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By

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ABSTRACT

STUDIES ON THE STABILITY OF DRIED SOYBEAN CURDS

By

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Soybean curds were prepared by conventional methods and modified by addition of either sesame milk or ground sesame. They were air dried, or frozen, aged, thawed and then air dried viz., dried frozen or freeze dried. The stability of the dried products with respect to lipid oxidation and non-enzymatic browning were studied.

Accelerated storage studies were conducted at 37°C and 50°C. Peroxide value, diene conjugation and TBA tests were used to follow the oxidative changes in lipids. High linear correlation was obtained between peroxide value and diene conjugation. Low correlation was obtained between either peroxide value or diene conjugation and TBA tests.

Sorption isotherms were established for the dried products at 37°C. Freeze-dried products had lower hysteresis values than dried-frozen products which in turn had lower values than air-dried products. This was consistent with quality as judged by lipid oxidation measurements. These showed freeze-dried, dried-frozen and air-dried products had the lowest, medium and the highest degree of oxidation, respectively, after drying.

Both freeze-dried and dried-frozen products had relatively poor stability during accelerated storage. Addition of sesame milk had no significant effect on stability while addition of ground sesame provided additional stability to the dried products. The rate of lipid oxidation increased on both sides of monolayer value in the water activity range. The rate of lipid oxidation also increased with increasing storage temperature.

During storage at 37^{o}C and 0.75 a_w, the greenness decreased and no appreciable changes in lightness occurred. The yellowness increased slightly for air-dried products but remained essentially constant for dried-frozen and freeze-dried products. It was suggested that browning might be occurring at the same time as bleaching. However, at 50^{o}C and 0.75a_w, the lightness decreased, the greenness vanished, while the redness and yellowness increased.

Although freeze-dried and dried-frozen products had lower browning indices than air-dried products after drying, these products were more susceptible to browning reactions during storage. The browning was more intense in the products with ground sesame than in the others. Maximum browning was obtained at a water activity of 0.62. The rate of browning decreased above and below this water activity and increased with increasing temperature. Both lipids and carbohydrates were involved in the browning

reactions. Lipids might provide carbonyl compounds from oxidative degradation of polyunsaturated fatty acids.

Browning was accompanied by the reduction of available lysine content. The loss of available lysine was correlated with browning. Higher losses of available lysine occurred in products with ground sesame than in the others. Maximum loss was found in products stored at the water activity of 0.62. Loss of available lysine also increased with increasing temperature. During accelerated storage, greater losses were found in freeze-dried and dried-frozen products than in air-dried products.

Oxygen appears to be the primary cause of product deterioration. The optimum water activity for storage with respect to lipid oxidation and browning was 0.22.

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INTRODUCTION

Two thirds of the world's people live in developing countries with burgeoning populations where malnutrition and starvation are basic problems and concerns. Proper diet and food costs become increasingly interdependent.

Soybeans are capable of producing the greatest amount of protein per unit of land of any major plant or animal source used as food by man. Thus they occupy an important place in global food and nutrition. Because of their composition, soybeans are an excellent source of oil and protein. The higher availability and lower cost of soybean protein compared with animal protein make soybeans more suitable as a protein source to meet nutritional needs in developing countries where animal products are generally too expensive.

Soybean curd is the major unfermented soybean food in East and South East Asia. The curd is rich in essential fatty acids and essential amino acids except methionine. Sesame seed is known to be rich in the amino acid methionine. Since soybean and sesame proteins are complementary, their combination may yield a high quality protein.

More than 80% of fresh soybean curd's total weight is water which makes the curd highly perishable. Dehydration is one preservation method that has been applied to soybean

curd. With the loss of water, the soybean curd becomes a highly concentrated source of protein and oil.

In these studies, soybean curd and soybean curd modified by addition of sesame were made. The curds were directly air dried or frozen, aged, thawed and then air dried or freeze dried. The purpose of the research was to study the stability of the dried soybean curd and the dried modified soybean curd with respect to lipid oxidation and non-enzymatic browning under various conditions of temperature and water activity.

LITERATURE REVIEW

The Use of Soybeans in Asia

The soybean (Glycine max, L.) originated in Eastern Asia where it has been utilized as a food source for centuries. It was cultivated for food in Asia long before the existence of written records (Waggle and Kolar, 1979).

Soybeans are important crops among the food legumes grown in Indonesia. Soybeans occupy fifth place among the other food crops after rice, cassava, maize and sweet potatoes (Somaatmadja and Guhardja, 1976).

In the Orient, soybeans are traditionally used as food in a variety of ways. They are used to make unfermented foods such as tofu (soybean curd), yuba (soymilk film), and kinako (roasted soyflour) (Ebine, 1976). In addition, roasted beans, tofu chips, and boiled seeds are eaten as snacks (Somaatmadja and Guhardja, 1976). Soybeans are made into various types of fermented products such as miso (soy paste), shoyu (soy sauce), natto, sufu and tempeh (Ebine, 1976).

Soybean Curd

Tahu (Indonesian), tofu (Japanese, dan fu (Vietnamese), teou fu or tou fu ho (Chinese) or soybean curd (English) is

a cottage cheese like product formed into a cake which is precipitated from soybean milk by calcium (Smith and Circle, 1978). This makes an excellent source of protein since the soybean curd consists primarily of the protein matter of the soybean. Soybean curd is also a good source of unsaturated fats, vitamins and minerals.

Soybean curd has a bland taste so it can be easily flavored with seasonings or blended with other food materials to produce a variety of tasty products. It can be used for replacing meat or fish in particular regions or countries where meat and fish are not available or too expensive. Moreover, soybean curd is an important source of high quality proteins for vegetarians (Thio, 1975).

Soybean curd is a perishable product and dehydration is one of the preservation methods which has been applied to soybean curd. Thio (1975) reported that sliced soybean curd can be dried with hot air at temperatures not exceeding $70^{o}C$. Sun-drying is not recommended as it may lead to decomposition of riboflavin. Dried soybean curd can be used in all kinds of dishes. When added to relatively dry dishes, which contain a little gravy, the dried slices should first be soaked in hot water for 15-30 minutes to make them softer. When added to wet dishes which contain a lot of broth or to vegetable soups,

it is not necessary to soak beforehand. In this case, the dried curd can be added to the dish and cooked together. Moreover, dried soybean curd can be fried and consumed as a snack (Thio, 1975).

Another form of dehydrated soybean curd is called dried-frozen tofu (Kori tofu). The processing starts out by making fresh soybean curd which is cut into slices, and frozen and aged at -1 to -2^{o}C for 2-3 weeks. After aging, the frozen tofu is thawed, dried, and packaged. The dried frozen tofu has the advantage of a shelflife of 6 to 12 months (Smith and Circle, 1978).

Tofu has been produced for centuries in Indonesia, China, and Japan on a home or cottage scale. In Japan, however, soybean curd is being manufactured on a relatively large scale, some of it in the freeze-dried form (Winarno and Karyadi, 1976).

Mechanism of Lipid Oxidation

Oxidation of lipids is usually initiated by peroxidation of unsaturated fatty acids. It results in a number of undesirable changes in flavor, color, and nutritional value. In addition, lipid oxidation products participate in reactions with nonlipid constituents of foods (Karel, 1968). Oxidation of fat is frequently alluded to as autoxidation because the rate of oxidation increases as the reaction proceeds. Unless mediated by other oxidants or

enzyme systems, oxidation proceeds through a free radical chain reaction mechanism involving three stages: "(1) initiation, formation of free radicals; (2) propagation, free radical chain reaction; and (3) termination, formation of non radical products (Dugan, 1976)." The mechanism may be depicted as (Boland and Ten Have, 1947):

$$\text{Initiation} : RH \longrightarrow R^{\cdot} + H^{\cdot}$$

$$\text{Propagation} : R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

$$\text{Termination} : ROO^{\cdot} + ROO^{\cdot} \longrightarrow ROOR + O_2$$

$$ROO^{\cdot} + R^{\cdot} \longrightarrow ROOR$$

$$R^{\cdot} + R^{\cdot} \longrightarrow RR$$

RH refers to any unsaturated fatty acid in which the H is labile by reason of being on a carbon atom adjacent to a double bond. $R^{\cdot}$ refers to a free radical formed by removal of a labile hydrogen.

Various extraneous influences may be present that affect the rate of oxidation. These factors are temperature, light, ionizing radiation, metals, enzymes and pigments (Lundberg, 1962).

In the initiation stage, an unsaturated hydrocarbon loses a hydrogen to form a radical, $RH \longrightarrow R^{\cdot} + H^{\cdot}$, and oxygen adds at the double bond to form a diradical:

$$R\text{-}\underset{H}{\overset{H}{C}}=\underset{}{C}\text{-}R^{\cdot} + O_2 \longrightarrow R\text{-}\underset{H}{\overset{H}{C}}\text{-}\underset{O\text{-}O^{\cdot}}{C}\text{-}R^{\cdot}$$

Alternatively, oxygen in the singlet state can apparently

interpose between a labile hydrogen to form a hydroperoxide directly ($RH + O_2 \dashrightarrow ROOH$) (Dugan, 1976). A trace amount of plant or animal pigments present in most sources of fatty acids was considered to be a sensitizer for photo-sensitized production of singlet oxygen (Rawls and Van Santen, 1970).

Measurement of Lipid Oxidation

Foods can become rancid as a consequence of lipid oxidation and this oxidative rancidity is a major cause of food deterioration. One way to define rancidity is the development of off flavor which makes the food unacceptable on a consumer market level (Labuza, 1971).

The acceptability of a food product depends on the extent to which deterioration has occurred. Thus some criteria for assessing the extent of oxidation is required. Many methods have been developed for measuring lipid oxidation, some of these are peroxide value, diene conjugation and thiobarbituric acid (TBA) tests.

Peroxide Value

It seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation since the primary products of lipid oxidation are hydroperoxides. The iodometric methods of Lea (1931) and Wheeler (1932) are widely used, and these are based on the measurement of iodine produced from potassium iodide by peroxides present

in the oil. According to Mehlenbacher (1960), the two principal sources of error in these methods are (a) the absorption of iodine at unsaturated bonds of the fatty material and (b) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated. Lea (1931) attempted to eliminate this error by filling the sample tube with nitrogen at the beginning of the test and assuming that the evolution of chloroform thereafter would prevent the reentry of oxygen into the tube. Wheeler (1932) used a homogeneous solution in an attempt to eliminate the need for shaking thereby minimizing the effect of oxygen.

Eskin and Frenkel (1976) developed a colorimetric method based on complex formation between titanium and hydroperoxides resulting in a colored complex that can be measured spectrometrically at 415 nm. Another spectrophotometric method has been developed by Takagi et al. (1978). In this method, after oxidation of iodide to iodine with the sample for 5 minutes under an inert atmosphere, an excess of iodide ion is immediately converted to a cadmium complex for protection from atmospheric oxygen. The iodine is measured spectrometrically at 358 or 410 nm and the peroxide value is calculated from the absorbance.

Diene Conjugation

Oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption. Fatty acids with

conjugated unsaturation absorb strongly in the region 240 to 375 nm, diene unsaturation at 234 nm, and triene unsaturation at 268 nm. The magnitude of change is not readily related to the degree of oxidation because the effects upon the various unsaturated fatty acids vary in quality and magnitude. However, the changes in the ultraviolet spectrum of a given substance can be used as a relative measurement of oxidation (Gray, 1978).

Oils containing linoleate or more highly unsaturated fatty acids are oxidized to conjugated diene systems that can be measured by ultraviolet absorption at 233 nm. Farmer and Sutton (1943) indicated that the absorption increased proportionately to the uptake of oxygen and to the formation of peroxides in the early stages of oxidation.

St. Angelo et al. (1975) studied the autoxidation of peanut butter by measuring the peroxide value and the increase in absorption at 234 nm due to diene conjugation. They concluded that the diene conjugation method can be used as an index of progressive staling in place of, or in addition to, peroxide value. The diene conjugation method is faster than the peroxide method, is much simpler, requires no chemical reagents, does not depend on chemical reaction or color development, and can be conducted on smaller samples.

Thiobarbituric Acid (TBA)

The thiobarbituric acid (TBA) test is one of the more commonly used methods for the detection of lipid oxidation. However, the popularity of a method is not in itself ample proof that the method fulfills all the requirements of a reproducible technique (Gray, 1978).

Kohn and Liversedge (1944) observed that animal tissue which had been incubated aerobically gave a red color when mixed with 2-thiobarbituric acid. Bernheim et al. (1947) found that the red color was formed from the oxidation products of unsaturated fatty acids and 2-thiobarbituric acid.

Investigations by Sinnhuber et al. (1958) helped to clarify the nature of the colorimetric reaction that occurs during the TBA test. They proposed that the chromagen was formed through the condensation of two molecules of TBA with one molecule of malonaldehyde. However no evidence was presented that malonaldehyde could be found in all oxidizing systems.

Dahle et al. (1962) postulated a mechanism for the formation of malonaldehyde, a secondary product in the oxidation of polyunsaturated fatty acids. They noted that only peroxides which possessed unsaturation, β, γ to the peroxide group were capable of undergoing cyclization with the ultimate formation of malonaldehyde. Such peroxides could only be produced from fatty acids containing three or more double bonds.

Pryor et al. (1976) studied the nature and mechanism of formation of the TBA-reactive material produced in the autoxidation of polyunsaturated fatty acids or their esters. They concluded that the TBA test detects malonaldehyde which arises at least in part from the acid-catalyzed or thermal decomposition of endoperoxides (2,3-dioxanorbornane compounds).

Water Activity

Low moisture foods are stabilized because the availability of water for microbial growth is reduced in these foods. This availability of water depends not only on its total amount but also to its binding to food components (Karel, 1975). The thermodynamic parameter which describes the state of water is the water activity, a_w, which can be defined as the ratio of the water vapor pressure of that food compared to the vapor pressure of pure water measured at the same temperature. a_w may also be thought as the relative humidity in equilibrium with a food divided by 100 (Rockland, 1969; Labuza et al., 1970). An expression of a_w could be presented as:

$$a_w = \frac{p}{p_o} = \frac{ERH}{100}$$

a_w = water activity.

p = partial pressure of water in food.

p_o = vapor pressure of water at the given temperature.

ERH = equilibrium relative humidity (%).

Water binding may be estimated by various methods, but the most successful method for study of the properties of water in foods is the determination of the water sorption isotherms, which are curves relating the partial pressure of water in foods to the moisture content. Usually, instead of partial pressure, one actually considers water activity (Karel, 1975).

The isotherm curve can be obtained in one of two directions. An adsorption isotherm is made by placing a completely dry material into various atmospheres of increasing relative humidity and measuring the weight gain due to water. The desorption isotherm is found by placing the initially wet material under the same relative humidities, but in this case measuring the loss in weight (Labuza, 1968).

Procedures for obtaining water vapor isotherms for foods have been described by Taylor (1961), Karel and Nickerson (1964), Loncin et al. (1968), and have been reviewed in detail by Gal (1975). In general, these methods utilize one of two techniques. The first is one in which the material is placed in vacuum desiccators containing saturated salt solutions which give a certain equilibrium relative humidity. The desiccators are then closed and evacuated and allowed to equilibrate for at least 24 hours (Heidelbaugh and Karel, 1975). Following equilibration, the samples are removed for weighing. In the second method, for example as used by Taylor (1961), the vapor pressure of water in

equilibrium with a food at a given moisture content is measured by a sensitive manometric system.

As the water activity of a product decreases, as it does during drying, the state of water changes. Water in food at low moisture content can be considered to exist in monolayer, multilayer, and capillary condensation states (Rockland, 1969; Labuza et al., 1970). In dried foods, this aqueous solution may be found in capillaries or held by swollen protein or polysaccharide gels. As the water activity decreases, the predominating form of water shifts to water hydrating hydrophyllic constituents. However, water in this region still acts as an aqueous phase down to the BET (Brunauer, Emmet, Teller) monolayer (Brunauer et al., 1938). Above this point, water acts to dissolve solutes, and mobilizes them to react within the aqueous environment. The BET monolayer thus describes the moisture content below which most reactions cease if they depend on being in the dissolved condition (Labuza et al., 1970).

It is noted that the isotherms for food materials determined by the desorption procedure do not coincide with the values generated by an adsorption procedure for attaining equilibrium moisture contents. As a result of this situation, a food can exhibit two different moisture contents at a single a_w, depending upon the method used for reaching equilibrium. The differences between the adsorption and desorption moistures has been defined as hysteresis

Rockland, 1969; Labuza et al., 1970; and Wolf et al., 1970).

a_w and Stability of Low Moisture Systems

Lipid oxidation and non-enzymatic browning are two chemical processes of deterioration which are important in the quality changes exhibited in processed and stored low moisture foods. In calssical studies, Henry et al. (1948) reported that the biological value of the proteins of dried skim milk of high moisture content (7.6%) decreased progressively during storage in air at 37°C. At a lower storage temperature (28.5°C), the changes were about six times slower than at 37°C. At lower moisture contents, the product was much more stable. The authors noted that most of the decrease in the biological value was accounted for by inactivation of lysine.

It has been reported that shelled walnuts are most stable at an optimum moisture content, above and below which they deteriorate at a more rapid rate. Under conditions of accelerated aging (140°C), maximum flavor and color stability were observed between the approximate limits of 1.4 to 4.5% moisture (Rockland, 1957). Kapsalis et al. (1964) reported that the rate of oxidation of freeze-dried beef was tripled on going from the dry state (a_w 0.01) to an a_w of 0.32 which is above monolayer. Kapsalis (1973) noted that a sharp maximum force for shearing freeze-dried beef corresponded to 0.80-0.85 a_w.

Martin (1958) studied the development of rancidity in crisp oatflakes prepared by being oven-dried, freeze-dried

or by a combination of both methods. The stability gradually increased as the time of oven drying was reduced, but, in contrast, the sample prepared by freeze drying had a relatively poor stability.

Studies by Fishwick and Zmarlicki (1970) on freeze-dried turkey muscle have showed that oxidation of sulfhydryl group was accompanied by a decrease in soluble nitrogen. They stated that the major deteriorative process at low moisture content was a type of lipid browning reaction which was dependent on oxygen and caused discoloration and objectionable odors. Fishwick (1970) showed that during the freeze drying process myoglobin was converted to a low-spin complex, ferromyochromagen, while ferrimyoglobin forms the corresponding ferrimyochromagen. During storage in the freeze-dried state, the iron complexes do not catalyze the autoxidation of unsaturated lipids.

Love and Dugan (1978) monitored lipid oxidation, changes in soluble proteins, reducing sugar content, extent of non-enzymatic browning and product color of instant navy bean powder during one month of accelerated storage at 37°C. Below the monolayer, i.e., a_w 0.11, greater lipid oxidation occurred in neutral lipids and phospholipids of air-packed samples than in nitrogen-packed samples. Indices revealed that monolayer samples browned more and lost more soluble protein than samples stored below the monolayer. Nitrogen-packed samples lost more reducing sugars and had darker

color than air-stored samples at each a_w.

Wolf et al. (1972) investigated water sorption hysteresis in freeze-dried beef, carrot, haddock and potato. They reported that the effect of increases in temperature was to decrease the amount of hysteresis and to limit its extent along the isotherm. The effect of storage was to increase the area of hysteresis loop, due mainly to a decrease of the adsorptive capacity of the material.

Lipid Oxidation in Low Moisture Systems

An important aspect related to lipid oxidation in low moisture systems is the potential for free radical production during freezing and drying. Munday et al. (1962) observed that free radicals were formed during freeze-drying of various meats. Free radicals were also reported in frozen bacteria, especially in the presence of oxygen (Swartz, 1971). The mechanism of free radical generation during freezing and drying is not firmly established. Some authors attribute it to the effects of removal of bound water, others to mechanically induced breakage of polymer chains and still others to the reaction with oxygen while in frozen and dehydrates states (Karel, 1975).

In early studies, it was found that as moisture content was lowered, the foods became rancid much sooner. Based on the fact that oxygen diffusion is 10^4 times faster in air than it is in water, Halton and Fisher (1937) proposed that the water retarded the diffusion of oxygen to the sites of

the unsaturated double bonds. Salwin (1959) suggested that at the BET monolayer, the water formed a protective barrier, preventing the oxygen from reaching the underlying unsaturated fats.

Studies describing the storage behavior of vacuum dried milk have showed that milk dehydrated to a low water content is highly susceptible to oxidation even at oxygen pressure corresponding to 1% concentration in container headspace, and that low water contents are prooxidant (Tamsma et al., 1961; Tamsma and Pallansch, 1964; Aceto et al., 1965). The importance of avoiding any contact between freeze-dried beef and molecular oxygen has also been stressed by Bengtson (1967).

Maloney et al. (1966) and Labuza et al. (1966) have investigated the effect of water in freeze-dried model systems based on cellulose and methyl linoleate. They reported that water inhibits oxidation by hydrogen bonding between water and hydroperoxides and deactivation of metal catalysts by hydration of their coordination shells.

Tjhio et al. (1969) investigated the effect of humidification on activity of catalysts in model systems containing lipids and additives. They noted that complexes of manganese and histidine show enhanced prooxidant activity at high water contents while those of cobalt and histidine become more antioxidant. The effectiveness of EDTA as a chelating agent is also enhanced as the moisture content is increased.

However, the effect of humidification is dependent on other components of the system including the nature of the hydrophilic support.

Labuza and Chou (1974) noted that at low metal content (10-50 ppm), as moisture increases the rate of oxidation increases due to the decreased viscosity, increased mobility and the swelling which exposes new catalytic sites. However, in systems containing high concentration of trace metals, the rate of high a_w decreases as a_w increases. The reason given is that at high metal concentration metal catalysis is a predominant force in inducing formation of free radicals. Thus any substantial decrease in the concentration depresses the rate steeply.

