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To the Graduate Council:

I am submitting herewith a thesis written by Wan Nan Chou entitled "A Comparison of Two Methods to Produce Soy Protein-Lipid Films." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

Hugh D. Graves
Major Professor

We have read this thesis and recommend its acceptance:

Ivon E. McCarty

David L. Coffey

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

A COMPARISON OF TWO METHODS TO PRODUCE SOY PROTEIN-LIPID FILMS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Wan Nan Chou

August 1973

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CRANES CREST

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ABSTRACT

The formation of soy protein-lipid film was studied with natural soymilk and model systems in attempts to optimize processing and yield by the traditional method and soy protein isolate method. Under optimum conditions, the system gave a film protein incorporation efficiency of over 80 percent based on the total protein in solution. Dispersion included 2 percent dispersible protein and 5 percent fat which emulsify and bind the protein-lipid complex to enhance yield. Associated protein of heavy particle weight, long aliphatic chain lipids, and a mixture of polar lipids favored the protein-lipid association and facilitated the film formation.

The isolate soy protein was prepared by isoelectric precipitation and then centrifuged 20 minutes at 796 G. The sediment was neutralized and was dried for one hour at 100°C. The yield of film was 36 percent compared to a yield of 30 percent by the traditional method. This film contains more protein and less fat than traditional film.

The finished film product by the traditional method and isolate method could be used as a food ingredient directly, or further fabricated into a textured food with enriched protein (52 percent protein in dry-basis in traditional film and 53.6 percent in isolate soy protein film). These two processing methods may be extended to many other protein and oilseed resources, such as cottonseed and peanut.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	4
	World Production of Major Oilseeds	4
	Utilization of Soybean Protein	5
	Tofupe: Protein-Lipid Soy Film	5
	Soybean Protein	6
	Behavior of soybean protein	7
	Heat denaturation of soybean protein	8
	The Protein-Lipid Phenomenon	10
	Flavor Problems	11
III.	MATERIAL AND METHODS	14
	Raw Material	14
	Methods of Analysis	14
	Preliminary soybean study	15
	Making soybean milk	15
	Soybean protein-lipid film processing by the traditional method	16
	Soybean protein-lipid film processing by the isolate method	16
	Color measurement for the traditional film and the isolate film	17
	TBA test for rancidity determination	17

CHAPTER PAGE

Sensory evaluation of film 18

IV. EXPERIMENTAL RESULTS 19

Soaking Time and Temperature of Soybean in Hot Water . . 19

Composition of Soymilk, Slurry and Residual Whey After the Formation of Films in the Traditional Method . . . 19

Film Yield from the Isolate Soy Protein Method 23

Recovery of Protein from Soymilk by Isoelectric Precipitation 23

Composition of Soy Protein-Lipid Films Made by the Traditional Method and the Isolate Method 24

Rancidity of Fat in the Traditional and Isolate Soy Protein Films 25

Color of the Traditional and the Isolate Soy Protein Films 25

Sensory Evaluation of Films Made by the Two Methods . . . 29

V. DISCUSSION 33

VI. SUMMARY 37

LITERATURE CITED 40

APPENDIX 44

VITA 51

LIST OF TABLES

TABLE		PAGE
I.	Composition of Soymilk, Residual Slurry and Residual Whey	21
II.	Recovery of Protein From One Liter of Soymilk by Isoelectric Precipitation	24
III.	Composition of the Isolate and the Traditional Films	24
IV.	TBA Absorbency at 532 mμ Measured in Soy Films	26
V.	The Chromaticity Coordinates x, y and Lightness Index for Soy Protein Films	26
VI.	T-Values for Sensory Comparison Between Fresh and Old Soy Protein-Lipid Films, Made by the Traditional and the Isolate Methods and Cooked by Four Cooking Methods	30
VII.	F-Values for Sensory Comparison of Soy Protein-Lipid Films Prepared by the Traditional and the Isolate Methods, Including Fresh and Old Films Cooked by Four Cooking Methods	31
VIII.	The Means of the Sensory Evaluation Score of the Traditional and the Isolate Soy Protein Films Used in 4 Cooking Methods	32
IX.	Formulas for the Calculation of C.I.E. Values From Color-Eye Colorimeter Tri-Stimulus Data (X, Y, Z, $\bar{x}$)	45
X.	The First Taste Panel Score Data on the Fresh Traditional Film in 4 Cooking Methods	46

TABLE PAGE

XI. The Second Taste Panel Score Data on the Fresh Isolate Soy Protein Film in 4 Cooking Methods 47

XII. The Third Taste Panel Score Data on the Old Traditional Film in 4 Cooking Methods 48

XIII. The Fourth Taste Panel Score Data on Old Isolate Soy Protein Film in 4 Cooking Methods 49

XIV. Equations for the Calculation of "t" and "F: Values 50

LIST OF FIGURES

FIGURE PAGE

1. Effect of Soaking Time on Water Uptake by 20 g. Soybeans at 65°C 20
2. Effect of Soaking Time of 100 g. Soybeans on the Protein-Lipid Film Yield 22
3. The (x, y)-Chromaticity Diagram of the C.I.E. System of the Fresh Traditional and the Isolate Soy Protein Films 27
4. The (x, y)-Chromaticity Diagram of the C.I.E. System of the Traditional and the Isolate Soy Protein Films Which Were Stored 5 Months at 34°C 28

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CHAPTER I

INTRODUCTION

About two thousand years ago the soybean was transplanted to China inland from the new territory, west of China proper, and was soon developed into an array of indispensable foods for daily consumption, ranging from green beans and bean sprouts used as vegetables to processed bean curd (tofu) and dried bean curd used in place of meats. Many of these foods are fairly well known in the West. Perhaps a less familiar soy based food is the more sophisticated "vegetable chicken" which is fabricated from the so-called soymilk skin. Its Chinese formal name is "Tofupe" or "Yutope" or the Japanese name, "Yuba" (26). This foodstuff is produced by the process of heating the soymilk in a water bath, during which a cream-yellow, bland flavored surface film forms. After picking up and putting the resulting films in teflon pans for drying at 100°C, the films are packaged in wax paper, put in paper bags, and then marketed or stored in dried sheets, sticks, and chips, or further fabricated into "vegetable chicken". The films can be consumed directly as an ingredient of soups, used as a sheet for wrapping and shaping ground pork, mixed with vegetables as a slice for salad, cooked with cubed pork for sweet-sour pork or roasted pork in various forms. The vegetable chicken is made by molding the films in an aluminum mold into the shape of a whole frying chicken, which is seasoned with soy sauce and different kinds of spices and shaped like a salami. The texture and taste of these foods are so

appealing to the oriental that they have become the favorite dishes of vegetarian gourmets as well as common people. Tofupe foods are also indispensable in religious holiday menus in oriental countries.

The major components of the soy film are protein and fat. Therefore, its nutritional value is equal to or even higher than tofu (soybean curd). The storage quality of the dry films is admirably good, and they may be stored for over a year without detrimental changes. The films are water insoluble and withstand heat treatment. Therefore, they may be steam-cooked or used as discrete particles in gravies. The author has steam-cooked them as porkroll or cooked as soup with meat and vegetables. Owing to their water-absorbing property, films may be seasoned with desirable flavors, such as sweet-sour pork, porkroll, soup and salad.

Although traditional and highly acceptable, despite the primitive mode of manufacture, the yield and quality of this type of product are quite variable. The use of isolated soy protein for the formation of film is an improved method. There have been few technical reports concerning the study of soybean films (tofupe). The oriental soybean foods such as tofu and miso have recently been studied and frequently refined in the United States, but little attention has been given to soybean films.

Soybeans, having been introduced into this country about half a century ago, were previously processed mainly for the economically important oil, and the defatted meal was primarily used as animal feed. Upon recognizing the nutritive value as well as the economic potential of soybeans, food processors and research institutes in the United States have

developed a number of new products and processes such as soy flour and grits, soy protein concentrates, soy protein isolates, spun soy protein fiber, and recently Texgran, a textured product made from defatted soy flour. The future of these versatile products is quite attractive. However, a lower cost and a more direct use of soybean as food may be even more helpful in the utilization of this crop, since the United States is the world leader in soybean production.

