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PURIFICATION AND CHARACTERIZATION OF PROTEIN CONCENTRATES FROM JOJOBA (SIMMONDSIA CHINESIS) PRESSED MEAL

The University of Arizona PH.D. 1983

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PURIFICATION AND CHARACTERIZATION OF PROTEIN CONCENTRATES FROM JOJOBA (*SIMMONDSIA CHINESIS*) PRESSED MEAL

by

Meganne O. Wiseman

A Dissertation submitted to the Faculty of the

DEPARTMENT OF NUTRITION AND FOOD SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN NUTRITIONAL SCIENCES

In the Graduate College

THE UNIVERSITY OF ARIZONA

1983

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Meganne O. Wiseman

entitled Purification and Characterization of Protein Concentrates from Jojoba (Simmondsia chinensis) Pressed Meal

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

James W. Berry	12/14/83 Date
K. Y. Lee	12/14/83 Date
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David E. Hall	14 December, 1983 Date
Ralph L. Pike	14 Dec 1983 Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Ralph L. Pike	14 Dec 1983
Dissertation Director	Date

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SIGNED: Virginia O Wilson

Dedicated to my family and friends,

with all my love and sincerest gratitude,

without whom this would never have been completed.

ACKNOWLEDGEMENTS

The author wishes to extend thanks and express sincere appreciation to Dr. Ralph Price for his support, guidance, and friendship, without whose encouragement, this endeavor would never have been done. Thanks are also extended to the Arizona Agriculture Experimental Station for the research assistantship which kept the author with a roof overhead and with the other essentials needed for survival.

Sincere appreciation is extended to Dr. David Lei and Dr. S. Sathe for their technical assistance in purifying proteins, to Dr. Bobby Reid for assistance with the HPLC and amino acid analysis, to Becky Pharisse and Angelo Longo for their assistance in analysis of amino acid content, to Dr. Charles Weber and Dr. James Berry for their assistance and encouragement during the research, and to Dr. Darrel Goll for his guidance and participation on the committee.

I would like to extend my thanks to Ann Cotgageorge for drawing the graphs included in the dissertation. Her assistance on short notice was greatly appreciated.

I would also like to thank my friends for a listening ear and encouragement throughout the trials and tribulations of the research project.

Lastly, I extend my deepest gratitude to my parents and brothers for their encouragement, and to my husband, Michael Finerty for his encouragement, emotional support and assistance with the photographs.

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ABSTRACT

Jojoba, Simmondsia chinensis, is a shrub native to Sonoran Desert, has seeds with a high percentage of oil. The oil, which has properties similar to sperm whale oil, is expressed with heat and pressure, leaving behind a pressed meal rich in protein and carbohydrate. High concentration of a cyanoglycoside, simmondsin, and polyphenolic compounds in the meal make it unusable for animal or human consumption. Commercial means of protein extraction were improved by washing the protein concentrate with methanol, acetone, and acidic methanol to remove sugars, polyphenolic components and simmondsin. A concentrate with 85% protein, less than 0.3% polyphenolic compounds, and less than 1% simmondsin resulted. The foamability, water absorption, oil absorption, gelation, emulsification and nitrogen solubility were comparable to other plant protein concentrates. Fewer than 15 proteins in the pressed meal and concentrates were detected using PAGE (12.5% T, 2.4% C) in a Laemmli discontinuous system. The proteins were deficient in the sulfur amino acids, and marginal in threonine and lysine. The amino acid imbalance might be partially responsible for poor weight gain and other toxicity symptoms reported previously.

INTRODUCTION

Jojoba, Simmondsia chinesis, is a desert shrub native to the Sonoran desert. The seeds, dark brown when dried and the size of coffee beans or small peanuts, contain a liquid wax (Elliger, Waiss, and Lundin, 1973). The liquid wax, monounsaturated long chain monoesters of long chain alcohols, has the properties of a fine lubricant such as sperm whale oil (Miwa, 1971, and Office of Arid Lands Studies, 1980, p.23). Currently, the wax is predominantly used by the cosmetic industry since the cost of the oil, approximately $50/gallon, is prohibitive for other industrial uses.

Mature shrubs can produce up to 12 pounds of clean, dry nuts per annum (NAS, 1977), but more commonly produce 5 to 10 pounds per annum (Miwa, 1971, and Yermanos, 1974). Verbiscar and Banigan (1978) report that the seeds contain approximately 50% oil, 15% protein, 4-5% moisture, 30% carbohydrate, and 1-2% ash.

For the past two decades, researchers have sought new protein sources for supplementing the diets of poor people in under-developed countries, for less expensive and more energy efficient protein staples than animal products,

and for use in formulated food products (Saio and Watanabe, 1978).

After removal of the wax by the expeller, a protein-rich meal is left (NAS, 1977, p.21). The meal, which contains about 10% residual oil, is of no current value, and is usually kept in storage awaiting further applications. The meal itself contains bitter polyphenolic compounds and simmondsin, a cyanide-containing glucoside, which prevent the direct use of the meal for animal feed. Therefore, a method of extracting the protein from the meal while leaving behind these compounds was sought.

Eggs are usually the standard for comparison of functional properties of proteins in formulated food products. Whipping, foaming, oil absorption, water absorption, gelation and solubility are the important functional properties in foods. Allergies, availability, convenience, and cost may prohibit the use of eggs. Therefore, researchers have been investigating vegetable proteins as possible substitutes (Baldwin, 1977). Soybean proteins have been successful in some applications. It is hoped, that if jojoba farming becomes economical, and jojoba meal is detoxified, that jojoba proteins will be used in animal feeds and in formulated food products.

This research project developed an isolation procedure that could be used on a large scale recovering 60% to 70% of the protein in the meal as a concentrate containing at least 50% protein and an isolate with 90% protein. Functional properties were investigated for possible use of the protein in a formulated food product. Vegetable protein researchers have been concerned about several anti-nutritional factors, i.e. trypsin inhibitors, phytate, tannins, polyphenolic compounds, and simmondsin. Therefore, these factors were quantitated, although not characterized.

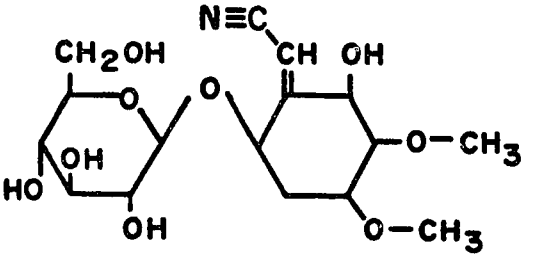
LITERATURE REVIEW

Introduction

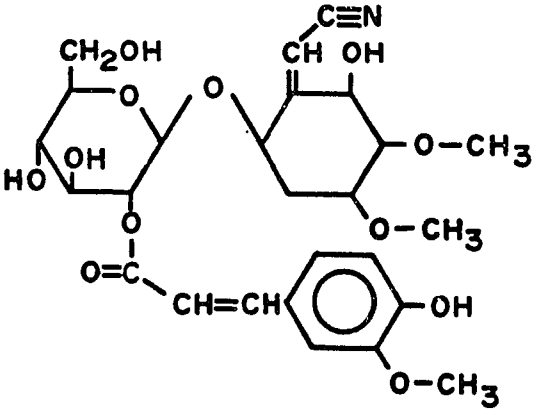
Jojoba, Simmondsia chinesis, a desert shrub native to the arid land regions of Arizona, southern California, and northern Mexico, has seeds rich in oil, protein and carbohydrate. The defatted meal could make a good animal feed or flour for human consumption (Utz, 1982 and Yermanos and Duncan, 1976). However, the meal contains several "anti-nutritional" factors, which currently are preventing the direct utilization of the meal (Elliger, Waiss, and Lunden, 1973; Booth, Elliger and Waiss, 1974, Williams, 1980, Verbiscar and Banigan, 1977, Verbiscar, et al., 1981). The literature deals predominantly with jojoba oil, and simmondsin, presumably the major toxicant. This literature review briefly discusses simmondsin and other toxic factors before discussing vegetable proteins, and jojoba proteins in particular.

Simmondsin

The focus of jojoba research has been on simmondsin and simmondsin-2'-ferulate, two cyanoglucoside metabolites found in the seed (Figure 1). Together, the two compounds make up to 3% of the seed on a dry weight basis and up to



SIMMONDSIN



CIS, TRANS SIMMONDSIN
2' FERULATE

Figure 1. The Structure of Simmondsin and Simmondsin-2'-ferulate.

7% of the meal on a dry weight basis (Verbiscar and Banigan, 1978).

Simmondsin, first isolated and characterized by Elliger, Waiss, and Lundin (1973), has been implicated as the cause of weight loss, depressed growth, loss of appetite and eventual death in rats and mice (Williams, 1980, Verbiscar and Banigan, 1978, and Booth, Elliger and Waiss, 1974). Booth, Elliger and Waiss (1974) reported that 15% jojoba meal in diets fed to rats caused death, apparently due to starvation, in less than two weeks. Depressed appetite resulting in weight loss and death in less than 3 months was observed in rats fed 5% and 10% jojoba meal in the diet. Rats fed more than 0.30% simmondsin in the diet also had depressed appetites and increased weight loss.

Administration of 2.5 gm/kg body weight simmondsin via a stomach tube to adult mice, caused no observable ill effects and injection intraperitoneally (i.p.) of 3.6 gm/kg body weight simmondsin caused weight loss, but no deaths. Histological abnormalities were not apparent after feeding rats 750 mg/kg body weight/day simmondsin via stomach tube for 5 days. However, 600 mg/kg body weight simmondsin injected (i.p.) into young adult mice caused immediate death. Prolonged feeding of jojoba meal for 35 days caused

severe testicular atrophy and cessation of spermatogenesis. Conn (1969) reported some of the possible degradative pathways of cyanogenic glycosides in general.

Williams (1980) further investigated the toxicity of simmondsin to mice. Since simmondsin contains a cyanide radical, cytochrome oxidases responsible for respiration could be blocked resulting in fat accumulation in the liver, low sodium levels in the kidney and decreased brain functioning resulting in eventual death. The young adult mice were dosed orally with a single injection of 97% pure simmondsin in amounts up to 12.95 gm/kg body weight and observed for 19 days with no weight loss or any signs of toxicity. Mice dosed daily with up to 750 mg/kg body weight simmondsin for 5 days showed weight changes not significantly different from the controls, and exhibited no outward toxicity symptoms.

