analytical technique in the field of general volatile analysis (Wright 1999). The extreme sensitivity of GC (picogram detection levels) makes it well suited for flavor research (Reineccius 1999). Gas chromatography-olfactemetry (GC/O) and gas chromatography-mass spectrometry (GC/MS) are both used in the study of flavor and aroma. The technique of GC/O allows both flavor chemists and sensory scientists a method of chemical analysis that provides sensory responses to odor-active chromatographically separated chemicals. GC/MS serves as a means of separation and characterization of chemicals whether odor-active or not, although determining which compounds in a sample are odor-active requires a bioassay (Friedrich and Acree 2000). So, in using GC for flavor analysis it must be determined which constituent or constituents are/is contributing to the characteristic aroma properties of the food being investigated (Teranishi 1998). GC/O can serve as a bioassay for the purpose of revealing odorants in terms of a pattern of smell-activity, thereby eliminating odorless compounds from consideration (Friedrich and Acree 2000).

GC/O is an important analytical tool in flavor research for it allows for the characterization of the odor of single compounds or complex mixtures of volatiles as they emerge from a sniffing port which is attached to a GC. GC/O is often used to detect trace amounts of character odor compounds or off-odors in foods (Mistry and others 1997). It is generally difficult to judge the sensory relevance of volatiles with a single GC/O run. It is for this reason that several techniques have been developed to objectify GC/O data and estimate sensory contributions of single aroma components. Dilution techniques and

time-intensity measurements are the two main GC/O methods used for this purpose (Blank 1997).

CharmAnalysis and aroma extract dilution analysis (AEDA) are two techniques based on odor detection threshold that involve sniffing GC effluent of a diluted series of aroma extract. With ChamAnalysis, the durations of aroma perception detected. This method is based on relative odor detection thresholds of volatiles in relation to known gas chromatographic retention indices of those compounds. In AEDA, the assessor indicates whether or not an aroma is perceived while also noting the sensory descriptor. Results are expressed as the flavor dilution (FD)-factor, and serve as the ratio of concentration of the odor-active compound in the initial extract to the concentration of the most dilute extract in which odor can be detected by the assessor. The FD-factor is a relative measure that represents the odor threshold of a compound at a given concentration. Data from AEDA are represented in a FD chromatogram which indicates retention indices and FD-factors in a logarithmic scale (Blank 1997). It is worth noting that both CharmAnalysis and AEDA are screening methods for potent odorants, meaning they are not corrected for losses occurring during isolation (Grosch 1993).

Osme is a technique developed for measuring the perceived odor intensities of compounds in detected from GC/O, as a time-intensity measurement used in GC/O analysis. The Osme technique allows human subjects to rate aroma intensity on a 16-point scale time-intensity device while also indicating the corresponding aroma characteristics. The technique ideally only requires one injection if working with well trained assessors (da Silva 1994).

Sensory Analysis of Flavor

Chemical flavor compounds contribute to flavors and aromas and greatly affect the overall perception of a particular food or ingredient. Human perception of foods is the result of complex sensory and interpretation processes (Lawless and Heymann 1998). Sensory evaluation, as a science, is a method used to evoke, measure, analyze and interpret responses to products as they are perceived through the senses of sight, smell, touch, taste, and hearing (Stone and Sidel 1993). Current sensory evaluation practices used in industry and academic research involve three classes of methods for testing acceptability and perception of products. Classes of evaluative sensory tests are based on the type of information that is needed about the product including discrimination, description, and/or affection. Discrimination and descriptive tests are analytical in nature while affective sensory tests are hedonic.

Analysis of discrimination tests involves the statistics of frequencies and proportions that are used to determine differences among a set of similar or control products. In descriptive analysis, sensory testing involves methods that are used to quantify perceived intensities of the sensory characteristics of a product. Descriptive analysis is considered to be the most comprehensive and informative sensory evaluation tool, with information from tests that can be applied to consumer acceptance information and instrumental measures by means of such statistical techniques as regression and correlation. Affective or hedonic tests attempt to quantify the degree of liking or disliking of a product. A nine-point hedonic scale, developed by the U.S. Army Food and Container Institute, has achieved widespread use in consumer testing of foods. The scale provides the opportunity to not only rate a product's acceptability, but also look for

segments of people who like different products or styles. As well, the nine-point hedonic scale may provide the opportunity to explore for more complex information having to do with an individual's reason for liking or disliking a product (Lawless & Heymann 1998).

Descriptive analysis is the primary sensory tool for specifying characteristics of complex aroma, fragrance, flavor or odorous mixtures of volatiles (Lawless 1999). Descriptive analysis techniques usually employ a group of highly trained panelists to examine flavor or texture of a product in order to provide a detailed descriptive evaluation or profile. A flavor profile describes the flavor and aroma of a food product. A flavor description names the perceptible factors, their intensities, the order in which they are perceived, aftertaste, and overall impression. Descriptive analysis is applicable to product development and provides complete descriptions of sample differences while also guiding product developers in the modification of a product to meet consumer demands. While training of profile panels requires time and a high degree of member motivation and interest, a panel can provide thorough and reliable descriptions of products in a short time (Larmond 1976).

A method of sensory evaluation called quantitative descriptive analysis (QDA) combines descriptive analysis and ratio scaling in order to obtain quantitative data for certain characteristics of a product. This method of sensory analysis employs a panel that, through consensus, develops reference standards, a standardized vocabulary to describe sensory differences among samples, and a sequence for evaluating each attribute. QDA panels begin training on in groups, with the actual evaluation being done individually (Lawless and Heymann 1998).

Once gathered, sensory test results are statistically analyzed by various techniques, which are chosen to best summarize the sensory data and allow the sensory scientist to make conclusions about the information acquired from an experiment. The use of statistics in the analysis and interpretation of sensory data serves three important functions. The first function of statistics is to provide a simple description of the results. In this capacity, statistics can be used to summarize the data in terms of an estimation of the most likely numbers to represent raw numbers collected from tests. There is also an inferential function of statistics, which can provide some kind of confidence or support for conclusions made about the products and variables that are being tested. A third function of statistics, measurement, can function to estimate strengths of relationships between variables, the size of experimental effects, and the degree of correctness of equations or models which are generated from collected data(Lawless and Heymann 1998).

Flavor Compounds in Soy Protein

Through the utilization of various techniques, many flavor and aroma components have been identified as contributing to the flavor of soy and soy protein products. Phenolic acids, found in plants, have a peculiar effect on foods made from plant materials (Arai and others 1965). In defatted soy flour, Arai and others (1965) investigated extract of soybean flour that had been systematically fractionated and detected the presence of seven phenolic acids which were considered to have influences on soybean flavor. The phenolic acids in defatted soy flour exhibited sour, bitter, astringent and phenol-like flavors, originated from raw soybean and were not decomposed or removed through process of oil-extraction. The seven phenolic compounds identified were syringic acid,

vanillic acid, ferulic acid, gentisic acid, salicylic acid, *p-coumaric* acid, and *p*-hydroxy-benzoic acid. In addition to phenolic compounds, isomers of chlorogenic acids presumed to be isochlorogenic and chlorogenic acids were found to be present in the extracted sample. In relation to flavor, chlorogenic compounds have sour, bitter, and astringent flavors that are exemplified in coffee (Arai and others 1965).

Qvist and von Sydow (1974) investigated model samples of unconventional proteins as aroma precursors. Included in this study was soy protein isolate which was analyzed following treatments that included heating the samples in water, heating in water with fat and starch, and no heat treatment. By using headspace analysis, researchers found SPI heated with water and heated with fat, starch, and water to 121º C to contained considerable amounts of detectable volatile components. In particular, heated samples were mostly found to contain considerably higher concentrations of aldehydes, furans, and hydrogen sulfide compared to unheated samples (Qvist and von Sydow 1974).

Lipid extracts of SPI and hexane-defatted flakes were analyzed following fractionation by silicic-acid chromatography. GC/MS and retention times of authentic standards with Kovat's indices were used for identification. Thirty eight compounds were identified and quantified, with twenty three being reported for the first time in SPI. Classes of compounds being reported for the first time included: butyl, methyl, ethyl, and esters of fatty acids, as well as phenols, diphenyls and phenyl esters. The presence of abeitic acid derivatives was also identified (Boatright and Crum 1997).

2-pentyl pyridine, a flavor compound associated with roasted and fried foods, has been reported in fractions from SPI. In the identification of compounds contributing to

undesirable flavors in SPI, 2-pentyl pyridine was found to be a major contributor of the characteristically throat catching and grassy flavor along with a fishy odor (Boatright and Crum 1997). Further investigation determined that the formation of 2-penyl pyridine in SPI was a synthesis reaction of 2, 4-decadienal and ammonium hydroxide during the process and preparation of SPI (Zhou and Boatright 2000)

Compounds contributing to the "beany" odor of soy protein isolate in aqueous solutions were studied by Boatright and Lei (1999). Undesirable odors in soy protein isolate were analyzed by GC/O and GC/MS, and AEDA. The two most powerful odorants found in this study were dimethyl trisulphide and trans-2,4-decadienal. Trans-2, 4-decadienal was reported to have a characteristically oxidized/fatty odor. (Boatright and Lei 1999). Dimethyl trisulphide has been reported to be a contributor to offensive odors of broccoli florets stored under modified atmosphere conditions (Hansen and others 1992).

