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Hae-Ran Lee Ashraf
Iowa State University

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FLAVOR AND COMPOSITION OF SOYMILK AS INFLUENCED BY ETHANOLIC SOAKING, HEATING AND PH CONTROL

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Flavor and composition of soymilk as influenced by ethanolic soaking, heating and pH control

by

Hea-Ran Lee Ashraf

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INTRODUCTION

The science and technology of soybean utilization for human consumption have been developing in parallel with the growing world protein problem during the last two decades. Remarkable achievements were made to convert soybeans to edible products. Even though unanticipated problems were encountered and resolved, many of the problems are still waiting to be solved.

Soymilk is one of the soybean products directly consumed by humans. The traditional Oriental soymilk is a simple water extract of soybeans and has excellent nutritional value comparable to cow's milk. Interest of food technologists and nutritionists in soymilk has been due not only to the economical and nutritional value of the product but also to the use of soymilk for infants allergic to cow's milk. Accordingly, many improvements were made in the methods of preparation as well as in the quality of the different types of soymilk.

However, the problem of an undesirable flavor, often described as beany, green or painty, limits the acceptability of soymilk, especially to the population that is not accustomed to soymilk. This undesirable flavor is believed to be caused by lipoxygenase which is inherent to soybeans.

The Oriental preparation of soymilk and most alternative processes use heating to inactivate this enzyme. Severe heating, however, impairs nutritional value of soymilk due to loss of lysine and to heat fixation of protein bodies that results in less protein content in soymilk.

Alcohol extraction of dry soybean products can improve the flavor. Soaking soybeans in ethanol at mild temperatures has been shown to be effective in inactivating lipoxygenase and in maintaining protein solubility.

The main objectives of the study are to use ethanol soaking for soymilk preparation and to study the flavor and composition of the resulting soymilk.

LITERATURE REVIEW

Utilization of Soymilk

Soybean, Glycine max (L.) Merrill, is one of the oldest cultivated crops. The earliest record of the soybean appeared in 2383 B.C. In the Far East, it is one of the most important legumes and has provided a large share of protein, fat and flavor in the diet for thousands of years. A Chinese materia medica written in about 450 A.D. even recommends using soybeans as a drug. Buddhism played an important role in the development of various soybean foods due to the Buddhist principle of excluding meat from the diet (Smith and Circle, 1972a).

Soymilk, a simple aqueous extract of whole soybeans, was introduced for the first time by Whai Nain Tse in China about twenty centuries ago and is widely consumed in these regions along with the other soybean products such as soybean curd, soy-sauce and fermented soybean paste (Wang et al., 1979).

Harry Miller, an American medical missionary in China in the early 1920's, produced soymilk and fed it to infants and patients in hospitals. Later, in 1940, he fortified soymilk with vitamins and minerals and introduced it to the United States for infants and for people who are allergic to cow's milk (U.S.D.A., 1961).

Heiner et al. (1964) reported that 7% of American infants are allergic to cow's milk and that soymilk is a palatable substitute. In the United States, 10% of infants were using soy based formulas in 1973 (Weisberg, 1974).

Soymilk has not gained wide acceptance among adult populations outside of the Orient mainly due to the characteristic beany flavor (Wilkens et al., 1967; Nelson et al., 1971; Wolf, 1975; Wang et al., 1979). In European countries, soymilk use is limited to proprietary types of infant foods.

In many of the developing countries where dairy industries are not well-established, need for nutritious infant and weaning foods, as well as food for adults, at low cost is very great. International organizations under the United Nations and many other institutions of developed and developing countries are introducing technology for producing soymilk. This technology ranges from small scale village processing to larger more sophisticated operations (Weisberg, 1974).

Soy beverages have been commercially produced in several developing countries since the early 1970's. In Latin America, Saci and Puma have been marketed in Brazil and British Guiana but not successfully (Wilding, 1970). Soymilk powders have been developed in Colombia and Mexico. In Africa, Morocco and Nigeria, there are attempts to introduce soymilk to the public (Wang et al., 1979).

More products are available in Asian countries than in the West; for instance: Vitasoy in Hong Kong, Super-D and Vegimil in Korea, Beanvit and Vitabean in Singapore and Malaysia, Philsoy in the Philippines and Vitamilk in Thailand (Elder and Weisberg, 1970). Pilot scale production of soy beverages in India and Sri Lanka has been attempted recently (Wang et al., 1979).

Soymilk has existed for more than two thousand years. It is one of the traditional foods in Asia in contrast to its limited use outside of the Orient. It has, however, the potential of becoming an important food and protein source in many cultures, if inherent flavor problems can be overcome.

Soymilk Preparation

The conventional Oriental soymilk is prepared by soaking whole soybeans in water for several hours and grinding with the addition of enough water to give the desired solids content in the milk. Filtering of this slurry is followed by boiling for 30 min.

Even though this process is simple, the flavor of the product is not accepted readily. Modifications of the process have been made during the last fifteen years to improve the flavor and the nutritive quality of the product. The addition of sugar, salts and flavoring agents to soymilk is commonly done to improve its flavor (Smith and Beckel, 1946).

Hackler et al. (1963) found that the insoluble residue removed in the preparation of soymilk had a higher protein efficiency ratio (PER) than soymilk. Thus, incorporation of more insoluble residue in the soymilk could improve its nutritional quality.

Hand et al. (1964) made a pilot plant study on soymilk production with the purpose of developing a soymilk process for Indonesia using whole soybeans. They found that soymilk from water-soaked whole soybeans had better flavor than from unsoaked dehulled soybeans.

Miles (1966) designed a soymilk process using soybean flakes to make a slurry. After pressure cooking, the slurry was homogenized and centrifuged. He claimed the elimination of soaking prevents the possible bacterial contamination during soaking.

Wilkens et al. (1967) pointed out that flavor defects of soymilk can be corrected either by removing volatile compounds after the soymilk is prepared or by preventing their formation through inactivating lipoxygenase. They suggested that prevention of volatile compound formation is more feasible since stripping the volatiles from the complex aqueous system can not be done satisfactorily. They developed a new process (hot grind) for an acceptable, bland soymilk. The new process includes grinding unsoaked, dehulled soybeans with water at temperatures above 80°C for instant inactivation of lipoxygenase as soon as the cells are broken.

Lo et al. (1968a) observed that soaking temperature affects the yield of the extracted solids. Eight hours soaking gives higher solids in soymilk than unsoaked beans or soy flour. Also, the highest solids content is obtained with extraction temperatures between 55 and 65°C regardless of the soaking time. There are difficulties in filtering soymilks when the extraction temperature is above 85°C.

Lo et al. (1968) reported on the difficulty of producing a sweetened, condensed soymilk due to the gel formation at over 27% solids content. This phenomenon is called the viscosity barrier and is attributed to the formation of disulfide bonds between cysteines in soy proteins. Wallace and Khaleque (1971), however, reported the possibility of sterilizing and canning concentrated soymilk containing up to 15.5% total solids (about 9% protein) if it has been forewarmed at 115°C for 5 min before concentration. Preheating increases the viscosity before sterilization but decreases viscosity in the final, sterilized product.

Soymilk from sprouted soybeans was attempted by Okumura and Wilkinson (1968). The sprouting step improves the vitamin content and removes a substantial amount of hulls. Sprouted beans are ground into a slurry and cooked. Addition of calcium sulfate and magnesium chloride precipitates protein which is removed from the slurry and washed with water. The protein is ground into a fine state, formulated by addition of ingredients to make the milk similar to cow's milk and homogenized.

Kon et al. (1970) used low pH to prevent the oxidative off-flavor formation during grinding of raw legume seeds. Suppression of off-flavor is possible at pH 3.85 and below, however, further acidification is necessary for maximum protein extraction. Bland legume milk is obtained by neutralization after heating. Inactivation of lipoxygenase at low pH was also attempted by Al-Kishtaini (1971).

Badenhop and Hackler (1970) investigated the effect of soaking soybeans in sodium hydroxide solution on the flavor, acceptibility and nutritional value of the soymilk. Soaking in 0.05N sodium hydroxide produces a soymilk with a pH of 7.4 and significantly improved flavor. A further increase in pH results in poorer flavor, less cystine content and lower PER values but less trypsin inhibitor activity and more available niacin. Later, Badenhop and Hackler (1973) reported successful results with methionine supplementation to compensate for cystine loss during alkaline soaking for soymilk preparation.

Khaleque et al. (1970) reported that soaking soybeans in sodium carbonate solution produces a soymilk with significantly less beany flavor than soaking in water or sodium hydroxide. They raised the question of lipoxygenase being solely responsible for the beany flavor in soymilk, since their results showed only a small difference in flavor between cold and hot grinding. Also, they found a large difference in flavor between low temperature extracts with sodium carbonate vs. sodium hydroxide while the lipoxygenase activities were very similar. However,

Bourne et al. (1976) explained that the small difference in flavor between cold and hot grinding was due to insufficient heat to inactivate lipoxygenase. They emphasized that the minimum temperature for the prevention of beany flavor is 80°C throughout the grinding process.

Bourne et al. (1976) investigated the effect on flavor and pH of the addition of sodium alkali and sodium salts to soymilk. Addition of sodium hydroxide to pH 7.0-7.5 was preferred over sodium carbonate or sodium bicarbonate. Further increases in pH with sodium hydroxide gave soapy flavor and less acceptability. They theorized that the improvement of flavor is related to the sodium ion concentration and not due to the changes in pH.

Bourne (1971) reported on an extensive study of soymilk production, acceptability and nutritional aspects in the Philippines. Hot grind soymilk is well-accepted and can supply as much as 50% of the recommended daily protein intake for a toddler. Bourne and Clemente (1971) found that among thirty Philippino varieties of soybeans, six are unsuitable for soymilk preparation due to poor flavor and two are unsuitable due to very low extractability.

Lo (1971) patented a process for a dried soybean beverage using a Wenger expansion cooker. The patent was assigned to a soybean product company in Hong Kong. In this process, dehulled flakes are preconditioned with steam at 100°C to give 18 to 20% moisture content and are extruded at high pressure. The pellets are ground to powder.

Mustakas et al. (1972) prepared a spray-dried soymilk powder. A slurry of full-fat flour is milled through a colloid mill with a clearance of 0.001 inch and emulsified using a homogenizer. The spray-dried product forms a stable emulsion when mixed with water.

Mustakas (1974) also patented a soymilk process using acid pH to inactivate lipoxygenase and to precipitate lipid-protein. Full-fat soybean flour is suspended in water and heated at a pH of 3.5-4.5 to give a lipid-protein precipitate. This is resuspended in water, adjusted with alkali to pH 9 and heated briefly. The slurry is then homogenized, neutralized and centrifuged.

Mital and Steinkraus (1976) investigated the flavors of the soymilks prepared from defatted soyflour and by the hot grind process. The latter is inferior to the former due to beany, objectionable flavor. They indicated that even though hot grind soymilk is considered to be much better than the traditional cold water extract, it requires further improvement before it can be acceptable to American consumers.

Nelson et al. (1976) developed an Illinois process which includes soaking and blanching whole soybeans in 0.5% sodium bicarbonate, grinding, homogenizing, neutralizing, diluting and adding sugar and flavor. Colloidal stability due to high pressure homogenization results in increased yields of soymilk through the elimination of solid separation. Kuntz et al.

(1978) observed that the soymilk from this process, however, suffers from chalkiness caused by the particles in the soymilk. They showed that alkalinity of the blanching solution and post-blanching processing is important for the improved mouth feel of soymilk because of the greater hydration and more extensive disruption of the particles.

Forster and Ferrier (1979) reported that soymilk by the Illinois process shows pseudoplastic and slight thixotropic flow characteristics. Increased solids content, inclusion of soy hulls and sodium bicarbonate blanching increase the viscosity, whereas double homogenization decreases viscosity.

Kapoor et al. (1977) reported the acceptability of hot grind soymilk by an Indian panel. However, they found that blending soymilk with skimmed cow's milk has better acceptability.

Johnson and Snyder (1978) carried out a comparison of processing methods on the yield and composition of soymilks. Heating before cell disruption decreased the solids and protein recovery due to loss of heat fixed protein bodies. High pressure (8,000 psi) and high temperature (75°C) homogenization are more effective than low pressure or cold homogenization for the recovery of solids.

Although numerous studies were made to examine and improve the quality of soymilk over the decades, only two processes (hot grind and Illinois) are well-established and used in

various areas in the world, especially in conjunction with the effort to introduce high protein low cost foods to the developing countries. Improvements in flavor and the quality of soymilk are definitely necessary to expand the use of soymilk in the developing countries as well as developed countries.

