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Effect of the use of lipoxygenase-free soybean line on the sensory attributes of soymilk and tofu

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Effect of the use of lipoxygenase-free soybean line on the sensory attributes of soymilk and tofu

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TABLE OF CONTENTS

ABSTRACT

Flavor is the most important factor limiting consumption of soy foods in western markets. Soybean lines lacking one or several of the lipoxygenase isozymes involved in flavor have recently been developed. Sensory evaluation provides relevant information related to actual perception which presents some challenges that must be addressed. Perception of flavor by people varies according to their background. The use of terminology to describe the sensory attributes must be addressed. Also, the dynamism of the perceived intensity of some sensory attributes must be considered for evaluation of soy products.

In the first part of the present study, three groups of panelists from the United States, Japan and China were trained to describe and compare soymilk or tofu made from lipoxygenase-free and normal soybean lines. Soymilk made from lipoxygenase-free soybeans had less cooked beany aroma, less cooked beany flavor and less astringency. Panelists noted no differences between lipoxygenase-free and normal soybeans for milky flavor, wheat flavor, thickness, chalkiness or aftertaste. Tofu made from lipoxygenase-free soybeans had less cooked beany flavor than that made from normal soybeans. There were no differences in cooked beany aroma, raw beany aroma, raw beany flavor, wheat flavor, astringency, hardness, darkness or yellowness.

The second part of the research comprised the description of the concept related to the 'beany' attributes. A panel of five judges was trained to describe and evaluate different soymilks. Descriptors used to describe 'beaniness' were 'raw as hexanal' for flavor or aroma, 'grassy' flavor, and 'sweet as green floral' flavor. Significant differences ($p<0.01$) were found among commercial soymilk and soymilks processed from normal or lipoxygenase-free soybean lines and lipoxygenase-free soybeans stored for 1 yr at 4°C.

The third part of the study addressed the changes in perception with time for bitterness and beaniness in soymilk. Time intensity was used to evaluate the effect of sugar (0, 2.5 % and 5%), temperature (5°C and 25°C) and soybean line (normal and lipoxygenase-free) on the intensity of bitterness and beaniness of soymilk. Temperature did not have any important effect on bitterness or beaniness. Total intensity of bitterness was reduced by the use of lipoxygenase-free soybeans or addition of sugar to the 2.5 % level. Beaniness maximum intensity was reduced with the combined effect of using lipoxygenase-free soybeans and 2.5 % sugar.

CHAPTER 1. GENERAL INTRODUCTION

It is believed widely that soybeans [*Glycine max.* (L.) Merril] originated in the north and central regions of China 4000-5000 years ago (Lui, 1997). Soybeans were used as a food by ancient Chinese cultures because of their remarkable nutritive qualities. Chinese civilizations have used soybeans as a source of oil and meal and also have developed a large variety of non-fermented and fermented soy foods. China was the world leader in soybean production and export until 1954. Since then, the United States has occupied first place in production and export of soybeans (Lui, 1997).

The first introduction of soybeans samples into the United States occurred in 1764 when Samuel Bower immigrated to Georgia (Lui, 1997). The soybeans were planted the next year. Despite early introduction of the crop into the United States, it was not until the early 1900's that a large-scale production occurred. The first harvest was in 1920 and the first processing plant of soybeans was opened in 1922. Today, the United States is the source of about 50% of the total world production of soybeans (Asbridge, 1995; Lui, 1997).

In contrast to the Chinese culture, where soybeans are used as an important source of human food, the United States used this crop exclusively for the production of oil and meal for animal feed. Soybeans in the United States, as well as in other markets, are the main oilseed. The success of this crop is based on several factors including high nutritional quality of the soy products and favorable economical considerations for the farmer and processor. The chemical composition of soybeans has made it a preferred crop among many other legumes and cereals. Soybeans have a combination of amino acids and fatty acids that make the crop ideal for production of high quality oil and protein. Those two components are highly concentrated in soybeans. The protein constitutes about 35% and oil 17%, on a wet basis. The remainder is carbohydrates (31%) and ash (4.4%).

Flavor is the most important factor limiting consumption of soy foods in western markets. Consumers aware of the health benefits of soy would also like soy products to taste good. Even though soymilk and tofu are vegetable products, consumers expect them not to have such strong plant-like flavors. Flavors in soy products are a complex mixtures of many different chemical compounds such as ketones, esters, alcohols and aldehydes. Some of these chemical compounds are already present in the beans, while others are the result of the breakdown by enzymes such as lipoxygenase present in soybeans.

Several approaches have been used to make the flavor of soy products more acceptable. Most have focused on processing methodologies to decrease objectionable flavors. Another approach to attack flavor problems in soybeans is the use of breeding to develop new soybean lines lacking one or several of the lipoxygenase isozymes. More recently, a line of soybeans lacking the three isozymes involved in flavor has been developed (Hajika et al., 1992). Soy-products from the new line are expected to have a more desirable 'bland' flavor.

Evaluation of the impact of lipoxygenase-free soybean lines on flavor of soy products needs to be addressed by instrumental and sensory methodologies. The gas chromatograph and the electronic nose are instruments used for flavor evaluation. These instruments do not provide information about how flavor is perceived by humans. Sensory evaluation provides information related to actual perception, but also presents some challenges that must be addressed. Perception of flavor by people varies according to the background of people. A potential problem in the evaluation of soy foods is the variation that can exist in perception of sensory attributes from panelists from different cultures. The use of terminology to describe the sensory attributes must be considered. Research is needed about the description of the attributes traditionally used to describe 'beany'. These terms and concepts need to be defined to properly evaluate soy foods. Finally, the dynamism of the perceived intensity of some sensory attributes must be addressed for evaluation of soy products. The perception of some sensory characteristics of these products presents the challenge of changing in intensity at different times during evaluation.

The objective of this research was to address the following three sensory areas in evaluation of soymilk or tofu to facilitate the better use of terms and sensory methodologies among industry and researchers.

A. Evaluation of effect of lipoxygenase-free soybean line in flavor attributes of soymilk and tofu and the differences in perception of flavor for panelists from different nationalities of origin.

B. Description of the sensory flavor of soymilk emphasizing objectionable flavors. The use of those descriptors in evaluation of soymilk processed from stored soybeans from normal and lipoxygenase-free soybeans.

C. Evaluation of sugar and reduced temperatures on the perception of bitterness and beaniness using time-intensity methodologies.

DISSERTATION ORGANIZATION

This dissertation contains a general introduction, including a literature review. The research is organized in three separate papers, one of which was published in the Journal of Food Science (Vol. 63: 6, pp 1084-1087, 1998), and the other two are presented in the format to be submitted to the same journal. Finally, a 'general conclusions' section is presented.

LITERATURE REVIEW

Consumption of Soy Products

Soybean oil is an important product of soybean processing, comprising 77% of total edible fat and oil consumption in the United States in 1992 (Asbridge, 1995). Price is the most important factor that makes soybean the leading oil. Also, a positive consumer attitude towards the product because of its high content of linoleic acid and a low saturated fatty acids content, which make it nutritionally desirable.

Soy protein meal accounts for two-thirds of the value of the bean (Asbridge, 1995). This product is an important factor in the economic success of the crop. The use of soy meal is almost exclusively for high-protein commercial animal feeds, especially for poultry and swine.

Soy protein is of high nutritional quality. Even though it is low in sulfur-containing amino acids, with methionine being the most significant limiting amino acid, followed by cysteine and threonine, it contains sufficient lysine, which is deficient in most cereal proteins. Despite its high nutritional value, only about 2% of soy protein produced goes into edible protein products in the United States (Asbridge, 1995). An increase in this percentage of soy protein for human consumption might be expected as research shows relevant results in chronic disease prevention. Popular health, home and food magazines are making consumers more aware of the benefits of soy proteins. United States Department of Agriculture food-intake surveys show that beef and pork consumption is decreasing and that United States consumers are eating more grain products (Demos, 1997). Meat consumption is at its lowest level in United States since the early 1960's. Even though there is not a trend towards vegetarianism, people are reducing their consumption of animal products. Soy flour and meat products with soy partially substituting meat, such as hot dogs, sausage, and bacon are slowly being incorporated into the diets of

United States consumers. Food companies producing alternatives to meat products report increasing retail sales and food service sales. Important multinational food companies such as Archer Daniels Midland Co. have sold vegetable protein-based burgers in the United States and around the world. They reported that sales grew by 40% in 1995 compared to 1994 and 26% in 1996 (Demos, 1997). The company that produced vegetable protein-based burgers was recently sold to Worthington Foods, which is successfully producing this product.

Other soy products that have recently been introduced into the U.S. markets are soymilk and tofu. These products are less acceptable to consumers because of their unfamiliar texture and flavor. In addition to these factors, these products are consumed directly instead of being incorporated into other food products as with the use of soy protein as a partial meat substitute. Soymilk and tofu were primarily introduced in health and natural-food stores but today more than half of the tofu and soymilk is sold in mainstream supermarkets (Demos, 1997). There is no marketing data showing what percentage of the final consumers are U.S. native or Asian-American. Nevertheless, the increase in sales coincides with an increase in consumer awareness of studies linking soy phytochemicals, such as isoflavones, to positive health effects.

Lipoxygenase Enzyme

The enzyme lipoxygenase (linoleate : oxygen oxidoreductase, EC 1.13.11.12) is widely distributed in nature, especially in legumes and cereals. The enzyme catalyzes the hydroperoxidation by molecular oxygen of polyunsaturated lipids that contain a cis,cis-1,4-pentadiene moiety (Schwimmer, 1981).

In plants the enzyme is physiologically important since the hydroperoxidation of linoleic acid is the first step in biosynthesis of growth-regulatory substances (jasmonic acid) and factors involved in wound healing (traumatin). An important physiological role of the enzyme is resistance to insects and pathogens. Jasmonic acid is involved in insect protection and is an antimicrobial compound. In mammalians the enzyme is also important because hydroperoxidation of arachidonic acid initiates syntheses of important substances such as leukotrienes and lipoxins (USDA, 1996).

Lipoxygenase has also applications in the food industry. It is of importance in bread making since it benefits wheat flour. The enzyme helps dough rheological properties, which decreases the use of energy during the process as well as the time for mixing. It also gives desirable nutty flavor to the bread and helps in producing a whiter crumb in bread since it bleaches carotenoids (Whitaker, 1994).

Lipoxygenase also has detrimental effects in foods. The enzyme causes destruction of the essential fatty acids linoleic, linolenic, and arachidonic acids. The free radicals produced damage other compounds including vitamins and proteins (Whitaker, 1994). Lipoxygenase also contributes flavor properties considered detrimental by consumers in western markets. The enzyme catalyzes the formation of hexanal, which has a low olfactory threshold and imparts undesirable flavor to certain food products (Hildebrand et al., 1990). Hexanal formation occurs through the action of lipoxygenase and subsequent cleavage of the product by hydroperoxide lyase. Soybeans produce relatively high levels of linoleic acid and lipoxygenase so the amounts of hexanal generated are likely to produce objectionable flavors and odors. The hydroperoxides and other breakdown products are also known to produce undesirable flavors. These hydroperoxides interact with protein and are released when soy flours, concentrates or isolates are used as ingredients in food preparation.

Genes from five lipoxygenases in soybeans have been cloned and sequenced. A sixth lipoxygenase has been partially characterized. There is evidence of at least one additional lipoxygenase gene that exist in the soybean genome (USDA, 1996). Soybean lipoxygenase (SBL) -1, -2 and -3 have been purified and characterized. SBL-1, -2, and -3 are found in mature soybean seeds at high levels. They are expressed only during seed maturation and are different from the lipoxygenases expressed in the embryo tissue at earlier development stages.

Seeds of commercial soybeans contain the three distinct lipoxygenase isozymes SBL-1, -2 and, -3. Soybeans have been screened for mutants without seed lipoxygenase and lines with non-detectable or very low amounts of lipoxygenase have been found (lipoxygenase-null). A lipoxygenase-free line (a triple null) or line without the three lipoxygenases has also recently been developed (Hajika et al., 1992). Several authors (Matoba et al., 1985; Davis et al., 1987) have examined the generation of hexanal by lipoxygenase-null mutants and concluded that SBL-2 was largely responsible for the generation of hexanal. However, other authors (Hildebrand et al., 1990) claim that a major effect on hexanal production was due to the presence or absence of SBL-3.

Factors Affecting Quality of Soy Products

The composition of soybeans has been reported to depend on factors such as the variety and climatic conditions (Perkins, 1995). The quality of soybeans is also affected by harvest, storage and handling. According to Erickson (1995), the quality of soybeans highly influences the attributes of the final products and processors must be aware of such variability in order to adjust for their processing practices.

Flavor is an important attribute affecting the acceptability of soy products. Several studies on the effect of the different lipoxygenase enzymes on the flavor attributes of soymilk and tofu have been conducted. Davies et al. (1987) analyzed the flavor improvement of soymilk prepared from soybeans lacking one or two of the lipoxygenases. Lower scores for beany, oily and rancid flavor and aroma as detected by a six-member sensory panel were found. Also, significantly lower scores were found for dairy and cereal flavor and aroma. Kobayashi et al. (1995) found that the yields of volatile compounds affecting flavor were highly reduced when soybeans lacking the lipoxygenases were used in soymilk preparation.

Wilson (1996) reported the effects of using several lipoxygenase-null soybean varieties on the flavor of tofu. Significant variations were found with the lipoxygenase null soybeans having the lowest beany flavor scores.

Texture of food products is greatly affected by the composition of the raw materials being used. The texture of tofu is attributable to processing conditions as well as to the composition of the soybeans. Soybean varieties from Japan and United States were compared by Smith et al. (1960) with the purpose of establishing whether or not the varieties were equivalent with regard to the quality of tofu. These soybean varieties were different in protein content of the beans and equivalent in yield of tofu. This study also reports a differences in texture and color between some of the U.S. and Japanese varieties.

Soybean quality has been reported to have an effect on texture of tofu. Skurray et al. (1980) reported variation in texture (hardness and cohesiveness) of tofu due to 7S and 11S proteins in soybeans, although the level of significance was not reported.