The high proportion of unsaturated fatty acids in soybeans and an abundance of lipoxygenases are factors that can lead to the development of undesirable flavors in soybean products (Wolf 1975). Lipoxygenases catalyze the oxidation of polyunsaturated fatty acids of soy lipids and produce hydroperoxides. The degradation of hydroperoxides leads to the formation of certain volatile compounds which are responsible for the beany, grassy taste of various soybean products, including soy protein products (Liu, 1997b). Of the various compounds produced from the degradation of hydroperoxides, aldehydes appear to be a source of objectionable beany and grassy odors and off flavors in soy protein products (Qvist and von Sydow 1974; Rackis and others 1979).

Flavor improvement through enzyme treatment and genetic alteration of soybeans has been researched as a means of eliminating objectionable flavors associated with soybean products. A reduction in the beany odor of raw soybean extracts was accomplished through treatment with bovine liver aldehyde oxidase (Takahashi and others 1979). Flavor improvement through the genetic removal of lipoxygenase has also been researched. Sensory evaluation of soymilk prepared with lipoxygenase-2 deficient soybeans revealed significantly higher flavor scores for aroma and flavor evaluations, with the most noticeable attribute being the lack of rancid flavor when compared to soymilk prepared with normal soybeans (Davies 1987). GC analyses of soybean milk preparations from normal and lipoxygenase deficient soybeans have also been performed to determine if the removal of the lipoxygenase results in flavor differences in finished soybean products. Results of research by Kobayashi and others (1995) showed that samples of soymilk prepared from lipoxygenase deficient soybeans had significantly lower aroma concentration levels of certain compounds thought to be products of the degradative oxidation of polyunsaturated fatty acids, specifically 1-penten-3-ol, (*E*)-2-hexenal, 2-pentylfuran, 1-pentanol, (*Z*)-2-pentenol, (*E*)-2-heptenal, hexanol, nonanal, 1-octen-3-ol, and (*E,E*)-2,4-nonadienal.

Sensory Aspects of Soy

A major problem in expanding the use of soy has been its characteristic "beany", grassy, and bitter flavors (Kinsella 1979). Efforts made in the marketing of soy protein materials for food purposes have usually focused on nutrition value, functionality, and price, and have underestimated the flavor problems associated with these products (Schutte and Ouweland 1979). Schutte and Ouweland (1979) addressed two major

problems in soy protein materials: first the off-flavors inherent in soy and second the absence of attractive, positive flavor such as those found in meat.

Much research has been performed to determine flavor and taste perceptions of soy. To provide a basis for further improvement, Kalbrener and others (1971) assembled and trained a 17-member panel to evaluate odors and flavors of commercial soy protein products including soy flours, concentrates, and isolates. Results showed that the most objectionable flavors were beany and bitter and, that through the use of threshold panel analysis, the undesirable taste constituents of soy are detectable and quite intense at very low concentrations (Kalbrener and others 1971).

Partial substitution of non-fat milk solids with other protein ingredients, mainly soy, may prove to be a means of lowering production costs and improving nutritional values of ice cream (Magdoub 1992). Addition of soy protein ingredients to soft-serve frozen desserts and the resulting flavor outcomes were studied by Simmons and others (1980). The study utilized soy flour (SF), soy protein concentrate (SPC) and soy protein isolate (SPI) at varying percentages in place of non-fat milk solids in vanilla soft-serve frozen dessert formulas. Flavor and odor of the soft-serve dessert formulas were evaluated along with other characteristics by an eight-member, in-house panel of judges over a period of five months. Results of sensory scores for flavor and odor were dramatically reduced when substitution of SF, SPC, and SPI reached 40% and above (Simmons and others 1980).

Undesirable flavor effects attributed to soy were encountered when defatted soy protein powder (DSPP) was added to ice cream to replace non-fat milk solids (MSNF) (Magdoub and others 1992). Standardized ice cream formulations using 0, 10, 20, 30, 40,

and 50% substitution of MSNF with DSPP were used in the study. Sensory evaluation was conducted by a staff member score panel to evaluate various perceptions that included flavor. Although results of the study indicated that DSPP can be successfully used as a substitute for MSNF at concentrations up to 50%, data displayed that substitution of DSPP for MSNF in increasing amounts led to a steady decline in flavor more than body, texture and appearance.

Raidl and Klein (1983) evaluated the effects of soy flour on sensory characteristics of chemically leavened quick breads. Substituting defatted soy flour for wheat flour in quick bread formulas at 5, 10, and 15% levels had an effect on various characteristics including flavor scores. The study involved an 8-11 member semitrained panel of women which were assembled to evaluate bread qualities and characteristics. Research data showed that odor, aftertaste, off taste, and taste desirability were similar to the control bread (0% soy flour) up 10%, and that at 15% substitution these qualities were significantly decreased, thereby offering evidence that the flavor and aroma constituents in soy flour definitely have a negative impact on sensory characteristics (Raidl and Klein, 1983).

As stated, the "beany" characteristic of soybeans have been an unacceptable quality of flavor in many products including soymilk. *Lactobacillus* fermentation of soy has shown to be effective in masking and modifying beany flavor (Wang 1974). Drake and others (2000) investigated dairy yogurt fortification with SPC. Sensory evaluation of yogurts with 1, 2, and 2.5% SPC resulted in increases in soy flavor. Although the mild, delicate flavor of fermented dairy was seen as promising in reducing the objectionable

characteristic flavor of soy, levels of dairy flavor attributes of dairy yogurt were decreased with increased levels of added SPC (Drake and others 2000).

Soy fortified low fat dairy ice cream

While various forms of soy protein have been investigated in respect to flavor and aroma-active chemical compounds, flavor aspects, and consumer acceptability of soy containing foods, there has been little research done in the area of soy fortification of traditional, well established food products. While food companies continue to explore new and innovative ways to develop and market products aimed at meeting consumer demands for acceptable products that provide health benefits, it is important to focus on the fortification of already acceptable products with soy protein.

Frozen foods, in particular, the frozen treats market has been and remains to be a leading, high gross food market, representing nearly $ 7 billion or 26% of the total frozen food sales in grocery stores (Anonymous 2002). Ice cream, including frozen yogurt/tofu and sorbet/sherbet, represents greater than two-thirds of consumer dollars spent on frozen treats (Anonymous 2002). With the establishment of ice cream as a popular consumer market and the fact that dairy is an ideal carrier for soy protein, there exists opportunity for food companies to develop and successfully market fortified frozen dairy desserts to consumers in the environment created by recent FDA approved soy health claims.

Factors to be addressed in undertaking such an endeavor will include research into specific factors affecting soy proteins acceptability as an ingredient in frozen dairy desserts, as well as product formulation requirements necessary to meet FDA guidelines for the labeling of with a soy protein/CHD health claim. The following research

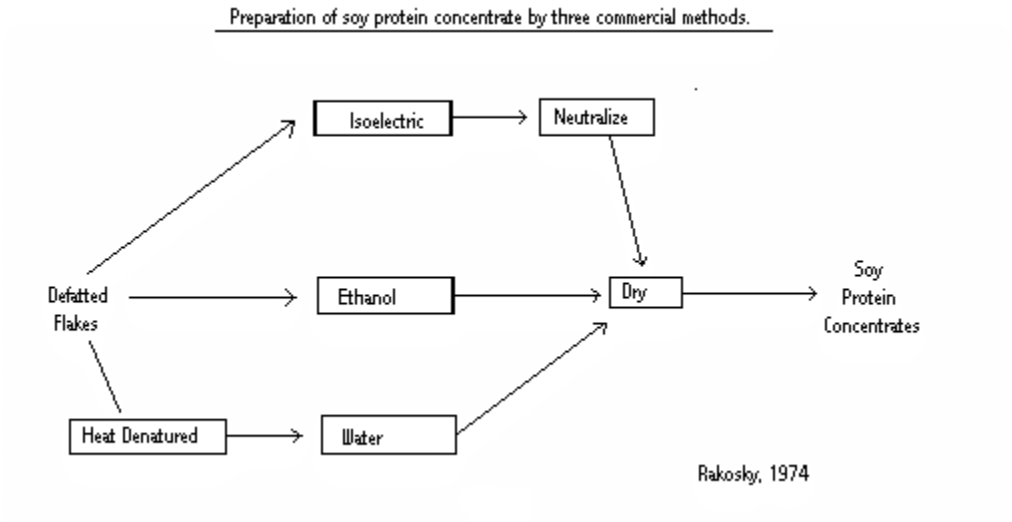
investigates flavor attributes and consumer acceptance of a low-fat ice cream formulation fortified with SPI.