Flavors of Soymilk

Green-beany flavor

The typical green-beany flavor of soymilk is considered the major deterrent to acceptability of soymilk. When raw legume seeds are ground with water, undesirable odor and flavor similar to drying oil develop very rapidly.

Wilkens et al. (1967) observed that soymilk prepared from whole soybeans develops beany and rancid flavors, whereas soymilk from defatted beans has a bland cereal-like flavor. They showed that lipoxygenase causes off-flavor, and rapid hydration and high temperature grinding prevents off-flavor development. Several studies have confirmed this concept (Mustakas et al., 1969; Kon et al., 1970; Wolf, 1975; Sessa and Rackis, 1977). Absence of the off-flavors in the properly blanched products emphasizes that the formation is due to enzymatic reaction rather than autoxidation of the oil (Snyder, 1973; Kalbrener et al., 1974; Sessa and Rackis, 1977).

Erickson (1967) showed that in pea, lipoxygenase and free or bound unsaturated fatty acids are located in all parts of the seed, and the possibility of enzyme, substrate and molecular oxygen coming in contact is very great. Gardner (1975) pointed out that when the beans are disrupted mechanically, lipoxygenase probably is the predetermining factor in off-flavor development.

Not all authors agree about lipoxygenase causing off-flavors. Sessa et al. (1969) reported that removal of volatile carbonyl compounds present in defatted flakes does not change beany flavor characteristics of these flakes. Rackis et al. (1970, 1972) also pointed out that some lipid degradation occurs during the dry processing of soybeans but causes no rancid odors or flavors. They observed no correlation between lipoxygenase activity and green-beany flavor during maturation of soybeans and suggested that characteristic raw beany flavor may preexist in the whole soybeans. They, however, indicated that extensive oxidation of lipids when large amounts of air come in contact with the lipids during wet processing of full-fat soybeans can cause objectionable flavors in addition to those originally present in soybeans.

Lipoxygenase (linoleate:oxygen oxidoreductase, E.C. 1. 13. 11. 12) catalyses the conversion of polyunsaturated fatty acids containing a 1,4-cis,cis pentadiene to L-hydroperoxy fatty acids by molecular oxygen (Hamberg and Samuelson, 1967).

Soybean lipoxygenase is actually a group of isozymes: lipoxygenase 1, the enzyme described originally by Theorell et al. (1947); lipoxygenase 2 (Christopher et al., 1970); and lipoxygenase 3 and 4 (Christopher et al., 1972).

These isozymes can give rise to isomers of monohydroperoxides due to their specificity for the position of oxygenation, C-9 or C-13, of the substrate. There is also steric specificity which yields L at C-13 and D at C-9 for all lipoxygenases (Hamberg and Samuelsson, 1967; Axelrod, 1974; Roza and Francke, 1973; Gardner, 1975). Christopher and Axelrod (1971) observed the ratio of 13- to 9- isomers of 95:5 and 50:50 by lipoxygenase 1 and 2, respectively. Christopher, 1973, as cited by Axelrod (1974) found that lipoxygenase 3 and 4 have the same positional specificity as lipoxygenase 2. However, this positional specificity varies somewhat with experimental conditions such as O_2 tension, pH and temperature (Christopher and Axelrod, 1971).

Kalbrener et al. (1974) reported that the flavor of linoleic acid hydroperoxide is predominantly grassy-beany, musty-stale and rancid, whereas linolenic acid hydroperoxide is grassy-beany, bitter and astringent.

Hydroperoxides undergo a series of reactions, enzymatic or autoxidative, resulting in many secondary decomposition products such as volatile carbonyls, polymers and oxygenated compounds with various flavors (Grosch et al., 1976; Gardner,

1975). Grosch and Laskawy (1975) and Grosch et al. (1976) reported on the differences in the amount and the ranges of volatile compounds formed by soybean lipoxygenase isozymes. Lipoxygenase 1 (optimum pH 9.0) produced 2-trans-hexenal, propanal and 2-trans-pentenal, whereas 2-trans-pentenal, 2-trans-hexenal, 2-trans,6-cis-nonadienal, 2-trans,4-cis-heptadienal, 3,5-octadien-2-one and 2,4,6-nonatrienal were produced by neutral lipoxygenases.

The flavors of the higher alk-2-enals and alk-2,4-dienals formed by neutral lipoxygenases were described as cardboardy, oily and painty (Sessa, 1978). On the other hand, n-heptanal, n-hexanal, 2-trans-heptenal and 3-cis-hexenal contribute to the green-beany flavor of soybean (Hoffman, 1961; Kinsella et al., 1967; Leu, 1974; Sessa, 1978).

Meijboom (1964) reported that the presence of decadienal and 2-trans-nonenal can completely mask the perception of 3-cis-hexenal even when the concentration of the latter is higher than the threshold value. He also pointed out that the occurrence, number and stereochemical configuration of double bonds, and the length of the hydrocarbon chain influence the flavor quality and intensity.

Badenhop and Wilkens (1969) identified 1-octen-3-ol in soymilk prepared with water soaked soybeans. This compound has mushroom-like flavor and is produced presumably by enzymatic reaction during the soaking of soybeans.

Mattick and Hand (1969) isolated ethylvinyl ketone which has a slight green-beany flavor from ground soybeans. Even though the peak of this compound on a gas chromatogram is small in relation to other components eluted, the odor is very intense and penetrating.

In their study of soymilk volatiles, Wilkens and Lin (1970) identified forty-one compounds positively and thirteen compounds tentatively. Several compounds are believed to contribute to the green-beany flavor of soymilk. Hexanal, contributing 25% of the volatile compounds and having an extremely low threshold, is considered one of the major compounds responsible for the off-flavors. Hexanol contributes a harsh, grassy odor and 2-hexenal, called the leaf aldehyde, possesses the odor of green foliage. Other compounds identified are 1-penten-3-ol, ethylvinyl ketone, amylvinyl ketone and 2-pentylfuran. They, however, could not isolate 3-cis-hexenal. They speculated that the presence of 2-trans-hexenal could result from isomerization of 3-cis-hexenal to 2-trans-hexenal. This isomerization was also shown by Stone et al. (1975).

Arai et al. (1970) reported that alcohols such as pentanol, hexanol and heptanol are distributed throughout the soybean tissues, with especially high concentrations in the hypocotyl.

Bitterness

Bitterness has been another flavor problem of soybean products. Sessa et al. (1974, 1976) found that strong bitterness develops during the autoxidation of purified soybean phosphatidylcholine. On the other hand, hydrogenated phosphatidylcholine does not develop bitterness indicating oxidized fatty acid as the cause of the flavor.

Sessa (1978) reported that nonvolatile fatty acids such as hydroxy-, dihydroxy-, oxo-octadecadienoic acids and hydroxy-epoxy- and tri-hydroxy octadecenoic acids are produced in model systems with soybean lipoxygenase and linoleic acid. Similar products are isolated from bitter-tasting soybean phosphatidylcholine. However, soybean lipoxygenase has very little activity on phosphatidylcholine.

Baur et al. (1977) identified bitter-tasting 9,12,13-trihydroxyoctadec-10-enoic acid and 9,10,13-trihydroxyoctadec-11-enoic acid in the system of linoleic acid and crude soybean lipoxygenase. Similar results were shown by Grosch (1978). Baur suggested that the occurrence of at least three hydroxy functions in the alkyl chain is essential for the bitterness characteristic.

Astringency

Soybeans contain a number of phenolic compounds which are related to the flavor or other sensory effects such as

astringency and throat-catching (Cowan et al., 1973). Phenolic compounds occur widely in the plant kingdom and include simple phenolics containing a single benzene ring, flavonoid compounds possessing a basic structure of C_6-C_3-C_6 and a third group of heterogeneous phenolic derivatives having high toxicity in animals (Singleton and Kratzer, 1973). Simple phenolics include phenolic amino acids, coumaric, cinnamic, caffeic, chlorogenic and gallic acid. The phenylpropane pattern is the principal structural unit of lignin. Flavonoid compounds consist of anthocyanins which are water soluble pigments of flowers, fruits and vegetables; anthoxanthins, many of which are bitter; catechins, substrates for enzymatic browning and tannins, responsible for astringency in many foods (Berk, 1976).

Arai et al. (1966) carried out the fractionation of an ethanol extract of defatted soybean flour. Fraction A, which has strong phenolic flavor, contains seven phenolic acids; syringic, vanillic, ferulic, gentisic, salisylic, p-coumaric and p-hydroxy benzoic, whereas fraction B contains chlorogenic acid and isochlorogenic acid and has sour, bitter and astringent flavor.

The presence of several isoflavones in the soybean has been demonstrated. They include genistein, daidzin and 4,7-dihydroxy,6-methoxy isoflavone (Naim et al., 1973).

Honig et al. (1969) investigated the characteristics of the fractions from defatted soybean flake lipid and found that the fraction containing isoflavones, sterol glycosides and phosphatidic acid has bitter, throat-catching, astringent, and biting tastes with a lingering aftertaste.

Kalbrener et al. (1974) reported that the linoleic and linolenic acid hydroperoxides formed by the action of lipoxygenase are astringent. Soy flour has even more intense astringency than the hydroperoxides.

Kalbrener et al. (1971) observed that astringency appears in commercial soy protein concentrate and isolate.

Nuttiness

Wilkins and Lin (1970) observed that some of the compounds identified from their soymilk have agreeable flavors. They identified benzaldehyde with cherry or almond-like aroma; octanol, nonanol and 1,1-diethoxypropane with a faint rose odor, aliphatic methyl ketones with fruity aromas.

In a study of sensory evaluation of soyflour, Moser et al. (1967) showed that autoclaving of soyflour produces nutty, sweet and toasted flavors intensifying with the increase in autoclaving time up to 10 min.

Manley and Fagerson (1970) reported the presence of alkylated pyrazines in a volatile fraction of hydrolyzed soy protein. Pyrazines have been isolated from various roasted

food products such as peanuts, cocoa beans, butter, coffee and potato chips. Newell et al. (1967) indicated pyrazines are generated from nonenzymatic browning reactions resulting in nut-like flavor.

In a study of volatile compounds from model systems containing protein and water with or without added fat and carbohydrate, Qvist and von Sydow (1974) identified more than 150 compounds. Using soy protein isolate, they found that the concentration of all volatiles, especially branched chain aldehydes of low molecular weight increases on heating, presumably due to the Strecker degradation. Addition of fat generally decreases the ketones and furans possibly due to the solvent effect of fat, whereas there is no marked difference in aldehyde content since some of the aldehyde is generated from the fat, and at the same time some is retained by the dissolved fat. The absence of pyrazine is probably due to the aqueous model system.

Cooked flavor

Van der Meer and Spaans (1970) isolated 4-vinylphenol and 4-vinylguaiacol from the volatiles of steamed soyflour dough. These compounds are derived from p-coumaric and ferulic acids respectively. However, the reproducibility of the soybean cooked flavor by mixing these two compounds with diacetyl, maltol, hexanol and other compounds is not successful, presumably due to the absence of various other compounds

in soybean.

Interactions between flavor compounds and other components

Interactions of flavor compounds with other components can have an effect on the overall flavor of the food.

Beyeler and Solms (1974) observed that soy protein has much stronger affinity with aldehydes and ketones than toward alcohols.

Franzen and Kinsella (1974) reported that the binding of volatile flavor compounds to soy proteins in model systems is influenced by the composition of proteins, the presence of moisture and endogenous compounds such as lipid, as well as the kinds of volatile compounds. They suggested the possibility of predicting flavor release or binding in fabricated foods by controlling these parameters. Thus, optimum flavor release might be achieved.

Anderson and Warner (1976) found that an acid-sensitive fraction of soy protein binds the grassy-beany, bitter and astringent soy flavor compounds. The irreversible binding and loss of volatility of the components result in the loss of the flavor characteristics (Sessa and Rackis, 1977). Stone et al. (1975) showed the isomerization of 3-cis-hexenal to 2-trans-hexenal as well as conversion of aldehydes to alcohols in crushed tomatoes. These changes were attributed to oxido-reductase systems in tomatoes and to heating, with heating

being more effective. This result indicates the possibility of flavor change on heating due to structural changes of flavor compounds.

Kalbrener et al. (1971) suggested the correlation of particle size to the flavor scores of water dispersions of soybean products. With bigger particles, less flavor component reaches the taste buds than with finer particles. Also, dried soy flour has less flavor intensity than a water dispersion.