By the aid of advanced technology, a more effective process developed for the making of highly acceptable soybean films may contribute not only to the increased utilization of soybeans but also to greater exportation of soybeans to overseas markets. The price of soybean films could be lowered through effective mass production techniques. The present price of "tofupe" made from imported American soybeans is about 25 cents a pound in Taiwan and the yield is about 30 percent by the traditional method. The price could be lowered and the yield could be increased if we could use the isolate method for making soy protein films in commercial practice. It could contribute to highly acceptable food products being developed from many other protein resources, such as cottonseed, sunflower seed, peanuts, single cell protein and leaf proteins. More extensive utilization of this oilseed through the concept of film formation could help to partly alleviate the world problem of protein malnutrition.

The purpose of this study was:

1. To investigate the traditional Chinese process of making soybean films and isolate soy protein films.

2. To compare the chemical compositions, yields and acceptability of the traditional film and the isolate soy protein film and the difference between the processing methods.

CHAPTER II

REVIEW OF LITERATURE

I. WORLD PRODUCTION OF MAJOR OILSEEDS

Milner (20) indicated that the world's production of oilseeds approximates 80 million metric tons of which the major oilseed crops, soybeans, cottonseed, and peanuts, constitute about 90 percent. He also mentioned that the potential contribution of the oilseeds to the world protein resources could be considered to be about equal to that of all the animal protein now available, 20 million tons per annum. Of the world soybean production, as pointed out by Fangauf (12), the U. S. grows 75 percent, China 19 percent, Brazil 2 percent, others combined 4 percent. The U. S. commands 89 percent of the world market, selling to Japan, Germany, Europe, Canada, and China. The production of cottonseed, distributed principally in the U. S., U. S. S. R., China, South Korea, Mexico, Egypt, and Brazil, totaled about 23.4 million metric tons in 1962. It is much more widely distributed over the world than soybeans and peanuts. Cottonseed is grown in practically all the areas of the world where there is a food deficit and prevalence of protein-calorie malnutrition. Parpia and Subramanian (25) listed the production of peanuts in some regions and selected countries of the world. Asia and Africa are the primary areas with India growing about 30 percent, Nigeria 9 percent, the Far East 43 percent, North America 5.6 percent, and Brazil 4 percent.

II. UTILIZATION OF SOYBEAN PROTEIN

The U. S. exports 27 percent, processes 59 percent, sows 6 percent, and puts in surplus 8 percent of its soybean production (20,21). Consumption is as food and feed, fats and oils and in the manufacturing of industrial products. Human nutritional use of the soy protein is appreciable only in the Far East. Soybeans are processed into milk, dairy products, soups, and sauces (12).

Milner (20) pointed out that, even though defatted heat-processed soybean meals are useful and cheap as protein supplements, they suffer certain limitations which additional research and process development must seek to remedy. Much challenge to research exists in finding more effective and economical ways to process soybeans, and particularly to improve their palatability and flavor. One expanded future market hinges upon a new process consisting of alkali dispersion of soy protein followed by extrusion at the isoelectric point to yield 95 percent protein fibers which may be formed into foods with meat-like consistency. Addition of color, aromatics, or flavors yields acceptable meat, fish and poultry analogues. Advantages are storage stability in the dried form, uniform quality, and ability to tailor nutritive value to specific consumer needs (12).

III. TOFUPE: PROTEIN-LIPID SOY FILM

The earliest description of tofupe (Japanese name--"yuba") in England appeared in 1922 (26): "In the preparation of tofupe, soymilk of the best quality is boiled for about one hour in a copper pan over a slow fire. A

small quantity of auramine is added which tends to produce a thick film. The film is separated from the milk by passing a stick underneath the surface; the film is thus removed folded over the stick. It is dried slowly on a galvanized net over a charcoal fire, resulting in a thin yellowish sheet. It is very brittle and is sold in sticks, sheets, or small flakes." In cooking, tofupe is used as a roll for the foods, cut into slices or small pieces, and either fried or used in soups. Tofupe is valued mainly because of its versatile textured form and high protein content which consists primarily of albuminoids combined with fats. The composition of tofupe is shown in the following:

Composition of Tofupe

Authority	Water	Protein	Fat	Carbohydrate	Ash
Oshima (24)	18.31%	49.65%	18.00%	11.82%	2.22%
Borgstrom (7)	--	52.0 %	24.0 %	--	--
L. C. Wu (35)	8.0 %	51.0 %	26.0 %	11.0 %	2.2 %

Recently, Wu (34) presented a method for making soy protein-lipid film wherein the soymilk was heated in flat, shallow, open pans at 90°C. After 3.5 minutes, a cream-yellow, bland flavored surface film gradually formed. The films were successively removed by glass rods from the surface, hung to air dry and marketed or stored as the dried form. The films could be cooked with meats or vegetables into various forms.

IV. SOYBEAN PROTEIN

Many studies (11) have shown that the majority of seed proteins are particle-bound proteins called protein bodies, or aleurones, which exist

mainly in storage form for the need of seed germination. Altschul (1) suggested that they may be named "aleurins" to distinguish them from the minor proteins of seed cytoplasm. Recently, Saio et al. (27) have indicated by chemical analysis that isolated protein bodies contain about 10 percent nitrogen, 0.8 percent phosphorus, 8.5 percent sugar, 7 percent ash, 0.48 percent RNA, and 9.5 percent water. The protein bodies are surrounded by a membrane believed to consist of phospholipids. Effects on protein bodies of processing variables such as flaking, heat and moisture normally used in processing soy products are still unknown. In contrast to wheat, mature soybeans contain little or no starch but a larger number of protein bodies (33).

Behavior of Soybean Protein

According to the review by Wolf (33), the bulk of soybean proteins are globulins, which are insoluble in water at their isoelectric points. They are soluble, however, in water or dilute salt solutions at pH values above or below the isoelectric points. Dispersion of the defatted soybean meal in water at pH 6.5 results in nearly maximum solubility of the proteins as compared to a pronounced minimum in solubility at pH 4.6. This effect of pH on solubility explains the apparent inconsistency in the use of water to extract soybean proteins and their classification as globulins, a group of proteins normally considered to be insoluble in water and soluble only in salt solutions. The high extractability of the proteins with water indicates that the membranes surrounding the protein bodies are easily broken.

Soybean proteins differ from the wheat proteins, gliadin and glutenin, in that they lack the dough-forming properties. However, due to action of lipoxidase on polyunsaturated fatty acids to form hydroperoxides, low heat-treated soybean flour has some bleaching properties in bakery products (33).

Heat Denaturation of Soybean Protein

Circle et al. (10) studied the effect of heat on soybean protein gelation and reported that heating solutions of soy proteinates in concentrations above 7 percent resulted in increased viscosity followed by gelation. In concentrations of 8 to 14 percent protein, gels formed within 10 to 30 minutes at 70° to 100°C. Disulfide cleaving reagents, such as cysteine and sodium sulfite, which act as solubilizing agents for isolates, reduced viscosity of both unheated and heated dispersions of the proteinates and prevented gelation.

Watanabe and Nakayama (31) reported from ultracentrifugal analysis of soybean globulin that it consisted of 2S, 7S, 11S, and 15S fractions, and that 7S and 11S comprised 70 percent of the soybean globulin. They reported that in dilute solutions of the water-extractable proteins, heating caused aggregation of the 11S and 15S fractions plus a part of the 7S fraction as indicated by ultracentrifugation and gel filtration patterns. In the study of gelation of soybean protein, Aoki and Sakurai (3) reported that gelation of gel precursors (protein paste) by heating was prevented by the addition of reducing agents having the ability to cleave disulfide bonds. Oxidizing agents also prevented gelation, but

their activity was not as great as the reducing agents. Urea and guanidine-HCl also prevented gelation generally, but the gelation of rather brittle precursors was promoted by the addition of low concentrations of these agents. There was no correlation between water-holding ability of paste or gels and the toughness of gels in the presence of these agents, while there was a correlation in the absence of these agents.