Williams (1980) followed the cyanide concentration in the blood of mice. The cyanide levels rose steadily during the five day period. Mice rapidly detoxified cyanide to thiocyanate in the mitochondria using thiosulfate. Since the thiosulfate reserve concentration was depleted on the fifth day and could not be replenished, the cyanide levels rose dramatically on day five. The damage observed in the liver and kidney may be due to the

cyanide. Williams (1980) concluded that simmondsin was probably acting synergistically with other substances causing the weight loss and eventual death in experimental animals fed jojoba meal.

Verbiscar, et al (1981) reported further toxicity studies and attempts at detoxification. Lactobacillus species grew well on jojoba meal at room temperature (21C and 26C) which supposedly decreased levels of simmondsin, but analysis was not explained. Animal studies using chicks, cattle, and sheep showed weight gains not significantly different from controls. No further testing was reported.

After the study by Williams (1980), another toxic factor was sought that could be responsible in part for the observed weight loss and eventual death observed in experimental animals. Some possibilities included tannins, polyphenolics, trypsin inhibitors, and phytate.

Tannins

Tannins and polyphenolic compounds bind to other macromolecules including protein and carbohydrates. These complex molecules are no longer completely metabolized and utilized, possibly resulting in weight loss (Glick and Joslyn, 1975; and Elias, DeFernandez and Bressani 1979). Equilibrium favors formation of a reversible

protein-polyphenolic complex via hydrogen bonds or an irreversible complex via oxidation and covalent condensation with N-substituted amines. Harborne and Van Sumere (1975) suggest that 30% or more of the protein may be tied up by phenolic compounds. Phenolic compounds outside the range 500 to 5000 molecular weight are ineffective in forming stable crosslinking and therefore may be absorbed, but form a low stability complex.

Harborne (1975) distinguishes two classes of tannins. The hydrolyzable tannins contain glucose and are water soluble. The condensed tannins are proanthocyanidins and complex with proteins. The phenolic compounds are almost always found in nature combined with protein, or bound to sugars as b-D-glucopyranosides.

Haslam (1975) defines vegetable tannins, using the old definition, as any material which can produce leather from hide. These tanning compounds, invariably found to be phenolic in nature, were until recently (Hagerman and Butler, 1980) quantitated using assays specific for phenols. Mistakenly all compounds detected by these phenol assays were called "tannins". As phenolic compounds, tannins can complex with iron salts, be oxidized by KMnO in alkali, and be easily substituted by electrophilic groups, i.e. coupled to diazonium salts and aldehydes. Most

determinations for tannins are based on their phenolic character and not based on their ability to precipitate protein.

Harborne and VanSumere (1975) report that the hydrolyzable tannins are strongly bound to proteins at pH 3 to 4, and loosely bound at pH > 5. The theory is that a strong hydrogen bond is formed by the unionized carboxyl groups of tannins and the weaker hydrogen bonds of the unionized phenolic -OH groups. The condensed tannins are bound independent of pH < 7 to 8. The phenol ionizes above pH 8 and can no longer serve as a proton donor for hydrogen bonds.

Harborne and VanSumere (1975) list possible methods of preventing o-quinone formation. The list includes:

1. Exclude oxygen by using a nitrogen atmosphere.

2. Remove polyphenols via extraction with acetone, methanol, phenol, or using sequestering agents i.e. PVP, BSA, polyethylene glycol, etc.

3. Inhibit polyphenoloxidase activity by extracting tissue in TCA, thioglycollate, metabisulphite, potassium ethylxanthate, or sodium diethyldithiocarbamate.

4. Reduce quinones via extraction in ascorbic acid.

5. Trap quinones via extraction in cysteine or benzenesulphinic acid.

Although these methods are reported throughout the patent and journal literature, the methods are usually only partially successful. Procedures used for analysis combine many aspects of the methods cited by Harborne and VanSumere (1975).

Blouin, Zarins and Cherry (1982) reported possible mechanisms involved in the discoloration caused by protein-polyphenolic interactions. One observation is that disruption of tissues by grinding, heating, or blending with other ingredients, i.e. food processing, creates an environment favorable for both enzymatic and nonenzymatic oxidation reactions responsible for discoloration. Use of soybean meal at 20% substitution in biscuits results in no discoloration, but use of sunflower, alfalfa leaf, and cottonseed flours does. The discoloration in sunflower flour biscuits is thought to be due to the presence of 2% to 3% esters of chlorogenic acid, a common plant phenolic acid, in the seed fresh. Chlorophyll and phenolics in alfalfa leaf flour and gossypol in cottonseed flour are thought to cause discoloration.

Sosulski (1979) reviewed the interaction of phenolic compounds and protein in plant flours and protein

isolates. The preferred substrates for polyphenoloxidase in oilseed products are cinnamic acids and caffeic acids. At alkaline pH, the protein isolate solution is brown or green. The color is not removed when precipitated at the pI (pH 4.7) or when washed.

Sabir, et al. (1972) and Sabir, et al. (1973) investigated the interaction of phenolic compounds and protein when purifying the sunflower seed protein on a gel filtration (Sephadex G-200) column eluted with a neutral salt solution. The major polyphenolic compound, chlorogenic acid, has an absorbance maxima at 328 nm. Dialysis removed the low molecular weight phenolic compounds. Since the fifth fraction, Fraction V, decreased in concentration, it supposedly had the polyphenolic compounds. Fraction V, (lowest molecular weight proteins), from the neutral salt elution reapplied to the Sephadex G-200 column and then eluted with a 7M urea in neutral salt solution gave typical absorption patterns at 280 nm and 328 nm (Figure 2). As shown in Figure 3, Fraction V separated into two peaks, the first, V1, absorbed at both 328 nm and 280 nm in the scanning ultraviolet spectra, while the second, V2, only absorbed at 280nm. Sabir, et al. (1973) concluded that the first subfraction has chlorogenic acid covalently bound to the low molecular weight protein.

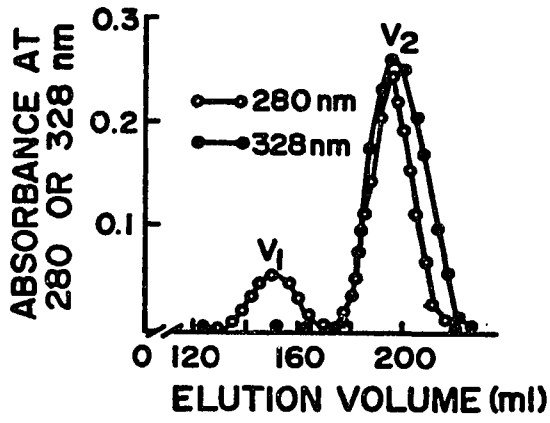


Figure 2. Absorption of Dissociated Fraction V Protein from Sunflower Meal at 328 nm and 280 nm. (Sabir, et al., 1973)

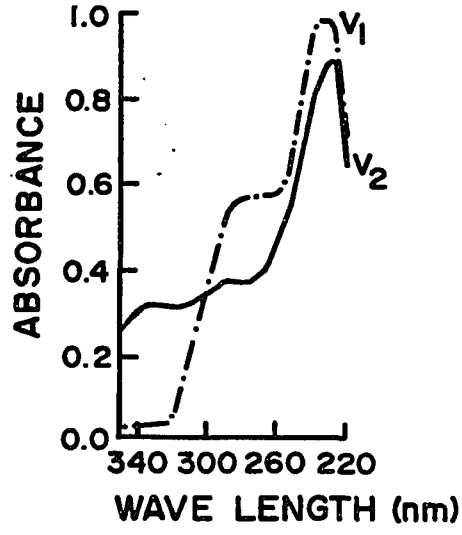


Figure 3. Scanning Ultraviolet Spectrum of the Two Subfractions of Fraction V from Sunflower Meal Gel Filtration. (Sabir, et al., 1973)

Sabir, et al. (1973) also reported observations about the amino acid content of the above fractions. Lysine, tyrosine, and glycine levels were in lower proportions in Fraction V than in the other fractions, or in the fractions of the unpurified meal. The %nitrogen was much lower in Fraction V. Sosulski (1979) reported that approximately 1/3 of the chlorogenic acid was covalently bound to polypeptides and oligonucleotides under 5000 molecular weight.

Poulter (1981) reported that the quantity of protein extracted by water was affected by tannins. Tannins reduce the quantity of proteins extracted by salt solutions. Therefore, true albumins and true globulins cannot be determined. These studies discussed above document and describe the problems caused by the protein-phenolic interactions, and their importance in protein purification. Also reported by Poulter (1981) was an inverse relationship between the concentration of tannin and the activity of trypsin inhibitor.

Trypsin Inhibitors

Trypsin inhibitors have been investigated extensively in soybean flours, concentrates and isolates. Liener (1977) reported that the trypsin inhibitors of legumes decreased digestibility of proteins. Trypsin

inhibitors cause hypertrophy of the pancreas resulting in loss of endogenous protein and depressed growth. Trypsin inhibitors act by blocking trypsin from acting on protein. As a result, the liver continues to stimulate production of more trypsin by the pancreas. Since trypsin is rich in cysteine, the supply of cysteine and methionine are used up. Methionine supplements counteract the hypertrophy.

Trypsin inhibitors are frequently inactivated by heat treatment normally given by processing, i.e. 1 hour at 100C to 125C with increased effectiveness at higher moisture contents.

Chang and Tsen (1981b) have reported trypsin inhibitors of different molecular weights for various cereals (Table 1). As in most characterized trypsin inhibitors, the levels of glycine, glutamic acid, proline, and leucine are high (Boisen and Djurtoft, 1981).

Chang and Tsen (1981a) reported that all of the cereal trypsin inhibitors had no free cysteine. Inactivation of trypsin inhibitors at 100C for 1 hour ranged from 7% of triticale trypsin inhibitor to 70% of red winter wheat trypsin inhibitor II. At 125C all of the trypsin inhibitors were inactivated.