Oxygen absorption is not the only factor correlated with lipid oxidation which can be affected by moisture content. As shown by Labuza et al. (1969) for model system and confirmed in potato chip studies by Warner et al. (1974), the production of pentane, an off-odor product of oxidation, decreases as a_w is increased from the dry state into the monolayer region.

Quast and Karel (1972) also found that water had an effect on the rate of light-induced oxidation. They reported that light has a greater prooxidant effect at a_w 0.40 than in the dry state. These authors also showed that at higher humidities, the rate of oxidation was less affected by decreases in oxygen level in the atmosphere. The reasons

are not known.

Antioxidants have been found to be very effective in pure oils and model systems but little evidence exists for their value in real foods. Walter and Purcell (1974) found nitrogen packing of dehydrated sweet potatoes to be very effective in reducing oxidation, whereas antioxidants had no effect. Labuza et al. (1969) found that antioxidants were less effective in the presence of proteins.

Non-Enzymatic Browning

In 1912, the French chemist L.C. Maillard observed the formation of brown pigments while heating a solution of glucose and glycine. The amino-carbonyl or the Maillard reaction is dependent on the reaction between amino groups of proteins or amino acids and carbonyl groups from reducing sugar or other carbonyl compounds in foods. The carbonyls may arise from lipid oxidation. Colors and flavors generated from the amino-carbonyl reactions may contribute to both the destruction or the improvement of many foods.

Hodge (1953) reviewed the chemistry of browning reactions in model systems. The author classified the overall non-enzymatic browning as occurring in three stages: "(1) the initial stage (colorless product with no absorption in near-ultraviolet) resulting from sugar-amine condensation, followed by Amadori rearrangement; (2) the intermediate stage (colorless or yellow products with strong absorption in near-ultraviolet) resulting from sugar dehydration, sugar

fragmentation and amino acid degradation; (3) the final stage (highly colored products) resulting from aldol condensation and aldehyde amine polymerization with the formation of heterocyclic nitrogen compounds."

In the reaction of aldoses with amines, aldosylamines and then the Amadori rearrangement products, 1-amino-1-deoxyketoses are formed and the latter decompose to 3-deoxyosones and other reactive carbonyl compounds and form polymers (Kato, 1963; McWeeny and Burton, 1963; Kato et al., 1969).

In the reaction of ketoses with amines, ketosylamines and Heyns rearrangement products are formed. The reaction between D-fructose and amino acids gives 2-(N-amino acid)-2-deoxy-D-glucose and -D-mannoses (glucose- and mannose amino acids) and lesser amounts of 1-(N-amino acid)-1-deoxy-D-fructose (fructose amino acid) (Heyns et al., 1957; Heyns and Breuer, 1958). However, aldose amino acids are considered to be more stable than fructose amino acids (Kato et al., 1969).

Adrian (1974) noted that, after an Amadori rearrangement, a 1-amino-1-deoxy-2-ketose type molecule was formed. This molecule provided a basis for the reaction, which can develop in three different directions: "(1) producing fission products, small carbonyl molecules such as diacetyl, acetol, pyruvaldehyde; (2) undergoing severe dehydration resulting in furfurals and hydroxymethylfurfural, (3) undergoing a

more moderate dehydration with formation of reductones and dehydroreductones." The reducing substances can react with still intact amino acids and transform them into aldehydes after decarboxylation. This is known as Strecker degradation, a phase typical of Maillard reaction. Some of the Maillard reaction products are water soluble and others are water insoluble. The soluble products are called premelanoidins and the insoluble ones melanoidins.

The first compounds of the Maillard reaction are characterized by enzyme-resistant linkages (Evan and Butts, 1948; Schroeder et al., 1955; Horn et al., 1968; Hagan et al., 1970). The amino acid thus linked to the sugar can not be hydrolyzed enzymatically; however, the link can be broken by chemical hydrolysis to regenerate the initial sugar and amino acid. Thus from a nutritional point of view, the amino acid is immediately lost and the Maillard reaction is irreversible from the beginning. For a chemist, on the other hand, the amino acid can be recovered and the Maillard reaction is reversible in the early stages.

The behavior of amino acids in the Maillard reaction is variable. Their action depends on the distance between the alpha carbon and amino group: gamma aminobutyric acid causes ten times more intense coloration than its alpha isomer. Lysine is six times more active than norleucine which has only one amino group which is in the alpha position (Lento et al., 1958; Underwood et al., 1959).

When amino acids are included in a protein chain, the destruction of protein by Maillard reaction varies with respect to the amino acids representing the proteins. First, the N-terminal units of the protein are damaged, then the basic amino acids, especially lysine, are destroyed. The S-containing amino acids (cystine and methionine) follow, and sometimes tryptophan is involved (Adrian, 1974).

Various factors such as temperature, pH, and water activity can affect the extent of browning. The Maillard reaction occurs during both storage and heat treatments. Heating is not indispensable to the development of the reaction. It is slow at room temperature but its intensity increases as the temperature rises (Lea and Hannan, 1949; Overby et al., 1959).

The Maillard reaction increases approximately linearly with increasing alkalinity from values at pH 3 up to pH 8 at least and probably up to pH 10 (Lea, 1950). This pH zone corresponds to a great extent to that of foods, thereby explaining the importance of the Maillard reaction in foods.

Lea and Hannan (1949) studied the effect of water activity on disappearance of amino nitrogen in a casein-glucose mixture. They found a maximum reaction at 65-70% equilibrium relative humidity. In dried milk, Loncin et al. (1965) found a maximum loss of lysine and in the rate of browning within the same range of water activities. Dried meat showed maximum browning at an equilibrium relative

humidity of 57% (Sharp and Rolfe, 1958) and pea soup mix at 70% (Labuza et al., 1970).

Another pathway to non-enzymatic browning derives its carbonyl reactants from autoxidizing lipids. Oxidized lipids emulsified in aqueous dispersions of proteins gave brown copolymers (Tappel, 1955; Venolia and Tappel, 1958) or complexes (Narayan and Kummerow, 1958, 1963). The results obtained by Tappel (1955) suggested that an amino-carbonyl condensation had occurred. Narayan and Kummerow (1958, 1963) considered that the precipitates obtained from egg albumin and oxidized lipids were complexes in which the attachment of the lipid to the denatured protein did not involve covalent bonds.

Lea (1958) reported on a study with stored herring meal. This product browned extensively when stored in air. There was a loss in the nutritive value of the protein. Storage under nitrogen delayed the process as did antioxidants, implying a role for lipid oxidation. Some reactions of oxidized lipids in systems similar to freeze-dried foods were reported by Koch (1962). The amino acids lost through these reactions were lysine, phenylalanine and glycine.

Lea et al. (1960) used propanal for the reaction with vacuum-dried cod muscle. Propanal was used as a model aldehyde of low molecular weight that could be formed in lipid oxidation. They used relatively high moisture (16%) to ensure maximum reactivity, and found that 35% of the

available lysine was lost in 48 hours at 37°C. Andrews et al. (1965) indicated, while studying the reaction of an autoxidized lipid with protein, that lipid intermediates react with the epsilon amino group of lysine, and also with phenylalanine and glycine, and the N-terminal amino group of insulin.

Tannenbaum et al. (1969) studied loss of methionine in a model system containing casein and methyl linoleate stored at relative humidities of 0, 33, and 75%. In each case, the loss of methionine was proportional to the amount of protein bound non-enzymatic browning pigment. They proposed that methionyl residues may act as peroxide decomposers with concomitant carbonyl compound formation which can lead to non-enzymatic browning.

Buttkus (1967) studied the reaction of myosin with malonaldehyde, an oxidation product of polyunsaturated fatty acids. The rate of reaction with epsilon-amino group of myosin was greater at -20°C than at 0°C and was almost as great as that at +20°C. The same relationship was observed when the decreasing malonaldehyde concentration was measured in the protein-malonaldehyde mixture. The increased rate of reaction in the frozen system is explained as a concentration effect and as a catalytic effect involving the ice mixture.

Crawford et al. (1967) reported the reaction of malonaldehyde with the epsilon-amino group of lysine and N-terminal amino aspartic acid groups on protein as judged by their

loss to reaction with 1-fluoro-2,4-dinitrobenzene. A nucleophilic 1,4-addition of free amino functions on the protein to the end carbon atom of α, β-unsaturated carbonyl system of the free enol of malonaldehyde to form an enamine is postulated to be the mechanism for this reaction.

Roubal and Tappel (1966a,b) noted that transient free radicals are produced in peroxidizing lipid-protein reaction systems. As the extent of oxidation progresses, soluble polymeric products, incorporating low level of lipid, give way to firmly cross-linked polymeric products containing moderate amounts of occluded and complexed lipid material. Among the most labile amino acids are methionine, histidine, cystine and lysine.

Roubal (1971) hypothesized that at low moisture content, radical attack and not aldehyde attack on protein is predominantly responsible for damage to protein. Zirlin and Karel (1969) noted that gelatin-linoleate interactions in the dry state can lead to scission of protein as well as to cross-linking.

Braddock and Dugan (1973) isolated fluroescent compounds, indicative of C=N functional groups, from an autoxidizing system consisting of sodium linoleate and Coho salmon myosin. Similar compounds were also present in extracts from freeze-dried salmon steaks and salmon kept frozen for one year. They also noted significant decreases in the amounts of histidine, lysine and methionine following

oxidation. The appearance of fluorescence compounds was also reported by Chio and Tappel (1969) in enzyme-lipid reaction mixtures. They proposed that the fluorescence is produced by intra- and inter-molecular cross-linking of the enzyme and that malonaldehyde is responsible for the cross-linking.

Jarenback and Liljemark (1975) indicated that linoleic acid hydroperoxides were ten times more effective than linoleic acid in reducing the amount of protein in KCl-extracts from incubated myofibrils of post-rigor cod muscle.

In a series of papers (Homma and Sakurai, 1967; Homma et al., 1969; and Homma et al., 1970), it was reported that browning of dried-frozen tofu (Kori tofu) is apparently caused by the interaction between oxidized oil and protein. They noted that unsaturated aldehydes and dicarbonyl compounds are reactive in the browning reaction. Damage of proteins by lipid peroxides and reactive carbonyl compounds are suggested.

Schwenke et al. (1975) studied modification of proteins by reaction with carbonyl compounds. They reported that stepwise blocking of the alpha- and epsilon-amino groups leads to modified protein with lowered isoelectric points and changes in the solubility, precipitation characteristic and electrophoretic behavior. There are relationships between nutritional value and blocking of lysine.

It is apparent from these statements that lipid-protein reactions are important in food systems. The oxidizing lipids have been shown to bring about change in proteins. There is the possibility that carbonyl-amine reactions seem similar to sugar amine reactions which led to the production of some of the brown pigments in the Maillard reaction.

Browning Products and Amino Acids as Antioxidants

Several articles have described an antioxidant effect of browning products. The antioxidant properties are believed to associate with the presence of reductones formed during the browning reaction (Griffith and Johnson, 1957).

Cooney et al. (1958) noted that reductones in fat systems show similarities in browning to reductones in aqueous systems. They suggested a mechanism of antioxidation by polyphenols and reductones in oils. Their study indicated that at least four possible reactions for the mechanism of antioxidation in oils: "a) air-oxidation of the enediols to alpha-dicarbonyl compounds, b) spontaneous reduction of the alpha-dicarbonyl compounds to enediols, c) spontaneous oxidation of the alpha-dicarbonyl compounds which is dependent of oxygen to produce deeper colored products, and d) oxidation-reduction between the colored compounds and fat peroxides."

Evans et al. (1958) stated that the outstanding features of the reductone-treated oils were long induction periods, slow absorption of oxygen and low rates of peroxide

development. Reductones are believed not to react directly with peroxides but to prevent peroxide formation by reacting with some precursors.

Anderson et al. (1963) studied the effects in cereals of certain dehydroreductones (maltol, isomaltol, cyclotene and kojic acid). It was found that each of these dehydroreductones has shown antioxidative activity when sprayed in alcohol on toasted cereals at levels of 20-100 ppm.

Kirigaya et al. (1968) showed a parallel correlation between the color intensity of browning and antioxidant activity in a model system of xylose, glycine and linoleic acid in 40% ethanol. Instead of reductones, they considered melanoidin pigments play an important role in the antioxidant activity.

The products of reaction between dihydroxy acetone (DHA), a triose sugar, with different amino acids have exhibited the most potent activity, followed by those from xylose, and then from glucose. Among the amino acids tested, methionine, leucine, isoleucine and valine gave browning products having pronounced antioxidant activity when reacting with DHA. These products have more potent antioxidant activity than butylated hydroxyanisole (BHA) (Itoh et al., 1975).

Eichner (1975) demonstrated that in low moisture and high viscosity systems, the 1,2-enaminols are at least in part responsible for retarding fat oxidation. These behave

like reductones and are readily oxidizable and capable of reacting with peroxides or radicals produced during fat oxidation.

The literature contains conflicting observations on amino acids as antioxidants for fats. Marcuse (1960) studied, manometrically, the effect of amino acids on herring oils. He noted that the antioxidant effect was strongest with histidine and that cysteine was prooxidant. It was noted that the effect was pH dependent and the amino acids acted synergistically with tocopherol. Further studies (Marcuse, 1962) showed that tryptophan and histidine are very functional whereas glycine and alanine exhibit weak activity. The antioxidant effect is enhanced and the pro-oxidant effect is lowered by addition of phosphate or emulsifier like Tween.

Farag et al. (1978a,b) studied the effect of various amino acids in oil-in-water emulsions, in oils and in freeze-dried systems. The amino acids tested were all pro-oxidants in liquid emulsions and in oils. This effect was attributed to the $H_3\overset{+}{N}$-R group. But in dried systems the amino acids, with the exception of cysteine, all proved to be antioxidants.

Recently, Riisom et al. (1980) measured oxygen absorption rate of emulsions containing safflower oil and various amino acids. The antioxidant effects of several amino acids were quite variable depending upon the type of

emulsifier used, the pH of the system and the presence of added sugar. Maillard reaction products obtained by heating dextrose with lysine showed little stabilizing effect. In freeze-dried emulsions, methionine, threonine, lysine and histidine all exhibited antioxidant activity.

Available Lysine

The nutritive value of a food protein depends not only on its content of essential amino acids but also on physiological availability. Amino acids are unavailable if they are in regions of a protein protected (chemically or physically) from action of proteolytic enzymes or if they are linked to other chemical moieties through bonds not readily broken by digestion (Finley and Friedman, 1973).

Lysine is the only essential amino acid that still has a free amino group when in condensed form within a peptide chain (Carpenter and Booth, 1973). This is the reason why the nutritive value of vegetable proteins and of animal protein of inferior quality has been directly related to the lysine in these proteins (Boyne et al., 1961; Mann et al., 1962). A loss of availability of amino acids especially lysine can occur by different mechanisms depending on the conditions of processing of the protein. Lysine reacts with glucose monohydrate in the presence of heat (Stevens and McGinnis, 1947). Overheating of casein lowers the biological availability of lysine (Greaves et al., 1938). A reaction between glucose and the free amino group in casein has been

reported (Lea and Hannan, 1949, 1950). Both reducing and non-reducing sugars react with lysine in proteins to render it unavailable. El-Nockrashy and Frampton (1967) showed that sucrose, raffinose and trehalose all react with lysine. Gossypol reacts with the free amino group of lysine in albumin or cottonseed protein (Lyman et al., 1959).

Cross-linking is also an important mechanism restricting biological utilization. Because of its epsilon-amino group, lysine is particularly susceptible to side reaction and cross-linking making it unavailable. Lysine may be buried in a protein matrix in a particular sequence or conformation which is slow to hydrolyze or is not hydrolyzed at all by animal proteases (Finley and Friedman, 1973). Lysine can be cross-linked to an aspartyl or glutamyl residue on another protein or in the same protein molecule (Holt and Milligan, 1970; Asquith and Otterburn, 1971; Harding and Rogers, 1971; Hurrell et al., 1976). Another reaction of lysine is cross-linking with dehydroalanine residues. Bohak (1964) and Patchornik and Sokolovsky (1964) reported that dehydroalanine is formed by heating serine or cysteine under alkaline conditions. The dehydroalanine thus formed, reacts with the epsilon-amino group to form lysinoalanine.

The nutritive quality of protein can be assessed in feeding trials with higher animals. However, since these tests are time-consuming and expensive, microbiological and chemical techniques for assessing availability of amino

acids, particularly lysine, have been developed. Much effort has been expended to establish reliable chemical methods for measuring available lysine because of the advantages of speed of analysis compared with other techniques, and also its suitability for routine assay.

The chemical estimation of lysine in foods can involve the measurement of total lysine or available lysine. Total lysine is generally determined after an acid hydrolysis treatment and does not necessarily reflect the amount of lysine that is in the nutritionally available form, while available lysine is usually regarded as those lysine residue possessing free epsilon-amino groups (Peterson and Warthesen, 1979).

The reagent 1-chloro-2,4-dinitrobenzene and 1-fluoro-2, 4-dinitrobenzene were used by Sanger (1945) to react with free amino groups which formed dinitrophenylamino acids with a yellow color that could be estimated colorimetrically. Carpenter and Ellinger (1955) used 1-fluoro-2,4-dinitrobenzene (FDNB) to react with the free epsilon-amino group of lysine in 15 samples of animal by products and showed a highly significant correlation between results obtained and a biological assay of protein quality with chicks. This reagent (FDNB) has also been used to evaluate fish protein (Bruno and Carpenter, 1957). Subsequently, the method was modified by avoiding interfering substances in the final measurement (Carpenter, 1960). Booth (1971) improved the

method by taking into account the recovery factor for materials rich in carbohydrate.

Roach et al. (1967) developed the "difference procedure" for determining available lysine. In this method, free lysine (rather than dinitrophenyl lysine) was estimated in the acid hydrolysates of the test materials that had been treated with FDNB. This value is taken as a measure of inaccessible or bound lysine which has not reacted with FDNB, but is released by acid hydrolysis. Available lysine by difference is then calculated as total minus bound lysine.

Kakade and Liener (1969) used 2,4,6-trinitrobenzene sulfonic acid (TNBS) instead of FDNB. The principle of the use of TNBS and FDNB are the same. First, the compounds are allowed to react with the lysyl side chain under mild conditions. Next, the protein is hydrolyzed, and the hydrolysis products are extracted with ether to remove excess reagent. Finally, the derivatives are measured spectrophotometrically.

Finley and Friedman (1973) employed a method based on alkylation of epsilon-amino group of lysine side chains by methyl acrylate. After incubation, the alkylated protein was subjected to hydrolysis and ion-exchange chromatography on an amino acid analyzer. Available lysine was measured by comparing lysine content before and after alkylation.

O-methylisourea reacts with epsilon-amino groups of lysine to form homoarginine units. On acid hydrolysis,

homoarginine is released and can be analyzed by amino acid analysis (Hurrell and Carpenter, 1974). The disadvantage is that o-methylisourea reacts slowly i.e. 4 days at room temperature, which is presumably the reason why this procedure has not been used extensively.

Details of a chemical method, using acetic acid and sodium nitrite, for determination of available lysine in plant materials are given by Allison et al. (1973). The method showed significant positive correlations with biological values and true digestibility when used on leaf protein concentrates. Other chemical methods used on these proteins have not been very successful in part due to the interference of plant pigments.

Methods using enzyme pronase to predict damage of amino acids in processed foodstuffs have been described by Provansal et al. (1975) and Rayner and Fox (1976). Pronase released lysine has been shown to have excellent potential in measuring available lysine in rapeseed meal (Rayner and Fox, 1976) and beef muscle damage by heat and glucose or heat alone (Rayner and Fox, 1978).

Several workers (Hurrell and Carpenter, 1975; Walker, 1979) have used dye-binding procedures for determining available lysine. The dyes which seem particularly convenient for this purpose are the acid azo-dyes. They combine at low pH with the basic group of lysine, histidine and arginine units and with terminal amino group of protein chains. If

the nature of the protein remains constant, the amount of dye bound depends on the quantity of protein in the samples. It is, of course, necessary to calibrate separately for different food materials because each material has its own characteristics amino acid pattern. Since lysine can combine through its epsilon-amino group with the dyes to form nutritionally unavailable derivatives, these may result in reduced dye binding by lysine units.

Recently, Peterson and Warthesen (1979) reported the use of high pressure liquid chromatography (HPLC) for determining total and available lysine. They followed the procedure of Carpenter (1960) through the hydrolysis step. The spectrophotometric procedure then requires ether extraction to remove alpha-DNP amino acids and dinitrophenol formed from excess FDNB. In addition, a blank is prepared by reaction of the hydrolysate with methoxycarbonyl chloride followed by ether extraction to remove absorbance due to DNP-lysine. With the HPLC procedure, the filtered hydrolysate containing DNP-lysine was injected directly on to the liquid chromatograph without extensive clean-up. When comparing the HPLC method to an FDNB spectrophotometric method, the latter gave higher results. The difference was attributed to compounds formed during hydrolysis that interfered with the spectrophotometric method. The authors noted that the peak for DNP-lysine was chromatographically separated from dinitrophenol, alpha-DNP amino acids, and other compounds formed

from carbohydrates during acid hydrolysis. In this procedure, total lysine was determined by reacting hydrolysates with dansyl chloride and then separation and quantification of didansyl lysine.

Lipid Extraction

Although lipid research is as old as any branch of organic chemistry, it is only in the 1950's with the work of Folch et al. (1957) and Bligh and Dyer (1959) that satisfactory extraction methods were developed that were applicable to all types of organic tissues. Folch et al. (1957) have successfully used a solvent system of chloroform-methanol 2:1, v/v.