In a study on the flavor of oxidized milk fat, Day et al. (1963) showed the additive interaction between two carbonyl compounds. For instance, the mixture of subthreshold concentrations of n-nonanal and n-decanal gives a detectable flavor. They concluded that the flavor of oxidized milk fat is the combined effects of the various carbonyl compounds.

Flavor of a food is very complex. In soymilk, a number of off-flavors are present. Green-beany or painty, the predominant flavor, has been the subject of numerous investigations and shown to be caused mainly by lipoxygenases and various carbonyl compounds. Other flavors of soymilk, however, have not been investigated, even though some flavor compounds were identified occasionally. The interactions among flavor components and effects of variables during soymilk preparation have not been extensively studied. More research has to be done before the nature and the improvement of the soymilk flavor can be established.

Lipoxygenase Inactivation

Heating has been the most successful process for the inactivation of undesirable compounds in soymilk including lipoxygenase. Farkas and Goldblith (1962) showed that the inactivation of lipoxygenase by heat follows a first order reaction rate for 90% of the reaction. The decreased heat sensitivity when lipoxygenase is suspended in 20% pea puree led them to conclude that a protective complex is formed. They also found that heat inactivation of lipoxygenase is very sensitive to pH. The rate of inactivation increases below pH 4 or above pH 8.

Christopher et al. (1970) observed that heat stability of lipoxygenase 1 is at least 36 times greater than lipoxygenase 2. Similar results were shown by Borhan and Snyder (1979).

Nelson et al. (1971) studied various methods of blanching whole soybeans to inactivate lipoxygenase. Blanching fully soaked beans at 99°C for 10 min, blanching drybeans for 20 min, or soaking and blanching in 0.5% sodium bicarbonate for tender beans were suggested depending on the ultimate use of the soybeans.

Baker and Mustakas (1973) showed that lipoxygenase is very heat sensitive, and complete inactivation is achieved by cooking soybeans in water at 83°C for 15 min. However, severe heat treatment insolubilizes the major soybean proteins and

hampers the functional properties such as solubility, gelation, foaming and emulsification (Kinsella, 1976). Especially in soymilk, heat fixation of protein bodies before disruption of soybeans results in less protein in the soymilk (Johnson and Snyder, 1978).

Although heat treatment of soybeans appears to be the best method to prevent the formation of off-flavor, heating can result in lowered protein content of soymilk. Therefore, a method to inactivate detrimental biological compounds efficiently and to maintain solubility of proteins is needed.

Alcohol Treatment

Beckel et al. (1948) observed improvement in color and decreased bitterness of soybean products by ethanol extraction. Mustakas et al. (1961) reported that 95% ethanol and 91% isopropanol are effective in removing bitterness and color from defatted soy flakes, whereas absolute ethanol is not effective indicating the necessity of moisture.

Eldridge et al. (1963) extracted phospholipid-like material from soybean protein isolate by aqueous alcohols but not with other methods. The alcohol washed proteins are improved in color and flavor, and they whipped and foamed similar to egg white. The alcohol extractables from soybean proteins include phosphatidyl choline, phosphatidyl ethanolamine, saponins, sitosterol glycoside and genistein (Nash et al., 1967).

Maga and Johnson (1972) observed that a polar solvent (hexane:ethanol) is more efficient in removing the residual lipid than nonpolar solvents. Extraction with polar solvents produces less beany and better products than extraction with nonpolar solvents.

Steinkraus (1973) developed a successive extraction method using 95% ethanol at 60-65°C for 2 hr followed by a 1:1 chloroform:ethanol mixture for 22 hr to produce a completely bland defatted soybean meal. He observed that residual bound fat, principally phospholipid, is the cause of the off-flavor. The ethanol treatment decreases bonding between the lipid and protein, and chloroform removes the lipids.

Mann and Briggs (1950) reported that hot and cold methanol or ethanol extraction of soybean meal reduces the extractability of all soybean proteins, especially globulins. Roberts and Briggs (1963) and Wolf et al. (1963) confirmed Mann and Briggs' finding. Both reported that the 7S component is most sensitive to the alcohol denaturation. Other proteins (11S and 15S) are also denatured but slowly, and the 2S component is not denatured. Isopropanol also denatures 7S protein more readily than the other soy globulins.

Smith et al. (1951) investigated the denaturation of soybean protein by various alcohols. A pronounced denaturation takes place at 40 to 60% aqueous solutions, and the major portion of denaturation is completed in less than 5 min.

Mitsuda et al. (1967) observed the reversible inhibition of lipoxygenase by saturated monohydric alcohols and found that the degree of inhibition increases with chain length of alcohols. Fukushima (1969) pointed out that denaturation of soy protein by organic solvents is of interest because of the presence of hydrophobic regions that are not altered by water. In general, the denaturing power of organic solvents depends on their hydrophobicity and their concentration in water. Short chain alcohols are stronger denaturants than other organic solvents. At low concentration, the denaturing power increases with hydrophobicities of alcohols, and at high concentrations the reverse is true. This phenomenon is explained by the structure of the globulin protein in which hydrophilic regions are exterior and hydrophobic regions are interior. Thus, disruption of the hydrophilic region has to take place by aqueous solutions before the hydrophobic region is attacked by alcohols.

Eldridge et al. (1977) observed that soybeans soaked in 40 to 60% alcohol give the best over all flavor score and minimum grassy, beany response as well as minimum lipoxygenase activity. They pointed out that the improvement of whole soybean flavor by alcohol treatment is parallel to the inactivation of lipoxygenase. This observation strongly supports the theory that the off-flavor compounds in soy products are the result of lipoxygenase action. They also suggested that

alcohol soaking of whole soybeans inactivates lipoxygenase in situ, or wet milling in aqueous ethanol can be used as an alternative method for lipoxygenase inactivation.

Borhan and Snyder (1979) investigated lipoxygenase destruction in whole soybeans by combinations of heating and soaking in ethanol. They observed that lipoxygenase assay at pH 9 is suitable for the detection of the most heat resistant lipoxygenase. They found that the concentration of ethanol required for complete inactivation of lipoxygenase in 24 hr soaking decreases as temperature increases. Higher nitrogen solubility was obtained with lower temperatures of soaking. Increasing pH using sodium carbonate and sodium bicarbonate is effective in rapid inactivation of lipoxygenase. With emphasis on protein solubility, they recommended 15 to 45% ethanol, 40 to 60°C and 2 to 6 hr for soaking soybeans.

Alcohol treatment has been shown to be effective for removal of residual lipids and flavor of soybean products and for inactivating lipoxygenase in soybeans. The usefulness of alcohol treatment over heating is in maintaining the high solubility of protein products.

MATERIALS AND METHODS

Materials

Reagent grade chemicals were used in all experiments. Soybeans of Amsoy 71 variety and seed bean quality with proximate composition of protein 37.1%, crude lipid 18.2%, carbohydrate 28.8%, and ash 5.2% (on a wet weight basis) and moisture 10.7% were used throughout the experiments. Water for chemical analyses and lipoxygenase assays was obtained from a commercial still, passed through a Barnstead standard mixed bed ion exchange resin and stored in a polyethylene tank. For soybean soaking and soymilk preparation, tap water was used. All the chemical analyses and enzyme assays were done in triplicate.

Methods

Treatment of soybeans

Soybeans were soaked in various ethanol concentrations at a ratio of 1:3 (bean:ethanol solution) for the desired time and temperature. For raising the pH of soaking solutions, Na_2CO_3, $NaHCO_3$ and NaOH were added to 15% ethanol to give final concentrations of 0.1M or 0.01M. An aqueous solution of 0.5% $NaHCO_3$ was used for soaking and blanching soybeans in the Illinois process. Soaked soybeans were drained, washed and resoaked in tap water at a ratio of 1:10 (dry bean:water) at

4°C for 18 hr to remove the residual ethanol.

Soymilk preparation

Soaked soybeans were ground with added tapwater in a Waring blender for 10 min. The temperature of the slurry was raised to 47°C during grinding. The weight of the slurry was adjusted to 9 times the original dry weight of soybeans with tap water (1:8, bean:water ratio) after grinding. The diluted slurry was homogenized in a Gaulin Model 15M 8TA homogenizer at 3,500 psi for the first stage and 500 psi for the second stage. Desludging was done by centrifugation in an International Equipment Co. refrigerated centrifuge at 352 x G for 30 min. A portion of soymilk was freeze-dried in a VirTis Cabinet model freeze-drier and dried further in a hot air oven. Freeze-dried soymilk was stored in a polyethylene bag in a desiccator for later analysis. Another portion of soymilk was stored at 4°C for the flavor evaluation.

Flavor evaluation

A six member taste panel was trained for eight weeks with distinctly flavored soymilks. Terminology (paintiness, grassiness, astringency, nuttiness and cereal flavor) was defined during the training. Soymilks with particular flavors were prepared by four different treatments of soybeans; painty flavored soymilk resulted from soaking soybeans in 15% ethanol at room temperature for 6 hr, grassy flavored soymilk resulted

from soaking in water for over night and dipping in boiling water for 10 seconds, nutty and astringent flavored soymilk resulted from soaking and blanching for 30 min in 0.5% $NaHCO_3$ (Illinois process), cereal flavored soymilk resulted from soaking and blanching in tap water for 20 seconds and grinding at over 80°C (hot grind process).

A quantitative descriptive method (Stone et al., 1974) was used for flavor analysis of soymilks (Fig. 1). Panelists made a mark on the scales according to the intensity of the flavors, nuttiness, cereal, paintiness, grassiness and astringency in the sample. The flavor ratings on the scales were converted to numerical ratings ranging from 0 (no flavor) to 10 (very strong) by the use of a template. The panelists were given 20 ml samples at 25°C in 50 ml glass beakers and were instructed to rinse their mouths between samples. In some instances, nutty and cereal flavored soymilks used during training were standardized for their flavor intensities by the taste panel and were used as references for these flavors. Flavor evaluation was done at 2 p.m. in a taste booth under red lights.

Lipoxygenase assay

An oxygen uptake method (Zimmerman and Snyder, 1974) was employed using a Yellow Springs Instrument Co. Model 53 Oxygen Monitor with a Sargent Welch SGR recorder. A plunger containing the oxygen sensor covered by a gas permeable Teflon

Fig. 1. Sensory evaluation sheet used for flavor analysis of soymilk

Panel members made a mark on the lines according to the intensity of the flavor. The marks were converted to numerical ratings ranging from 0 (no flavor) to 10 (right end of the line, very strong).

The figure is reduced in size. The original length of each line was 6 inches.

SENSORY EVALUATION SHEET

Tester _____

Date _____

Sample No. Weak Moderate Strong

Nuttiness

Cereal

Paintiness

Grassiness

Astringency

Sample No.

Nuttiness

Cereal

Paintiness

Grassiness

Astringency

Sample No.

Nuttiness

Cereal

Paintiness

Grassiness

Astringency

membrane occupied all the space in the reaction chamber above the reaction mixture, and thus kept the mixture out of contact with air. The reaction mixture which consisted of 3 ml of 0.2M tris buffer (pH 9), 10.5 µl of sodium linoleate and 10-100 µl of enzyme source was stirred magnetically. The concentration of oxygen in the buffer solution was taken to be the same as in air saturated water at the same temperature (240 µM at 25°C). After addition of buffer and sodium linoleate to the reaction chamber and adjustment of the recorder to 100% saturation of oxygen, soymilk as the lipoxygenase source was injected into the reaction mixture. The lipoxygenase activity was calculated as the initial rate of oxygen consumption in micromoles oxygen per min per ml of soymilk.

TBA number

The distillation method by Tarladgis et al. (1960) was used for determination of lipid oxidation in soymilk. A 100 ml mixture at pH 1.5 containing appropriate volumes of soymilk and deionized water and HCl solution was distilled until 50 ml of distillate was collected. Antifoaming agent (3 drops) was used to prevent extensive foaming during distillation. The distillate (5 ml) was reacted with 5 ml TBA reagent in a 50 ml stoppered glass test tube by boiling for 35 min. The solution was cooled under running tap water for 10 min, and the absorbency was determined at 538 nm. A

standard curve was established with 1,1,3,3-tetraethoxy propane.

Solids determination

Solids content of soymilk was determined by freeze drying a 2 g sample in a pre-dried and weighed aluminum pan followed by drying in a hot-air oven at 100-105°C.