Samac and Storey (1981) studied trypsin inhibitor activity in germinating jojoba seeds. Trypsin inhibitor

Table 1. Molecular Weights of Cereal Trypsin Inhibitors Isolated by Affinity Chromatography (Chang and Tsen, 1981b).

Inhibitor	Molecular Weight	CHO
Rye I	44,000	
RyeII	17,000	+
Rye III	5,300	
Triticale I	19,500	+
Triticale II	5,400	
Hard Red Winter Wheat I	36,800	+
Hard Red Winter Wheat II	21,000	-
Hard Red Winter Wheat III	5,000	
Durum Wheat I	38,500	+
Durum Wheat II	22,500	
Durum Wheat III	------	

activity was found to decrease with germination and was heat labile. It was still present in commercially prepared meals and the albumin fraction of seed proteins. Trypsin inhibitor activity was measured by the amount of meal or albumin needed to inhibit 5 ug bovine trypsin; 110 mg meal and 190 ug albumin were required. The conditions required for inactivation were not reported.

Ngou Ngoupayou (1982) reported at least 3.64 trypsin inhibitor units per gram of untreated deoiled meal and 0.12 to 0.72 trypsin inhibitor units per gram of the albumin fraction. Treatment at 100C for 15 minutes reduced the activity by 1/2. Total denaturation and inactivation was achieved at 100C for 45 minutes and 15% moisture.

Phytate

Wheeler and Ferrel (1971) reported a method for determination of phytic acid. Phytic acid might prevent the availability of phosphorus to monogastrics, might interfere with calcium and iron absorption in humans, and could be removed from cereal products during processing. The method of Wheeler and Ferrel detected phytate via the ferric ion binding to the phosphate groups in a known ratio, i.e. 4 iron/6 phosphorus molecules/phytic acid group. Omosaiye and Cheryan (1979) report that

ultrafiltration could decrease the level of phytic acid in protein concentrates and isolates.

Protein Extraction

Protein isolation methods for vegetable proteins must obtain reasonable yield, at least 70% protein purity, and minimize the levels of antinutritional factors (Anderson, Rackis and Tallent, 1979). The protein concentrate or isolate must then perform some function in formulated food products (Martinez, 1979). Vegetable protein isolation procedures are numerous in the literature. However, the procedures are usually modifications of those procedures used by the industry for soybean protein concentrates and isolates (Waggle and Kolar, 1979).

Seal (1980) reported the composition of decorticated soybean as 42% protein, 20% oil, 12% soluble carbohydrate, 13.5% insoluble carbohydrate, 4.5% ash, and 8% water. The industrial isolate process involves extracting the meal in an aqueous solution (pH 7 to 8.5), leaving behind the insoluble polysaccharide and protein residue. The protein is precipitated from solution after adjusting the pH to 4.2, the isoelectric point, leaving the sugars and whey proteins in solution. Only 10% of the soybean protein remains soluble at the isoelectric point.

The precipitated protein is usually neutralized and then spray dried.

Sometimes a concentrate with a lower protein concentration is used instead of an isolate. This process involves immobilizing the protein, so impurities can be removed, followed by extraction and subsequent spray drying. Seal (1980) reported three industrial methods including moist heat/water leach, aqueous leach, and dilute mineral acid leach. The moist heat process involves heating the protein in a moist environment until the protein is denatured and precipitates.

The alcohol leach involves using 20 to 80% alcohol to precipitate the protein, removing soluble carbohydrates. Smith and Circle (1978) discuss alcohol denaturation of protein. The lower molecular weight alcohols denature the protein least, and aqueous alcohol solutions denature proteins more. Proteins can be denatured in as little as 5 minutes. The globulin proteins, the major fraction of proteins in soybeans, are more susceptible to denaturation by alcohols.

The last method does not denature the protein in the processing. Dilute mineral acid solutions at the isoelectric point precipitate most of the protein, and remove the albumins, minerals, and soluble carbohydrate.

The use of concentrates and isolates depend on the desired function of the protein in the specific food product. For example, emulsions, which are made from 10% concentrate, 30% water and 60% fat, are incorporated into sausages in order to control the reduction of cooking loss. The alcohol extracted concentrates are used in the bakery industry, since the water absorption properties are improved leading to prolonged shelf-life.

Experimental knowledge of functional properties of a concentrate are important. For instance, solubility data is used for protein solvation in beverages; water absorption data for absorbing and entrapping water in cakes and breads, and preventing drip in sausages; gelation data for fabricating meats and cheese; emulsification formation and stabiization data for fabricating sausage and formulating soups, sauces and cakes; and fat absorption data for making sausages and donuts. One isolate function is forming stable films to entrap gas in toppings, chiffon desserts, and angel cakes.

Numerous variations of the isolation methods have been written about in the literature. In the preliminary investigations chapter, more about these other procedures, that were adopted and rejected will be discussed. The

remainder of the literature review will discuss the proteins in jojoba.

Jojoba Proteins

Only two publications have been published about jojoba protein. Samac, et al. (1980) extracted the decorticated seed with chloroform/methanol to remove the lipids and lipoproteins, the defatted meal was then extracted for proteins based on solubility. The first fraction isolated was the globulin fraction, which upon dialysis was separated into the globulin and albumin fraction. A 50 mM sodium phosphate buffer was used to extract the protein with 1N NaCl. The prolamines were isolated with 50% n-propanol and the glutelins with 50mM borate buffer, pH 10. No prolamines were found, and proteins were 33% glutelins, 29% globulins, and 35% albumins.

Samac and associates (1980)investigated the trypsin inhibitor activity in the meal, protein fractions, and the germinating cotyledons. They reported that the albumin fraction had all of the activity of the meal. The trypsin inhibitor was completely inactivated by heat. The inhibitor was probably a polypeptide or low molecular weight protein, since it "was eluted with the void volume in gel filtration chromatography". The parameters for the

gel filtration were not specified. The large molecular weight proteins are eluted with the void volume in gel filtration, therefore this last conclusion may be invalid.

The second study, Cardoso (1980) also characterized jojoba protein via solubility. Albumins were the major fraction, 65% of the total seed protein, and globulins 21%, glutelins 6% and prolamines 8%. Cardoso extracted the seeds with distilled water to recover the albumins, followed by a 10% NaCl extraction to recover the globulins and 70% ethanol to recover the prolamines, and $Ca(OH)_2$ to recover the glutelins.

Cardoso (1980) investigated the functional properties of the various fractions of protein. As was expected from the literature, the albumins and globulins were the useful fractions. The albumins were still 50% soluble at their pI, while the globulin fraction was only 10% soluble at the pI. Solubility of both the globulins and albumins at pH above 8 was at least 80%. The solubility increased in a nearly linear relationship from the minimum solubility to pH 8 and 80% soluble. This relationship is similar to most other plant proteins. Fat absorption (mL of oil/gm of protein) was 4 for albumin and 3.2 for globulin. This exceeds that of soybean concentrates and isolates. The foamability and foam stability of a 1%

dispersion of albumin exceeded, although not statistically different from, the standard proteins of bovine serum albumin and ovalbumin. The globulin fraction expanded similarly to the albumin, but was not as stable. Expansion of 100% was achieved, but after 2 hours, more than 60% expansion in the albumin and 30% in the globulin fraction remained. Emulsion stability and capacity of the albumin and globulin fractions were similar to oat and alfalfa, but less than the commercial soy isolates and concentrates. At least 7% dispersions of albumins and at least 10% dispersions of globulins were required for gelation. This is comparable to many plant proteins. Sathe and Salunkhe (1981) reported that protein concentrates from great northern bean will gel at 8%, protein isolates at 12%, albumins at 18% and flour at 10%.

PRELIMINARY INVESTIGATIONS

In the introduction and literature review, the problems associated with isolation of protein from plant sources were discussed. In this chapter, preliminary experiments using most of the methods mentioned in the literature review for removal of polyphenolic compounds will be briefly described. Although the problem of polyphenolic compounds are not unique to jojoba, the level found in jojoba is high in comparison with beans, cereals, and some other oilseeds. A complication in the interpretation of results is that "tannins" were quantitated by two unrelated methods, the standard method (Horowitz, 1980) using the Folin-Denis reagent, and the method of Price and Butler (1977) using the Prussian blue reaction. Neither method is specific for tannins, but both are indicative of phenolic compounds. For this reason, polyphenolics are reported, and not "tannins".

One further complication with the interpretation of the data acquired in this preliminary work is that the method of protein quantification varied between experiments. Three methods, biuret (Bailey, 1967), microkjeldahl (Bailey, 1967), and Bradford (Biorad) assay

(Bradford, 1976) were used. In most cases, the biuret method was used.

Experimental

The methods first used were described by Johnson (1976) and Seal (1980). Johnson summarized the current industrial methods for soybean concentrates and isolates as water leach, alkali leach, acid leach, alcohol leach, and acid precipitation of alkali leach. The first problem was deciding which portion to save, the soluble or the residual portion. In the case of alcohol leach, the residue is retained and in all the other cases, the supernatant was retained.

Although filtration through Whatman No. 1 paper is considered rapid for scientific purposes, it is slow when volumes are large and the flour is as small as 60 mesh. Therefore, filtration through a polyester interfacing or centrifugation at 2000 rpm using a Universal bucket centrifuge were used.

Sample Preparation

The pressed meal was deoiled by hexane extractions until the hexane was no longer yellow. The meal was ground while being extracted on the Sorvall Omni Mixer (Ivan Sorvall, Inc., Newtown, Conn.) at the 5 setting resulting

in a 40 to 60 mesh meal. The meal was desolventized at 20C overnight prior to milling. A hammermill increased the flour mesh size to at least 60 mesh.

Five samples of 100 grams of deoiled meal were extracted for two hours at room temperature with 2000 mL of one of the 5 solutions (pH 8.5 water, 50% alcohol, 80C water, pH 4 water, or alkali extract later acidified to precipiate the protein), filtered or centrifuged, and lyophilized all five residues and filtrates.