Recently, Wolf (33) indicated that when a 0.5 percent solution of 11S globulin was heated at 100°C (pH 7.6, 0.5 ionic strength), the solution quickly became turbid and a precipitate formed on continued heating. The aggregation was responsible for turbidity of the solution and was the precursor for the precipitate, which reached maximum value after 7 minutes of heating. Accompanying the disappearance of the 11S component was the formation of a slow-sedimenting fraction of 4S and the transient appearance of the 7S intermediate. The 4S fraction reached a maximum concentration in 5 to 7 minutes and was stable to continued heating for more than 30 minutes. He explained that heating at 100°C obviously disrupted the subunit structure of the 11S molecule. One group of subunits showed a pronounced tendency to aggregate and precipitate, whereas the other groups remained soluble. Although sensitive to heating at 100°C, the 11S protein retained its ability to react with antibodies to the 11S protein when heated at 80°C for one hour. Ultracentrifugal behavior of the protein when heated at lower temperature is not known, but the rapidity of the reactions observed at 100°C suggested that disruption of the subunit structure also occurs at lower temperatures, but

perhaps at a slower rate. Wolf (33) also emphasized in his conclusion that more information is needed about the proteins, other than the 7S and 11S globulins, and possible interactions between the various proteins. Likewise, interactions between protein and nonproteins, such as polysaccharides and lipids, must also be studied. As new food products are developed, new problems will be encountered and more research will be required.

V. THE PROTEIN-LIPID PHENOMENON

Cornwell and Horrocks (11) mentioned that electrostatic, hydrogen, and hydrophobic forces all contribute significantly to the stability of protein-lipid complexes in biological systems. There is little evidence for covalent bonds between protein and lipid although lipopeptides may represent a contribution of this type. When lipids and proteins have isoelectric points near physiological pH (around pH 7.0), then this contributes significantly to the electrostatic force (8,32).

In addition, Cornwell (11) showed that a number of hydrogen bond interactions were important in the organization and structure of protein-lipid molecular complexes. These interactions were thought to include bonding between lipids in their associated lipid complexes, intra- and intermolecular bonds with protein chains, and specific bonds between the lipid and protein moieties of a complex. Some lipid micelles and films were stabilized by hydrogen bondings.

The oil-water interface has a profound effect on the structure of globular proteins. Cheesman and Davies (9) suggested that unfolding

occurs at this interface, converting the protein to a partial random coil, forming intramolecular hydrogen bonds between protein chains and between protein and lipid. Biswas and Haydon (6) examined protein films at the oil-water interface and concluded that intermolecular hydrogen bonds were responsible for the observed viscosity and elasticity. For example, lipid anions were shown to interact with phenolic hydroxyl and amide groups in the protein. Since water molecules compete in hydrogen bond reactions (16,27,28,29), the interactions described above require the additional stabilization effect of electrostatic and hydrophobic bonds. As stated by Kauzmann (16), hydrophobic bonds between nonpolar side chains in proteins and alkyl side chains in lipids are important in intra- and intermolecular bonding with protein-lipid complexes. A large gain in entropy results when nonpolar groups leave the aqueous environment. The free energy change in this transfer is about 3000 to 5000 cal/nonpolar amino acid side chain or 1000 cal/CH_2 group (30). These interactions alter protein structure by decreasing intramolecular hydrophobic bonds (13,15,16,30) and explain partial unfolding at the oil-water interface. Hydrophobic interactions are thought to be important in protein aggregation. It would seem that they are highly significant in stabilizing complexes formed between associated lipid phases and protein.

VI. FLAVOR PROBLEMS

Nelson et al. (22) reported that soybean off-flavor development is caused by the enzyme, lipoxidase, which is almost instantaneous in action. Once this off-flavor has developed, it is practically impossible to eliminate or mask it.

Several reports have afforded us appreciable information about the definite components which are responsible for the "green bean" flavor of soybeans. First, Matlick and Hand (19) reported that a volatile component which developed in soybeans during comminution contributed to the raw bean odor and flavor. Existing in small amounts, but being strong and sharp, this compound was isolated and identified as ethyl vinyl ketone by gas chromatography and mass spectrometry. They demonstrated that this component may be eliminated by grinding soybeans with water at a temperature of 90°C. Soymilk prepared in this manner had no raw bean odor. When ethyl vinyl ketone was added to this milk to give a concentration of 5 ppm, the raw green bean odor was judged to be present. A possible mechanism of formation of this component by oxidation of linolenic acid was postulated. They also pointed out that if this theory of autoxidation of unsaturated fatty acids, together with the action of lipoxidase in soymilk, held for the formation of ethyl vinyl ketone, then anyl vinyl ketone would likewise be formed by a similar mechanism in oxidized milk fat. Secondly, Badenhop and Wilkens (5) found that soaking of soybeans in water resulted in the production of significant quantities of 1-octen-3-ol. They indicated that at 50°C the amount increased with the length of soaking time and reached a maximum after approximately 6 hours. The flavor threshold of 1-octen-3-ol in soymilk was between 0.5 and 1.00 ppm. It is very likely that Matlick's assumption (19) will be proved to be true since 1-octen-3-ol is a reduced form of anyl vinyl ketone. Third, Nelson, et al. (22) devised a treatment in which the soybeans were soaked in tap water for about 4 hours to obtain

a 100 percent weight increase. Enzyme inactivation could then be accomplished by blanching, e.g., heating in tap water at about 210°C for about 10 minutes, cooling by tap water and blending 3 minutes at high speed. This soymilk had no beany flavor. The reason given was the inactivation of lipoxidase.

CHAPTER III

MATERIAL AND METHODS

I. RAW MATERIAL

One lot of Hill variety soybeans was used in all studies. The planting area was Blount County, Tennessee. The planting time was the 1st of June and the beans were mechanically harvested the 20th of September. An analysis of this lot indicated 45 percent protein, 17 percent fat, 23 percent carbohydrate, 11 percent moisture and 4 percent ash.

II. METHODS OF ANALYSIS

Protein nitrogen was determined by the Kjeldahl method (2.024, A.O.A.C., 10th Ed., 1965) (2). Throughout the study a conversion factor of 6.25 was used for the calculation of protein content from the nitrogen content of all samples analyzed.

Total fat was determined by extraction with ether solvent in a Goldfisch extractor. After extraction the fat was ascertained by determining the amount of fat contained in the organic solvent. This was accomplished by evaporating the organic solvent containing the fat in a tared vessel and determining the fat weight by difference.

The ash of a sample is the material remaining after oxidation at 600°C for 3 hours (A.O.A.C. 22.010) (2).

Moisture of samples was determined in a vacuum oven by drying at 70°C for 16 hours in an aluminum drying dish (A.O.A.C. 13.070) (2).

Carbohydrate was measured by difference [100% - (% protein + % fat + % ash + % moisture)].

pH values were read from a Beckman Zeromatic pH-meter.

Preliminary Soybean Study

Fifty g. of clean whole soybeans were ground in a Waring blender for 3 minutes and put into a 200 ml. beaker. Forty g. of the ground soybeans were removed for 4 fractions for determining moisture in a vacuum oven. Protein, fat, and ash were determined after drying in the vacuum oven. Each analysis was performed in duplicate.