Crude lipid determination

Crude lipid content of freeze-dried soymilk was determined by extraction with hexane in a Goldfisch apparatus (AACC, 1969 method 30-20). The sample (1 g) was placed in a pre-dried and weighed thimble and extracted for 12 hr. The crude lipid content was calculated from the weight loss during extraction.

Protein determination

A modified micro-Kjeldahl method (AOAC, 1970, 38.012) was used to determine the protein content of freeze-dried soymilk. The sample (100 mg) was digested with sulfuric acid, catalyst copper selenite and potassium sulfate on a Lab. Con. Co. digestion apparatus. Distillation was carried out for 3 min in a Lab. Con. Co. distillation apparatus with addition of 40% (w/v) sodium hydroxide. Boric acid (4%) was used to trap ammonia. Standardized HCl was used for titration with Tashro's indicator. Protein content was calculated using the conversion factor of 6.25.

Phenolic compound determination

The method by Hammerschmidt and Pratt (1978) was used to determine phenolic compounds in defatted freeze-dried soymilk. A sample of 100 mg was placed in a 50 ml stoppered glass test tube, 10 ml of methanol was added, and the sample was shaken vigorously using a vortex mixer. The sample was extracted for 2 hr at room temperature. Extraction was followed by filtration using Whatman #1 filter paper. The extract (0.3 ml) was added to 6 ml 2% Na_2CO_3. After 2 min, 0.3 ml 50% Folin-Ciocalteau reagent was added, and the extract was incubated at room temperature for 30 min. Absorbency at 750 nm was read in a Bausch and Lomb Spectronic 20 using the red filter. Chlorogenic acid was used as the standard.

RESULTS AND DISCUSSION

Lipoxygenase Inactivation

Effect of ethanol and temperature

Lipoxygenase is inactivated by heat (Farkas and Goldblith, 1962; Christopher et al., 1970) and alcohol (Mitsuda et al., 1967; Eldridge et al., 1977). The combined effect of heat and ethanol was also reported (Borhan and Snyder, 1979). Borhan and Snyder (1979) reported that the most efficient treatment of soybeans for high nitrogen solubility with complete inactivation of lipoxygenase was soaking whole soybeans in 15% ethanol at 50°C for 5 hr. These conditions for soaking soybeans before preparing soymilk were used in the present experiment to see if they would give a palatable product.

The results (Table 1) showed that inactivation of lipoxygenase due to soaking in water at 50°C for 6 hr without ethanol was 15.3% of the total lipoxygenase, and 97.7% lipoxygenase activity was inactivated by soaking in 15% ethanol at 50°C for 6 hr. This result did not agree with Borhan and Snyder's finding that complete inactivation occurred by soaking in 15% ethanol at 50°C for 5 hr. The difference was that they used a diluted enzyme preparation, and I used a full strength soymilk as enzyme source.

Borhan and Snyder (1979) also observed that as the temperature of the soaking solution decreased a greater

Table 1. Effect of soaking soybeans in water and 15% ethanol at 50°C for 6 hr on lipoxygenase activity

Conditions	Residual lipoxygenase activity $\mu mol\ O_2, min^{-1}, ml^{-1}$ soymilk
Soaking in water at 4°C 6 hr (Total lipoxygenase activity in soybeans)	42.5
Soaking in water at 50°C for 6 hr	36.0
Soaking in 15% ethanol at 50°C for 6 hr	1.01

concentration of ethanol was required for lipoxygenase inactivation. Therefore, more rapid and complete inactivation of lipoxygenase was attempted using higher ethanol concentrations and higher temperature. At 50°C, ethanol concentration was increased to 20 and 25%. Ethanol concentration of 15% was maintained at 60°C.

Table 2 shows the lipoxygenase activity under these soaking conditions. It was observed that 15% ethanol at 60°C was more effective than 25% ethanol at 50°C. Complete inactivation was shown in 6 hr by soaking in 15% ethanol at 60°C, whereas a large amount of lipoxygenase still remained in soybeans soaked in 20 or 25% ethanol solution for 6 hr.

Table 2. Changes in lipoxygenase activity during soaking soybeans in 15% ethanol at 60°C, 20 and 25% ethanol at 50°C

Soaking conditions	Residual lipoxygenase activity $\mu mol\ O_2, min^{-1}, ml^{-1}$ soymilk
20% ethanol at 50°C for 2 hr	6.48
" 4 hr	3.78
" 6 hr	1.35
25% ethanol at 50°C for 2 hr	6.30
" 4 hr	2.34
" 6 hr	1.08
15% ethanol at 60°C for 2 hr	4.14
" 4 hr	0.09
" 6 hr	0

Effect of addition of $NaCO_3$, $NaHCO_3$ and NaOH in 15% ethanol

Various reports showed that pretreatment of soybeans with sodium alkali or sodium salts for soymilk preparation improved the flavor of soymilk (Badenhop and Hackler, 1970; Khaleque et al., 1970; Nelson et al., 1976). Borhan and Snyder (1979) pointed out that a reasonable explanation for the improvement of these treatments was the rapid inactivation of lipoxygenase by the increased pH of the soaking solution.

In this experiment, 0.1 M and 0.01 M Na_2CO_3, $NaHCO_3$ and NaOH were tested as additions to 15% ethanol solutions to inactivate lipoxygenase more rapidly. The pHs of the 15% ethanol solutions were 11.6, 9.0 and 13.1 by adding 0.1M Na_2CO_3, $NaHCO_3$ and NaOH, respectively. Figure 2 shows the changes in lipoxygenase activity during soaking soybeans under these conditions at 60°C. The NaOH-ethanol solution completely inactivated lipoxygenase in 4 hr and the Na_2CO_3-ethanol solution in 5 hr but the $NaHCO_3$-ethanol solution failed to inactivate lipoxygenase completely in 5 hr.

It was also observed from Fig. 2 that the rate of inactivation was slower in the first hour of soaking than later. This phenomenon possibly was due to the time required for the soaking solutions to be imbibed in entire cotyledons as well as the temperature of soaking solution to equilibrate with the water bath.

The effect of the concentration of added salts was investigated by using 0.01M Na_2CO_3 and $NaHCO_3$ in 15% ethanol solution in comparison to 0.1M concentrations (Table 3). The initial pHs of 0.01M solutions were close to that of 0.1M solutions. As the soaking time elapsed, pH differences between 0.1M and 0.01M solutions became larger, and lipoxygenase activity was greater with 0.01M solutions.

Flavor of soymilks prepared from soybeans receiving these treatments will be discussed later.

Fig. 2. Changes in lipoxygenase activity during soaking soybeans in 15% ethanol and 15% ethanol containing 0.1M Na_2CO_3, $NaHCO_3$ and NaOH at 60°C

Lipoxygenase activity was expressed in μmol O_2, min^{-1}, ml^{-1} soymilk.

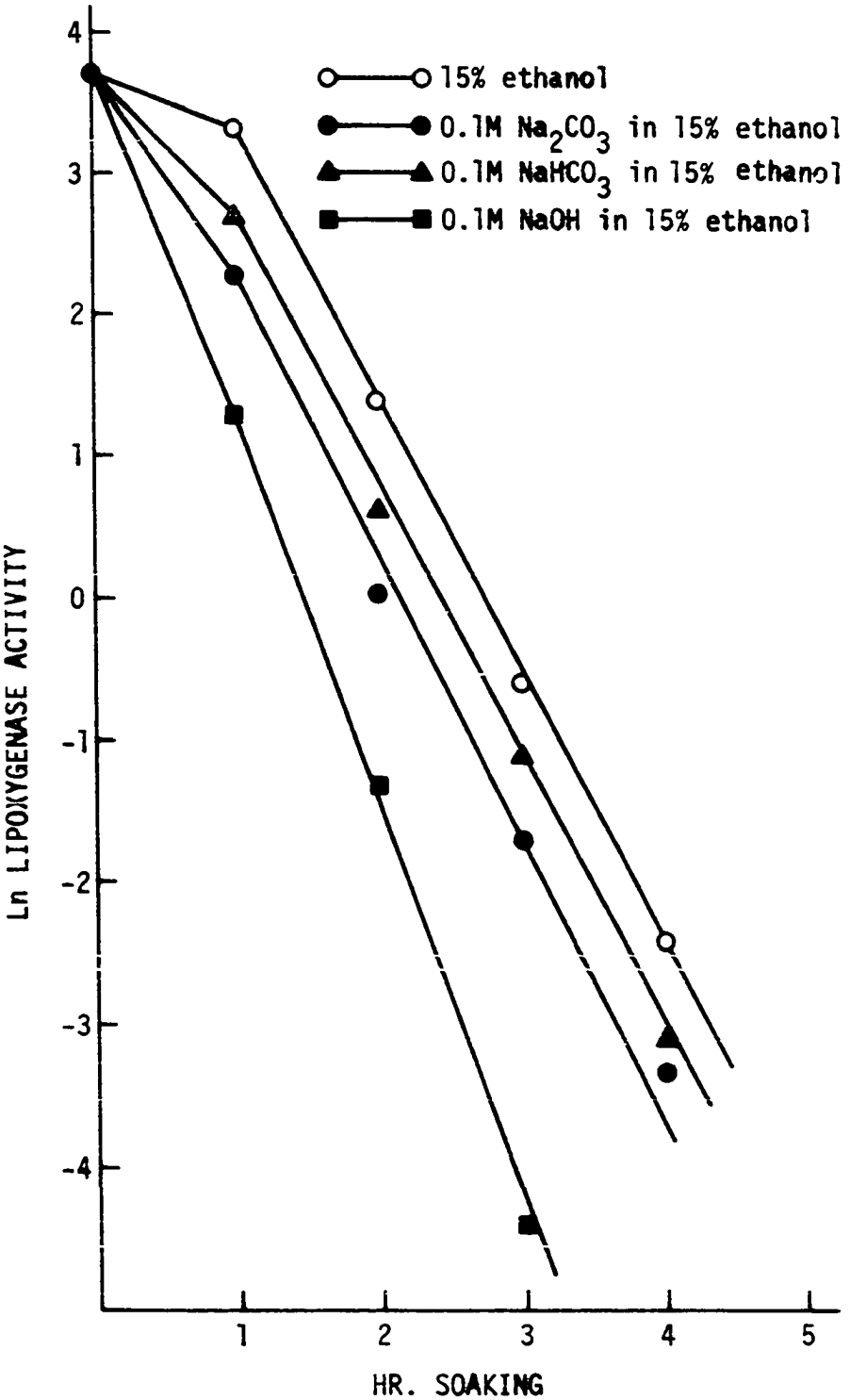

15% ethanol
0.1M Na_2CO_3 in 15% ethanol
0.1M $NaHCO_3$ in 15% ethanol
0.1M NaOH in 15% ethanol
Ln LIPOXYGENASE ACTIVITY
HR. SOAKING
4
3
2
1
0
-1
-2
-3
-4
1
2
3
4
5

Table 3. Changes in lipoxygenase activity during soaking soybeans in 15% ethanol solution containing 0.1M and 0.01M Na_2CO_3 and $NaHCO_3$

Concentration of salts	Initial pH of soaking solution	Hr soaking	Final pH of soaking solution	Lipoxygenase activity $\mu mol\ O_2,\ min^{-1},\ ml^{-1}$
0.1M Na_2CO_3	11.6	1	10.3	9.53
		3	10.0	0.18
		5	9.6	0
0.01M Na_2CO_3	11.3	1	8.6	10.80
		3	7.8	0.24
		5	7.6	0.02
0.1M $NaHCO_3$	9.0	1	8.7	14.58
		3	8.2	0.34
		5	8.2	0.01
0.01M $NaHCO_3$	8.8	1	8.3	18.36
		3	7.4	0.38
		5	7.1	0.12

Composition of Soymilk

Soymilks were prepared from whole soybeans that had been soaked in 15% ethanol containing 0.1M Na_2CO_3, $NaHCO_3$ and NaOH at 60°C for 1,2,3,4 and 5 hr and resoaked in water overnight. Composition of these soymilks was determined to examine the changes brought about by the different soaking conditions and to learn about possible correlations between any of the components and the flavor of soymilk. The pH of the soymilks and the solids content were measured on fresh soymilk, and protein, crude lipid and phenolic compounds were determined on the freeze-dried soymilk (Table 4).

pH

The pH of soymilks increased with time of soaking soybeans in 0.1M Na_2CO_3 and $NaHCO_3$. Soaking in 0.1M Na_2CO_3 resulted in a rapid increase in pH to 9.3 from 7.9 for 5 hr whereas only a 0.6 pH unit increase came from soaking in 0.1M $NaHCO_3$ during the same period of time. Soaking in 0.1M NaOH, however, gave the reverse effect. The initial pH of 8.0 after 1 hr soaking decreased to 6.9 after 5 hr soaking.