Protein Purification

Ion exchange chromatography was performed with Biorad AG-1-X8, acetate form anion exchanger (Biorad Co., Richmond, Calif.) in a Pharmacia K26x40 column (Pharmacia Fine Chemicals, Piscataway, N.J.). The following solutions were used sequentially for three hours in a batchwise process: aqueous solutions adjusted to pH 9 with no salt, pH 8 with 0.1N NaCl, pH7 with 0.2N NaCl, and pH 6 with 0.5N NaCl. All solutions contained 0.05% sodium azide as a preservative. Williams and Wilson (1981) and Needleman (1970) were used as references for chromatography.

These isolates were applied to a Sephadex G-100 gel filtration column (Pharmacia K26x40) and eluted with a 0.5M phosphate buffer, pH 7.6 at a flow rate of 20 mL/hour. Buffers at pH 6.5 and 8.5 were also used. The eluant was

monitored at 280nm with an ISCO monitor and detector (ISCO, Inc., Lincoln, Neb.), and was collected in tubes at a rate of 3 tubes/hour. The fractions for each peak were pooled and lyophilized. The dry weight and the protein content (determined using the biuret method) were recorded. The fractions were later applied to disc electrophloresis (12.5%T, 2.4%C gels). Since SDS gels were unsuccessful, i.e. poor resolution or undetectable bands, gels without SDS, urea, or 2-mercaptoethanol were used. Tris buffers were used according to the method of Laemmlli (1970). The gels were run for 3.5 hours at 40 ma total. Both Amido Black T and Coomassie Blue stains were used. Solutions were made according to the Laemmlli disc electrophloresis system (Laemmlli, 1970 and Catsimpoolas, 1977). The gels were stained for at least 4 hours. Destaining was with 7% glacial acetic acid/methanol (1:1) followed by 7% glacial acetic acid, until the bands were clearly visible.

Tannin Analysis and Purification

The two methods used to analyze the tannin content, were the colorimetric method using the Folin-Denis reagent (Horowitz, 1980), and the Price and Butler (1977) method using the Prussian blue test. The Price and Butler method was modified by using 10 mg samples, and only 1 mL of each reagent (ferric chloride, and potassium ferricyanide). A

standard curve using 10 to 1000 ug tannic acid was used. The standard was prepared fresh each week.

Characterization of the phenolic compounds in jojoba pressed meal was done by repeating the procedure of Krygier, Sosulski, and Hogge (1982). Phenolics were classified as those freely soluble, those esterified to glycosides, and those insoluble and bound to proteins. Further separation was not done. Fractions were lyophilized, weighed, and quantitated using the Folin-Denis reagent.

Since the Folin-Denis procedure for quantification of tannins required extraction with phenol/acetone/water (1:1:1) or phenol/acetic acid/water (1:1:1), removal of the polyphenolic compounds from the meal was attempted with these solvents. Other tested solvents were replacement of phenol with methanol in the above systems; including up to 1% ascorbic acid, BHT, or TenoxII in the aqueous extraction solution to reduce the phenols and prevent them from oxidizing; and affinity chromatography using BSA; and including up to 1% polyvinyl pyrrolidine (PVP), gelatin, guar gum and agar as scavengers as part of the extraction medium. These methods were cited by Harborne (1975) and Harborne and VanSumere (1979).

Optimization of Extraction

Temperatures were varied from 4C, 20C, 37C, 55C, 80C. Extraction ratios used were 1:10, 1:20, and 1:40. The pH and ionic strength were varied, i.e. KOH (0.045N, 0.45N, and 4.5N), NaOH (0.06N, 0.6N, and 6N), NH_4OH (0.06N, 0.6N, and 6N), Tris (0.025M, 0.05M, 0.5M, and pH 7.5, 8.5), and phosphate buffers (pH 6.5, 7.5, and 8.5). NaCl was also varied (0, 0.05N, 0.10N, 0.15N, 0.20N, 0.25N, 0.50N, and 1.00N). One gram samples were extracted with 20 mL solvent at room temperature. The samples were filtered or centrifuged and analyzed with the biuret method using BSA (Sigma Chemical Co., St. Louis, Mo.) as the standard protein. Appropriate reagent and solvent blanks were used, as well as BSA in the appropriate solution. The best methods for elimination of polyphenolic compounds were combined with the best of the above solubility studies. Protein content in samples was analyzed using the biuret method. Extracts from the most successful methods were purified on gel filtration and electrophoresis as described above.

Results and Discussion

Yield and Purity

The results using the biuret method showed that the water leach recovered 50% of the protein with a 60% purity. The results were similar for alkali leach, 59% purity and 60% recovery, and for acid leach, 53% purity but only 30% recovery. These results were disappointing since the commercial methods reported 70% purity, with "good" or unspecified yields (Johnson, 1976 and Seal, 1980). Since the biuret measures only soluble protein, the microkjeldahl method of analysis was also used. This was also disappointing, because the isolates again had between 50 and 60% purity, in agreement with the biuret method.

Polyphenolic Compounds

In the literature review several references were cited, which reported that tannins and polyphenolic compounds were bound to protein. This might explain the discrepancy between the relative amounts of albumins and globulins reported by Cardoso (1980) and Samac, et al (1980). Polyphenolic compounds, like carbohydrates in glycoproteins, may increase the water solubility of the protein, while maintaining solubility in low ionic strength solutions. The binding of polyphenolic compounds to

protein might account for the lower than expected purity of the concentrates.

Affinity chromatography using BSA was slow (Hoff and Singleton, 1977). If the process could have been incorporated into a hollow fiber ultrafiltration apparatus, it might have been more practical, but for the time being it was impractical. The only scavenger with potential for eliminating "tannins" was guar gum, however, 50 ug of the protein concentrate did not show up on a 12.5% T, 2.46%C PAGE gel using Amido Black T or Coomassie Blue R250 stains. Total acrylamides of 10% and 15% also were inadequate. It was not discovered until later in the research project that much higher quantities of protein were required for detection. The guar gum concentrate did not gel using 12% protein dispersion, and other concentrations were not tested. Ascorbic acid was the best reducing agent, since it improved the purity to approximately 70%, but it did not significantly reduce the polyphenolic level.

After repeating the study by Krygier, Sosulski and Hogge (1982), it was determined that 33% of the polyphenolic compounds, using the Folin-Denis assay, were bound to protein and therefore insoluble. Of the remaining

polyphenolics, 15.3% were soluble, and 51.6% were esterified to glucosides.

Since polyphenolics dis-associate from the protein above pH 8, pH 8.5 should be adequate to isolate the protein, while minimizing denaturation. Other effects of pH on protein-polyphenolic interaction are that low pH can cleave the bonds between phenolic compounds and sugars or proteins (Sodini and Canella, 1977), and that storing sorghum with strong bases (6N NaOH, 4.5N KOH, 6N NH OH) for several days reduced the polyphenolic content, ammonia was best (Price, et al, 1979).

Solubility

At this point solubility and precipitation properties were studied to optimize a method of isolation. Extraction temperatures were varied (4C, 20C, 37C, and 55C). Extraction time required at 4C increased, but time seemed to be irrelevant at the other temperatures studied. No PAGE or gel filtration was done to determine denaturation. Although the minimum extraction time at room temperature was 30 minutes, usually extraction was for 2 hours. Increasing ionic strength, by increasing the amount of NaCl, increased solubility up to 0.20N NaCl. The pH had a greater influence than ionic strength on solubility.

Extraction using Tris, NaOH, KOH, NH_4OH, and phosphate buffers were all effective.

Temperatures under 80C did not denature the protein causing a reduction in yield. Precipitation using HCl was more effective than using glacial acetic acid. Although precipitation could also be accomplished with 70% saturated ammonium sulfate, much more salt was required than acid. The acid could be later neutralized, but the salt had to be dialyzed out. Both salt and acid could denature protein, although salt denaturation is usually temporary.

Cogan, et al (1967) and Gheyasuddin, Cater, and Mattil (1970) also reported no significant improvements from longer extractions times than 30 minutes, extraction of finer particles, more than two extractions, or meal/solvent ratios between 1:10 and 1:20. Cogan, et al (1967) reported that hot processing of the soybean oil resulted in a decrease of 10% of the protein recovered, and toasting resulted in a decrease of 25%.

% Yield = [N] Vextracted (100)/[N] Wmeal,

where V is volume, W is weight of meal and N is the amount of nitrogen determined by the kjeldahl analysis.

Optimization and Purification

Samples of a concentrate from pH 8 aqueous-ascorbic acid extract and a concentrate from 70C aqueous 1% guar gum

extract were chromatographed on a Utrogel A6 column (LKB 2.6 cm (i.d.) x 90cm), and eluted with a pH 8.9 NaOH adjusted aqueous solvent. The eluants were monitored at 280 nm.

Ion exchange chromatography did not work well. The proteins eluted with the first solvent as one peak. The other solvents eluted proteins by gel filtration. Therefore, a cation exchange column might have worked better.

These concentrates were tested for functional properties, beginning with gelation, but they did not gel using 12% dispersions. When 50 ug of each sample was applied to PAGE gels, using the above parameters, Amido Black T detected no bands. Limited success was obtained with Coomassie blue stain. Examples of the non-dissociating slab gels obtained from the experiments discussed in the next chapter are shown (Figures 4 and 5). The gels are included here since they show the degradative effect of temperature and spray drying; and the loss of protein after purifying using ion exchange, gel filtration, and ultrafiltration. The apparent loss of protein may be protein degradation, insufficient amount of protein, or proteins outside the mass/charge range for 12% gels.

