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DEVELOPMENT AND PACKAGING OF A SOYNUT BUTTER

By

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ABSTRACT

DEVELOPMENT AND PACKAGING OF A SOYNUT BUTTER

By

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Expanding upon the work of Pichel and Weiss (1967), the development of a commercially viable soynut butter was pursued. The objectives were to determine the required processing parameters, optimum packaging and antioxidant combinations needed during distribution. Sensory evaluation studies indicated that consumers preferred smooth soynut butter containing 3.0% fructose. Stabilization of processing temperatures was achieved through the addition of 25% vegetable oil per batch, a 5-minute pre-cutting step, and a consistent rate of delivery of the pre-cut mix into the final grinding process. Product stability was assessed by conjugated diene absorbance, peroxide and thiobarbituric acid (TBA) values. Results showed that samples containing 0.02% TBHQ/CA had slower rates of autoxidation than control samples within the first two months of storage. High density polyethylene tubs generally provided protection equal to that afforded by glass upon the addition of antioxidant to the product.

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Romans 8:32 (NIV)

"He who did not spare His own son, but gave Him up for us all, how will He not also, along with Him, graciously give us all things?"

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Like the peanut the soybean is a legume, however the soy bean is richer in various amino acid residues. It was thought that the food-buying public would be receptive to a nutritious soynut spread, and that such a development might have a significant impact on the utilization of Michigan-grown soybeans.

The production of a nut-butter from soybeans was described by Pichel and Weiss (1967), but the questions of optimum antioxidant and package combinations were not studied. Optimum processing parameters for small-scale batch production, and optimum sweetener levels also remained unanswered. The present study was designed to gain knowledge and to propose future courses of action for the processing and packaging of a commercially acceptable soynut butter. Peroxide value, TBA value, conjugated diene and TBA (distillation) absorbances were measured to follow autoxidative changes in the sample spreads. Four antioxidant blends were evaluated: BHA/BHT, BHA/PG, TBHQ, and TBHQ/CA. Three package systems - glass jars, high density polyethylene (HDPE) tubs, and tubs in evacuated nylon/Saran^{R} pouches - were used to study effects of differing barrier on product stability.

Sensory evaluation of added sweeteners according to type and concentration was accomplished using two tests. The first evaluation utilized a degree of preference test to measure the ability of respondents

to differentiate statistically significant differences in concentrations of various sweeteners. The second evaluation was a degree of preference test which is a hedonic measurement.

LITERATURE REVIEW

Soybeans as Food

The soybean is one of the earliest crops cultivated by man, some 4,800 years ago in China. The legendary emperor of China, Shang Nung, taught his subjects how to use a plow and sow grain for harvesting (Shih, 1918). The first historical reference of soybean cultivation is 2,207 B.C., as there was no accurate dating system prior to this time (Smith, Circle, 1978). Soybeans were prescribed in a materia medica around 450 A.D., as a drug (Smith, Circle, 1978) and are closely associated with Buddhism. They constitute a major protein source in the diet of Chinese today.

The soybean of commerce is the variety Glycine Max (L.) Merr., although there are approximately 100 named varieties which have been registered with the Crop Science Society of America. There are three major U.S. markets for soybeans: raw beans, soybean oil, and soybean meal. In 1979 there were 61 million metric tons of soybeans produced by more than 650,000 farmers in 27 states (Ray, 1981). America has become the major exporter of beans to world markets, and is responsible for 80% of the beans involved in international commerce (Smith, Circle, 1978).

Most of the soybean crop in the U.S. is solvent extracted since soybean oil is in great demand for use in margarine, shortenings and as a salad oil. The residual cake is processed into high protein animal feed. In Asia soybeans are an important ingredient in many foods for

human consumption and are often utilized following fermentation. The young green pods are sometimes eaten as a vegetable (Elsevier, 1981). Defatted soy-flour is the starting material in the production of soy protein isolate, the collection of soy proteins precipitated at low pH. Soy-protein isolate may subsequently be textured and processed to simulate meat and poultry foods in chewiness and flavor (Smith, Circle, 1978). The protein is "spun" and the emerging fibers are coagulated in an acid bath, and stretched to a desirable size and strength (Campbell, 1981). Upon the addition of various binders, fats, flavorings and colors, the product is heat set to form the finished meat analog (Campbell, 1981). The concept of a total soy-protein replacement for meat has failed because the analogs were as expensive as the meat they replaced, and lacked the flavor of real meat.

Nutritive Value of Soybeans

The soybean seed consists of proteins, lipids, carbohydrates, minerals and crude fiber (Krober and Carter, 1962). Sixty percent of the seed consists of protein and lipid. The majority of the protein resides in the aleurone bodies, which are subcellular structures between 2-20 microns, and which account for 60-70% of the protein in the seed (Orthoefer, 1978). The lipid components are found in subcellular structures called spherosomes, and are 0.2-0.5 microns in diameter.

The soybean has the following approximate whole bean composition: (Krober and Carter, 1962).

Protein	40%
Lipid	20%
Cellulose and hemicellulose	17%
Sugars	7%

Crude fiber	5%
Ash (dry weight basis)	6%

The proximate composition of soybean components as found in seed parts (Wolf and Cowan, 1971) are:

Seed part	Percentage of whole bean	Percent composition of soybean components			
		Protein	Fat	Carbohydrate	"ash"
Cotyledon	90	43	23	29	4.9
Hull	8	9	1	86	4.3
Hypocotyl	2	41	11	43	4.4

Defatted soya flour is very high in most of the essential amino acids. Soy protein isolate is rich in isoleucine, leucine, lysine, and valine as compared to peanut isolate.

g/100 g protein (W.H.O. Tech. Rep. Ser., No. 37 (1965))

AMINO ACIDS:	Soy Isolate	Peanut Isolate
Tryptophan	1.2	1.0
Threonine	3.8	2.5
Isoleucine	4.7	4.3
Leucine	7.5	6.7
Lysine	6.2	3.0
Methionine	1.4	1.0
Cystine*	1.0	1.2
Phenylalanine	4.8	5.6
Tyrosine*	3.6	Not reported
Valine	5.0	4.5

*Not essential but often limiting or lacking.

Protein Efficiency Ratio (PER) is one method of determining protein quality. PER = weight gain ÷ weight protein consumed and is the only method currently approved by the Food and Drug Administration for nutritional labeling of protein quality of foods (Labuza, 1977). Two procedures commonly used to measure the degree of protein denaturation in soya are: the nitrogen solubility index (NSI), and the protein dispersibility index (PDI). The NSI value measures the percentage of the total nitrogen in the sample that is soluble; the PDI value, the percentage of total protein soluble in a sample. In the evaluation of a food protein, anti-nutritional factors such as trypsin inhibitors, hemagglutenins and phytic acid must also be considered (Nelson, Steinberg, Wei, 1978).

The primary function of a protein is to furnish the essential amino acids and nitrogen required for the normal function and growth of an organism (Hopkins, Steinke, 1981). There are many ways to measure this "protein quality" in humans such as weight changes, nitrogen balance, serum protein concentrations in the blood and growth rates. Nutritional studies have shown that there is a favorable nutritional impact of soybean protein as a component of a mixed diet or as a protein supplement (Ibid). When combined with cereal grains in the diet, a more favorable amino acid complement is achieved.

The overall protein intakes of lower socioeconomic groups in developing countries are often deficient for proper nutrition. Calorie intake may also be deficient among such groups. In developing countries that have cereal-based diets, there has been a marked decrease in the availability and consumption of legumes. Soybeans have contributed to food systems as sources of calories, as supplementary protein and as

complementary protein because of their relatively good essential amino acid pattern (Bressani, 1981).

The future of soy protein supplements in the diets of developing populations is still in doubt. The major obstacles are not only political, but also cultural, economic and technical. Many countries import U.S. soybeans for animal feeding but do not yet have the available technology to make better economic use of the protein for human consumption. Soy products are just beginning to become popular in the U.S. and may become more accepted worldwide if people will accept the flavor of soy products and alter their social and cultural habits of eating.

The lipid components of soy include triglycerides, phospholipids, lipid-soluble pigments, tocopherols and sterols. Soybean oil contains a significant level of linolenic acid (Pryde, 1980). This polyunsaturated acid is readily oxidized by enzymatic, thermal, photo- and metal catalysed autoxidation with the production of highly undesirable reversion or rancid flavor and odor. The development of flavor reversion is characteristic of soybean oil and other linolenate-containing oils and occurs at low levels of oxidation. The flavor of reverted soybean oil is described as beany and grassy at the early stages and as fishy or painty at the more advanced stages (Frankel, 1980). Chang et al. (1966) attributed the flavor of reverted soybean oil to α-pentyl furan, which they assumed to be derived from linoleate oxidation. Another linolenic acid derivative implicated as a cause of flavor reversion are oxidative polymers. Oxidative polymers are a complex mixture of oxygen-containing compounds formed by polymeric decomposition of linolenate hydroperoxides. These high molecular weight compounds can easily decompose and generate volatile aldehydes and other

compounds contributing to flavor deterioration occurring even in the absence of oxygen and low temperatures (Frankel, 1980). Dimeric compounds with carbon-carbon linkages between fatty acid molecules were produced by thermal decomposition of linolenate hydroperoxides (Frankel et al., 1960). A mixture of allylic 14-, 15-, 16- and 17-hydroperoxides would be produced from the free radical oxidation in a fatty acid with a single double bond at carbon 15. Decomposition of these hydroperoxides would produce a mixture of aldehydes and alcohols (Frankel, 1980) (Figure 1). These compounds have been cited among the more than 70 compounds identified in oxidized soybean oil (Selke et al., 1970). Crude soya oil contains substantial amounts of natural antioxidants such as tocopherols and phospholipids (Buck, 1981). The principal phospholipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol (Orthoefer, 1978).

Soybeans contain approximately one-third carbohydrate which consists primarily of the sugars sucrose, raffinose, and stachyose (Ibid). The insoluble polysaccharides consist of galactomannans, acidic polysaccharides, xylan hemicellulose, and cellulose (Ibid). The seed coat is the major site of insoluble saccharides and lignins. Also present in soy are various enzymes, of which lipoxygenases are of importance from the aspect of flavor deterioration.

Lipoxygenases catalyze the oxidation of unsaturated fatty acids containing 1,4-pentadiene systems in the cis configuration (Ibid). Other lipid related enzymes present are lipoperoxidase and lipases. Proteases are also present, and they cleave interior peptide bonds of proteins (Ibid). Alpha and beta amylases are present, but have an unknown effect as mature soybeans reportedly do not contain starch.

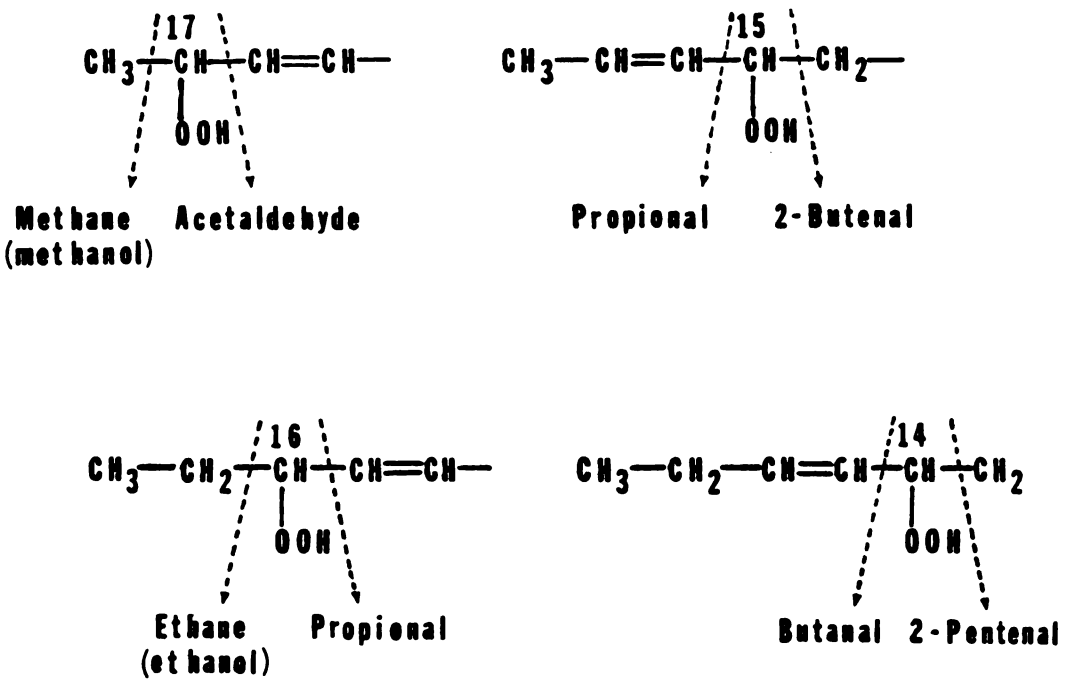


Figure 1. Decomposition of linolenate dimer hydroperoxides (Frankel, E.N. Prog. Lipid Res. 19:1 (1980) p. 16)

Peanut Butter Technology

Before discussing the manufacture of soynut spread, it is helpful to look at the processing of peanuts into peanut butter. Peanuts are the fruit or pod of Arachis hypogaea of the Leguminosae family. The annual per capita consumption of peanuts in the United States exceeds eight pounds in terms of a farmers' stock sold in the shell (Woodroof, 1981). Consumption of peanut butter per capita has increased from about 2½ lb in 1950, to 3½ lb in 1970, and about 4 lb in 1980 (Ibid).

The U.S. Department of Agriculture has set standards for grades of peanut butter in Section 52.3061-.3073. Legal peanut butter is a "cohesive, comminuted food product prepared from clean, sound, shelled peanuts by grinding properly roasted mature peanut kernels from which the seed coats have been removed and to which salt is added as a seasoning agent", U.S. standard for grades (1962). There are three defined textures of peanut butter: smooth, regular, and chunky. "Smooth" peanut butter means that there is a very fine and even texture with no perceptible grainy peanut particles. "Regular" peanut butter has a definite grainy texture with perceptible peanut particles not more than 1/16 in. in any diameter. "Chunky" peanut butter has partially fine and partially grainy particles with substantial amounts larger than 1/16 in. in diameter. Three grades of peanut butter exist: U.S. Grade A, U.S. standard, and substandard, which fails to meet the requirements of U.S. standard.

Peanut butter manufacture consists of shelling, dry roasting, and blanching the peanuts, followed by fine grinding (Woodroof, 1981). Peanut butter may be produced from any variety of peanuts, but for

optimum consistency a blend of two parts Spanish or Runner peanuts with one part Virginia peanuts is suggested (Ibid). By federal regulations, 90% of peanut butter must be peanuts. Artificial flavors, artificial sweeteners, chemical preservatives, natural or artificial color, purified vitamins, or minerals are prohibited. The fat content may not exceed 55% including the natural oil in the peanuts. Hydrogenated vegetable oils are added in small amounts as emulsifiers to prevent oil separation, and to improve spreadability. Dextrose, sugar, or honey are the commonly used sweeteners (Ibid).

Peanuts may be dry roasted by one of two methods: batch or continuous. Batch-roasted peanuts are roasted in 400-lb lots in a revolving oven heated to a temperature of 800^{o}F. The peanuts are heated to 320^{o}F (Beattie, 1936) and held for one hour to achieve a satisfactory roast. All the nuts in the batch must be uniformly roasted so that there is complete development of color from the center to the surface of each kernel, without scorching, excessive oiliness, or decomposition of surface fats (Woodroof, 1981). Continuous roasting involves a conveyor-fed batch of peanuts with a counter-current flow of heated air. The peanuts are constantly agitated to improve the transfer of heat and extraction of moisture and volatiles (Ibid). The moisture content is reduced from an average of 5.0% to 0.5%. The color change is due to absorption of lipid by the cell walls. The roasted peanuts leave the roaster and are discharged into a perforated metal cylinder, where cooled air is forced through the mass by suction fans.

The next step in the peanut butter making process is dry blanching, which removes the hearts and skins. The peanuts are passed through

the blancher in a continuous flow and brushes or ribbed rubber belting remove the skins. The skins are blown into porous bags and the hearts are separated from the cotyledons by screening (Woodroof, 1981). The blanched nuts are inspected and screened to remove scorched and rotten nuts. Discolored peanuts are removed by electric eye, and metal parts by magnets (Ibid). Peanut butter is usually made by two grinding operations. The first grinding reduces the nuts to a medium ground texture. The final grind gives a smooth and fine texture to the peanut butter. The nuts are forced between the grinding surfaces by an impeller mounted on a rotor shaft. Openings between the rotor and stator are from 3-5 mils for regular peanut butter (Weiss, 1983). The peanuts must be kept under a constant pressure from the start to the finish of the grinding process to assure uniform grinding and protect the product from air bubbles. Swept-surface heat exchangers such as the Votator™, are used to cool peanut butter from 170° to 120°F, before it is packaged. Oil separation is determined primarily by the nature and amount of crystals present in the peanut butter. One procedure for preventing separation involves shock-chilling the hot peanut butter to produce finely divided crystals, followed by a slow tempering process (Woodroof, 1981).

Heat processing of peanuts will generally improve the flavor, aroma and texture, but will reduce the shelf-life of the oil by destroying natural antioxidants such as tocopherols. At the time of manufacture the oil in peanut butters remains relatively stable to oxidative rancidity (Willich et al., 1954), and seems to remain stable after storage in the dark at 80°F for two years. It has been observed that

autoxidation initially proceeds rapidly for about 3 months, or until available internal and headspace oxygen is depleted, whereupon stability remains relatively constant for more than two years (Freeman et al., 1954). Methods for preventing autoxidation in peanut butter include evacuating the headspace above the product or by addition of an approved antioxidant or antioxidant blend to function as a free radical terminator. Light, especially shorter wavelengths, also catalyzes lipid oxidation in peanut butter. Experimental data (Woodroof, 1981) indicated a measurable difference in flavor of peanut butter stored in the dark, or in amber, green, or blue colored jars. The differences noted are reduced oil separation, better aroma and flavor, but no major differences in peroxide or other chemical determinations of lipid oxidation. In 174 samples, free fatty acid values (FFA) averaged 0.373 for samples stored in the light and 0.345 for those in dark; the peroxide values averaged 4.06 for samples in the light and 4.05 for those in the dark (Ibid).

Most peanut butter is packed in glass jars for retail distribution. Opaque polyethylene and transparent XT polymer jars and polystyrene cups with polyethylene lids have also had limited use (Anon., 1950; Anon, 1965; Russo, 1968). The shelf-life of peanut butter in plastic containers is about 9 months to one year. In glass jars the normal shelf-life is about two years (Weiss, 1983). The decreased stability is due to permeation of oxygen through the plastic jar wall which results in increasing off-flavors. Another defect in peanut butter known as "pull-away" occurs when the butter loses its ability to adhere to the wall of a glass jar. Moisture in the processing area will cause jars to become damp and accentuate the pull-away. Jars for peanut

butter are designed without a shoulder, which would act as a starting point for pull-away. The bead at the rim of the jar must be above the peanut butter surface for the same reason as stated above (Ibid).

Soynut Butter Technology

The roasting of soybeans to make a "soynut" product began in the 1940's. Soynuts became a specialty item on U.S. candy counters (Heller and McCarthy, 1944). Soynuts are prepared at home by oven-roasting soybeans in soybean oil, and with the addition of a little salt they have been claimed to compare favorably in taste and texture with more expensive nuts. Compared to the peanut, the soynut has substantially less fat, more protein and are less expensive than peanuts. Soybeans generally have a characteristic undesirable "beany" flavor due to early low level development of unusual compounds from autoxidation (reversion) unlike peanuts which have a desirable "nutty" flavor and aroma. Pichel and Weiss (1967) attempted to overcome these problems in the process for preparing a nut butter from soybeans. Roasting or frying alone naturally does not modify this off-flavor since the reactions will have occurred prior to heating. Pichel discovered that by treating the dehulled soybeans with hot water, either by soaking or by steaming, practically eliminated the "beany" or "grassy" flavor without rendering the product tastless. This may be due in part to thermal inactivation of lipoxygenases. After moisturizing the beans, they were fried in oil and reduced to a fine paste. Pichel also found that he could achieve a greater throughput rate and smoother butter by comminuting whole fried beans with sufficient added oil in an Urschel Micro-cut mill with a shaving head (Weiss, 1983). The palatability of the soybean spread may

be enhanced by incorporating sugar, salt, hardfat stabilizer and oil. The enhanced spread is smooth, homogenous, with no noticeable "beany" or "grassy" flavor. The final soynut butter has a consistency similar to that of peanut butter (Pichel, 1963).