Increased pH of soymilks from soybeans soaked in Na_2CO_3 and $NaHCO_3$ might be due to a reaction between $CO_3^{=}$ and the components of soybeans whereby these ions stay in the soybeans. On the other hand, the decreasing pH of soymilks from NaOH soaked soybeans may be due to the solution imbibing

Table 4. Composition of soymilks prepared from soybeans soaked in 15% ethanol solution containing 0.1M Na_2CO_3, $NaHCO_3$ and NaOH at 60°C

Chemicals added in 15% ethanol	Hr soaking	pH	Solids content %	Protein content %	Lipid content %	Phenolic compounds content (as chloregenic acid mg%)
0.1M Na_2CO_3	1	7.9	6.6	3.2	2.1	6.8
"	3	9.0	6.6	3.3	2.2	8.5
"	5	9.3	6.5	3.0	2.2	8.3
0.1M $NaHCO_3$	1	7.0	6.7	3.3	2.1	5.0
"	3	7.5	6.6	3.4	2.2	8.9
"	5	7.6	6.5	3.4	2.2	8.5
0.1M NaOH	1	8.0	6.8	3.3	2.2	9.4
"	3	7.9	6.6	3.2	2.2	9.8
"	5	6.9	6.6	3.1	2.1	10.8

nature of soybeans. It took 10 hr for soybeans to absorb equal wt of 0.5% $NaHCO_3$ solution at 21°C (Johnson and Snyder, 1978). Therefore, one hour soaking in 0.1M NaOH at 60°C could result in just peripheral imbibition, and OH^- might migrate toward the center of the beans on resoaking in water. However, 5 hr soaking could be enough for NaOH solution to reach the center of the beans, and it might be easily washed out on resoaking.

Solids

There was no variation among the soymilks that received different soaking treatments. Approximately 40.2% of the solids of soybeans were recovered in soymilks.

Protein

The protein content of the soymilks changed very little during soaking of soybeans or by addition of different chemicals to the soaking solution. This indicated that soluble proteins were not affected during 5 hr soaking in spite of a large reduction in lipoxygenase activity. Approximately 48.0% of the protein in soybeans was recovered.

Crude lipid

No difference in crude lipid content among soymilks was observed. Most (65.5%) of the oil in soybeans was recovered in the soymilks.

Phenolic compounds

The total phenolic compound content was determined to examine any possible relation between total phenolic compounds and astringency in soymilk. It was observed that total phenolic compounds increased with time of soaking soybeans.

Flavor of Soymilk

Normal flavor of soymilk has not been a subject of extensive investigation, even though there are many reports on the disagreeable flavors of green-beany or painty. In the beginning of this investigation, soymilks were prepared by the hot grind process and the Illinois process, and examined for their flavor. It was found that these soymilks have distinctly different flavors. Hot grind soymilk had a definite cereal flavor, and Illinois process gave a strong nutty flavor to soymilk. Soymilk flavors were influenced by the processing method.

Ethanol soaking has the advantage in a flavor study of soymilk that lipoxygenase can be inactivated without boiling or severe heating. Hence, flavors produced due to heat can be distinguished.

During the training of the taste panel, five terms to describe the flavors of soymilk were defined. They are paintiness, grassiness, nuttiness, cereal flavor and astringency. Paintiness included terms such as rancid or cardboard which describe

oxidative rancidity or drying oil odor. Grassiness included green or beany. The panel preferred grassy to green or beany which are more commonly used. Nuttiness was to describe a typical flavor of soymilk produced by the Illinois process. Cereal described the flavor of soymilk prepared without any addition of chemicals but with appreciable heat treatment such as the hot grind process or the traditional Oriental process. Astringency described a mouth catching or mouth drying sensation that was perceived after soymilk was removed from the mouth. It took two months of training for the panel to agree on the terms and to be able to identify the flavors skillfully.

The standard deviations of all flavor scores (paintiness = 0.67, grassiness = 0.95, astringency = 1.52, nuttiness = 1.44 and cereal = 1.34, flavor score range = 0 to 10) show that there was a high degree of individual difference in identifying the intensity of flavors, and the difference depended on the characteristics of the flavors. Paintiness which was very strong and penetrating showed the least deviation, whereas the mild nutty and cereal flavors showed more deviation. Astringency which could accumulate in the mouth as the panel tasted several samples in one session showed the greatest deviation, even though a water rinse was used between samples.

Soymilks used in the flavor study were prepared identically to the soymilks used for the composition study.

Paintiness

Paintiness of soymilk decreases sharply with the time of soaking (Fig. 3). In the literature, paintiness has not been investigated as a prominent flavor. Instead, green-beany has been the main subject of flavor problem in conjunction with lipoxygenase. Paintiness, however, was the major disagreeable flavor and was examined separately from grassiness even though they both disappeared on heating.

In soymilk prepared from soybeans soaked in 20 or 25% ethanol at 50°C and in 15% ethanol at 60°C, paintiness decreased with decrease in lipoxygenase activity (Table 5). There was a very close positive correlation ($r = 0.965$) between lipoxygenase activity and paintiness. This result indicated that paintiness could be caused by lipoxygenase and agreed with the results by Wilkens et al. (1967), Mattick and Hand (1969), Kalbrener et al. (1974), Wolf (1975), Grosch et al. (1976), Eldridge et al. (1977), and Borhan and Snyder (1979) except that none of these investigators separated paintiness from grassiness.

Also, it was observed that even at very low lipoxygenase activity (0.21% residual activity) paintiness was perceivable showing the high potency of lipoxygenase activity (Wolf, 1975; Sessa and Rackis, 1977).

Soaking soybeans in ethanolic solutions containing Na_2CO_3, $NaHCO_3$ and NaOH, however, did not confirm the relationship

Fig. 3. Changes in paintiness of soymilk with time of soaking at 60°C

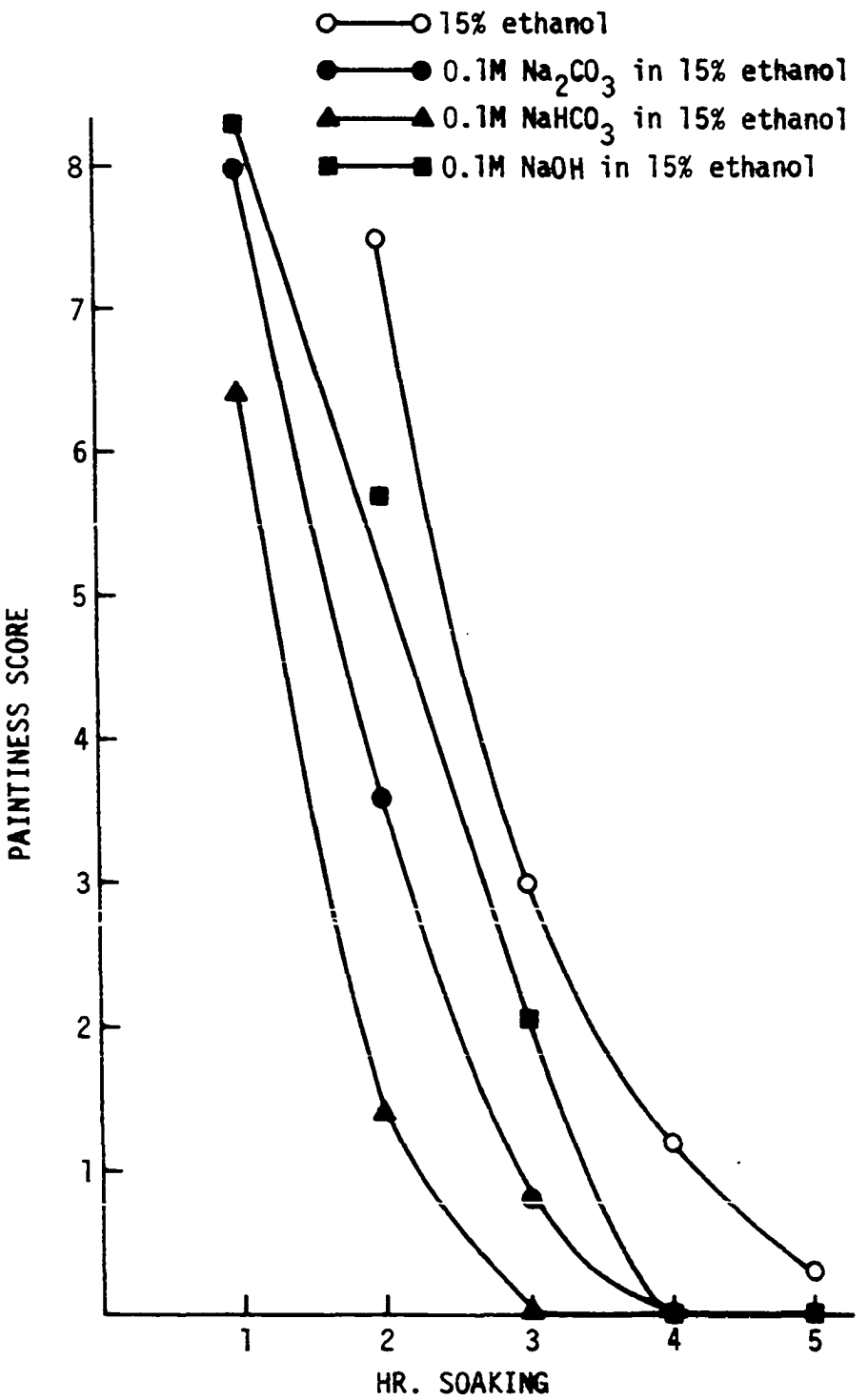

15% ethanol
0.1M Na_2CO_3 in 15% ethanol
0.1M $NaHCO_3$ in 15% ethanol
0.1M NaOH in 15% ethanol
PAINTINESS SCORE
8
7
6
5
4
3
2
1
1
2
3
4
5
HR. SOAKING

Table 5. Changes in paintiness and lipoxygenase activity in soymilks prepared from soybeans soaked in ethanol solutions

Lipoxygenase activity (μmol O_2, min^{-1}, ml^{-1})	Soaking condition	Paintiness[a]
6.48	20% ethanol at 50°C for 2 hr	6.9
6.30	25% ethanol at 50°C for 2 hr	6.0
4.14	15% ethanol at 60°C for 2 hr	2.5
3.78	20% ethanol at 50°C for 4 hr	4.7
2.34	25% ethanol at 50°C for 4 hr	2.5
1.35	20% ethanol at 50°C for 6 hr	1.3
1.08	25% ethanol at 50°C for 6 hr	0.9
0.54	15% ethanol at 60°C for 3 hr	1.0
0.09	15% ethanol at 60°C for 4 hr	0.4
0.07	15% ethanol at 60°C for 5 hr	0
0.0	15% ethanol at 60°C for 6 hr	0

[a]Intensity of paintiness in these samples were much more intense than the samples presented later in this thesis even though the scores are the same. Two and five tenths in this table is equivalent to 7.5 in the later tables or figures.