Tsai et al. (1981) analyzed soybean varieties from the United States and Taiwan for strength, softness and chewiness in different kinds of tofu. A comparison between varieties was not established in this study with regard to physical properties and the yield of tofu was reported to be the same for United States and Taiwan varieties.

Soybeans from five soybean varieties from United States and five varieties from Japan grown under the same experimental conditions were analyzed by Wang et al. (1983). Varietal differences were found to affect composition and color of the tofu as well as protein and oil contents of the beans. Differences among the varieties were not attributable to country of origin. A positive correlation between protein content of soybeans and the resulting tofu was found. Also, significant variation in the hardness of tofu was found among soybean varieties.

Studies on the effect of soybean varieties lacking one or several of the lipoxygenase isoenzymes on the physical properties of tofu are not abundant. Recently, Wilson (1996) reported the effect of using soybean lipoxygenase-2-free (SBL-2) and SBL-2,3 soybean varieties on textural attributes of tofu. Tofus from SBL-2 and SBL-2,3 soybeans were softer than tofus from traditional varieties as measured by a sensory panel. Nevertheless, no significant difference was found when physical properties were measured as hardness, fracturability, cohesiveness and gumminess and color.

Panelists' Background

U.S. consumers have been slow to accept soy in food products because of the off-flavors (Wilson, 1996). Development of a soybean line with less intense flavor characteristics may be useful in marketing food products to such consumers, but there has been little evaluation of foods made with lipoxygenase-free lines of soybeans.

Cultural differences in consumption of soy products might account for some differences in perception of sensory attributes. Marketing new soybean lines and soy products for export may require calibration of panels across cultures. The need for using sensory panelists from the targeted markets for successful product development has been pointed out by Hollingsworth (1998). Studies using panels from different countries to evaluate the same product may give some indication about whether such differences should be considered. Sensory panels from different countries have been used to determine whether the underlying perceptual dimensions characterizing samples of milk chocolate were stable across British and Norwegian panelists (Risvik, 1992).

Time-Intensity

In the common methods of sensory scaling, panelists rate intensity by giving a single (uni-point) measurement of perceived intensity. This requires that the panelists 'time-average' or integrate the changing sensation to provide a single intensity value or estimate only the peak intensity (Lawless and Heymann, 1998). In some cases, single point measurements of intensity may be appropriate to quantify the perceived intensity. These measurements have been shown to correlate well with peak time-intensity values. However, time-intensity allows the sensory scientist to see and compare sensory changes over time for a perceived attribute. This information can be missed when uni-point measurements are conducted.

Parameters extracted from the time-intensity curve are presented in Chapter 4 and they may include measurements such as time to maximum intensity, peak intensity, total duration of intensity, area under the curve or total intensity, increase angle, increase area, decrease angle, decrease area, and initial intensity. The degree of correlation among those parameters for the attribute under study gives an indication of whether the use of time-intensity technique is useful to the sensory scientist. When the parameters are highly correlated, they are redundant and it is not worth the time and effort required to conduct the time-intensity study. This is a very important criteria to consider before using the technique.

According to Lawless and Heymann (1998), other factors that must be considered in deciding whether to apply the time-intensity technique over conventional scaling are: whether the attribute to analyze is known to change over time, if the products differ in time course as a function of ingredients or processing variables, if the variation will occurs in such a way that will probably not be captured by single measurements.

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CHAPTER 2. SENSORY CHARACTERISTICS OF SOYMILK AND TOFU MADE FROM LIPOXYGENASE-FREE AND NORMAL SOYBEANS

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ABSTRACT

Soymilk made from lipoxygenase-free soybeans had less cooked beany aroma, less cooked beany flavor and less astringency and was rated darker and more yellow than that made from soybeans with normal lipoxygenase. Sensory descriptive panelists noted no differences between lipoxygenase-free and normal soybeans for milky flavor, wheat flavor, thickness, chalkiness or aftertaste. Tofu made from lipoxygenase-free soybeans had less cooked beany flavor than that made from normal soybeans. There were no differences in cooked beany aroma, raw beany aroma, raw beany flavor, wheat flavor, astringency, hardness, darkness or yellowness. Native-born Japanese, Chinese and U.S. descriptive panelists differed in responses to flavor, texture and color of soymilk and tofu.

Key Words: Soymilk, tofu, flavor, lipoxygenase, soybean

INTRODUCTION

Lipoxygenases produce off-flavors in soybeans [*Glycine max* (L.) Merr.] by hydroperoxidation of fatty acids and by interaction with protein in flours, concentrates and isolates (Rackis et al., 1979). The undesirable flavors, characterized as beany, green, grassy, painty, astringent, and bitter, reduce acceptance of soy products by many consumers who may prefer a bland flavor in soy products.

Normal mature soybean seeds contain three lipoxygenase isozymes important for flavor (SBL-1, SBL-2, and SBL-3) (Axelrod et al., 1981); other isozymes also have been described in soybeans (Shibata, 1996). The three isozymes have been associated with the production of hexanal and other aldehydes, ketones and alcohols that contribute to off-flavor. Hexanal, an undesirable flavor compound of raw soybeans, is a breakdown product of the hydroperoxides produced from oxidation of linolenic and linoleic acids. Matoba et al. (1985) determined that the SBL-2 isozyme was responsible for n-hexanal formation by using free linoleic acid as the substrate and comparing soybeans lacking SBL-1, SBL-2, SBL-3, and SBL-1,3 isozymes. Furuta et al. (1996) reported that lipoxygenase-free soybeans had lower 1,3-diethyl-2-thibarbituric acid (an index for hydroperoxide production) and n-hexanal levels than soybeans with lipoxygenase or lacking SBL-1, SBL-2, or SBL-3 isozymes. Several researchers identified soybeans lacking one or more lipoxygenase isozymes (Hildebrand and Hymowitz, 1981; Kitamura et al., 1983; Kitamura et al.,1985; Davies and Nielsen,1986; Hajika et al., 1992). Hildebrand et al. (1990) noted that soybeans containing the SBL-3 isozyme had reduced hexanal formation.

Genetic removal of the lipoxygenase isozymes may reduce or eliminate off-flavors of soy and increase consumer acceptability in some markets. Removal of the SBL-2 isozyme produced lower scores for beany, rancid and oily flavor and aroma attributes in soymilk and soy flour and higher scores for dairy and cereal flavor and aroma (Davies et al., 1987). Soymilk made from soybeans lacking SBL-2,3 and SBL-1,2,3 had less volatiles including hexanal, 1-penten-3-ol, 2-hexenal, 2-pentylfuran, 1-pentanol, 2-pentanol, 2-heptanal, hexanol, nonanal, 1-octen-3-ol, and 2,4-decadienal (Kobayashi et al., 1995). According to Wilson (1995), the objectionable flavor and odor of soymilk are formed by oxidation of specific unsaturated fatty acids by lipoxygenase enzyme during grinding of the seed. Soymilk flavor has an impact on the sensory attributes of tofu since it is an intermediate product. Wilson (1996) reported that tofu made from lipoxygenase-2 null lines of soybeans were less beany than their respective controls as evaluated by a sensory panel.

U.S. consumers have been slow to accept soy in food products because of the off-flavors (Wilson, 1996). Development of soybean lines with less intense flavor characteristics may be useful in marketing food products to such consumers, but there has been little evaluation of foods made with lipoxygenase-free lines of soybeans.

Cultural differences in consumption of soy products might account for some differences in perception of sensory attributes. Marketing of new soybean lines and soy products for export may require calibration of panels across cultures. The need for using sensory panelists from the targeted markets for successful product development has been pointed out by Hollingsworth (1998). Studies using panels from different countries to evaluate the same product may give some indication about whether such differences should be considered. Sensory panels from different countries have been used to determine whether the underlying perceptual dimensions characterizing samples of milk chocolate were stable across two different cultures (Risvik et al., 1992).

The objectives of this research were to compare soymilk and tofu made from normal and lipoxygenase-free soybeans, and to use three different panels equally trained to evaluate the products to indicate differences in sensory perception across cultures. Trained panelists from the United States, Japan or China evaluated 15 color, texture and flavor characteristics of soymilk and tofu.

MATERIALS & METHODS

Preparation of Soymilk

Soybean lines homogeneous for the three alleles controlling normal lipoxygenase and lines homogeneous for the three null alleles controlling absence of the lipoxygenase were grown at the Agricultural Engineering and Agronomy Research Center of Iowa State University during the summer of 1996. The BC_1F_2:4 lines were selected from a backcross population developed by the transfer of the three null alleles into the cultivar IA2020. The donor parent for the backcross was a lipoxygenase-free genotype obtained from Keisuke Kitamura at the National Agriculture Research Center, Yatabe, Tsubuka, Japan in 1992. The agronomic and seed traits of the soybean lines lacking seed lipoxygenase isozymes were described by Narvel et al. (1997).

Each line was harvested separately and 25 individual seeds of each were evaluated by a colorimetric assay (Suda et al. 1995) to confirm that the lines were homogeneous for presence or absence of the lipoxygenase alleles. To obtain sufficient seeds for the soymilk and tofu tests, it was necessary to combine the seeds of 7 normal or 7 lipoxygenase-free lines. The seeds were stored at 4°C until the soymilk and tofu were processed. A sample (3.5 kg soybeans) was soaked with tap water at room temperature for 12 h, drained, sorted to remove beans that were not hydrated, and washed with fresh water. The hydrated beans were combined with 26 kg tap water and ground twice with a Stephan grinder (A. Stephan u.Sohne GmbH & Co., West Germany).

Soymilk was prepared from the slurry with an Automatic Soymilk Plant (Takai Tofu & Soymilk Equipment Co., Japan). The maximum temperature reached by the soymilk was 95°C. Soymilk was homogenized with a double-stage homogenizer (Gaulin Corporation, Everett, MA) and stored for 18 h at 4°C until evaluation.

Preparation of Tofu

Tofu was made from soymilk prepared as described. A solution of calcium sulfate was added to soymilk at 80-82°C so the final concentration of coagulant was 0.02N. The soymilk and coagulant mixture was allowed to stand for 2 min for formation of curds. The resulting coagulum was cut and drained 5 min for separation of the whey. Curds were placed in a 43 X 49-cm perforated press box covered with cheese cloth and pressed with a hydraulic press. Pressure was applied 3 times for 5 min at 2 kg/cm^2, 5 min at 4 kg/cm^2, and 5 min at 6 kg/cm^2. Tofu was cooled to 20°C in a fresh water bath and 2.5 cm was trimmed from the edge of the tofu block. The remaining tofu was cut into 12.7 X 7.3-cm pieces. Only pieces from the outside of the block were sampled; pieces located in the 4 corners and the middle were not considered because of differences in firmness (Torres and Reitmeier, unpublished data). Tofu was stored in water-filled plastic containers at 4°C for 18 h until texture evaluation and 48 h until sensory evaluation.

Sensory Evaluation

Sensory training and evaluation of soymilk were conducted in a 5-wk period followed by a 5-wk period of training and evaluation of tofu according to procedures described by Meilgaard et al. (1991). Soymilk or tofu samples were presented in partitioned booths for evaluation, under white light and at room temperature. Soymilk (30 mL) or tofu (15g) was presented in clear plastic cups labeled with 3-digit random numbers. Cups were covered with lids to avoid volatilization of aroma compounds. The samples were presented in a counterbalanced order. Samples from lipoxygenase-free soybeans were presented first. Two samples of tofu or soymilk were evaluated per session. Tofu blocks were steamed in a covered pan for 5 min and cut into 1-cm cube samples. Four cubes per cup were presented to panelists. The two samples (normal and lipoxygenase-free) of soymilk (or tofu) were evaluated with a 15-cm intensity line scale anchored from none to intense for each attribute. Judges were instructed to swallow the samples and rinse with water between samples. For aroma evaluation, judges smelled the samples in two short sniffs. Hardness of tofu was evaluated as the force required to masticate when the sample was put between molars. Thickness of soymilk was evaluated as the force to draw the sample between the lips from the spoon and the rate of flow across the tongue.

The sensory panel consisted of 3 groups of different native-born nationalities. In preliminary sessions, each group developed terminology to analyze the products. Separate sessions were conducted for each product. There were 12 U.S.-born judges, 11 Chinese-born judges, and 9 Japanese-born judges for soymilk evaluation (32 total) and 12 U.S.-born judges, 10 Chinese-born judges, and 7 Japanese-born judges for tofu evaluation (29 total). American judges were monolingual English speakers, Chinese were Chinese-English bilingual and Japanese were bilingual in Japanese and English. All judges were ISU graduate students educated in their language at the university level. Taste and aroma attributes were determined by presenting judges pilot-plant soymilk (or tofu) and asking for descriptors. The judges indicated those terms which they thought were present in the products. Also, a preliminary list of the terms most frequently reported and published, was selected by the researchers and presented to the judges to be considered as descriptors as well. The terms that were more frequently mentioned and discussed by the judges and that the panelists in the three groups agreed were appropriate to describe the products were selected. The same protocol was repeated for tofu evaluation.

After selection of the attributes, groups were trained separately, but by similar procedures and by the same person. Two 1-h training sessions were conducted for each group and for each of the 2 products. Groups were trained separately to give more attention to individuals and to avoid influence of one group to the other. The same protocol was repeated for tofu evaluation. The panelists were the same for tofu and soymilk except 3 panelists dropped out of the tofu evaluation.

Flavor attributes evaluated for both soymilk and tofu were cooked beany aroma, cooked beany flavor, raw beany aroma, raw beany flavor, milky flavor, wheat flavor, and astringency. The viscosity of soymilk was evaluated by thickness and the texture by chalkiness and aftertaste. Tofu samples were evaluated for hardness. Color attributes were yellowness and darkness for both soymilk and tofu.

Judges were presented with standards to identify maximum intensity (15 cm on the scorecard) for each attribute. Judges compared commercial and pilot plant-prepared soymilk (or tofu) samples. The standards were soymilk with lipoxygenase present and overheated at 97°C for 15 min for cooked beany aroma and cooked beany flavor, uncooked soymilk slurry with lipoxygenase present for raw beany aroma and raw beany flavor, whole dairy milk for milky flavor, 'Cream of Wheat' (Nabisco Inc, East Hanover, NJ) for wheat flavor, an alum solution (2 g/L) for astringency, 'Eden Soy Extra' Soymilk (Eden Foods, Inc., Clinton, MI) for darkness and aftertaste and eggnog (Borden Inc, Columbus, OH) for yellowness and thickness, and antacid tablets (Chateau, Chaska, MN) for chalkiness.