Bligh and Dyer (1959) reported a lipid extraction procedure in which cod fish tissue was blended with chloroform-methanol-water, 1:2:0.8, v/v/v, a monophasic system and then diluted with chloroform and water to a biphasic system. The advantages of this method were that it was quantitative, the lipid components were concentrated in the chloroform layer and the non-lipid contaminants were concentrated in the methanol-water layer. The method stands today with that of Folch et al. (1957) as one of the two standard methods for the extraction of lipids from organic matter (Nelson, 1975).

Palmer (1971) pointed out disadvantages of Bligh and Dyer's method by showing that the acidic phospholipids (phosphatidyl serine, phosphatidyl inositol and phosphatidic

acid) were adsorbed on proteins when the homogenate was diluted to a biphasic system. He showed that low concentration of divalent ions (100 micro equivalents) suppressed the readsorption of phosphatidyl serine and phosphatidic acid, but that higher concentration of divalent ions (500 micro equivalents) were required to suppress readsorption of phosphatidyl inositol. Palmer recommended that the proteins be removed from the homogenate before it was diluted to a biphasic system.

Ostrander and Dugan (1961) who modified the procedure of Bligh and Dyer, helped correct the problems pointed out by Palmer. Their method adds zinc acetate to the monophasic homogenate to aid in precipitating the proteins, and the residue is separated from a monophasic homogenate and reextracted with chloroform. The final filtrate is a biphasic system with the advantages of the original Bligh and Dyer method. The addition of zinc acetate should help suppress readsorption of acidic phospholipids.

Melton et al. (1979) while comparing various methods for lipid extraction from soy products, noted that addition of zinc acetate did help precipitate the soy proteins. In addition, they found that the chloroform-methanol-water 1:1:0.8, v/v/v, ratio in the monophasic homogenate of the Ostrander and Dugan method could be altered to 1:1:0.5 without loss of lipid quantity extracted.

MATERIALS AND METHODS

Raw Materials

Soybeans (*Glycine max, L.*) were provided by the Central Soya Co., Decatur, Indiana. Dehulled sesame seeds (*Sesamum indicum, L.*) were purchased from a local store.

Proximate analyses were conducted on soybeans as well as sesame seeds. These included determination of moisture (AOAC procedure 14.004, 1980), ash (AOAC procedure 7.009, 1980), nitrogen (AOAC procedure 47.021-47.023, 1980) and lipids (Ostrander and Dugan method, 1961). Carbohydrate content was calculated by difference.

Preparation of Soybean Milk

Soybean milk was prepared according to the procedure of Thio (1975) with slight modification. 250 g soybeans were soaked in 1000 ml distilled water for 18 hours to soften the beans. The soaked beans were washed with 1000 ml distilled water and were then transferred into a Waring Blendor. 250 ml distilled water was added and the mixture was blended at high speed for 10 minutes. The slurry was diluted to 2 liters with distilled water and heated at 100^{o}C for 10 minutes. The slurry was filtered through 4 layers of cheese-cloth and the residue was wrapped with the cloth and pressed by a Dick hand-press. The filtrate obtained was a

little less than 2 liters, it was then diluted to volume with distilled water in order to make sure that 2 liters of soybean milk was derived from 250 g soybeans. The soybean milk obtained was mixed with those from other batches in a 20 liter capacity milk tank in order to obtain a homogenous soybean milk. The milk was placed in a refrigerated room to avoid deterioration until it was used to prepare soybean curd although the soybean curd was prepared the same day.

Preparation of Sesame Milk

The preparation of sesame milk was similar to that of soybean milk but without previously soaking the seeds, since the seeds were already soft. In addition, the loss of water soluble substances to the soaking water can be avoided.

Preparation of Soybean Curd and its Modified Forms

A procedure was devised by modification of procedures published by Thio (1975) and Shurtleff and Aoyagi (1975a,b). Modified tofu was made by addition of either sesame milk or ground sesame with a ratio of soybean:sesame of 10:1 (w/w).

To three liters of soybean milk was added 300 ml distilled water or 300 ml sesame milk or 37.5 grams finely ground sesame suspended in 300 ml distilled water. The mixture was heated to 80-85^{o}C with constant stirring. While it was maintained at a temperature of about 80^{o}C, 75 ml of 10% calcium chloride solution (calculated as anhydrous $CaCl_2$) were added and the mixture gently stirred until a good

coagulum formed. It was then filtered through a cheese-cloth using a suitable tofu mold. The cloth was folded over the filter cake, a weight (1.5 kg per 200 cm^2) placed on it to squeeze out the whey and allowed to stand for 30 minutes. The soybean curd thus obtained was cut, soaked in cooled distilled water, and kept in a refrigerator at 1.7°C for 30 minutes. The curd was then sliced in pieces 2-3 mm thick which were then frozen in a freezer or directly air-dried.

Air-Dried Soybean Curds

The freshly sliced soybean curds were spread on perforated stainless steel trays and placed in a cabinet dryer (Proctor & Schwartz Inc., Philadelphia). Drying was performed with air circulation at 60°C until the material became crisp. This required 6-7 hours.

Dried-Frozen Soybean Curds

The freshly sliced soybean curds were frozen in a freezer, and were allowed to age in a frozen state for 2 weeks. They then were thawed and spread on perforated stainless steel trays. Dehydration was conducted in the cabinet dryer at a temperature of 60°C for 3-4 hours at which time the material became crisp.

Freeze-Dried Soybean Curds

The freshly sliced soybean curds were first frozen for 24 hours. Freeze drying was conducted in a RePP Sublimator (RePP Industries, Inc., Gardiner, N.Y.) with glycol setting

at 26.7°C and refrigeration setting at -51.1°C. The time required for freeze drying at this condition was 48 hours.

Dried Soybean Curd Powder

The dried soybean curd obtained from these three methods of treatment and drying were converted into powder with the aid of a high-speed mechanical blendor. In order to get a homogenous sample for each dried product, the powder derived from one batch was mixed with those from other batches of similar treatment in a Waring Blendor.

Proximate Analyses of Soybean and Sesame Products

Proximate analyses were made on soybean milk, sesame milk, and dried curds. These included the determination of moisture, ash, nitrogen and lipids. The method used were the same as those for raw soybeans and sesame seeds.

Sorption Isotherm Determination

The sorption isotherm was determined at 37°C by exposure under vacuum to water-vapor over constant humidity salt solutions (Bull, 1944; Karel and Nickerson, 1964; Mizrahi and Karel, 1977). All samples for the adsorption isotherm were stored in an evacuated desiccator containing Drierite ($CaSO_4$) for one month at room temperature and the moisture content was then determined.

The sample was removed from the desiccator, and 4-gram portions were spread out in evaporation dishes. Duplicate samples were placed in each of a series of vacuum desiccators

equilibrated to different relative humidity levels. The relative humidity levels were achieved by employing saturated salt solutions under the desiccator plate according to Rockland (1960) and Heidelbaugh and Karel (1975). The desiccators were closed, thoroughly evacuated and placed in a constant temperature chamber (cubicle) at 37°C. They were allowed to equilibrate, as judged by constant weight in subsequent weighing. The final moisture content of the product was established by taking the weight gained, and adding it to the weight of water in the sample before storage was initiated.

The desorption isotherm was derived in a similar manner; however these samples were first humidified and then placed under the same relative humidities but, in this case, the loss in weight was measured.

Storage Conditions

All samples were stored in open vials. Four grams of sample were placed in each vial and these were placed in desiccators containing saturated NaCl which have a_w of 0.75. The desiccators were evacuated and placed in a constant temperature chamber at 37°C for two days to allow equilibration with the constant humidity solution. The desiccators were then filled with air and were further stored in the chamber in the dark for 8 weeks. Samples were taken for analysis at intervals.

Effect of Temperature

Dried soybean curds were used to study the effect of temperature. They were treated in a similar manner, however, the desiccators containing these samples were stored in an oven at a temperature of 50°C and 0.75 a_w.

Effect of Water Activity

Dried soybean curds with added sesame milk were used to study the effect of water activity. They were also treated in a similar manner, but in this case, they were placed in a series of desiccators equilibrated to different relative humidity and stored at 37°C.

Lipid Extraction

Lipids were extracted according to the method of Bligh and Dyer (1959) as modified by Ostrander and Dugan (1961). Each 5 gram sample was macerated with 49.5 ml distilled water for 2 minutes in a Waring Blendor at high speed with voltage regulator set at 70-80 volts. To the sample was then added 130 ml of absolute methanol and an approximate 1-inch cube of dry ice. The mixture was blended for 5 minutes at high speed. To the mixture, 65 ml chloroform were added and blended at high speed for 5 minutes, followed by addition of 65 ml chloroform again and the mixture was blended for 30 seconds. Added were 65 ml distilled water and 1 g zinc acetate. The mixture was blended for 10 seconds. The homogenate was filtered through Whatman No. 1 filter paper on a

Buchner funnel under suction. A stream of nitrogen gas was directed over the content of the funnel to minimize oxidation during filtration.

The residue was removed and the funnel was wiped with a half piece of facial tissue. The residue, filter paper and tissue were reblended with 100 ml of chloroform for 2.5 minutes. The mixture was then filtered as above using 50 ml of chloroform for rinsing. The filtrate was poured into a 500 ml graduated cylinder and 10 ml of saturated sodium chloride solution was added if an emulsion formed. After allowing a few minutes for complete separation and clarification, the volume of the chloroform layer was recorded. The chloroform layer was separated from the alcoholic layer by a separatory funnel. An aliquot was taken to determine the percentage of fat by evaporation to dryness. The solvent was removed in a flash evaporator and the evaporated samples were stored under nitrogen at -20^{o}C.

Preparation of Methyl Esters

Methyl esters were prepared by the procedure of Morrison and Smith (1964). Approximately 30-50 mg of oil were weighed in a centrifuge tube provided with a Teflon-lines screw cap. Four ml of boron fluoride-methanol reagent were added under nitrogen and the tube closed with the screw cap. Boron fluoride-methanol reagent was prepared by mixing 25% BF_3 (140 g BF_3 per liter of methanol), 20% benzene and 55% methanol. The tube was then heated in a boiling water bath

for 30 minutes, cooled and opened. The esters were extracted by adding 2 volumes of pentane, then 1 volume of water, shaking briefly, and centrifuging until both layers were clear. After separation and drying of the pentane layer with anhydrous sodium sulfate, the pentane was driven off and the samples redissolved in petroleum ether.

Gas Liquid Chromatographic Analysis of Methyl Esters

The chromatographic analysis of fatty acid methyl esters was performed using a Hewlett Packard 5830A Gas Chromatograph equipped with a hydrogen flame detector. A coiled glass column 6 feet long and 1/4 inch inside diameter packed with 15% (w/w) DEGS on 80/100 mesh Chromosorb W. (Supelco Inc.) was used for methyl ester separation.

The column oven temperature was 175^{o}C, the injection temperature was maintained at 170^{o}C and the detector at 180^{o}C. The nitrogen carrier gas was adjusted to 50 ml per minute. The flow rate of hydrogen and oxygen were 45 ml per minute and 190 ml per minute respectively. The emerging peaks were identified by comparing retention time for each to those of a standard mixture of known fatty acid methyl esters (Supelco Inc.). Peak areas were integrated by a 18850A GC Terminal Hewlett Packard integrator and the percentage of total fatty acids were determined.

Diene Conjugation

About ten mg of oil in duplicate were weighed accurately into 10 ml volumetric flasks. Spectro-grade isooctane

(2,2,4-trimethylpentane) was added to the samples which were shaken vigorously to assure complete dilution and then diluted to volume. Conjugated diene absorption was measured at 233 nm using isooctane as a blank. If the absorption was too high, dilution was accomplished by taking 1 ml of sample solution and mixing with 9 ml of pure isooctane in order to bring the absorbance within the proper limites. Diene conjugation was calculated at a dilution of 1:1000 (w/v).

Determination of Peroxides

Peroxides in the extracted oils were measured by the iodometric method of Wheeler (1932). About 0.5 g oils in duplicate were weighed and transferred into Erlenmeyer flasks where the samples were dissolved in 30 ml of glacial acetic acid - chloroform solution (3/2, v/v). Then 0.5 ml of saturated potassium iodide solution was added and the flasks swirled to provide mixing. The flasks were kept in the dark for 10 minutes after which 30 ml of distilled water was added and the liberated iodine was titrated with standardized sodium thiosulfate solution. Starch indicator (1%, w/v) was added when near the end point. The solution was titrated to disappearance of the blue color. The peroxide content was determined as meq/kg of oil.

Thiobarbituric Acid (TBA) Test

The TBA test was performed by the method of Sidwell et al. (1954) as modified by Heidelbaugh and Karel (1970).

A 2 g sample of soybean curd in duplicate was weighed out in a tared 12-ml centrifuge tube and 4 ml of distilled water was added. The mixture was stirred by a Fisher mini-shaker and allowed to stand for 10 minutes. Added was 4 ml of trichloroacetic acid (TCA), prepared by dissolving 10 g of TCA in distilled water and bringing to 100 ml. The tubes were centrifuged at 2000 rpm for 10 minutes.

Next, 4 ml of the supernatant was placed in a test tube and reacted with 1.25 ml of 0.75% TBA for 30 minutes at 100°C. The 0.75% TBA was made by dissolving 0.75 g of TBA in 75 ml of glacial acetic acid with 2 ml of concentrated HCl, heating until dissolved, and making up to 100 ml with glacial acetic acid. Absorbance of the resulting solution was read at 530 nm on a Beckman DU spectrophotometer. The blank was 2 ml distilled water carried through the procedure in place of 2 grams of sample.

Determination of Tocopherols by HPLC

The method described by Carpenter (1979) was used to determine alpha- and gamma- tocopherols present in oils extracted from soybeans, sesame seeds and dried curds. One gram of oil was weighed into a 10 ml volumetric flask. The sample was brought to volume with 1.5% isopropyl alcohol in HPLC grade hexane. The solution was filtered through a 0.45 µm Gelman Millipore filter just before injection. The injection volumes were 40-50 µl.

The liquid chromatograph was assembled from a Waters Associates Model-6000A pump, a U6K Injection Port, a Waters Model 440 Absorbance Detector and a Recorder (Linear Instruments Corp.). The chromatographic separation was performed on a Whatman Partisil PXS 10/50 column No. 2B, with a mobile phase of 1.5% isopropyl alcohol in hexane at a flow of 2 ml per minute and a pressure of 600 psi. The UV detector was set at 280 nm.

For peak identification and quantitation (by peak height), alpha- and gamma-tocopherols (Sigma Chemical Co.) in 1.5% isopropyl alcohol in hexane at concentration 0.01% (w/v), were used as standards. With a chart speed of 16 inches per hour, alpha- and gamma-tocopherols were eluted in 5.3 and 7.1 minutes respectively. A straight line relationship between peak height and concentration of each standard was obtained.

Determination of Sugars by HPLC

Sugars were extracted according to the method of Black and Bagley (1978) with a slight modification. The samples were first defatted by petroleum ether extraction in a Soxhlet extractor for 24 hours. The fat-free residue was then allowed to stand under a hood for about 1-2 hours and was dried over Drierite in a desiccator overnight.

About 4-5 g defatted sample in duplicate was weighed and transferred quantitatively into a centrifuge bottle. The sample was thoroughly mixed with 20 ml of ethanol-water

(80:20, v/v). The sample was held in a water bath at 80°C for 30 minutes with frequent stirring and then centrifuged with a centrifuge (International Equipment Co.) at 2000 rpm for 10 minutes. The extraction was repeated three more times, each time combining the extracts in another centrifuge bottle.

The combined extract was deproteinized with 2 ml of 10% lead acetate and centrifuged. The precipitate was washed with 10 ml of the ethanol solution and recentrifuged. The supernatants were combined. The extract was concentrated to ca 10 ml on a rotary evaporator. Excess lead was precipitated with 10% oxalic acid until the extract was free of lead. The extract was then centrifuged to remove lead oxalate, and the clear extract was quantitatively transferred into a 25 ml volumetric flask and brought to volume with deionized water. The solution was then passed through a Florisil cartridge Sep-Pak (Waters Associates, Inc.) to remove traces of lipids. Just before the injection onto the column, it was filtered through a 0.45 μm Gelman Metricel membrane filter.

The liquid chromatograph system was assembled from a Waters model M-45 Delivery System, a U6K Injection Part, a Waters model R401 Differential Refractometer and an Aminex Carbohydrate HPX87 column (Bio-Rad Laboratories). The column had a dimension of 30 cm long and 7.8 mm inside diameter. The column was jacketed with hot water which was automatically

supplied from and circulated back to a controlled-temperature water-bath (Precision Scientific Corp.).

The mobile phase was degassed, deionized water supplied from a reservoir which was constantly heated to a temperature of 50°C. Separation of sugars was accomplished in the column at 80°C as controlled by the water bath. With a flow rate of 0.6 ml per minute and a pressure about 600 psi, stachyose, raffinose, sucrose and glucose were eluted in 6.8, 7.4, 8.4 and 10.2 minutes respectively. Volumes injected ranged from 5-10 µl. For peak identification and quantitation by peak height, glucose, sucrose, raffinose and stachyose (all from Supelco, Inc.) were used as external standards. The standards were prepared in aqueous solution containing 2-3 mg sugar per ml. A straight-line relationship between peak height and quantity of each sugar was obtained.

Non-Enzymatic Browning Pigments

The brown pigments were measured by the method of Choi et al. (1949) as modified by Karel and Labuza (1968). Two gram samples in duplicate were dispersed in 20 ml of water, and 2.5 ml of a 10% freshly prepared suspension of trypsin were added. After one hour of incubation at 45°C, 2 ml of 50% trichloracetic acid and 0.1 g of Celite filter aid were added. After mixing and filtration, the absorbance at 400 nm was measured on the clear solution, with the enzyme blank set at 100% transmittance. The results were reported as (absorbance per gram solids) x 100.

Color Analysis

Product color changes were monitored on a Hunter tristimulus colorimeter model D25-2 (Hunter Associates Laboratories Inc.). A 12.0 g sample of soybean curd powder was analyzed and the yellow plate which has L (lightness) value of 78.2, a (redness or greenness) value of -1.9 and b (yellowness or blueness) value of 25 was used as a standard.

Nitrogen Determination by Micro-Kjeldahl

Nitrogen was determined by the Micro-Kjeldahl method according to AOAC 47.021-47.023 (1980). Samples containing approximately 15 mg protein were digested for 2 hours in duplicate. Two ml sulfuric acid of 1.84 specific gravity were used for digestion and 1.9 g potassium sulfate and 40 mg HgO were added to increase the boiling point and as catalyst respectively. After cooling the flasks, the sides were rinsed with deionized water and the digestion was continued for an additional hour. After cooling, the digest was quantitatively transferred into a Micro-Kjeldahl distillation apparatus by using deionized water for rinsing. The digestion mixture was neutralized with 15 ml NaOH-$Na_2S_2O_3$ solution. The solution was prepared by dissolving 60 g NaOH and 5 g $Na_2S_2O_3 \cdot 5H_2O$ in deionized water and was diluted to 100 ml. The released ammonia was steam-distilled into 5 ml of saturated boric acid solution containing 4 drops of methyl red - methylene blue indicator mixture. The mixture was prepared by mixing 2 parts of 0.2% alcoholic methyl red solution with

1 part 0.2% alcoholic methylene blue solution. The distillation was continued until the volume of the receiving flask reached 50 ml. The ammonium-borate complex was titrated with standarized 0.02 N HCl. A blank determination was conducted simultaneously with that for the sample.

Available Lysine Determination by HPLC

The available lysine was determined by the method of Peterson and Warthesen (1979). Approximately 500 mg sample were accurately weighed and placed in a 250 ml boiling flask. Boiling chips along with 12.5 ml of 8% sodium bicarbonate in water were added. After the sample was wetted by the sodium bicarbonate solution, 15 ml of an ethanol solution containing 0.4 ml of 1-fluoro-2,4-dinitrobenzene (Eastman Kodak Co.) was added. The sample was shaken for 4 hours at room temperature by a Burrell wrist action shaker (Burrell Corp.) and the ethanol was then evaporated in a rotary evaporator until a weight loss of 12.5 g was obtained, by weighing the flask before and after evaporation. If the weight loss was more than 12.5 g distilled water was added while when the weight loss was still less than 12.5 g the evaporation was continued.

Thirty ml of 8.1 N HCl were added and the sample was refluxed for 16 hours. After hydrolysis, the sample was filtered through a 0.2 μm membrane filter (Gelman Metricel). Separation and quantitation of DNP-lysine was then accomplished by HPLC.

The liquid chromatograph used in this study consisted of a Waters Associates Model-6000A pump, a U6K Injection Port and a Water Model 440 Absorbance detector fitted for determination of wavelength of 436 or 254 nm. The detector output was recorded on a Linear Instrument Corp. recorder.

The wavelength used to detect DNP-lysine was 436 nm. The separation was accomplished on a μBondapak C_{18} column (3.9 mm id x 30 cm, Water Associates) with a mobile phase of 20% nanograde acetonitrile (Mallinckrodt) and 80% 0.01 M acetate buffer, pH 4.0.

With a flow rate of 2.0 ml per minute, DNP-lysine eluted in 13-14 minutes. The usual volume injected was 40 μl. For peak identification and quantitation, DNP-lysine HCl (Sigma Chemical Co.) was used as an external standard and at least four injections of different volumes were made each day. A 2,4-dinitrophenol standard was also used to identify it together with the NDP-lysine. The 2,4-dinitrophenol was eluted in 10 minutes.