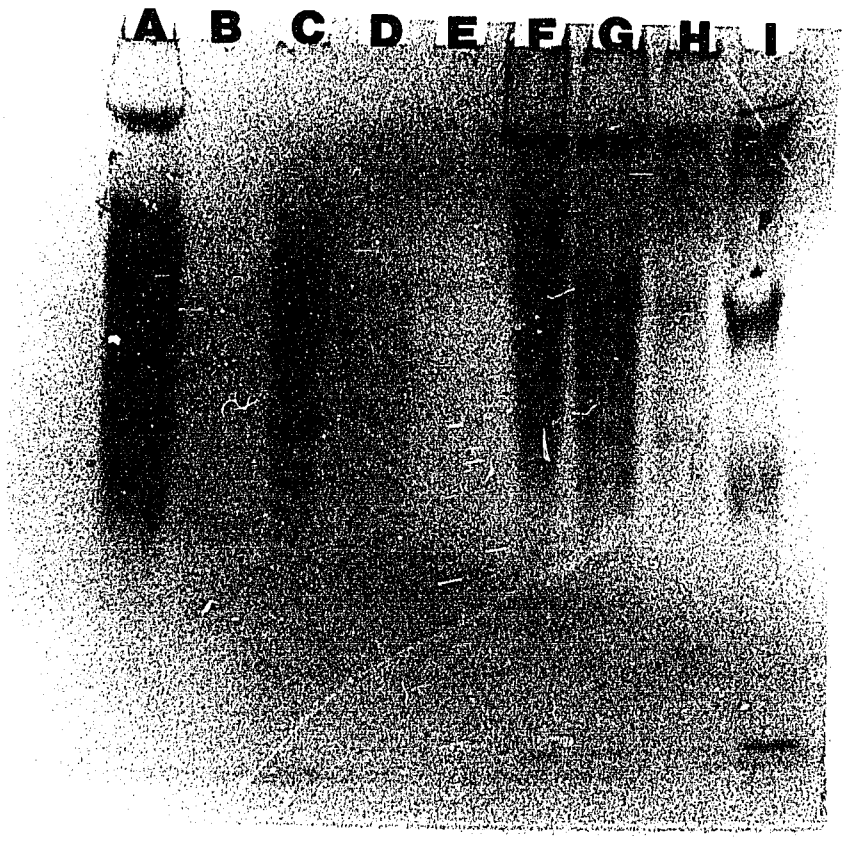


Figure 4. Polyacrylamide Gel Electrophoresis Showing Denaturation of Jojoba Meal Proteins.
From left to right: 150 ug samples of spray dried alkaline soluble ion exchange peak 3 (A), albumin (20C) (B), SDS treated albumin (C), albumin (70C) (D), pH 8.9 extract-peak 3 from ion exchange (E), methanol/acetone washed albumin (F), methanol/acetone washed concentrate extracted with 0.003M $Ca(OH)_2$ (G), spray dried alkaline extracted meal (H), BSA, b-glucosidase, and trypsin inhibitor standards (I).

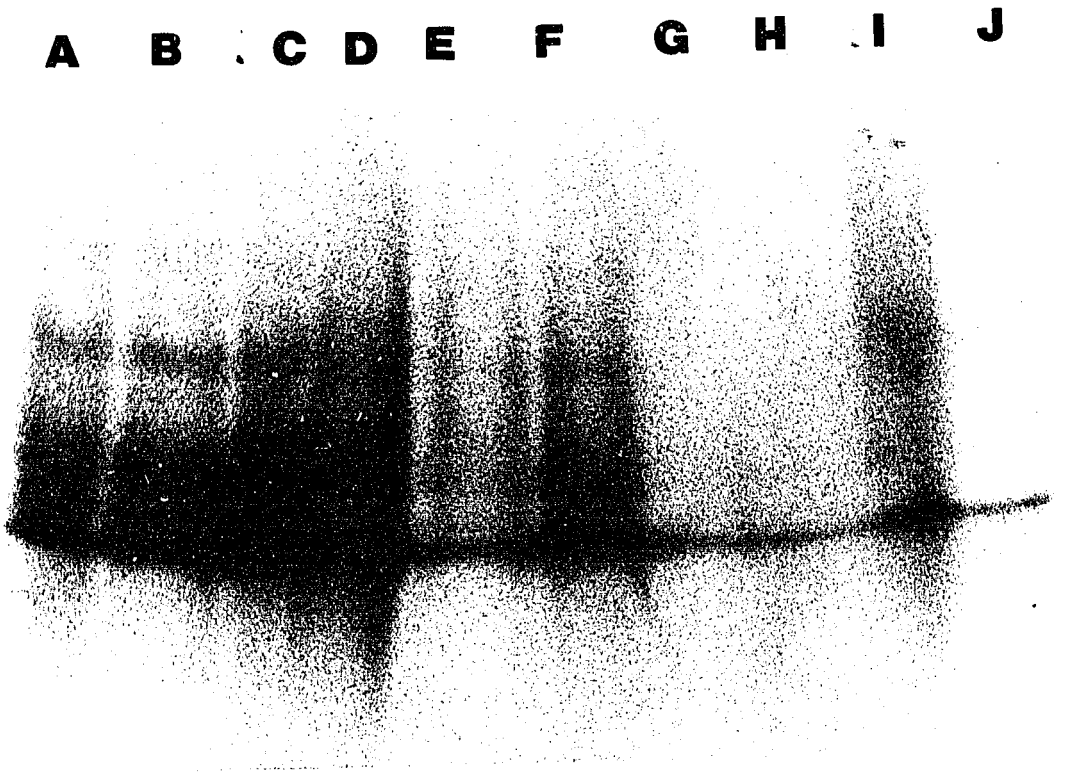


Figure 5. Polyacrylamide Gel Electrophoresis Showing the Separation of Preliminary Protein Extraction Methods of Pressed Jojoba Meal.
From left to right: 2.5 mg samples of yeast extracted meal for 1/2 hour (A), 1 hour (B), and 2 hours (C), protein from Sephadex G-25 (D), globulin (E), albumin (F), ultrafiltered albumin (G), spray dried albumin purified by ion exchange (H), and by Sephadex G-25 (I), and BSA, b-glucosidase, and trypsin inhibitor standards (J).

Further modifications of these methods were made to develop concentrates with desirable properties. These modifications are discussed in the next chapter.

ISOLATION AND CHARACTERIZATION OF PROTEIN FROM PRESSED MEAL OF SIMMONDSIA CHINESIS

Introduction

Jojoba, Simmondsia chinensis, is a dioecious desert shrub native to the Sonoran desert. The oil from its seed is a fine lubricant used mostly in the cosmetic industry. The seed is approximately 40 to 50% oil, 15 to 25% protein, 4-5% moisture, and up to 30% carbohydrate (Yermanos and Duncan, 1976, Cotgageorge, 1978, Verbiscar and Banigan, 1978). However, jojoba meal contains up to 3% simmondsin, a cyanoglucoside. Williams (1980), Verbiscar and Banigan (1978), and Booth, Elliger and Waiss (1974) discuss the toxicity of simmondsin and jojoba meal. The study by Williams (1980) indicated that simmondsin by itself was not responsible for the deaths of laboratory mice fed the meal, but other compounds, i.e. polyphenolic compounds might have acted as synergists to cause the toxicity and weight loss. The deaths appeared to be due to starvation. Because of the toxicity of the meal, it was desirable to isolate the protein free from simmondsin and polyphenolic compounds.

The goal of this investigation, was to isolate and characterize the protein from the pressed meal. Currently

the meal is a waste product, but it is a rich source of protein and carbohydrate suitable for an animal feed or food product. For the past two decades, researchers have concentrated on developing new vegetable sources of protein to help feed the poor. Jojoba meal has that potential.

Many problems encountered in the isolation of protein from pressed meal. Solubility tests using various alkalis, acids and salts indicated maximum solubility at pH greater than pH 8.5 and low ionic strengths (no salt or 0.15M NaCl). These results are similar for other plant protein isolation procedures (Seyam, et al, 1983; Gheyasuddin, et al, 1970; Blaicher, et al, 1983; and Seal, 1980).

Experimental

Samples

The pressed meal was extracted with hexane until the solvent was no longer yellow-colored, and then left at 20C overnight to desolventize. The meal was ground to at least 60 mesh with a hammermill. This meal was used for all subsequent experiments. Protein concentrates were obtained at room temperatures using solvents in a 1:20 w/v ratio. Extractions were with water (albumin), 0.15M NaCl (globulin), washed 3 times with methanol/acetone (1:1)

followed by extraction with water (SDI), and methanol/1N HCl (98:2) washes of SDI concentrate (SDII). The extractions were either lyophilized (Virtis, Model 50-SRC-5, Gardiner, New York) or spray dried (inlet 200C, outlet 80C, Niro Mobile Minor Atomizer, Copenhagen, Denmark). All solvents were ACS certified or reagent grade, and all chemicals were reagent grade.

Fermentation of the meal to remove carbohydrate was investigated using Saccharomyces cerevisae (Fleishmann's baking yeast), and glucose oxidase (No. G-6500, Type V, in 0.1M acetate buffer, pH 4, 1000 Units, Sigma Chemical Company, St. Louis, Mo.). Yeast/meal/pH 6.5 phosphate buffer (1/100/2000) were incubated for 2 hours at 37C. The yeast was removed using a glass fiber filter (No. 09-804-90A, Grade III, Fisher Scientific, Pittsburg, Pa.) and the filtrate was lyophilized. The glucose oxidase has been used in food products (Beck and Scott, 1974; Hill and Sebring, 1977; and Schwimmer, 1981). The glucose oxidase was added to meal (1000 units/100 gm) and incubated with 2000 mL citrate-phosphate buffer, pH 6.5 at 37C for 2 hours. The filtrate from a Whatman No.50 filter was lyophilized.

Protein Analysis

The various concentrates were analyzed for protein using the methods of biuret, microkjeldahl, (Bailey, 1967, pp 341, 346-348), using BSA (No. A-4378, Sigma Chemical Co., St. Louis, Mo.) as a standard, and the method of Bradford (1976) marketed by Biorad (San Diego, Calif.) as a sensitive colorimetric assay based on the affinity of protein for Coomassie blue. The Bradford (1976) assay was modified by diluting the reagent 1:10 instead of 1:4 (recommended by Biorad), to improve the accuracy. Both the biuret and Bradford methods were read at 30 minutes. The readings were made in a Bausch and Lomb Spectronic 20 (Bausch and Lomb, Inc., Rochester, New York). All samples were done in triplicate, with the appropriate controls and blanks. The %protein determined by microkjeldahl method was calculated using the formula:

%protein = DF x 90.24 x mL HCl/mg sample x 6.25.

DF is the dilution factor, which was 5 in all of these experiments. The 90.24 takes into consideration the N of the standard acid, the N of the alkali and mL of alkali, multiplied by 100 and by 14 gm/mole nitrogen.