Commercial soynut butter spreads to date have not been widely accepted due to continuing tactual and flavor defects. Consumer demand for a soynut butter spread appears to be greatest in the health-food and specialty market. The use of soynut butter as an ingredient for confectionary items has begun recently. The idea for the research reported in this thesis originated from early interest of a Michigan-based soybean processor to create various products based on the attrition or grinding of Michigan produced soybeans and flavored with various additives. The reasoning behind such research is that the food-buying public would be receptive to a nut butter spread with a more complete amino acid array and that soybeans are lower in cost than peanuts. If a commercially acceptable, flavored, soy spread could be developed, it could have a significant impact on the utilization of Michigan-grown soybeans.

Mechanism of Oxidative Rancidity

The oxidation of lipids, both mechanisms and oxidation products, has been well reported in numerous scientific papers. For this research a brief review of mechanisms of autoxidation, oxidation products, and antioxidants is presented. Rancid off-flavors are produced through autocatalytic processes with oxygen in a refined fat or oil. This type of rancidity is known as oxidative rancidity. Off-flavors produced through reactions catalyzed by lipases from food or from microorganisms is known as hydrolytic rancidity. Oleate, linoleate, linolenate and other polyunsaturated fatty acids are the lipid moieties responsible for the formation of products of lipid oxidation (Labuza, 1971). Lipid autoxidation proceeds through a free-radical chain mechanism involving initiation, propagation, and termination steps. These steps can be schematically represented by:

Initiation

$$RH + O_2 \xrightarrow{\text{initiators}} R^{\cdot} + {}^{\cdot}OOH$$

(fat molecule) (free radical)

Propagation

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$

(peroxy free radical)

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

(hydroperoxide)

Termination

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

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

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow ROOR + O_2$$

In this presentation RH refers to any unsaturated fatty acid in which the H is labile by reason of being bound to a carbon atom alpha to a double bond. Various agents such as radiation and heavy metals like copper ion are the principal initiators of autoxidation. The primary products of lipid oxidation are hydroperoxides (ROOH) which are generally known simply as peroxides. The oxidation process becomes extremely complex as the peroxides undergo scission, dismutation and other interactions through thermal instability or reactions with other materials to form more free radicals (Dugan, 1961). Secondary reaction products involving hydroperoxides include alkoxy free radicals which dismutate to give numerous aldehydes, ketones, and alcohols varying in carbon chain length. It is these secondary reaction products which are primarily responsible for the off-flavors in oxidized polyunsaturated lipid systems. Different hydroperoxides are formed when light and photosensitized oxygen molecules are present. The production of singlet oxygen is believed to be the mechanism for the production of allylic hydroperoxides in which the double bond has been shifted (Hamilton, 1983). The off-flavors produced from photooxidation are sometimes characterized as having a "grassy" aroma and flavor.

Measurement of Lipid Oxidation

The acceptability of a food product depends on the extent to which the oxidative deterioration has occurred. There are five known stages of autoxidation of a fat; the induction period, peroxide formation, peroxide decomposition, polymerization, and degradation stage. In the induction period hydroperoxides increase very slowly in amount and are not measurable. At the end of the induction period, there is a sudden

increase in peroxide content. Hydroperoxide concentration will reach a maximum, then decrease as a result of peroxide decomposition. As oxygen is absorbed total carbonyl content increases as well the viscosity of oils. Monitoring the various precursors and products of autoxidation at each of these stages is accomplished with different chemical tests. Susceptibility tests measure the stability of a lipid under conditions favor oxidative rancidity and include tests such as the Schaal oven test and active oxygen methods. Some of the tests which measure the extent of oxidation in a lipid system include: peroxide value, anisidine value, thiobarbituric acid test, Kreis test, conjugated diene absorption method and sensory evaluation.

Peroxide Value

Measurement of peroxide value is useful up to the stage at which extensive decomposition of hydroperoxides begins. Many analytical procedures for the measurement of peroxide value have been developed. In all cases the accuracy of the test depends on the specific experimental conditions, as the method is highly empirical (Rossell, 1983). The most common procedures are iodometric methods developed by Lea (1931) and Wheeler (1932). The method is based on the measurement of the iodine liberated by oxidation of potassium iodide by the peroxides present in the oil. The iodine is liberated in a stoichiometric ratio of two atoms of iodine for each atom of active oxygen in the system. The iodine is titrated with sodium thiosulfate in the presence of soluble starch indicator. The peroxide value (P.V.) is expressed in milliequivalents of "peroxide oxygen" per kilogram of fat. The assumption is that a high P.V. indicates that oxidation has begun, and the fat is in or

past the induction period and oxidation will accelerate rapidly (Gunstone, Norris, 1983). Mehlenbacher (1961) has suggested the two principal sources of error in these methods are (a) the absorption of iodine at unsaturated bonds of the fatty acid, and (b) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated. Lea (1931) attempted to eliminate this latter error by filling the sample tube with nitrogen at the beginning of the test and making the assumption that the vaporization of chloroform would prevent the re-entry of oxygen into the tube. Other possible sources of error in iodometric methodologies include variation in weight of sample, the type and grade of solvent used, and variation in reaction conditions such as time, temperature, and reactivity of the peroxides being titrated (Gray, 1978).

Anisidine Value

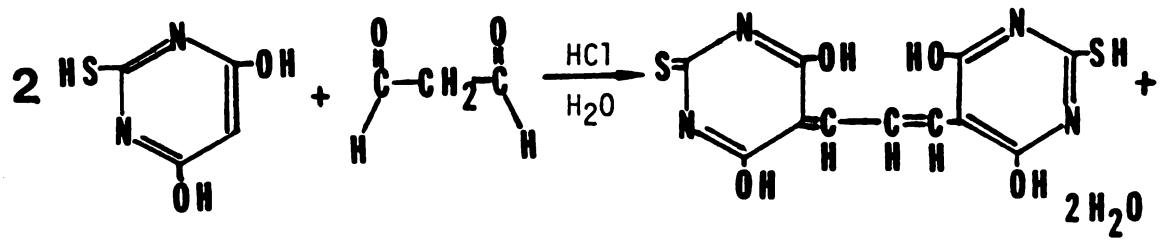
This method is used for the measurement of high molecular-weight carbonyl compounds, which indicate the prior oxidative deterioration of a fat. The IUPAC standard method II.D.26 defines anisidine value (A.V.) as 100 times the absorbance of a solution resulting from the reaction of 1 g of fat or oil in 100 ml of a mixture solvent and p-anisidine, measured at 350 nm in a 100 mm cell under the conditions of the test. A fat that had undergone extensive oxidation could show a low P.V. after deodorization, but the A.V. would presumably be little changed, and would warn of this past history. The A.V. is seldom used in the U.S. because most oils are domestically produced and fresh (Gunstone, Norris, 1983).

Thiobarbituric Acid (TBA) Test

The Thiobarbituric Acid (TBA) test is a common method for the detection of lipid oxidation. Early studies by Sinnhuber et al.

helped to clarify the nature of the colorimetric reaction that occurs during the TBA test. Two moles of 2-thiobarbituric acid condense with one mole of malonaldehyde to yield a red pigment. The intensity of the red (pink) color produced is proportional to the amount of malonaldehyde in the oxidized oil and within a certain range can be linearly related to the extent of oxidation of the fat (Figure 2). The main absorption maximum is at 532∿535 nm.

Proposed TBA reaction (Sinnhuber, 1958)



TBA Malonaldehyde TBA chromagen (pink-red)

Figure 2. Proposed TBA reaction (Sinnhuber, 1958).

Malonaldehyde is a dicarbonyl compound formed in oxidized polyunsaturated lipid systems. A disadvantage of the TBA test is that malonaldehyde can combine with amino acids such as lysine to form a Schiff's base, an intermediate step in non-enzymatic browning. The obvious limitation is that not all the malonaldehyde produced will be available to react with the thiobarbituric acid to produce the pink chromagen. Another limitation is that oxidative rancidity in lipids containing little or no fatty acids of the linolenate or higher unsaturation would not be expected to show significant TBA values even though these lipids gave a high peroxide value (Sinnhuber, Yu, 1977). Pohle et al. (1964) found that flavor score

could not be estimated for any given fat from the TBA value since the relative level varied from product to product. The change in flavors in an oil and their relationship to TBA value would have to be established before the TBA value could be used as an index of off-flavor development (Gray, 1978).

Distillation of Malonaldehyde

In 1955 Sidwell et al. described a steam distillation procedure for dried milk in which the malonaldehyde was distilled from the acidified milk. An aliquot of the distillate was then reacted with TBA, and the color was read directly. Tarladgis et al. (1960) described a simplification of Sidwell's technique by directly heating samples (meat slurries) on a Kjeldahl distillation rack. This procedure allows the simultaneous distillation of multiple samples with equipment generally available in most food laboratories. The distillation procedure offers several important advantages over other methods. The malonaldehyde is obtained in a clear aqueous solution so that its reaction product with thiobarbituric acid does not require a long solvent extraction procedure. The distillation heat treatment uses acid to effect the liberation and distillation of malonaldehyde from the sample, which means that there is less likelihood of fat oxidation occurring during the test itself (Ibid).

Kreis Test (Rancidity Index)

The Kreis Test was one of the first tests used to evaluate the oxidation of fats, and is based on the production of a red color when phloroglucinol reacts with epoxyaldehydes and their acetals in acid solutions (Rossell, 1983). The standardized method involves reacting the sample with phloroglucinol in diethyl ether solution. The products

are next extracted with HCl, and a red aqueous solution is obtained if the sample material is rancid. The red color is quantitated with a Lovibond colorimeter in a 1 in. glass cell. Color readings up to 3 red units indicates incipient rancidity. A color reading between 3 and 8 units indicates that the rancidity is occurring towards the end of the induction period. Color readings of over 8 units indicates definite rancidity. One limitation of the Kreis test is that some food additives, such as vanillin, can interfere with the test (Ibid).

Conjugated Diene Absorption Method

Oxidation of polyunsaturated fatty acids is accompanied by an increase in ultraviolet absorption due to the formation of conjugated diene and triene hydroperoxides. Conjugated unsaturations of fatty acids absorb strongly in the region 230 to 375 nm. The magnitude of change in absorbance is not directly related to the degree of oxidation because the effects upon various unsaturated fatty acids can differ in magnitude and quality (Holman and Burr, 1946).

Oils which contain linoleate or higher unsaturated fatty acids are oxidized to produce conjugated diene systems that can be quantitated by ultraviolet absorption at 233 nm. Absorption will increase proportionately to the uptake of oxygen and to the formation of peroxides in the early stages of oxidation (Farmer, Sutton, 1943).

St. Angelo et al. (1975) studied the autoxidation of peanut butter by measuring the peroxide value as subsequent increase in absorption at 234 nm due to diene conjugation. Samples were analyzed by modifications of both the peroxide value and conjugated diene method A.O.C.S. official method Ti 1a-64. St. Angelo concluded that the modified conjugated diene

hydroperoxide (CDHP) method can be used as an index of progressive staling in place of or in addition to, the peroxide value. The (CDHP) method requires smaller samples and is more rapid, and simpler than the peroxide value method. The (CDHP) method does not require additional reagents and does not depend upon chemical reaction (Ibid). This method is applicable for the analysis of conjugated diene production in soynut butter which contains a high amount of linoleic and lower, but significant, levels of linolenic acid.

Antioxidants

An antioxidant is a substance that is added to fats or fat-containing foods to retard oxidation and thereby prolong their wholesomeness and palatability.

Ideally an antioxidant should: (1) have no harmful physiological effect; (2) not contribute any objectionable odor or taste to the fat or food in which it is used; (3) be fat soluble; (4) be effective at low concentrations; (5) be readily available; (6) be economical; (7) be legal; (8) persist following processing to provide effective protection to food in which it exists, (i.e. "carry-through" properties) (Dugan, 1976).

Primary antioxidants function by inhibiting or interrupting the free radical chain mechanism. Their ability to interrupt the free radical is usually based on the phenolic configuration within their molecular structure (Sherwin, 1976). Antioxidants such as ascorbic acid function by being preferentially oxidized and they afford relatively poor protection.

An antioxidant AH apparently reacts with radicals produced during autoxidation according to the scheme:

$$RH \longrightarrow R^{\cdot} + H^{\cdot}$$

$$R^{\cdot} + AH \longrightarrow RH + A^{\cdot}$$

$$RO^{\cdot} + AH \longrightarrow ROH + A^{\cdot}$$

$$ROO^{\cdot} + AH \longrightarrow ROOH + A^{\cdot} \quad \text{(termination reactions)}$$

$$R^{\cdot} + A^{\cdot} \longrightarrow RA$$

$$RO^{\cdot} + A^{\cdot} \longrightarrow ROA$$

$$A^{\cdot} + A^{\cdot} \longrightarrow AA$$

The preceding reaction diagram shows how antioxidants interfere with the free-radical mechanism. Boozer et al. (1955) proposed a different mechanism, involving complex formation, as follows:

$$ROO^{\cdot} + AH_2 \longrightarrow (RO_2AH_2)^{\cdot}$$

$$(RO_2AH_2)^{\cdot} + ROO^{\cdot} \longrightarrow \text{stable product}$$

Peroxide decomposers act as catalysts to decompose peroxides initially present as well as those that are formed during further oxidation. An important feature of this scheme is that the primary stable products are not free radicals (Tranggono, 1978). This naturally rules out the decomposition of peroxides by metals such as copper, cobalt and iron (Dugan, 1963). Some antioxidants provide increased protection as the concentration increases whereas others have optimal levels and higher levels are sometimes prooxidant. The correct balance must be achieved to provide maximum stabilization without intensifying oxidation.

Among the most widely used commercial antioxidants are: (BHA) butylated hydroxyanisole (a mixture of isomers of 2-t-butyl-4-methoxyphenol and 3-6-butyl-4-methoxyphenol); (BHT) butylated

hydroxytoluene (2,6-di-t-butyl-4-methylphenol); esters of gallic acid; and (TBHQ) di-tert-butyl-hydroquinone. In the U.S. the Federal Food and Drug Administration requires that the phenolic antioxidant content may not exceed 0.02% of the fat or oil content of a food including the essential (volatile) oils. When various mixtures of antioxidants are used, the concentration of any single primary antioxidant may not exceed 0.01% of the fat or oil content of the food. When antioxidants or synergists are added, the combined total may not exceed 0.025%, with no single antioxidant exceeding 0.01% of the fat or oil content of the food. The amount of antioxidants permitted in a food lipids is never greater than one hundredth of the L_D50 established for that antioxidant.

In some cases it is found that a specific combination of two or more antioxidants is more effective in inhibiting oxidation than the equivalent quantity of a single antioxidant. This phenomenon is known as positive synergism. Not all combinations of antioxidants display this synergistic effect. Although BHA and BHT are synergistic and BHA and propyl gallate (PG) are synergistic, the combination of BHA with PG results in a decreased stability of a fat than expected from the sum of the effectiveness of each antioxidant if used alone. This effect is known as a negative synergism.

TBHQ (di-tert-butyl-hydroquinone) was first permitted as an antioxidant for food use in 1972 in the U.S. (Coppen, 1983). TBHQ has been shown to be very effective as an antioxidant for vegetable oils and is more effective than PG. Unlike some of the gallic acid esters, TBHQ is quite soluble in oil and will not discolor in the presence of iron and water. Crude soybean oil without added antioxidant possesses

a high degree of oxidative stability and treatment with TBHQ increases the stability of the crude oil considerably (Sherwin, Luckadoo, 1969). The high degree of stability in the crude soybean oil may be due to naturally occurring antioxidants in the oil such as tocopherols. The activity of the various tocopherols has been compared and found to vary inversely with the order of vitamin E activity. Studies by Dugan and Kraybill (1956) observed that cooking may destroy or modify tocopherols, and that gamma tocopherol, which is a better antioxidant than alpha tocopherol, is also a better carry-through antioxidant. Tocopherols found in soybean oil are mostly gamma tocopherols. Lecithin or mixtures of phosphatides have some antioxidant activity, as do several flavones, sterols and sulfhydryl compounds. Warner and Frankel (1985) found that the most effective combination of antioxidants in soybean oil was achieved with TBHQ and citric acid (CA). The combination of TBHQ and CA increased the induction period from 1 day in the soybean oil without antioxidants, to 9 days, as measured by direct gas chromatography of head-space volatiles. TBHQ/CA is an important antioxidant combination used in soya oil, and should be just as important in products containing soybeans or soybean oil as ingredients.

Organoleptic Evaluation

Sensory evaluation plays a critical role in the development of foods and beverages for human consumption. Consumers will often reject foods from which they could derive nutritional benefits because the food has a poor taste or because the particular consumers have not had previous experience with the food item (Moskowitz, 1984). Most aspects of quality are measurably only through trained sensory panels that evaluate foods

by the senses of taste, smell, touch, and hearing when a food is eaten (Larmond, 1977). Sensory evaluation panels can be grouped into three types: highly trained experts, routine laboratory panels, and large consumer panels. Preparation for sensory panel analysis involves setting up a special training area so that distractions can be minimized and conditions can be controlled (e.g. light, sound). Selection of the proper test, development of a statistically consistent language for odor, texture, and appearance, and proper sample preparation are all part of the difficulties involved in obtaining accurate sensory data. In the case of the trained expert panel, a difficult and long process occurs when educating and screening each panelist so they can properly identify a particular threshold of a certain stimulus consistently over an extended period of time. When people are used as a measuring instrument, it is necessary to rigidly control all testing methods and conditions to overcome errors caused by psychological factors. "Errors" may include all kinds of extraneous influences (Ibid).

The area of sensory analysis concerned with the judgements people make such as "good-bad" or "like-dislike" is known as hedonic measurement. Hedonic characteristics determine our behavior when it comes to choosing foods for the first time and repeating an initial choice upon continued exposure (Moskowitz, 1984). Hedonics are concerned with: attitudes versus behavior; measuring likes by classifying responses; measurement of degree of liking; ratio scales; and time preference measures of liking. The degree of overall "liking" of a food product represents the key evaluative criterion against which the researcher judges all other variables (Ibid). An order of preference test as used

in this thesis is a hedonic measurement tool. In a multiple comparisons test, a known reference or standard sample is presented to the panelist and compared to the reference on the basis of some named characteristics. Multiple comparisons may be used to evaluate the effects of replacing or changing an ingredient, of packaging material, of changing a specific process, or of storage. Small differences between the reference sample and control can be detected. Information about the direction and magnitude of the change is also obtained (Larmond, 1977).

Packaging of Legume Spreads

The legume spread of major importance is peanut butter. Recently almond butter, cashew butter, and sesame butters have also received more attention in consumer markets. As previously discussed most peanut butter is packed in glass jars for retail distribution. In glass jars the shelf-life of peanut butter is about two years. In opaque polyethylene containers, the decreased shelf-life of 9 months may be extended by incorporating an antioxidant into the plastic. Since packaging a fat or fatty food allows fat from the food to be dispersed over the surface of the packaging material, there is an increased tendency for oxidative rancidity to occur. The use of BHA or BHT in packaging materials increases the shelf-life of lard or butter by a factor of 2 to 3 and has an improving effect on margarine stability (Eastman Chemical Products, Inc., 1953). Antioxidants such as BHA or BHT have sufficient volatility to migrate into the package atmosphere and contact the material in the package for significant improvement in stability. Till et al. (1982) studied the migration of BHT from high density polyethylene (HDPE) in a variety of foods and food simulants.

Migration of BHT was found to be more rapid into oils and fatty foods than into aqueous materials.

Another important consideration in packaging of legume spreads is the effect of light tansmission, especially U.V. light, upon the stability of the product. As previously mentioned, when peanut butter is stored in the dark or in colored jars, there is reduced oil separation and better aroma and flavor. Polyethylene containers for milk that have been pigmented with titanium dioxide results in substantial reduction in U.V. light transmission (Nelson, Cathcart, 1983).