Amino Acid Analysis

Amino acid analyses were performed on HCl hydrolyzates of protein using a Durrum Amino Acid/Peptide Analyzer. Fat-free samples consisting of approximately 10 mg of protein were weighed in duplicate into hydrolysis tubes provided with a Teflon-lined screw cap. Two ml of 1 mM norleucine was added as internal standard. Ten ml of 6 N HCl and 2 ml 12 N HCl were then added to each sample. A stream of nitrogen

gas was run gently into each tube for 30 seconds. The tube was capped quickly and fastened tightly. The tubes were then autoclaved for 16 hours.

After hydrolysis, the tubes were opened and the hydrolysate was filtered through a Whatman No. 2 filter paper. The residue was washed well but the volume should be kept small. A special deionized water which had been passed through five deionizer columns, was used throughout the analysis. The filtrate was evaporated just to dryness using a rotary evaporator with a 55^{o}C water bath. Ten ml of deionized water was added to dissolve the residue and it was then evaporated to dryness again. This step was repeated one more time.

The residue was dissolved by addition of 4 ml 0.01 N HCl, and the solution was then transferred carefully to a small vial and it was frozen in a freezer. Later, after thawing, the solution was diluted (1:4, v/v). The solution was filtered through a 0.20 μm Gelman Metricel filter and the filtrate obtained was ready for injection to the amino acid analyzer.

Determination of Tryptophan

Tryptophan is very labile during acid hydrolysis, therefore, it was determined colorimetrically after hydrolysis with the enzyme pronase according to the procedure W. described by Spies (1967). The pronase hydrolytic solution contained 10 mg of pronase (Sigma Chemical Co.) per ml of

0.1 M phosphate buffer, pH 7.5. The solution was prepared on the day it was used. The pronase suspension was shaken gently for 15 minutes and clarified by centrifugation.

A 10 mg fat-free sample in duplicate was weighed directly into a 2 ml glass vial with a screw cap. To the sample were added 100 µl of pronase solution and one drop of toluene to act as a preservative. The vials were stoppered and incubated for 24 hours at 40°C. After incubation, 0.9 ml of 0.1 M phosphate buffer, pH 7.5 were added to each vial. The uncapped vials were placed into 50 ml Erlenmeyer flasks containing 9.0 ml of 21.2 N sulfuric acid and 30 mg of dimethylaminobenzaldehyde (DAB) (Sigma Chemical Co.). The vials were tipped over and the contents were mixed quickly by swirling. The flasks were cooled to room temperature and placed in the dark at 25°C for 6 hours. To each flask was then added 0.1 ml of 0.045% sodium nitrite. After 30 minutes, absorbances were read at 590 nm using a Beckman DU spectrophotometer. The blank solution contained all components but protein and pronase.

Duplicate samples of the pronase hydrolytic solution, without protein, were treated and analyzed as described above. The tryptophan content of the pronase solution was subtracted from the total tryptophan.

A standard curve from zero to 120 µg tryptophan was prepared according to the procedure E. described by Spies and Chamber (1948). 2.4 mg tryptophan were dissolved in 20 ml

21.2 N sulfuric acid containing 60 mg of DAB. 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of this solution were made up to 10 ml with solution of 21.2 N sulfuric acid containing 3 g DAB per liter, and placed in 50 ml Erlenmeyer flasks. The mixture was kept in the dark at 25^{o}C for 6 hours. To each flask was then added 0.1 ml 0.045% sodium nitrite. After 30 minutes, absorbances were measured at 590 nm, using a Beckman DU spectrophotometer. A straight-line relationship was obtained between absorbance and the tryptophan content.

RESULTS AND DISCUSSION

Proximate Analyses

The chemical composition of plant products usually varies somewhat with different varieties, geographical locations, and climatic conditions. The proximate analyses of soybeans, sesame seeds and their products are shown in Table 1. As can be seen in the table, the protein content of soybean is 42.54% and is almost twice the amount of the lipids. Chang and Murray (1949) reported the composition of soybean in % dry matter to be 43.7% protein, 22.2% fat, 4.9% ash and 29.9% carbohydrate. Other data (Horan, 1974) gave 40.4% protein, 22.3% crude fat, 4.9% ash, 31.9% N-free extract and fiber. Thus, the composition of soybeans used in this study was in close agreement to values in the literature.

Sesame seeds are different from soybean in that they contain 56.2% lipid and the protein content is less than one half that of the lipid. Krishnamurthy et al. (1959) reported the chemical composition of white sesame seed in % dry basis as 22.0% protein, 60.5% fat, 3.02% fiber, 1.12% Ca, 0.50% phosphorus and 1.8% oxalic acid. In the present study, small differences from values in the literature especially in the lipid content might be due to the difference in growth

Table 1. Proximate analyses of soybeans, sesame seeds and soybean curd products.

Sample	Moisture %	Total Solid %	Protein (Nx6.25) % TS	Lipid % TS	Ash % TS	Carbohydrate (by difference) % TS
1. Soybean	9.70	90.30	42.54	21.96	4.58	30.92
2. Sesame seeds	7.81	92.19	23.44	56.20	2.83	17.53
3. Soybean milk	93.67	6.3	49.12	22.75	4.80	23.33
4. Sesame milk	97.70	2.30	25.80	57.49	2.15	14.56
5. Soybean curd	81.40	18.60	59.18	30.24	2.69	7.89
6. Soybean curd with added sesame milk	80.80	19.20	58.62	31.18	2.71	7.49
7. Soybean curd with added ground sesame	81.20	18.80	52.81	34.38	2.86	9.95

% TS = % Total Solids.

conditions or varieties of sesame seeds.

The chemical composition of soybean milk and soybean curd vary greatly. Miller et al. (1952) analyzed soybean milk and soybean curd obtained from three different factories in Hawaii. A great variation in composition was obtained. Chang and Murray (1949) reported the composition of soybean curd to be 63.7% protein, 30.0% fat, 3.6% ash and 2.7% carbohydrate calculated as % dry basis. Shurpalekar et al. (1961) obtained different values viz., 46.7% protein, 27.3% oil and 20.0% carbohydrate, expressed as % dry matter. The differences reported in the literature may be due either to raw material or processing factors. The raw material factor may be caused by differences in soybean varieties and growth conditions. The processing factor may involve extraction, ratio of beans to water, temperature and length of heating time, kind and amount of coagulant used.

The composition of soybean milk, sesame milk, soybean curd and its modified forms are presented in Table 1. Since it is difficult to extract the lipid directly from these products, especially the milk, the products were first freeze-dried. Determination of protein, lipid, moisture and ash were then accomplished on the freeze-dried products.

The composition of dried soybean curds obtained from different methods of drying are presented in Table 2. As can be seen in the table, the method of drying did not seem to have a significant effect on the quantity of the protein,

Table 2. Proximate analyses of dried soybean curds.

Sample	Drying method	Constituent (% dry matter)			
		Protein (Nx6.25)	Lipid	Ash	Carbohydrate (by difference)
I. Soybean curd	AD	59.30[a]	29.76[a]	2.75[a]	8.19[ab]
	DF	58.58[a]	31.19[a]	2.62[a]	7.71[a]
	FD	59.18[a]	30.24[a]	2.69[a]	7.89[ab]
II. Soybean curd with added sesame milk	AD	58.90[a]	30.85[a]	2.79[a]	7.46[a]
	DF	58.04[a]	31.55[a]	2.73[a]	7.68[a]
	FD	58.62[a]	31.18[a]	2.71[a]	7.49[a]
III. Soybean curd with added ground sesame	AD	52.56[b]	34.43[b]	2.78[a]	10.23[cd]
	DF	52.19[b]	34.61[b]	2.90[a]	10.30[d]
	FD	52.81[b]	34.38[b]	2.86[a]	9.95[bcd]

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

[a]All numbers in the same column sharing the same superscript are not significantly different at 5% level.

fat, ash and carbohydrate present in the products. However, with respect to quality, significant differences were noted in properties such as appearance, color, and ability of these products to be reconstituted as checked by qualitative tests. In these regards, freeze drying produced the best quality product.

Fatty Acid Composition of Oils

Fatty acid composition of oils extracted from soybeans, sesame seeds and their products was determined by gas liquid chromatography. The results are presented in Table 3. The ranges of values in percentage for fatty acid composition of soybean oil tentatively adopted by the Food and Agriculture Organization/WHO Codex Alimentarius Committee on Fats and Oils is palmitic (16:0) 7.0-12.0, stearic (18:0) 2.0-5.5, oleic (18:1) 19-30, linoleic (18:2) 48-58, and linolenic (18:3) 4-10 (Spencer et al., 1976). Thus the fatty acid composition of soybean oil extracted here is within the range of values reported in the literature.

As shown in Table 3, the major fatty acids present in sesame seed oil are oleic and linoleic which occur in almost equal amounts. The content of linolenic acids is only 0.61%. The Codex ranges in percentage for sesame oil are palmitic 7-12, stearic 3.5-6, oleic 35-50, linoleic 35-50, linolenic less than 1, and arachidic also less than 1. Therefore, the contents of most of the fatty acids fall within the Codex ranges.

Table 3. Fatty acid composition of extracted lipids.

Sample	Drying method*	[a]Fatty Acid (%)					
		16:0	18:0	18:1	18:2	18:3	20:0
1. Soybean	-	10.93	4.78	22.78	52.51	9.00	-
2. Sesame	-	10.67	6.25	39.16	43.10	0.61	0.20
3. Soybean milk	-	11.08	5.00	23.79	51.27	8.86	-
4. Sesame milk	-	10.80	6.20	40.23	41.90	0.51	0.30
5. Soybean curd	AD	12.85	4.83	20.92	52.78	8.23	-
	DF	12.46	4.65	21.10	53.16	8.30	-
	FD	11.93	4.50	21.26	53.32	8.56	-
6. Soybean curd with	AD	11.97	5.08	24.20	51.40	6.70	-
added sesame milk	DF	11.93	4.57	23.93	52.27	7.06	-
	FD	11.40	4.89	23.86	52.56	6.91	-
7. Soybean curd with	AD	11.51	4.71	24.87	52.54	6.04	-
added ground sesame	DF	11.43	4.70	24.48	52.54	6.23	-
	FD	11.45	4.59	24.88	52.65	6.18	-

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

[a]The notation used to describe fatty acids is number of carbon atom:number of double bonds.

It was evident that the relative percentage of saturated fatty acids of soybeans was lower than that of soybean milk which in turn was lower than that of soybean curds. It was noted that oxidation of unsaturated fatty acids occurred during the processing of soybean curd. However, the extent of oxidation was small since the dried soybean curds still contain high percentages of unsaturated fatty acids. It was noted that modification of soybean curd by addition of either sesame milk or ground sesame did alter the fatty acid composition. This was especially true for soybean curd with added ground sesame.

Tocopherols in Soybean Curds

Tocopherols provide some antioxidant activity in vegetable oils in decreasing order from delta, gamma to alpha respectively, but this order may be influenced by temperature and light conditions (Sherwin, 1976).

Table 4 shows the tocopherol content of oils extracted from soybeans, sesame seeds and dried soybean curds. Carpenter (1979) reported that soybean oils contain 39-70 µg/g alpha-, 600-924 µg/g gamma-, 235-370 µg/g delta-tocopherols. As shown in Table 4, the oil extracted from soybean had 46 µg/g alpha- and 507 µg/g gamma-tocopherols. However, neither alpha- nor gamma-tocopherols were present in sesame oil.

It was noted that alpha-tocopherol survived better than gamma-tocopherol during soybean curd processing. Vitamin E

Table 4. Content of alpha- and gamma-tocopherols in oil extracted from soybeans, sesame seed and dried soybean curds.

Sample	Drying method*	Tocopherols (μg/g oil)	
		alpha	gamma
1. Soybeans	-	46	507
2. Sesame seed	-	0	0
3. Soybean curd	AD	20^{a}	59^{a}
	DF	21^{a}	63^{a}
	FD	31^{b}	84^{b}
4. Soybean curd with added sesame milk	AD	32^{b}	76^{b}
	DF	34^{bc}	80^{b}
	FD	41^{cd}	84^{b}
5. Soybean curd with added ground sesame	AD	39^{bcd}	77^{b}
	DF	43^{d}	83^{b}
	FD	45^{d}	85^{b}

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

[a]All numbers in the same column sharing the same superscript are not significantly different at 5% level.

activity is attributed mainly to alpha-tocopherol (Sherwin, 1976). Since alpha-tocopherol in soybean curd is present in very small amounts, it may contribute little to nutritional value.

Cort (1974) indicated that alpha- and gamma-tocopherols at a concentration of 0.02% had low antioxidant activity in stripped soybean oils. Based on this, the presence of tocopherols in soybean curds may not provide much antioxidant activity.

Sorption Isotherm

The sorption isotherm of dried soybean curds and dried soybean curds with added sesame milk or ground sesame at 37°C are presented in Table 5. Figure 1 shows the water sorption isotherm for air-dried soybean curd. It is noted that the values determined by the desorption procedure do not coincide with the values generated by an adsorption procedure for attaining equilibrium moisture content. This phenomenon will be discussed later in the section regarding hysteresis. The BET monolayer value can be calculated from the BET transformation equation:

$$\frac{a_w}{(1-a_w)V} = \frac{1}{V_m C} + \frac{(C-1)a_w}{V_m C}$$

in which

a_w = water activity

V = moisture content (cc/g solids or g/g solids)

Table 5. Sorption isotherm of dried soybean curds at 37°C.

Sample	Drying Method*	Desorpt. Adsorpt.**	Moisture (g/100 g solids) at a_w of					
			0.11	0.22	0.32	0.43	0.53	0.62
I. Soybean curd	AD	D	3.46^{a}	4.68^{abcd}	5.95^{a}	6.86^{b}	7.49^{bcd}	8.42^{a}
		A	2.92^{a}	3.85^{a}	4.72^{a}	5.41	6.34^{d}	7.64^{a}
	DF	D	3.47^{a}	4.70^{bcd}	6.00^{a}	6.56^{a}	7.54^{cd}	8.47^{a}
		A	3.26^{cd}	3.92^{a}	5.12^{ab}	5.60^{a}	6.93^{b}	7.88^{bc}
	FD	D	3.50^{a}	4.56^{a}	5.90^{a}	6.62^{a}	7.52^{cd}	8.37^{a}
		A	3.35^{d}	4.35^{e}	5.34^{c}	5.83^{b}	7.04^{bc}	7.93^{cde}
II. Soybean curd with added sesame milk	AD	D	3.52^{a}	4.75^{cd}	6.00^{a}	6.85^{b}	7.49^{bcd}	8.35^{a}
		A	3.04^{a}	4.12^{c}	5.01^{a}	5.69^{ab}	6.57^{a}	7.70^{ab}
	DF	D	3.45^{a}	4.83^{e}	5.95^{a}	6.89^{b}	7.30^{a}	8.28^{a}
		A	3.25^{c}	4.34^{de}	5.40^{cd}	6.22^{de}	6.93^{b}	7.97^{de}
	FD	D	3.52^{a}	4.80^{de}	5.90^{a}	6.80^{b}	7.32^{a}	8.27^{a}
		D	3.39^{d}	4.58^{ab}	5.47^{cd}	6.32^{e}	6.95^{b}	7.99^{de}
III. Soybean curd with added ground sesame	AD	D	3.47^{a}	4.58^{ab}	6.14^{a}	6.83^{b}	7.44^{bcd}	8.34^{a}
		A	3.18^{bc}	4.06^{bc}	5.17^{b}	5.72^{ab}	6.65^{a}	7.78^{ab}
	DF	D	3.39^{a}	4.61^{ab}	6.06^{a}	6.80^{b}	7.39^{ab}	8.30^{a}
		A	3.25^{c}	4.18^{cd}	$5.5.^{de}$	5.99^{c}	7.04^{bc}	8.00^{de}
	FD	D	3.50^{a}	4.66^{abc}	6.16^{a}	6.87^{b}	7.41^{ab}	8.32^{a}
		A	3.37^{d}	4.36^{e}	5.63^{e}	6.12^{cd}	7.15^{c}	8.07^{e}

* AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

** Desort. (D) = desorption Adsorpt. (A) = adsorption

[a] Moisture values obtained by a desorption process at the same a_w sharing the same superscript are not significantly different at 5% level. Moisture values obtained by adsorption process at the same aw sharing the same superscript are not significantly different at 5% level.

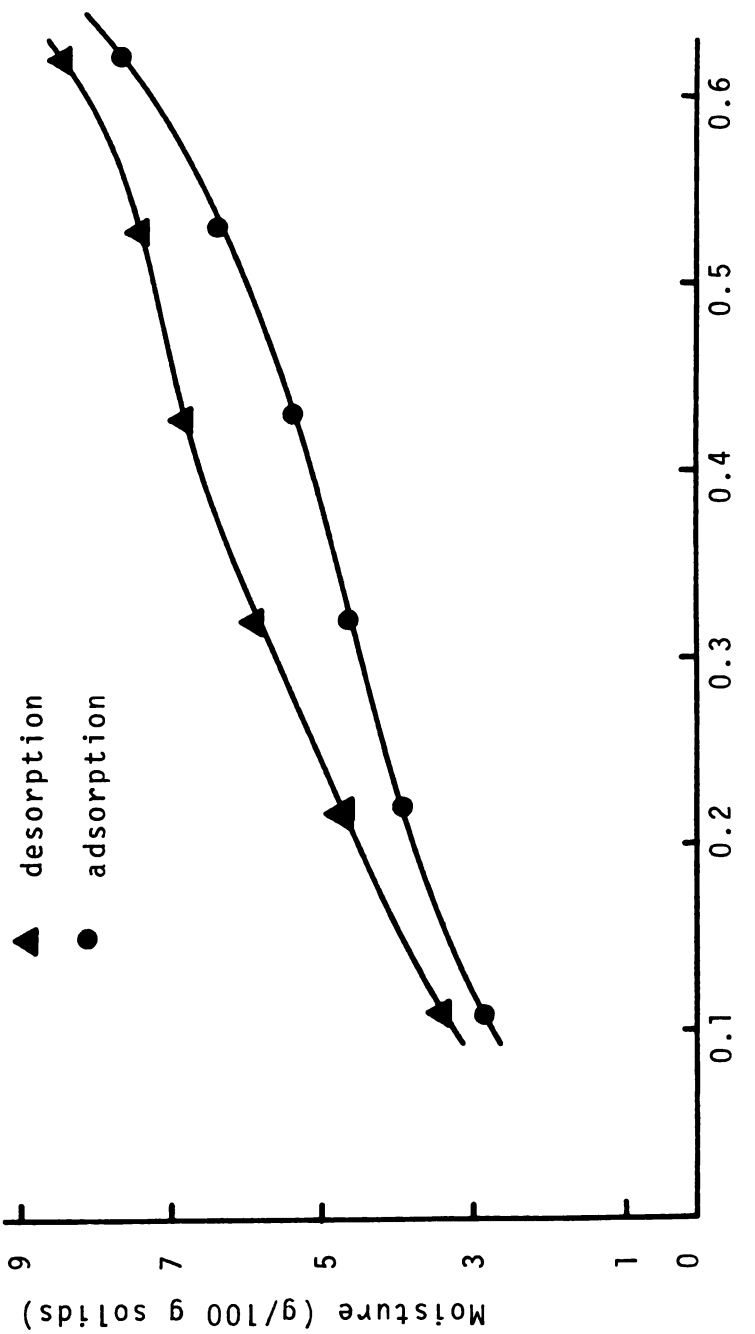

Figure 1. Sorption isotherm for air-dried soybean curd at 37°C.

V_m = monolayer value (cc/g solids or g/g solids)

C = constant related to heat of sorption

The BET equation can only be applied with the following assumptions (Labuza, 1968):

"(a) Q_1, the heat of sorption of first layer, is constant and equal to the total ΔH_v (heat of vaporization) plus a constant heat due to site interaction; (b) Q_1 for all layers above a monolayer is equal to ΔH_v, the heat of vaporization; (c) sorption occurs only on specific sites."

A plot of $a_w/(1-a_w)$ V versus a_w gives a straight line as displayed in Figure 2 for air-dried soybean curd. Since the assumptions are not entirely true for most materials, the BET isotherm holds only between water activities from 0.1 to about 0.5. From the slope and intercept of the line, the monolayer coverage can be calculated. The slope values were calculated to be 22.83 for desorption and 28.27 for adsorption. The intercepts were 0.90 and 0.95 respectively. The monolayer values were 4.21% moisture (dry basis) for the desorption process and 3.42% moisture for the adsorption process. The water activities which correspond to these monolayer values are 0.18 for desorption and 0.17 for adsorption.