Making Soybean Milk

Five hundred g. of soybeans were washed twice in tap water (18°C) and then soaked in 65°C tap water in a stainless steel container for one hour (35) and then divided into 3 portions to accommodate the capacity of the Waring blender. Each grinding consisted of about 170 g. of beans and 800 ml. of soaking water and was blended at high speed for 5 minutes. The blended portions were combined and passed through a screw expeller fitted with a 0.02 inch screen. The first press cake retained by the screen was then divided into two portions, each of which was ground with about 500 ml. of water for 3 minutes. These slurries were also combined and passed through the expeller, and the resulting press cake again comminuted with 500 ml. of water for 2 minutes. The last press cake was mixed with 400 ml. of water and was blended an additional 1 minute. The total volume of water used was 8 liters for 500 g. soybeans with four successive extractions which had blending times of 5, 3, 2, and

1 minutes, respectively. This soymilk was passed through a cheesecloth to obtain the soymilk free from insoluble material. A portion of the soymilk and press cake were vacuum oven dried and analyzed for protein, fat, moisture, ash, and carbohydrate.

Soybean Protein-Lipid Film Processing by the Traditional Method

The prepared soymilks were stored at 35°F and portions of them were used in subsequent studies. A 2.5 liter portion of the soymilk was transferred into a 3 liter shallow aluminum pan which was then placed in a large water bath and surrounded with hot water (80-90°C). An electric fan sent room air over the surface of soymilk. Upon heating, a film formed gradually on the surface of the soymilk. After 7 minutes, the film became strong enough to be picked up by passing a glass rod underneath the film and lifting it in the middle. On lifting, the film folded and hung on the glass rod. The film was drained for one minute and put in a teflon pan and was then oven-dried for 20 minutes at 100°C. After drying the films were weighed to determine the yield and put in paper bags which were stored at 34°C, 37°C, and 0°C for sensory evaluation and rancidity determinations. A portion of the films was ground into powder for 3 minutes in a Waring blender at highest speed and stored in glass bottles at room temperature for subsequent chemical analysis, color quality and rancidity determinations.

Soybean Protein-Lipid Film Processing by the Isolate Method

Two and one-half liters of prepared soymilk were transferred into 3 one-liter beakers and adjusted with 0.5 N NaOH solution to pH 8.0. It

was then adjusted with 0.5 N HCl to pH 4.6. The soymilk was then centrifuged 20 minutes at 796 G. and the sediment was collected and neutralized with 0.5 N NaOH solution to pH 6.6. Two hundred ml. of sediment slurry was spread in a teflon pan and oven-dried at 100°C for one hour, after which the film was sufficiently dry for removal and was then put in paper bags. These films were stored at 34°C, 37°C and 0°C for sensory evaluation and rancidity determinations, and a portion of the films was ground into powder in the Waring blender for 3 minutes at high speed, and stored in glass bottles for subsequent chemical analysis, color quality and rancidity determinations.

Color Measurement for the Traditional Film and the Isolate Film

The Color Eye Colorimeter, Model D-1, was used to measure the tristimulus values of the samples. Thirty g. of ground film were packed as uniformly as possible into a 2.5 inch diameter plexiglass cylinder. The bottom, which was made of optical glass, was fitted over the sample port and held tightly with rubber bands. The colorimeter values were converted to the Commission Internationale de I'Eclainage (C.I.E.) X, Y, and Z values, from which the chromaticity coordinates x and y and lightness index were calculated. Purity and dominant wavelengths were obtained by reference to the C.I.E. chromaticity diagram.

TBA Test for Rancidity Determination

Five g. of ground film were blended with 50 ml. distilled water for 2 minutes. This slurry was put into a beaker with 50 ml. distilled water and then 1 ml. TCA (Trichloroacetic acid) solution (1 g./ml/) was added

to each beaker. After heating 15 minutes at 60°C in a water bath, 2 ml. 95 percent ethanol was added to each beaker and heating was continued 15 minutes. These solutions were filtered through 2 layers of number 1 filter paper 6 times. Four ml. of filterate was measured into a test tube and 0.5 ml. TBA (2-thiobarbituric acid) solution (1.4 g./100 ml. ethyl alcohol) was added. The tubes were held 60 minutes at 60°C, cooled, and centrifuged at high speed. The supernantant was retained and the absorbency at 532 mu was measured with a double-beam grating spectrophotometer, P. and E. Model 124. This procedure followed the method of King (17).

Sensory Evaluation of Film

One test method was used to evaluate the sensory attributes and overall acceptability of the samples. The scoring difference test was conducted to determine if there was a significant difference between the samples and to provide an indication of the degree of acceptability. Values ranging from one to six were used in the scoring test. The sensory test was replicated four times with four different cooking methods. Both traditional film and isolate film were tested with four cooking methods: soup, salad, roll, and sweet-sour.

Statistical analysis was used for the calculation of analysis of variance on sensory evaluation data. Differences between means were analyzed by the Duncan's Multiple Range test. Equations used to calculate "t" and "F" values are listed in the Appendix, Table XIV.

CHAPTER IV

EXPERIMENTAL RESULTS

Soaking Time and Temperature of Soybean in Hot Water

The amount of water absorbed by soybeans upon soaking increased with holding time at a given temperature. Twenty g. of clean and whole soybeans were soaked at 65°C in water for one hour. It appeared that there was a maximum water uptake, around 23 g. per 20 g. soybeans at 65°C for one hour (Figure 1). Wu (35) had mentioned that soybeans soaked at 85°C and 65°C for 2 to 3 hours gave a maximum water uptake, around 1.33 g. per gram soybeans.

Composition of Soymilk, Slurry and Residual Whey After the Formation of Films in the Traditional Method

The composition of soymilk, slurry and residual whey will vary with the variety of beans used, with the method of manufacture and with the quantity of soymilk. Five hundred g. of soybeans were soaked for one hour in 65°C water and they were then extracted with water four times. The total soymilk was 8003 g., the slurry was 450.6 g., and the residual whey was 875 g. after the formation of films by the traditional method. The composition of soymilk, slurry and the residual whey is shown in Table I.

From the above results, the soymilk and slurry were rich in nutritive substances. In China, the soymilk is extensively used for infant feeding.

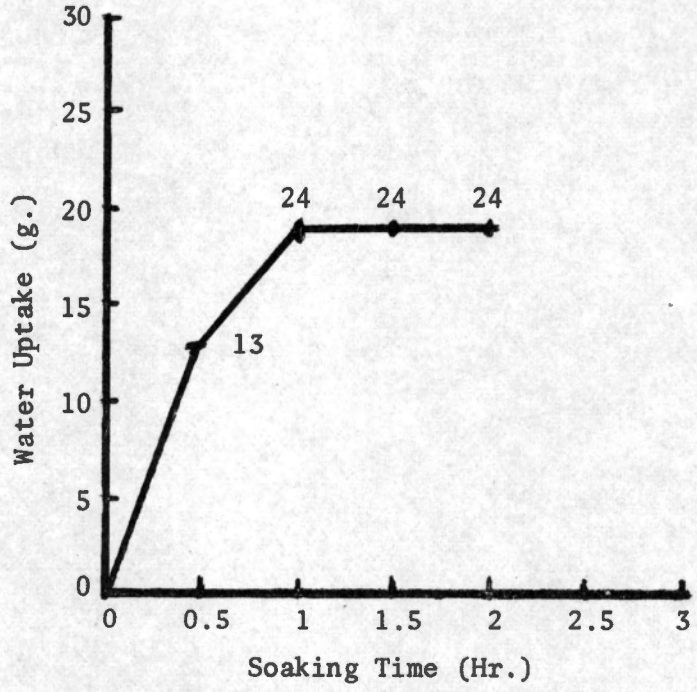


Figure 1. Effect of soaking time on water uptake by 20 g. soybeans at 65°C.

The slurry is cooked with meat or vegetables for the important menu of farmers or for feeding animals.