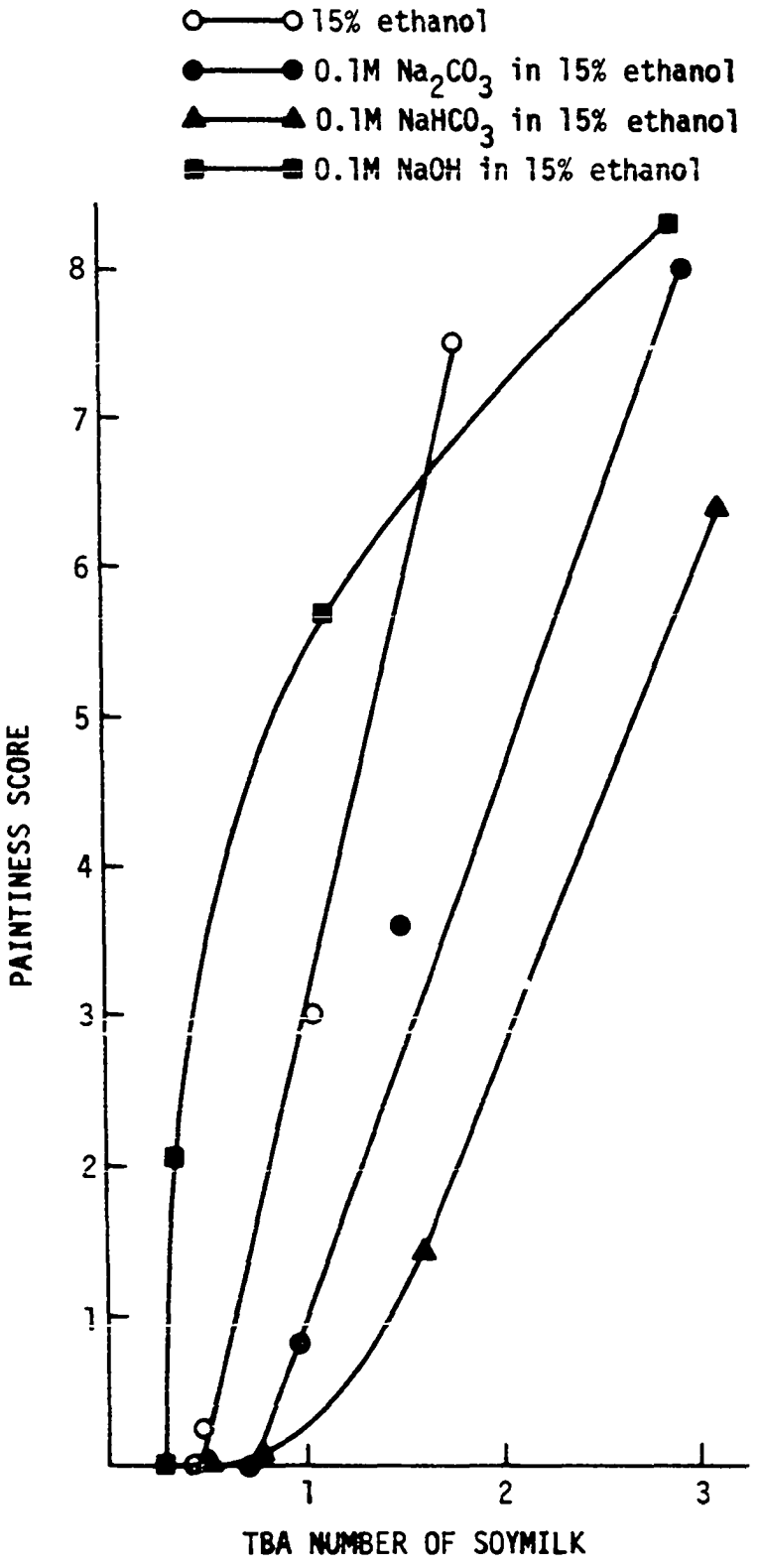
between lipoxygenase activity and paintiness. The 0.1M NaOH-ethanol treatment which had the least lipoxygenase activity among these treatments showed greatest paintiness, and 0.1M $NaHCO_3$-ethanol treatment which had the highest lipoxygenase activity showed the least paintiness (Fig. 2 and Fig. 3).

Since there was the possibility of autoxidation of lipid during preparation of soymilk, the total lipid oxidation was measured by TBA numbers. The relationship between paintiness and TBA number is shown in Fig. 4. The NaOH-ethanol treatment had the most paintiness at the same TBA numbers. The Na_2CO_3-ethanol treatment had more paintiness at the same TBA numbers than the $NaHCO_3$-ethanol treatment.

Similar results were reported by Khaleque et al. (1970). They observed that soymilk prepared from beans soaked in 0.4M Na_2CO_3 at room temperature for 24 hr had significantly better flavor than the soymilks from soybeans soaked in 0.2M NaOH solution, even though lipoxygenase activity of the latter was less than the former. Also, in spite of no lipoxygenase activity in the hot ground soymilk, NaOH treated soymilk had much more beany flavor than soymilk treated with Na_2CO_3. Thereby they raised the question of lipoxygenase being a cause of beany flavor. Bourne's et al. (1976) answer to this question was the temperature of hot grinding was not sufficient to inactivate lipoxygenase. Borhan and Snyder (1979) also stated that the effect of sodium alkali and sodium salts on inactivation of

Fig. 4. Changes in paintiness with TBA number of soymilk

Soymilks were prepared from soybeans soaked in 15% ethanol, and solutions containing 0.1M Na_2CO_3, $NaHCO_3$ and NaOH in 15% ethanol.


15% ethanol
0.1M Na_2CO_3 in 15% ethanol
0.1M $NaHCO_3$ in 15% ethanol
0.1M NaOH in 15% ethanol
PAINTINESS SCORE
8
7
6
5
4
3
2
1
1
2
3
TBA NUMBER OF SOYMILK

lipoxygenase is the high pH which brings about rapid inactivation of the enzyme. However, from the results of the present experiment, it is observed that in the absence of sodium hydroxide and sodium salts, paintiness correlates with lipoxygenase activity, but with the addition of these chemicals, paintiness does not confirm the lipoxygenase theory.

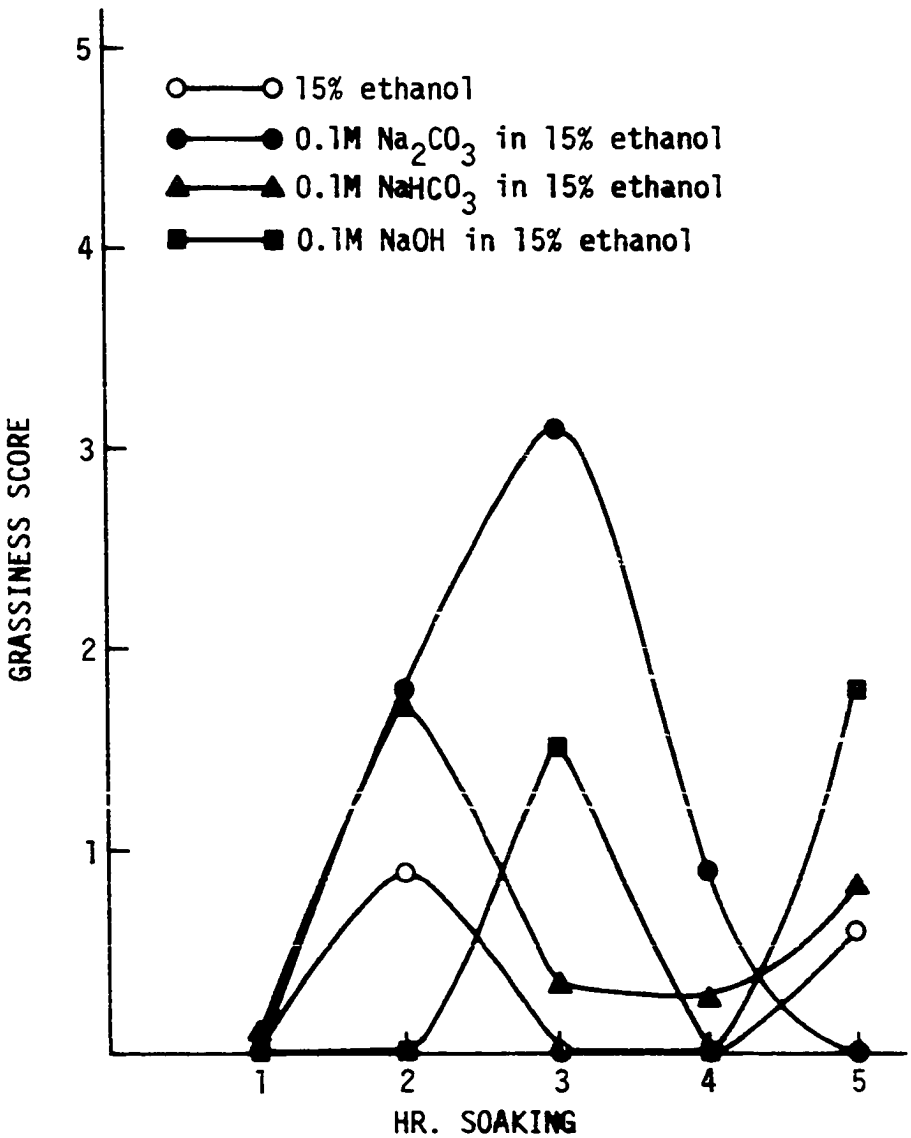
Of the sodium salts investigated, $NaHCO_3$ has the greatest effect on reducing painty flavor, but NaOH had the greatest effect on destroying lipoxygenase, and the least effect on controlling paintiness.

Grassiness

Figure 5 shows the changes in grassiness with time of soaking. It was observed that grassiness increased until it reached maximum intensity. Then it decreased to almost no perceivable intensity followed by reappearance in 5 hr. Grassiness is believed to be produced by lipoxygenase activity. However, the changes in grassiness did not correspond to the lipoxygenase activity at all. Possibly due to the very intensive and penetrating characteristics of paintiness, other mild flavors were masked. Therefore, when paintiness was strong, grassiness was not detectable even though the grassiness was strong. When paintiness decreased, grassiness was detected with increasing intensity until paintiness decreased to near zero. Then when paintiness disappeared completely, even a small amount of grassiness appeared distinctively.

Fig. 5. Changes in grassiness of soymilk with time of soaking at 60°C

Soaking solutions included 15% ethanol and solutions containing 0.1M Na_2CO_3, $NaHCO_3$ and NaOH in 15% ethanol.


15% ethanol
0.1M Na_2CO_3 in 15% ethanol
0.1M $NaHCO_3$ in 15% ethanol
0.1M NaOH in 15% ethanol
GRASSINESS SCORE
1
2
3
4
5
HR. SOAKING

This appearance of grassiness in the absence of painti-ness raised a question of lipoxygenase causing these two flavors at the same time. This phenomenon could be due to the presence of lipoxygenase isozymes. Lipoxygenase 1 which is more heat resistant (Christopher et al., 1970; Borhan and Snyder, 1979) produces 2-trans-hexenal, propanal and 2-trans-pentenal whereas less heat resistant lipoxygenase 2 and 3 generate 2-trans-pentenal, 2-trans-hexenal, 2-trans,6-cis-nonadienal, 2-trans,4-cis-heptadienal, 3,5-octadien-2-one and 2,4,6-nonatrienal (Grosch and Laskawy, 1975). The flavor of n-hexanal, 3-cis-hexenal,n-heptanal and 2-n-pentylfuran was reported as green-beany (Hoffman, 1961; Kinsella et al., 1967; Leu, 1974; Sessa, 1978) whereas higher alk-2,4-dienals have painty flavor (Sessa, 1978). Therefore, lipoxygenase 1 might generate mainly grassiness and by being more heat stable produce the flavor until the last trace of enzyme is destroyed. On the other hand, lipoxygenase 2 and 3 might contribute paintiness as well as grassiness and being destroyed more rapidly, the paintiness disappeared earlier than grassiness.

Astringency

Astringency was detected in all of the soymilks prepared by different processes: hot grind, Illinois process, traditional Oriental method as well as ethanol soaking. However, there was no report in the literature on taste panel scoring of astringency in soymilk.

Kalbrener et al. (1974) reported that linolenic acid hydroperoxide flavor is described as grassy-beany followed by bitter and astringent. They also observed that soyflour had more astringency than hydroperoxides. Kalbrener et al. (1971) observed that astringency is detectable in a soy protein concentrate and an isolate.