Statistical Design and Analysis

Data from soymilk and tofu evaluation were analyzed using Proc GLM (SAS, 1988). One replicate of this experiment consisted of processing a batch of the product and having panelists evaluate the sensory properties. The Americans, Chinese and Japanese were represented by 12, 11 and 9 panelists respectively for soymilk evaluation (or 12, 10 and 7 for tofu). This protocol was repeated 3X for each product. An average value was determined for each line, nationality and replication. Resulting averages were used as data in a randomized complete block analysis where replications were blocks and 6 treatments were a factorial combination of 2 lines and 3 nationalities. When F values were significant ($p<0.05$), least significant differences (LSD) were calculated. Main effect means (line and panelist nationality) were reported when no interactions were present. Interaction means were reported when an interaction occurred between line and panelist nationality, i.e., for raw beany aroma and raw beany flavor of soymilk and milky flavor of tofu.

RESULTS & DISCUSSION

Effect of Soybean Type

Soymilk made from the lipoxygenase-free soybeans had less cooked beany aroma, less cooked beany flavor and less astringency than that from the normal line (Table 1). Sensory panelists reported low values for raw beany aroma (3.8) and raw beany flavor (4.6) and raw aroma and flavor were not expected to be strong in cooked soymilk samples. Less astringency was expected since the lipoxygenase enzymes may contribute such flavors (Rackis et al., 1979). Flavonoids and other phenolic compounds contribute astringency to soymilk. A concern that bland (lipoxygenase-free) soyfoods may allow the astringency to become more pronounced (Wilson, 1996) was not supported by our results.

There were no differences between lipoxygenase-free and normal soybeans for milky flavor, wheat flavor, thickness, chalkiness or aftertaste (Table 1). The similar genetic background of the soybean types, except for absence of lipoxygenases, was expected to yield products with similar characteristics other than beany flavor. Lipoxygenase-free soymilk was rated darker and more yellow by visual appearance than soymilk made from normal soybeans (Table 1), although measurement of color by Hunter spectrocolorimeter indicated no differences between normal (L = 83.50, a = 1.45, b = 10.28) and lipoxygenase-free (L = 83.00, a = 1.53, b = 11.59) soymilk.

Sensory panelists rated tofu from lipoxygenase-free soybeans had less (p = 0.06) cooked beany flavor than that from the normal cultivar (Table 2). There were no differences between tofu samples in cooked beany aroma, raw beany aroma, raw beany flavor, wheat flavor, astringency, hardness, darkness or yellowness (Table 2). Hunter color values for normal (L = 88.26, a = 0.25, b = 13.16) and lipoxygenase-free (L = 88.71, a = 0.31, b = 13.53) soymilk were not different. Tofu generally has been perceived as milder in flavor than soymilk. Removal of the whey during the processing of tofu probably eliminates water-soluble compounds that contribute to flavor (Watanabe and Kishi, 1984); however, no studies have identified such flavor compounds that are removed.

An interaction occurred between soybean cultivar and panelist nationality for raw beany aroma and raw beany flavor of soymilk (Table 3). U.S. panelists rated the soymilk from lipoxygenase-free soybeans as having less raw beany aroma than that from normal soybeans. Chinese panelists rated the soymilk from lipoxygenase-free beans with more raw beany aroma than the soymilk from the normal line. For raw beany flavor, U.S. panelists found less intense flavor in the soymilk made from lipoxygenase-free soybeans, Chinese panelists found more flavor and the Japanese panelists found no differences. The low intensity of the raw aroma and flavor would make differentiation between soymilk from normal and lipoxygenase-free soybeans difficult.

Effect of Panelist Nationality

Results indicated that panelists were using different parts of the linescale to evaluate soymilk and tofu from normal and lipoxygenase-free soybeans. They responded similarly to the normal and lipoxygenase-free soymilk (i.e., there was no interaction between soybean type and panelist type), except for raw beany aroma and raw beany flavor (Table 3). For cooked beany aroma and flavor of soymilk, Chinese and Japanese panelists used the rating scale similarly and higher than U.S. panelists (Table 4). Japanese panelists rated soymilk higher than or equal to U.S. panelists in milky flavor, astringency, chalkiness and aftertaste. Both Japanese and U.S. panelists rated these soymilk characteristics higher than did the Chinese panelists. Japanese panelists rated the soymilk darker than did the U.S. and Chinese panelists. For yellow color of soymilk, U.S. and Japanese ratings were similar and Japanese and Chinese ratings were the same. Panelists were not different in ratings of soymilk for wheat flavor or thickness.

Japanese panelists rated raw beany aroma and raw beany flavor of tofu higher than U.S. panelists; the Chinese responses were the same as the U.S. for raw beany aroma, but less than the U.S. responses for raw beany flavor (Table 5). Cooked beany aroma responses were not different. Chinese and Japanese panelists were similar in response to cooked beany flavor whereas U.S. panelists scores were less than the Japanese but the same as the Chinese scores.

Japanese scores were the same or greater than U.S. responses for wheat flavor and astringency and the Chinese responses were lower than the U.S. (Table 5). Hardness of the tofu was scored highest by the Chinese, followed by the Japanese, then the U.S. Japanese consumers usually eat pasteurized and refrigerated silken tofu that is soft and includes the whey; U. S. consumers prefer a smooth, cheese-like texture for tofu (Murphy et al., 1997). The U.S. panelists gave higher values for darkness than did the Chinese and the Japanese. There was no difference in responses to tofu yellowness.

An interaction occurred between soybean type and panelist nationality for milky flavor characteristic of tofu (Table 3). The U.S. and Chinese found no difference between tofu from normal beans and that from lipoxygenase-free beans, but the Japanese rated the tofu from lipoxygenase-free beans as having less milky flavor than that from normal beans.

Responses of the Japanese to sensory characteristics of soymilk and tofu generally were higher than the responses of U.S. panelists. Chinese panelists rated the soy products lower in flavor intensity than U.S. panelists. There was some concern that Japanese consumers of traditional soy foods such as soymilk and tofu may find the products without lipoxygenase too bland (Wilson, 1996). The intensity of flavors, except cooked beany flavor, did not decrease notably in the lipoxygenase-free soy products.

The term beany has been extensively used concerning evaluation of soy products. Use of this term may be inappropriate since it is complex and has different meaning for people from different cultures. The use of a single standard for beany may not be valid. The use of more specific terms may be necessary to align the beany concept within and among panels. As Ishii and O'Mahony (1987) indicated, the use of several standard stimuli may be necessary for definition of a taste concept. More extensive descriptive analysis for the attributes related to beany assessment may be necessary to more effectively describe this term. Investigation on the use of flavor profile methodology to describe beaniness of soy products is needed.

CONCLUSIONS

There was a reduction in cooked beany aroma and flavor of soymilk and tofu made with soybeans without the SBL-1, SBL-2, and SBL-3 lipoxygenase isozymes. Cultural differences may have accounted for some of the variations in panelist responses by nationality. Cultural differences in response to soy products may exist and should be considered when marketing lipoxygenase-free soybean products.

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Table 1- Normal and lipoxygenase-free lines main effect sensory differences for soymilk[z]

Sensory attribute	Normal	Lipoxygenase-free	p-Value
Flavor			
Cooked beany aroma	7.3	5.4	< 0.01
Cooked beany flavor	7.5	6.3	< 0.01
Milky flavor	2.6	3.0	0.23
Wheat flavor	4.2	3.4	0.08
Astringency	4.6	3.7	0.04
Viscosity			
Thickness	3.1	3.5	0.09
Texture			
Chalkiness	2.3	2.0	0.17
Aftertaste	5.2	4.8	0.30
Color			
Darkness	3.9	5.4	< 0.01
Yellowness	6.1	8.0	< 0.01

[z] Main effect means are responses of 32 panelists and 3 replications

Table 2- Normal and lipoxygenase-free lines main effect sensory differences for tofu[z]

Sensory attribute	Normal	Lipoxygenase-free	p-Value
Flavor			
Cooked beany aroma	7.3	6.5	0.27
Raw beany aroma	2.7	3.1	0.42
Cooked beany flavor	6.9	6.0	0.06
Raw beany flavor	2.5	2.4	0.76
Wheat flavor	3.7	3.3	0.24
Astringency	3.0	2.7	0.33
Texture			
Hardness	8.1	7.5	0.08
Color			
Darkness	2.9	3.3	0.14
Yellowness	5.1	5.6	0.53

[z] Main effect means are responses of 29 panelists and 3 replications.

Table 3- Significant sensory interaction effects of nationality of origin by line for soymilk and tofu[z]

Sensory attribute	U.S. Normal	U.S. Lipoxygenase-free	Chinese Normal	Chinese Lipoxygenase-free	Japanese Normal	Japanese Lipoxygenase-free
Soymilk						
Raw beany aroma	4.7 **c**	2.7 **ab**	2.0 **a**	3.2 **b**	5.4 **c**	4.5 **c**
Raw beany flavor	6.1 **d**	4.1 **b**	2.5 **a**	4.2 **bc**	5.3 **c**	5.5 **d**
Tofu						
Milky flavor	2.0 **b**	2.2 **b**	0.1 **a**	0.0 **a**	4.3 **c**	3.1 **b**

[z] Interaction means (values within a row) are responses of 12 U.S., 11 Chinese and 9 Japanese panelists for soymilk and 12 U.S., 10 Chinese and 7 Japanese panelists for tofu in 3 replications. Means within a row followed by the same letter are not significantly different ($p<0.05$).

Table 4- Nationality of origin main effect sensory differences for intensity ratings of soymilk samples[z]

Sensory attribute	U.S.	Chinese	Japanese	p-Value
Flavor				
Cooked beany aroma	4.7 **b**	7.2 **a**	7.1 **a**	< 0.01
Cooked beany flavor	5.7 **b**	7.7 **a**	7.4 **a**	< 0.01
Milky flavor	3.6 **a**	1.1 **b**	3.7 **a**	< 0.01
Wheat flavor	4.0	3.6	3.9	0.72
Astringency	4.5 **a**	3.2 **b**	4.7 **a**	0.02
Viscosity				
Thickness	3.3	3.0	3.6	0.11
Texture				
Chalkiness	2.0 **b**	1.3 **c**	3.1 **a**	< 0.01
Aftertaste	5.0 **b**	4.0 **c**	6.1 **a**	< 0.01
Color				
Darkness	4.5 **b**	4.3 **b**	5.2 **a**	0.01
Yellowness	7.8 **a**	6.2 **b**	7.1 **ab**	0.02

[z] Main effect means are panelist responses for normal and lipoxygenase-free soybeans in 3 replications. Means within a row followed by the same letter are not significantly different ($p<0.05$).

Table 5- Nationality of origin main effect sensory differences for intensity ratings of tofu samples[z]

Sensory attribute	American	Chinese	Japanese	p-value
Flavor				
Cooked beany aroma	6.0	7.2	7.6	0.17
Raw beany aroma	2.7 **b**	1.7 **b**	4.4 **a**	< 0.01
Cooked beany flavor	5.5 **b**	7.3 **a**	6.5 **ab**	0.02
Raw beany flavor	2.4 **b**	0.7 **c**	4.2 **a**	< 0.01
Wheat flavor	3.7 **a**	2.9 **b**	3.9 **a**	0.03
Astringency	3.3 **b**	1.0 **c**	4.2 **a**	< 0.01
Texture				
Hardness	6.0 **c**	9.0 **a**	8.3 **b**	< 0.01
Color				
Darkness	4.0 **a**	3.0 **b**	2.2 **c**	< 0.01
Yellowness	5.6	5.0	5.5	0.83

[z] Main effect means are panelist responses for normal and lipoxygenase-free soybeans and 3 replications. Means within a row followed by the same letter are not significantly different ($p<0.05$).

CHAPTER 3. DESCRIPTIVE ANALYSIS OF SOYMILK FLAVOR

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ABSTRACT

A descriptive analysis panel developed terms to describe flavor of soymilk. Panelists evaluated a commercial soymilk and soymilks processed with stored beans from the normal or lipoxygenase-free lines and with the beans that were not stored. Descriptors used to describe 'beaniness' were 'raw as hexanal' for flavor or aroma, 'grassy' flavor, and 'sweet as green floral' flavor. High correlations were found among those attributes. Judges were consistent in the use of terminology. Significant differences were found between commercial soymilk, soymilk processed from normal or lipoxygenase-free soybean lines and lipoxygenase-free soybeans stored for 1 yr at 4°C. Stored soybeans from the normal soybean line produced soymilk more stable to flavor changes than the soymilk from the lipoxygenase-free line.

Key words: soymilk, flavor, lipoxygenase, storage.

INTRODUCTION

The flavor of soymilk is the main factor limiting its consumption by the western population. Development of the lipoxygenase-free line of soybeans [*Glycine max* (L.) Merr.] lacking three isozymes (SBL-1, SBL-2 and SBL-3) has been one of the most recent approaches towards the decrease of objectionable flavors.

Sensory evaluation of the impact of any breeding or new processing technique on flavor of soy products requires the appropriate terminology as well as appropriate standards to evaluate objectionable flavors. Description of the sensory attributes characterizing aroma and flavor of soymilk has not been thoroughly investigated. Difficulty in describing the attributes has led to a variety of terms to describe the concept related to the flavor characteristics of soybean products. The term 'beany' has been adopted as a general descriptor by most researchers. More specific terms, such as green, grassy, painty, rancid, astringent, and bitter, have been used to refer to the same concept. Whether each term refers to one single attribute or to a variety of closely related attributes is not clear. More recently, the terms 'raw beany' and 'cooked beany' have been developed by sensory descriptive panels from different nationalities (Torres-Penaranda et al., 1998). A significant interaction between panelists and soybean line was found with the term 'raw beany'. This may be attributed to cultural differences in perception or to an inappropriate use of terminology or standards. Ishii and O'Mahony (1987) have indicated that the use of several standard stimuli may be necessary to define some sensory concepts.