TABLE I

COMPOSITION OF SOYMILK, RESIDUAL SLURRY AND RESIDUAL WHEY

Product	Protein	Fat	Carbohydrate	Moisture	Ash
Soymilk	1.82%	0.97%	0.92%	95.5%	0.41%
Slurry	1.26%	0.61%	8.9 %	88.7%	0.36%
Residual Whey	0.61%	0.29%	5.32%	92.8%	0.27%

Film Yield of Extracts from Soaked Soybeans in the Traditional Method

Soaking in 65°C water for one hour produced soymilk which gave the highest film yield, whereas soaking times longer than one hour decreased film yield sharply (Figure 2). One hundred g. of soybeans soaked in 65°C water for one hour and made into soymilk produced 30 g. soy films. As shown in Figure 2, it might be concluded that the optimum time at a given temperature for soaking soybeans for subsequent production of soymilk and film was one hour at 65°C. Lo (18) mentioned that to minimize soaking loss, soaking should be continued only long enough to permit the soybeans to about double their initial dry weight, at which condition the power input for grinding was greatly reduced. One hundred g. of soybeans were soaked in tap water (18°C) for 4 hours which resulted in water uptake of 120 g. per 100 g. soybeans. These were blanched 10 minutes at 100°C with soaking water and the film yield was 11.7 g. per 100 g. soybeans. This film contained about 72 percent fat and 0.4 percent protein; it was unsatisfactory film.

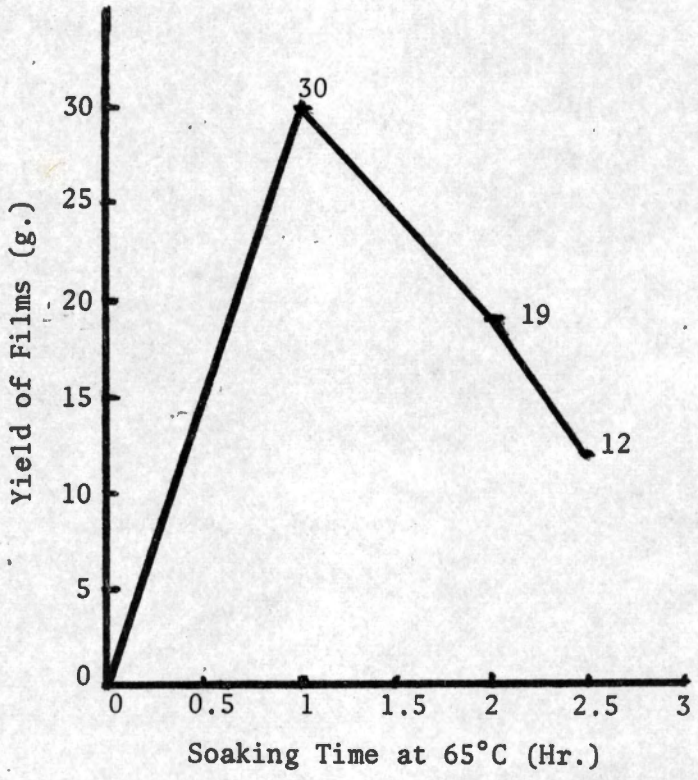


Figure 2. Effect of soaking time of 100 g. soybeans on the protein-lipid film yield.

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Film Yield from the Isolate Soy Protein Method

Two and one-half liters of soymilk were equally divided into five one-liter beakers and the protein was isolated with sodium hydroxide solution and hydrochloric acid, then centrifuged 20 minutes at 796 G. The yield of dry sediment (100°C/1 hour) varied with pH: pH 4.4 - 34.7 g., pH 4.6 - 36.3 g., pH 4.8 - 34.5 g., pH 5.0 - 33.9 g., pH 5.2 - 33.5 g. Sediments were neutralized and adjusted to pH 6.6. Two hundred ml. of the sediment was put into a teflon pan and dried for 1 hour in a 100°C oven. The yield of film was 36.3 g./100 g. soybeans at pH 4.6. This yield was about 6 percent higher than the traditional film yield. In the Chinese fashion, the soy protein is precipitated by heat coagulation in the presence of calcium or magnesium salts. The water-soluble nonprotein fraction is left in solution and rejected after collection of the protein precipitate.

Recovery of Protein from Soymilk by Isoelectric Precipitation

The soy protein was extracted by sodium hydroxide solution and precipitated by hydrochloric acid, then centrifuged 20 minutes at 796 G. The sediments and supernantants prepared at pH 4.4, 4.6, 4.8, 5.0, and 5.2 were collected and analyzed. The protein content is shown in Table II.

From the results given in Table II, the optimum pH value was 4.6 for precipitation of the soy protein. This sediment was dried for one hour at 100°C. Analysis of this sediment indicated that it contained 53.6 percent protein and 27 percent fat.

TABLE II

RECOVERY OF PROTEIN FROM ONE LITER OF SOYMILK BY ISOELECTRIC PRECIPITATION

pH Value	Protein (g.) Sediment	Supernantant
4.4	16.40	1.86
4.6	17.63	0.64
4.8	16.81	1.46
5.0	15.99	2.28
5.2	15.58	2.69

Composition of Soy Protein-Lipid Films Made by the Traditional Method and the Isolate Method

The isolate soy protein film always contained more protein and less fat than the traditional film. The composition of isolate and traditional films is shown in Table III.

TABLE III

COMPOSITION OF THE ISOLATE AND THE TRADITIONAL FILMS

Sample	% Protein	% Fat	% Carbohydrate	% Ash	% Moisture
Isolate Films					
Average	53.6	27.5	6.0	2.8	9.8
Range*	53.2-54.1	26.3-27.8	5.9-7.1	2.5-2.9	9.7-10.3
Traditional Films					
Average	52	32	9.1	2.0	5.1
Range*	50.7-52.2	27.5-33.8	8.7-9.8	1.8-2.3	4.8-5.4

*Fifty samples.

From the results shown in Table III, it was concluded that the isolate films contained more protein and less fat than the traditional films.

Rancidity of Fat in the Traditional and Isolate Soy Protein Films

The acid extract TBA method was used to measure the oxidative rancidity of the samples. The absorbency was an indicator of the degree of oxidation of unsaturated fatty acids in the isolate soy protein films and the traditional films. The red compound of malonaldehyde was formed by reaction of 2-thiobarbituric acid (TBA) with the unsaturated oxidized lipids. TBA absorbency at 532 mμ was measured for the isolate soy protein and the traditional films and in shown in Table IV.

The absorbencies in Table IV show that there was little difference in fat oxidation between the traditional film and the isolate soy protein film. The oxidation was not influenced significantly by storage time nor by storage temperature.

Color of the Traditional and the Isolate Soy Protein Films

The colorimeter values from the Color Eye Instrument were converted to the Commission Internationale de I'Eclainage (C.I.E.) X, Y, and Z values, from which the chromaticity coordinates x and y, and lightness index were calculated. The values of the fresh traditional and isolate soy protein films, and the old traditional and the isolate soy protein films are shown in Table V.

From Table V, the x, y values plotted on a chromaticity diagram of the C.I.E. System (Figure 3 and Figure 4) depicts the uniformity of color and the narrow ban of dominant wavelengths of the traditional film and isolate soy protein film. Figure 3 indicates that the color of the traditional film was darker than the isolate soy protein film. From Figure 4,

TABLE IV

TBA ABSORBENCY AT 532 mμ MEASURED IN SOY FILMS

Sample	Absorbency	
	Average	Range*
Fresh traditional film	0.213	0.210-0.220
Fresh isolate soy protein film	0.183	0.180-0.188
Stored 15 days at 0°C		
Traditional film	0.199	0.196-0.203
Isolate soy protein film	0.185	0.185-0.186
Stored 15 days at 37°C		
Traditional film	0.222	0.220-0.230
Isolate soy protein film	0.203	0.200-0.205
Stored 5 months at 34°C		
Traditional film	0.216	0.210-0.220
Isolate film	0.186	0.183-0.188

*Twenty-five samples.

TABLE V

THE CHROMATICITY COORDINATES x, y AND LIGHTNESS INDEX FOR SOY PROTEIN FILMS

Sample	X_{CIE}	Y_{CIE}	Z_{CIE}	x^*	y^*	Lightness Index*
Fresh						
Traditional film	43.1	42.3	25.2	0.389	0.382	6.495
Isolate soy protein film	49.8	50.6	36.9	0.363	0.368	7.12
Old (34°C for 5 months)						
Traditional film	42.6	42.1	24.9	0.380	0.375	6.492
Isolate soy protein film	48.7	49.9	36.5	0.351	0.357	7.10

*Mean of twenty samples.