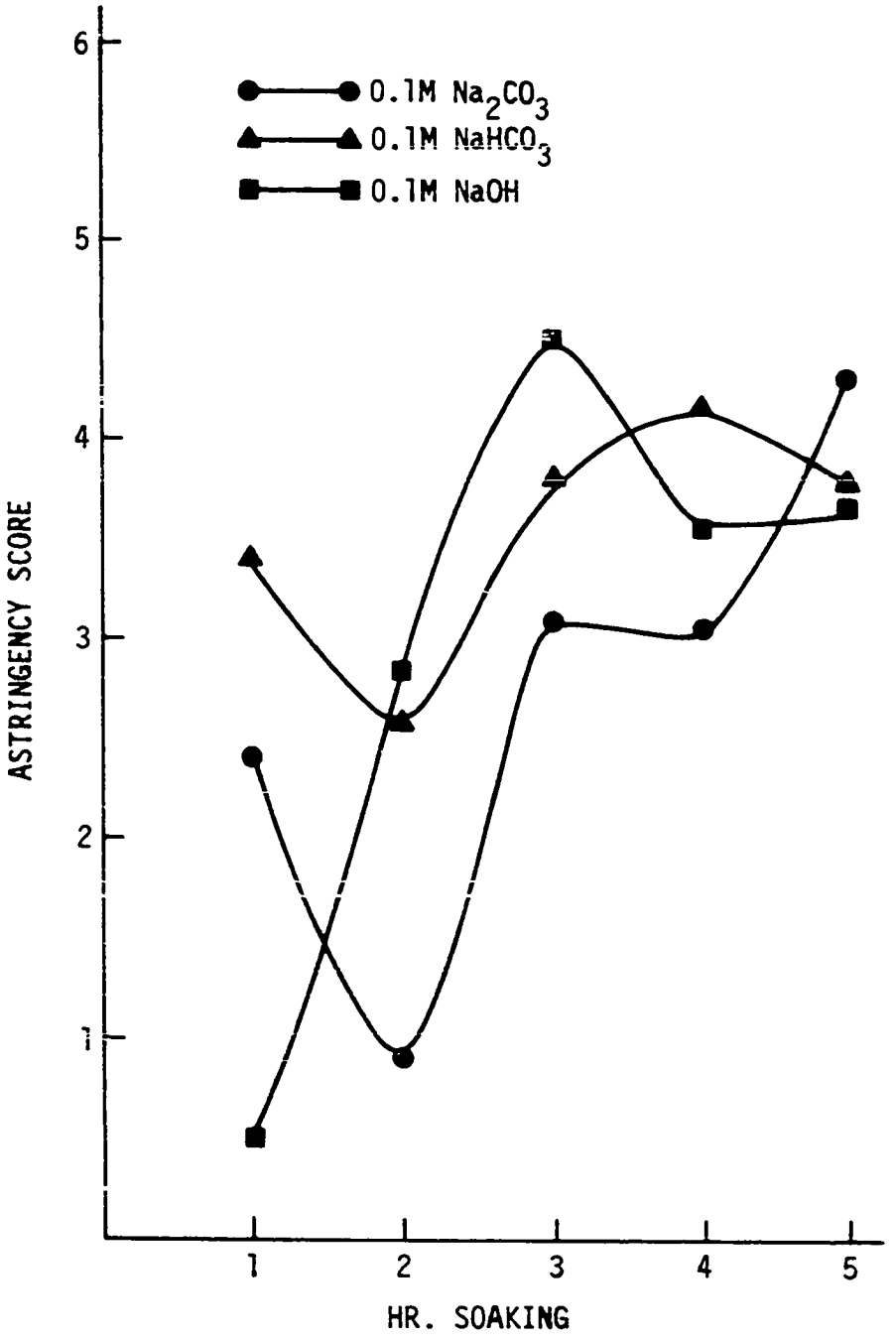
Honig et al. (1969) detected astringency in one of the lipid fractions which contains isoflavones, isoflavone glycosides, sterol glycosides and phosphatidic acid. Arai et al. (1966) isolated two isomers of chlorogenic acid whose flavor is sour, bitter and astringent.

It was assumed that astringency in soymilk could be caused by phenolic compounds, and phenolic compound content in soymilk was determined using chlorogenic acid as standard. But no correlation ($r = 0.069$) was observed between these two factors. However, the changes of these two factors were similar. That is, they generally increased after about 3 hr soaking, then decreased very little in 5 hr soaking (Fig. 6 and Table 4).

It was difficult for the taste panel to identify mild flavors in the presence of paintiness. Astringency, especially, could be confused since both paintiness and astringency are associated with bitterness. The taste panel commented that paintiness had an aftertaste similar to bitterness. In most of the literature, astringency occurred with bitterness (Honig

Fig. 6. Changes in astringency of soymilk with time of soaking at 60°C

Soaking solutions contained 0.1M Na_2CO_3, $NaHCO_3$ and NaOH in 15% ethanol.


0.1M Na_2CO_3
0.1M $NaHCO_3$
0.1M NaOH
ASTRINGENCY SCORE
HR. SOAKING
1
2
3
4
5
6

et al., 1969; Kalbrener et al., 1971; Kalbrener et al., 1974).

Nuttiness

Figure 7 shows the changes in nuttiness of soymilk with time of soaking. Nuttiness increased with time of soaking which can be expressed as time of heating. Similar results were shown during steaming soyflour by Moser et al. (1967).

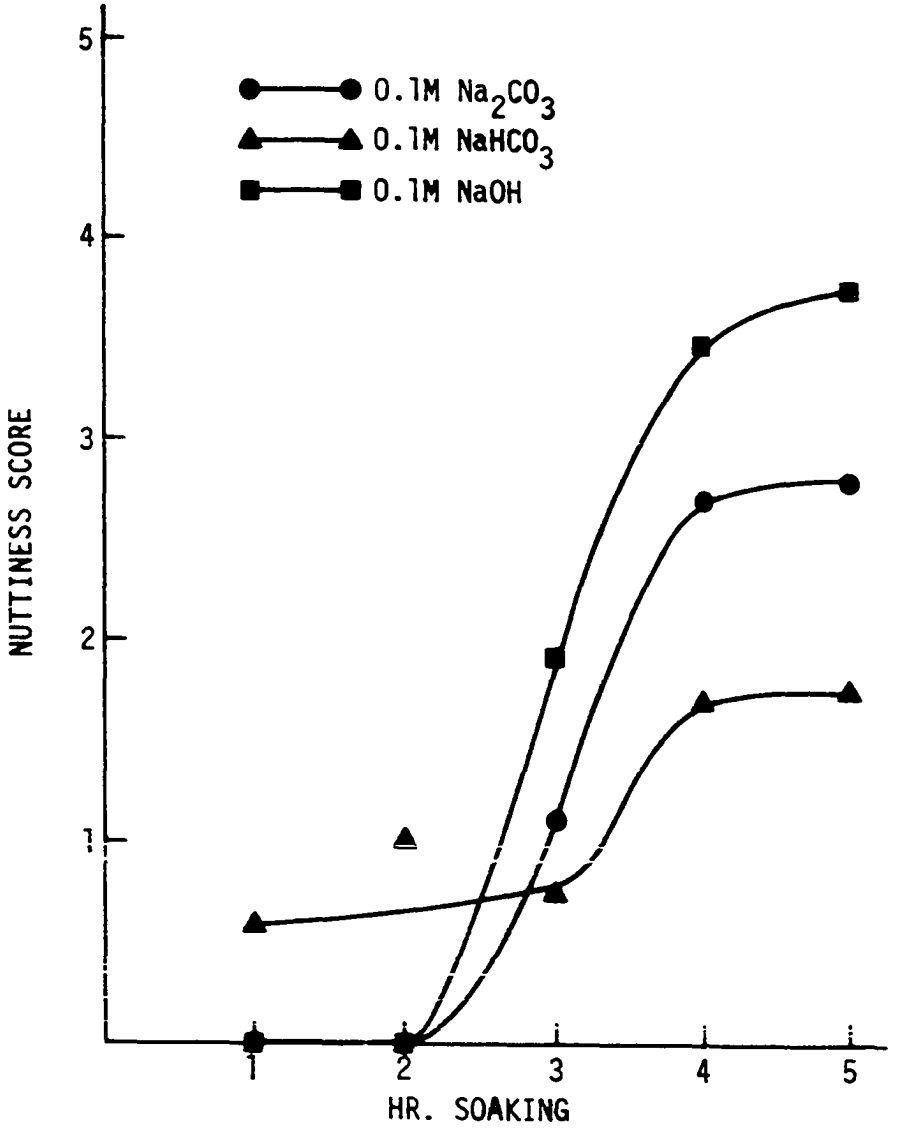
It was observed that the pH of the soaking solution was a very important factor affecting nuttiness of soymilk. NaOH-ethanol solution (initial pH 13.1) gave the most nuttiness and Na_2CO_3-ethanol solution (initial pH 11.6) had more nuttiness than $NaHCO_3$-ethanol solution which had an initial pH of 9.0. Manley and Fagerson (1970) reported that alkylated pyrazines, products of nonenzymatic browning in hydrolyzed soy protein, have roasted nut-like flavor. They made the point that the flavor is not present in neutral solutions of hydrolyzed soy protein but is clearly shown at alkaline pH.

A similar observation was made with soymilk in this experiment. Soymilk from the hot grind process with no adjustment of pH did not have nutty flavor. Soymilk from the Illinois process in which 0.5% $NaHCO_3$ solution is used for soaking and blanching had very strong nuttiness, and this soymilk was used as the nutty flavor reference.

During soaking soybeans in 15% ethanol at 45°C for 24 hr, approximately 67% of the sugars are leached out (Borhan and

Fig. 7. Changes in nuttiness of soymilk with time of soaking at 60°C

Soaking solutions contained 0.1M Na_2CO_3, $NaHCO_3$ and NaOH in 15% ethanol.


0.1M Na_2CO_3
0.1M $NaHCO_3$
0.1M NaOH
NUTTINESS SCORE
HR. SOAKING
1
2
3
4
5

Snyder, 1979). However, some sugars remain in soybeans and can participate in browning on hydrolysis. By increasing the pH above 7, the conditions become more favorable for non-enzymatic browning. Enough pyrazines to produce a nutty flavor could be formed, and with increased time, a more intense flavor could result.

It was observed among the ethanol treated soybeans that nuttiness increased with an increase in lipid content of the soymilk (Fig. 8). Probably nuttiness in these soymilks was not the same nuttiness as in alkaline treated soymilks; however, it was expressed as nuttiness by the taste panel.

Cereal flavor

Cereal flavor appeared as a characteristic flavor of soymilk prepared by heat treatment without addition of any chemicals. Also, this flavor remained in soymilk when all other flavors were missing. Cereal flavor, however, was very mild and was masked by most of the flavors in soymilk. In the presence of paintiness and grassiness, this flavor was not easily identified and often was confused with nuttiness.

Figure 9 shows the changes in cereal flavor in the soymilks with time of soaking. Cereal flavor generally increased with time of soaking. Cereal flavor was probably present from the beginning, but due to paintiness, grassiness and nuttiness, the intensity of this flavor appeared to change with time of soaking.

Fig. 8. Changes in nuttiness with lipid content of soymilk

Soymilks were prepared from soybeans soaked in 20, 25 and 30% ethanol at 50°C and 15% ethanol at 60°C.