Simmondsin Analysis

Simmondsin was quantitated using a u porasil C18 column eluted with methanol/water (25:75) and detected at

217 nm on a Spectra Physics 8000B HPLC. The samples were extracted directly with water and filtered through sintered glass fiber filter (No. 09-804-90A, Grade III, Fisher Scientific Products, Pittsburg, Pa.) and applied to the column. The method used was a combination of the methods of Verbiscar, et al (1980) and Williams (1980). Standards (simmondsin purified by Williams (1980)) were run before and after the one run of samples.

Polyphenolic Compound Analysis

Polyphenolics were quantitated using the method of Price and Butler (1977) modified by using 10 mg samples, and 1 mL of both reagents, ferric chloride and potassium ferricyanate. Absorbance was read at 10 minutes at 720 nm using a Bausch and Lomb Spectronic 20. A new standard curve using 10 to 1000 ug gallotannic acid was made for each run.

Amino Acid Analysis

Amino acid content of the concentrates was analyzed by the addition of 25 mL 5.6N HCl to 100 mg sample and 100 mg sodium thioglycollate in a 250 mL round bottom flask, covered with a 50 mL beaker and autoclaved for 17 hours at 250C and 20 psi. The digests were evaporated to dryness on a rotary flash evaporator, reconstituted with exactly 10 mL pH 2.2 citrate buffer, filtered through a Whatman No. 5

filter paper and then diluted to make approximately a 0.5% protein solution. One mL samples were applied to a Beckman automatic amino acid analyzer Model 121 (Beckman Instruments, Palo Alto, Calif.). Quantification of amino acids used these formula:

K = standard area/.2 umole

umoles AA/gm conc = 10 x sample area x dilution/K.

g/16 g N = umole AA/gm conc x gm conc/gm prot. x

ug AA/umole AA x 100 gm prot/16 gm N x gm/1,000,000 ug

Chromatography

The protein was purified on a Utrogel A6 column (molecular weight cutoff 2,000,000), 90cm x 2.6 cm i.d. LKB column with a 20 mL/hr flow rate using pH 8.9 aqueous solution with 0.05% sodium azide as a preservative, at room temperature. Eluents were monitored at 280 nm with an ISCO monitor and detector (ISCO, Inc., Lincoln, Neb.) The effluents were frozen daily and lyophilized weekly to minimize degradation of the protein. The standard proteins were BSA, b-glucosidase from almond extract, and trypsin inhibitor (Calbiochem, SanDiego, Calif.). Blue dextran was used to determine void volume (Sigma Chemical Co., St. Louis, Mo.).

Electrophloresis

For confirmation of data, the protein was separated on slab polyacrylamide gel electrophloresis using the method of Laemmlli (1970). The gel contained 12.5% T, 2.46% C, 0.067% TEMED, and 0.083% ammonium persulfate in 0.375 M Tris adjusted with 1N HCl to pH 8.9. The reservoir contained 0.025M Tris and 0.192 M Glycine adjusted to pH 8.3 with 1N HCl. The gels were run at 40 ma for 3.5 hours. The indicator, bromphenol blue was added to the samples, which were solubilized in 50% sucrose. All chemicals, Tris, N,N'methylene-bis-acrylamide, acrylamide, ammonium persulfate, and N,N,N',N'tetramethylethylenediamine, and glycine were obtained from Sigma Chemical Co., St. Louis, Mo.

Trypsin Inhibitor Analysis

Trypsin inhibitors were quantified using the method of Kakade, et al (1974) with the modifications by Chang and Tsen (1979). a-N-benzoyl-DL-arginine-p-nitroanilide HCl was the substrate. All of the reagents were acquired from Sigma Chemical Company, St. Louis, Mo.

Phytic Acid Analysis

Phytic acid concentration in 500 mg samples was quantified using the method of Wheeler and Ferrel (1971).

Results and Discussion

Extraction and Yield

The protein extracted by the methods described in the experimental section, were separated successfully by chromatography and electrophloresis. If the extractions were further modified by extracting with pH 8.5 water instead of pH 6 to 7 water, the yield would again increase. This was shown with small samples, but when large kilogram samples were prepared, it was much easier to extract with large quantities of tap water rather than constantly being concerned with adjusting the pH of the water.

Better yields were achieved from 100 gm samples than from kilogram samples (Table 2). The larger samples were those that were tested and characterized. The recovery was about 2/3 of the total possible protein in large batches, and about 3/4 of the total in smaller batches.

The enzyme glucose oxidase, an oxidoreductase, is used industrially (Richter, 1983) for removing either glucose or oxygen from the system. Glucose oxidase has been successfully used in food products (Beck and Scott, 1974; Hill and Sebring, 1977; and Schwimmer, 1981). In this case, it was added to convert the glucose, and other

Table 2. Yield and Purity of Protein in Concentrates of Jojoba Pressed Meal.

	% Protein			Percent of Protein Recovered	
Sample	Biorad	Biuret	Kjeldahl	100 gm	1 kg
GOX treated	60.5	41.3	73.5+/-2.3	60	nd[1]
SDI	74.4	55.9	80.5+/-4.9	75	67
SDII	60.7	22.0	84.8+/-2.0	75	65
Albumin	55.1	59.6	68.2+/-0.3	72	70
Globulin	37.7	37.6	67.2+/-0.5	73	66
Meal	20.5	19.5	20.3	-	-

1. nd - not done.

pyranosides to their aldonic acid and peroxide. The preparation of glucose oxidase, which was used also contains catalase, which reduces peroxide to water and oxygen. The GOX/catalase system would hopefully increase the methanol solubility of the phenolic compounds and sugars. The glucose oxidase is a dimer, with subunits about 90,000 daltons. The enzyme has optimum pH 4.5 to 6.5, and optimum temperature at 30C to 50C.

A b-glucosidase may also help cleave the ester linkages between the phenolic compounds and sugars and between the sugars and proteins in glycoproteins. Cotgageorge (1978) had limited success with the enzyme.

The GOX/catalase system should have at least prevented oxidation of the phenolic compounds present and thereby reduce discoloration, even if it only oxidized monomer sugar units, but not esters or glycoproteins. Glycoproteins, which frequently contain much glucose and galactose, are probably the major proteins in jojoba meal since jojoba meal is mostly albumin (Cardoso, 1980).

The Saccharomyces cerevisae treated meal was recovered in low yields, therefore, no further investigations were made with it. Since the yield of GOX-treated protein was greater than the yeast treated meal, it was included in the rest of the experiments.

Protein Content

The values of protein varied greatly depending upon the method of determination. The two colorimetric determinations determine the soluble protein. The biuret reacts with fewer interfering compounds than the Lowry, and therefore was utilized in preference to the Lowry method. The biuret is affected by ionic strength, phosphates, tris, and polyphenolic compounds. The use of proper controls minimizes but does not eliminate the undesirable interactions. The BSA was not purified prior to use as a standard, and may contain 1% to 3% globulin. This may also be part of the observed inaccuracy.

The Bradford method is similar to the microbiuret method, in that only 100 uL samples are used, and therefore has the potential for greater error. The sensitivity limit is 200 ug protein per sample. Larger sample sizes exceed the linear range for quantitation. This sensitivity of Coomassie blue has been useful in gel electrophloresis when detection of low levels of protein is required. Coomassie blue attaches to the N-amino grooup and protonated amino groups in the protein. Therefore the quantity of basic amino acids and the chain length of protein are important factors. The Bradford reagent has advantages over the biuret since tris, phosphates, or polyphenolic compounds do

not interfere. The major drawback of the Bradford method is that greater precision in measurements must be used, to achieve the same accuracy as in the biuret or kjeldahl.

If the protein is not very soluble, the value determined by Bradford or biuret assays will be much lower than the kjeldahl value, which includes non-protein nitrogen and insoluble protein. In preliminary investigations, very little nitrogen was left in the supernatant after precipitation of the protein with 30% trichloroacetic acid. It is therefore assumed that since there is little non-protein nitrogen, the kjeldahl value was the most accurate. According to Ockerman (1978, p.745), most oilseeds have a protein factor af 5.3 instead of 6.25. Therefore, the reported kjeldahl value may be higher than the actual kjeldahl value and protein.

The kjeldahl analysis of the protein concentrates shows that the percentage of protein in each of the protein concentrates is significantly different from the others and from the pressed meal at the 95% confidence level. From that data, the SDII fraction has the greatest purity. The biuret and Bradford analyses are not in agreement with the kjeldahl presumably due to interferences. The Bradford (1976) method is in closer agreement presumably because the interferences are fewer.

Nitrogen Solubility

Nitrogen solubility was determined by dividing the soluble protein in citrate/phosphate and Tris buffers in the Biorad assay (Bradford, 1976) by the total protein determined by the microkjeldahl assay and multiplied by 100 for percent. The conversion factor for the microkjeldahl was 6.25 (16% nitrogen in the proteins was assumed). From the nitrogen solubility curves, the isoelectric points can be seen (Figure 6). The albumins and GOX-treated protein concentrates in solution have isoelectric points near pH 7, the two purified protein fractions have pI near pH 3. Cardoso (1980) reported the pI of jojoba seed albumins and globulins to be pH 4 and pH 3 respectively. Rahma and Narasinga Rao (1979) reported a pI near pH 3 for sunflower proteins extracted with salt solutions, but that the pI of sunflower proteins shifted to near neutrality after purification using alcohol or acid for removal of the polyphenolic compounds.