Table 1. Food uses of soybean proteins

Protein From	*Uses*
Defatted flours and grits	Baked goods
	Ground meat extenders
	Meat analogs
	Nonfat dry milk replacers
	Breakfast cereals
	Infant foods
	Diet foods
	Soup mixes
	Confections
Concentrates	Processed meats
	Frozen meat dinners
	Breakfast foods
	Infant foods
Isolates	Whole milk replacers
	Coffee whiteners
	Cake mixes
	Beverage Products
	Confections
	Processed meats
	Meat analogs
	Infant formulas

(Mounts and others., 1987)

Figure 1. Soy Protein Concentrate Preparations



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Manuscript

Soy Protein Fortification of a Low Fat Dairy-Based Ice Cream

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Abstract

Since the inception of the 1998 FDA approved health claims linking soy and health, soy protein has been investigated as a potential ingredient in many foods. Products containing soy protein have been characterized as having beany or woody off-flavors, which are unacceptable by many American consumers. Frozen dairy dessert products, in comparison, are appealing to many consumers and may potentially serve as carriers for functional ingredients such as soy protein. Understanding the flavor and sensory impact of soy protein in a dairy-based frozen dessert is key to designing soy fortified dairy products with high market acceptability. The goals of this research are to identify and characterize aroma active compounds contributing to off flavors in soy protein fortified dairy-based frozen desserts, and assess associated sensory impacts.

Descriptive sensory analysis was conducted on frozen dairy dessert mixes formulated with 0, 2, and 4% soy protein isolate (SPI). Duplicate samples (750g) containing an internal standard were distilled by high vacuum transfer followed by extraction of the distillate with diethyl ether. Extracts containing volatile compounds were separated into neutral-basic and acidic fractions and analyzed by gas chromatography-mass spectroscopy (GC-MS) and gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA). Comparison of retention indices, odor properties, and GC-MS data against reference standards was performed for identification of compounds. SPI fortified dessert mixes displayed different textural and color properties in comparison to the 0 % SPI control. Soy fortification increased mouth viscosity and mouthcoating. Green/grassy and doughy/fatty flavors increased in intensity with added SPI. GC-O analysis revealed higher concentrations of hexanal(green), (*Z*)-4-heptanal (fishy/oily), 2-acetyl-1-pyrroline (nutty/popcorn), and

(*E,E*)-2,4-decadienal (fatty/frier oil) in the SPI fortified samples compared to controls. Consumer testing revealed lower acceptability of SPI fortified ice creams in conjunction with lower intensities of pleasing ice cream flavors with added SPI. Chocolate flavored SPI fortified ice creams received higher acceptability scores than vanilla flavored SPI fortified ice creams. Consumers indicated a general knowledge of the healthfulness of low fat dairy desserts and soy foods. This information will aid in the design and optimization of an acceptable soy fortified low fat dairy ice cream.

Introduction

Soybeans have served as a major source of dietary protein for many people throughout Asia for over 1,000 years. In other parts of the world, however, soybeans have been sought after mostly for their oil. Though the soybean's protein content is greater than 50%, soybean meal from oil processing has been used mostly as animal feed in the Western world (Hettiarachchy and Kalapathy 1997). Recent developments in processing technology and a need to meet demands of new soyfood consumers has brought on the development of a new class of soyfoods, known as "the second generation of soyfoods" (Liu, 1997a). This "second generation of soyfoods" includes among others soy sausages, soy yogurt, and soy cheeses, and soy-based dairy analogs. These foods utilize protein ingredients derived from defatted soybean meal including soy protein concentrate (SPC), soy protein isolate (SPI), and texturized soy protein (Liu 1997a).

Consumption of soyfoods has been on the rise since the establishment of the October, 1998 U.S. FDA-approved soy health claim, which links the intake of products high in soy protein with positive health benefits such as lower risk for heart disease (Henkel 2000; Federal Register 1999). Recently, the market for SPI has seen expansive growth due to the nutritional properties of SPI which make it useful in multiple food applications, such as nutritional beverages, supplements, nutraceuticals, and nutrition bars (Parle 2000). Though this market has enormous growth, there still remains a major problem in expanding the use of soy protein, its characteristic "beany", grassy, and bitter flavors (Kinsella 1979). Primary efforts made in the marketing of soy protein materials for food purposes have usually focused on nutritional value, functionality, and price, and have underestimated the two main flavor

problems associated with these products: the inherent off flavor and the lack of attractive, positive flavors such as that of meats and dairy (Schutte and Ouweland 1979).

Previous research has investigated flavor characteristics of soy protein derivatives. Compounds contributing to the "beany" odor of aqueous soy protein isolate solutions have been studied. Trans-2, 4-decadienal was found to be a major odor contributor to the oxidized, fatty off-aroma of SPI (Boatright and Lei 1999). The high proportion of unsaturated fatty acids in soybeans and an abundance of lipoxygenases are factors that can lead to the development of undesirable flavors in soybean products (Wolf and Cowan 1975). Lipoxygenases catalyze the oxidation of polyunsaturated fatty acids of soy lipids and produce hydroperoxides. The degradation of hydroperoxides leads to the formation of certain volatile compounds which are responsible for the beany, grassy taste of various soybean products, including soy protein products (Liu 1997b). Hydroperoxide degradation, as a subsequent action of lipoxygenase activity, is thought to produce aldehydes (Boatright and Crum, 1997). Aldehydes appear to be the source of beany, grassy off flavors in soy protein products (Boatright and Crum 1997; Davis and others 1987; Qvist and von Sydow 1974; Rackis and others 1979).

Soy-based dairy analogs such as soymilk, soy yogurt, and soy ice cream are available; however there are few soy-fortified dairy-based food products that might appeal to a more traditional dairy consumer (Berry 2002). Drake and others (2000; 2001) evaluated soy protein fortification of dairy yogurts. Although physical and sensory properties were altered, consumer studies indicated an interest and a potential market for soy fortified dairy yogurts and other foods (Drake and Gerard 2003). Frozen foods represent another opportunity for soy protein addition. The frozen treats market has been and continues to be a leading, high gross

food market, representing nearly $ 7 billion or 26% of the total frozen food sales in grocery stores (Anonymous 2002a). Ice cream represents greater than two-thirds of consumer dollars spent on frozen treats (Anonymous 2002b). Currently, there is no literature encompassing the soy protein isolate fortification of a low fat dairy based ice cream for the purpose of meeting any soy protein regulatory labeling requirements. The purpose of this research was to investigate the chemical and sensory effects of soy protein isolate fortification on a low fat dairy-based frozen dessert.

Materials and Methods

Soy Protein Isolate and Low fat ice cream mixes

Prior to initiating experiments, several commercial SPIs (5) were received and screened for overall flavor profiles in low fat ice cream mix, and the presence of soy-associated off flavors. A commercial SPI, Prolisse™ from Cargill Soy Protein Solutions (Wayzata, MN) was chosen for its bland flavor profile. Low fat ice cream mixes were formulated using conventional methods (Arbuckle 1977) and prepared on a 33% (w/w) solids basis and 2% milk fat (w/w) using 4% fat (w/w) milk (50% w/w), sucrose (12% w/w), skim milk (13% w/w), nonfat dry milk powder (15% w/w), and stabilizer (0.45% w/w). All ingredients were obtained from the North Carolina State University dairy plant. The stabilizer AO565-DR1-1 (Danisco, New Century, KA) consisted of mono and diglycerides, guar gum, polysorbate 80, locust bean gum, carageenan, calcium sulfate, and silicon dioxide. Low fat ice cream mixes with 0, 2, and 4% (w/w) added SPI were made by substituting SPI for nonfat dried milk and adjusting water content in order to keep the total solids content constant. Milk, water and dry ingredients were weighed and mixed in a blender (Oster™

6664, Sunbeam Products, Inc, Boca Raton, FL) at low speed for 2 minutes to insure thorough incorporation. Low fat ice cream mixes were then heat treated at 68 °C for 30 minutes, followed, again by blender mixing on low speed for one minute and cooled in an ice bath, prior to placing product in plastic containers for storage. Low fat ice cream mixes were made in duplicate 1.63 kg batches and stored at 4 °C. Volatile aroma components, physical measurements (color, viscosity) and descriptive analysis was conducted on low fat ice cream mixes.

Physical Measurements

Solids and fat were tested on a Mojonnier™ Tester (Mojonnier Bros. Co., Chicago, IL) (Atherton and Newlander 1977). Viscosity of low fat ice cream mixes was conducted at 4 °C using a serrated, concentric CC 25 couette cylinder with a Stress Tech controlled stress rheometer (ATS Rheosystems. Bordenton, NJ/Rheologica Instruments AB, Lund, Sweden). A 10-sec pre-shear at 10 s^{-1} was used with all samples and apparent viscosity was measured over shear rate from 1 to100 s^{-1}. Color was evaluated with a Spectrogard Color System colorimeter (BYK Gardener, Silver Springs, MD) using the CIE color scale (L*, a*, b*). Duplicate evaluations of each low fat ice cream batch were performed.