Flavor and aroma of soymilk is formed by a complex combination and interaction of chemical compounds, some of which have decreased in concentration with the development of new lines lacking one or several lipoxygenase isozymes. Despite improvements in flavor, the lipoxygenase-free lines still contain many of the same chemical compounds (Kobayashi et al., 1995). Aroma constituents of soymilk from normal soybeans and those lacking two or three of the lipoxygenases have been identified by gas chromatography (GC), and the GC effluents have been analyzed using an odor sniffing system (Kobayashi et al., 1995). Hexanal, which contributes to the odor of soymilk from the normal line, completely disappears in the lipoxygenase-free line. This is believed to be the main reason for flavor improvement.

Storage conditions on farms and elevators may be an additional source of changes in flavor from these lines. Soybeans are stored in steel bins or concrete silos after harvest under conditions ranging from very low temperatures in winter to, in some cases, high temperatures in summer. According to Liu (1997), when temperatures are kept at ambient levels, moisture content of 13 % or below is considered necessary to ensure stability of soybeans for several months. Agrawal and Saddiqui (1973) reported that the optimum temperature and percentage seed moisture for storage of soybeans are 5°C and 11 %, respectively. Changes in soybean quality when storage conditions are not appropriate have been reported. Lambrecht et al. (1996) reported that soybean storage above 25°C and 50 % relative humidity for three months adversely affect the pH and solids content of soymilk produced from SBL-2 and SBL-3 null soybeans. Flavor changes of the lipoxygenase-free lines in storage has not been investigated.

In this research, we have described the 'beaniness' concept using descriptive analysis. The judges were exposed to a variety of chemical compounds present in soymilk to find those chemicals suitable for standards. Using the flavor profile developed by the panel, we quantified the differences between soymilks processed with stored beans from the normal or the lipoxygenase-free lines and with the beans that were not stored.

MATERIALS & METHODS

Sample preparation

Soybean lines homogeneous for the three alleles controlling normal lipoxygenase and lines homogeneous for the three null alleles controlling absence of lipoxygenase were grown at the Agricultural Engineering and Agronomy Research Center of Iowa State University during summers of 1996 and 1997. The lines were selected and harvested as described by Torres-Penaranda et al. (1998).

The treatments evaluated were a commercial soymilk powder and soymilks prepared from: normal line, normal line stored 1 yr, lipoxygenase-free line, and lipoxygenase-free line stored 1 yr.

The commercial soymilk was 'Soyabean Drink, Instant' (Guang XI Cereals Oils and Foodstuffs Import and Export Corporation, Wuzhou, Guang XI, China). The commercial soymilk was prepared by mixing the soymilk powder, which contained 5% sugar cane, with 230 g water and blending at medium speed for 30 sec. Soymilks samples (10 ml) were poured into 4-oz white plastic coded cups with lids 2 h before serving to equilibrate to room temperature.

The storage treatment consisted of the 1996 normal or lipoxygenase-free soybean lines which were stored at 4°C for one additional year compared to the 1997 normal or lipoxygenase-free soybean lines. The moisture content of the soybeans was 10%. The soybeans from 1997 normal or lipoxygenase-free lines were stored at 4°C after harvest until soymilks were processed. Soybeans from each treatment were soaked with tap water at room temperature for 12 h, drained, sorted and washed with fresh water. A sample of 200 g of the hydrated beans were combined with 1.4 L tap water and ground for 1 min at medium speed with a blender. Soymilk was prepared by cooking the slurry on a stove with constant stirring for seven min until the temperature reached 95°C and for seven additional minutes at that temperature. Soymilk was filtered through four layers of cheesecloth and stored for 20 h at 5°C until 2 h before evaluation.

Sensory evaluation

Difference tests

A triangle test was performed for each of the two lines of soybeans in order to determine differences between soymilks processed from 1996 soybeans stored for one year and beans from the 1997 harvest. Thirty-five panelists Iowa State University students and staff with previous experience evaluating soy products and familiarity with triangle tests performed the evaluation. Panelists received an orientation about the triangle test and the characteristics of the samples. Twenty mL of the samples were presented under red light in 4-oz white plastic cups coded with 3-digit numbers with lids on at room temperature. Panelists were instructed to examine the samples by smelling in two short sniffs, to swirl the sample in their mouths for 3 sec and then swallow. The procedure was repeated with the other two samples. Panelists were instructed to select the odd sample. Tests for treatments from each line of soybeans were performed in two separate sessions to avoid fatigue. An equal number of the six possible combinations of both samples were prepared and presented in random order.

Selection of panelists for descriptive analysis

The judges were Iowa State University students and staff with previous experience evaluating soy products. They were pre-selected on the basis of interest, availability, and ability to articulate. Pre-screening questionnaires (Meilgaard et al.,1991) for aroma and for flavor were initially used to screen individuals. Three additional screening tests were used: a basic taste test, an odor recognition test and intensity ranking test, as described by the ASTM (1981a; 1981b) for flavor profile. The interpretation of the screening tests was done according to the same reference.

Training of panelists for descriptive analysis

Panelists were trained for a period of 3 months in 1-hr sessions three times a week. Judges were exposed to a variety of soymilks including the treatments to be evaluated, as well as commercial brands. Initially, they were asked to evaluate sensory differences among samples and make a list of the descriptors for these differences. They considered aroma, flavor, mouthfeel, and aftertaste. The descriptors generated after initial consensus are listed in Table 1.

With the purpose of finding descriptors to define the term 'beany, panelists were presented with a series of chemical compounds which have been found in the headspace of soymilks from normal or lipoxygenase-free lines (Kobayashi et al.1995) or recommended as standards by ASTM (1996). These chemicals (Table 2) were put into safflower oil in a concentration of 50 ppm and evaluated for aroma. Panelists were asked to indicate whether each compound was related to the sensation of beaniness in soymilk. During the process of defining standards and preliminary evaluation of the samples, the initial list of descriptors was modified (Table 3).

Panelists developed standards for each of the attributes. After further exposure to the products and more familiarity with standards, panelists arrived at consensus for the descriptors and the type of standards to use. Standards were presented in different concentrations until an appropriate dilution was found to anchor the ends of the scale for each attribute. Panelists also defined the type of scale to use for evaluation. A 10-cm line scale anchored from 'not detectable' to 'intense' was defined.

Statistical analysis

Difference test

Results were analyzed using the statistical tables for the critical number of correct answers for difference tests from Meilgaard et al. (1991).

Descriptive analysis

Four samples were used for evaluation by descriptive analysis. One commercial sample plus the three samples that were significantly different according to the triangle test.

Six of the attributes were evaluated for the 4 samples in eight replications, followed by eight replications for evaluation of the other six attributes. Each replication was a separate session. The four samples were presented in a different random order for each judge during each replication. Data were analyzed using analysis of variance and principal component analysis of the correlation matrices was performed for the significant attributes (SAS, 1988).

The interaction of judges with the soymilk factor was divided into two parts. One part was a linear component of the soymilk factor with judges and the other a deviations component of the soymilk factor with judges. The soymilk treatment means were used to define quantitative levels for determining linear and deviations components of interaction with judges.

RESULTS & DISCUSSION

The terminology for soymilk description generated by panelists by initial consensus consisted of a more common consumer language (Table 1), while the final list of terms (Table 3) produced after judges were extensively exposed to the samples and standards showed the use of more technical vocabulary. As mentioned by Civille and Lawless (1997), the fractionation of a perception into its component sensory impressions may be very difficult. The term 'beany' that appears on the initial list was better defined later using more specific attributes. The use of the terms 'raw as in hexanal' aroma or flavor, 'grassy' flavor and 'sweet as in green floral' flavor shows the ability of trained panelists to develop a vocabulary that specifically describes a concept, generally known as 'beany'.

According to Lawless and Heymann (1998), the terms used for sensory description of products should be orthogonal, i.e. not correlated to each other. There was a high positive correlation (Table 7) among the terms generated to describe 'beaniness' which shows their close interrelation and therefore the difficulty of their definition by untrained panelists. The high correlation explains the fact that the principal component 1 (Fig 1) accounts for most of the variation (89.7%). On the other hand, the interaction term for judge x soymilk for the terms related to 'beaniness' was not significant (Table 5). This shows that judges were highly consistent with regard to the use of the terminology.

A set of terms in descriptive analysis should enable differentiation among products (Civille and Lawless, 1997). Figure 1 shows that 'beaniness' for the normal soymilk is better described by the terms 'raw aroma as in hexanal' and 'raw flavor as in hexanal', while 'beaniness' for soymilk from the lipoxygenase-free line is better described by the terms 'sweet as green floral' and 'grassy' flavors. The terms related to beaniness which were developed by a intensively trained panel can also be used as an illustration of the 'beany concept' when evaluation is done by less trained sensory panels using a series of terms and standards (multiple standards) to define the concept. The use of multiple standards to illustrate the 'beany concept' would allow less trained panelists the evaluation of soymilk using better criteria and broader knowledge of the concept. Multiple standards would also facilitate judgement of 'beaniness' when samples with different kinds of 'beaniness' are tested, as occurs in soymilks from normal and lipoxygenase-free lines.

With the exception of the term 'painty', there was no judge x soymilk interaction for any of the attributes (Table 5). Even though the term 'painty' was developed by the panelists and they established the standard, judges were not consistent in the use of the term. The lack of consistency may be either the result of the judges not understanding the term or that most of the samples were not significantly different for 'painty' (Table 6). Table 5 also shows that replication was not a significant source of variation except for the attribute 'bitter' which exhibited a difference across replications at the 5% level.

Descriptive analysis showed significant differences across the soymilks for all the attributes (Table 6). The commercial soymilk was significantly different from the others for all the attributes. None of the attributes related to 'beaniness' are good descriptors to characterize the commercial soymilk (Fig. 1). Aroma of the commercial soymilk can be better described as 'starch as flour' and 'sweet as dairy caramelized'. The commercial product has a very intense 'sweet as dairy caramelized' flavor which is expected since sugar was contained in the soymilk powder. The absence of bitter and metallic flavors and very low astringency may be due to the addition of sugar. This soymilk had a lower painty flavor score as compared to the other soymilks, and a significantly higher intensity of mouthcoating. Differences between the commercial soymilk and those prepared at the laboratory may be due to several factors including the processing techniques, which are unknown for the commercial product. Drying of the soymilk may have caused volatilization of flavor compounds. Also, the commercial soymilk has been stored after processing which may have caused changes in flavor.

Soymilks from normal and lipoxygenase-free soybeans were significantly different with regard to all the attributes except for the 'painty' and 'astringent' flavors (Table 6). Soymilk from lipoxygenase-free soybeans had a lower intensity in the attributes 'raw as hexanal' aroma and 'raw as in hexanal' flavor. The intensity of the 'raw as hexanal' attributes was lower for the soymilks from the lipoxygenase-free soybeans as expected from the breeding modifications of this new soybean line. The decrease of these two attributes may have enhanced the 'grassy' and 'sweet as green floral' flavors. Aroma of the soymilk from the lipoxygenase-free soybeans was characterized by the term 'starch as in flour'. Soymilk from the lipoxygenase-free soybeans was significantly higher in the 'metallic' and 'bitter' attributes and equally 'painty' as compared as the soymilk from the normal soybean line.

There were significant sensory differences between soymilks from the storage treatment and the non-stored treatment for the lipoxygenase-free line. In contrast to the lipoxygenase-free line, soymilks from the normal line did not exhibit sensory differences between the storage and non-storage treatments (Table 4). Changes in flavor between the soymilks stored and the non-storage treatments may be due to the fact that the soybeans were produced in different years. Lambrecht et al. (1996) found that the null lines lacking lipoxygenase-2 and lipoxygenase-3 were more stable to adverse storage treatments than the normal soybeans with regard to changes in pH and solids content of the soymilks produced from the stored beans. They found that the higher the temperature and relative humidity, the greater the reduction in solids of the soymilk. No changes in flavor were analyzed in that study.

Soymilk from lipoxygenase-free lines processed after the storage of the beans at 4°C exhibited changes in aroma and flavor (Table 6). The intensity of two of the aroma attributes decreased significantly, including the 'starch as in flour'. The 'raw as hexanal' flavor remained the same as the non-storage treatment for lipoxygenase-free soymilk. The 'grassy' and 'sweet as green floral' flavor attributes decreased to the same intensity as the soymilk from the normal line. The 'painty' flavor and the attributes related to mouthcoating underwent no significant changes in storage for lipoxygenase-free soybeans. There was a significant decrease in the 'metallic' attribute and a significant increase in bitterness of soymilk processed after storage of lipoxygenase-free beans.

CONCLUSIONS

The use ot the term 'beany' for soymilk comprises a series of different related attributes and must be properly defined when sensory evaluation of soymilk is conducted. The terms 'raw as hexanal' aroma or flavor, 'grassy' flavor and 'sweet as green floral' flavor, with the appropriate use of standards, can be used to describe sensory differences in beaniness among soymilk samples when a descriptive analysis panel is properly trained. Because of the high correlation among these terms, their use may also serve as a tool to illustrate the sensory concept of 'beaniness' when that characteristic is analyzed as a whole. The standards can be used as multiple standards to define the multidimensional character of the concept 'beany'.

Relevant significant differences detected between the commercial soymilk and the soymilk processed from lipoxygenase-free line suggest that sugar addition to soymilk would produce important desirable changes in flavor, especially in decrease of bitterness, astringency and the attributes related to 'beaniness'.

Soymilk processed with lipoxygenase-free soybeans exhibited changes in the type of perceived beaniness in comparison to the soymilk from normal soybeans. Beaniness in the normal soymilk was perceived more as 'raw as hexanal', while in the lipoxygenase-free beaniness is perceived more as 'grassy' and 'green floral'. 'Painty' flavor remained the same when the new soybean line was used, and there was an increase in 'metallic' and 'bitter' flavors. 'Cooked beany' aroma and flavor attributes significantly decreased in soymilk when lipoxygenase-free soybeans were used, as evaluated by 32 panelists from different nationalities (Torres-Penaranda et al., 1998).

Despite a decrease in the intensity of the 'grassy' and 'sweet as green floral' flavor attributes, changes in flavor for soymilks processed from stored lipoxygenase-free beans are important considering that the storage treatment significantly increased bitterness. Even though aroma score was lower for the 'raw as hexanal' attribute as compared as the non-stored lipoxygenase-free soymilk, the 'raw as hexanal' flavor remained the same.