The barrier characteristics of a plastic container are critical when packaging a high-fat legume spread. Shelf-life can be defined as the length of time that a container or a material will remain in a saleable or acceptable condition under specified conditions of storage. When discussing the influence of barrier on shelf-life the term "permeability" is important to understand. The permeability of a material is the flux or the rate at which a quantity of permeant gas or vapor passes through a unit surface area in unit time, and is dependent upon the partial pressure of the permeant, the film thickness, the surface area, and often the temperature at which permeation occurs. The mechanism of permeation is a complex topic and is based on mass-transfer theory. The permeation mechanism is based on: the size of the permeant molecule; the molecular structure of the permeant molecule; the thickness and density of the package material; the concentration gradient of the permeant molecule, and temperature of the environment. Water vapor transmission rate, as expressed in terms of weight gain, is defined as mg/package x 24 hours x mm Hg. The oxygen transmission rate

through a packaging material is expressed in cc/m^2x24 hours. From each rate a permeability constant may be calculated for a certain barrier material under specific test conditions. The advantages of measuring the permeability are that the integrity of the closure, and the influences of machine processing and distribution can be quantified. Once a standard container's permeability constant is calculated over a range of temperature and humidity values, the packaging engineer has a protective tool which can help eliminate ineffective container design and selection.

MATERIALS AND METHODS

Soybeans

Soybeans procured for soynut production were dehulled, cleaned, and obtained from Diehl Fields, Inc. of Dansville, MI. The soybeans are packaged in 3-ply paper and polyethylene bags. The moisture content of the incoming soybeans was between 13 and 15%.

Oils

The pre-boiled soybeans were roasted in Gordon Food Service (GFS) Red Label™ solid vegetable fat. The GFS fat consists of 100% hydrogenated soya oil, 0.25% lecithin, .002% β-carotene, 0.02% TBHQ, .0005% citric acid, and 0.001% antifoam (dimethyl polysiloxane). The TBHQ in the roasting vegetable oil means that there will be a small level of antioxidant in every sample batch. This implies that the "control" samples may have contained some antioxidant, and that some unplanned synergisms, negative or positive, may have occurred with other antioxidants.

The oil added to the soynut butter to control body and spreadability was a commercial soybean oil (Bunge Edible Oil Co.) and did not contain antioxidants (AH). The antioxidant-free oil was specifically produced for this project for INARI, Inc. and shipped to their production site. Production of an antioxidant-free oil is difficult and expensive as most oil is produced with antioxidants in it, and to remove all residual antioxidant from the machinery is very time-consuming. Therefore traces of antioxidant might be present in the oil.

Stabilizer

A peanut butter stabilizer (hardener) composed of semi-solid partially hydrogenated palm oil, was used to harden the soynut butter and prevent oil separation.

Antioxidants

Four antioxidants were used in this study. Commercial antioxidant or blends were obtained from UOP, Inc. and consisted of Sustane 20™, a commercial name for di-tert-butyl hydroquinone with a citric acid synergist; Sustane 3™, a commercial name for a mixture of mono-tertiary-butyl-4-hydroxyanisole (BHA) and n-propyl-3,4,5-trihydroxybenzoate (propyl gallate) and citric acid; Sustane 6™, a commercial name for a mixture of BHA and 2,6-di-tert-butyl-para-cresol (BHT); Sustane 20A™, a commercial name for a mixture of TBHQ, citric acid, and propylene glycol.

Batch Production Procedures

Pilot plant batches for chemical analysis for studies one and two consisted of 11 and 15 pounds of roasted soybeans, 4 and 6 pounds of antioxidant-free vegetable oil, 0.18 and 0.55 pounds of dextrose, 0.045 and 0.022 pounds of salt and 0.179 and 0.176 pounds of "hardener" (partially hydrogenated vegetable fat) respectively. A control was made for each by these formulations and each succeeding batch contained a preweighed amount of a particular antioxidant which was dissolved in the oil. The amount of antioxidant that was added was the legal maximum of 0.02% by weight of the total lipid in the final spread.

The ingredients were "pre-cut" in a Hobart VCM 2-speed grinder at 1,800 r.p.m. for approximately 5 minutes. The premix had a "gritty"

"soupy" consistency. The precut was next sent through an Urschel Comitrol 1700™ microcut grinder for the final grind. The gritty precut is spun inside the microcut blade head at speeds up to 13,000 r.p.m. The primary grind (pre-cut) has a final temperature of about 80°F, while the final grind temperature should be about 140-180°F. Temperatures in excess of 195-200°F will cause the outcoming soynut butter to scorch, forming dark brown streaks in the product.

Packaging of Soynut Butter

Two different packaging studies were undertaken; in the first soynut spread was packed in wide mouth pint jars and stored under the following conditions: dark/100°F, dark/ambient 22°C and under fluorescent light/ ambient temperature. The second study involved packaging of soynut butter in wide-mouth pint glass jars with metal dome lids, high density polyethylene (HDPE) pint tubs with pry-off HDPE lids. The third package system consisted of the same tubs placed in nylon/Saran® plastic pouches which were evacuated prior to sealing. Storage conditions were identical to those used in the first study. Each container was filled to approximately the same level in which the glass jars contained an average of 255 g soynut butter per container; while the HDPE tubs contained an average of 275 g soynut butter per container. The average headspace was controlled by filling to the same point when filling each container. A sufficient amount of spread was packaged in each of the containers for both study one and study number two. The experimental design for the studies is shown in Table 1.

Table 1. Experimental Design of Chemical Analyses

Study #1 (All samples in Ball wide-mouth glass jars)

	Storage Conditions				
100°F/Dark	Initial	2 weeks	4 weeks	12 weeks	(P.V. & T.B.A.)
Dark/Ambient	"	"	"	"	"
Light/Ambient	"	"	"	"	"

(samples: control no antioxidants, BHA/BHT, TBHQ, TBHQ/Ca, BHA/PG.) (70 total samples)

Study #2 (3 packaging systems used: A=HDPE tubs; B=HDPE tubs in evacuated nylon/Saran^{R} pouches; C=Ball - wide-mouth glass jars)

	Storage Conditions								
	2 weeks	4	6	8	10	13	17	24	(T.B.A. & C.D.A.)
100°F/Dark	A.B.C.	A.B.C.	A.B.C.	A.B.C.	A.B.C.	A.B.C.	A.B.C.	A.B.C.	
Dark/Ambient	"	"	"	"	"	"	"	"	
Light/Ambient	"	"	"	"	"	"	"	"	

(samples: control no antioxidants, and TBHQ, Sustane 20^{TM}/CA) (144 total samples)

Analysis of Samples Prepared in Study #1

Lipid Extraction

A twenty gram sample of spread was homogenized in a Waring blender with 750 ml. of a 2:1 chloroform/methanol mixture. The homogenate was stirred and one part distilled water was added to form a two phase system (Bligh, Dyer, 1959). The mixture was filtered through Whatman #1 paper in a Buchner funnel under water aspiration. The filtrate was refrigerated for one hour and the upper phase was removed by water suction. The lower phase, which contained the lipid, was placed on a rotary evaporator for 15 minutes or until there was no detectable solvent odor in the oil. Oil samples were transferred into 100 ml. volumetric flasks, flushed with nitrogen, placed on dry ice and refrigerated until the time of analysis.

Peroxide Values

Peroxide values were determined by weighing 5.0 g of extracted oil into a 250 ml. Erlenmeyer flask and dissolved in 30 ml. of 2:1 acetic acid/chloroform. After swirling the flask, 0.5 ml. of saturated potassium iodide solution was added followed by 3 drops of starch indicator solution. Thirty ml. of distilled water were added and the sample was immediately titrated to a clear end point with 0.01 N sodium thiosulfate. The peroxide value is calculated as milliequivalents of peroxide oxygen per 1,000 g of fat (meq/kg).

Thiobarbituric Acid Values

Thiobarbituric acid (TBA) values were determined by weighing 3.0 g of extracted oil into 250 Erlenmeyer flasks and dissolved in 10 ml. of benzene. The contents were stirred and 10.0 ml. of freshly prepared TBA

reagent added to the flasks and the contents mixed thoroughly. The entire mixture was transferred to a 125 ml. separatory funnel and the layers were allowed to separate. The lower aqueous layer was collected in screw cap test tubes and heated in a boiling water bath for five minutes. Each sample was transferred into a clean glass cuvette and the absorbance was measured at 532 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. The TBA value was calculated as the average absorbance x 100.

Analysis of Samples Prepared and Stored in Study #2

Having determined the optimum combination of antioxidant the second study was undertaken to observe the effect of three different package systems. Two different methods of analysis were used, a modification of the conjugated diene hydroperoxide (CDHP) method as described by St. Angelo et al. (1972), and a modification of the distillation method of Tarladgis et al. (1960) for TBA values was used to determine lipid oxidation during storage.

Conjugated Diene Absorption

Following the basic procedure of St. Angelo et al. (1975), 1.2 g samples of soynut spread from the head-space surface of each container were weighed into large centrifuge tubes. The product/head-space interface is where oxidation of lipid material would be most pronounced. Thirty ml. of spectrophotometric grade hexane was added to each tube and the contents stirred. The samples were covered with aluminum foil and allowed to stand for one hour. The samples were then centrifuged in an International Equipment Co. model HR-1 high-speed refrigerated centrifuge at 12,000 r.p.m., for 15 minutes at $4^{o}C$. The supernatants were carefully

decanted into separate beakers and 0.2 ml. aliquots were pipetted into glass test tubes. Following addition of 3.0 ml. of spectrophotometric grade hexane, the test tubes were shaken and duplicate samples transferred into 1.00 cm square cuvettes. Absorbance was read at 233 nm in a Bausch and Lomb Spectronic 2,000^{TM} dual beam spectrophotometer. Conjugated diene absorbance was reported per 6.5 mg of fat (per cuvette) extracted from a 1.2 g sample. Pure spectrophotometric grade hexane was used as a blank. The absorbance values are reported directly, although St. Angelo (1975) calculated concentrations of CDHP as μmoles CDHP g of sample, based on an e_{max} of 24,500.

Purification of TBA

To 5 g of TBA was added 200 ml. of distilled water in a clean, 400 ml beaker. The mixture was heated to 80^{o}C to dissolve the TBA. Activated charcoal was added and the solution was filtered through a Buchner funnel. The filtrate was returned to a beaker placed in an ice bath in order to recrystallize the TBA. Then the contents of the beaker were filtered with a Buchner funnel. The filtration apparatus was allowed to run for 30 minutes to dry the TBA crystals. The TBA was refrigerated in a brown bottle for later use in preparing reagent.

TBA Reagent Preparation

Recrystallized TBA was weighed (0.7208 g) and transferred quantitatively to a clean 250 ml. volumetric flask. Twenty five ml. of glacial acetic acid was then added. Distilled water was added to the mark and the volumetric flask was placed in an ultrasonic bath for 5 minutes or until the TBA crystals were completely dissolved. The TBA reagent was freshly prepared on the day the analyses were made.

TBA Distillation Method

Using a modification of the procedure of Tarladgis et al. (1960) as described by Moerick and Ball (1974), the distillation of malonaldehyde from each sample for further reaction with thiobarbituric acid was achieved. A 5.0 g sample of soynut butter was transferred from a polyethylene weighing dish to a clean Kjeldahl flask. The weighing dish was rinsed with 50 ml distilled water and added to the flask in order to remove any residual soynut spread. An additional 47.5 ml. of distilled water was added to each flask with 2.5 ml. of 4 N HCl. Antifoam agent (Thomas Antifoam Spray^{TM}) and a few clean glass boiling beads were added to each flask. The flasks were then placed on the Kjeldahl distillation apparatus and 50 ml. of distillate was collected in a clean Erlenmeyer flask. The distillate was thoroughly mixed and a 5.0 ml aliquot was pipetted into a clean 30 ml. glass screw-cap test tube. Recovery of malonaldehyde was calculated and reported per 0.5 g test sample. All samples were analyzed in duplicate. A 5.0 ml. amount of the TBA reagent was pipetted into the tubes, the caps tightened, and the tubes were then heated for 35 minutes in a boiling distilled water bath and then cooled for 10 minutes under running tap water. Each sample was transferred into a clean glass cuvette and the absorbance measured against a reagent blank at 532 nm in a Bausch and Lomb Spectronic 20^{R} spectrophotometer. Absorbance was based on a 0.5 g sample.

Sensory Analysis of Optimum Levels of Sweetener

A multiple comparisons test was used to determine the optimal level of sweetener in test batches of soynut butter. The six different batches under test contained: 1.5% and 3.0% dextrose, 1.5% and 3.0% fructose, 2.0%

and 4.0% corn syrup solids, 8.1% and 9.6% of a pear concentrate. A multiple comparison sensory evaluation method was used to examine the effect of changing the amount and type of sweetener as compared to the standard sample of 1.5% dextrose labeled "R". Fifty people volunteered for a consumer taste panel to identify the degree of difference from R and the order of preference for each test soynut spread. Each sample was stored in separate glass beakers covered with aluminum foil and kept in a darkened refrigerator. On the day of test the test spreads were thawed for one hour at room temperature to enhance spreadability. A spoonful of each spread was spread on a salt-free cracker. Two trays of four samples each were spaced on a sheet of clean, white paper with a code number by each sample. Reference samples marked "R" were also placed on each tray for the comparison between samples. Each panelist was instructed to rinse his/her mouth completely with cool distilled water containing 0.5% lemon juice, with a minimum of one minute wait before tasting the next sample. A sample questionnaire is shown in Figure 3. The data was analyzed by analysis of variance testing as described by Larmond (1977).

Methylation of Fatty Acids

Methyl esters were prepared by a rapid procedure described by Morrison and Smith (1964). Two drops (14-16 mg) of extracted oil sample were transferred into a 1.0 ml. Reactivial™ using a micropipette. One ml. of borontrifluoride-methanol was added to the Reactivial™ which was then purged with nitrogen and capped. The Reactivials™ were placed in a heating block at 100°C and allowed to cool for 15-20 minutes. Samples were transferred to clean glass centrifuge tubes and 2 ml. of hexane and 1 ml. of distilled water were added. The tubes were capped

Figure 3. Sensory evaluation instrument (multiple comparisons of relative soynut butter sweetness)

NAME _____ PHONE #_____

DATE _____ TIME _____

You are receiving samples of soynut spread to compare for sweetness. You have been given a reference sample, marked R, with which you are to compare each sample. Taste each sample and determine whether it is more sweet, equal to, or less sweet than the reference. Please indicate how much you like or dislike each sample. Please rinse your mouth completely with water and allow a minimum of one (1) minute before tasting the next sample. You may retaste the reference sample as often as you wish.

(PLEASE PLACE A CHECK MARK (X) ON THE APPROPRIATE LINE)

Sample number	____	____	____	____	____	____	____	____
More sweet than R	____	____	____	____	____	____	____	____
Equal to R	____	____	____	____	____	____	____	____
Less sweet than R	____	____	____	____	____	____	____	____

AMOUNT OF DIFFERENCE

Sample number	____	____	____	____	____	____	____	____
None	____	____	____	____	____	____	____	____
Slight	____	____	____	____	____	____	____	____
Moderate	____	____	____	____	____	____	____	____
Much	____	____	____	____	____	____	____	____
Extreme	____	____	____	____	____	____	____	____

2

ORDER OF PREFERENCE

Sample number	____	____	____	____	____	____	____	____
Like extremely and would purchase	____	____	____	____	____	____	____	____
Like very much and would purchase	____	____	____	____	____	____	____	____
Like moderately and would purchase	____	____	____	____	____	____	____	____
Like slightly and would purchase	____	____	____	____	____	____	____	____
Dislike slightly and would not purchase	____	____	____	____	____	____	____	____
Dislike moderately and would not purchase	____	____	____	____	____	____	____	____
Dislike very much and would not purchase	____	____	____	____	____	____	____	____
Dislike extremely and would not purchase	____	____	____	____	____	____	____	____

and centrifuged for 2-3 minutes. The upper phase was removed, and the lower phase containing the hexane and methylated fatty acid was transferred into small vials and sealed with a foil-lined cap. The vials were stored at $0^{o}C$ until gas chromatography was performed.

Gas Chromatography

Gas chromatography was accomplished on a 5840A Hewlett Packard gas chromatograph. The gas chromatograph consisted of a flame ionization detector (F.I.D.) with an injection temperature of $275^{o}C$. An SP-2300 Supelco column packed with cyanosilicone stationary phase was used to separate the fatty acid methyl esters. Supelco fatty acid standard RM-3™ contained a mixture of C_{14}, C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, C_{20}, $C_{20:1}$, C_{24} fatty acids in methylene chloride.

Oxygen Transmission Studies

The rate of oxygen transmission was determined for the HDPE tubs using an adaptation of the American Society for Testing and Materials (A.S.T.M.) D-1434, standard test method. This method measures oxygen gas transmission through flat plastic film and sheeting using a coulometric sensor. The test apparatus used was a Modern Controls, Inc. MoCon Ox-tran 100. The adapted method for intact packages was found in Appendix D of the MoCon Ox-tran 100 manual. The Ox-tran 100 was calibrated before actual testing using a standard polyester (mylar) reference film.

The test HDPE tubs (empty) were fitted with two sections of 1/8 in. O.D. copper tubing which were inserted in two holes in the bottom of each tub, cemented in place with Duro™ epoxy and allowed to harden for 48 hours. Each copper tube section was fitted with a copper washer, nut and ferrule for attachment to the Oxtran test apparatus. The test package was attached to the "package test attachment" by removal of the stainless steel bypass tube which normally connects nitrogen inlet and outlet ports on the Ox-tran, and by threading of the copper nuts on the tubing of the test package in its place. A resinous putty was wrapped around each copper tube at the nut and base of the tub to prevent leakage of nitrogen. Packages were tested using a driving force or gradient established by normal levels of oxygen in ambient air.

The package was purged slowly (10-20 cc/min.) with nitrogen carrier gas to insure that the package is completely purged of any residual oxygen. Oxygen permeating through the package wall is carried by the

nitrogen stream through the nitrogen outlet to the coulometric sensor. A 24 hr. conditioning period is allowed prior to testing to allow for transmission of oxygen to reach an equilibrium state. The coulometric sensor is based on the fundamental electrochemical relationship of Faraday's law that each oxygen molecule causes the transfer of four electrons, so that one mole of atmospheric oxygen is equivalent to four Faraday units. Sensor output is recorded on a variable speed strip-chart recorder. The recorder readings are converted from millivolt readings to a package transmission rate by use of a load resistor which is inserted across the "sensor outlet" terminals. Recorder response in millivolts directly converts (1:1 relationship with oxygen transmission rate) to cc O_2/24 hr./package (1 mv = 1 cc oxygen per 24 hours per package). Since HDPE is considered a poor to intermediate oxygen barrier, a 5.3 mv load resistor was used to measure the response of oxygen permeating the HDPE container in a shorter time period.

The strip chart recorder range was set to the 10 mv range (full scale) so that 1" on the chart equaled 1 mv of response or 1 cc O_2/m^2/24 hr. A chart speed of 1 in./hr. was selected and a base line established. The testing conditions were 72°F (22°C), 0% R.H. To insure that the package was at equilibrium the coulometric sensor was taken off sensor-bypass and adjusted to insert sensor setting for a few seconds and returned to the bypass position. If equilibrium was complete the recorder pen returned to the base line, establishing a zero reference level. When the recorder trace stabilized at constant level with the sensor inserted, an equilibrium transmission rate was established. The measured peak height converted in millivolts

was multiplied by the range setting to find cc oxygen/24 hr./package. The final value must be multiplied 4.8 to account for the 21% oxygen concentration in air. The transmission rate for 100% pure oxygen varies (100% ÷ 21% = 4.8) by this correction factor.

Evacuation of Containers

To increase the oxygen barrier properties of the HDPE tubs, pouches made of nylon laminated with Saran^{R} were drawn tight around the tubs. This will be hereafter referred to as evacuation (designated "B", see Table 1). The pouches were obtained from Koch, Inc. and had a reported oxygen transmission rate of 9 cc/m^2/24 hr. at 100^{o}F, 37.0^{o}C. The pouches were selected for their excellent barrier to both oxygen and moisture. After placing filled and capped HDPE containers in the pouches they were simultaneously evacuated and heat-sealed on a Smith Equipment Co. SuperVac^{R} machine.