A: Fresh Traditional Film

B: Fresh Isolate Soy Protein Film

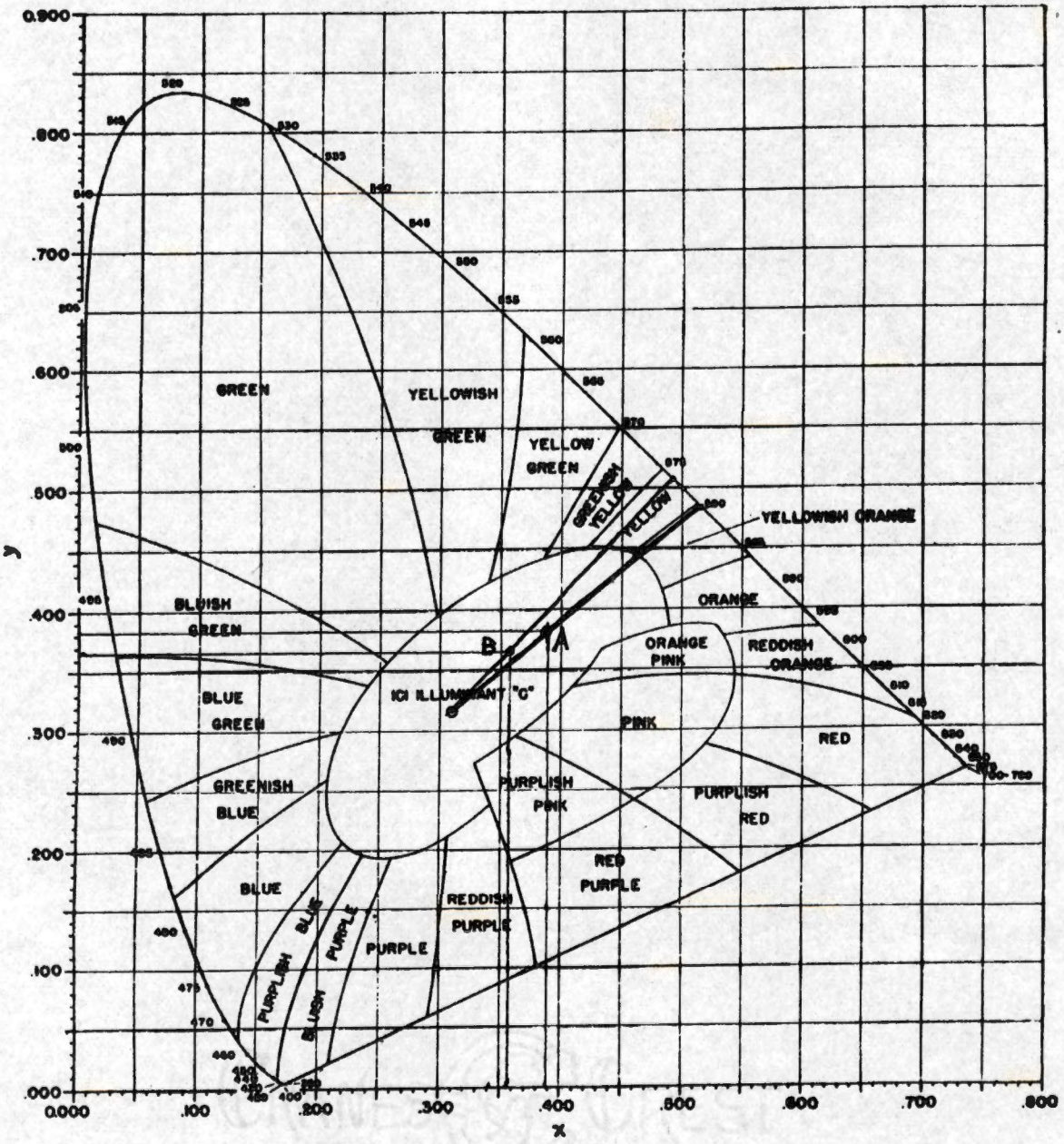


Figure 3. The (x, y)-chromaticity diagram of the C.I.E. system of the fresh traditional and the isolate soy protein films.

A: Traditional Film

B: Isolate Soy Protein Film

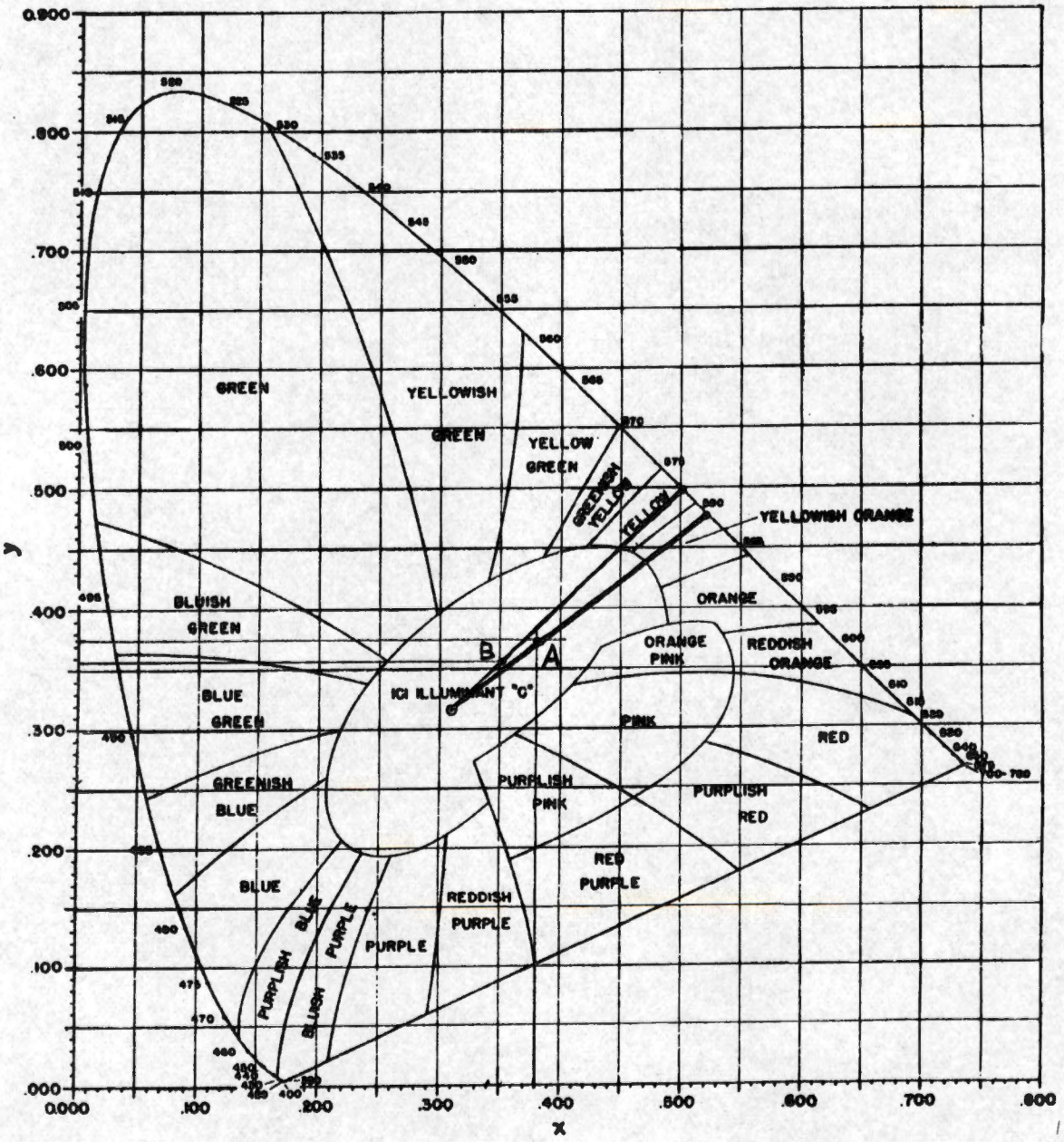


Figure 4. The (x, y)-chromaticity diagram of the C.I.E. system of the traditional and the isolate soy protein films which were stored 5 months at 34°C.

the storage time had little influence on the color of the films. Stored samples were very little darker than fresh films.