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Table 1. Descriptors developed by panelists by initial consensus

Term	Description
Aroma	
Starch	flour
Pasta	cooked pasta
Sweet	sugar
Dairy	dry milk
Dairy sour	evaporated milk
Beany	soy, bean slurry, grass, green peas, earthy, raw beans, pungent
Flavor	
Sweet	sugar
Sweet	legume
Vanilla	vanillin
Dairy	dry milk dissolved in water
Dairy cooked	cooked milk
Oxidized	aged oil
Bitter	coffee
Astringent	dry, clean tongue
Beany	soy, green, grass, fresh vegetables, raw beans, pungent, unpleasant
Mouthfeel	
Coating	film coating the teeth
Aftertaste	
Astringent	dry, clean tongue
Bitter	coffee
Beany	raw beans

Table 2. Chemicals used by panelists to match 'beaniness'

Chemical compound *	Relation with 'beaniness'
hexanal	yes
1-hexen-1-ol	yes
1-octen-3-ol	no
1-octen-3-one	no
(E)-2-nonenal	no
(E,E)-2,4-nonadienal	no
(E,E)-2,4-decadienal	no

* Source: Sigma-Aldrich, Bellefonte, PA

Table 3. Sensory attributes for description of soymilk

Descriptor	Standard	Standard concentration for 'intense'
Beany aroma		
Raw as hexanal	Hexanal in safflower oil	50 ppm
Other aromas		
Starch as flour	Flour in water	1:10
Sweet as dairy caramelized	Carnation sweetened condensed milk	1:3
Beany flavor		
Raw as hexanal	Hexanal in safflower (as illustration by aroma)	50 ppm
Grassy	1-hexen-1-ol (as illustration by aroma)	50 ppm
Sweet as green floral	Fresh bean sprouts blended in water	1:60
Other flavors		
Painty	Soybean oil aged for 4 yr, 25°C, in safflower oil	1:10
Sweet as dairy caramelized	Carnation sweetened condensed milk in water	1:3
Metallic	Iron pills, not dissolved.	-
Bitter	Caffeine in water	0.1 %
Mouthfeel		
Astringent	Alum in water	0.05 %
Mouthcoating	Whole milk	100 %

Table 4. Number of correct judgements for a difference triangle test of soymilk from normal line or lipoxygenase-free line

Treatment	Correct judgements out of 35
Normal line vs. normal line stored	7 NS
Lip-free line vs. lip-free line stored	17 *

NS, *: not significantly different, significantly different at $p<0.05$ respectively.

Table 5. Significance of the replication factor and of interaction judge x soymilk for sensory attributes

Source of variation	Judge x soymilk	Replication
	P>F	
Sensory attribute		
Aroma		
Raw as hexanal	0.53	0.504
Starch as flour	0.41	0.075
Sweet as dairy caramelized	0.29	0.618
Flavor		
Painty	<0.001	0.978
Raw as hexanal	0.51	0.741
Grassy	0.12	0.536
Sweet as green floral	0.66	0.976
Sweet as dairy caramelized	0.96	0.924
Metallic	0.35	0.051
Bitter	0.29	0.044
Mouthfeelling		
Astringent	0.71	0.995
Mouthcoating	0.85	0.900

Table 6. Sensory evaluation of commercial soymilk and soymilk processed from normal and lipoxygenase-free and lipoxygenase-free stored for one year. [z]

Sensory attribute	Soymilk				P>F
	Commercial	Normal	Lipoxygenase-free	Lipoxygenase-free stored	
Beany aroma					
Raw as hexanal	3 d	66 a	51 b	36 c	< 0.01
Other aromas					
Starch as flour	82 a	29 c	41 b	22 c	< 0.01
Sweet as dairy caramelized	92 a	22 b	13 c	7 c	< 0.01
Beany flavor					
Raw as hexanal	2 c	75 a	54 b	44 b	< 0.01
Grassy	1 c	38 b	66 a	31 b	< 0.01
Sweet as green floral	8 c	37 b	53 a	28 b	< 0.01
Other flavors					
Painty	11 b	35 a	39 a	35 a	< 0.01
Sweet as dairy caramelized	91 a	13 b	4 c	7 cb	< 0.01
Metallic	0 d	37 b	54 a	21 c	< 0.01
Bitter	0 d	18 c	32 b	47 a	< 0.01
Mouthfeeling					
Astringent	7 b	37 a	42 a	41 a	< 0.01
Mouthcoating	58 a	13 c	29 b	30 b	< 0.01

[z] Main effect means within a row are responses of five panelists and eight replications.

Table 7. Correlation matrix for the attributes related to 'beaniness'

	Raw aroma	Raw flavor	Sweet green flavor	Grassy flavor
Raw aroma	1.000	0.995	0.832	0.783
Raw flavor		1.000	0.794	0.745
Sweet green flavor			1.000	0.995
Grassy flavor				1.000

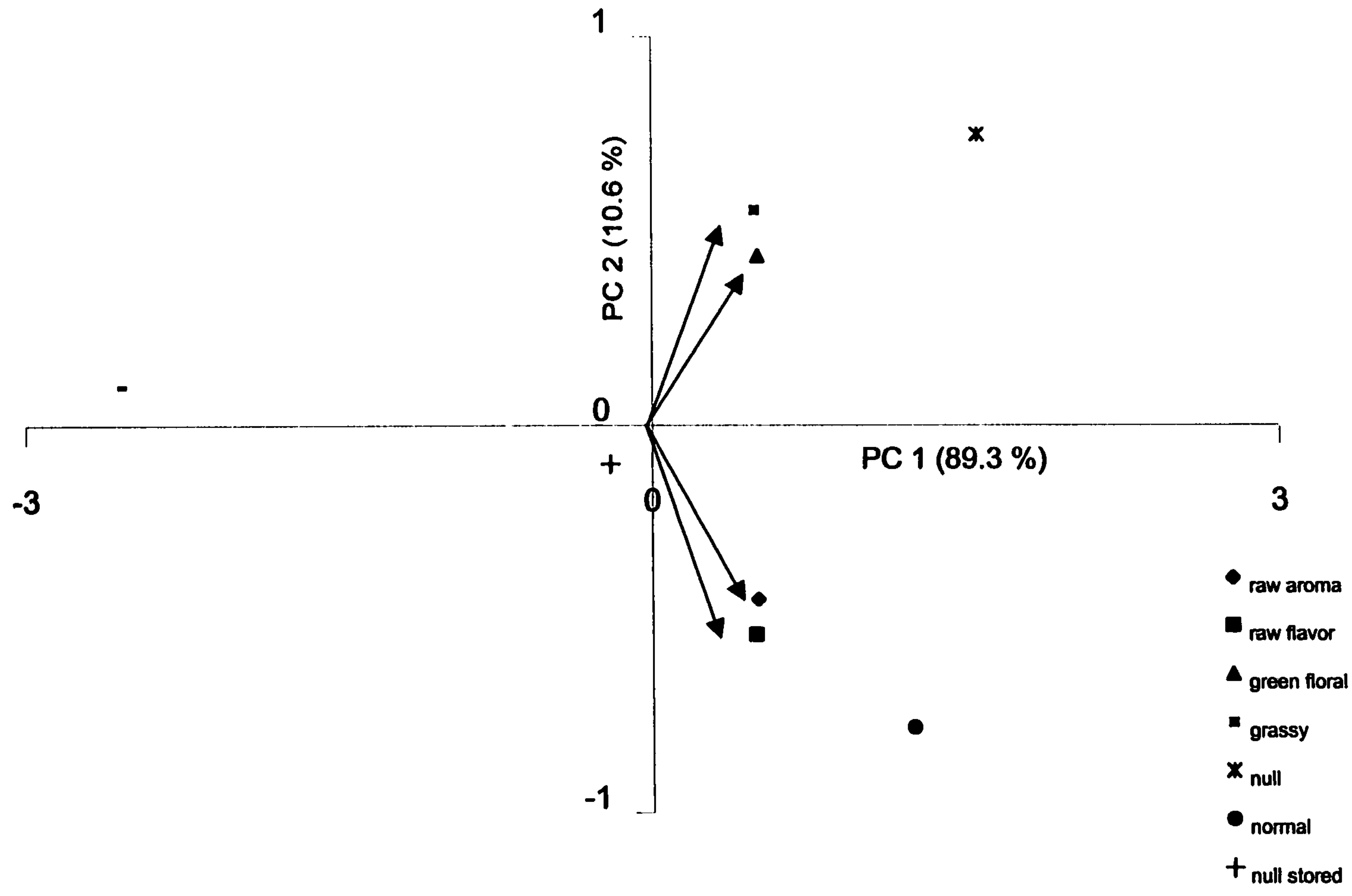

Figure 1. PCA for attributes related to beany flavor

CHAPTER 4. TIME DEPENDENCY OF INTENSITY OF BITTERNESS AND BEANINESS AS A FUNCTION OF SUGAR LEVEL AND TEMPERATURE IN SOYMILK

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Key words: soymilk, lipoxygenase, time-intensity, bitter, beany.

ABSTRACT

Time-intensity (T-I) was used to evaluate the effect of sugar (0, 2.5 and 5%), temperature (5 and 25°C) and soybean line (normal and lipoxygenase-free) on the intensity of bitterness and beaniness of soymilk. Treatments were also compared with a commercial soymilk. Temperature did not have any significant effect on bitterness or beaniness. Total intensity of bitterness was significantly reduced by the use of lipoxygenase-free soybeans or addition of sugar at the 2.5 % level. Most T-I parameters except for initial intensity were highly correlated among themselves for bitterness. Principal Component 1 (PC1) for bitterness described 83.3% of the variance and separated the treatments on the basis of sugar concentration and soybean line. The effect of soybean line varied when maximum intensity or area under the curve was used to evaluate bitterness. For beaniness, the effect of sugar or soybean line was different for maximum intensity or area under the curve. Beaniness maximum intensity was reduced with the combined effect of using lipoxygenase-free soybeans and 2.5% sugar. Decrease angle and increase area did not correlate with the rest of the T-I parameters when beaniness intensity was measured.

INTRODUCTION

Perception of flavors in foods is a dynamic phenomenon rather than static, i.e., perception of intensity of some attributes change from moment to moment. When panelists are asked to rate intensity with a single measurement, they have to integrate their changing perceptions to estimate one single value or to estimate only the peak intensity (Lawless and Heymann, 1998).

Flavor in soy products is complex due to the complex nature of the food and time-dependency of the perception of the sensory characteristics makes their evaluation even more complex. Most sensory measurements of flavor in soy products have been conducted using unipoint measurements of intensity. Some sensory differences among treatments during the time that the intensity of the attribute lasts may be missed when single-point measurements of intensity are conducted. Use of time-intensity methodologies to assess time-related sensory attributes in soy products may be a means to provide additional information not contained in single-point measurements.

Bitterness is a basic taste that is time dependent. It has been associated with the undesirable flavor of soy products by western consumers. The chemical components responsible for bitterness of normal soybeans have been found to be mainly saponins (Okubo et al., 1992). Attenuation of bitterness by a variety of methods has received much attention (Roy, 1997). Sugar has commonly been used to mask bitterness. In soymilk, addition of 3% sugar decreased bitterness and raw beany flavor when panelists used uni-point measurements of intensity (Lee, 1995). Perception of taste is not only dependant upon the compounds present but also upon the physical and chemical properties of the medium of presentation (Thorngate, 1997). Suppression of bitterness by sugar may also be related to temperature of the samples under analysis. The effect of low temperature on bitterness perception is not clear. No suppression of bitterness for saccharin was found in a time-intensity study when temperature of samples was lowered to 3°C (Larson-Powers and Pangborn, 1978). Other studies have found that cooling of both the tongue and the sample suppresses bitter taste, although the reduction was not significant (Green and Frankmann, 1987).

In this study, we used time-intensity methodology to evaluate the effect of sugar and reduced temperature on the perception of bitterness and beaniness of soymilk. Soymilk processed from normal or lipoxygenase-free soybeans was also analyzed and all treatments were compared to a commercial soymilk.

MATERIALS & METHODS

Sample preparation

Soybean lines homogeneous for the three alleles controlling normal lipoxygenase and lines homogeneous for the three null alleles controlling absence of the lipoxygenase were grown at the Agricultural Engineering and Agronomy Research Center of Iowa State University during 1998. The lines were selected and harvested as described by Torres-Penaranda et al. (1998).

The soybeans were stored at 4°C until soymilks were processed. Soybeans from both lines were soaked with tap water at room temperature for 12 h, drained, sorted and washed with fresh water. Two hundred g of the hydrated beans were combined with 1.4 L tap water and ground for 1 min at medium speed with a blender. The blended slurry was cooked on a stove with constant stirring for 7 min until temperature reached 95°C and for seven additional min at that temperature. The soymilk was filtered through four layers of cheesecloth and sugar added in the concentrations indicated in Table 1. Soymilks were stored 20 hr at 5°C until evaluation.

The commercial soymilk used in this study was 'Soyabean Drink, Instant' (Guang XI Cereals Oils and Foodstuffs Import and Export Corporation, Wuzhou, Guang XI, China). The processing methods for this soymilk were unknown. The commercial soymilk contained dehydrated soymilk processed from soybeans plus 5% sugar cane. This soymilk was selected among other commercial soymilks because it contained only soybeans and sugar. It was prepared by mixing the soymilk powder contained in a 22 g package with 230 g water, and blending at medium speed for 30 sec. Soymilks samples (10 mL) were poured into 4 oz white plastic coded containers with lids 2 hr before serving. Samples were stored at 25°C or 5°C until served.

Sensory evaluation

All training and testing sessions were conducted at the sensory facilities at the Center for Food Design to Improve Nutrition at Iowa State University. Compusense Five (version 2.2), a computerized time-intensity (T-I) system (1996 Compusense Inc., Guelph, Ontario, Canada), was used to record temporal perception. Each judge used an individual computer terminal with a digital pad to record the changes in perception of intensity of each attribute using the Single Attribute Time Intensity (SATI) method. The computer was programmed to collect responses every second during a total time of 2.5 min for each sample with a 5 min delay between samples. Judges were in separate booths under white light. They were instructed to take the entire 10-mL sample into their mouths, swallow after 15 sec, and record before and after swallowing until the perceived intensity (bitterness or beaniness) reached extinction. Judges rinsed with water between samples. Judges evaluated five samples in each of nine sessions for bitterness. The same procedure was repeated for beaniness with a 2-wk period between the evaluation of each attribute. The ten judges were Iowa State University students and staff with previous experience evaluating soy products.