Polyphenolic Compounds

Although tannins are the polyphenolic compounds that bind to and precipitate proteins, it is much easier to quanitate polyphenolic compounds in general. Using the Price and Butler (1977) assay, the levels of polyphenolic compounds decreased in the concentrates washed with

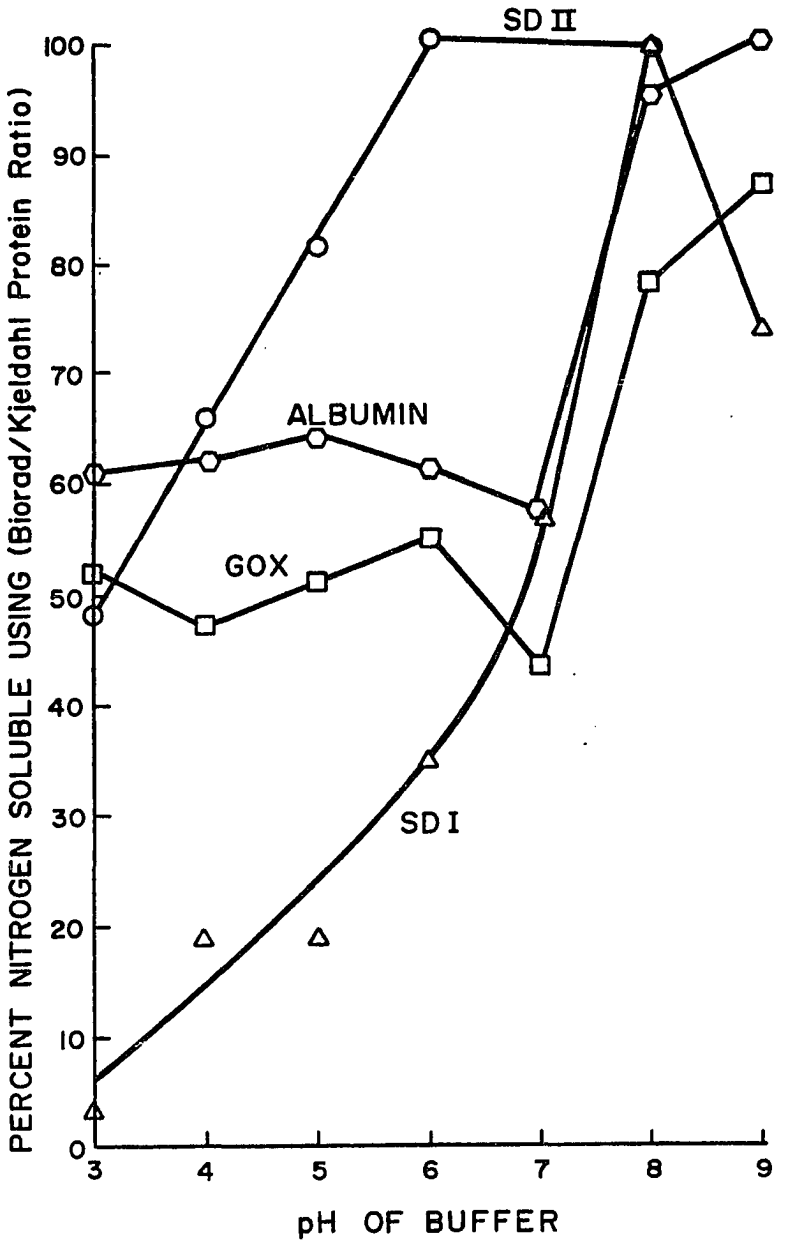


Figure 6. Nitrogen Solubility Curve of Protein Concentrates from Jojoba Pressed Meal.

methanol/acetone and methanol/1N HCl (Table 3). The higher concentrations of polyphenolic compounds in the globulin fraction than in the albumin fraction was probably due to the increased solubility of protein including the low molecular weight proteins bound to the polyphenolic compounds. The enzyme, glucose oxidase, reduced the total amount of polyphenolic compounds, although the solvents were more effective. Combination of the enzyme and solvents might have worked better than either method did alone.

The toxic level of tannins in the diet has not been determined (Tan, 1983). Tan reported 0.3 mg/gm to 7.5 mg/gm catechin equivalents in the winged bean, using the vanillin test, were not found to be nutritionally significant. In comparison, the concentration of polyphenolics found in jojoba meal was up to 10 fold higher than that reported for winged beans and consequently might be nutritionally detrimental. The level found in the SDII fraction is within the range of winged beans reported by Tan (1983) to be of little nutritional importance. Therefore, the protein isolated by this method may be safe to incorporate into feeds.

Sodini and Canella (1977) obtained a colorless protein isolate from sunflower meal after 8 extractions

Table 3. Percentage of Polyphenolics and Simmondsin, and Trypsin Inhibitor Activity of Jojoba Pressed Meal and its Protein Concentrates

Sample	Simmondsin %	Polyphenolics %	Trypsin Inhibitor TIU/10 gm
GOX treated	5.5	2.31+/-.090	249+/-148
SDI	1.7	0.66+/-.022	225+/-52
SDII	1.0	0.20+/-.148	163+/-49
Albumin	3.9	1.51+/-.027	223+/-8
Globulin		2.13+/-.032	84+/-10
Meal		8.07+/-.076	45+/-4

with acidic butanol. The level of chlorogenic acid was lowered from 2.6% to 0.1%. The treatment moved the pI to pH 5-6, increased the solubility at pH above 7 to greater than 90%. Because of this report, the methanol/HCl washes were used to purify the SDII concentrate. Since 3 washes reduced the polyphenolic levels, increasing the number of washes with acidic methanol might further reduce the color and polyphenolic content.

Simmondsin

The simmondsin levels were quantitated using a combination of the methods of Williams (1980), and Verbiscar and Banigan (1978). Verbiscar, et al (1980) analyzed simmondsin with acetonitrile/water (9:1) at 220 nm with simmondsin eluting at 8.0 minutes and simmondsin-2'-ferulate at 5.5 minutes using a flow rate of 1.0 mL/minutes. Williams (1980) used gradients of methanol/water with the simmondsin eluting between 20 to 26% methanol in water. The purified standards used by Williams (1980) were used to quantitate simmondsin. Standards were run before and after the one run of extracts from the protein concentrates. Absorbance at 217 nm was used for detection since there were fewer peaks than at 230 nm or at 320 nm, two other wavelengths absorbed by polyphenolic compounds. The 217 nm absorbs phenols and

aromatic nitriles (Silverstein, Bassler, and Morrill, 1974). Due to technical difficulties with the HPLC, only one run was made on each sample. Therefore, there may be variability between runs and the confidence of the values cannot be certain.

However, the value of simmondsin in the GOX treated and albumin fractions exceeded that found in the pressed meal. This indicated that the simmondsin was co-extracted with the protein. Both the methanol and acetone washes removed simmondsin, but simmondsin also remained with the protein concentrates. The residues were not analyzed for simmondsin. The organic washes were rotary evaporated, lyophilized and analyzed for simmondsin. Simmondsin comprised approximately 85% of the methanol extract solids and 90% of the acetone extract solids. Twenty four times as much simmondsin was recovered in the methanol than in the acetone. The methanol and acetone extracts were for separation and purification of the tannins (Hagerman and Butler, 1978), but very little tannins were recovered. This agrees with the solubility studies done by Cotgageorge (1978), who found that water and methanol were the best solvents to partially remove simmondsin. Since both the simmondsin and the total polyphenolics were decreased,

the toxicity of the protein should also decrease. However, toxicity studies need to be done.

Trypsin Inhibitor Content

The third anti-nutritional factor investigated was the presence of trypsin inhibitor. Both Samac, et al (1980), and NgouNgoupayou (1982), reported trypsin inhibitor (TI) activity. Samac and associates were working with germinating seeds and found inhibitors in the albumin fraction of commercial pressed meal. The inhibitors inhibited trypsin, chymotrypsin, and pepsin. The activity of the trypsin inhibitor decreased with germination. The activity is measured in trypsin inhibitor units (TIU), which is defined as a change in absorbance of 0.01 absorbance units using the standard method of Kakade, et al. (1974). NgouNgoupayou worked with the meal, and found 3 to 6 TIU/gm untreated deoiled meal, and 0.12 to 0.72 TIU/gm in the albumin fraction. The activity (TIU) for the meal (Table 3) agree with NgouNgoupayou, with 4.5 TIU/gm.

The activity found in the albumin fraction indicated, that the water extraction removed the low molecular weight proteins, including the trypsin inhibitors. This is contrary to NgouNgoupayou's results. Extraction with water at 80C for 30 minutes reduced the trypsin inhibitor activity to a value within the error of

the determination. Since activity was reduced in the SDII concentrate and not in the albumin and SDI concentrates, the methanol/1N HCl washes either remove or inactivate the trypsin inhibitor. The differences between the GOX treated, the SDI, and the albumin fractions were not significantly different at the 95% confidence level. The other samples were significantly different.

Phytic Acid Analysis

The amount of phytate was below detectable levels in the concentrates. Tan (1983) found 6.1 to 7.5 mg phytate phosphate per gram winged beans, a level comparable to that in soybeans, and therefore not considered to be harmful. The decorticated meal and seed had lower levels, 0.07 and 0.10 mg/gm, respectively. In the jojoba study, the concentration of phytate in the protein concentrates in the preliminary study were below the level of detection, and also below the level found in soybeans. Therefore further quantification was deemed unnecessary.

Amino Acid Analysis

Table 4 summarizes the amino acid composition of jojoba protein concentrates. As was also reported by Cardoso (1980), and NgouNgoupayou (1982), the acidic amino acids (ASX, GLX) were the most plentiful, and the sulfur

Table 4. Amino Acid Composition of Jojoba Pressed Meal Protein Concentrates in umole/gram and gm/16gm N.

Amino Acid	Albumin		Globulin		SDI		GOX	
	umole gm	gm 16gmN	umole gm	gm 16gmN	umole gm	gm 16gmN	umole gm	gm 16gmN
LYS	74.4	1.59	22.3	0.49	72.5	1.31	7.38	1.47
HIS	37.8	0.86	11.5	0.26	40.0	0.77	3.90	0.82
ARG	133	3.39	50.3	1.30	123	2.66	13.4	3.17
ASX	261	5.07	58.2	1.15	253	4.16	252	4.54
THR	122	2.12	29.2	0.52	136	2.01	121	1.95
SER	102	1.57	34.8	0.54	112	1.46	106	1.50
GLX	232	4.98	58.2	1.27	205	3.73	235	4.67
PRO	243	4.09	63.4	1.08	132	1.89	156	2.44
GLY	252	2.77	77.4	0.86	251	2.34	251	2.56
ALA	103	1.35	29.1	0.39	125	1.38	95.8	1.16
CYS	0.46	.088	0.55	0.52	4.6	0.52	0.97	.016
VAL	133	2.35	29.7	0.52	149	2.16	126	2.00
MET	20.6	0.45	7.01	0.15	17.9	0.33	16.2	0.33
ILE	77.6	1.70	20.1	0.39	88.6	1.44	77.3	0.29
LEU	158	3.03	35.1	0.68	1.82	2.97	142	2.53
TYR	65.9	1.26	18.8	0.51	67.6	1.52	58.0	1.43
PHE	79.6	1.31	16.8	0.41	98.1	2.01	68.5	1.54

amino acids were deficient. Table 5 shows the amino acid composition of eggs and milk (Ockerman, 1978) for comparison. The arginine, proline and leucine contents are high. The ratio of arginine to lysine ranged from 1.7 to over 2, a relationship commonly found in plant proteins.