Sensory Evaluation of Films Made by the Two Methods

The analysis of variance summaries for the effect of the film processings on the sensory panel scores in 4 cooking methods are given in Table VI and Table VII.

The t-test was used to test the difference between two means. The first taste panel had 10 panelists and the second taste panel had 12 panelists, so a t-test was required for analysis of the variances which went into Table VI. From the tabulated results, there was no significant difference between the fresh and the old films. Time of storage did not affect sensory evaluation results either.

From observation of the results in Table VII, there was no significant difference between the traditional films and the isolate soy protein films prepared by 4 cooking methods. This indicated that processing methods of film made no difference in flavor.

The four cooking methods were in soup, salad, meat roll, and sweet-sour pork. From the results in Table VIII of the cooking methods, the panelists preferred meat roll and sweet-sour over soup and salad when the films were cooked with meats and vegetables. The means of the sensory evaluation scores of the traditional and the isolate soy protein films in 4 cooking methods are shown in Table VIII.

TABLE VI

T-VALUES FOR SENSORY COMPARISON BETWEEN FRESH AND OLD SOY PROTEIN-LIPID FILMS, MADE BY THE TRADITIONAL AND THE ISOLATE METHODS AND COOKED BY FOUR COOKING METHODS

Old \ Fresh	A_{11}	A_{12}	A_{13}	A_{14}	A_{21}	A_{22}	A_{23}	A_{24}
B_{11}	0.307	--	--	--				
B_{12}	--	0.976	--	--				
B_{13}	--	--	0.807	--				
B_{14}	--	--	--	0.694				
B_{21}					0.217	--	--	--
B_{22}					--	1.092	--	--
B_{23}					--	--	0.880	--
B_{24}					--	--	--	0.739

*Significant at the 0.05 level of probability, t = 1.725.

A_{IJ}

A: Fresh Films
I: Methods of Processing
I = 1, 2
I = Traditional Method
2 = Isolated Method

B_{IJ}

B: Old Films
J: Cooking Methods
J = 1, 2, 3, 4
I = Soup 2 = Salad
3 = Meat Roll 4 = Sweet-Sour

TABLE VII

F-VALUES FOR SENSORY COMPARISON OF SOY PROTEIN-LIPID FILMS PREPARED BY THE TRADITIONAL AND THE ISOLATE METHODS, INCLUDING FRESH AND OLD FILMS COOKED BY FOUR COOKING METHODS

Traditional Film / Isolate Soy Protein Film	A_{11}	A_{12}	A_{13}	A_{14}	B_{11}	B_{12}	B_{13}	B_{14}
A_{21}	2.098	--	--	--				
A_{22}	--	0.133	--	--				
A_{23}	--	--	1.00	--				
A_{24}	--	--	--	1.00				
B_{21}					1.973	--	--	--
B_{22}					--	0.144	--	--
B_{23}					--	--	1.017	--
B_{24}					--	--	--	1.008

*Significant at the 0.05 level of probability, F = 3.0280.

A_{IJ}

A: Fresh Films
I: Methods of Processing
I = 1, 2
1: Traditional Method
2: Isolated Method

B_{IJ}

B: Old Films
J: Cooking Methods
J = 1, 2, 3, 4
1 = Soup 2 = Salad
3 = Meat Roll 4 = Sweet-Sour

TABLE VIII

THE MEANS OF THE SENSORY EVALUATION SCORE OF THE TRADITIONAL AND THE ISOLATE SOY PROTEIN FILMS USED IN 4 COOKING METHODS

Sample	Means			
	Soup	Salad	Meat Roll	Sweet-Sour Pork
Fresh				
Traditional Film	1.500	2.083	1.500	1.500
Isolate Soy Protein Film	1.917	2.167	1.250	1.750
Old				
Traditional Film	1.900	2.500	1.500	1.700
Isolate Soy Protein Film	2.000	2.500	1.500	1.500

CHAPTER V

DISCUSSION

As noted in the previous section, soaking of the whole soybeans at 65°C for one hour prior to making soymilk was the optimum condition for the highest film yield, the highest water uptake and reduction of the grinding power input. It could also be the optimum condition for the extraction of the lipid, protein and carbohydrate in the soymilk for making film. A water-to-beans ratio of 16:1 was preferred for the soymilk since it produced the highest yield of the film. This ratio provided soymilk of a suitable soluble solids content for the formation of film.

A short soak period, such as one hour, evidently did not cause a substantial formation of 1-octen-3-ol, an off-flavor compound produced by enzyme action. Soaking at room temperature overnight according to the traditional Chinese practice was not an effective process to avoid the development of green bean off-flavor. When soymilk was so prepared for making film, the bean flavor was present. If the film was dried in 100°C oven for 20 minutes in the traditional method or one hour in the isolated soy protein method, and the film was cooked with meat or vegetables and seasonings for taste panels, the panelists could not detect bean flavor. It may be that enzyme action was controlled by heating at 100°C for a period of time. Owing to the film's water-absorbing property, when they were seasoned with desirable flavors, the bean flavor present may have been masked.

From the taste panel results, there was no significant difference between the traditional and the isolate soy protein films. In addition, flavor was not affected by storage time. In sweet-sour and salad cooking methods, the panelists felt that the films were moderately tough. This may have been due to soaking the films in a medium containing vinegar. In later taste panels, when the vinegar was added later, the films were not as tough.

Lipid oxidation leading to rancidity proceeded very slowly in the soy protein-lipid films. There was no significant difference in TBA absorbency of the films made by the traditional and the isolate soy protein methods, nor between the fresh and the old films during the storage time. The TBA absorbency tested the degree of oxidation of unsaturated fatty acids in the products. The isolate method films did contain a little less fat, but probably not enough less to affect TBA results. There was no significant difference of the TBA absorbency between the fresh films and the old films. Probably the presence of natural antioxidants in soybean seed contributed to the storage stability of the films. Markley and Goss (14) indicated the probable role of the phosphatides as natural antioxidants in soybean oil. They concluded, on the basis of experimental work prior to 1940, that relatively pure lecithin and its principal nitrogenous component, choline, possessed little or no antioxygenic activity. But they also indicated that lecithin and cephalin apparently act synergistically with certain tocopherols for the natural antioxidants of soybean oil.

From the results, the color of the isolate soy protein film was lighter than the traditional film. The light brown color of these products arises from two systems. One chemical reaction is between acid and chlorophylls and carotenes. Aronoff (4) indicated that chlorophyll reacted with acid and produced the olive-brown pheophytin:

$$\text{Chlorophyll} + 2H^{+} \rightarrow \text{Pheophytin} + Mg^{++}.$$

All cis form of the carotenes was caused by acid and they form a weak color. Nonenzymatic browning, which involves the reaction between carbonyl and amino compounds, increased the color of the films during heating in the oven. Soy protein-lipid films are complex mixtures of lipids, carbohydrates, proteins, metals and water. This combination promoted chemical changes during storage. There were some minor changes of the dominant wavelength and lightness of the products during storage. Nonenzymatic browning and some chemical reactions might have occurred in the sample during storage. These factors might have been responsible for a little darkening of the sample during storage, and the small differences between the two production methods.

The composition of the traditional film showed a protein-to-fat ratio of approximately 5:3 and a protein-to-carbohydrate ratio of 5:1. In the isolate soy protein film, the ratio of protein-to-fat was about 2:1, and protein-to-carbohydrate was 6:1. Food and nutritional grades of the isolate soy protein have become available commercially. They are prepared with a minimum of chemical modification and with no apparent loss of amino acids. The isolate soy protein film should be suitable as a protein source in nutrition.