A similar method was applied to the sorption data of other samples. The results are exhibited in Table 6 which shows the BET equations, the monolayer values and their corresponding water activities for nine samples of soybean

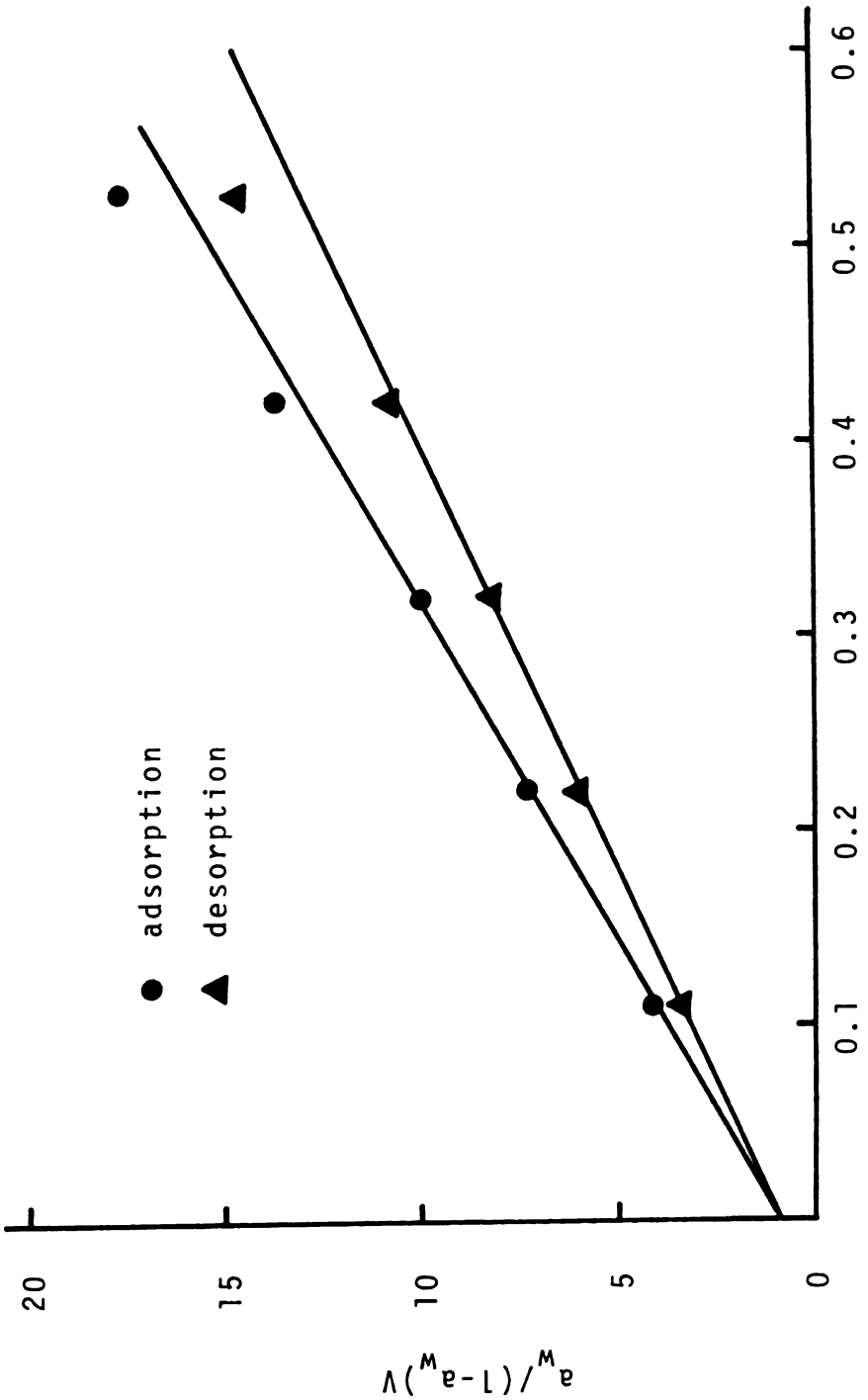

Figure 2. BET transformation for air-dried soybean curd at 37°C.

Table 6. BET equations and monolayer values of soybean curds.

Sample	Drying method*	Desorption/ Adsorption**	BET equation[a]	Monolayer value[b]	
				V_m	a_w
I. Soybean curd	AD	D	Y = 0.90 + 22.83 X	0.0421	0.18
		A	Y = 0.95 + 28.27 X	0.0342	0.17
	DF	D	Y = 0.70 + 22.60 X	0.0429	0.18
		A	Y = 0.70 + 27.40 X	0.0356	0.16
	FD	D	Y = 0.72 + 23.79 X	0.0408	0.17
		A	Y = 0.75 + 25.83 X	0.0376	0.16
II. Soybean curd with added sesame milk	AD	D	Y = 0.80 + 22.04 X	0.0438	0.19
		A	Y = 0.80 + 27.67 X	0.0351	0.16
	DF	D	Y = 0.85 + 22.82 X	0.0422	0.17
		A	Y = 0.85 + 25.67 X	0.0377	0.16
	FD	D	Y = 0.78 + 23.45 X	0.0413	0.17
		A	Y = 0.80 + 25.11 X	0.0386	0.16
III. Soybean curd with added ground sesame	AD	D	Y = 0.99 + 22.77 X	0.0421	0.18
		A	Y = 1.00 + 27.02 X	0.0357	0.16
	DF	D	Y = 1.20 + 20.34 X	0.0464	0.22
		A	Y = 1.20 + 26.04 X	0.0367	0.16
	FD	D	Y = 0.90 + 22.64 X	0.0425	0.18
		A	Y = 0.94 + 25.83 X	0.0374	0.16

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

**D = desorption A = adsorption

[a] $Y = a_w/(1 - a_w)\ V$ $X = a_w$, water activity V = moisture content (g/g solids)

[b] V_m = moisture content at monolayer (g/g solids)

curd used in this study. Monolayer values varied from 3.42 to 4.64% moisture (dry basis) which correspond to water activity of 0.16 to 0.22. No significant effect on monolayer values appeared from either drying method or supplementation with sesame.

It is noted that the effect of drying was more significant upon adsorption rather than desorption. Both freeze-dried and dried-frozen products had higher adsorption capacity than air-dried products.

Hysteresis

As can be seen in Figure 1, the isotherm obtained by placing a dry sample in an atmosphere of increasing water activity, lies below that obtained from wet material subjected to decreasing water activity. Thus, the moisture at a certain water activity will be greater for a desorption history than for an adsorption history. The difference in moisture between the two is called hysteresis.

According to Labuza (1975) and Wolf et al. (1972), hystersis has a significant effect on food stability. Ayerst (1965) stated that hysteresis is often quite large, but this situation rarely occurs under practical conditions. In the present experiment, hysteresis occurred in the samples used. Figure 3 displays a plot of the hysteresis against water activity of dried soybean curds. It is interesting to note that the hysteresis value of air-dried samples was

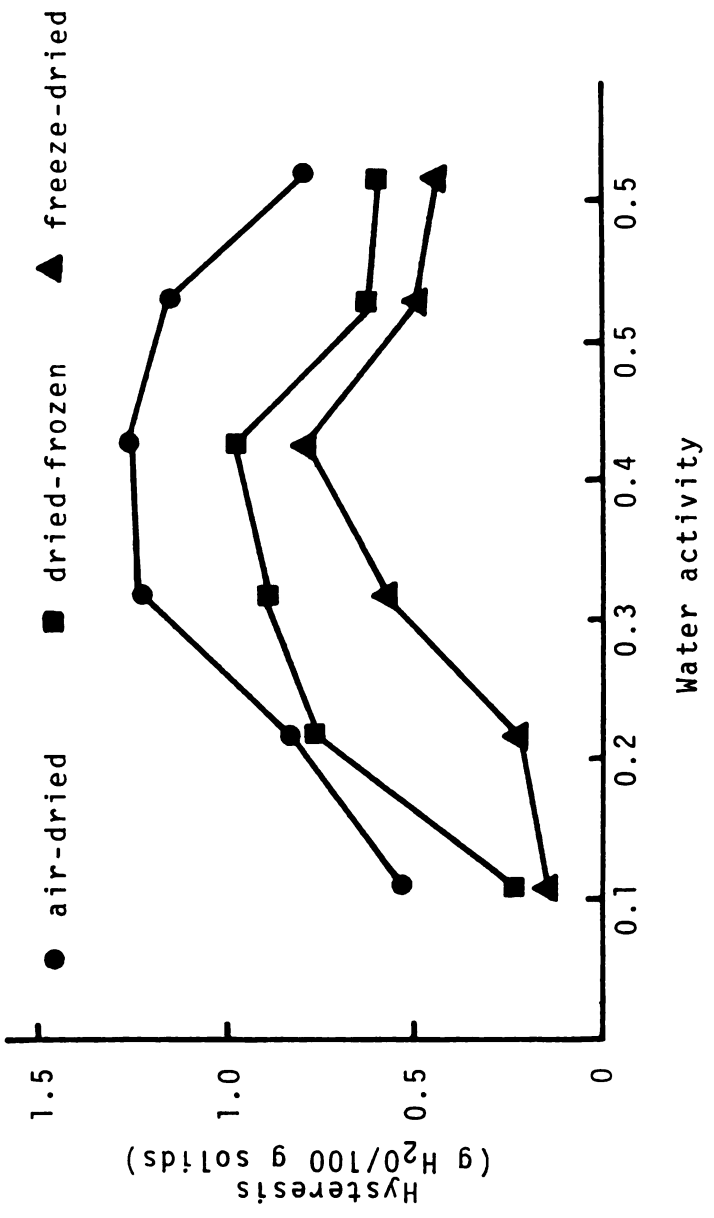

Figure 3. The hysteresis values of dried soybean curds at 37°C.

greater than that of dried-frozen samples which in turn was higher than that of freeze-dried samples. This phenomenon may be due to the fact that the outer layers of particles change in moisture content more rapidly than the inner layers and, during subsequent internal equilibration, the direction of water exchange in the outer layers is reversed. Since freeze-dried or dried frozen soybean curd probably had a more porous structure, these samples had higher hygroscopicity than that of air-dried samples. The porosity of the hygroscopic samples may cause higher adsorption and a more rapid internal equilibrium with the result of less hysteresis than that of air-dried samples.

Wolf et al. (1972) suggested the possibility of using hysteresis as an index of quality deterioration. The present study supports the suggestion that a high quality in terms of appearance, color, low degree of lipid oxidation and ability to be reconstituted of processed food as represented by the freeze-dried soybean curds exhibits lower hysteresis than a low quality one as depicted by air-dried soybean curd. Therefore, the greater the area of hysteresis loop, the lower the quality of the dried soybean curd products.

A similar effect of drying method on the extent of hysteresis was also obtained on samples containing sesame milk or ground sesame as shown in Table 7. The effect of composition on hysteresis has been reported by Wolf et al. (1972). They noted that the composition affects the

Table 7. Hysteresis values of dried modified soybean curds at 37°C.

Sample	Drying method*	Hysteresis (g/100 g solids) at a_w of					
		0.11	0.22	0.32	0.43	0.53	0.62
I. Soybean curd with	AD	0.48	0.63	0.99	1.16	0.92	0.65
added sesame milk	DF	0.20	0.49	0.55	0.67	0.37	0.31
	FD	0.13	0.22	0.43	0.48	0.37	0.28
II. Soybean curd with	AD	0.29	0.52	0.97	1.11	0.79	0.56
added ground sesame	DF	0.14	0.54	0.55	0.81	0.35	0.30
	FD	0.13	0.30	0.53	0.75	0.26	0.25

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

distribution of hysteresis along the isotherm, the a_w at which hysteresis reached a maximum value and also the total amount of hysteresis. The addition of sesame seemed to have an effect on the hysteresis value. This may be due to the difference in composition and/or properties of the components between samples with and without sesame. It was noted that maximum hysteresis was achieved at a water activity of 0.43, which is within the multilayer region.

Lipid Oxidation

Determination of peroxides is one of the most widely used chemical methods to measure the deterioration of fats and oils. Oxidation of polyunsaturated fatty acids produces lipid peroxides and the double bonds shift to a conjugated form which absorb in the ultraviolet region of the spectrum and have absorption maxima at 233 nm. The usefulness of the diene conjugation test has been indicated in some foods. Many investigators use the TBA procedure for estimating the extent of oxidative rancidity particularly in fish, meats and their products. In dried systems, several investigators used monometric systems (Labuza et al., 1969) and peroxide value (Martin, 1958; Rockland et al., 1961 and Loncin et al., 1968) to follow the rate of lipid oxidation.

Table 8 shows the development of peroxide values in lipids of dried soybean curds with and without added sesame during accelerated storage at 0.75 a_w and temperature of 37°C for 8 weeks. It was observed that the freeze-dried

Table 8. Peroxide values of dried soybean curds during storage at 0.75 a_w and 37°C.

Sample	Drying method*	Peroxide value (meq/kg oil)				
		0 weeks	2 weeks	4 weeks	6 weeks	8 weeks
I. Soybean curd	AD	11.2^{c}	22.5	35.3^{a}	41.6^{a}	52.6^{b}
	DF	9.6^{b}	28.2^{b}	41.9^{c}	50.3^{bcd}	55.2^{c}
	FD	2.4^{a}	27.5^{ab}	42.0^{c}	51.8^{d}	56.4^{c}
II. Soybean curd with	AD	10.8^{c}	21.2	36.4^{a}	40.4^{a}	51.5^{bc}
added sesame milk	DF	9.3^{b}	28.0^{ab}	41.2^{bc}	50.7^{cd}	55.0^{c}
	FD	2.6^{a}	27.2^{ab}	41.7^{c}	50.3^{bcd}	54.9^{c}
III. Soybean curd with	AD	10.6^{c}	17.5	22.7	34.6	40.8
added ground sesame	DF	8.5	27.2^{ab}	39.0^{b}	48.8^{b}	50.4^{a}
	FD	2.3^{a}	26.9^{a}	36.4^{a}	49.0^{bc}	52.3^{ab}

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

[a]All numbers in the same column sharing the same superscript are not significantly different at 5% level.

samples had lower initial peroxide values than those of dried-frozen and air-dried samples. Therefore, lipid oxidation during freeze drying was less than in samples prepared by other drying methods. It was noted that freeze-dried and dried-frozen samples had porous structures.

The peroxide values of dried soybean curds as well as dried modified soybean curds increased throughout the storage time. After about 2 weeks under the accelerated storage condition, dried-frozen and freeze-dried samples had higher peroxide values than the air-dried samples. Roth et al. (1965) reported that exposure to oxygen appeared to be the most significant factor in the degradation of freeze-dried products stored at elevated temperature. Martin (1958) noted that freeze-dried outflakes cereal had relatively poor stability. Since both dried-frozen and freeze-dried samples probably had a more porous structure, the rate of oxygen uptake and the diffusivity of oxygen in these products can be expected to be high.

It is interesting to note that samples with added ground sesame were more stable with respect to lipid oxidation than samples with added sesame milk or samples without added sesame. Sesame seeds contain two minor constituents i.e. sesamin and sesamolin. Sesamol, a phenolic antioxidant, usually present in traces, is formed from sesamolin under processing conditions. Sesamol has been reported to possess marked antioxidant activity in lard and also exhibits a

pronounced protection for vegetable oils (Budowski, 1950; Budowski, 1964).

The high stability of samples with added ground sesame can be accounted for by one or more of the following possibilities. First, it may be due to the presence of sesamol, a natural antioxidant. Second, it may be due to compounds formed as a result of non-enzymatic browning since it was found that the samples had higher browning than the others. Finally, some effect may derive from the difference in fatty acid composition as shown earlier in Table 3. The sample with added ground sesame had less linolenic acid than the others.

Table 9 shows changes in TBA value expressed as absorbance at 530 nm per gram oil measured on dried soybean curd and dried modified soybean curds during storage at 0.75 a_w and a temperature of 37^{o}C for 8 weeks. It was indicated that dried-frozen and freeze-dried samples reached maximum TBA values at 2 weeks storage period while air-dried samples reached the maximum at 4 weeks storage period. Beyond these times, the TBA values decreased continuously until the end of storage. Therefore, the pattern of TBA value change during accelerated storage was different from that of peroxide value.

The principal TBA reactant is considered to be malonaldehyde which is soluble in water. The red pigment produced in the TBA test was found to result from the condensation

Table 9. TBA values of dried soybean curds during storage at 0.75 a_w and 37°C.

Sample	Drying method*	TBA**				
		0 weeks	2 weeks	4 weeks	6 weeks	8 weeks
I. Soybean curd	AD	0.338	0.447^{a}	0.661^{c}	0.456^{a}	0.375^{b}
	DF	0.288^{d}	0.732	0.682^{c}	0.617^{b}	0.397^{b}
	FD	0.239^{ab}	0.903	0.667^{c}	0.605^{b}	0.425^{c}
II. Soybean curd with	AD	0.289^{d}	0.458^{a}	0.609^{b}	0.404	0.316^{a}
added sesame milk	DF	0.265^{cd}	0.657	0.585^{b}	0.564	0.404^{bc}
	FD	0.241^{bc}	0.853	0.600^{b}	0.512	0.506
III. Soybean curd with	AD	0.289^{d}	0.380	0.489^{a}	0.326	0.259
added ground	DF	0.211^{a}	0.492	0.433	0.449^{a}	0.313^{a}
sesame	FD	0.182	0.631	0.484^{a}	0.449^{a}	0.316^{a}

* AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

**Calculated as absorbance at 530 nm per g oil.

[a] All numbers in the same column sharing the same superscript are not significantly different at 5% level.

of two molecule of TBA with one molecule of malonaldehyde (Sinnhuber et al., 1958). The participation of malonaldehyde in the carbonyl-amino reaction may be a factor that caused the different pattern between TBA and peroxide changes.

Regardless of the pattern of difference from that of peroxide formation, TBA tests show that the stability of freeze-dried samples after storage was lower than dried-frozen samples which in turn was lower than air-dried samples. Supplementation with sesame appeared to increase the stability. Kenaston et al. (1955) found that oxidized linolenate produced 60-100 times as much color as oxidized linoleate whereas oleate produced no color when measured at the same level of oxidation indicated by peroxide value. Thus the lower content of linolenic acid in supplemented samples probably is responsible for the lower TBA values observed in these samples compared to samples without added sesame. In addition, the presence of sesamol and browning products as discussed earlier may also account for the stability.

Table 10 shows the diene conjugation value of lipids from dried soybean curds with and without added sesame during storage at 0.75 water activity and temperature of 37^{o}C. The trend was similar to that of peroxide value in which the values increased continuously throughout the storage period. Samples with added ground sesame had higher stability than other samples. Also, freeze-dried and

Table 10. Diene conjugation values of dried soybean curds during storage at 0.75 a_w and 37°C

Sample	Drying method*	Absorbance at 233 nm**				
		0 weeks	2 weeks	4 weeks	6 weeks	8 weeks
I. Soybean curd	AD	0.523^{d}	0.725	0.975	1.093	1.213^{a}
	DF	0.496^{bc}	0.830	1.098^{b}	1.230^{bc}	1.350^{de}
	FD	0.361^{a}	0.775^{b}	1.128	1.250^{c}	1.375^{d}
II. Soybean curd with added sesame milk	AD	0.514^{cd}	0.694^{a}	0.900	1.052	1.190^{a}
	DF	0.485^{b}	0.775^{b}	1.046^{a}	1.240^{c}	1.303^{ce}
	FD	0.366^{a}	0.750	0.075^{b}	1.232^{c}	1.316^{cd}
III. Soybean curd with added ground sesame	AD	0.502^{bc}	0.625	0.714	0.892	0.970
	DF	0.490^{b}	0.690^{a}	1.022^{a}	1.185^{ab}	1.260^{b}
	FD	0.360^{a}	0.706^{a}	1.034^{a}	1.201^{ab}	1.260^{b}

* AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

**Calculated at a dilution of 1:1000 (w/v).

[a]All numbers in the same column sharing the same superscript are not significantly different at 5% level.

dried-frozen samples which probably had a more porous structure seemed to have poor stability as measured by this method.

Cannon et al. (1952) and Privett et al. (1953) isolated mixed hydroperoxides in high purity from autoxidation of linoleate. Infrared spectra showed that the hydroperoxides assumed the cis-trans and trans-trans configurations, the latter form being more prevalent at higher temperatures and levels of autoxidation. Using the molar absorptivity for cis-trans conjugated octadecadienoate, both groups of workers estimated about 90% conjugation in their hydroperoxide preparations.

In the present study, the similarity between the peroxide value and that of diene conjugation is an expected consequence of oxidation of linoleic and linolenic acids since each act of a hydroperoxide formation involves shifting of double bonds into conjugated form and even beyond peroxide formation some of the decomposition products have conjugated dienes.