Judges were trained during five 1-hr sessions for each of the two attributes. Judges were tested for genetic sensitivity to 6-n-propylthiouracil (PROP) to select for 'taster' individuals. Solutions of 0.1% caffeine and 0.1% citric acid were presented to determine the distinction between sour and bitter. Training and evaluation of bitterness was conducted first. A 0.1% caffeine solution was used as a standard to anchor the scale for maximum intensity of bitterness. Judges were also trained in the evaluation of T-I with paper for recording and with the computer to learn how to use the digital pad to record changes on intensity over time. Training for evaluation of beaniness was conducted before the evaluation of that attribute and consisted of the use of multiple standards to illustrate the concept of beaniness, as determined in Chapter 3. The standards used were: a freshly prepared solution 1:60 of bean sprouts in water for 'green floral', 50 ppm of hexanal in safflower oil for 'raw as in hexanal', and 50 ppm of 1-hexen-3-ol in safflower oil for 'grassy'. The two chemicals were used only for aroma and the bean sprouts for tasting.

Statistical analysis

Each treatment was evaluated by sensory panelists in three replications. Each replication was conducted in three incomplete blocks, five treatments per block. One of the 15 treatments evaluated is not reported since it was beyond the objectives of this study. The 14 treatments reported are described in Table 1. The attributes analyzed are described in Table 2. Treatments were randomly assigned to blocks with a different randomization used in each replication. Treatments in a block were presented in the same random order for each judge.

Soymilks were evaluated in two separate experimental designs for bitterness and beaniness. Data were evaluated using analysis of variance for treatments 1 to 12. Treatments for the commercial soymilk were compared to the others using a model for contrast. Principal component analysis (PCA) of the correlation matrices was conducted for the parameters showing significant differences (SAS, 1988).

RESULTS & DISCUSSION

Bitterness

Results for the nine time-intensity (T-I) parameters analyzed for bitterness for the main factors: replication, soybean line, temperature, sugar level and judges as well as the interactions between some of the factors and the contrasts for comparison of the commercial soymilk with the other treatments are summarized in the ANOVA results (Table 3). No significant differences were found for replication across most of the parameters analyzed. Judges were a significant source of variation, which indicates that they differed among each other in perception over time. The temperature effect was not a significant source of variation, except for the increase area (iare), which changed with temperature. No significant interactions were found for temperature and sugar factors or for sugar and soybean line. The temperature*sugar interaction was significant for total time and area under the curve. A significant difference was found between soymilks from the two soybean lines for seven of the nine T-I parameters. The time to reach maximum intensity (tmax) and the rate of decrease of bitterness (dang) were not affected by sugar. Nevertheless, sugar addition significantly affected the perception of bitterness for most of the T-I parameters.

The means of the significant parameters for soybean line and sugar concentration (pooled over the other factors) as well as the means for the commercial soymilk are shown in Table 4. When sugar was added at the 2.5% level, a significant decrease in bitterness was observed. Increase in sugar to the 5% level did not cause any significant change in the T-I curve for perception of bitterness (Table 4). Contrasts of the commercial soymilk with each of the treatments showed a significant difference for the T-I parameters with the exception of time for maximum intensity (tmax), decrease angle (dang), and initial intensity (iint) (Table 3). Commercial soymilk in general was perceived as significantly lower in bitterness as compared to the other treatments.

Using the T-I parameters that significantly varied across the treatments, Principal Component Analysis (PCA) was performed to summarize the T-I responses for bitterness (Figure 1). PC 1 accounted for most of the variation (83.3%) and separated the treatments on the basis of sugar concentration and soybean line. The significant attributes were highly correlated with each other, except for initial intensity. The PCA graph (Fig. 1) showed that addition of sugar to soymilks from any of the two soybean lines decreased bitterness (closer to the bitterness of the commercial soymilk). It also showed how soymilk from the lipoxygenase-free soybeans was closer to the commercial soymilk than soymilk from the normal line. Soymilk from lipoxygenase-free line plus 5% sugar was the treatment that most resembled the commercial soymilk with regard to bitterness.

Consistent with the PCA, Figures 2 and 3 illustrated the effects of the lipoxygenase-free soybean line or sugar on maximum intensity or area under the curve for bitterness, as well as the comparison of those treatments with the commercial soymilk. Results obtained for the effect of soybean line differed when maximum intensity or area under the curve was used as a parameter to measure bitterness intensity. When no sugar was added, maximum intensity of bitterness was not significantly different for soymilks from the normal and the lipoxygenase-free lines. In contrast, areas under the curves were different.

Beaniness

A summary of the ANOVA results for the main effects (replication, soybean line, temperature, sugar level and judges), one-way interactions and contrasts for commercial soymilk with the other treatments is presented in Table 5. Replication was not a significant source of variation for any of the T-I parameters analyzed and judges were a significant source of variation. No significant variation was found for the effect of temperature for any of the T-I parameters. None of the interactions analyzed (temperature*sugar, temperature*line or sugar*line) were significant.

The effect of soybean line on perception of beaniness was significant for most of the T-I parameters, except for time for maximum intensity (tmax), duration (ttot) and initial intensity (iint). All the significant T-I parameters showed significantly lower beaniness over time for lipoxygenase-free lines when the means for line were pooled over the other effects (Table 6). Sugar addition was also a significant source of variation for seven of the nine T-I parameters.

To characterize the T-I responses, the parameters maximum intensity (imax) and area under the curve (area) were used. Figures 4 and 5 show the relationship among each of these two parameters, the level of sugar and the soybean line. The reduction of perception of maximum intensity of beaniness with lipoxygenase-free soybeans varied depending on the sugar level used (Fig. 4). When no sugar was added, the reduction in beaniness with the use of lipoxygenase-free soybeans was not significant. This effect was significant for both levels of sugar. Nevertheless, no significant decrease in maximum intensity of beaniness was found when the sugar level increased from 2.5 to 5 %. Figure 4 also shows the similarity in maximum intensity between the commerciai soymilk and all the treatments with the normal soybean line. It also shows the combined effect of lipoxygenase-free soybeans and sugar which produced soymilk significantly lower in beaniness maximum intensity as compared to the normal soymilk with sugar or without sugar. The area under the curve (which represents the total intensity of the sample) was lower when lipoxygenase-free soybeans were used and sugar was added at 2.5% or more (Fig. 5). Addition of sugar at the 5% level for the lipoxygenase-free soybean line made the total intensity of beaniness lower than the commercial soymilk.

Figure 6 summarizes the overall T-I responses for beaniness for all the treatments and the significant T-I parameters. The first principal component describes 73.4% of the variance. PC 1 separated the normal line without sugar from the lipoxygenase-free with sugar and located them at opposite ends of PC 1. These were the soymilks highest or lowest in beaniness for most of the T-I parameters. The loading of the eingenvectors shows how most of the T-I parameters were highly correlated. Other parameters (dang, iint or iare) were not correlated which also explains part of the variation among treatments. The high correlation among the other four parameters (imax, iang, area, or dare) suggests that the treatments may be discriminated by beaniness using any of those parameters. Nevertheless, a closer look at the parameters area and maximum intensity (Fig. 4 and 5) show how each give information about differences in treatments not explained by the other.

CONCLUSIONS

Use of the lipoxygenase-free soybean line yielded soymilk with reduced bitterness when intensity was measured as a function of time. In contrast, low temperature did not cause any significant change in perception of bitterness or beaniness over time as compared to room temperature. Addition of 2.5% sugar produced an additional decrease in bitterness perception. The effect of sugar or soybean line on bitterness or beaniness varied when maximum intensity or area under the curve was used as a parameter. Soymilk made from the lipoxygenase-free soybean line or with 2.5% sugar caused an effect on the total intensity of bitterness or beaniness as measured by area under the curve. This did not occur when maximum intensity was used as a parameter. Maximum intensity of beaniness decreased only with the combined effect of using lipoxygenase-free soybeans and 2.5% sugar but did not decrease by the sole use of lipoxygenase-free soybeans or the addition of sugar to soymilk from the normal line.

Time-intensity is a useful method to measure intensity of beaniness in soymilk when different treatments are tested since it provided parameters which give unique information and which could not be accomplished with the use of single point measurements of intensity. The low correlation of some of the parameters such as initial intensity or decrease angle with the other T-I parameters was also an indication of the validation of the use of T-I methodology in contrast to single-point measurements.

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Table 1. Description of the treatments

Soymilk	Temperature (°C)	Sugar concentration (w/v%) 0	2.5	5
		Treatment number		
Normal line	25	1	2	3
	5	4	5	6
Lipoxygenase-free line	25	7	8	9
	5	10	11	12
Commercial	25	-	-	13
	5	-	-	14

Table 2. Time intensity parameters and definitions

Parameter	Abbreviation	Definition
Time to maximum intensity	tmax	The time to reach maximum intensity (in seconds)
Maximum intensity	imax	The maximum intensity of an attribute
Duration	ttot	Total time for the perception of the attribute (in seconds)
Area under the curve	area	The total area under the time intensity curve
Increase angle	iang	The angle of increase to maximum intensity (rate of onset of the attribute)
Increase area	iare	The area under the increasing portion of the curve
Decrease angle	dang	The angle of decrease from maximum intensity (the rate of decrease of the attribute)
Decrease area	dare	The area under the decrease portion of the curve
Initial intensity	iint	The first intensity response that the panelist records

Source: adapted from Zimoch and Findlay (1998)

Table 3. F ratios from analysis of variance for main effects of the T-I parameters for bitterness

		TI parameters								
Source of variation	**df**	**tmax**	**imax**	**ttot**	**area**	**iang**	**iare**	**dang**	**dare**	**iint**
Replication	2	5.0**	1.9	1.0	0.6	7.4***	2.6	1.5	1.8	3.2*
Line	1	0.1	14.6***	4.0*	9.5**	5.4*	4.4*	0.5	7.1*	5.1*
Temperature	1	2.9	0.0	3.2	1.3	1.0	4.0*	0.0	0.1	0.2
Sugar	2	0.6	25.6***	7.5***	23.5***	14.7***	13.0***	0.6	18.4***	5.6**
Judge	9	4.1***	17.7***	18.0***	10.6***	8.5***	2.1*	24.4***	15.1***	10.9***
Temp*sugar	2	1.9	2.5	5.6**	4.0*	0.5	1.9	2.0	2.9	1.6
Temp*line	1	0.5	2.4	1.4	1.4	0.0	0.0	0.2	1.9	0.2
Sugar*line	2	1.6	0.5	0.5	0.2	0.2	0.5	0.1	0.5	1.3
Contrasts [z]										
Contrast commercial vs. Lipoxygenase-free	1	3.9	46.7***	32.1***	50.5***	33.7***	21.0***	0.7	36.2***	1.4
Contrast commercial Vs. Lipoxygenase-free 2.5 % sugar	1	0.5	16.9***	17.7***	24.1***	11.7**	2.4	0.0	23.8***	0.3
Contrast commercial vs. Lipoxygenase-free 5% sugar	1	2.0	15.6***	16.2***	15.2***	9.9**	5.3*	1.1	10.3**	0.1
Contrast commercial vs. normal	1	0.4	75.8***	62.7***	93.6***	51.3***	28.9***	0.8	75.0***	4.0
Contrast commercial vs. normal 2.5% sugar	1	2.2	32.4***	27.6***	33.6***	23.3***	10.3**	2.0	25.4***	1.1
Contrast commercial vs. normal 5% sugar	1	4.9	22.7***	23.4***	25.7***	13.4**	7.0*	0.4	20.8***	0.4

*, **, *** Significant at $p < 0.05$, 0.01, and 0.001, respectively
[z] Calculated in a separate statistical model

Table 4. Pooled mean values for significant T-I parameters for bitterness.

	Line (n=180)		Sugar concentration (w/v%) (n=120)			Commercial (n=60)
Parameter	**Normal**	**Lip-free**	**0**	**2.5**	**5**	
imax	69.8 a	62.2 b	76.0 a	62.2 b	59.7 b	39.8
ttot	77.8 a	71.9 b	82.7 a	72.2 b	69.6 b	51.3
area	2874.7 a	2412.5 b	3355.6 a	2406.6 b	2168.7 b	889.4
iang	66.6 a	63.7 b	69.9 a	63.9 b	61.8 b	53.6
iare	923.6 a	806.3 b	1065.6 a	757.3 b	771.8 b	469.9
dare	1946.6 a	1608.7 b	2898.4 a	1653.2 b	1381.4 b	479.1
iint	7.7 a	5.5 b	8.8 a	6.0 b	5.0 b	5.6

Means with different letters within rows for line or sugar concentration are significantly different ($p < 0.05$)

Table 5. F ratios from analysis of variance for main effects of the T-I parameters for beaniness

		TI parameters								
Source of variation	**df**	**tmax**	**imax**	**ttot**	**area**	**iang**	**iare**	**dang**	**dare**	**iint**
Replication	2	2.2	0.0	0.1	0.6	1.2	0.6	1.6	1.3	0.6
Line	1	3.8	34.6***	1.4	26.8***	25.7***	17.5***	4.8*	18.7***	0.1
Temperature	1	0.0	0.8	0.4	0.6	0.1	0.0	0.3	0.4	0.1
Sugar	2	0.9	8.4***	2.2	12.0***	6.4**	11.1***	0.6	6.8**	4.8**
Judge	9	4.7***	27.8***	10.0***	9.4***	13.8***	9.4***	29.0***	6.9***	5.9***
Temp*sugar	2	1.8	0.7	0.3	0.4	0.8	0.0	0.3	0.6	2.0
Temp*line	1	0.4	0.0	3.3	2.1	0.1	1.8	0.6	1.8	0.0
Sugar*line	2	0.7	1.2	2.6	0.0	0.9	0.5	0.7	0.2	0.2
Contrasts[z]										
Contrast commercial vs.Lipoxygenase-free	1	0.1	0.0	0.7	0.3	1.3	0.1	0.3	0.2	0.4
Contrast commercial vs. Lipoxygenase-free 2.5 % sugar	1	0.2	8.2**	2.0	3.4	0.4	8.0**	0.3	0.2	0.4
Contrast commercial vs. Lipoxygenase-free 5% sugar	1	0.0	9.8**	0.5	6.7*	2.8	4.5*	0.1	2.5	0.9
Contrast commercial vs. normal	1	1.2	2.9	2.7	15.2***	5.8*	5.3*	1.3	11.2**	0.5
Contrast commercial vs. normal 2.5% sugar	1	0.3	1.0	0.2	1.0	5.1*	0.0	0.2	1.5	0.0
Contrast commercial vs. normal 5% sugar	1	4.0	0.0	1.8	0.1	0.9	0.0	0.3	0.3	1.2

*, **, *** Significant at $p < 0.05$, 0.01, and 0.001, respectively.