Light Transmission Studies

The light-barrier property of the container was a critical consideration in the selection of a material for soynut butter. Decomposition of fatty acids through lipid autoxidation depends on the intensity, duration and wavelength of the radiant energy as well as the penetrability of the container material. Those wavelengths in the U.V. region (200-380 nm) are particularly effective in accelerating the decomposition of peroxides in foods containing conjugated unsaturated fatty acids (Lundberg, 1962). To study the effect of light, the barriers selected were opaque HDPE and clear glass jars.

A Perkin-Elmer Lambda 3B UV/Vis double beam Spectrophotometer was used to determine the percent light transmission for the HDPE

containers. The glass jars will allow nearly total transmission of all U.V. above 300 nm and all visible wavelengths. Two samples of HDPE measuring 1 in. x 3/4 in. were taken from the sidewalls of two different containers. A wavelength scan was begun at 850 nm with percent transmission recordings taken at 25 nm intervals down to 200 nm. The thickness of each HDPE specimen was measured with calipers and recorded next to the percent transmission readings. Data collected show average transmission values and will be discussed later.

Measurement of Water Vapor Transmission Rates

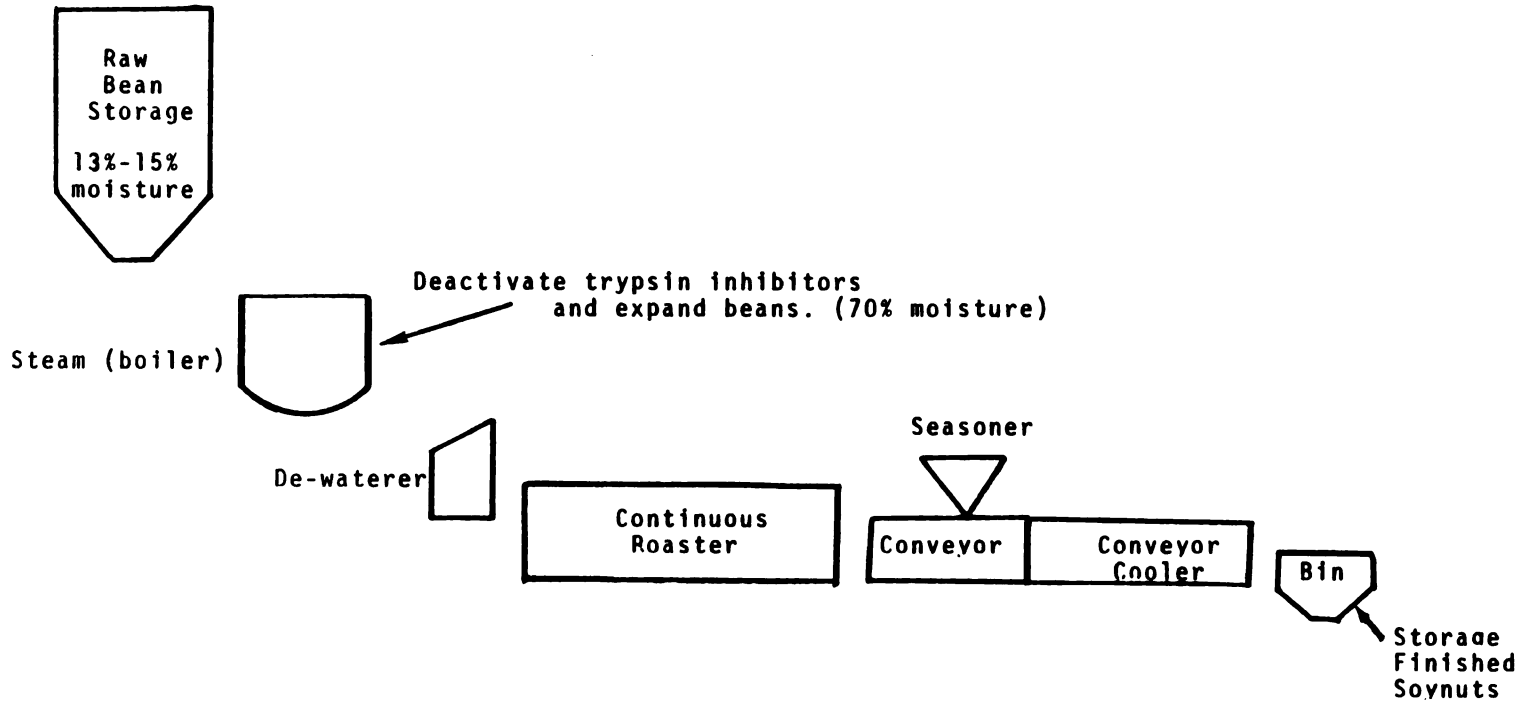
The standard test method used to measure water vapor transmission of materials, in sheet form, was the gravimetric method described by A.S.T.M. E-96.66. In this procedure a desiccant with a high affinity for water vapor is sealed in an aluminum cup by the test film using molten wax to secure the film to the cup ledge. The cup is placed in a constant temperature and relative humidity chamber and the gain in weight is measured and plotted as a function of time. An equivalent method may be used to determine water vapor transmission rates of a package (Giacin, 1978). Drierite^{TM} ($CaSO_4$) desiccant which maintains low water vapor pressure was poured into each tub to a depth of 1/4 in. and the caps were sealed tightly. Empty control tubs were also placed with the samples in a 37^{o}C, 86% R.H. chamber and the weight measured on a Mettler AE 160^{TM} analytical balance. Weighings were made over a period of 3 weeks and net weight gain in mg was plotted as a function of elapsed time in hours. The slope of the straight line portion of the graph is equal to the rate of water vapor transmission for the tub container. The water vapor permeability constant for each tub was calculated by knowing the

water vapor transmission rate, the saturation vapor pressure at test temperature (mm Hg), and the relative humidities of the internal package environment R_1 and external chamber environment R_2.

RESULTS AND DISCUSSION

Processing

The unique high protein and fiber content of soynuts as compared to that of the peanut calls for micro particle grinding. A product flow chart for soynut production can be seen in Figure 4. With the advent of the Urschel Comitrol 1700^{R} a semi-smooth soynut spread was prepared. A two-step operation was required. The whole-roasted soynuts were simultaneously reduced, and combined with optimal amounts of partially hydrogenated vegetable oil, salt, sugar, stabilizer and other flavorings in a Hobart VCM 2-speed grinder at 1,800 r.p.m. for approximately 5 minutes. The first stage grind has a "gritty", "soupy" consistency. The primary grind was next sent through the Urschel comitrol unit for a final grind. The microcut head consists of a ring of closely spaced tungsten carbide blades which are individually positioned to produce ultrafine particles. The gritty primary soyspread was rotated inside this ring of blades at speeds up to 9,600 r.p.m. Centrifugal force causes pressure on particles between the rotating impeller, and against the blades, equal to several thousand times the weight of the product. The pre-cut mix reached a processing temperature of about 85-90^{o}F, and the final grind temperature was 140-180^{o}F. Product temperature, pressure, particle size, expansion and moisture vaporization can be regulated by physical adjustments such as: type of impeller used, impeller speed, and the spacing between



Product Flow

1. Inspection of raw soybeans
2. Deactivate trypsin inhibitors and expand beans
3. De-water beans
4. Continuous oil roast
5. Salting or seasoning applied
6. Cooling
7. Finished product inspection
8. Storage in bin for packaging or soynut butter processing

Figure 4. Product flow for production of soynuts.

the blades on the microcut head. Temperatures in excess of 195-200^{o}C will cause the outcoming soynut butter to scorch, forming dark brown streaks in the product. Three factors appeared to be critical in maintaining constant, reproducible temperature: a minimum of 25% vegetable oil to achieve desirable viscosity, a primary grinding time of five minutes, and the rate of delivery of the pre-cut mix to the Urschel Comitrol. The proposed product flow would next enter a scraped-surface heat-exchanger, like a Votator^{TM}. Small amounts of moisture must be removed before the butter is packaged to reduce high viscosity and pastiness. The moisture in the warm butter can be stripped off along with residual air in a deaerator. This could be used in addition to a swept-surface heat-exchanger. A proposed flow chart for soynut butter can be seen in Figure 5. The cooled soynut butter would then be filled automatically into jars or tubs, moving on a conveyor belt, and the lids placed and tightened on each container. Filling temperatures should be maintained below the point at which the polymer becomes soft $\triangleq$ 250-300^{o}F. The containers would pass through a metal detector before the lids were in place, be code dated, and once closed could be packed into corrugated shippers with partitions and stapled shut. Corrugated trays are another possibility with a saran-coated low density polyethylene shrink-wrap over the containers and tray.

Adams Foods, a division of International Multifoods, Inc., produces old-fashioned peanut butter and found leakage of oil from plastic containers to be a major problem (Hess, 1985). The peanut butter, without stabilizers, released oil to the product surface where it worked its way through the snap-on lid during shipment. This caused rejection

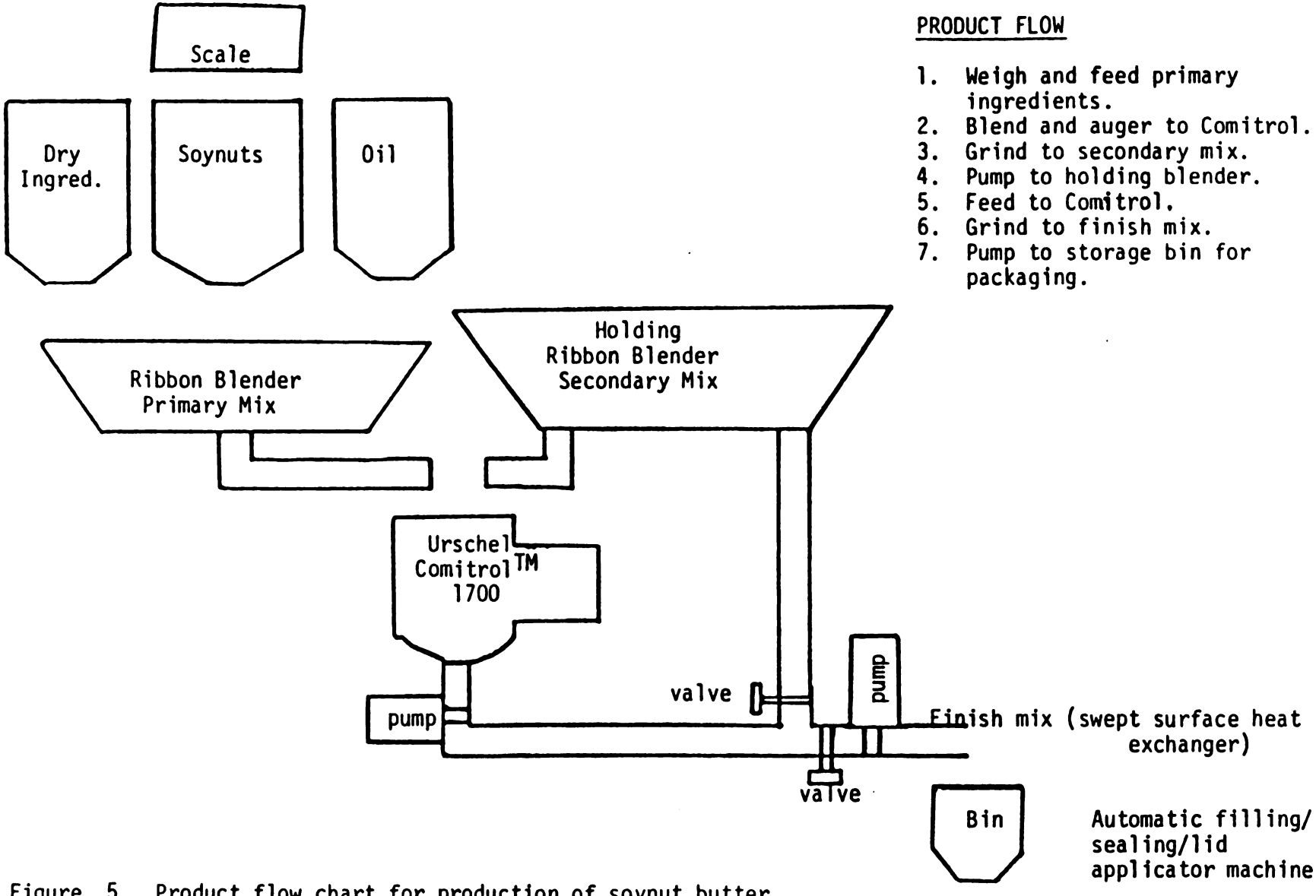


Figure 5. Product flow chart for production of soynut butter

of individual containers and surrounding intact containers in the shipping case which were stained by the leakage. A two-head automatic filling and sealing machine was used to apply a clear polyester film innerseal over the container tops before lidding. Adams also used a tamper evident feature on its plastic pails, in which two tabs on the shoulder rim must be broken in order to lift the lid. In the case of soynut butter, oiling-off is reduced by the addition of hardener in the formulation, but high temperature storage at 100°F over a period of 1 week did cause some oil to leak to the surface. The system used by Adams Foods would therefore be equally as effective in the packaging of soynut butter, especially in the reduction of leaking containers. Adams projected product shelf-life at 6 months to 1 year maximum, because of the high oil content of the product and the oxidative, thermal, and light transmission stresses applied during the distribution cycle.

A swept-surface heat exchanger, such as the Votator™, provides a system in which there is rapid cooling under agitation. Such a system is used by peanut butter manufacturers to crystallize the fat in the mix providing improved filling capabilities while limiting air bubbles in the final product. Initial soynut butter production runs indicated that air bubble formation was a persistent problem at normal processing temperatures of 180°F. Some measure of control was achieved by allowing containers of warm butter to stand for 30 minutes with occasional shaking to force the trapped air bubbles out of the product. This procedure would be highly impractical for high-speed filling operations and would necessitate the use of a heat exchanger such as the Votator™. Also critical was the ability to control the rate of addition of the

pre-cut mix into the Urschel unit. A constant flow over a short period of time gave the coolest processing temperatures, below 160^{o}F.

Among the objectives of processing soynut butter was to develop a commercial spread with sufficient added sweetener to mask any bean flavor. In considering improvement of flavor the related changes in palatability were also observed. With the addition of 3.0% powdered dextrose there was no significant change in the viscosity of the soynut butter with the added vegetable oil composing 25% of the formula, and the soynuts composing 71% of the same batch. Added salt and partially hydrogenated palm oil respectively provided additional flavor and helped prevent excess oil-off. When dextrose was added in excess of 7.0%, fructose in excess of 3.0%, or pear concentrate in any amount, a corresponding percentage of vegetable oil had to be added to prevent clogging of the Urschel Comitrol grinder. Product with a 4.0% fructose of 7.5% dextrose and containing only 25% vegetable oil would exit the finish grind "scorched" at temperatures of 200-220^{o}F. Dextrose, fructose, and corn syrup solids are reducing sugars and can react with free amine groups in the soy protein. This reaction is accelerated by heat and is especially pronounced in peanut protein at 180^{o}F (Weiss, 1983). This same effect was seen in sample batches of soynut butter in excess of 180^{o}F and resulted in increased viscosity and frictional heat. Another undesirable effect occurs when hydrated dextrose in a nut butter dehydrates during grinding. The moisture is transferred to the soy protein and causes the butter to thicken considerably. Dextrose can then rehydrate forming larger crystals and a grainy texture (Ibid). The solution may be to use an anhydrous dextrose, increasing the pre-mix

grinding time, or adding normal dextrose monohydrate to the butter after it has cooled following the final grind (Ibid). The same logic would apply when adding other reducing sugars to warm soynut butter. It was observed that when pear extract was added before final grinding, even in small quantities, excess of 40% added vegetable oil per batch was necessary to achieve appropriate processing temperatures and palatability. Fructose added before grinding at a concentration of 3% required approximately 35% added vegetable oil per batch. The implications of these processing parameters will be critical in future product scale-up for commercial production runs. It must be emphasized that through the optimization of the amounts of these necessary ingredients, i.e. vegetable oil, sweetener, soynuts, stabilizer and/or emulsifier and salt, a spread of good consumer acceptability has been produced. The discussion of consumer preference is discussed in the Results of Sensory Evaluation section. The high percentage of added vegetable oil required to achieve palatability will be of major concern to health-conscious consumers who seek to avoid consumption of high-fat foods. However, both peanut or soy spreads are by nature high fat foods. The high fat content is also of major concern in protection of the product from off-flavor development and the necessary packaging to protect the product from autoxidation.

In the work of Pichel and Weiss (1967) a similar process was used which included the step of conditioning of the soybeans by moisturizing them with an amount of water which was reported to affect removal of "beany" or "grassy" flavor when the beans were properly heated. The added moisture appeared to penetrate and permeate the bean

to an extent that the "grassy" or "beany" flavor constituents of the bean were volatilized along with the water, and most likely lipoxygenases were inactivated. In the subsequent heating step Pichel fried the moisturized bean in hydrogenated vegetable oil at 140-180^{o}C for about 2-5 minutes. Subsequent grinding in a high speed attrition mill with an impeller speed of 7,500 r.p.m. was found to give the most efficient subdivision of soybeans.

Results obtained in processing procedures paralleled much of Pichel's findings with the exception that the soynut butter was grainy despite the addition of 25% vegetable oil. When the added vegetable oil content exceeded 25%, based on the total batch weight, the soynut butter could be processed through the Urschel grinding unit at temperatures below 180^{o}F. The product still had a grainy mouth-feel. With the addition of 35-40% extra vegetable oil the spread had very little grainy texture. The high oil content of the soynut butter made it feel smooth on the palate. Pichel added enough bland, edible oil to increase the oil content to about 35-60% based on the weight of beans (Ibid). Separation of oil is a major concern so that various monoglycerides, partially hydrogenated palm oil, and other commercial hardeners must be added.

Although beany flavor is substantially reduced after boiling the raw, dehulled beans, the bean-flavor was still present in the soynut butter. Hawley (1966) realized this problem and stated that it is sometimes necessary to add 10% to 15% of flavoring materials based on total product weight. Among the flavoring components were peanut oil extract, sesame seeds and various sweeteners. Experimentation with

various sweeteners has shown that the addition of 3.0% fructose significantly improved the flavor of the spread and ameliorated off-flavors. Addition of fruit flavored extracts also improved the flavor but required the addition of even greater amounts of added vegetable oil to achieve the same smooth consistency in the product. An independent laboratory report on the differences between peanut butter and soynut butter is seen in Table 2. These data indicate that the base amounts of carbohydrates, protein and fat are virtually identical, but as previously seen, soy protein contains a richer amino acid array than peanut protein.

Table 2. Soynut Butter Comparison to Peanut Butter, Based on 32 g Samples.

	Soynut butter	Peanut butter
Calories	189	200
Protein	9.6 gm	9.0 gm
Carbohydrates	5.0 gm	6.0 gm
Fat	14.5 gm	16.0 gm

Results of Study #1

The Effect of Antioxidant Type Upon Lipid Stability

The type of and concentration of antioxidant plays a significant role in the stabilization of fats and oils, depending on the chemical composition of the lipid system. Sherwin and Luckadoo (1969) reported that .02% TBHQ provided greater protection than .02% BHA and .02% PG in soybean oil stored at 140^{o}F over a 40 week time period as measured by peroxide value. Reported peroxide values were less than 10 meq/kg after 12 weeks of storage in both control samples, and those containing antioxidants.