Effect of Temperature on Lipid Oxidation

Thermal energy affects the rate of chemical reactions including lipid oxidation. The rate of lipid oxidation can be expected to be higher with increase in temperature. Figure 4 displays the effect of storage temperature on peroxide value of dried soybean curds stored at a_w of 0.75. It is noted, that at 50^{o}C storage temperature, peroxide value increased during the first 4 weeks storage period

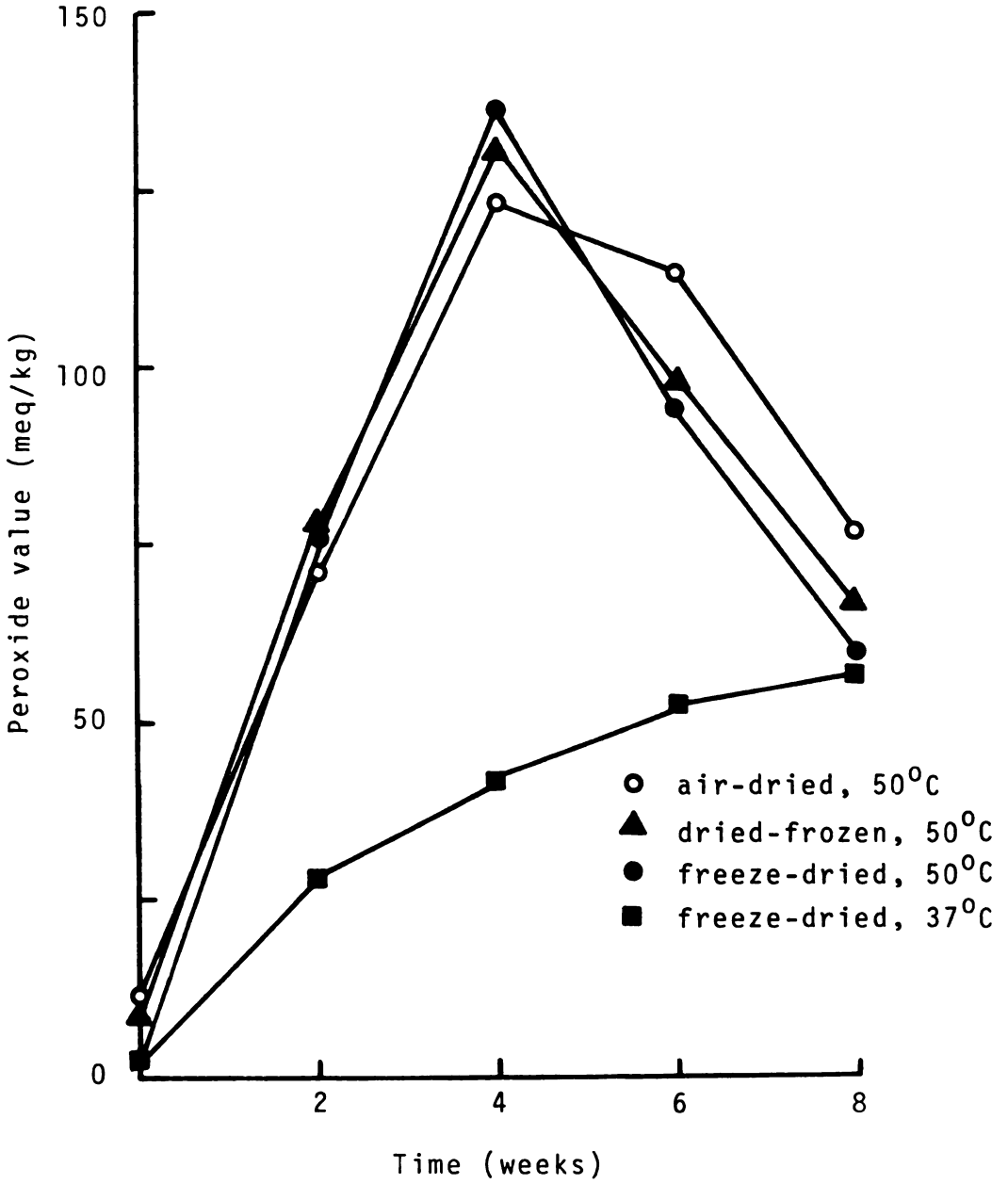

Figure 4. Effect of storage temperature on peroxide value of dried soybean curds at 0.75 a_w

after which the value decreased again. Increasing temperature may accelerate not only the chain propagation reaction, but also peroxide decomposition, thereby making a greater concentration of free radicals available for the initiation and propagation of reaction chains. Up to 4 weeks storage period at 50°C, peroxide formation was higher than peroxide decomposition. After this period the condition was reversed.

Table 11 shows the development of diene conjugation value measured on the samples stored at 0.75 a_w and temperature 50°C. Maximum values of diene conjugation were reached at the 4 week storage period for all samples used. However, the rate of diene conjugation value change of one was different from the others. In the first 4 week period, the rate of increasing conjugated diene absorption in the freeze-dried sample was higher than that of dried-frozen sample which in turn was higher than that of air-dried sample. In the subsequent storage period, the decrease of conjugated diene absorption was more rapid in the freeze-dried sample than in other samples. This resulted in freeze-dried, dried-frozen and air-dried samples having the lowest, medium and the highest conjugated diene absorption, respectively, when the storage was terminated.

Effect of Water Activity on Lipid Oxidation

Water activity plays an important role in the mechanism of lipid oxidation. The role may involve both antioxidant and prooxidant effects. The antioxidant effect may include

Table 11. Diene conjugation value of dried soybean curds during storage at 0.75 a_w and temperature of 50°C

Storage time (weeks)	Diene conjugation (Abs. at 233 nm)**		
	Air-dried	Dried-frozen	Freeze-dried
0	0.523[a]	0.497[a]	0.360
2	1.601	1.734[a]	1.695[a]
4	2.571	2.684	2.825
6	2.370	2.113	2.025
8	1.687	1.535	1.365

*Calculated at a dilution of 1:1000 (w/v).

[a]Values in the same row sharing the same superscript are not significantly different at 5% level.

hydration and/or dilution of trace metals, hydrogen bonding of hydroperoxides and providing radical recombination or reaction with other components. The prooxidant effects may be due to reduced viscosity thereby promoting the mobility of reactants, and to dissolve precipitated catalysts which in turn lead to increased reaction rates. In addition, water can cause the swelling of solid matrices which may aid in exposing new catalytic surfaces (Labuza, 1975).

Figure 5 shows the development of diene conjugation on freeze-dried soybean curd with added sesame milk during storage at various water activity at 37°C for 8 weeks. It can be seen that the rate was high at the dry state, i.e. a_w near zero. The rate reached a minimum in the monolayer region i.e. 0.22 a_w. As the water activity increased from the monolayer to 0.43, the rate of lipid oxidation increased. A further increase occurred when the product was stored at 0.75 a_w. Table 12 shows the development of peroxide values measured on the sample. It can be seen that lipid oxidation as measured by peroxide value was similar to that measured by conjugated diene absorption.

The amount of water which represents a monolayer according to the BET theory may be regarded as a protective film which protects the particles of food from attack by oxygen. Salwin (1959) stated that the statistical monolayer may not in fact represent a continuous film but rather corresponds to the available reactive adsorption sites in the protein,

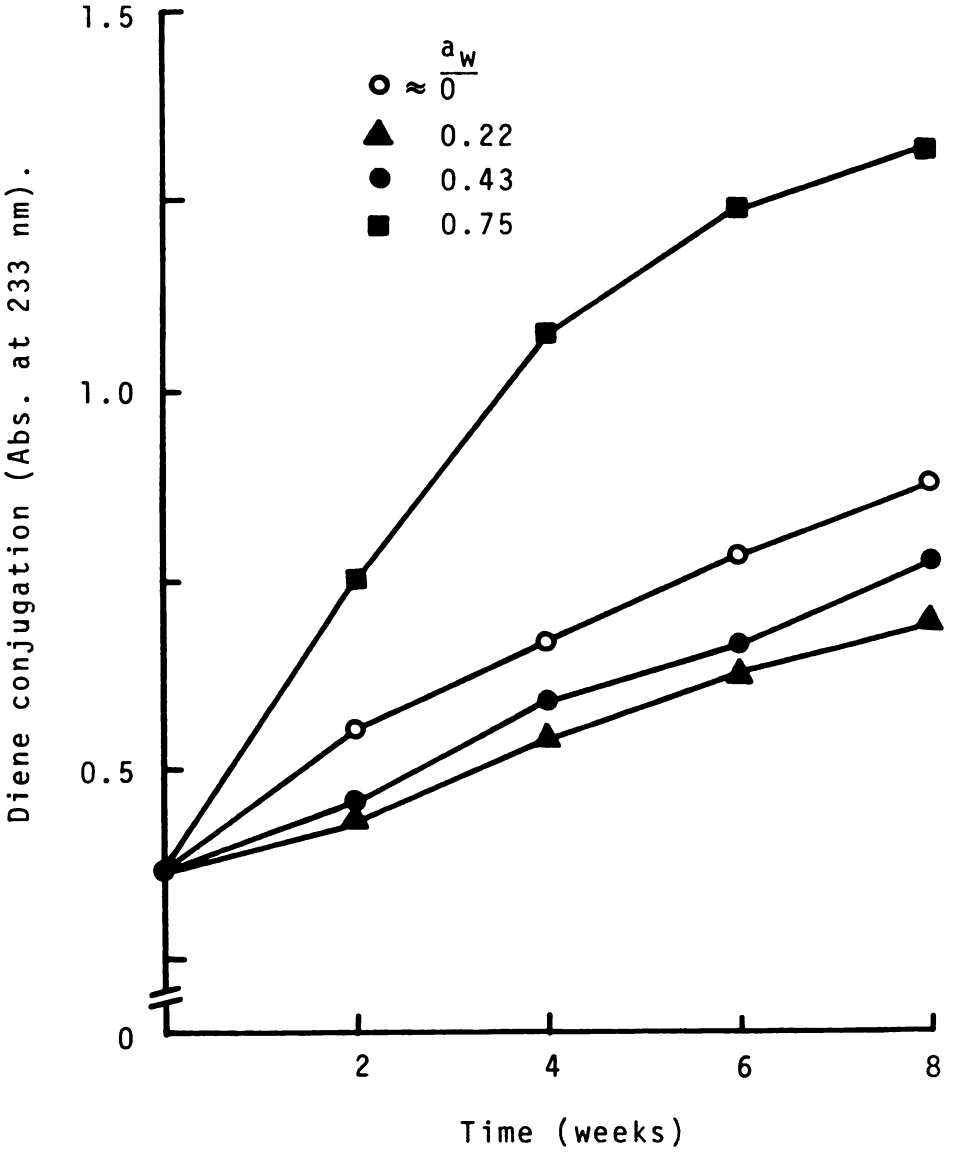

Figure 5. Effect of a_w on diene conjugation value of freeze-dried soybean curd with added sesame milk at 37°C.

Table 12. Peroxide value of freeze-dried soybean curd with added sesame milk during storage at various a_w and temperature of 37°C.

Water activity	Peroxide value (meq/kg)				
	0 week	2 weeks	3 weeks	6 weeks	8 weeks
≈0	2.6	12.6	20.5	26.4	28.5
0.22	2.6	6.5[a]	11.7[a]	16.9[a]	20.3[a]
0.43	2.6	6.7[a]	11.8[a]	17.7[a]	22.4[a]
0.75	2.6	27.2	41.7	50.3	54.9

[a]Values in the same column sharing the same superscript are not significantly different at 5% level.

carbohydrate and fat components of food. When the amount of water is adequate for combining these functional groups, they are protected from the reaction with oxygen. These theories may explain why the rate of lipid oxidation at the monolayer as observed in soybean curd with added sesame milk was less than that below the monolayer adsorption state.

Table 13 shows the diene conjugation value of air-dried, dried-frozen and freeze-dried soybean curds with added sesame milk stored at various a_w at the end of the storage period. Here again, as the water activity increased from near zero the lipid oxidation decreased, reached minimum values at the water activity of 0.22 and increased again at water activity beyond monolayer.

Changes in Fatty Acid Composition

Changes in the ratio of unsaturated and saturated fatty acids (U/S ratio) were used as a measure of changes in lipid composition of stored products. These U/S ratios were calculated from the GLC analyses. They are shown in Table 14. Since lipid oxidation is associated almost exclusively with unsaturated fatty acids, it can be expected that the U/S ratio decreases as a result of lipid oxidation during storage. The U/S ratios vary with the products, drying method and also storage temperature. It is noted that for the same drying method the initial U/S ratio of soybean curd was somewhat less than that of soybean curd with added sesame milk or

Table 13. Diene conjugation value of dried soybean curds with added sesame milk stored at various a_w and temperature of 37°C for 8 weeks.*

Water activity	Final value		
	Air-dried	Dried-frozen	Freeze-dried
≈ 0	0.783	0.794[b]	0.875
0.11	0.705[a]	0.778[ab]	0.734
0.22	0.626	0.647	0.693
0.43	0.689[a]	0.747[a]	0.775
0.62	0.987	1.167	1.105
0.75	1.190	1.303	1.316
Initial value	0.514	0.485	0.366

*Expressed as absorption at the wave length of 233 nm and calculated at a dilution of 1:1000 (w/v).

[a]Values in the same column sharing the same superscript are not significantly different at 5% level.

Table 14. Changes in the U/S ratio of dried soybean curds stored at 0.75 a_w for 8 weeks.

Sample	Drying method*	U/S ratio**			% change	
		Initial	Final (37°C)	Final (50°C)	37°C	50°C
I. Soybean curd	AD	4.63	4.16	1.41	10.15	69.54
	DF	4.83	4.32	1.30	10.56	73.08
	FD	5.06	4.42	1.18	12.64	76.68
II. Soybean curd with	AD	4.83	4.49	-	7.04	-
added sesame milk	DF	5.05	4.58	-	9.31	-
	FD	5.12	4.48	-	12.50	-
III. Soybean curd with	AD	5.14	4.79	-	6.81	-
added ground sesame	DF	5.16	4.73	-	8.33	-
	FD	5.22	4.64	-	11.11	-

* AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

**Ratio of unsaturated fatty acids to saturated fatty acids $(C_{18:1} + C_{18:2} + C_{18:3})/(C_{16:0} + C_{18:0})$.

ground sesame, but the soybean curd had a higher linolenic acid content which is more easily oxidized than linoleic or oleic acids. This is probably the reason why the percentage change of the U/S ratio of soybean curd was slightly higher than those of products with sesame for the same drying method. The other possible reason is due to the presence of natural antioxidant in products with sesame. This was in agreement with lipid oxidation measurements obtained by peroxide value, diene conjugation and TBA tests.

As discussed earlier, one method of drying gave products which had a different oxidation rate from those of the others. Consequently, a difference in the U/S ratio change of products from one method from the others was obtained. As shown in the table, freeze-dried products had higher U/S ratio changes than products obtained from the other drying methods. Again, this was in agreement with lipid oxidation measurements.

Increases in temperature from 37^{o}C to 50^{o}C led to corresponding increases in % changes of the U/S ratio. These results are as expected since lipid oxidation would proceed faster at higher temperature.

Changes in Dried Soybean Curd Color

Color is an appearance property attributed to the spectral distribution of light and is important in the evaluation of foods. The color of the dried soybean curds during storage was measured with a Hunter Color Difference

Meter D-25 model adjusted with the yellow standard.

Table 15 shows the color changes in terms of L, a and b values of dried products during storage at 37°C and 0.75 a_w. The L values measure lightness of the color from 100 for white to zero for black. The a values measure redness when plus, gray when zero and greenness when minus. The b values measure yellowness when plus, gray when zero and blueness when minus. Figure 6 displays changes in lightness of dried soybean curds during storage at 0.75 a_w at either temperature of 37°C or 50°C. The air-dried products had initial higher L values than those of either dried-frozen or freeze-dried products. During the storage at 37°C, 0.75 a_w, the L values decreased with increasing storage time which indicated that these products became darker. However, the L values of either dried-frozen or freeze-dried products slightly increased with time, denoting that the products became lighter. This may be due to the bleaching of coloring matters naturally present in the products. Thus two processes may have occurred concurrently, i.e. browning which reduced L value and bleaching of natural pigments which increased the L value.

All products had negative a values initially. As the time of storage increased, the value of a also increased. These indicated that the greenness of the products decreased. The yellowness as indicated by positive b values increased for air-dried products but remained essentially constant for

Table 15. Color of dried soybean curds during storage at 0.75 a_w and 37°C.

Sample	Drying method*	Color axis**	Hunter color values				
			0 week	2 weeks	4 weeks	6 weeks	8 weeks
I. Soybean curd	AD	L	73.95	70.00	70.20	71.10	71.00
		a	- 1.65	- 0.55	- 0.50	- 0.40	- 0.25
		b	19.40	20.50	20.55	20.75	20.80
	DF	L	67.55	58.90	58.70	69.20	69.75
		a	- 1.00	- 0.95	- 0.80	- 0.70	- 0.45
		b	22.70	22.65	22.60	23.00	22.70
	FD	L	67.15	68.65	69.05	69.40	69.40
		a	- 1.75	- 0.95	- 0.70	- 0.40	- 0.30
		b	22.80	23.15	23.45	23.30	23.35
II. Soybean curd with added sesame milk	AD	L	74.80	71.55	70.60	71.05	70.50
		a	- 2.00	- 0.95	- 0.65	- 0.45	- 0.50
		b	18.45	20.05	20.55	20.20	20.35
	DF	L	67.95	71.90	71.95	71.25	69.10
		a	- 1.05	- 1.00	- 0.80	- 0.75	- 0.55
		b	22.60	21.80	21.90	22.45	22.95
	FD	L	67.40	69.15	69.50	70.20	70.00
		a	- 0.90	- 0.75	- 0.75	- 0.60	- 0.55
		b	22.95	23.00	23.10	23.25	23.20
III. Soybean curd with added ground sesame	AD	L	72.30	67.35	66.65	66.95	69.70
		a	- 2.15	- 1.35	- 0.90	- 0.70	- 0.80
		b	20.20	22.05	22.20	22.40	22.40
	DF	L	63.90	64.75	64.75	64.65	64.55
		a	- 0.40	- 0.30	- 0.25	- 0.10	0.05
		b	22.90	22.80	23.50	23.75	23.60
	FD	L	63.60	65.25	64.65	64.75	64.75
		a	- 0.45	- 0.35	- 0.25	- 0.20	- 0.10
		b	23.35	23.50	23.60	23.65	23.75

* AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

**L, a and b stand for lightness, greenness or redness and yellowness respectively.

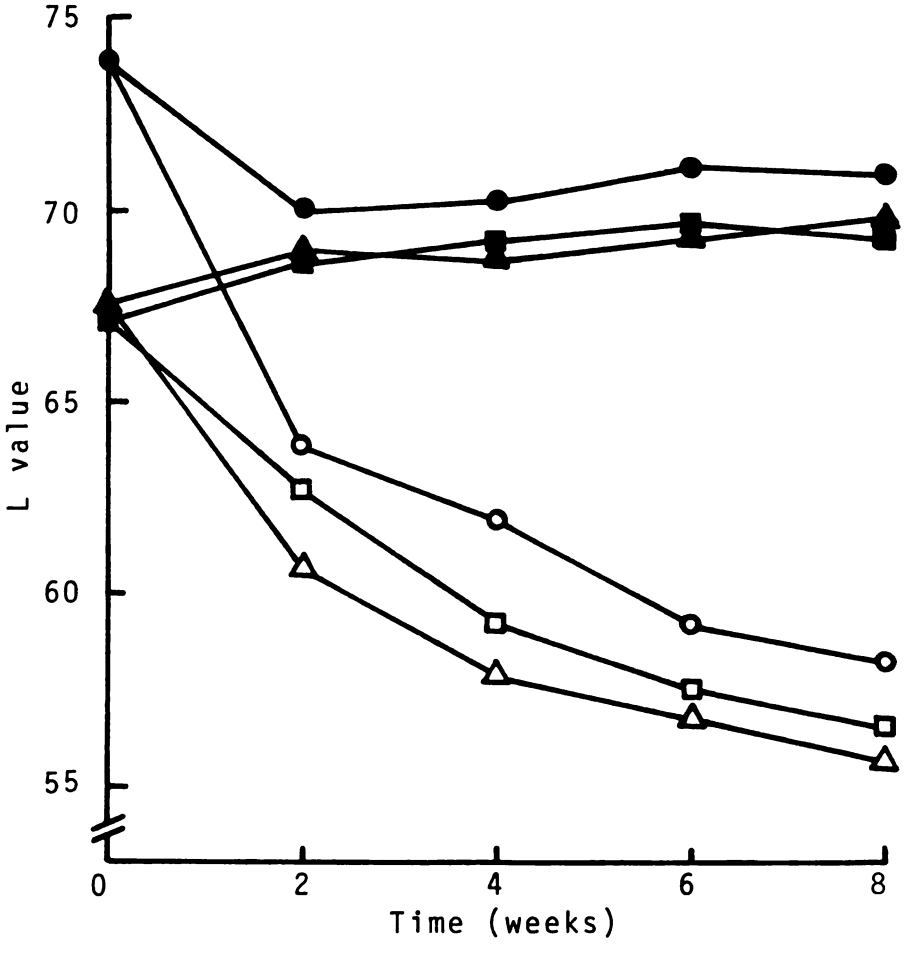

Figure 6. Changes in lightness of dried soybean curds during storate at 0.75 a_w.

● air-dried (37°C) ○ air-dried (50°C)
▲ dried-frozen (37°C) △ dried-frozen (50°C)
■ freeze-dried (37°C) □ freeze-dried (50°C)

dried-frozen and freeze-dried products.

More significant changes were obtained when the products were stored at 50°C. As shown in Table 16, the negative a values changed to positive values as storage progressed. Therefore, the greenness vanished while the redness appeared. High increases in yellowness were obtained for samples stored at this temperature. As displayed in Figure 6 and in Table 16, the rate of decrease in L values was greater at 50°C than 37°C. The L values of all samples decreased throughout the storage period. Thus at this temperature the rate of browning was presumably higher than the rate of bleaching. Also, the amount of bleachable material may be fixed and when it is used up, no more bleaching effect would be noticeable.

It was noted that the addition of sesame milk had little effect on the color of the dried products. However, the addition of ground sesame caused a darker color in the dried products obtained.

Changes in the Browning Index

Non-enzymatic browning occurs when aldehydes or ketones react with amino groups. The possible reactants for non-enzymatic browning in dried soybean curds may arise from lipids or carbohydrates which provide the aldehydes or ketones and proteins which provide the amino groups.

The soluble browning pigments formed in the dried soybean curds were measured spectrometrically after

Table 16. Color of dried soybean curds during storage at 0.75 a_w and temperature of 50°C.

Drying method	Color axis*	Hunter color values				
		0 week	2 weeks	4 weeks	6 weeks	8 weeks
Air-dried	L	73.95	63.80	62.00	59.30	58.30
	a	- 1.65	3.05	3.40	5.65	6.60
	b	19.40	21.35	25.15	28.80	29.80
Dried-frozen	L	67.55	60.60	57.90	56.80	55.55
	a	- 1.00	3.95	4.75	5.50	7.95
	b	22.70	23.65	25.30	26.90	30.80
Freeze-dried	L	67.15	62.70	59.20	57.50	56.50
	a	- 1.75	3.00	3.70	5.35	7.50
	b	22.30	23.05	26.50	30.10	31.20

*L = lightness a = greenness or redness b = yellowness

incubation with trypsin at 45°C for one hour. The results are presented in Table 17. It can be seen that the initial browning index of air-dried samples was higher than that of dried-frozen samples which in turn was higher than that of freeze-dried samples. The browning indices of stored products were higher than those of unstored products. The changes in the browning index vary with the product, storage temperature and drying method. The percentage changes were higher in soybean curds with added ground sesame than the others. As storage temperature increased, the formation of brown pigments also increased. The rate of browning in freeze-dried or dried-frozen products was higher than that in air-dried products. Tuomy et al. (1970) noted that temperature, vacuum and storage time significantly affect oxygen uptake of dried foods, but the relative importance of the three factors was different for each product. The higher rate of browning in freeze-dried and dried-frozen products may be related to higher accessible oxygen in these porous products. Although the browning reaction itself did proceed without oxygen, other reactions such as lipid oxidation which could provide substrate for the browning reaction were oxygen dependent.