[z] Calculated in a separate statistical model

Table 6. Pooled mean values for significant T-I parameters for beaniness

	Line (n=180)		Sugar concentration (w/v%) (n=120)			Commercial (n=60)
Parameter	**Normal**	**Lip-free**	**0**	**2.5**	**5**	
imax	65.9 a	52.9 b	65.8 a	57.0 b	55.4 b	61.6
ttot	46.9 a	44.8 a	47.7 a	46.4 ab	43.4 b	42.4
area	1580.9 a	1172.0 b	1647.4 a	1270.4 b	1211.5 b	1338.5
iang	66.8 a	59.5 b	66.3 a	63.2 ab	59.9 b	60.8
iare	696.4 a	540.7 b	741.2 a	573.3 b	541.2 b	638.5
dang	69.5 a	66.4 b	68.6 a	68.4 a	66.9 a	67.3
dare	893.8 a	632.5 b	914.2 a	727.6 b	647.7 b	691.0
iint	6.9 a	6.5 a	8.7 a	6.9 ab	4.5 b	7.1

Means with different letters within rows for line or sugar concentration are significantly different ($p < 0.05$)

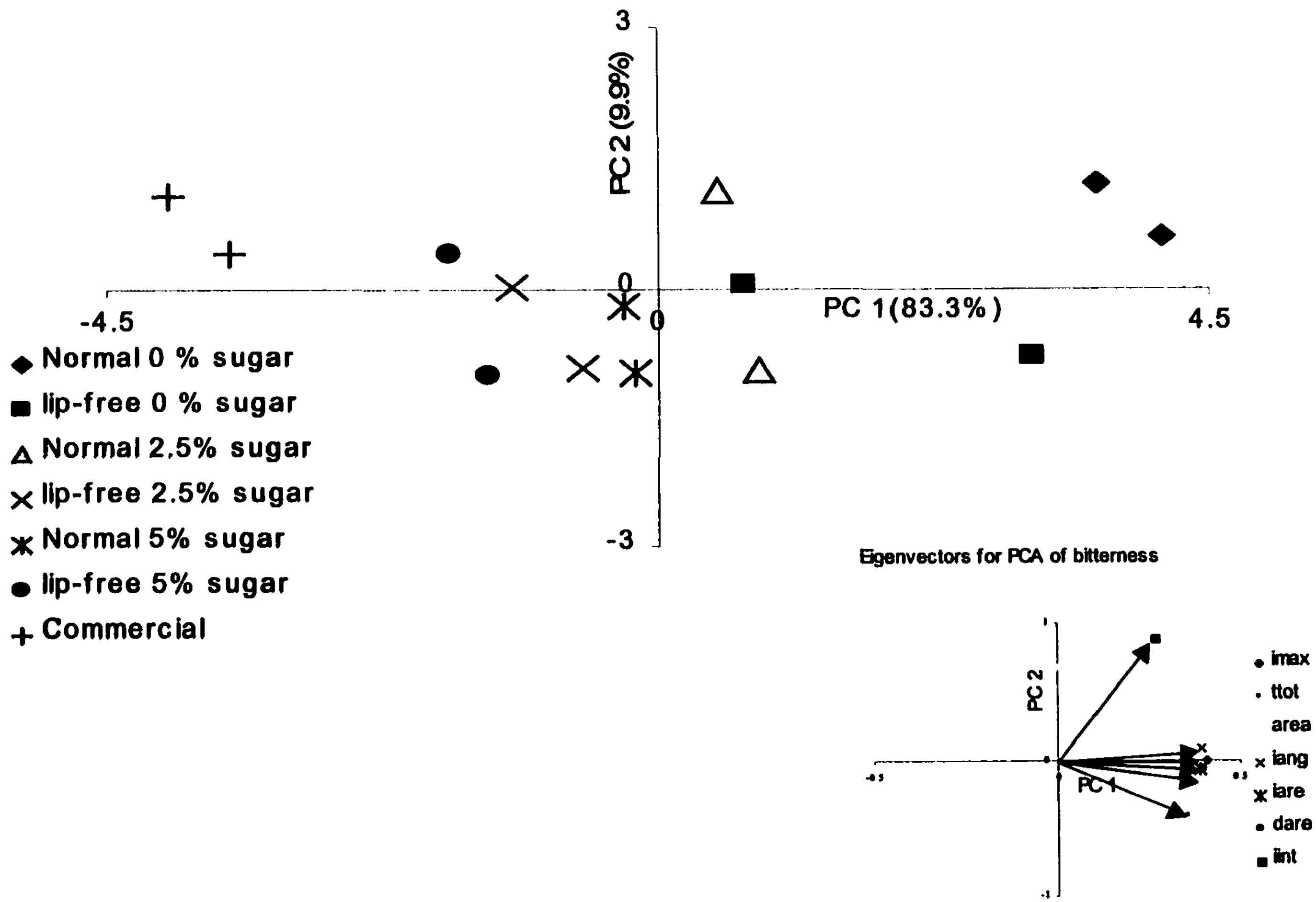


Figure 1. PCA for bitterness of soymilk

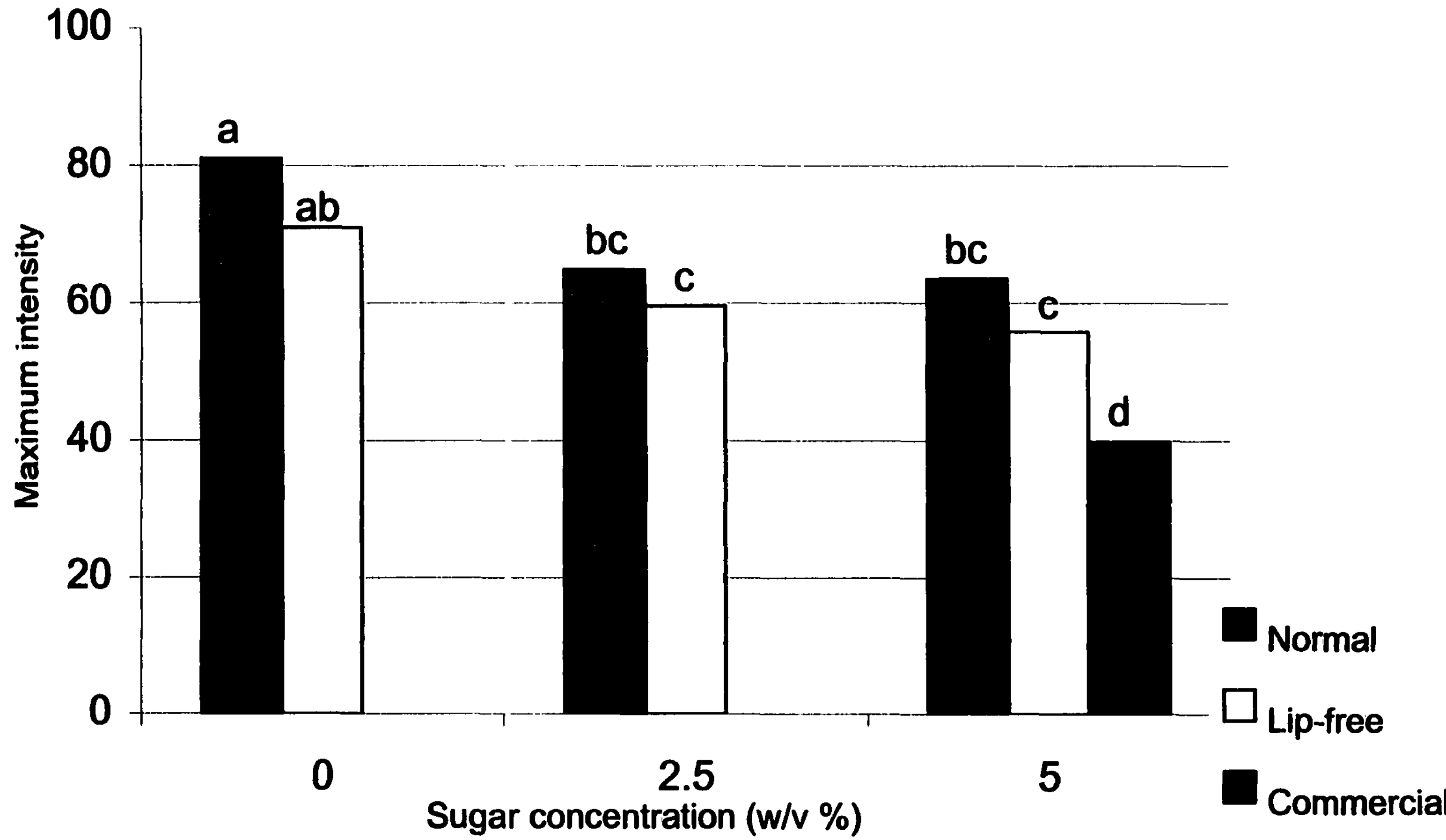


Figure 2. Bitterness maximum intensity of soymilks

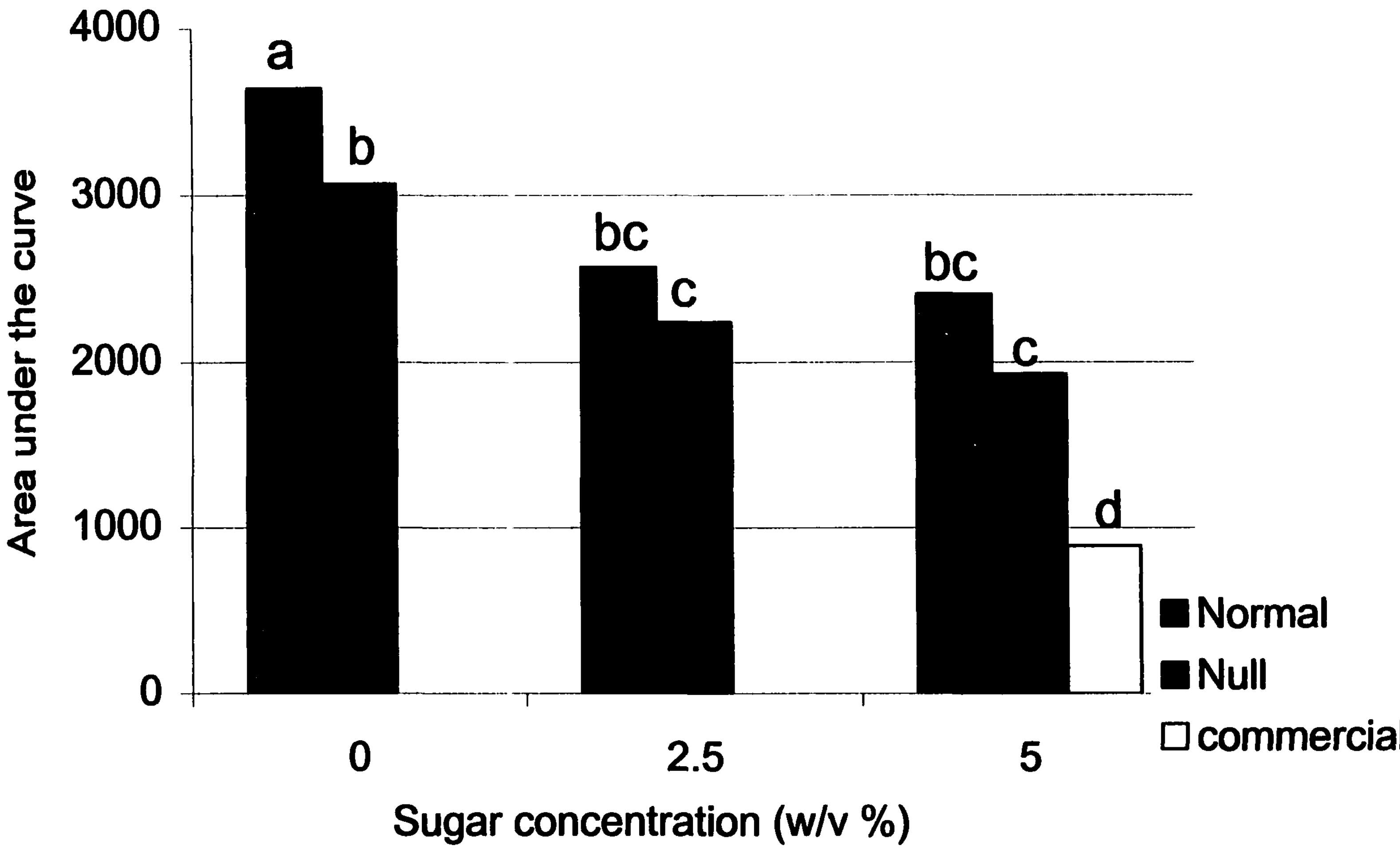


Figure 3. Bitterness area under the T-I curve of soymilks

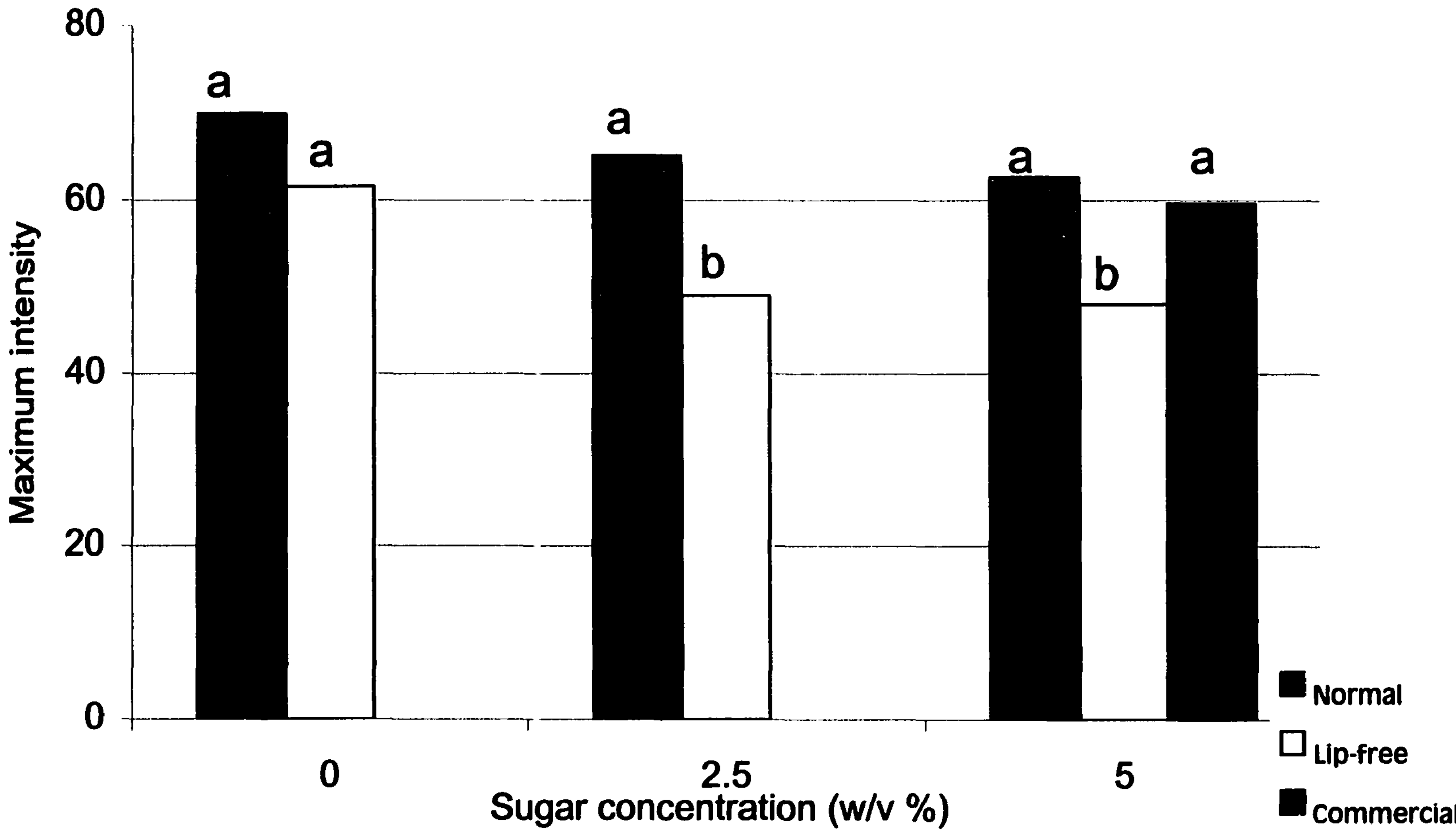


Figure 4. Beaniness maximum intensity of soymilks

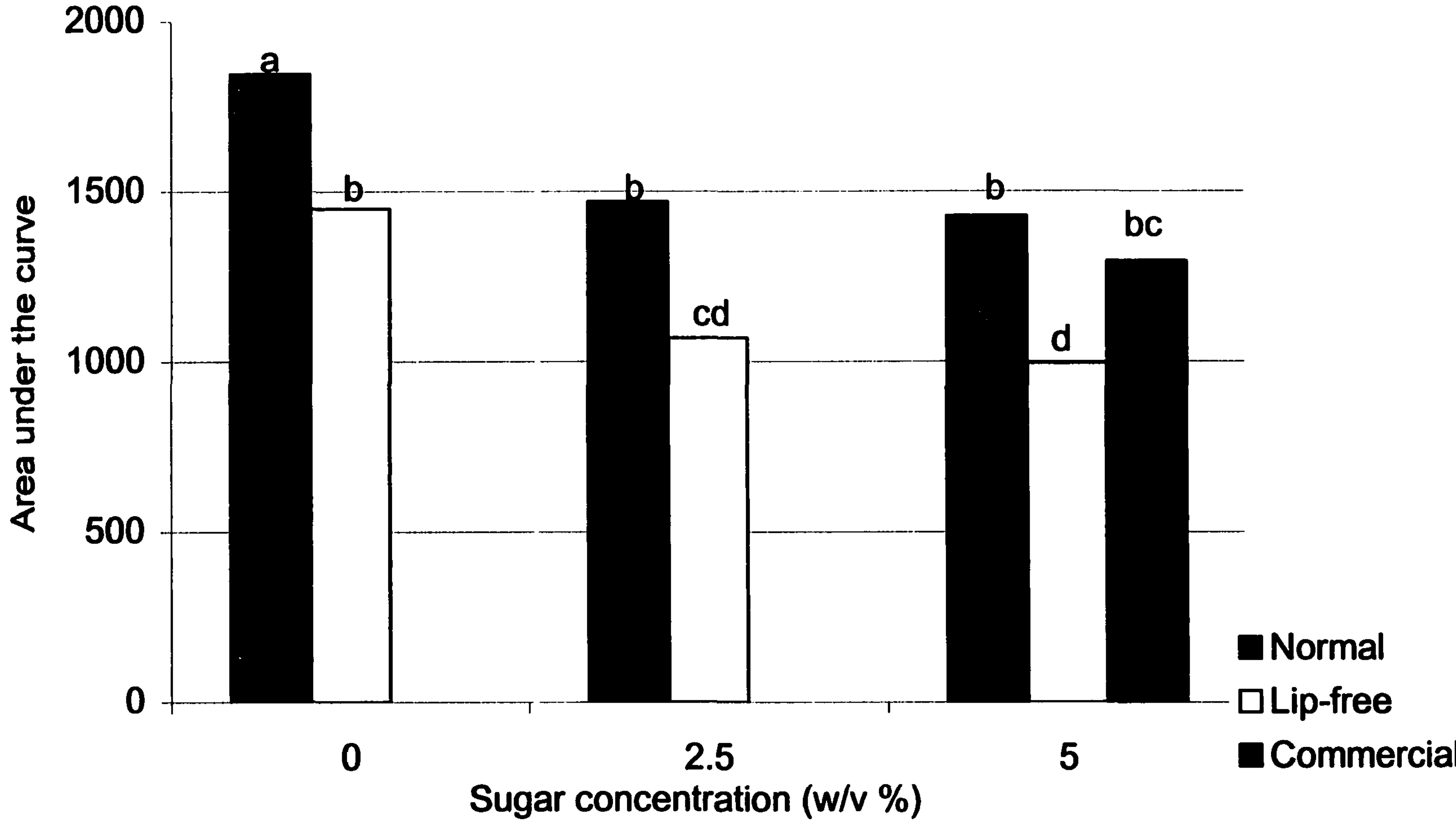
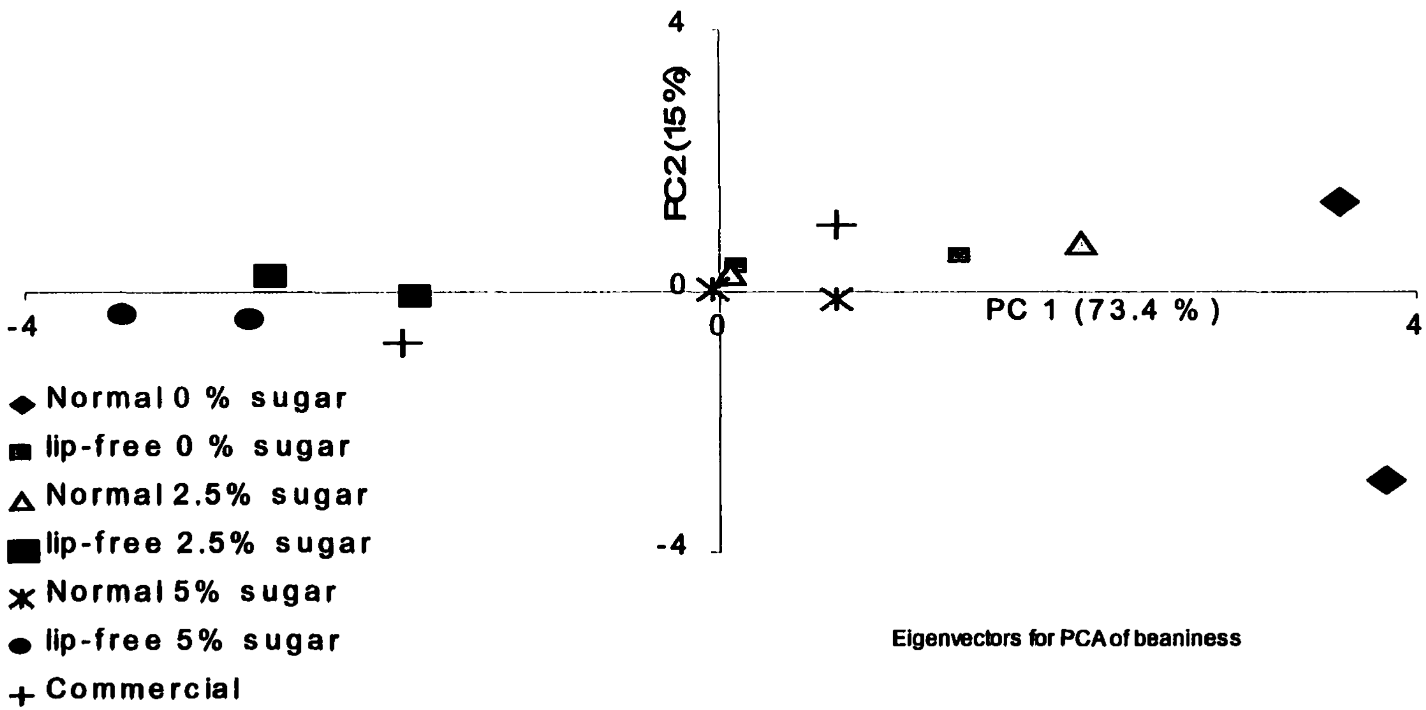


Figure 5. Beaniness area under the T-I curve of soymilks



Eigenvectors for PCA of beaniness

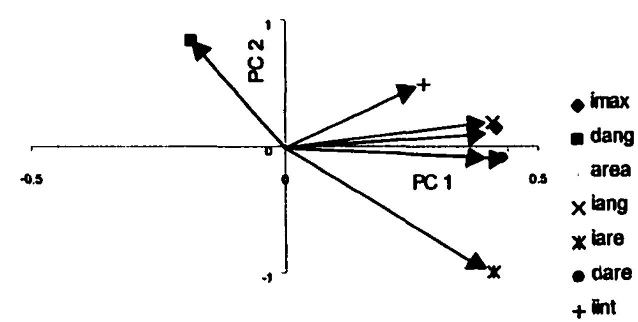


Figure 6. PCA for beaniness of soymilk

CHAPTER 5. GENERAL CONCLUSIONS

Description of the sensory attributes of soymilk was performed by different methods. Some differences could be observed when the different sensory methodologies were used. Perception of bitterness using unipoint measurements in soymilk evaluated by a panel of five members intensively trained was different from the perception of bitterness using time intensity by a panel of 10 members. When uni-point measurements of bitterness intensity were conducted, soymilk processed from lipoxygenase-free soybeans was more bitter than from the normal line. The opposite happened when time-intensity methodology was used. With this methodology, the total intensity of the sample is detected from the onset of the attribute to its extinction.

The studies conducted showed significant differences in the attributes related to beaniness between the two soybean lines evaluated. The attributes related to 'beaniness' were present in the soy products from any line. There was a reduction in 'cooked beany aroma' and 'cooked beany flavor' of soymilk and tofu made with soybeans lacking the SBL-1, SBL-2, and SBL-3 isozymes. Cultural differences may have accounted for some of the variations in panelist responses by nationality. Cultural differences in response to soy products may exist and should be considered when soy products are analyzed.