Sensory Evaluation

Nine trained panelists were selected based on interest, time available, and knowledge of dairy associated flavors. Each panelist (2 male, 7 female) had at least 40 hours of previous training on the evaluation of dairy products using the Spectrum™ descriptive analysis technique (Meilgaard and others 1999). Four 30-min training sessions were conducted to focus on sensory properties of soy fortified frozen dairy dessert mixes. Flavor and texture terms identified and selected by the panelists are listed in Table 1. Color was also evaluated.

Panelists scored responses on 15-point numerical intensity scales anchored on the left with "none" and on the right with "very", and "light" on the left and "dark" on the right for color. Descriptive analysis was conducted by each panelist in triplicate on duplicate batches in a randomized block design for control (0%), 2, and 4% SPI fortified low fat ice cream mixes (Meilgaard and others 1999). Samples (30 g) were served in 59-ml plastic cups fitted with plastic lids (Sweetheart Cup Co., Owings Mills, MD) and labeled with three digit codes. Samples were served at 4 ± 2 °C.

Volatile Flavor Components

High Vacuum Distillation

Glassware used in high vacuum transfer (HVT) was baked at 160 ºC for at least two hours prior to use. Five serial HVT distillations of individual 150 g samples were conducted to distill a total of 750 g of ice cream mix for each treatment (control, 2% SPI, 4% SPI). Low fat ice cream mixes (~150 g) were placed in a 1 L round-bottomed flask, with the first 150 g sample containing 20 µL internal standard which consisted of 2-methyl-3-heptanone (5.44 µg/µL) and 2-methylpentanoic acid (6.18 µg/µL) in methanol. The sample flask was then immersed in a Dewar flask containing liquid nitrogen and swirled to allow freezing of the sample to occur on sides of the flask, increasing surface area for adequate and thorough evaporation of volatile containing moisture. A rough pump/diffusion pump vacuum source, fitted with a receiving tube and waste tube was connected to the sample flask. The HVT unit was equivalent to that used by Karagul-Yuceer and others (2001, 2002). Receiving and waste tubes were immersed in liquid nitrogen-filled Dewar flasks throughout the entire distillation. Vacuum (ca. 10^{-5} torr) was applied to the system for 4 h, with the flask being held at room temperature for the first 2 h., and then 50 ºC in a water bath for the remaining 2 h. Following distillation, aqueous volatile distillate was recovered from the first receiving tube.

Solvent Extraction

Each aqueous distillate was split into three equal portions in odor free 50 mL glass test tubes fitted with PTFE-faced, rubber-lined caps (Fischer Scientific, Fair Lawn, NJ) and washed by shaking for 2 minutes with diethyl ether (3 x 90 mL, EM Science, Gibbstown, NJ). The solvent layer, containing volatile compounds, was collected following each wash. Solvent extracts from each of the 5 distillations for each sample were pooled and stored at -

20 ºC. Pooled extracts were then concentrated to 100 mL at 38 ºC using a Vigreux column (150 × 15 mm, VWR Scientific Products, St. Louis, MO). Extracts were further concentrated to 20 mL under a gentle nitrogen gas stream. After concentration, extracts were washed with aqueous 0.5 M sodium carbonate (2 × 3 mL, Fischer Scientific, Fair Lawn, NJ) and a saturated solution of sodium chloride in water (3 × 1 mL, Fischer Scientific) to solubilize and precipitate acidic compounds (aqueous phase) from the neutral/basic compounds in the solvent phase. The solvent phase, containing the neutral/basic volatiles, was drawn off and collected after each wash. The neutral/basic solvent phase was then dried over anhydrous sodium sulfate (Fisher Scientific) and then concentrated to 0.5 mL under a gentle stream of nitrogen gas. The remaining aqueous (acidic) phases were collected and pooled after each wash, followed by pH adjustment with 18 % hydrochloric acid (Fisher Scientific) to pH 2-2.5. The acidified fraction was then washed with diethyl ether (3 x 5 mL). The acidic compound-containing solvent phase was drawn off of the aqueous phase and collected after each wash, followed by drying and concentrating, as was carried out for the neutral/basic phase. For GC-MS and GC-O analysis of the extracts, a 200 µL portion was taken from the 0.5 mL concentrated extract (netral/basic, acidic), put into a separate measured vial, and further concentrated to 50 µL under a gentle nitrogen gas stream.

Gas Chromatography-Olfactometry (GCO)

The GCO system consisted of a HP5890 series II GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID), a sniffing port, and an on-column injector. Neutral/basic and acidic fractions of each extract (2 µL) were injected into a polar capillary column (DB-WAX 30 m length x 0.25 mm i.d. x 0.25 µm film thickness (df); J & W Scientific, Folson, CA) and a nonpolar column (DB-5ms 30 m length x 0.32 mm i.d. x

0.25 μm df; J & W Scientific, Folson, CA). Column effluent was split 1:1 between the FID and sniffing port using deactivated fused silica capillaries (1 m length x 0.25 mm i.d.). GC oven temperature was programmed from 40 ° C to 200 ° C at a rate of 10 °C /min with initial and final hold times of 3 and 20 min, respectively. A temperature of 250 °C was maintained at the FID and sniffing port. Humidified air at 30 mL/min was supplied to the sniffing port. Two experienced panelists conducted aroma extract dilution analysis of each fraction (AEDA) (Grosch 1993). Extracts containing the neutral/basic and acidic volatiles were diluted at a ratio of 1:3 (v/v) stepwise with diethyl ether. The dilution procedure was performed until no odorants were detected by GCO. The highest mean dilution for each panelist was defined as the flavor dilution (FD) factor (Grosch 1993).

Gas Chromatography-Mass Spectrometry (GC-MS)

The system consisted of an HP5890 Series II GC/HP 5972 mass selective detector (MSD, Hewlett-Packard, Co.). Separations were performed on fused silica capillary column (DB-5ms 30 m length x 0.25 mm i.d. x 0.25 μm df, J&W Scientific). Helium at a constant flow of 1 mL/min, was used as the carrier gas. Oven temperature was programmed from 40 °C to 225 °C at a rate of 4 °C /min with initial and final hold times of 5 and 30 min, respectively. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33-350 a.m.u; EM voltage (Atune+200 V); scan rate, 5 scans/s. Each extract (2 μL) was injected in the on-column mode. Duplicate analyses were performed on each sample.

Identification of Odorants

Identifications of odorant compounds in samples were made by comparison of retention indices (RI), mass spectra data, and odor properties of unknowns with those of

authentic standards analyzed under identical conditions. Tentative identifications were based on comparison of the mass spectra of unknown compounds with those in the National Institute of Standards and Technology (NIST 1992) mass spectral database or on the matching of RI values and odor properties of unknowns to those of authentic standards. Retention indices were calculated by using an n-alkane series (Van de Dool 1963).

Low fat ice creams with added SPI

Mix Composition

Vanilla and chocolate flavored low fat ice creams were prepared with 0 and 4% SPI added in 113.6 L batches. A fortification level of 4% SPI was chosen for consumer acceptance testing for the reason that it contained a significant amount of soy protein (2.64g / 66g serving) to qualify as a "good source" of soy protein, whereas the 2% SPI formula did not. Formulations for flavored ice creams were similar to that used for ice cream mixes prepared for instrumental and descriptive sensory analysis with the exception of the addition of cocoa powder, and liquid flavoring agents. Vanilla flavored low fat ice creams were prepared on a 33% (w/w) solids basis and 2% milk fat (w/w) with 4% fat (w/w) milk (50% w/w), sucrose (12% w/w), skim milk (13% w/w), nonfat dry milk powder (15% w/w), and stabilizer (0.45% w/w) which were all obtained from the North Carolina State University dairy plant. Low fat ice creams with 0 and 4% added SPI and cocoa were made by substituting SPI for nonfat dried milk and adjusting whole milk (4% fat w/w) and water content in order to keep the total solids and fat content constant. Cocoa powder (10/12 alkali processed, Benjamin P. Forbes Co., Cleveland, OH) was added at a rate of 2.7% w/w to the chocolate mixes.

Ingredients were thoroughly mixed for ten minutes in a 189.3 L mixing vat. Mixes were then HTST pasteurized (APV, Tonawanda, NY) at 76 º C for 20 s and homogenized in a two-stage homogenizer (Manton Gaulin, Everett, MA). Pressures were 12.4 and 3.4 MPa for the first and second stages, respectively. The mixes were then rapidly cooled to below 10 º C and aged at 4 º C for 24 hours. Mixes were frozen using a continuous freezer (Tetra Pak Hoyer, San Giuliano Milanese, Italy). Single fold pure Bourbon vanilla

extract (VA01, Virginia Dare, Brooklyn, NY) extract was added to all 4 ice creams at a 0.80 % w/w before freezing. Chocolate flavoring (#15114, David Michael & Co., Philadelphia, PA) was also added to the chocolate mixes prior to freezing at a usage rate of 0.20% w/w. Additionally, the 4% SPI chocolate and vanilla mixes contained a soy masking ingredient (Prosweet MM95, #13068, Virginia Dare, Brooklyn, NY) which was added at 0.05% w/w prior to freezing. The mixes were frozen to -4 º C with 80-90% overrun. Ice cream for consumer sensory analysis was packed into 11.4-L paperboard containers (Huhtamaki, Fulton , NY) with lids. Ice creams were hardened at -28 º C for 48 hours, followed by storage at -20 º C.