The Maillard reaction is low in the anhydrous state, it is maximal at a relative humidity of 40-70%, it then decreases as the aqueous dilution increases (Lea and Hannan, 1949; Hannan and Lea, 1952; Loncin et al., 1968). Similar results

Table 17. Browning indices of dried soybean curd stored at 0.75 a_w for 8 weeks.

Sample	Drying method*	Storage temp. °C	Browning index**			% Change	
			0 week	4 weeks	8 weeks	4 weeks	8 weeks
I. Soybean curd	AD	37	7.22^{bc}	8.01^{a}	9.35^{a}	10.9	29.5
	DF	37	6.26^{a}	8118^{ab}	9.53^{ab}	29.4	52.2
	FD	37	6.17^{a}	8.07^{ab}	9.83^{c}	30.8	59.3
	AD	50	7.22	11.64^{a}	12.75^{a}	61.2	76.6
	DF	50	6.26^{a}	12.05^{b}	13.17^{a}	92.5	110.4
	FD	50	6.17^{a}	11.94^{ab}	13.88	93.5	125.0
II. Soybean curd	AD	37	7.25^{c}	8.10^{ab}	9.75^{bc}	11.7	34.5
with added	DF	37	6.35^{a}	8.24^{ab}	9.87^{c}	29.8	55.4
sesame milk	FD	37	6.17^{a}	8.30^{b}	10.00^{c}	34.5	62.1
III. Soybean curd	AD	37	8.71	10.12^{c}	12.11^{d}	16.2	39.0
with added	DF	37	7.12^{bc}	10.30^{c}	12.46^{e}	44.7	75.0
ground sesame	FD	37	7.06^{b}	10.25^{c}	12.33^{de}	45.2	74.6

* AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

**Was expressed as 100 x absorbance at 400 nm per gram solids.

[a] All values in same column and same storage temperature sharing the same superscript are not significantly different at 5% level.

were obtained from this study as shown in Figure 7. The browning in freeze-dried soybean curd with added sesame milk was low at water activities near zero to 0.22 which may be due to insufficient mobility of the reaction groups. It then increased with increasing water activity up to 0.62, and declined at water activity level beyond that point which may be accounted for by increasing mobility of reactants and by a dilution effect respectively.

Table 18 reveals the browning indices for air-dried and dried-frozen soybean curds with added sesame milk stored at various water activities at 37^{o}C for 8 weeks. The trend of browning development for each product seems to be similar to that discussed above.

Changes in Carbohydrate

Both reducing and non-reducing sugars may participate in the browning reaction, although the latter have a lower rate. In the present study, changes in sugars were analyzed with high pressure liquid chromatography. The results are displayed in Table 19. The sugars present in soybeans were in agreement with those reported by several authors (Horan, 1974; Black and Bagley, 1978; Macrae and Moghaddam, 1978).

Sugars in soybeans have been implicated as factors responsible for flatulence. Flatus is produced by bacterial fermentation of dietary carbohydrate in the lower intestine (Rackis et al., 1970). As shown in the table, the sugar content of soybean curd was much lower than in whole soybean.

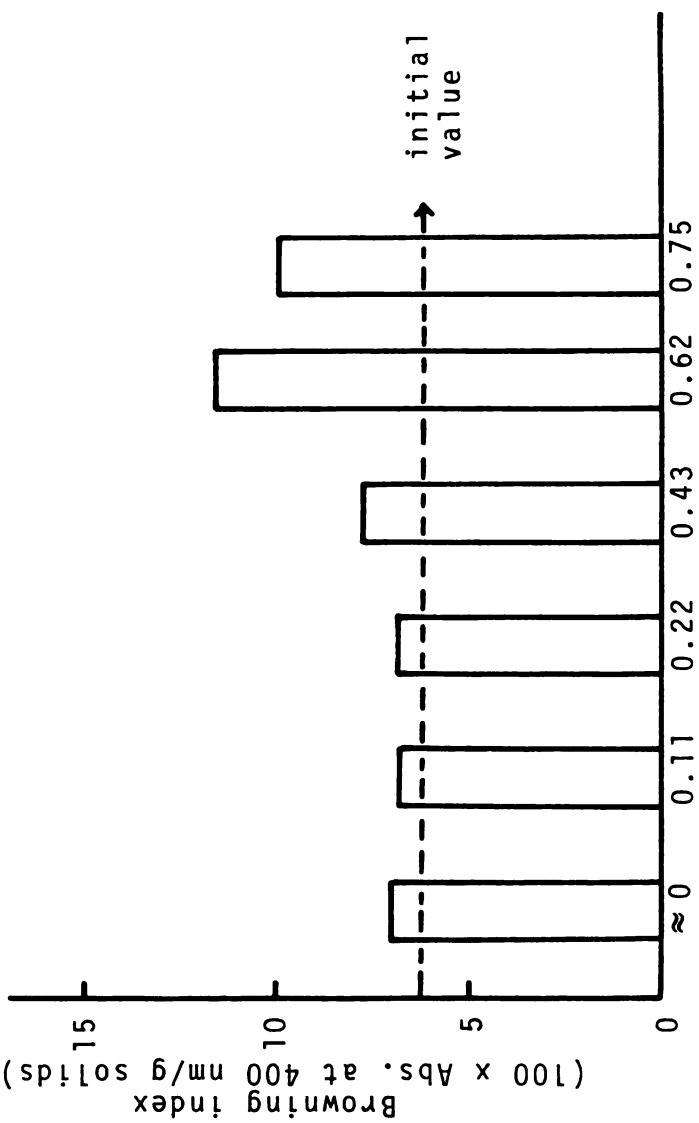

Figure 7. Browning indices of freeze-dried soybean curd with added sesame milk stored at various a_w and 37°C for 8 weeks.

Table 18. Browning indices of air-dried and dried-frozen soybean curds with added sesame milk stored at various a_w and 37°C for 8 weeks.*

Water activity	Final value	
	Air-dried	Dried-frozen
≈ 0	7.84[a]	6.96[a]
0.11	7.82[a]	7.00[a]
0.22	7.76[a]	6.99[a]
0.43	8.45	7.68
0.62	9.88[b]	10.57
0.75	9.75[b]	9.87
Initial value	7.25	6.35

*Was expressed as 100 x absorbance at 400 nm per gram solids.

[a]Values in the same column sharing the same superscript are not significantly different at 5% level.

Table 19. Sugar composition of soybean, sesame seed and dried soybean curds.

Sample	Drying method*	Storage temp. (°C)	Storage time (weeks)	Sugar (% dry matter)			
				Glucose	Sucrose	Raffinose	Stachyose
I. Soybean	-	-	-	tr.	4.68	1.77	3.83
II. Sesame seed	-	-	-	tr.	2.33	2.01	0.94
III. Soybean curd	AD	-	0	tr.	1.14	0.21	0.78
		37	8	tr.	0.90	0.17	0.71
		50	4	tr.	0.71	0.12	0.59
		50	8	tr.	0.50	0.10	0.55
	DF	-	0	tr.	1.28	0.24	0.89
		37	8	tr.	1.01	0.20	0.79
		50	4	tr.	0.56	0.16	0.67
		50	8	tr.	0.39	0.12	0.57
	FD	-	0	tr.	1.25	0.25	0.82
		37	8	tr.	0.91	0.18	0.74
		50	4	tr.	0.58	0.15	0.63
		50	8	tr.	0.44	0.11	0.53
IV. Soybean curd with added sesame milk	AD	-	0	tr.	1.09	0.19	0.83
		37	8	tr.	0.76	0.15	0.73
	DF	-	0	tr.	1.14	0.18	0.85
		37	8	tr.	0.75	0.13	0.76
	FD	-	0	tr.	1.18	0.20	0.86
		37	8	tr.	0.67	0.17	0.75
V. Soybean curd with added ground sesame	AD	-	0	tr.	1.03	0.22	0.76
		37	8	tr.	0.74	0.18	0.69
	DF	-	0	tr.	1.08	0.24	0.81
		37	8	tr.	0.69	0.16	0.70
	FD	-	0	tr.	1.10	0.25	0.78
		37	8	tr.	0.68	0.17	0.67

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

With respect to this matter, soybean curd is a better food than whole soybean.

During the very first stages of the Maillard reaction, sugar-amino linkages are formed. The products that are formed remain colorless although their amino acids are already lost nutritionally (Lea and Hannan, 1950; Lewis and Lea, 1950). This case might occur in processing of soybean curds since total sugar content of soybean curd was less than that calculated by difference as previously displayed in proximate analysis (Table 2). It is proposed that some of the sugars incorporate into a lipid-protein matrix through resistant linkages when coagulation of protein occurs.

El-Nockrashy and Frampton (1967) noted that sucrose, raffinose and trehalose react with lysine. The reaction causes a reduction in the optical rotation of the non-reducing sugars. They proposed that the 1-2 glycosidic linkage in sucrose and in raffinose undergoes aminolysis in the presence of the epsilon amino group of lysine and lysine is diminished. The alpha amino group of amino acids is not involved, but a group liberated on the aminolysis of the 1-2 glycosidic linkage may react with the alpha amino group.

As shown in Table 19, the sugars and particularly sucrose decreased during storage at 0.75 a_w. The decrease in the sugar content was higher with increasing storage temperature. There was a possibility that both 1-2- and 1-6-glycosidic linkages of these sugars underwent aminolysis

since the soybean protein is rich in lysine. The liberated monosaccharides presumably reacted directly with amino acids in the browning reaction.

Amino Acid Composition and Available Lysine

Table 20 shows the amino acid composition of freeze-dried soybean curds with and without addition of sesame. Amino acid requirements for man have been estimated by two expert groups, the FAO/WHO of the United Nations (Anonymous, 1973) and the Food and Nutrition Board of the National Academy of Sciences (Williams et al., 1974). For adult man it is now accepted that 8 amino acids are essential: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The infant requires histidine as well as the above 8 amino acids (Anonymous, 1973).

As shown in Table 20, most of the essential amino acids in soybean curds meet the patterns suggested by the two groups. It is the methionine plus cystine that is limiting in meeting these requirements. Although some methionine and cystine may be oxidized during acid hydrolysis, even under nitrogen, it can be seen in the table that the addition of sesame increased the content of these S-containing amino acids. Thus the deficiency can be minimized by combining sesame with soybean. Unfortunately the equipment was not available for determination of methionine and cystine separately. For comparison, the literature value of methionine in soybean curd was 1.3 g/100 g protein (Standahl,

Table 20. Amino acid composition of freeze-dried soybean curds and amino acid requirements for man

Amino acid	Amino acid of soybean curds (g/100 g protein)			Requirements (g/100 g prot.)	
	without sesame	+ sesame milk	+ ground sesame	FAO/ WHO*	FNB**
Aspartic	11.75	11.23	10.89		
Threonine	3.78	4.09	4.07	4.0	3.5
Serine	4.54	4.65	4.59		
Glutamic	19.49	18.27	15.54		
Proline	5.49	6.17	7.04		
Glycine	3.16	3.57	3.11		
Alanine	3.96	4.15	4.09		
Valine	5.05	5.28	5.09	5.0	4.8
Cystine	0.42	0.54	0.56	3.5[a]	2.6[a]
Methionine	1.16	1.20	1.29		
Isoleucine	5.27	5.23	5.11	4.0	4.2
Leucine	8.66	8.32	8.14	7.0	7.0
Tyrosine	3.73	4.30	4.39	6.0[b]	7.3[b]
Phenylalanine	5.42	5.61	5.84		
Lysine	6.10	6.00	5.71	5.5	5.1
Histidine	2.53	2.48	2.46		1.7[c]
Arginine	7.34	8.08	9.04		
Tryptophan[d]	1.45	1.39	1.43	1.0	1.1

* Food and Agric. Org./World Health Org. (Anonymous, 1973).

**Food and Nutr. Board, Natl. Acad. Sci. (Williams et al., 1974)

[a]methionine + cystine [c]tyrosine + phenylalanine

[b]essential only for infants [d]was determined spectrometrically

1967).

Scrimshaw and Young (1979) examined the effect of methionine supplementation on nitrogen balance when soybean protein isolates were fed at level of 0.51 g protein/kg/day. At this low level of isolate intake, supplementation with 1.1% methionine gave a positive response that was equal to that obtained with egg protein. However, addition of 1.6% methionine resulted in a lower nitrogen balance than with zero supplementation. On the other hand, when the level of soybean isolate was increased to 0.8 g/kg/day to meet the dietary allowance for total protein, additional methionine had no significant effect. They concluded that under normal usage in adults, methionine supplementation of properly processed soybean protein products is unnecessary and probably undesirable. In the present study, a combination of soybean and sesame 10:1 (w/w) did not seem to increase the methionine to an excessive level. However, regardless of the addition of sesame, it is important to note the fact that amino acids that may be limited in other foods may be present in more than enough quantity in soybean curds.

As stated, one of the most important deterioration reactions in dried foods is non-enzymatic browning. The chemical aspects of the non-enzymatic browning involve blocking and destruction of amino acids, lysine in particular. The blocking results in the amino acid becoming unavailable. In the present study, the available lysine

was determined by high pressure liquid chromatography. The results are shown in Table 21. The initial value of available lysine in freeze-dried products was less than total lysine as determined by the amino acid analyzer. Thus, some of the lysine was already unavailable at the beginning of storage. The rate of decrease of available lysine was greater in freeze-dried or dried-frozen products than in air-dried products. Higher storage temperature ($50^{o}C$) resulted in greater loss of available lysine. These results agree with those reported by Tsao et al. (1978) who stated that the thermal loss of available lysine in a rice meal system can be described by a monomolecular reaction and that temperature has a positive effect on reaction rate. It seems the products with added ground sesame had slightly higher rates of loss in available lysine. This phenomenon will be discussed later in the general discussion section.

Water activity is an important factor affecting the extent of browning and the loss of amino acids. Burvall et al. (1978) noted that no visible browning occurred in lactose-hydrolyzed dried milk although the available lysine loss reached 40%. The effect of water activity upon loss of available lysine in freeze-dried soybean curd with added sesame milk is displayed in Figure 8. The loss was low at low water activities then, as the water activity increased, the loss increased and reached a maximum at 0.62 a_w and then declined at water activities above this level. Both

Table 21. Available lysine in dried soybean curds stored at 0.75 a_w for 8 weeks.

Sample	Drying method*	Storage temp. °C	Available Lysine (g/100 g protein)				
			0 week	2 weeks	4 weeks	6 weeks	8 weeks
I. Soybean curd	AD	37	5.20^{bcd}	4.90^{c}	4.67^{d}	4.53^{c}	4.42^{c}
		50	5.20^{f}	4.30^{f}	3.64^{f}	3.17^{f}	2.87^{f}
	DF	37	5.30^{de}	4.81^{bc}	4.53^{bcd}	4.32^{b}	4.18^{b}
		50	5.30^{f}	4.32^{f}	3.45^{f}	3.00^{f}	2.80^{f}
	FD	37	5.34^{e}	4.72^{ab}	4.50^{bc}	4.37^{bc}	4.26^{bc}
		50	5.34^{f}	4.22^{f}	3.40^{f}	2.90^{f}	2.70^{f}
II. Soybean curd with added sesame milk	AD	37	5.12^{bc}	4.83^{bc}	4.63^{cd}	4.51^{c}	4.40^{c}
	DF	37	5.25^{cde}	4.68^{a}	4.45^{b}	4.29^{b}	4.16^{b}
	FD	37	5.30^{de}	4.70^{ab}	4.55^{cbd}	4.43^{bc}	4.35^{c}
III. Soybean curd with added ground sesame	AD	37	4.97^{a}	4.65^{a}	4.43^{ab}	4.27^{b}	4.14^{b}
	DF	37	5.10^{ab}	4.68^{a}	4.30^{a}	4.01^{a}	3.91^{a}
	FD	37	5.10^{ab}	4.60^{a}	4.29^{a}	3.98^{a}	3.86^{a}

*AD, DF and FD stand for air-dried, dried-frozen and freeze-dried respectively.

[a]All values in same column and same storage temperature sharing the same subscript are not significantly different at 5% level.

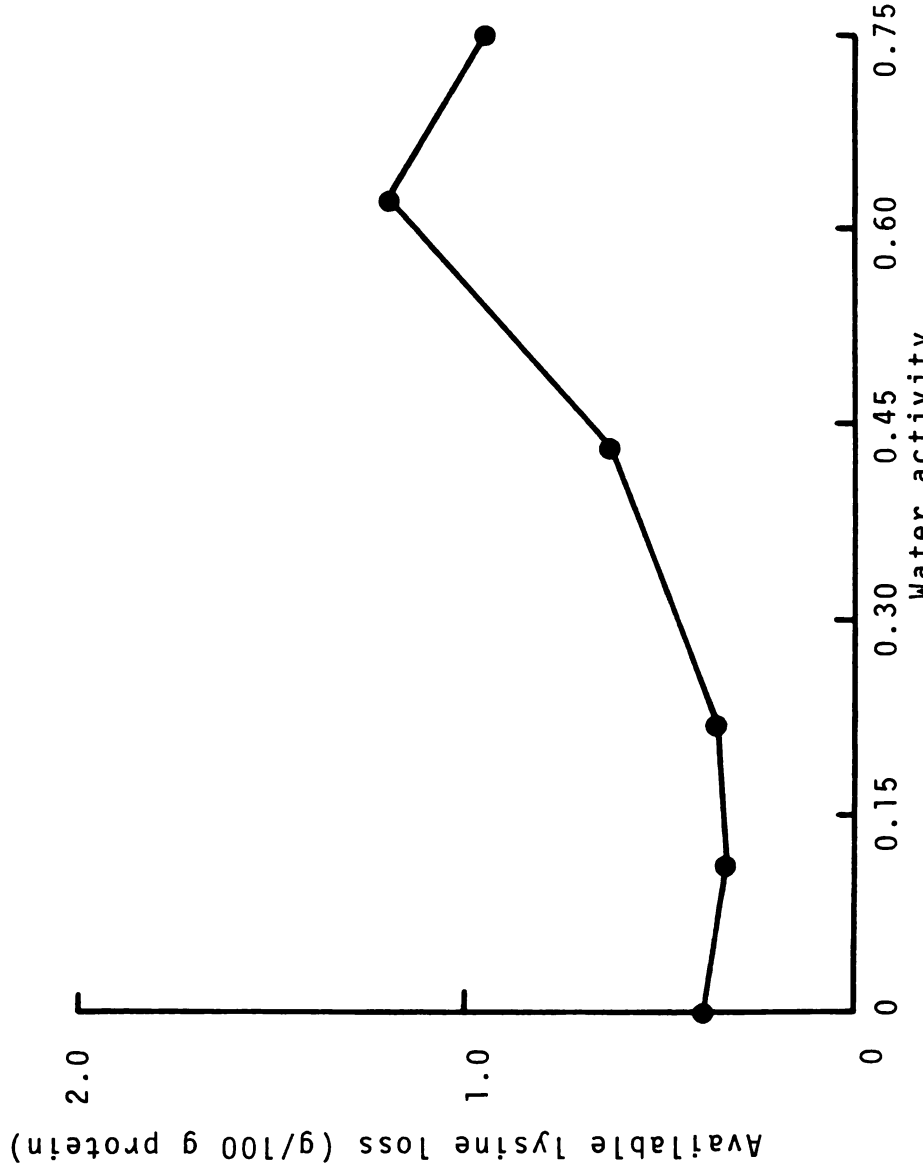

Figure 8. Available lysine loss in freeze-dried soybean curd with added sesame milk stored at various a_w and 37°C for 8 weeks.

accelerating and inhibiting effects of water were caused by the fact that the reaction needs water to ensure mobility of the reactants. At low water activities, the loss of available lysine was low due to solubility limitation. On the other hand, at water activities of about 0.4-0.6 the browning reaction was favored due to high mobility of the reactants. Then the loss declined due to dilution effects. The loss of available lysine along the water activity range was in agreement with losses reported for milk powder (Loncin et al., 1968), pea soup mix (Labuza et al., 1970) and lactose-hydrolyzed dried milk (Burvall et al., 1978). The available lysine content of air-dried and dried-frozen soybean curds with added sesame milk stored at various a_w and 37°C for 8 weeks are shown in Table 22. The trend of available lysine loss as a function of water activity in these products seems similar to that of the freeze-dried product discussed above.

Relationship Among Lipids and Browning Parameters

To test for possible linear correlations among lipids and browning parameters in the present experiment, a linear regression model was applied to the data obtained. St. Angelo et al. (1975) plotted and fitted a linear regression model to test for possible correlation between peroxide value and diene conjugation while studying shelf life stability of peanut butters. They found a high correlation coefficient which indicated the results of the two methods

Table 22. Available lysine of air-dried and dried-frozen soybean curds with added sesame milk stored at various a_w and 37°C for 8 weeks.

Water activity	Available lysine (g/100 g protein)	
	Air-dried	Dried-frozen
≈ 0	4.87[b]	4.95[b]
0.11	4.80[ab]	4.97[b]
0.22	4.92[b]	4.98[b]
0.43	4.63[a]	4.70
0.62	4.04	4.00[a]
0.75	4.40	4.16[a]
Initial value	5.12	5.25

[a]Values in the same column sharing the same superscript are not significantly different at 5% level.

were highly correlative. Dahle et al. (1962) found a linear relationship among peroxide value, TBA value and diene conjugation value while studying autoxidation of polyunsaturated fatty acid methyl esters.