In this study, control soynut butter samples were compared to samples containing four different antioxidants: BHA/BHT, BHA/PG, TBHQ and TBHQ/cirtic acid. The results are shown in Figures 6, 7 and 8 were based on peroxide values obtained from extracted oil in a 5 g sample of soynut butter. All of the figures indicate increasing peroxide values in each storage condition which suggests that the extent of peroxide decomposition was not achievable. Samples stored in the dark at ambient conditions had the slowest rate of increase in peroxide value. The dark, 37^{o}C environment proved to be the most detrimental, with storage in fluorescent/ambient conditions were of intermediate severity. Samples containing TBHQ and TBHQ/CA provided greater protection to soynut butter than did BHA/PG or BHA/BHT at dark, 37^{o}C and fluorescent, ambient (22^{o}C). BHA/BHT provided protection approaching that of TBHQ only in a dark ambient environment. BHA/PG provided the least protection of any of the antioxidants and control samples had the highest rate of increase of peroxides over 12 weeks total

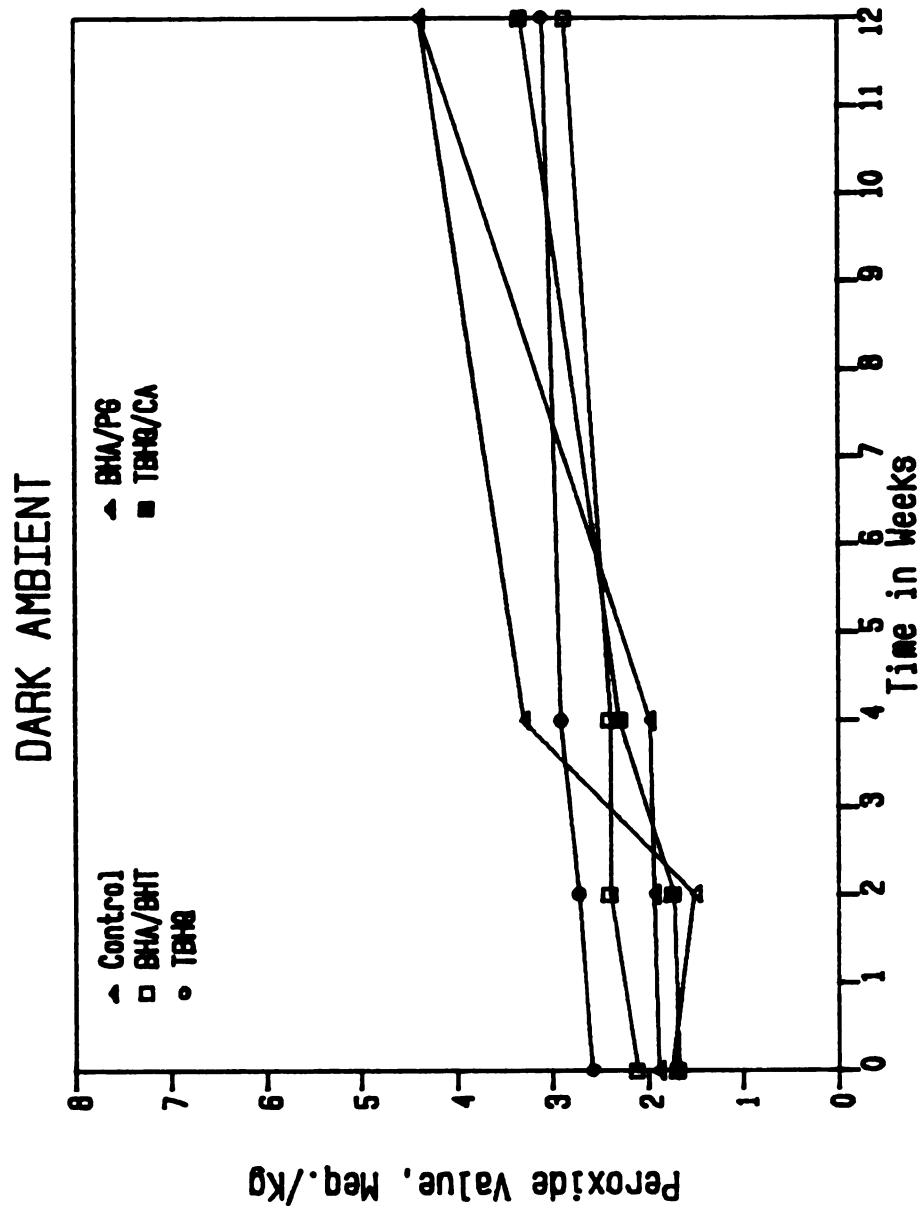


Figure 6. Peroxide value vs time for soynut butter during storage in dark, 22°C (ambient) condition.

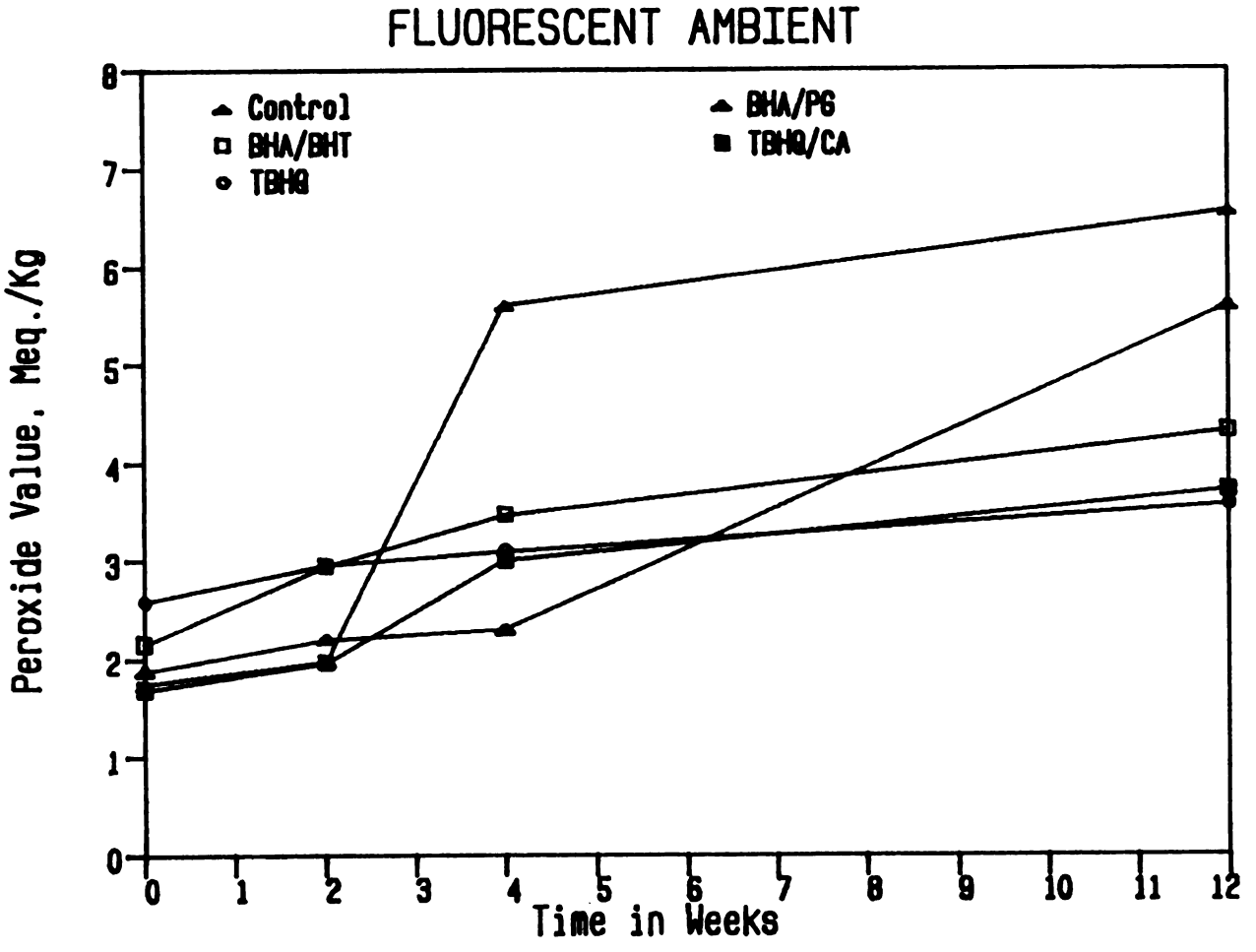


Figure 7. Peroxide value vs time for soynut butter during storage in fluorescent light, 22°C (ambient) condition.

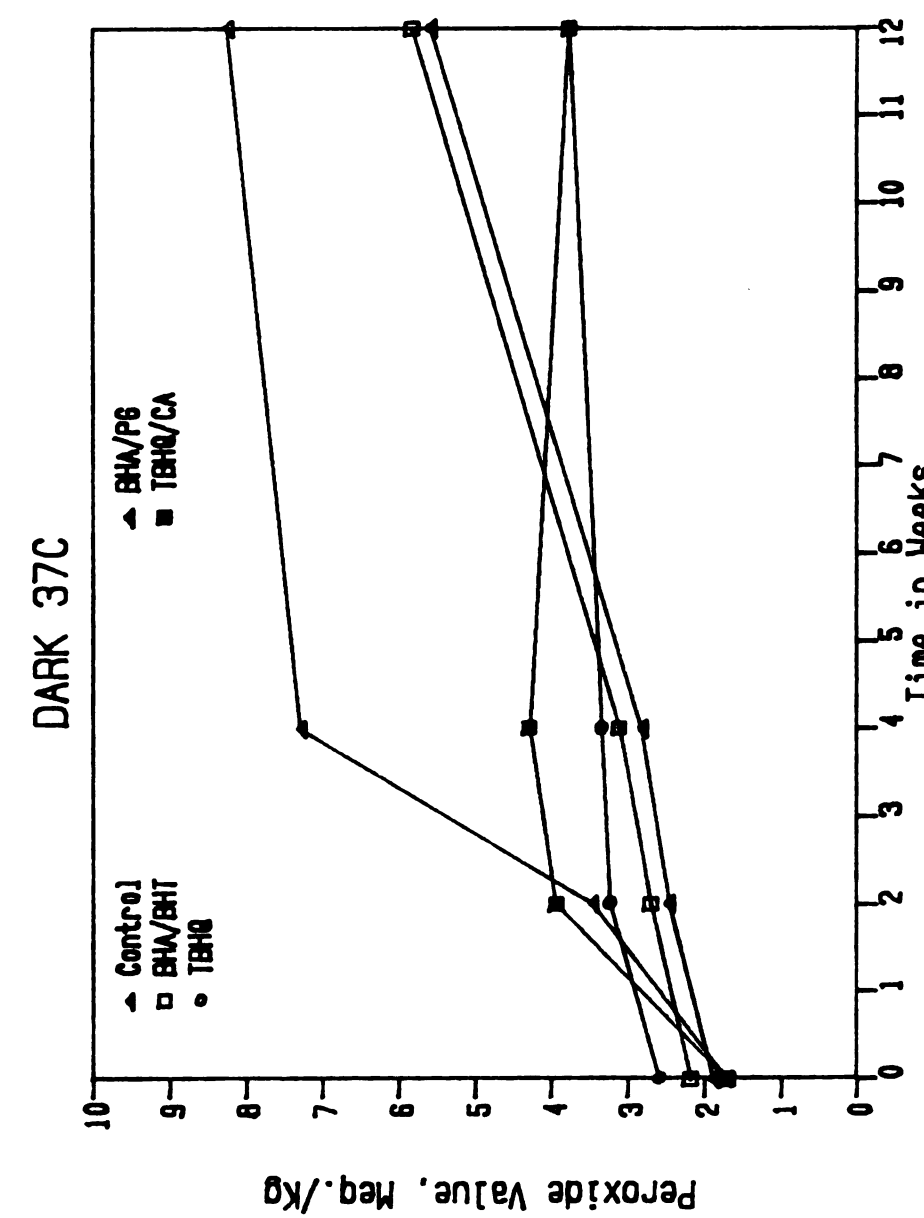


Figure 8. Peroxide value vs time for soynut butter during storage in dark, 37°C condition.

time. Propyl gallate exhibits antioxidant properties in soya oil, but its usage is hindered by low oil solubility and a tendency to complex with metal ions and to cause discoloration (Buck, 1981). As previously indicated, the antioxidant free radical which forms is stable, and most importantly, is usually not involved in propagating further oxidation of the fat or oil. Maximum efficiency of primary antioxidants can only be achieved if they are intimately mixed or dissolved in the oil (Sherwin, 1976). If this is done, there should be a significant delay in the onset of the final stages of autoxidation in which the oxidation breakdown products responsible for rancidity are formed (Ibid).

Figures 9 and 10 show the changes in TBA value of soynut butter stored for 12 weeks in fluorescent light at ambient ($22^{o}C$), and $37^{o}C$ respectively. TBA absorbances increased rapidly over the first four weeks of storage in each environment. After four weeks TBA values stabilized and in some cases decreased over the remaining at weeks of the test. It has been recognized that malonaldehyde and other secondary lipid peroxidation products will react with amino acids, esters, and amines to form fluorescent conjugated Schiff's bases (Frankel, 1984). Another limitation of this test is that the TBA reaction will also occur with lipid oxidation products other than malonaldehyde. Frankel (1984) demonstrated a more specific procedure to determine malonaldehyde by using a dilute HCl solution in methanol to cleave lipid oxidation products and form stable acetals suitable for gas chromatography. Any malonaldehyde formed was converted to a tetramethyl acetal derivative, and was quantitated on a GC/MS (gas chromatograph/mass spectrometer).

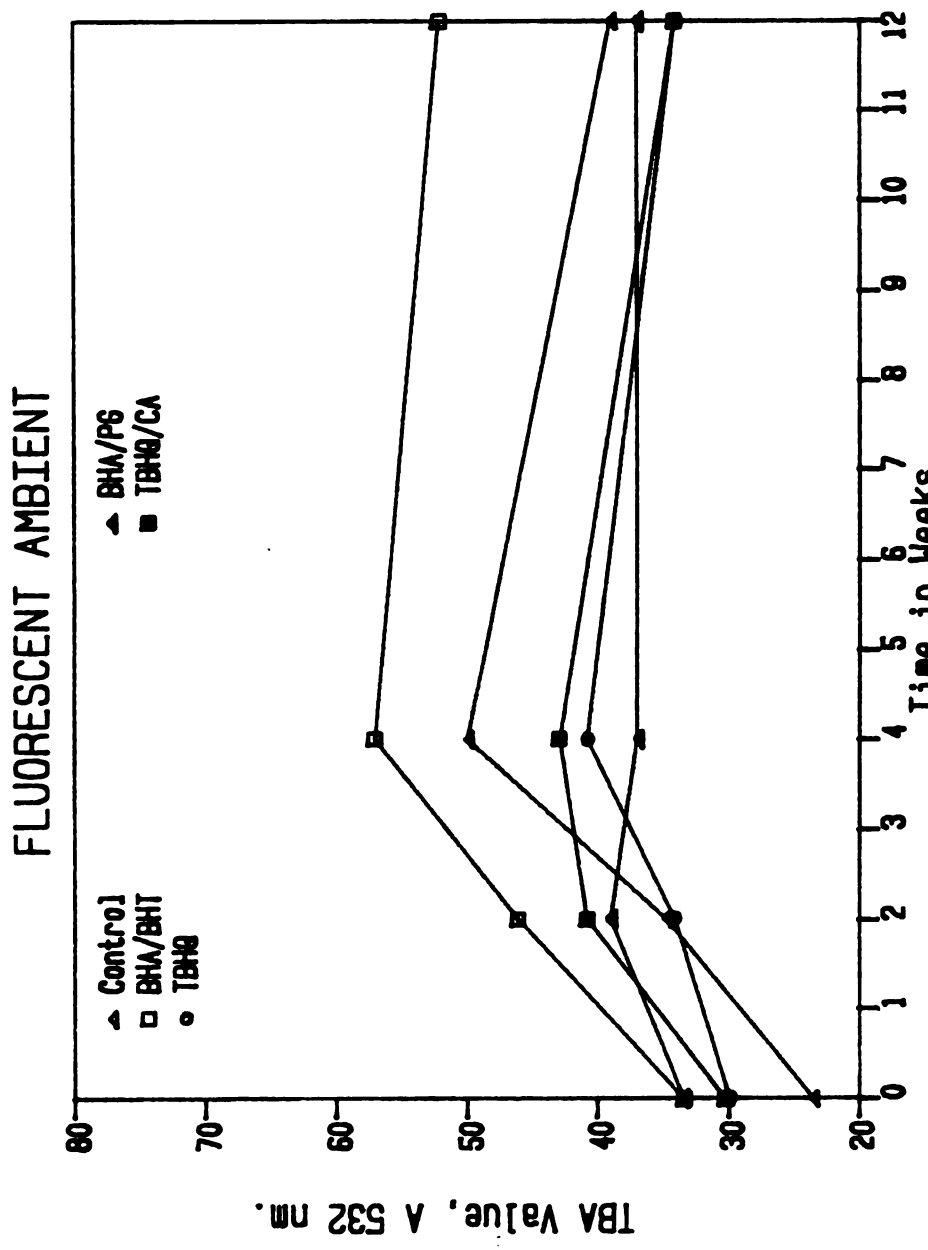


Figure 9. TBA value, absorbance 532 nm, vs time for soynut butter during storage in fluorescent light, 22oC (ambient) condition.

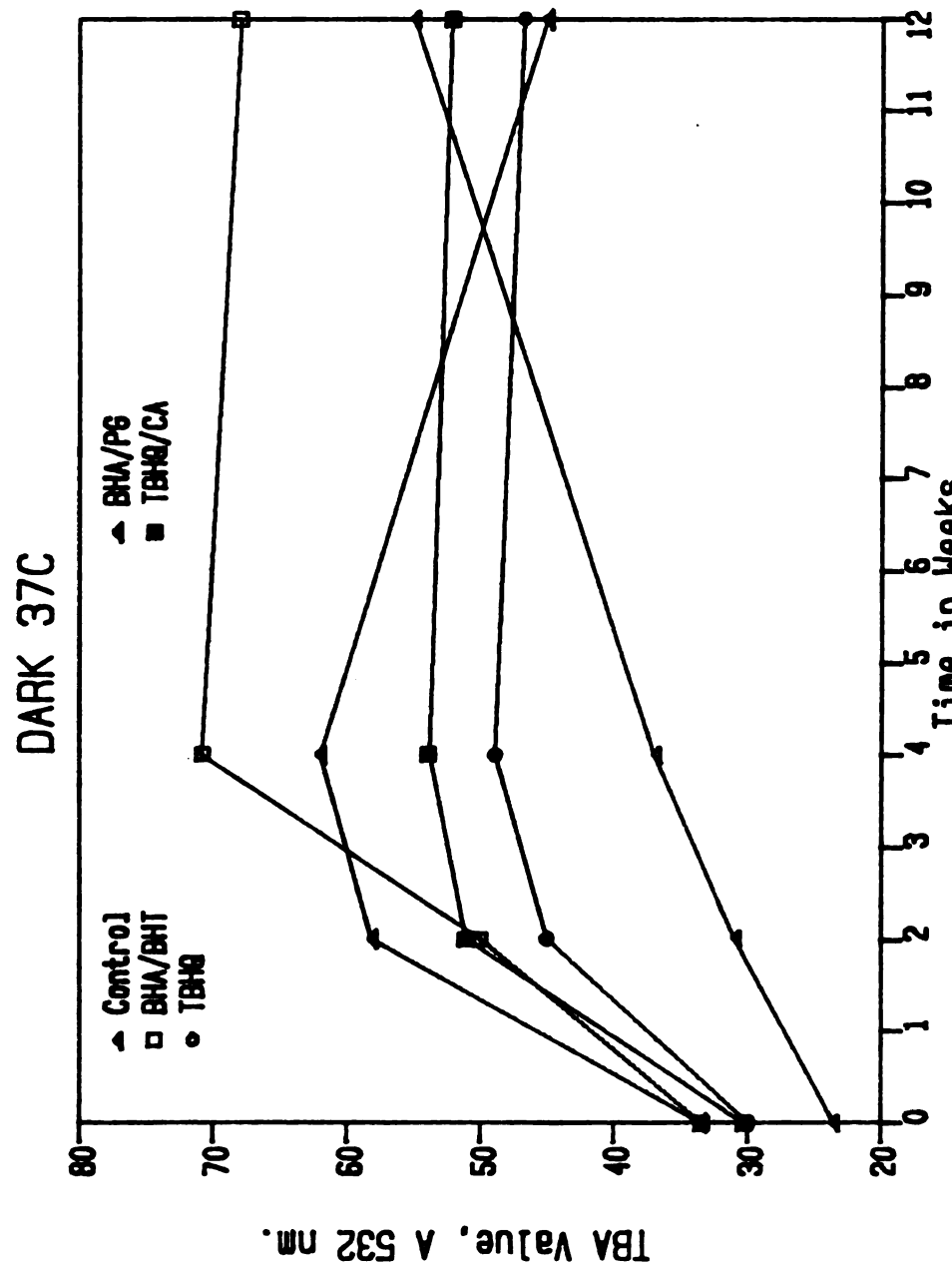


Figure 10. TBA value, absorbance 532 nm. vs time for soynut butter during storage in dark, 37°C condition.