Consumer acceptance testing

Consumer evaluation of ice cream was approved by the university institutional review board. An informed consent form listing ingredients and potential ingredients in the ice creams was signed by participants prior to tasting and filling out questionnaires (Fig 1). Faculty, staff, and students of the university community participated in the study. Ice creams were evaluated one week after manufacture. Consumers evaluated all four treatments on one day. Ice creams were tempered at -15 º C for 24 hours and samples (50 ml) were dispensed into 118-ml plastic cups fitted with lids (Sweetheart Cup Co., Owings Mills, MD) and held at -15 $\pm$ 2 º C until samples were served to panelists for tasting. Cups were labeled with random three-digit codes. Ambient temperature water and unsalted crackers were provided to consumers to cleanse the palate before evaluation and in between samples. Each consumer tasted ice creams monadically in a randomized order and evaluated intensities of flavor (vanilla or chocolate), sweetness, and overall dairy flavor on a nine-point hedonic scale anchored on the left with “low” and on the right with “high”. Additionally, appearance,

sweetness, texture, overall flavor, and overall acceptance were measured on a nine-point hedonic scale anchored on the left with "dislike extremely" and on the right with "like extremely" (Fig 2) (Meilgaard 1999).

All participants filled out questionnaires after tasting in order to reduce panelist bias that might originate from questions referring to soy. Panelists were asked questions to determine gender, age, shopping habits, frequency of purchase of low fat/reduced fat foods, types of low fat/reduced fat foods purchased, frequency of consumption of dairy products, frequency of consumption of frozen dairy desserts, manner in which frozen desserts are consumed, types of frozen desserts consumed, factors influencing choice of frozen desserts, and attitudes towards the healthfulness of low fat dairy ice cream and soy foods (Fig 3). To determine attitudes towards low fat dairy ice cream and soy foods, consumers were asked in to best indicate how they felt about the two following statements: 'Low fat ice cream is a healthy food' and 'Soy foods are healthy foods'. Consumers were asked to mark one of the following five possible answers: strongly agree, agree, neither agree nor disagree (don't know), disagree, or strongly disagree. Consumers were also asked a question in order indicate purchase intent of a low fat soy fortified ice cream. Purchase intent was determined by asking the consumer if the price per container were the same and flavor/texture were the same or better, would they purchase a low fat dairy ice cream fortified with soy protein.

Statistical Analysis

Statistical analysis of viscosity, color, descriptive and consumer sensory data were evaluated by analysis of variance (PROC GLM) with means separation. Significantly different means ($P<0.05$) were separated using least square means. Demographic data was

evaluated by frequency distribution with chi square tests (PROC FREQ) (SAS version 8.2, Cary, NC).

Results and Discussion

Physical Measurements

Total solids content of low fat ice cream mixes was 32.89 $\pm$ 1.53 % across all treatments. Total fat for all low fat ice cream mixes was 1.62 $\pm$ 0.21 %. Low fat ice cream mixes had a pH of 6.71 $\pm$ 0.26. Significant viscosity differences were observed by instrumental analysis. All low fat ice cream mixes exhibited shear thinning (Fig 4). Differences in viscosity were observed across a shear rate increase from 1 to 100 s^{-1}, with the SPI fortified mixes exhibiting significantly higher viscosities than that of the control at the lowest (1 s^{-1}) and highest (100 s^{-1}) shear rate (Table 2).

Color differences among low fat ice cream mixes with different levels of soy protein isolate were observed by instrumental analysis. The 4% SPI mix had a significantly lower L* value (76.40) than the 0% control (80.22), indicating ice cream mix fortified with SPI was less white in comparison to that of a typical low-fat ice cream (Table 3). Values for a*, which signify red (+) and green (-), were also significantly different among treatments. The a* values for the low fat ice cream mixes decreased with increasing SPI, demonstrating that the SPI mixes had more green color compared to the control. The b* value, an indicator of blue (-) and yellow (+), was also different among treatments. The b* value for the control increased with increasing levels of SPI, which demonstrates that the mixes increased from blue to yellow in color with increased SPI.

Descriptive Sensory Analysis

Descriptive analysis showed significant differences among treatments for flavor and textural characteristics (Table 4). Low fat ice cream mixes would be expected to have sweet taste and delicate dairy-associated flavors. Sweet taste and sweet aromatic were the highest perceived flavor associated attributes among all mixes. Higher intensities of green/grassy and doughy/fatty were detected in the 2 and 4% SPI mixes. The highest intensities of green/grassy and doughy/fatty were noted in the 4% SPI fortified mixes. Sweet taste, salty taste, and cooked/sulfur decreased with increasing SPI.

Sensory-perceived textural and color differences were also observed. Mouth coating and residual mouth chalkiness increased with the addition of SPI in the low fat ice cream mixes, an attribute that was also noted with soy protein concentrate fortified dairy yogurts (Drake and others 2000). Descriptive sensory analysis also revealed that the SPI fortified mixes exhibited higher thickness/viscosity and were darker in color, in agreement with instrumental analysis.

Volatile Flavor Analysis

Internal standard recovery was consistent between treatments and individual treatment replications, as determined by relative abundance. Flavor contributors in all low fat ice cream mixes were non-specific to either dairy or soy, but were common aroma contributors in both commodities (Bendall 2001; Stephan and Steihart 1999). Control mixes were characterized by lower overall intensities of aroma-active compounds, while the SPI fortified mixes exhibited higher overall intensities of compounds including aldehydes, lactones, and some heat generated compounds. Based on results of AEDA and GC-MS analysis (Table 5), several aroma-active compounds contributed to the development of soy

associated off-flavors in the soy-fortified samples. Aldehydes, and heat induced flavor compounds such as pyrrolines and thiazoles, were the most potent odorants in the SPI-fortified mixes as demonstrated by their higher mean intensities and FD factors in the 2 and 4 % SPI fortified mixes.

The high proportion of unsaturated fatty acids in soybeans and an abundance of lipoxygenases are factors that can lead to the development of undesirable flavors in soybean products (Wolf, 1975). Additionally, headspace volatiles of heated and unheated SPI samples analyzed by GC-MS were found to contain considerable amounts of detectable volatile components, such as aldehydes, furans, and hydrogen sulfide (Qvist and von Sydow, 1974). Soy protein isolate and other defatted soy protein products contain a negligible amount of fat (0.5-1.0 %) (Hettiarachchy and Kalapathy 1997). Additionally, the SPI used in the study has lecithin added prior to spray drying for enhanced dispersibility (Beck 2003). It would be expected then that a higher concentration of lipid oxidation compounds would be found in a dairy product containing SPI compared to a dairy product without added SPI.

Hexanal was positively identified by GCO and GC-MS in all low fat ice cream mixes. The aroma of hexanal was described as green or grassy. Hexanal flavor (green/cut leaf) was also detected by sensory analysis in all low fat ice cream mixes. Hexanal is known to be a volatile constituent in both dairy (Shiratsuchi and others 1994; Karagul-Yuceer and others 2001) and soy protein products, such as SPI's (Boatright and Crum 1997; Boatright and Lei 1999). It is although notable that the highest intensity of green aromatic detected by GCO (Table 5) and sensory evaluation (Table 4) was observed in the 4% SPI mix.

(*Z*)-4-heptenal, a compound with an aroma described as "doughy", "fishy", and (*E,E*)-2,4-decadienal (doughy/fatty) have also both been shown to be volatile components of both milk and soy products (Karagul-Yuceer and others 2001; Boatright and Crum 1997). Trans-2,4-decadienal was also reported to be one of the most powerful odorants in commercial SPI, having a oxidized/fatty odor (Boatright and Lei, 1999). AEDA of low fat ice cream mixes revealed that, though present in all samples, increasingly higher concentrations of both (*Z*)-4-heptenal and (*E,E*)-2,4-decadienal were detected in the 2 and 4% SPI samples. Decanal, having an aroma characteristic described as fatty/sweet or "stinkbug-like" was positively identified by GCO and GC-MS in all sample mixes. Nonanal, also having a fatty/sweet aroma, has been shown to be an aroma-active component of nonfat dry milk (Karagul-Yuceer and others 2001) and commercial SPI (Boatright and Crum 1997). Nonanal was detected by GCO and positively identified by GC-MS in all ice cream mix samples. As with many other aldehydes found with AEDA, decanal and nonanal had higher perceived intensities and higher concentrations in the 2 and 4% SPI samples in comparison to the control mixes. Octanal was tentatively identified by GCO. It is also interesting to note the high FD factors for octanal in the 4% SPI sample only which may be some consequence of an abundance of octanal in the sample. AEDA performed by Boatright and Lei (2001) revealed octanal to be an odor-contributing compound in aqueous SPC slurries.