Table 23 shows the relationships among various parameters used in this study. A plus or minus sign is attached to the correlation coefficient according to whether the slope of the fitted regression line is positive or negative.

A high correlation coefficient between peroxide value and diene conjugation was obtained which proved that the results from the two methods were highly correlative. This was in agreement with St. Angelo et al. (1975) and Dahle et al. (1962). On the other hand, very poor linear correlation was obtained between either peroxide value or diene conjugation and TBA values. Thus in dried soybean curds and possibly also in the other dried food systems, the TBA value development was not in agreement with that of pure lipid systems as reported by Dahle et al. (1962).

Heidelbaugh and Karel (1970) noted that the peroxide value test reflects oxidative changes better than the TBA test while studying changes which occurred in pouched heat processed foods. In the present experiment, it is apparent that either peroxide value or the diene conjugation test was a better method for monitoring lipid oxidation in dried soybean curds than the TBA test. Since soybean curds contain relatively high polyunsaturated fatty acids, the diene

Table 23. Correlation coefficients among lipids and browning parameters of dried soybean curds stored at 0.75 a_w.*

Storage temp.(°C)	Parameter		Regression equation		Correlation coefficient
	X axis	Y axis	Y intercept	Slope	
37	Diene conj.	Peroxide value	- 15.932	54.161	+ 0.98
	Diene conj.	TBA	0.310	0.166	+ 0.30
	Diene conj.	Browning index	5.373	3.784	+ 0.73
	Avail. lysine	Diene conj.	4.109	- 0.697	- 0.88
	Peroxide value	Avail. lysine	5.270	- 0.020	- 0.89
	Browning index	Avail. lysine	6.286	- 0.186	- 0.79
	TBA	Avail. lysine	4.794	- 0.467	- 0.20
	Browning index	Peroxide value	- 37.408	7.947	+ 0.75
	TBA	Peroxide value	19.136	31.569	+ 0.30
	TBA	Browning index	8.052	1.616	+ 0.14
50	Peroxide value	Avail. lysine	4.936	- 0.015	- 0.69
	Peroxide value	Diene conj.	0.311	0.018	+ 0.97
	Peroxide value	Browning index	8.179	0.042	+ 0.67
50	Peroxide value	L value	67.133	- 0.085	- 0.71
	Avail. lysine	Diene conj.	3.817	- 0.560	- 0.68
	Browning index	Avail. lysine	7.634	- 0.358	- 0.99
	L value	Avail. lysine	- 6.637	0.170	+ 0.92
	Diene conj.	Browning index	6.970	2.297	+ 0.72
	Diene conj.	L value	68.731	- 4.408	- 0.67
	L value	Browning index	38.278	- 0.447	- 0.91

*Values from dried soybean curds both with and without added sesame were pooled to calculate correlation coefficient.

conjugation test is presumably more advantageous than the peroxide value because the method is simpler and the structure remains in many molecules after peroxides have decomposed.

As stated, the characteristic pink color formed in the TBA test is a result of reaction between malonaldehyde and thiobarbituric acid. In dried soybean curds, amlonaldehyde may have formed since the products contain trienoic acid. The malonaldehyde thus formed probably reacted with amino groups of protein and this caused a poor correlation between either peroxide value or diene conjugation and TBA tests. Moreover, the carbonyl compounds that may participate in the carbonyl-amino reaction are not only malonaldehyde, but also other carbonyl compounds derived from lipids and carbohydrates. In addition, it has been reported that the intermediate products of carbonyl-amino reaction viz., reductones interfere with color development in the TBA test (Evans et al., 1958). Also, the TBA test does not fulfill all the requirements of a reproducible technique and has been criticized on several points (Lea, 1962; Gray, 1978). These may account for the poor correlations between TBA value and other parameters.

No appreciable changes in color were noted during storage at 37^{o}C except the greenness which faded gradually. However, at 50^{o}C significant changes in a and b values occurred and consequently, the L value decreased. At this

temperature a good correlation between L value and browning or available lysine content was obtained. Also, there was a good correlation between L value and peroxide value or diene conjugation.

A quite high correlation between browning index and available lysine content was found. It appears, that the increase in the carbonyl-amino reactions relates in part to the loss of available lysine. Since there was also a high correlation between lipid oxidation as measured either by peroxide value or diene conjugation and available lysine content, it is obvious that the loss of available lysine might be accounted for partly by lipid-protein interaction. One of the main requirements for the carbonyl-amino reaction is the presence of reducing groups, and these are readily provided by the oxidative degradation of polyunsaturated fatty acids in lipids.

General Discussion

Soybean curd has made a substantial contribution to nutrition due to its high content of protein and fat. However, the nutritional value of soybean curd protein is limited by low content of the sulphur-containing amino acids methionine and cystine. Sesame protein is rich in methionine. Almquist and Grau (1944) observed that lysine was the limiting amino acid in sesame protein and protein rich in lysine like soybean and fish proteins, supplemented sesame protein markedly. In the present study, sesame milk

or ground sesame was added in the preparation of modified soybean curd.

Since fresh soybean curd contains more than 80% moisture, it is perishable, fragile, and of course, difficult to transport for long distance. Thus more stable products should be developed. In this study, drying was used as a method of preservation. As in other methods of preservation, the problem encountered drying is the destruction of the constituents. It is very hard to make a dried product without any damage to the constituents. Three methods of drying were used. First, the products were directly air-dried which required 6-7 hours at temperature 60^{o}C. Second, before air drying the products were first frozen and aged for 2 weeks. Aging gave the products a porous sponge-like structure after thawing. According to Fukushima and Hashimoto (1979) this characteristic structure is brought about by the molecular interaction in the locally condensed protein solution among the ice crystals. The bonds responsible for the polymerization of soybean milk protein causing the characteristic structure might be hydrophobic bonds and S-S bonds. Due to the porous structure, the time required for drying was about one-half that of air drying i.e. 3-4 hours at a temperature of 60^{o}C. Third, the products were first frozen and were then dried in a freeze drier. Because the products were frozen and were at low temperature during freeze drying, 48 hours were needed to dry the products.

With regard to processing cost, the air-dried product was cheaper than either dried-frozen or freeze-dried products. However, in terms of quality such as color, appearance and ability to be reconstituted, the freeze-dried, dried-frozen and air-dried products were the best, medium and the poorest respectively.

The protein content of the dried products varied from 52.81 to 59.30% and the oil content also varied from 29.76 to 34.38%. These values were much higher than those of the original soybeans. The dried soybean curd was a concentrated source of proteins and lipids. Amino acid analyses indicated that most of the amino acids present in the dried products meet the patterns suggested by FAO (Anonymous, 1973) and Food and Nutrition Board, National Academy of Sciences (Williams et al., 1974). The content of saturated fatty acids in soybean curd was low. The essential fatty acid linoleic acid was present in about 50% of the total fatty acid in soybean curd. In addition, about 30% of total fatty acids were oleic and linolenic acid.

The most important deteriorative reactions in dried foods are lipid oxidation and carbonyl-amino reactions. There were two sources of carbonyl compounds present in soybean curds, viz., lipids and carbohydrates. Homma and Sakurai (1967) did not consider carbohydrates while studying browning of kori-tofu (dried-frozen soybean curd) since the presence of reducing sugar was negligible. Thus they

considered only lipid and their derivatives as active reactants for the browning reaction. They noted that the browning of kori-tofu is caused by reaction between protein and carbonyl compounds or other degradation products from lipids.

It is typical for legumes such as soybeans, peanuts, navy beans, cow peas etc., to have very low reducing sugars. Love and Dugan (1978) showed that instant navy bean powder contains only 0.2% total reducing sugars and they noted that the reducing sugars decreased during accelerated storage at 37^{o}C in both oxygen and nitrogen gas atmosphere even at low water activity.

In the present study, it was true that the presence of reducing sugar was negligible since glucose was only present in trace amounts. However, since the content of oligosaccharides in stored products were less than in unstored products, there was a possibility that these oligosaccharides were involved in the browning reaction. Many investigators (El-Nockrashy and Frampton, 1967; Frangne, 1972) noted that oligosaccharides react with amino acids although at slower rates than those of monosaccharides. In addition, there is the possibility that complex sugars may be broken under storage condition. Frangne (1972) also showed that sugar reactivity depends on the type of protein involved in which the same sugar caused different lysine losses according to whether the protein was lactalbumin or soybean globulins. El-Nockrashy and Frampton (1967) suggested that the reduction

in the availability of lysine in oilseed meals such as cottonseed meal, peanut meal or soybean meal was a consequence of reaction between the epsilon amino groups of lysine and the 1-2-glycosidic linkage in the non-reducing constituents of the seeds. The released monosaccharides from the reaction may readily react with alpha amino acids. Similarly, therefore, the involvement of non-reducing sugars in the dried soybean curds could be a possibility.

Lipid oxidation plays an important role in foods containing lipid because the products of oxidation may cause off flavor and changes in color. Lipid oxidation will be minimized in the presence of antioxidants in the systems. In dried soybean curds, there were many compounds that might act as antioxidants. Browning products such as reductones, dehydroreductones (Griffith and Johnson, 1957; Coonet et al., 1958; Evans et al., 1958) and melanoidins (Kirigaya et al., 1968) have been reported to act as antioxidants. Reductones are resonance-stabilized enediols, which are strong reducing agents in acidic as well as in neutral and alkaline aqueous media (Evans et al., 1958). In some respects, reductones behave in anhydrous fat systems as they do in aqueous systems (Cooney et al., 1958). Like other antioxidants, the activity of the reductones probably depends on the transfer of a hydrogen from the reductones to the chain-propagating free radicals to form inert products. The transfer of hydrogen results in a chain termination step. According to Dugan

(1963), peroxy radicals predominate in the termination step, both in natural and antioxidant terminations. Since peroxy radicals are precursors of peroxide, the reactions thus prevent peroxide formation. Kirigaya et al. (1968) showed that browning reaction products inhibited formation of peroxides and/or carbonyl compounds although on detailed reaction mechanism was unknown. Since the browning products are more soluble in water than in lipids, the lipid water interface must be taken into consideration as one of the places where they act as antioxidant. Morita et al. (1976) showed that both extractable and non-extractable components of browning products by ethyl acetate inhibit oxidation at a water-lipid interface while only the extractable components inhibit the oxidation in the lipid phase. The fact that products with added ground sesame which had higher browning, had a lower rate of lipid oxidation, suggested that these browning products might have a role in minimizing lipid oxidation.

Another possible antioxidant present in soybean curd with added sesame is sesamol, a characteristic antioxidant in sesame seeds. However, its carry-through properties was still questionable although it was reported that oil processing conditions such as bleaching and hydrogenation may lead to an increase in the content of free sesamol. The increase is caused by splitting of free sesamol from its bound form, sesamolin (Budowski, 1950).

L values of products did not change appreciably throughout a storage period of 8 weeks at 37°C. The greenness decreased while the yellowness increased for air-dried products but remained essentially constant for dried-frozen and freeze-dried products. However, at 50°C the lightness decreased, the greenness disappeared while the redness and yellowness increased. Loncin et al. (1968) noted an increase in yellow index of milk powder kept at 40°C for 10 days. This suggested that browning might be occurring at the same time as bleaching of coloring matters present in the soybean curds. A similar situation was observed by Martinez and Labuza (1968) who reported a decrease in the lipid soluble pigment astacene during storage of freeze-dried salmon.

High temperature accelerated both lipid oxidation and browning. Consequently the color became darker and a greater loss of available lysine was observed. This agreed with the report by Daza (1979) who noted that thermal losses of available lysine in soybean flour, concentrate and isolate should be considered when processing is planned since temperature had a positive effect on the reaction rate. Greater loss of available lysine with increasing temperature has also been observed in heated fortified rice meal by Tsao et al. (1978).

It was noted that dried-frozen and freeze-dried products had relatively poor stability with respect both to lipid oxidation and non-enzymatic browning. Both freeze-dried and

dried-frozen soybean curds had a porous sponge-like structure. The porous structure in the products might lead to greater accessibility of oxygen. This would then cause lipid oxidation and production of carbonyl compounds which in turn could be involved in carbonyl-amino reaction. In addition, although most of the pathways in non-enzymatic browning are oxygen independent, there is a part of Strecker degradation (Hodge and Osman, 1976) that requires oxygen. Although Strecker degradation is not primarily concerned with pigment production, it provides reducing compounds essential for their formation. The oxidative degradation of amino acids carried out during the Strecker degradation results in the formation of the corresponding aldehyde containing one carbon atom less, lost as carbon dioxide. Since oxygen was probably the main deteriorative factor, these products should be protected from oxygen to minimize oxidation. In other words, good packaging is necessary for these products.

It is noted that all samples had BET monolayer values corresponding to water activities between 0.16-0.22. Freeze-dried products had lower hysteresis values than dried-frozen products which in turn had lower values than air-dried products. This fact suggested a possible use of hysteresis as a tool for measuring quality deterioration of dried products. This was consistent with the degree of lipid oxidation measured after drying in which the lowest occurred for freeze-dried products, intermediate for

dried-frozen products and the highest for air-dried products.

Dried soybean curds with added sesame milk were most stable at a water activity of 0.22 with respect both to non-enzymatic browning and lipid oxidation. Water present as monolayer is tightly bound and can not act as an aqueous-phase reaction medium. Thus the rate of non-enzymatic browning, which requires water as a solvent, was quite slow at this water activity and below it. Just above the monolayer, solutes could become mobile, but the rate of movement was still slow (Duckworth, 1962). As the a_w increased, phase viscosity decreased (Lea and Labuza, 1975) and a distinct rapid mobilization point became evident. This increased the browning rate observed at water activities of 0.43 and 0.62. At a water activity of 0.75, the rate of browning was less than at 0.62 due to a dilution effect on the reactants.

The rate of lipid oxidation in the products increased on both sides of the monolayer value of the water activity range. Therefore, water had an effect upon the rate of reaction in the lipid phase. This, as noted by Labuza (1980) may be attributed to "(1) change in hydration of trace metal catalysts which become more active (decrease activation energy) as they lose water, (2) changes in availability and mobility of metal catalysts which can move to the lipid interface and increase oxidation rates, (3) hydrogen bonding of peroxide intermediates at the aqueous

phase, taking them out of reaction and (4) increasing the rate of reaction of free radicals with other species, such as protein, in the aqueous phase."

In this study, lipids apparently were involved in the browning reaction. Lipid oxidation caused a decreased U/S ratio due to the oxidation of unsaturated fatty acids present in the products. It is known that lipid oxidation causes production of carbonyl compounds. Homma and Sakurai (1967) and Homma et al. (1969) had identified benzaldehyde, methyl ethyl ketone, crotonol, acetaldehyde, alpha-keto-nonanal, alpha-keto-heptanal, hexane-2,3-dione, alpha-keto-pentanal, alpha-keto-butanal, pyruvaldehyde, and glyoxal in the ether extract of browned kori-tofu. Many of these carbonyls are very active in browning reactions (Burton et al., 1963).

Samples with added ground sesame had greater browning and greater loss of available lysine than other samples. Lipid oxidation rates in these products were less than those in the others. Dark color and other undesirable characteristics such as high fiber contents, presence of oxalate and selenium have been noted in oil-free sesame meal by Guerra and Park (1975). Adrian (1974) noted that sugar reactivity depends on the type of protein involved. The higher browning in samples with ground sesame might be due to the increased reactivity of sugar in the presence of sesame protein. It was noted that for the same drying method, samples with added ground sesame had slightly higher

loss of sucrose than the others during storage. There seemed to be no significant difference in raffinose and stachyose content between products with and without added ground sesame.

The Maillard reaction may involve amino acid blocking and loss. Homma et al. (1971) noted, that radicals formed from lipid oxidation might easily attack the sulfur containing groups, aromatic rings and imidazole groups of amino acid residues of protein. In the present study, it was apparent that some lysine was rendered unavailable. Since available lysine reflects protein quality, the reduction in available lysine content during storage caused a decrease in protein quality. This was especially true when the storage was conducted at high temperature and/or high water activities.

From a nutritional point of view, the fate of essential amino acids such as lysine is very important. Adrian (1974) noted that damage to certain amino acids such as lysine caused a protein to have a different amino acid balance, making restoration of protein quality very difficult. Moreover, numerous observations showed that the Maillard reaction involved phenomena that prevent complete restoration of protein damaged by heat treatment.

The addition of sesame, as expected, increased methionene content of the products obtained. From a nutritional point of view this is important since methionine is the

limiting amino acid in soybeans. The addition of ground sesame provided some additional stability while the addition of sesame milk had no effect upon the stability with respect to lipid oxidation of the products.

Lipid oxidation can cause off-flavor and a decrease in nutritional value in terms of the loss of essential fatty acids. Browning reactions are also deleterious to nutritional value of the products as well as cause a change in color. In the present study, it is clear that these reactions can occur during processing as well as during storage of the dried products. It is, therefore, imperative to arrest these reactions, thereby not only preventing nutritional changes but also other changes such as flavor and color which might render the products unacceptable to the consumer. A combination of lowering storage temperature and conducting the storage at water activities corresponding to monolayer together with appropriate packaging should be able to minimize these deteriorations.

As a member of developing countries, Indonesia has emphasized the utilization of legumes to meet the protein needs of its population. Soybean curd is by far the most important way of using soybeans as a daily food. However, growing incomes have created the need for convenience foods such as prepared by drying. Since the dried soybean curd is neither perishable nor fragile, it is well suited to centralized, large-scale production and nationwide

distribution. It is true, however, that high temperature and high relative humidity exist in Indonesia and these cause problems in the storage of dried products. In this study, it is clear that both lipid oxidation and non-enzymatic browning proceed faster at high temperature and high relative humidity. Therefore, the dried products must be carefully packaged to prevent any quality and nutritional losses. The package must act as a barrier to both oxygen and water.

SUMMARY AND CONCLUSIONS

Soybean curd and its forms modified by addition of either sesame milk or ground sesame were made and air dried, or frozen and aged for 2 weeks then thawed and air dried or freeze dried.

Proximate analyses indicated that addition of sesame milk did not appreciably alter the composition of the products obtained. However, the addition of ground sesame resulted in products with less protein but higher fat content. Gas liquid chromatography indicated that addition of ground sesame altered fatty acid composition to a state of less unsaturation. The method of drying did not seem to have a significant effect on the proximate analysis of the various products. Most tocopherols present in soybeans were lost during processing of soybean curds. Amino acid analyses indicated higher S-containing amino acids content of soybean curd with added sesame.

The dried products had BET monolayer values corresponding to water activities of 0.16 to 0.22 or 3.42 to 4.64% moisture (dry basis). Freeze-dried products had lower hysteresis than dried-frozen products which in turn had lower hysteresis than air-dried products.

The stability of the dried products with respect to lipid oxidation and non-enzymatic browning were studied during accelerated storage at 37°C and 50°C. Peroxide value, TBA and diene conjugation tests were used to follow oxidative changes of lipids. Freeze-dried, dried-frozen and air-dried products had the lowest, intermediate and the highest in both initial lipid oxidation and initial browning index at the beginning of storage. Peroxide value and diene conjugation tests were found to be good methods for monitoring lipid oxidation in these products. However, the TBA test was found not to be a good indicator for lipid oxidation presumably due to the involvement and loss of malonaldehyde in carbonyl-amino reactions. Both peroxide value and diene conjugation continuously increased during storage at 37°C and 0.75 a_w. However, at 50°C and 0.75 a_w, both peroxide value and diene conjugation reached maximum values at 4 weeks storage and then declined with further increase in time of storage.

Water activity had a significant effect on the rate of lipid oxidation and browning. The rate of lipid oxidation increased on each side of the BET monolayer value in the water activity range. The rate of non-enzymatic browning increased with increasing water activity up to 0.62 and then declined at water activity greater than this. Both the rate of lipid oxidation and browning increased with increasing temperature.

No appreciable changes in lightness of these products were detected by color measurement during storage at 37°C and 0.75 a_w. However, the greenness seemed to decrease. The yellowness increased in air-dried products but remained essentially constant in dried-frozen and freeze-dried products. When storage was conducted at 50°C and 0.75 a_w, the lightness decreased, the greenness disappeared, while the redness and the yellowness increased.

During storage, blocking of the amino acid lysine occurred and rendered it unavailable. The blocking was affected by temperature, water activity and time of storage. The loss of available lysine increased with increasing temperature or time of storage. The loss of available lysine over the water activity range was similar to the change in non-enzymatic browning.

Both lipids and carbohydrates might be involved in non-enzymatic browning. The involvement of sugar might be due to aminolysis of 1-2- and/or 1-6-glycosidic linkages of oligosaccharides. The liberated monosaccharides could then react readily with amino acids. The involvement of lipids might include reactions between various carbonyl compounds with amino acids.

Oxygen might be the most important factor determining the stability of the products in terms of lipid oxidation and non-enzymatic browning. It was observed that products with high accessible oxygen as in freeze-dried and

dried-frozen products had relatively poor stability.

Addition of sesame milk did not seem to have an appreciable effect on the stability with respect to lipid oxidation and non-enzymatic browning. However, samples with ground sesame exhibited some additional stability with respect to lipid oxidation. Samples with ground sesame had greater browning immediately after drying and the subsequent storage.

In conclusion, porous sponge-like products such as freeze-dried and dried-frozen products need more protection against oxygen attack than air-dried products. A good packaging is necessary of all these products. Storage at water activities of 0.22 and lowering the temperature during storage of these products can help to minimize both lipid oxidation and non-enzymatic browning.

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