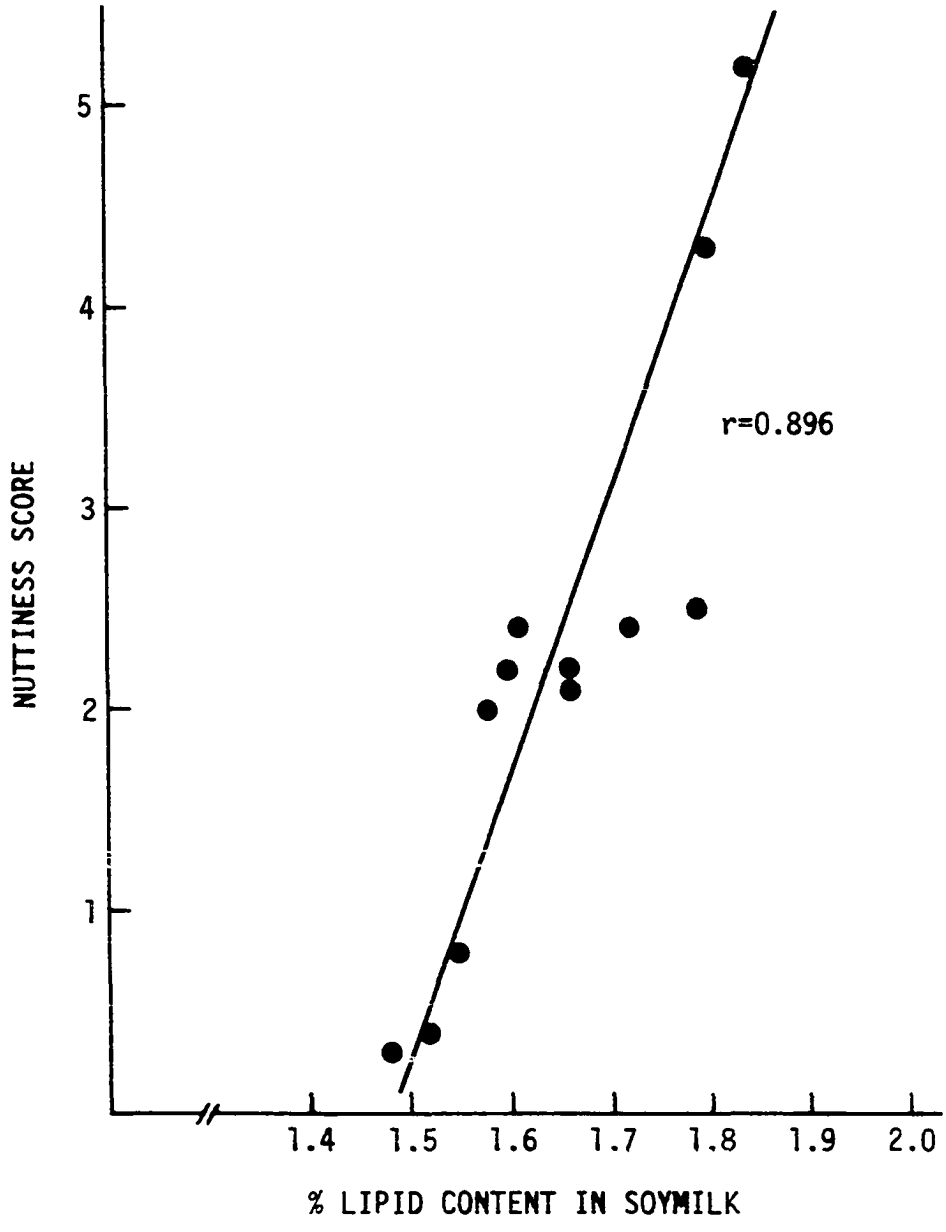
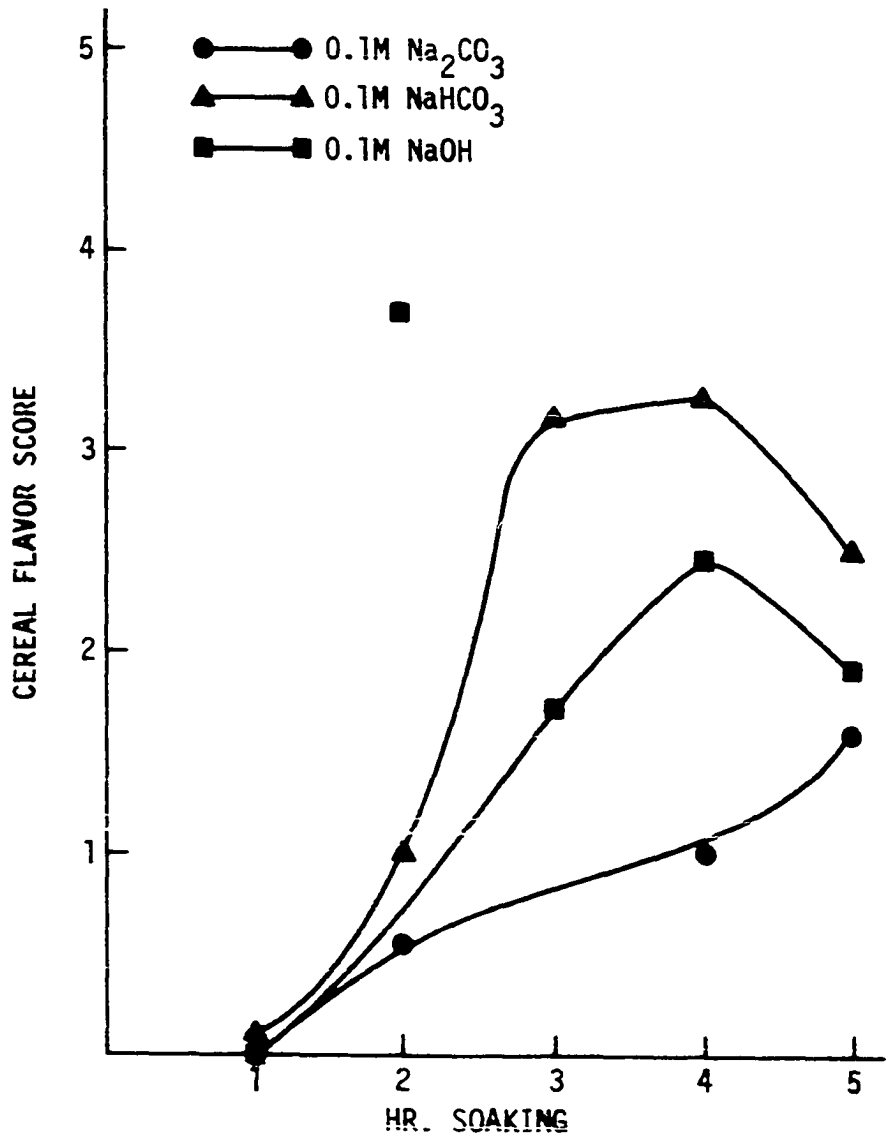

NUTTINESS SCORE
5
4
3
2
1
r=0.896
1.4
1.5
1.6
1.7
1.8
1.9
2.0
% LIPID CONTENT IN SOYMILK

Fig. 9. Changes in cereal flavor of soymilk with time of soaking at 60°C

Soaking solutions contained 0.1M Na_2CO_3, $NaHCO_3$ and NaOH in 15% ethanol.


0.1M Na_2CO_3
0.1M $NaHCO_3$
0.1M NaOH
CEREAL FLAVOR SCORE
HR. SOAKING
1
2
3
4
5

No compound possessing cereal flavor has been reported in soymilk so far.

Effect of heating soymilk

Heating exerts several effects on the flavor of food. Heating can produce flavor compounds by the interaction of the constituents of food, can cause isomerization of flavor compounds and can cause loss of flavor due to evaporation of volatile flavor compounds.

In dairy plants, a treatment of fluid milk using a vacuum system to remove objectionable flavors has become a general practice to obtain a uniform flavor throughout the year.

In this experiment, change of soymilk flavor due to heating was observed. The soymilk was made by the Illinois process which includes soaking and blanching 30 min in 0.5% $NaHCO_3$. This process was chosen because of the absence of paintiness and grassiness which mask other flavors and the presence of a strong nutty flavor. Soymilk was heated at 95°C for 5 min and was compared at 25°C with unboiled soymilk (Table 6).

The intensity of nuttiness and cereal flavor decreased by more than 1 unit. Heating after processing reduced these flavors even though extensive heating was done during preparation of the soymilk. No new flavor was produced by heating. Astringency was increased by 0.2 unit, so the compounds

Table 6. Comparison of flavors between unboiled and boiled soymilks

	Flavor scores	
	Unboiled soymilk	Boiled soymilk
Nuttiness	3.6	2.5
Cereal	3.8	2.4
Astringency	3.1	3.3

responsible for astringency are not volatile under these conditions.

Effect of Hulls

The hulls amount to 8.3% of the whole soybean weight and contain 86.2% N-free extract and fiber which includes 49.3% cellulosic type material, 22.6% pentosans, and 4.5% lignin (Smith and Circle, 1972b). These cellulosic materials probably make up the bulk of soymilk sludge which is removed during soymilk preparation.

Lignin, whose structural unit is phenolic, is suspected of affecting the flavor of soymilk because lignins are partly hydrolyzed by alkali and heating. The Illinois process in which soybeans are soaked and blanched 30 min in 0.5% $NaHCO_3$ showed strong astringency, and this soymilk was used as the astringency reference for taste panel training. Hence, the presence of

lignin in the hulls and the possibility of hydrolysis of lignin during preparation of soymilk was the reason for experimenting with hulls.

Two samples of soymilks were prepared by the Illinois process, one with hulls included and the other with hulls removed after blanching the soybeans.

Composition of soymilk

Table 7 shows the composition of the two soymilks. Solids, protein and crude lipid contents were increased, and phenolic compound content was also increased very slightly, showing that there is no correlation between astringency and the components of hull.

Table 7. Composition of soymilk from whole soybeans and dehulled soybeans

	% on wet basis	
	Whole soybeans	Dehulled soybeans
Solid content	7.2	7.8
Protein content	3.4	3.8
Crude lipid content	2.4	2.8
Phenolic compounds content	21.5 mg	22.9 mg

Flavor of soymilk

Table 8 shows the flavor scores of the soymilks. It was observed that soymilk without hulls showed reduced nuttiness and astringency but increased cereal flavor. Increased cereal flavor was possibly due to the reduced nuttiness.

More emphasis was put on astringency in this experiment since astringency was the main problem after paintiness and grassiness were eliminated in soymilk. Two attempts were made to determine if the molecules causing astringency were dialyzable and if astringency changes by diluting soymilk with cow's milk. A mixture of soymilk with cow's milk in 1:1 ratio showed a reduced astringency score from 3.1 to 1.8.

Astringency in dialyzed soymilk, however, was increased to 3.7 from 3.1 indicating that astringency causing factors are not only not dialyzable, but also there could be some other factors that are dialyzable and decrease the astringency of soymilk.

Table 8. Flavor scores of soymilk from whole soybeans and dehulled soybeans

Flavors	Flavor scores	
	Whole soybeans	Dehulled soybeans
Paintiness	0	0
Grassiness	0	0
Astringency	3.1	2.8
Nuttiness	3.6	3.0
Cereal	3.8	4.9

SUMMARY AND CONCLUSIONS

Soymilk prepared from soybeans soaked in 15% ethanol at 60°C showed no lipoxygenase activity after 6 hr. More rapid inactivation of lipoxygenase resulted from increasing the pH by adding Na_2CO_3 or NaOH to the 15% ethanol solution. Addition of 0.1M NaOH, which increased the pH of the ethanol solution to 13.1 from 5.6, inactivated lipoxygenase in 4 hr, and 0.1M Na_2CO_3, which increased the pH of the ethanol solution to 11.6, inactivated lipoxygenase in 5 hr. A $NaHCO_3$ solution (0.1M) was not effective in making the inactivation of lipoxygenase more rapid than 15% ethanol solution, even though the pH of the solution plus $NaHCO_3$ was raised to 9.0.

Approximately 48% of the protein and 65% of the oil in soybeans were recovered in the soymilk prepared from soybeans soaked in 15% ethanol solution containing 0.1M Na_2CO_3, $NaHCO_3$ or NaOH at 60°C, and these soymilks contained an average of 3.2% protein and 2.2% lipid. Very little differences in solids, protein and lipid contents were observed among the soymilks prepared from soybeans soaked different periods of time. Total phenolic compounds in soymilks increased with time of soaking soybeans.

Paintiness of soymilk prepared from ethanol soaked soybeans decreased with decrease in lipoxygenase activity ($r = 0.965$) indicating that paintiness could be caused by

lipoxygenase. However, the paintiness of soymilks prepared from soybeans soaked in ethanol containing Na_2CO_3, $NaHCO_3$ or NaOH did not confirm the theory that lipoxygenase causes paintiness of soymilk. Even though NaOH had the greatest effect on inactivating lipoxygenase, it had the least effect on controlling paintiness. On the other hand, $NaHCO_3$ had the least effect on inactivating lipoxygenase and the greatest effect of reducing paintiness.

Grassiness of soymilk increased with time of soaking as the intensity of paintiness decreased; then, grassiness also decreased when paintiness disappeared. The surprising appearance of grassiness as paintiness decreased could be explained by the presence of isozymes of lipoxygenase. The more heat resistant lipoxygenase 1 might generate grassiness and less heat resistant lipoxygenase 2 might generate paintiness and grassiness.

The astringency of soymilk increased with time of soaking. Phenolic compound content in soymilk also increased but no correlation was observed with astringency of soymilk.

Nuttiness of soymilk increased with time of soaking. Soaking soybeans in ethanol containing NaOH gave the highest intensity of nuttiness, and the Na_2CO_3-ethanol soaking gave more nuttiness in soymilk than the $NaHCO_3$-ethanol soaking. The soymilk from the Illinois process which uses $NaHCO_3$ in soaking and blanching solutions had a strong nutty flavor

according to the sensory panel, whereas the hot grind process in which water alone is used during processing was devoid of nuttiness. The latter process gave soymilk with definite cereal flavor, indicating that the flavor of soymilks could be affected by the chemicals used for their preparation.

Cereal flavor of soymilks from sodium salt-ethanol treatments increased with time of soaking; however, the cereal flavor was very mild and was masked by most of the other flavors of soymilks.

A definite decrease in nuttiness and cereal flavor was noted by the sensory panel after heating soymilk at 95°C for 5 min, and no new flavors were generated during this period of heating.

Soymilk prepared from dehulled soybeans contained more solids, protein and lipid than soymilk prepared from whole soybeans. Cereal flavor in soymilk from dehulled beans was more intense than in soymilk from whole soybeans. No effect on astringency of soymilk was observed by removing the hulls from the soybeans.

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