Methional, a Stecker degradation compound of methionine (Ballance 1961), and the key odor component in boiled potatoes (Mikael and others 1998) was tentatively identified by GCO analysis in the 2 and 4% SPI samples. Sniffers detected higher mean intensities and a higher FD factor in the 4% SPI sample compared to the 2%. Methional has been found to be an off flavor in stored nonfat dry milk (Karagul-Yuceer 2002), and a contributor to meaty-

brothy odors in low-fat cheddar cheese (Milo and Reineccius 1997). (*E*)-2-nonenal, having a characteristically cardboard/paper-like aroma, has been identified as a volatile flavor compound in skim milk powder (Shiratsuchi and others 1994). (*E*)-2-nonenal was tentatively identified by AEDA in all samples with higher perceived concentrations being detected in the 2 and 4% SPI mixes compared to the control. This compound is thought to be an enzymatic or chemical auto-oxidation product of linoleic acid (Pascal and Dubourdieu 1998; Lermusieau and others 2001); it is not surprising to find this compound at high concentrations in a product like SPI and a SPI-fortified dairy product.

Other aroma-active compounds, including 2-acetyl-1-pyroline, thiazolines, and 2-isopropyl-3-methoxypyrazine have also been isolated in liquid cheddar whey (Karagul and others 2003) and farmhouse Cheddar cheese (Suriyaphan 2001). Additionally, 2-isopropyl-3-methoxypyrazine has been reported to be an aroma-active component of nitrogenous bases of SPI and milk protein isolates (Terenina and others 1990) and soybean lecithins (Stephan and Steinhart 1999). 2-acetyl-1-pyrroline, thiazoles and 2-isopropyl-3-methoxypyrazine were tentatively identified in all low fat ice cream samples but had higher FD values in the SPI fortified mixes. These compounds exhibit nutty, toasted, grainy, vegetable-like aroma characteristics. The increased concentrations of these compounds in the SPI fortified samples likely contributed to typical nutty, grainy notes in SPI and soy-associated flavors in 2 and 4% SPI mixes. 3-methyl indole (skatole), having a mothball-like aroma was tentatively identified in only the 4 % mix. Boatright and Crum (1997) reported skatole, to be a component of commercial SPI samples. The lactones, γ-octalactone, γ-nonalactone, δ-decalactone, and δ-undecalactone were all tentatively identified and had high FD factors in the 4% SPI only, while the lactones δ-undecalactone and γ-dodecalactone were only detected in the control

and 2 % SPI mixes. Lactones are non-specific to many foods containing fat, like dairy and soy, and have been identified as aroma-active components of soybean products (Stephan and Steinhart 1999) and dairy products (Bendall 2001, Schiratsutchi 1994).

Flavored ice cream mixes

Total solids content of vanilla and chocolate 0 and 4 % SPI added low fat ice cream mixes was 32.97 $\pm$ 1.71 % across all treatments. Total fat for all flavored low fat ice cream creams was 1.78 $\pm$ 0.17 %.

Consumer attitudes towards low fat dairy and soy

Participating consumers (43 male/58 female) at the university location ranged from < 18 y to 65 (Table 6). Demographic information and consumption characteristics showed that a majority (41%) of consumers agreed with the statement "low fat dairy ice cream is a healthy food", though 30 % chose "neither agree nor disagree" and 21 % disagreed. In response to the statement "Soy foods are healthy foods", 14 % agreed strongly, 68 % agreed, and 17 % chose "neither agree nor disagree". A large percentage of participants (47 %) purchased low fat foods 2-4 times per month, with the most participants (82 %) purchasing low fat dairy items. Fifty nine percent of participants in the university study consumed dairy products > once a week, and a majority (58 %) consumed frozen dairy desserts 2-4 times a month, with 89 % of participants choosing to consume frozen dairy desserts on their own as opposed to as an accompaniment to a dessert. The most popular choice of frozen desserts consumed by participants were premium and super premium ice creams (84 %), low fat/fat free ice creams (52 %), and frozen novelties (44 %). The most important factors influencing frozen dessert choices of participants were flavor (94 %), price (69 %), and texture (51 %). A great number of participants (43 %) indicated they "probably would buy" a low fat dairy ice

cream fortified with soy protein if the price per container were the same and flavor/texture were the same or better.

Consumer acceptance of low fat ice cream with and with out SPI

Consumers detected no significant differences in appearance or overall texture liking among the chocolate and vanilla ice creams (Table 7). Significant differences were observed in consumer perception of flavor intensity between all treatments, with the 0 % SPI chocolate being scored the highest, followed by the 0 % SPI vanilla, 4 % SPI chocolate, and the 4 % SPI vanilla being scored the lowest ($p < 0.05$) in flavor intensity. Both ice creams without soy protein were rated higher in overall dairy intensity than either SPI fortified samples ($p < 0.05$) indicating dairy flavor was diminished with added SPI. Drake and others (2001) also reported decreases in dairy flavor of yogurts with the addition of SPC. Significant differences were also seen in consumer perceived sweetness intensities ($p < 0.05$), with sweetness intensities being scored lower in the 4 % chocolate and vanilla SPI mixes. Significant differences in sweetness liking were also demonstrated with ice creams containing SPI exhibiting lower sweetness liking in conjunction with lower perceived sweetness ($p < 0.05$). The 0 % SPI vanilla and chocolate were rated highest in overall flavor liking and overall acceptance, and the 4 % SPI vanilla had the lowest rating in both overall flavor liking and overall acceptance (Table 7).

Conclusions

Descriptive analysis, instrumental viscosity, and color testing revealed significant differences in SPI fortified mixes compared to the control mixes. SPI fortified mixes

exhibited higher viscosities and darker color. Doughy/fatty and green/cut leaf flavors increased as did chalkiness with the addition of SPI. Additionally, there were contrasting levels of aroma-active compounds that differentiated soy fortified mixes compared to control mixes. On the basis of FD factors obtained from AEDA, aldehydes such as hexanal, *(E,E)*-2,4-decadienal, decanal, nonanal, (*E)*-2-nonenal, and methional were likely major contributors to "green", "doughy", and "fatty/fried" flavors in SPI-fortified mixes. The heat generated compounds 2-acetyl-1-pyrroline and 2-isopropyl-3-methoxypyrazine were also thought to be key contributors to soy associated flavors in dairy-based low fat ice cream mixes fortified with SPI. Consumer sensory testing revealed no significant differences in appearance and texture in frozen 0 and 4 % vanilla and chocolate SPI fortified low fat dairy ice creams, though flavor effects resulting from the addition of SPI were evident. Decreased intensities of (vanilla/chocolate) flavor, dairy flavor, sweetness, as well as decreased sweetness liking, overall flavor liking, and overall acceptance were seen as a result of SPI addition, with the 4 % SPI vanilla being scored the lowest in overall acceptability. Consumer acceptability scores for the 4 % SPI chocolate ice cream were slightly higher indicating that chocolate flavor may have a greater impact on masking soy flavor than vanilla. Low fat dairy ice cream fortified with soy protein isolate may be a means of positively presenting soy protein to a broader market of American consumers.

Table 1. Preparation of reference materials for descriptive sensory evaluation of soy protein fortified low fat low fat ice cream mixes

Term	Definition	Reference
Sweet Aromatic	Aroma associated with sweet/milky flavors	Vanilla extract in milk (1% in 100 mL skim milk)
Green/Cut leaf	Aromatic associated with hexanal	Hexanal in MeOH (1000 ppm) on filter paper in sniff jars
Cooked/Sulfur	Sulfurous aromatic associated with cooked milk and boiled eggs	Boiled Egg
Astringent	Shrinking, drawing or puckering of the oral epithelium as a result of exposure to substances such as alum or tannins	Tea solution (6 tea bags soaked in 1 qt hot water for 1 hr)
Doughy/Fatty	Aromatic reminiscent of old fryer oil and/or biscuit dough	2, 4-decadienal in MeOH (1000 ppm) on filter paper in sniff jars
Salty Taste	Taste sensation associated with salt	(0.2 % NaCl in water)
Sweet Taste	Taste sensation associated with sugar	(10 and 11.875 % sucrose in water)
Ropiness	Degree to which a strand/rope will form when spoon is dipped into product and slowly pulled out	Assignment by panel
Thickness/ Viscosity	Force required to move spoon back and forth in product	1 = Water 3 = Cream 9 = Syrup 14 = Sweetened Condensed Milk
Color	The intensity or strength of a color from light to dark	0 = Dannon nonfat plain yogurt 14 = Pancake syrup

Definitions and references taken from Meilgaard and others 1999

Table 2 . Viscosity values (Pa s) of low fat ice cream mixes with varying levels of Soy Protein Isolate.

Shear Rate	0% SPI	2% SPI	4% SPI
1 s^{-1}	0.67[a]	5.53[b]	16.55[c]
100 s^{-1}	0.15[a]	0.54[b]	0.82[c]

[a,b,c] Means within a row without common superscripts differ ($P < 0.05$).
Mixes were evaluated at 4 ° C.