Despite the deficiencies in the TBA test, Figure 9 shows that the presence of antioxidant did have a protective effect with TBHQ and TBHQ/CA having lowest TBA values at 12 weeks. Samples containing BHA/BHT had the highest TBA values in fluorescent light and at 37°C (Figure 10). Min and Wen (1983) observed that the effectiveness of preventing the disappearance of free oxygen in soybean oil was in the order of TBHQ, PG, BHT and BHA, with BHT and BHA generally being least effective. These results agreed with the reported results of the relative effectiveness of these antioxidants as measured by AOM, active oxygen methods (Eastman Chemicals, 1980). Samples with TBHQ/CA and TBHQ had slightly higher TBA values at 37°C than at fluorescent 22°C light, but generally gave lower TBA values as compared to the other antioxidants (Figure 9 and 10).

Despite their empirical nature and variability, the composite results of TBA values and peroxide values indicated that the soynut butter containing .02% TBHQ or .02% TBHQ/CA did provide the greatest product stability during the initial stages of lipid autoxidation. Because an optimum level of added vegetable oil had not been completely established, each initial product batch (study #1) had a slightly different processing temperature. The batches produced containing TBHQ and TBHQ/CA received the highest temperatures of approximately 183°F during processing as compared to the average temperature of the other antioxidant test batches $\sim$ 168°F. Such variability was unavoidable with the pilot plant equipment used. This inconsistency did not appear to affect the performance of the TBHQ or TBHQ/CA (Figures 6-10).

Effect of Light and Heat on Shelf Life

Bright light sources, especially those with ultraviolet wavelengths, is detrimental to peanut butter (Woodroof, 1983). As previously stated in the literature review of this thesis, Woodroof observed peroxide values that averaged 4.06 for samples stored in light and only averaged 4.05 for those stored in the dark, over a 600 day period. However his data indicated significant differences in flavor of peanut butter stored in the dark compared to that stored in light in glass containers. Differences were seen in terms of reduced oil separation, and in aroma and flavor observations by 200 sensory evaluations (Ibid). Changes in the flavor of soynut butter were evaluated on an informal basis in the second study of this thesis. A major difficulty in using an expert panel for soynut butter is circumvention of preconceived dislikes for any product containing soy. The samples used for chemical analysis were produced with little sweetener and still contained the characteristic "bean" flavor, which is not a result of simple lipid autoxidation but of complex reversion products as previously mentioned. Many panel members may associate "bean flavor" as being the rancid off-flavor characteristic of lipid autoxidation as occurring through the steps of free radical chain mechanism and chain scission to form the characteristic secondary break-down compounds.

The data in Tables 3 and 4 indicate the presence of the characteristic bean flavor in each sample but also show that there are differences in samples stored in light or dark conditions. Only those samples stored in the fluorescent light showed production of grassy and lemony flavors and flavors seemed to be more pronounced in

Table 3. Informal Laboratory Sensory Evaluation of Soynut Butter Flavor after 13 Weeks Time

Condition/sample	13 weeks (control)	13 weeks (.02% TBHQ)
Dark, 22°C		
A	acidic/bean flavor	slight bean flavor
B	slight bean flavor	slight bean flavor
C	oily/bean flavor	oily bean flavor
Light, 22°C		
A	grassy flavor	slight-bean flavor
B	mild grassy flavor	slight-bean flavor
C	strong grassy flavor	moderate bean flavor
Dark, 37°C		
A	oily/mild bean flavor	oily/slight bean flavor
B	oily/moderate bean flavor	oily/mild bean flavor
C	oily/strong bean flavor	oily/mild bean flavor

Table 4. Informal Laboratory Sensory Evaluation of Soynut Butter Flavor after 24 Weeks Time (for Selected Conditions)

Condition/sample	24 weeks (control)	24 weeks (.02% TBHQ)
Dark, 22°C		
A	acidic/sour bean flavor	oily/strong bean flavor
C	oily/moderately rancid	dry/rancid
Light, 22°C		
A	lemony/grassy-sour	bean-grassy
C	mod. lemony/grassy-painty (isoprenoid)	slightly lemony/grassy
Dark, 37°C		
A	very oily/rancid bean	very oily/moderate bean flavor
B	-	very oily/rancid flavor
C	very oily/sour, strongly rancid	very oily/strongly rancid bean flavor

glass jars at both 13 and 24 week intervals. HDPE tubs provided some protection at 13 weeks but apparently some light wavelengths approaching 400 nm caused deterioration in the flavor by 24 weeks. The light transmission characteristics of HDPE tubs are seen in Table 5. Those samples stored in the dark, 22°C environment had the least amount of oil separation and off-flavor development as compared to the other conditions (Tables 3 and 4).

Woodroof (1983) also observed that the rate of change at which peanut butter becomes rancid is not great, and only those samples stored at about 85°F for one year could be called "rancid". He reported the average peroxide numbers for samples stored at 50°, 60°, 70° and 80°F as being 5.2, 5.6, 5.6 and 8.3 meq/kg at each of the respective temperatures after one year of storage. Soybeans contain a significantly greater amount of linolenic acid than peanuts, and may be more susceptible to autoxidation because of the increased quantity of polyunsaturated moieties. At 12 weeks time the average peroxide values for control samples at dark, 37°C and light, 22°C were 8.22 meq/kg and 6.56 meq/kg respectively. Samples with .02 TBHQ/CA averaged 3.74 meq/kg in both environments. Informal sensory results seemed to agree with this result, showing increased oil separation in samples stored 37°C and stronger rancid flavor development at 24 weeks storage time. In all cases more pronounced off-flavors and odors were noted in those samples stored without added antioxidant. Stability of the samples was greatest in dark, ambient conditions in which antioxidant was added, and least in those samples at 37°C without .02% TBHQ/CA. Further discussion of sensory analysis is limited to the sensory work done to assess optimum levels of sweetener in Study #2.

Table 5. Light Transmission Characteristics of HDPE Tubs

Wavelength, λ	Percent transmitted light	
	Sample #1 (39.4 mil)	Sample #2 (39.2 mil)
850	100.0	100.3
825	98.5	98.6
800	95.6	95.8
775	91.5	91.8
750	87.5	87.8
725	84.4	84.8
700	81.1	81.4
675	77.6	78.0
650	74.4	74.8
625	71.4	71.9
600	68.2	68.7
575	65.1	65.6
550	61.9	62.4
525	58.7	59.2
500	55.3	55.8
475	52.1	52.7
450	48.6	49.2
425	44.7	45.3
400	3.3	3.1
375	0.0	0.0
350	0.0	0.0
325	0.1	0.1
300	0.0	0.0

Results of Study #2

The Effect of Varying Packages on Lipid Autoxidation

Knowing that the optimum antioxidant combination was .02% TBHQ/CA from Study #1, the second study was designed to determine an optimum package barrier. The results over 24 weeks time indicated a series of step-wise increases and decreases in absorbance values, by both conjugated diene and TBA methods. Figure 11 shows the changes in conjugated diene absorbance of samples held for 24 weeks in the dark, 37°C. Those samples without TBHQ/CA had an average conjugated diene absorbance of 0.725 at 24 weeks, as compared to 0.645 for those samples with antioxidant as measured at 233 nm. Tubs packed in nylon/Saran^{R} pouches exhibited slightly higher absorbance values possible due to pressure cracks in the tubs. The tubs were flexible enough to buckle under the vacuum created by the SuperVac machine, but cracks formed gradually over time. There was still enough residual oxygen within the pouch to have a considerable effect on the stability of the soynut butter. The rising and falling of values may be related to laboratory technique, fluctuations in storage temperatures, and might possibly be the result of condensations of free amino groups in the soy protein with conjugated diene and malonaldehyde species to yield a Schiff's base (Day, 1962).

Absorbance values increased in a linear fashion between 0 and 8 weeks, which could be useful for determining specific differences between package systems. Best straight lines were drawn from regression equations of the data points for the first 8 weeks by a computer/plotter. In samples held in fluorescent light (Figures 12, 13, 14) only a slight increase in TBA absorbance values was noted in each

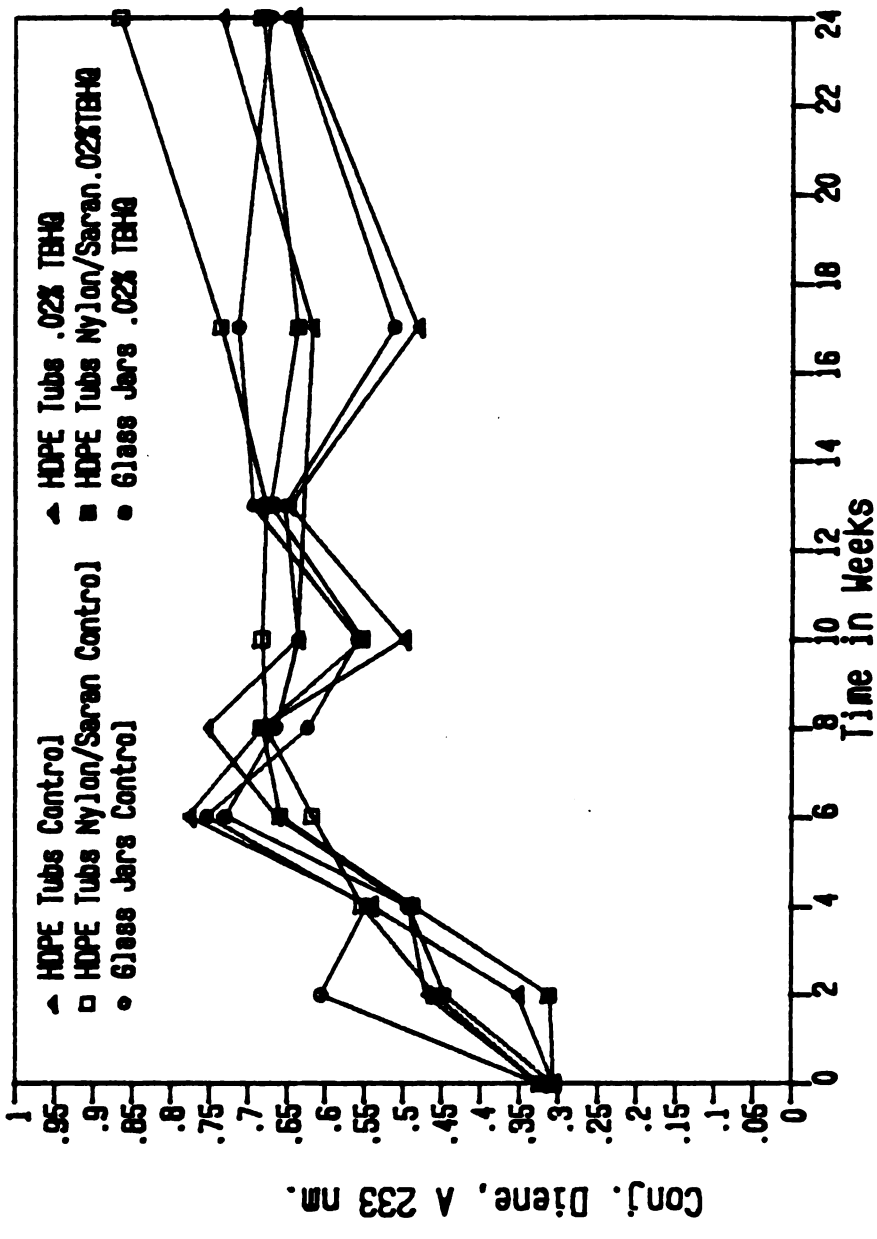


Figure 11. Conjugated diene absorbance, absorbance 233 nm. vs time for soynut butter during storage in dark, 37°C condition.

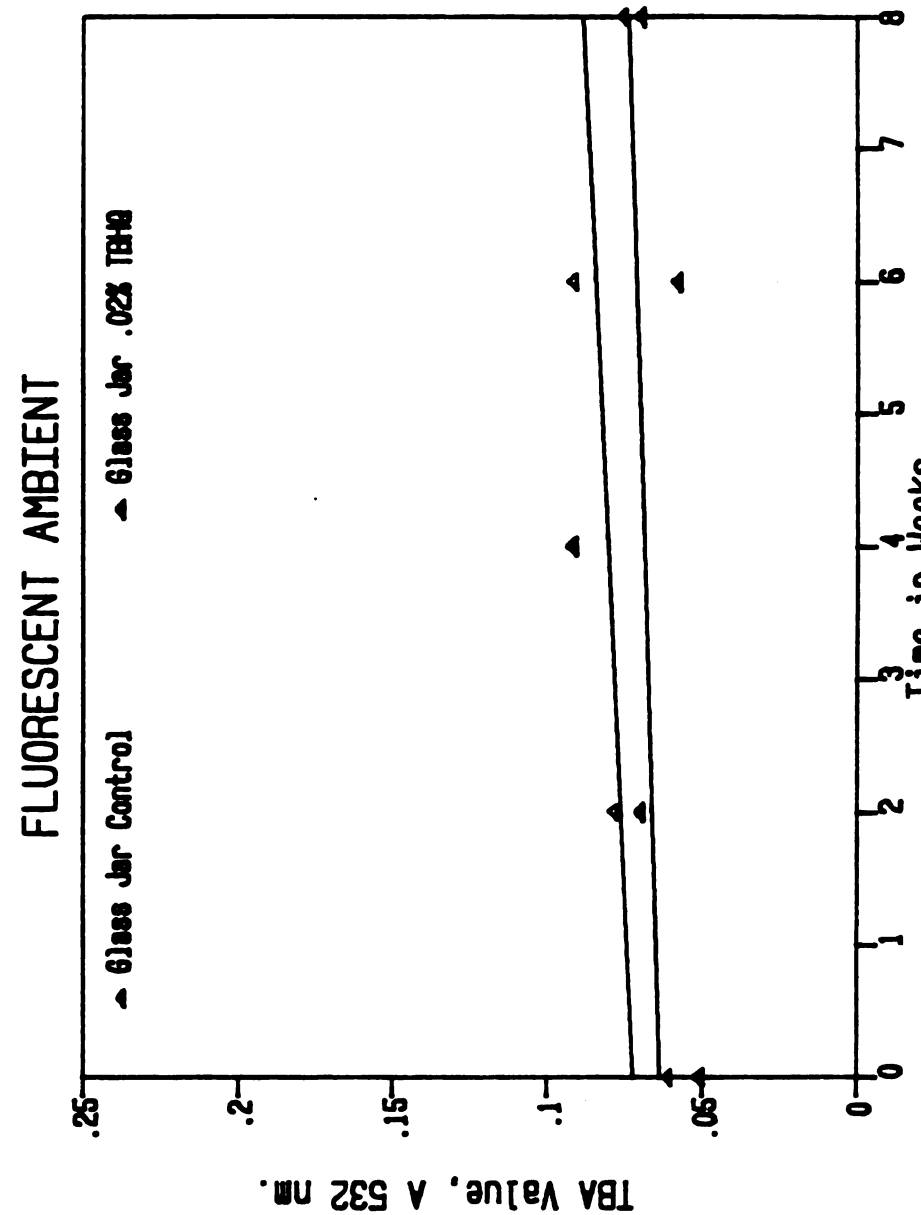


Figure 12. TBA, absorbance 532 nm. vs time for soynut butter during storage in fluorescent light, 22°C (ambient) condition in glass jars.

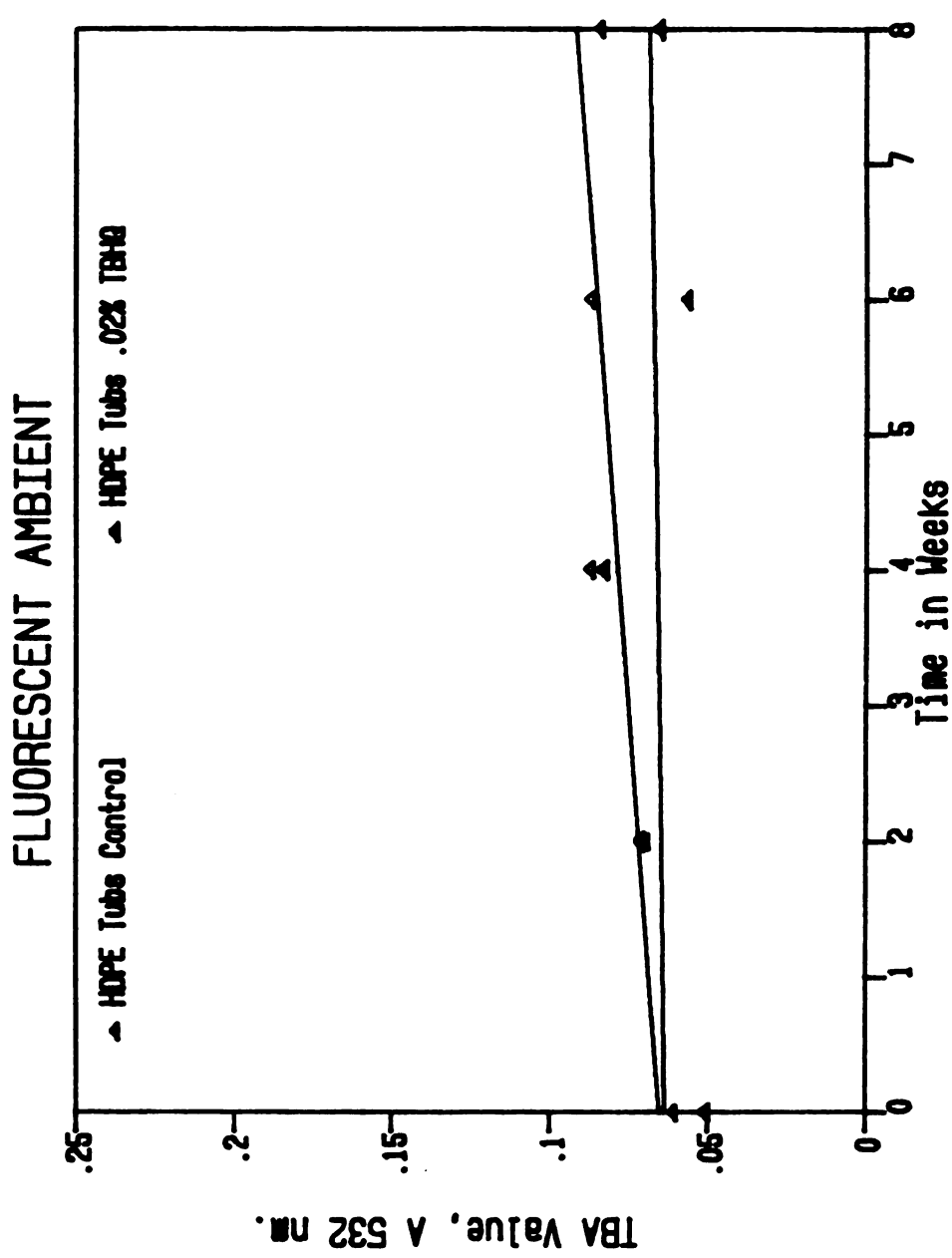


Figure 13. TBA, absorbance 532 nm. vs time for soynut butter during storage in fluorescent light, 22°C (ambient) condition in HDPE tubs.