Terminology for description of the 'beany' concept was developed in this study. The terms: 'raw as hexanal' aroma or flavor, 'grassy' flavor and 'sweet as green floral' flavor, with the appropriate use of standards, are recommended to describe sensory differences in beaniness among soymilk samples when a descriptive analysis panel is properly trained. Because of the high correlation among the terms, they may also serve as a tool to illustrate the sensory concept of 'beaniness' when it is analyzed as a whole, and when the respective standards are used as multiple standards to define the multidimensional character of the concept 'beany'.

Changes in flavor for soymilks processed from stored beans are important considering that the product significantly increased in bitterness, despite a decrease in the intensity of the 'grassy' and 'sweet as green floral' flavor attributes.

Time-intensity was a good method to measure intensity of beaniness in soymilk since it provided parameters with unique information. This could not be accomplished with the use of single-point measurements of intensity. The low correlation of some of the parameters such as initial intensity or decrease angle with the other T-I parameters was also an indication of the validation of this methodology in contrast to single-point measurements. Highly correlated parameters may indicate that they are redundant in explaining the intensity of the attribute.

The addition of sugar to soymilk produced important desirable changes in flavor, especially in decrease of bitterness, astringency and the attributes related to 'beaniness'. The use of the new soybean line produced soymilk with reduced bitterness when intensity was measured as a function of time. In contrast, temperature did not cause any change in perception of bitterness or beaniness over time. The addition of 2.5% sugar produced an additional decrease in bitterness perception. The effect of sugar or soybean line on bitterness or beaniness varied when maximum intensity or area under the curve was used as a parameter. The use of the lipoxygenase-free soybean line or 2.5 % sugar alone caused an effect on the total intensity of bitterness or beaniness as measured by area under the curve. This did not happen when maximum intensity was used as a parameter. Maximum intensity of beaniness decreased only with the combined effect of using lipoxygenase-free soybeans and sugar but did not decrease by the sole use of lipoxygenase-free soybeans or the addition of sugar to soymilk from the normal line.

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