Table 3. Color values (L*, a*, b*) [1] of low fat ice cream mixes with varying levels of SPI (Soy Protein Isolate).

Color Values	0% SPI	2% SPI	4% SPI
L*	80.22[a]	78.39[a, b]	76.40 [b]
a*	4.12[a]	2.99[b]	2.12[c]
b*	10.47[a]	11.11[b]	11.99[c]

[a,b,c] Means within a row without common superscripts differ (P < 0.05).

[1]L* = Black (0) to white (100); a* = green (-) to red (+); b* = blue (-) to yellow (+).

Table 4. Sensory characteristics of low fat ice cream mixes with and without soy protein isolate.

Attributes	*Control (0% SPI)*	*2% SPI*	*4% SPI*
sweet aromatic	2.3[a]	2.0[a]	1.9[a]
green	0.4[a]	0.88[b]	1.2[c]
cooked/sulfur	1.19[a]	0.9[b]	0.77[b]
doughy/fatty	1.17[a]	1.75[b]	2.29[c]
salty	1.7[a]	1.0[b]	0.7[c]
sweet	10.1[a]	9.3[b]	8.4[c]
astringency	1.4[a]	1.81[a,b]	1.88[b]
chalkiness	1.0[a]	1.28[b]	1.61[c]
mouth coating	1.75[a]	2.37[b]	2.99[c]
ropiness	4.78[a]	4.56[a]	3.7[b]
thickness/viscosity	3.84[a]	5.18[b]	6.94[c]
color	1.74[a]	3.0[b]	3.45[c]

[a,b,c] Means within a row without common superscripts differ ($P < 0.05$). Intensities were scored on a 15-point numerical intensity scale.

Table 5. Aroma-active compounds in low fat ice cream mixes with and without soy protein isolate.

No.	Chemical	Fraction [c]	Odor [d]	Mean Intensity [e]			RI [f]		Log_3 FD Factor [g]		
				0%	2%	4%	DB 5	DB Wax	0%	2%	4%
1	Acetic Acid [a]	A	Sour/ Vinegar	2.5	2.25	1.5	710	1439	1.5	<1	<1
2	Butanoic Acid [b]	A	Rancid cheese	2.5	2	2	836	1632	4	2.5	1.5
3	Diacetyl [a]	N/B, A	Buttery	1	1	2	628	968	<1	<1	<1
4	Hexanal [b]	N/B	Green	1.5	2	2.75	812	1059	<1	2.5	3
5	(Z)-4-heptanal [a]	N/B	Fishy/Rancid/ Fatty	3	3	4.25	914	1236	<1	<1	1.5
6	Methional [a]	N/B	Potato	nd	2	4	923	1437	-	<1	2
7	2-acetyl-1-pyrroline [a]	N/B	Corn Chip	3.75	4	5.25	935	1320	2	3	4.5
8	1-octen-3-one [a]	N/B, A	Mushroom	1.75	2.25	3.25	991	1300	<1	2.5	3
9	Hexanoic Acid [b]	A	Sour	1.5	-	1	1012	1785	<1	-	<1
10	octanal [b]	N/B	Fatty	nd	3.5	2.5	1010	-	-	-	4
11	2-acetylthiazole [a]	N/B	Popcorn/Corn Chip	nd	1.5	3	1029	-	-	<1	1
12	Unknown	N/B	Coconut/Fruity	nd	nd	2.75	1051	-	-	-	1
13	Unknown	N/B	Mushroom/Earthy	1.75	3.5	3	1063	-	<1	2.5	3.5
14	2-isopropyl-3-methoxy pyrazine [a]	N/B	Earthy/Green pepper	1.25	2	3.5	1096	-	<1	<1	3
15	Nonanal [b]	N/B	Fatty/Sweet/Stale	2	3	3	1117	1314	<1	<1	3.5
16	2-acetyl-2-thiazoline [a]	N/B	Corn Chip/Popcorn	nd	2	2.75	1126	-	-	<1	1.5
17	2-phenylethanol [a]	N/B	Rosey?Cereal/ Herbal	2	3.5	5	1168	-	<1	3	7.5
18	(*E*)-2-nonenal [a]	N/B	Old Book/Cucumber	2.25	2	4.5	1176	-	<1	1	4.5
19	Decanal [b]	N/B	Fatty/Sweet/ Stink-bug	2	2.5	3.5	1217	1493	<1	2.5	4.5
20	γ-octalactone [a]	N/B	Coconut	nd	nd	2.75	1276	-	-	-	1.5
21	Decanol [a]	N/B	Hay/Straw/ Tobacco/ Sweet	2	2.5	3.5	1294	-	1	1.5	3.5
22	(*E,E*)-2,4-decadienal [b]	N/B	Fatty/Grain/Fried/ Oatmeal/Sweet	1.5	2	3	1330	1728	<1	2.5	5.5
23	γ-nonalactone [a]	N/B	Coconut	nd	nd	3	1372	-	-	-	2.5
24	Unknown	N/B	Fatty/Sweet/ Cereal	2	3	4.5	1405	1773	<1	4	1
25	Skatole [a]	N/B	Mothball	nd	nd	3.5	1428	-	-	-	1.5
26	β-ionone [a]	N/B	Hay/Sweet/ Perfume/ Fruity	1	2	3.5	1493	1914	<1	1	3.5
27	δ-decalactone [a]	N/B	Sweet/Coconut/ Lactone	2	1.5	4.25	1564	-	<1	2	5.5
28	Unknown	N/B	Plastic	nd	nd	2.75	1611	-	-	-	1
29	δ-undeca-lactone [a]	N/B	Coconut/Lactone	3.25	3.75	nd	1623	2233	2	2.5	-
30	γ-dodeca-lactone [b]	N/B	Sweet/Fruity/ Peach	nd	nd	3.75	1692	-	-	-	4.5
31	γ-dodecalactone [a]	N/B	Peach/Sweet/ Solvent/ Fruity	1.5	1.5	nd	1729	2138	<1	<1	-

[a,b] Compounds were identified by comparison with authentic standards on the following criteria: [a] retention index (RI) on DB-5 and DB-Wax columns and odor property at the sniffing port; or [b] retention index (RI) on DB-5 and DB-Waxcolumns and odor property at the sniffing port mass spectra in the electron impact mode. [c] N/B, neutral/basic fraction; A, acidic fraction. [d] Odor description at the GC-sniffing port GC-O. [e] Mean odor intensity sniffed at GC-sniffing port. [f] Retention indices were calculated from GC-O data . [g] FD Factor, flavor dilution factors on DB-5 and DB-Wax column for neutral/basic and acidic fractions, respectively.

Table 6. Demographic information and consumption characteristics of participant in the study (N = 101)

Gender (% male / female)	43/58	
Age Group	2 %	< 18 y
	35 %	18 to 25 y
	37 %	26-35 y
	14 %	36-45 y
	7 %	46-55 y
	6%	56-65 y
Shop for Household (% yes / no)	95 / 5	
"Lowfat ice cream is a healthy food"	8 %	strongly agree
	41 %	agree
	30 %	neither agree nor disagree
	21 %	disagree
	1 %	strongly disagree
Purchase of low/reduced fat foods	2 %	never
	23 %	< once a month
	47 %	2-4 times a month
	29 %	More than once a week
Low/reduced fat items purchased	51 %	snacks (chips/cookies)
	53 %	Condiments/accompaniments (mayonnaise , salad dressing)
	82 %	Dairy (milk, cheese, yogurt, ice cream, etc.)
	44 %	Meats (hot dogs, luncheon meats, etc)
	51 %	Convenience items (low fat frozen / ready to eat entrees)
	30 %	Breads/Cakes
Frequency of consumption of dairy products	1 %	do not consume
	8 %	< once a month
	29 %	2-4 times a month
	59 %	> once a week
	3 %	> once a day
Frequency of frozen dairy dessert consumption	5 %	never
	11 %	< once a month
	58 %	2-4 times a month
	26 %	more than once a week
Manner in which dairy desserts consumed	89 %	on its own (bowl of ice cream, ice cream cone)
	11 %	as an accompaniment (cake and ice cream, pie a la mode, etc.)
Types of frozen desserts consumed	84 %	premium, super premium ice cream
	52 %	low fat/ fat free ice cream
	44 %	Novelties (ice cream sandwhich, etc.)
	35 %	sherbet
	32 %	Frozen confections (popsicles, fruit juice bars, etc.)
	23 %	non-dairy desserts (sorbet, fruit ice, tofu/soy frozen products)
Factors influencing frozen dessert choices	69 %	price
	94 %	flavor
	24 %	availability
	51 %	texture
	37 %	health
"Soy food are healthy foods"	14 %	strongly agree
	68 %	agree
	17 %	neither agree nor disagree
	2 %	disagree
"Would purchase low fat ice cream fortified with soy protein if price and flavor/texture was the same or better"	27 %	definitely would buy
	43 %	probably would buy
	28 %	maybe would buy
	3 %	would not buy

Table 7. Consumer Acceptability of ice cream with and without 4 % SPI (n = 101)

Attributes	*0 % SPI Vanilla*	*0 % SPI Chocolate*	*4% SPI Vanilla*	*4% SPI Chocolate*
Appearance	6.68 [a]	6.72 [a]	6.73 [a]	6.79 [a]
Flavor Intensity	6.67 [b]	7.32 [a]	4.78 [d]	6.20 [c]
Overall Dairy Flavor Intensity	5.95 [a]	5.66 [a]	4.95 [b]	5.24 [b]
Sweetness Intensity	6.70 [a]	6.20 [b]	5.26 [c]	5.49 [c]
Sweetness Liking	6.6 [a]	6.47 [a]	4.93 [c]	5.99 [b]
Overall Flavor Liking	6.65 [a]	6.53 [a]	4.27 [c]	5.81 [b]
Overall Texture Liking	6.53 [a]	6.65 [a]	6.27 [a]	6.38 [a]
Overall Acceptance	6.69 [a]	6.69 [a]	4.71 [c]	5.98 [b]

[a,b,c] Means within a row without common superscripts differ ($P < 0.05$).