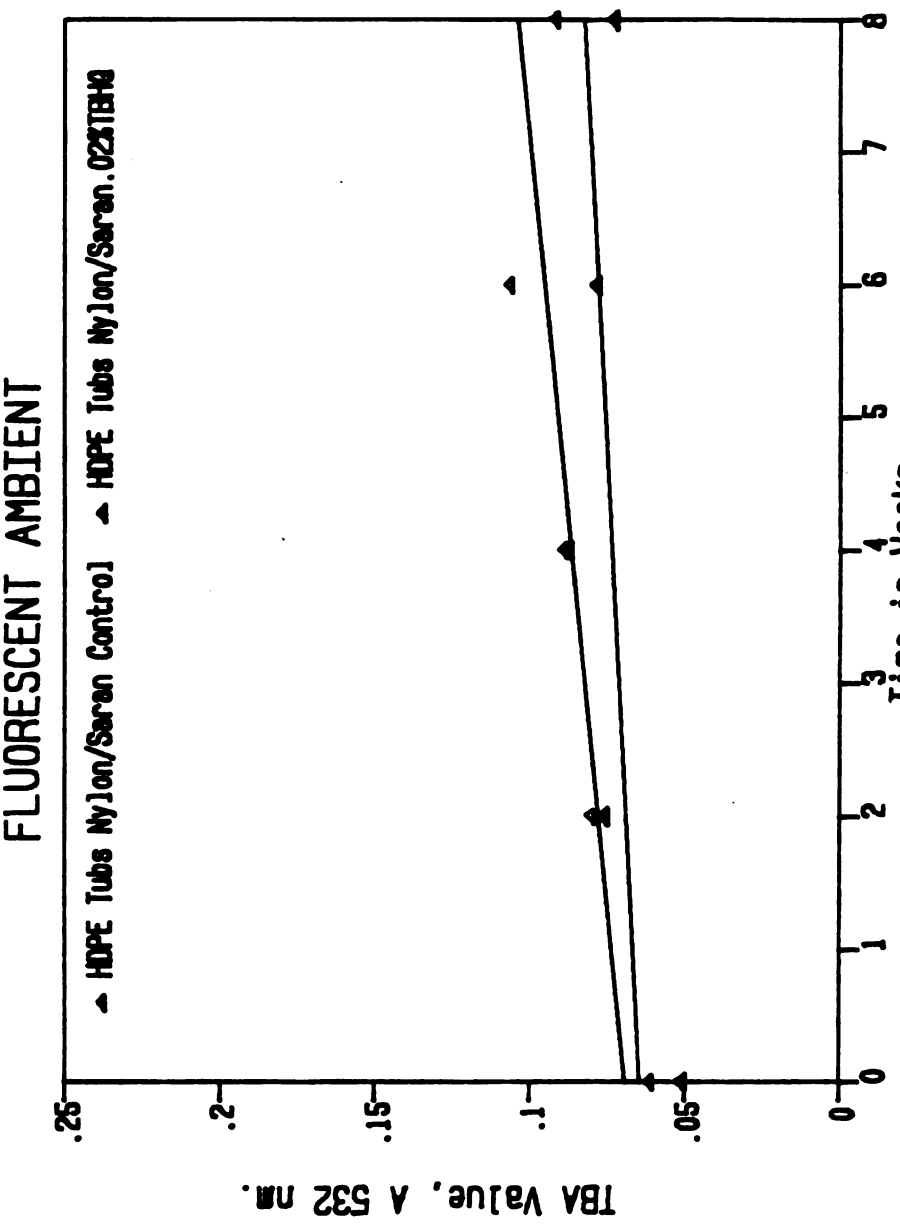


Figure 14 . TBA, absorbance 532 nm. vs time for soynut butter during storage in fluorescent light, 22°C (ambient) condition in HDPE in (nylon/saran pouches).

package system. The presence of TBHQ/CA did have a stabilizing effect during the first 8 weeks in each container. Tubs with TBHQ/CA had an average absorbance of 0.066 and glass jars with TBHQ/CA had an average of 0.071. These were the lowest values and suggest that HDPE tubs may provide protection similar to glass jars when antioxidant is added to the product at an optimum level. Similar results were seen at dark, ambient and at dark, 37°C conditions in Figures 15 and 16. Figure 15 shows that soynut butter packed in HDPE tubs without antioxidant has a steady increase in absorbance values. With TBHQ/CA the line is essentially horizontal indicating virtually no change in absorbance. At dark, 37°C there is an upturn in absorbances in both control and antioxidant containing samples. Dark, 37°C was the environment which appeared to be the most conducive to increased rates of lipid autoxidation as measured by TBA and conjugated diene absorbance, as well as peroxide value in Study #1. At high temperatures hydroperoxide decomposition and secondary oxidations occur at extremely rapid rates (Nawar and Witchwoot, 1980). The amount of a given decomposition product at a given time during the autoxidation process is determined by: hydroperoxide structure, temperature, the degree of autoxidation, and the stability of the decomposition products themselves, and therefore exerts a major influence on the final quantitative pattern (Ibid).

Conjugated diene absorbance values of samples stored in dark, 37°C increased at a faster rate than TBA absorbances (Figures 17, 18 and 19). Those samples without TBHQ/CA had an average conjugated diene absorbance of 0.683 by 8 weeks time, as compared to 0.678 for those samples with antioxidant as measured at 233 nm. Samples in HDPE tubs

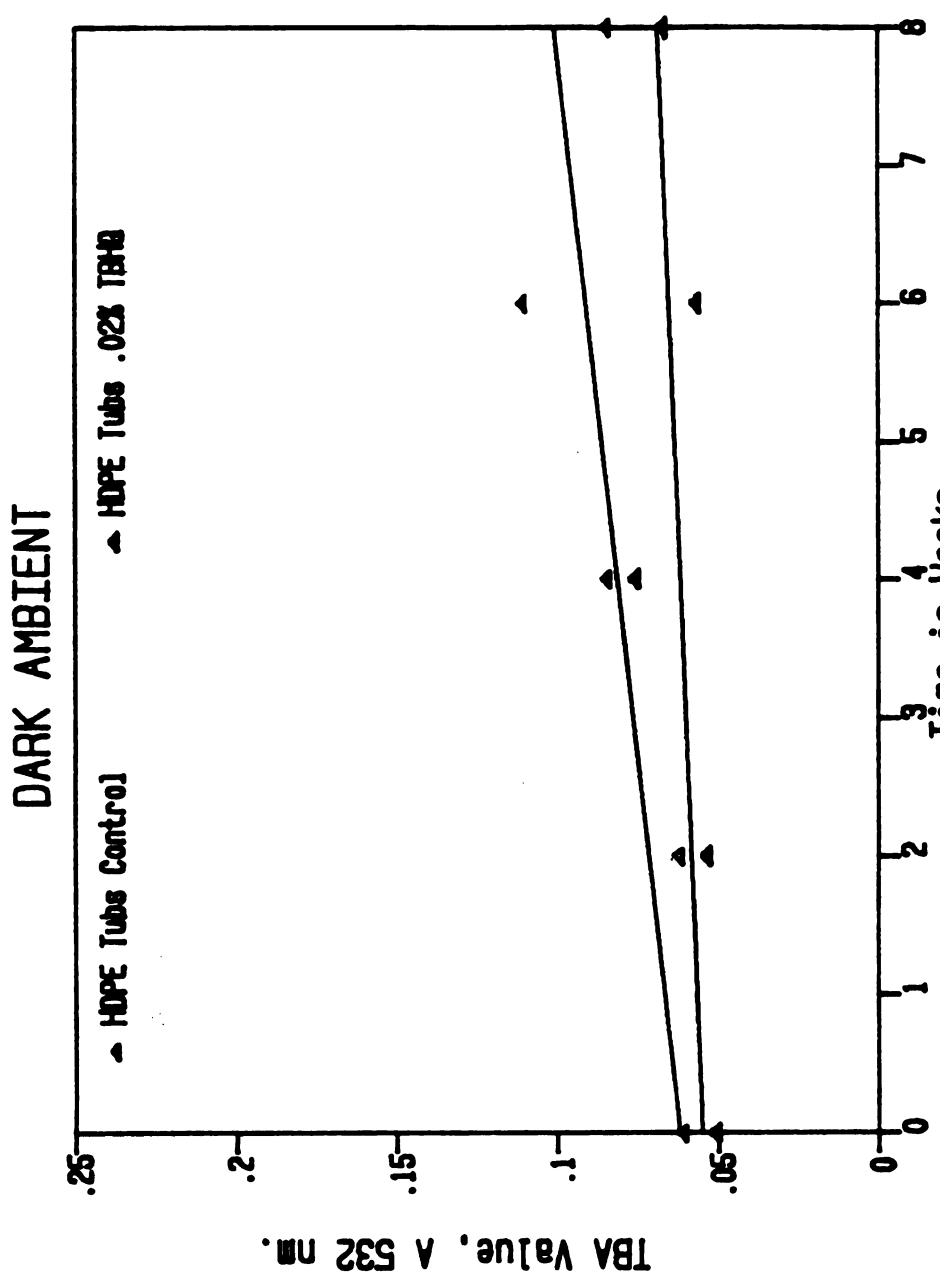


Figure 15. TBA, absorbance 532 nm. vs time for soynut butter during storage in dark, 22°C (ambient) condition in HDPE tubs.

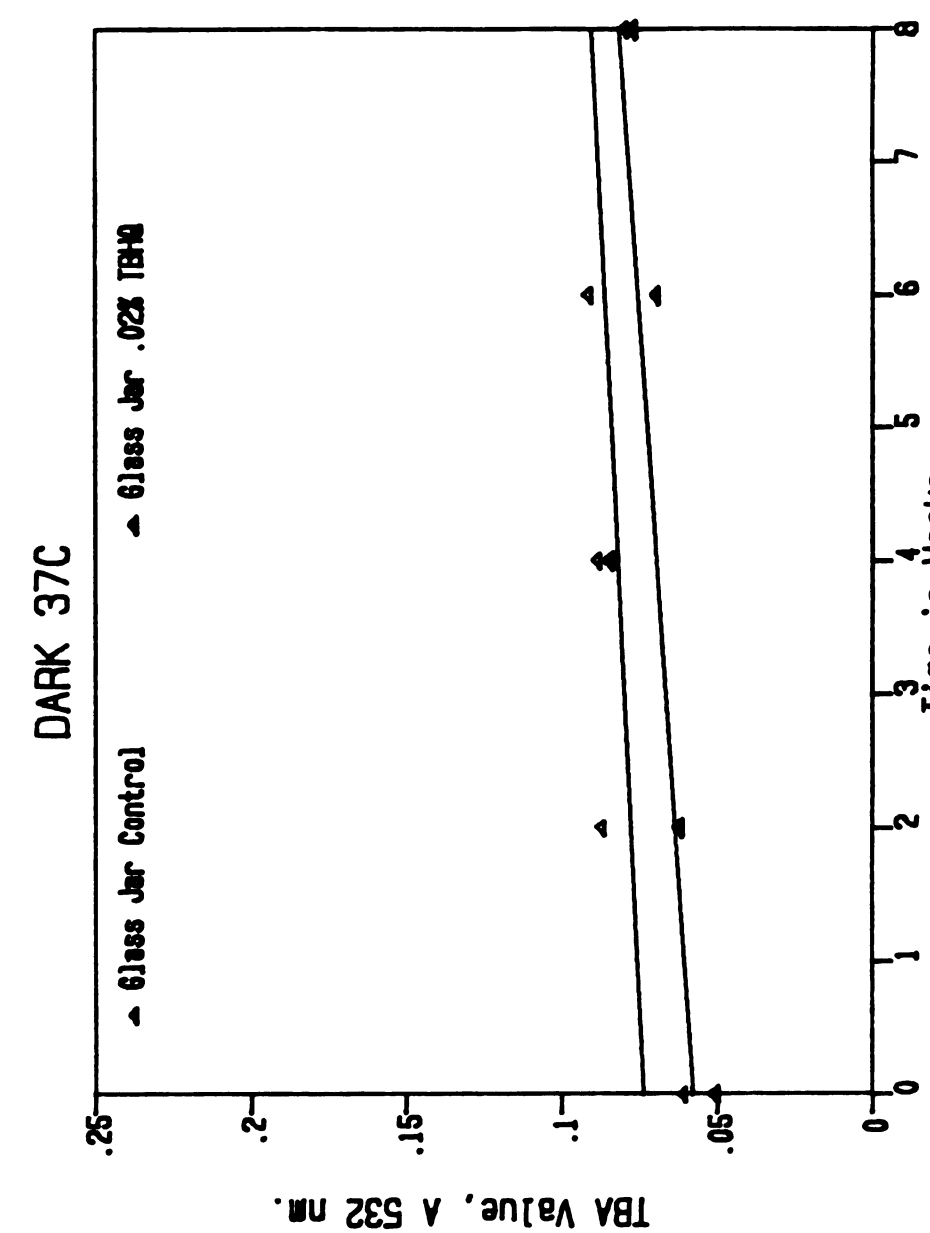


Figure 16. TBA absorbance 532 nm. vs time for soynut butter during storage in dark, 37°C condition in glass jars.

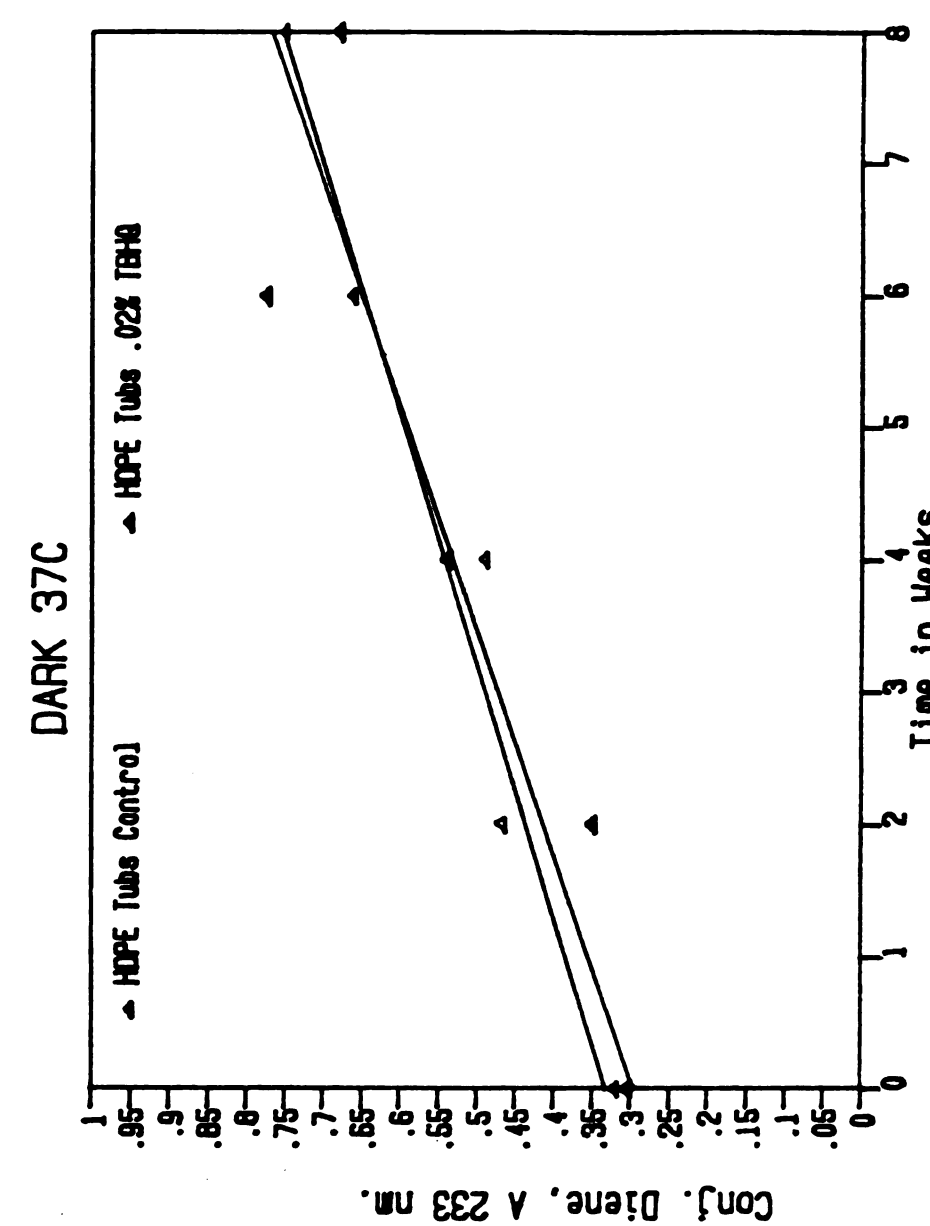


Figure 17. Conjugated diene absorbance 233 nm. vs time for soynut butter during storage in dark, 37°C condition in HDPE tubs.

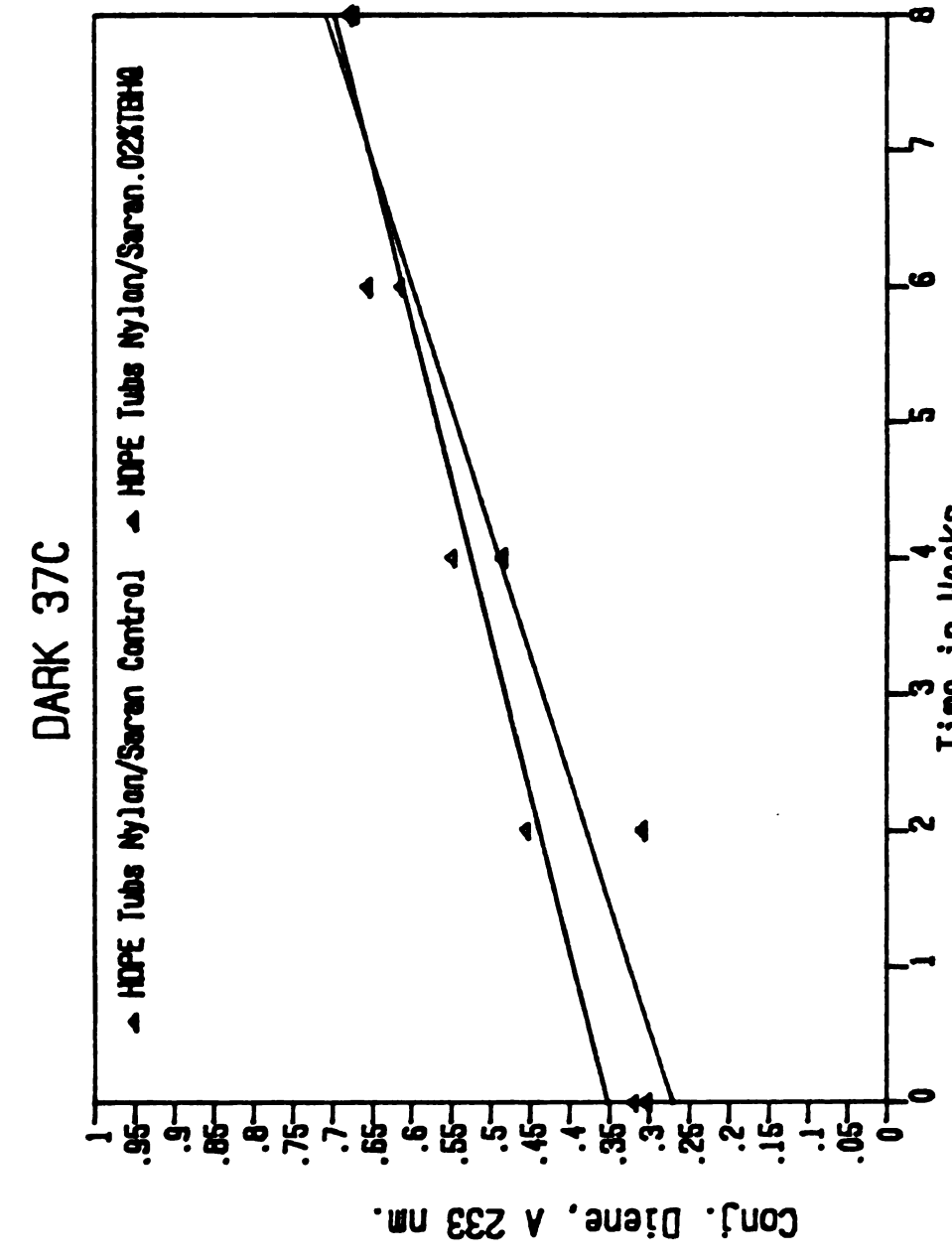


Figure 18. Conjugated diene absorbance 233 nm. vs time for soynut butter during storage in dark, 37°C condition in HDPE in (nylon/saran pouches).