The isolate method was developed in order to provide a method to manufacture a traditional product on a large scale. The isolation procedure gave a sedimented, high protein concentrate which had a lower proportion of fat and carbohydrate than the film made by the traditional method. When the isolated concentrate was oven dried as a film, its properties were similar to those of the traditional films. It would appear quite feasible to mechanize the production of the film using the isolate concentrate. On the other hand, the hand labor required in the traditional method limits its potential for expansion.

CHAPTER VI

SUMMARY

Whole soybeans were soaked in 65°C water for one hour. Then the soaked beans were ground witb a total water-to-beans ratio of about 16:1 by weight. Four extractions at a high speed of blending made soymilk. The soymilk was adjusted to pH 8.0 with 0.5 N NaOH solution. The globular protein dissociated into small molecular subunits and then the pH was lowered to 4.6 by the addition of 0.5 N hydrochloric acid and the protein precipitated. It was then centrifuged 20 minutes at 796 G. The sediment was neutralized with 0.5 N sodium hydroxide solution to pH 6.6. This slurry was dried in a thin layer in a teflon pan at 100°C for one hour, then put in paper bags for storage. This was the procedure used to produce "isolate soy protein film."

The natural soymilk was heated at 80°C-90°C in a shallow pan for making the traditional film. That pan was held in a water bath for getting equal temperature for heating.

Based on the results of this study, the following conclusions may be made:

1. In the isolate method, processing was in glass equipment and the sediment was used in making the isolate soy protein film. This should result in a cleaner product than the traditional film, because the traditional film is made in open metal pans and some foreign material may drop into the soymilk.

2. The isolate soy protein film contained more protein and less fat than the traditional film and it produced a higher yield than the traditional film.

3. It might be stored longer than the traditional film, because the isolate soy protein film contained less fat than the traditional film and should give a cleaner product.

4. The thickness of the isolate soy protein film could be adjusted by the quantity of sediment spread over the pan, so film thickness could be varied.

5. In the isolate soy protein film, the elimination of the insoluble, indigestible carbohydrate, which has great swelling ability, removes a substance with undesirable physical properties. The swollen, insoluble carbohydrate makes any food into which it is incorporated mushy, and it produces an undesirable, indigestible mass in the digestive system of man (23).

6. The isolate soy protein film would be adaptable to large-scale production in commerce for the following reasons:

   a. It produced less waste from soymilk.
   b. The isolate soy protein film can be stabilized by suitable drying for the utility of the consumer.
   c. The price of the film would be cheaper than the traditional film because of mechanization.

Finally, if soy protein-lipid films are desired in order to meet the consumer's dietary requirements and to avoid rancidity and bean off-flavor development, the film product could be produced by the isolate method.

This study may provide some information not only relating to soy protein-lipid film processing but also to design of experiments with systems other than soymilk such as cottonseed, peanuts, fish protein concentrates, and single-cell proteins. It can produce more protein and less fat in human food. This suggests the extension of soy protein-lipid film processing to the other systems would be quite feasible.

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APPENDIX

APPENDIX

TABLE IX

FORMULAS FOR THE CALCULATION OF C.I.E. VALUES FROM COLOR-EYE COLORIMETER TRI-STIMULUS DATA (X, Y, Z, $\overline{x}$)

Formulas
1. C.I.E. X = (0.783)X + (0.197)$\overline{x}$
2. C.I.E. Y = Y
3. C.I.E. Z = (1.18)Z
4. Chromaticity coordinates: $x = \frac{\text{C.I.E. X}}{\text{C.I.E. X + C.I.E. Y + C.I.E. Z}}$ $y = \frac{\text{C.I.E. Y}}{\text{C.I.E. X + C.I.E. Y + C.I.E. Z}}$
5. Lightness index = $\sqrt{Y}$

TABLE X

THE FIRST TASTE PANEL SCORE DATA ON THE FRESH TRADITIONAL FILM IN 4 COOKING METHODS

Panelists	Soup	Salad	Meat Roll	Sweet-Sour
P_1	1	2	1	1
P_2	1	2	1	1
P_3	3	4	2	2
P_4	1	3	1	2
P_5	1	1	1	1
P_6	2	1	1	2
P_7	2	2	1	1
P_8	1	2	1	1
P_9	1	2	1	2
P_{10}	1	2	1	3
P_{11}	1	2	4	1
P_{12}	3	2	3	1

TABLE XI

THE SECOND TASTE PANEL SCORE DATA ON THE FRESH ISOLATE SOY PROTEIN FILM IN 4 COOKING METHODS

Panelists	Soup	Salad	Meat Roll	Sweet-Sour
P_1	1	2	1	1
P_2	1	2	1	2
P_3	3	3	2	3
P_4	2	2	1	1
P_5	2	2	2	3
P_6	2	1	1	1
P_7	1	2	1	2
P_8	1	2	1	1
P_9	2	1	1	2
P_{10}	2	3	1	3
P_{11}	2	3	2	1
P_{12}	4	3	1	1

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TABLE XII

THE THIRD TASTE PANEL SCORE DATA ON THE OLD TRADITIONAL FILM IN 4 COOKING METHODS

Panelists	Soup	Salad	Meat Roll	Sweet-Sour
P_1	2	3	1	2
P_2	1	2	1	1
P_3	3	1	3	1
P_4	2	1	2	2
P_5	2	3	1	1
P_6	1	2	1	1
P_7	2	2	1	1
P_8	1	1	1	2
P_9	1	1	1	1
P_{10}	1	3	2	1

TABLE XIII

THE FOURTH TASTE PANEL SCORE DATA ON OLD ISOLATE SOY PROTEIN FILM IN 4 COOKING METHODS

Panelists	Soup	Salad	Meat Roll	Sweet-Sour
P_1	2	1	1	1
P_2	1	2	1	1
P_3	3	3	2	2
P_4	1	2	1	1
P_5	2	2	1	1
P_6	1	1	1	1
P_7	1	1	1	2
P_8	1	2	1	1
P_9	2	1	2	1
P_{10}	2	2	1	2

TABLE XIV

EQUATIONS FOR THE CALCULATION OF "t" AND "F" VALUES

Formulas
1. $\sigma^2 = \frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{r_1 + r_2 - 2}$
2. $\sigma^2_{\bar{x}_1 - \bar{x}_2} = \sigma^2(\frac{1}{r_1} + \frac{1}{r_2})$
3. $S_{\bar{x}_1 - \bar{x}_2} = \sqrt{\sigma^2_{\bar{x}_1 - \bar{x}_2}}$
4. $\delta = u_1 - u_2$
5. $t = \frac{(\bar{x}_1 - \bar{x}_2) - \delta}{S_{\bar{x}_1 - \bar{x}_2}}$
6. Correction factor (CF) = (Total)^2/Number of responses (No. of tasters x no. of samples).
7. S.S. of samples = (S.S. of the total for each sample/No. of judgments for each sample) - CF.
8. S.S. of panelists = (S.S. of the total for each panelist/No. of judgments by each panelist) - CF.
9. Total S.S. = S.S. of each judgment - CF.
10. df = r - 1.
11. M.S. = S.S./df.
12. F = M.S. of samples/M.S. of error.

VITA

The author was born on December 10, 1940, in Hu-nan, China. From 1959 to 1962 he attended The Provincial Kaoushing High School in Kaoushing, Taiwan. In June, 1962, he entered The National Taiwan University and was graduated with a Bachelor of Science degree in Agricultural Extension in 1966. He served in the Republic of China Marine Corps from July, 1966, to July, 1967. From September, 1967, to June, 1969, he taught biology and general chemistry in high school. He studied biology in The Northeast Missouri State College from September, 1969, to June, 1970. Since Fall, 1970, he has been studying at The University of Tennessee, Knoxville, in the Department of Food Technology and Science and working to complete the requirements for the degree of Master of Science.