Figure 1. Information provided to participants

INFORMED CONSENT / LOW FAT ICE CREAM CONSUMER PANEL

If you are allergic to any type of dairy product do not participate in this sensory panel.

The low fat ice cream samples may contain one or more of the following ingredients:

mono/diglycerides	**oat flour**
vanilla extract	**chocolate flavor**
cocoa powder	**soy protein**
whey protein	**milk**

Please sign and date below. Your signature below indicates that you have read this consent form and are aware of possible ingredients in these samples:

_____ _____

sign your name print your name

_____

date

Figure 2. Consumer Questionnaire Screener Form

CONSUMER LOW FAT ICE CREAM QUESTIONNAIRE

Please check the appropriate answer for the following demographic information:

1. Sex _____male _____female

2. Age group
   _____18 years or younger
   _____19 – 25 years
   _____26 – 35 years
   _____36 – 45 years
   _____46 – 55 years
   _____56 – 65 years
   _____65 years or older

Please answer the following questions. There are no right or wrong answers. We want to know about you
and what you think. Please ask if you have any questions!

3. Do you shop for your household, even if it is you alone? _____yes _____no

4. Check one letter that best indicates how you feel about the following statement:
   "Low fat ice cream is a healthy food"

   a. ___ strongly agree
   b. ___agree
   c. ___neither agree nor disagree (don't know)
   d. ___disagree
   e. ___strongly agree

5. How often do you purchase low or reduced fat foods? (check one)

   a. ___Never
   b. ___less than once a month
   c. ___2-4 times a month
   d. ___More than once a week

6. What reduced fat or low fat foods do you routinely purchase? (check all that apply)

   a. ___Snack foods (chips/cookies)
   b. ___Condiments/Accompaniments (mayonnaise, salad dressing, etc.)
   c. ___Dairy (milk, cheese, yogurt, ice cream, etc.)
   d. ___Meats (luncheon meats, hot dogs, etc.)
   e. ___Convenience items (low fat frozen entrees, low fat ready to eat entrees)
   f. ___Breads/Cakes

7. How often do you consume dairy products? (milk, cheese, yogurt, ice cream etc.) (check one)
   a. ___I do not eat dairy products
   b. ___less than once a month
   c. ___2-4 times a month
   d. ___More than once a week
   e. ___More than once a day

8. How often do you consume frozen dairy desserts (ice cream, frozen yogurt)? (check one answer)
    a. ___Never
    b. ___Less than once per month
    c. ___2 to 4 times per month
    d. ___More than once a week

9. How do you usually consume frozen desserts? (Check one)

    a. ___On its own (bowl of ice cream, ice cream cone, etc.)
    b. ___As an accompaniment (Pie a la mode, cake and ice cream etc.)

10. What types of frozen desserts do you consume? (Check all that apply)

    a. ___ Premium, Super-premium ice cream (Eddy's, Breyers, Ben & Jerry's, etc.)
    b. ___ Low fat /fat free ice cream (Ben & Jerry's/Haagen Dazs Low fat,etc.)
    c. ___ Novelties (Ice cream sandwiches, Klondike bars, etc)
    d. ___ Sherbet
    e. ___ Frozen Confections (popsicles, Fruit juice bars)
    f. ___ Non-dairy frozen desserts (Sorbet, Fruit Ice, Soy/Tofu frozen products)

11. What factors influence your choice of frozen dessert products? Check all that apply:
    _____Price _____Texture
    _____Flavor _____Health
    _____Availability

12. Check <u>one letter</u> that best indicates how you feel about the following statement:
    "Soy foods are healthy foods" (check one)
    a. ___Strongly Agree
    b. ___Agree
    c. ___Neither disagree nor agree (don't know)
    d. ___Disagree
    e. ___Strongly disagree

13. If the price per container were the same and the flavor/texture were the same or better, would you purchase a low fat ice cream fortified with soy protein? (check one)

    a. ___Definitely would buy
    b. ___Probably would buy
    c. ___Maybe would buy
    d. ___Would not buy

Figure 3. Consumer Scoring Ballot

Please bite a piece of saltine cracker and sip some water to rinse your palette. Taste the ice cream samples with the sample number indicated. After you have tasted the product, please circle your response for the questions below. **PLEASE ANSWER ALL QUESTIONS. We want to know what you think!!**

Sample _____

Appearance

1 2 3 4 5 6 7 8 9
Dislike Extremely — Neither like nor dislike — Like Extremely

Flavor (Vanilla *or* Chocolate) Intensity

1 2 3 4 5 6 7 8 9
Low — Moderate — High

Overall Milk/Dairy Flavor Intensity

1 2 3 4 5 6 7 8 9
Low — Moderate — High

Sweetness Intensity

1 2 3 4 5 6 7 8 9
Low — Moderate — High

Sweetness Liking

1 2 3 4 5 6 7 8 9
Dislike Extremely — Neither like nor dislike — Like Extremely

Overall Flavor Liking

1 2 3 4 5 6 7 8 9
Dislike Extremely — Neither like nor dislike — Like Extremely

Overall Texture Liking

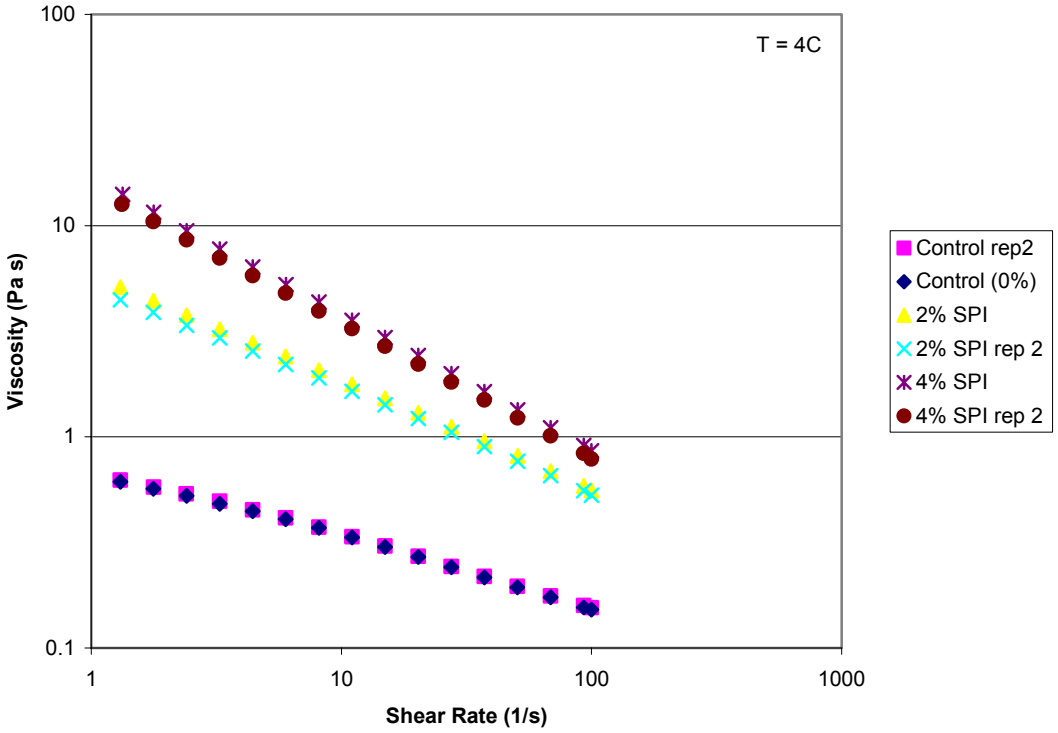
1 2 3 4 5 6 7 8 9
Dislike Extremly — Neither like nor dislike — Like Extremely

Overall Acceptance

1 2 3 4 5 6 7 8 9
Dislike Extremely — Neither like nor dislike — Like Extremely

Comments:

Figure 4. Viscosity of low fat ice cream mixes



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