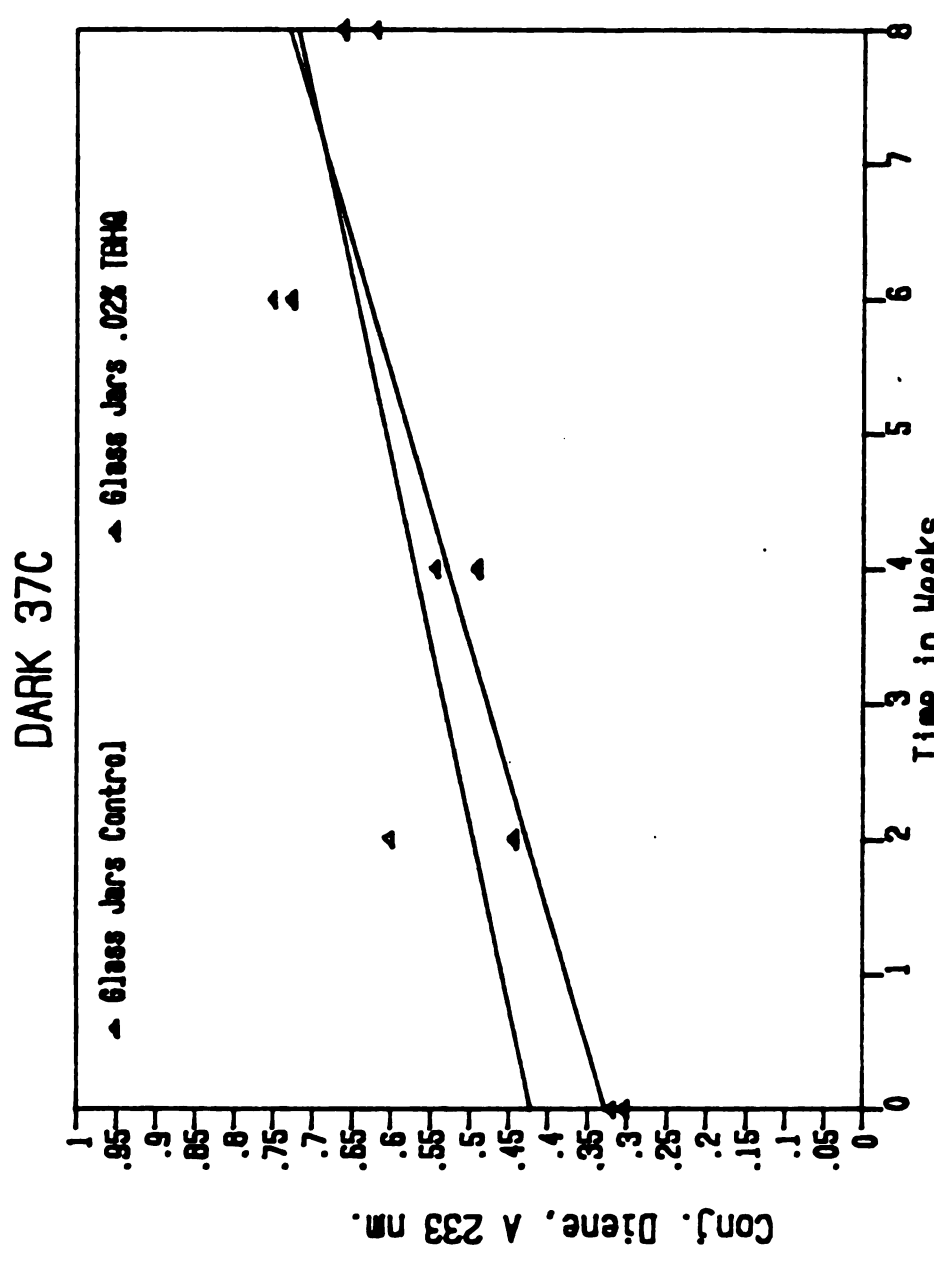


Figure 19. Conjugated diene absorbance 233 nm. vs time for soynut butter during storage in dark, 37°C condition in glass jars.

TBHQ/CA had an average of 0.670. Control samples showed that HDPE tubs without TBHQ/CA had an average absorbance of 0.754 as compared to 0.621 for control spread packed in glass jars. The soynut butter packed in tubs in evacuated nylon/Saran^{R} pouches with TBHQ/CA had an average conjugated diene absorbance of 0.684 by 8 weeks time. The oxygen transmission rate of the HDPE tubs alone is 21.1 cc/pkg.x24 hr. and the nylon/saran pouches have a transmission rate of 9.0 cc/pouchx24 hours. The plot of water net weight gain vs. time in hours can be seen in Figure 20. The WVTR of the HDPE tubs is 0.15 mg H_2O/pkgx24 hr. x mm Hg.

The original expectation was to see increased lipid autoxidation in the soynut butter stored in the tubs alone as compared to that stored in tubs in nylon/saran pouches, and glass jars. During processing and packaging of soynut butter samples there were varying amounts of dissolved oxygen in the form of air bubbles in the spread. There were also varying amounts of residual oxygen in the headspace above each sample. Apparently there was enough internal oxygen in each sample that the barrier characteristics of the packages could not be easily differentiated. Currently though, the same HDPE tubs are used for a clarified butter product sold in grocery stores. Tubs of narrower thickness are also used for peanut butter. Cecil (1948) believed the main factor in preventing oxidation of peanut butter is proper packaging (Woodroof, 1983). Vacuum packaging is commonly used as an aid in minimizing air in the headspace, but even without evacuation, a completely filled and sealed jar will contain insufficient oxygen to cause rancidity other than in the layer in direct contact with the

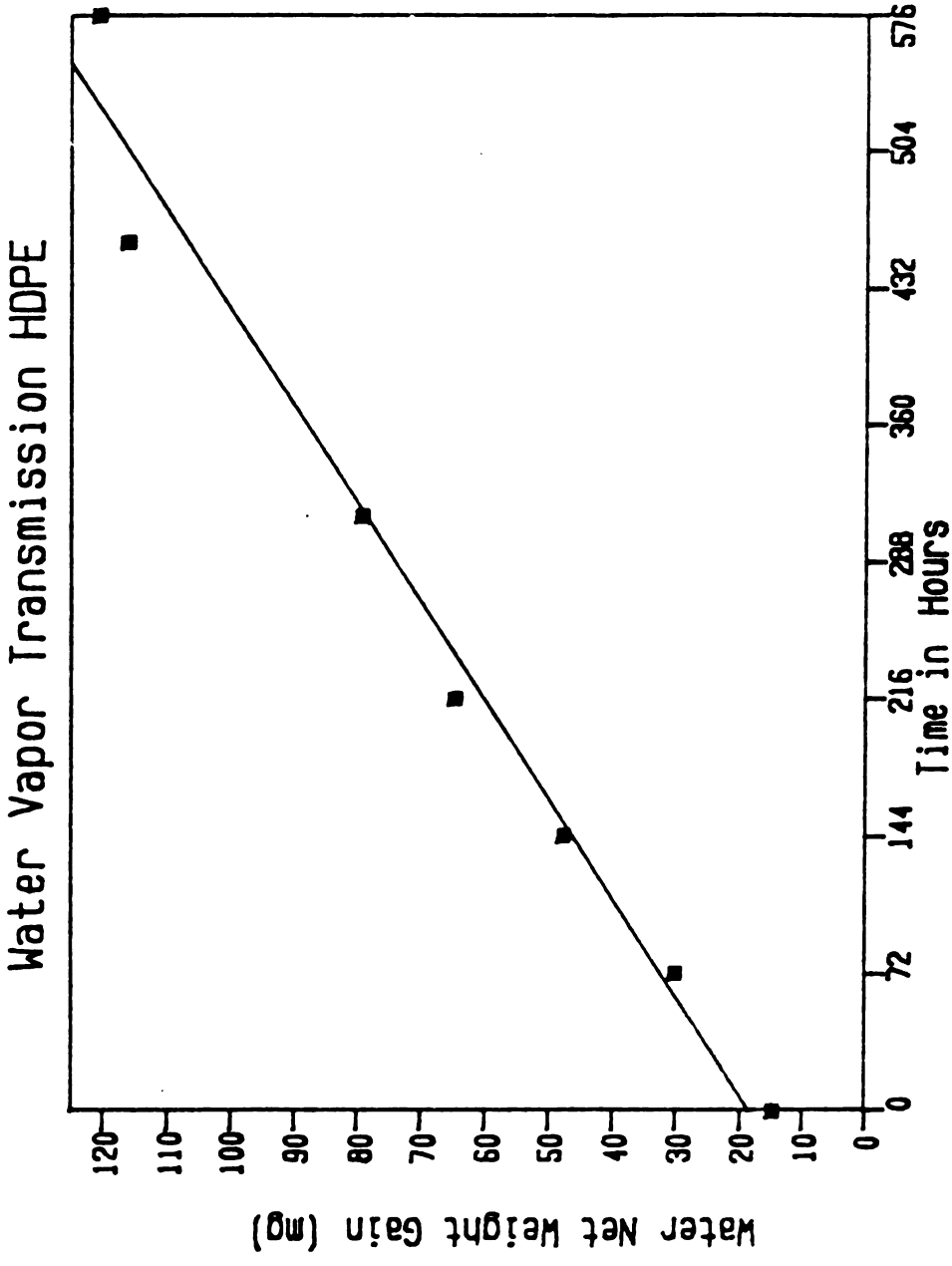


Figure 20. Net weight gain of water (mg) vs time in hours for HDPE tubs.

headspace (Ibid).

As previously seen in Tables 3, 4 and 5, there were more pronounced lemony and grassy off flavors in those samples stored in glass jars in fluorescent light. HDPE tubs with .02% TBHQ/CA added to the sample provided better stability despite only slight differences in conjugated diene absorbance values between packages (Figures 12, 13, 14). Storage in opaque or colored containers, as compared to clear jars, can be seen in terms of reduced oil separation, better aroma and flavor, but is not significant for chemical determinations (Ibid). The shelf-life of peanut butter without antioxidants in polyethylene jars has been determined to be 9 months to 1 year (Ibid). It may be projected that soynut butter with .02% TBHQ/CA in HDPE tubs, can be stored in a controlled ambient environment from 9 to 12 months as well due to the antioxidant protection. Soynut butter stored in glass jars in a dark, ambient environment may be stored from 12 to 16 months. Soynut butter stored in plastic (polyethylene) pails for 1 year in the dark was tasted by an informal panel alongside month old samples stored in glass jars in the dark. Panelists could not perceive any major differences between the two sample soynut butters in terms of rancid flavor. Additional sensory work, in terms of optimum sweetener levels, was studied to further product acceptability by consumers. The importance of these results is that a significant cost-savings can be realized by using lighter HDPE tubs for packaging of soynut butter.

Fatty acid composition of extracted oil samples from soynut butter showed that after 24 weeks of storage in 37^{o}C, dark unsaturated fatty acids 18:1, 18:2, and 18:3 were most protected with .02% TBHQ/CA

added (Tables 6, 7, 8 and 9). Linolenic (18:3) acid was 9.0% of the total fatty acid array measured in the soynut butter in HDPE tubs with antioxidant, 6.7% in tubs in nylon/saran pouches without antioxidant, and 5.0% in glass jars, control and antioxidant samples. Increased linolenic acid protection may be related to decreased production of secondary lipid oxidation products; aldehydes, ketones, alcohols. HDPE tubs are slightly poorer in protection of oleic (18:1) and linoleic (18:2) acids which are also responsible for secondary oxidation products.

Sensory Analysis of Optimum Levels of Sweetener

Of the fifty volunteers, 45 responses were accepted in both the degree of difference test and the degree of preference test. An analysis of variance was performed on the data received from the degree of difference test, and can be seen in the ANOVA Table 10. The calculated F value (33.60) exceeds the F values at the 5% and 1% levels of significance. The conclusion is that consumers can detect significant differences in the relative sweetness between soynut butter samples at the 1% level of significance. Since there was a significant difference among the samples, the ones that were different were determined using Tukey's Test. Sample means were arranged according to magnitude and the least significant difference was calculated as 0.756 (Table 11). Any two sample means that differ by 0.756 or more are significantly different at the 5% level. Results indicate that sample containing 9.6% pear concentrate is significantly sweeter than the other seven samples. Samples with 8.1% pear concentrate and 3.0% fructose were significantly sweeter than samples containing dextrose

Table 6. Fatty Acid Composition of Original Oils

Fatty Acid**	Fatty acid (%)		
	"Hi-Tone" veg. oil*	Extracted Hi-Tone Control	Extracted Hi-Tone (.02% TBHQ)
16:0	10.0	11.6	12.2
18:0	6.0	7.5	7.9
18:1	49.0	36.5	38.9
18:2	31.0	33.3	33.9
18:3	2.0-5.0	3.8	4.4
20:0	-	-	0.6
22:0	-	3.7	2.0
24:0	-	2.8	-

*Values Courtesy Bunge Edible Oil Co.

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

Table 7. Fatty Acid Composition of Extracted Oil at 24 Weeks, 37°C, Dark.

Fatty Acids**	Fatty acid (%)	
	HDPE tubs (control)	HDPE tubs (.02% TBHQ)
16:0	11.1	11.5
18:0	7.4	8.1
18:1	35.6	38.3
18:2	32.3	33.0
18:3	5.5	9.0
22:0	8.0	-

Table 8. Fatty Acid Composition of Extracted Oil at 24 Weeks, 37°C, Dark.

Fatty Acid**	Fatty Acid (%)		
	Glass jars (control)	Glass jars (.02% TBHQ)	HDPE tubs nylon/saran (control)
16:0	12.1	11.6	12.1
18:0	8.7	7.6	8.7
18:1	38.9	40.3	38.5
18:2	34.0	35.4	33.9
18:3	5.0	5.0	6.7
22:0	-	-	-

Table 9. Fatty Acid Composition of Extracted Oil at 24 Weeks, 37°C, Dark

Fatty Acid**	Fatty acid (%)	
	HDPE tubs (control)	HDPE tubs (.02% TBHQ)
16:0	11.1	11.5
18:0	7.4	8.1
18:1	35.6	38.3
18:2	32.3	33.0
18:3	5.5	9.1
22:0	8.0	-

Table 10. Degree of Sweetness (ANOVA) (n=45)

Source of variance	df	ss	ms	f
Samples	7	318.92	45.56	33.60*
Judges	44	126.29	2.87	2.12
Error	308	417.58	1.36	
Total	359	862.79		

$F_{\alpha.05}$ (7/308) = 2.0392, $F_{\alpha.01}$ (7/308) = 2.7084, (SE) = $\sqrt{(1.36/45)}$ = 0.174

(*33.60 > 2.7084, The difference between samples is significant at the 1% level)

Table 11. Comparison of Sample Means (Tukey's test (Snedecor, 1956))

n=45

Sample	9.6% P	8.1% P	3.0% Fructose	1.50% Fructose
Sample avg.	7.82	6.58	6.44	5.89

Sample	3.0% Dextrose	4.0% C.S.S.	2.0% C.S.S.	1.5% Dextrose (R)
Sample avg.	5.55	5.13	4.96	4.89

C.S.S. = Corn Syrup Solids

Significant studentized range at the 5% level (8/308) = 4.347

Least significant difference = 4.347 x 0.174 = 0.756

(Any two sample means that differ by 0.756 or more are significantly different at the 5% level.)

and corn syrup solids. The reference sample (R) was 1.5% dextrose and was judged to be the least sweet of all the samples.

Degree of Preference Test

The degree of preference test is a hedonic measurement used to determine the likes and dislikes, and the degree to which the consumer registers the sensory qualities of the product being analyzed. Consumers may say they prefer a certain product, but when it comes to behavior they may or may not follow what they say. For simple tastes, most individuals rated sweet as pleasant, salt as pleasant at low and middle levels but unpleasant at high levels, and sour and bitter as unpleasant at most concentrations (Engel, 1928). In most applied sensory research, overall "liking" represents the "bottom line" or key evaluative criterion against which the researcher judges all other variables (Moskowitz, 1984). The data in Table 12 show percentages of the 45 person sample liking and disliking the soynut butter samples with different levels of sweetener.

The words "would not purchase" were added to the dislike category, and the words "would purchase" were added to the category of like responses. Samples containing 3.0% fructose showed that one third of the 45 people polled indicated they liked it moderately and would purchase it. 17.8% of the respondents said they liked the product very much or more and would purchase it. In contrast, the sample with 1.5% dextrose only rated 11.1% like responses at the "moderate" level and 17.8% at the "slight" level. The most disliked product contained 1.5% dextrose with 71% of the 45 consumers indicating dislike for the product. The 3.0% fructose sample was least disliked with only 31%

Table 12. Degree of Preference (% of 45 responses)
n=45

	Dislike and Would Not Purchase				Like and Would Purchase			
	extreme	very much	moderate	slight	slight	moderate	very much	extreme
1.5% Dextrose	4.4	4.4	22.2	40.0	17.8	11.1	0.0	0.0
3.0% Dextrose	2.2	4.4	8.9	37.8	28.9	17.8	0.0	0.0
1.5% fructose	0.0	4.4	6.7	40.0	13.3	6.7	0.0	0.0
3.0% Fructose	0.0	0.0	8.9	22.2	17.8	33.3	15.6	2.2
8.1% Pear	2.2	2.2	17.8	24.4	31.1	13.3	8.9	0.0
9.6% Pear	6.7	0.0	24.4	24.4	20.0	8.9	15.6	0.0
2.0% Corn Syrup Solids	4.4	8.9	13.3	33.3	28.9	6.7	2.2	2.2
4.0% Corn Syrup Solids	0.0	4.4	11.1	44.4	24.4	15.6	0.0	0.0

disliking it. Textural parameters were not analyzed in these sensory tests but ultimately played a part in the respondents' evaluation of each sample. A higher amount of added vegetable oil, and presentation of soynut butter on saltless crackers was quite effective in registering the consistent responses. Initial soynut butter production for consumer markets should use a sweetener like fructose at the 3.0% level. The disadvantages of fructose are its high cost, and its reducing sugar characteristics which will affect Maillard browning. Citric acid may help to slow the browning reactions. Dextrose is used by peanut butter manufacturers as well as corn syrup solids (Weiss, 1983).

SUMMARY

Development and packaging of a soynut butter was studied based on the work of Pichel and Weiss (1967). Optimum antioxidant combinations and optimum package systems were evaluated in two separate studies. In the initial study the peroxide value and TBA value were utilized to follow the oxidative changes in soynut butter samples containing various antioxidants. Results of the first study indicated that 0.02% TBHQ/CA was the most effective of the antioxidants used in terms of lower peroxide and TBA values. Control samples without antioxidant, or containing BHA/PG, were least effective in inhibiting autoxidation in the soynut butter.

In a subsequent study conjugated diene absorbance and TBA (distillation) absorbance methods were employed to evaluate lipid autoxidation in soynut spreads in various packaging systems during storage for 24 weeks. Results of the best straight lines at eight weeks indicated increased conjugated diene and TBA absorbance numbers for control samples without TBHQ/CA. There was little difference in packages as measured by the two chemical determinations, but significant differences were detected in terms of light catalyzed flavor for samples stored in fluorescent light. Soynut butter in opaque high density polyethylene (HDPE) tubs had decreased light catalyzed off-flavor production as compared to glass jars. A substantial cost savings may be realized if soynut butter is packaged in HDPE tubs, with a resultant shelf-life

of 9 to 12 months under controlled storage. Savings would result from lower distribution costs due to decreased weight per pallet and decreased package costs.

Sensory evaluation indicated that consumers prefer a spread containing 3.0% fructose as a sweetener, and they they are able to differentiate various levels of sweetener in soynut butter at the 1% level of significance. Production studies showed that with a minimum of 25% added vegetable oil, a pre-cut time of 5 minutes, and a steady rate of addition of the pre-cut mix to the Comitrol™ grinder, smooth throughput and temperatures below 180°F were achieved.

RECOMMENDATIONS

Further work in the study of autoxidation rates in soynut butter is recommended. Headspace volatiles such as hexanal, and reversion compounds such as α-pentyl furans could be concentrated on a Tenax™ GC trapping system and quantitated over time during storage in various environmental conditions (Doi et al., 1980). A full production scale-up study should be undertaken, with an uninterrupted flow from raw ingredients to final processing and packaging. A swept-surface heat exchanger such as the Votator™ is recommended to give a smooth and cool product without air bubbles. A distribution study with soynut butter packed in various containers to the points of retail sale would help to further confirm the final package selection. Further market testing as to ethnic and geographical preferences is also recommended to assure that the perceived market actually exists.

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