Since tyrosine was approximately 3% of the amino acids in the various concentrates, and therefore approximately 2% of the concentrate, the residual polyphenolics might actually be the tyrosine reacting in the Prussian blue test. Cysteine (and half cystine) content increased from the albumin to the SDI fraction from 0.022% to 0.04%. The increase in cysteine might have resulted from cleavage of hydrogen bonds of polyphenolic compounds and protein. This same mechanism may also be responsible for the increased tyrosine. The hydrogen bonds between amino acids and phenols are reversible, although the bonds between phenolic compounds and the amides of glutamine, asparagine, arginine, lysine, and histidine are irreversible (Harborne and Van Sumere, 1975).

Chromatography and Electrophoresis

The final attempts to characterize proteins were with gel filtration and gel electrophoresis. Interpretation of gel filtration and electrophloresis data (Table 6) require assumptions for molecular weight

Table 5. Amino Acid Composition of Milk, Egg, and Wheat Protein (Ockerman, 1978, pp 455, 651, 917).

Amino Acid	Wheat g/16gN	Egg (Whole, as is) %	Casein %
Ala	3.25	0.64	
Arg	4.69	0.78	
Asp	5.09	0.95	
Cys	1.97	0.27	0.38
Glu	28.50	1.48	
Gly	3.88	0.42	
His	1.92	0.30	
Ile	3.90	0.72	6.6
Leu	6.48	1.01	10.0
Lys	2.74	0.84	8.0
Met	1.76	0.40	3.1
Phe	4.42	0.61	5.4
Pro	9.85		
Ser	5.06	0.92	
Thr	3.02	0.63	4.3
Trp	1.09	0.22	1.3
Tyr	3.10	0.54	5.8
Val	4.50	0.88	7.4

Table 6. Comparison of Interpolated Molecular Weights of Pressed Jojoba Meal Proteins in the Meal and Protein Concentrates.
(from electrophloresis data (X) and from gel filtration data (O))

Retention	MWT x1000	Meal	GOX	SDI	SDII	Alb.	Glob.	Yeast	Hot
14	75.3	OX		OX	OX	OX	O		
27	65.6	X				OX			
37	59.1	X				OX	OX		OX
40	57.3				OX	X	O		OX
47	53.2	X	X			X	O	X	
55	48.9			X	OX	X	OX		OX
60	46.3	X		X	OX	X	X		OX
67	43.0		X	X	OX	O	X	X	X
77	38.7	X	X	X	O	O	X	X	
85	36.3	OX			O	O	O		
100	30.3	O	X	OX	O	O	OX	X	
110	27.3	OX	X	OX			X	X	
	¼22	O				O			O

determination. Gel filtration separates proteins on the basis of the Stokes radius. Therefore, if all of the standard proteins, and the sample proteins are all globular, then the results are valid. Otherwise, the interpolation of molecular weight may be drastically different from reality.

Numerous studies have been reported on the use of gel filtration and PAGE for characterization of plant proteins. One study using a seed similar to jojoba is by Kabirullah and Wills (1983) on sunflower meal proteins. Three sunflower protein isolates were obtained based on their solubility in 1N NaCl, pH 11, and pH 6.5 water. Chlorogenic acid was a part of all fractions. The lower molecular weight fractions had more chlorogenic acid than the other fractions. The salt soluble sunflower proteins gave the lowest yield of protein. The water soluble protein fraction separated into 5 or 6 peaks on gel filtration columns. The alkali soluble protein fraction had an additional peak. The range of molecular weight for sunflower protein was 10,000 to 400,000 daltons. The major peaks were at 125,000 and 180,000.

Calibration with PAGE requires pretreatment of the samples with urea for dissocciation, 2-mercaptoethanol for cleaving disulfide bonds, and SDS for solubilization and

attaching ionic groups at regular intervals. All fractions should then have the same mass/charge ratio. Once the charge is no longer a variable, then the separation will be on the basis of molecular weight. The retention time is proportional to the log of molecular weights. Standard proteins were used for comparison.

Since the resolution on SDS gels was much poorer, the gels that were used in this study were non-denaturing gels. The proteins in their native structure, which were probably already denatured by the processing methods, were resolved on the gels. Keeping in mind that the criteria for molecular weight determination using electrophloresis were not met, the comparison of peaks between gel filtration and electrophloresis correspond very well. Since the jojoba proteins are slightly acidic, the proteins should migrate from the cathode to anode. If the proteins are basic, the poles would have to be altered for separation to occur. If any of the proteins were basic, they would have migrated off the gel using the non-dissociating gels.

The circles on Table 6 indicated the approximate molecular weight of the peaks from gel filtration. Usually a small peak was detected shortly after the void volume with a molecular weight approximately 75,000, followed by 1

or 2 other major peaks, whose molecular weight varied with the concentrate. The major bands on the electrophloresis (represented as X in Table 6) appear to have molecular weights corresponding to the major peaks in gel filtration. Figure 7 shows a representative electrophoresis chromatogram of the pressed jojoba meal protein concentrates. Quantitation of the protein bands was not carried out.

From the data collected there appears to be less than 15 major proteins in the pressed meal of jojoba seeds. The proteins in the meal and concentrates appear to be relatively low molecular weight proteins. The major proteins separated by gel filtration were at 75,000; 30,000 to 54,000; 20,000; and 18,000; and those separated by gel electrophloresis were at 48,000, 37,000, 31,000, 22,500, and 18,700. Since there were many proteins of similar size in the gel electrophoresis data, the proteins were coeluted in the gel filtration.

The electrophoresis chromatograms (Figures 4, 5 and 7) show the many causes of protein degradation complicating this study. Enzymes and yeast degraded the protein similarly (Figure 7, samples A, B). Hot water degraded the protein (Figure 4, sample D), but not as much as the enzymes did. Purification using methanol, acetone, and HCl

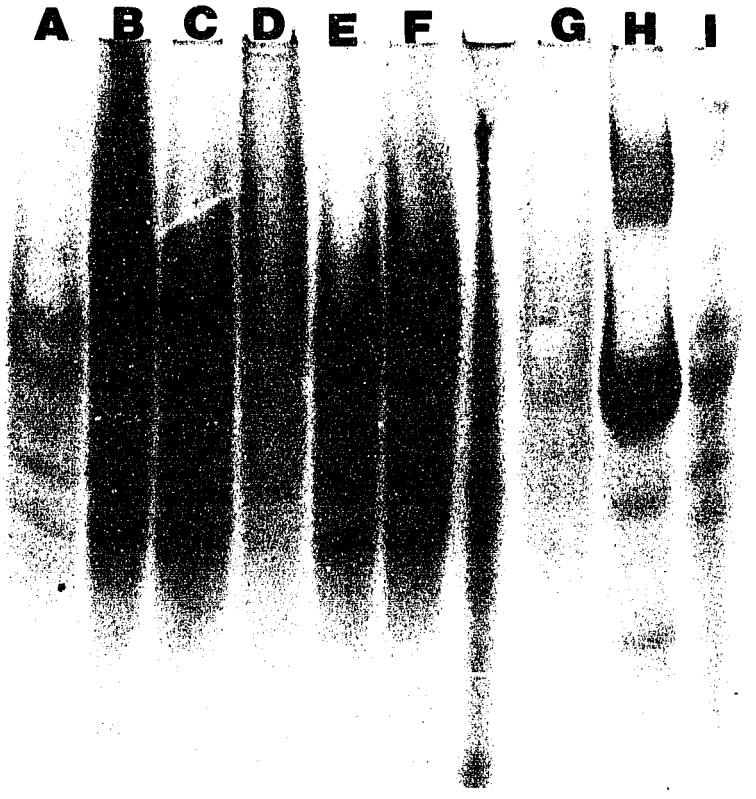


Figure 7. Polyacrylamide Gel Electrophoresis of the Protein Concentrates from Pressed Jojoba Meal.
From left to right: 7.5 mg samples of yeast extracted (2 hours) (A), GOX treated meal (2 hours) (B), albumin (C), spray dried SDI (D), globulin (E), kg albumin extract (F), spray dried SDI (G), BSA, b-glucosidase, and trypsin inhibitor standards (H), and meal (I).

(Figure 7, samples D and H) seem to degrade the major proteins. Contrary to expectation, the molecular weight of the albumin proteins appear to be larger than those of the globulin. Cardoso (1980) also reported finding more higher molecular weight proteins in the jojoba seed albumin fraction than the globulin fraction. The low molecular weight of the jojoba proteins, are partially due to the partial denaturation of protein due to the hot pressing process, since even the meal had lower molecular weight protein than expected. SDS electrophloresis showed nondistinct band less than 40,000 daltons for all of the samples, and therefore was of little value. The SDII fraction had all 5 major proteins on the electrophloresis gel. All extractions removed some of the bands in the meal.

Conclusions

Electrophoresis and chromatography confirmed that protein was present, but that the proteins were of the lower molecular weights. None of the proteins appeared to be larger than 70,000, but since the molecular exclusion limit of the PAGE was approximately 70,000 and the conditions were not met for accurate quantification, this conclusion may not be reliable. Although the solvent

treatments were known to be methods for denaturing protein, the 5 major bands were present in all concentrates.

All of the concentrates were at least 50% protein, with the SDII as 60% protein. The polyphenolic content was reduced from the level found in the meal in all of the concentrates. However, the polyphenolic content was reduced from 81 mg/gm meal to 2 mg/gm SDII concentrate. The simmondsin was reduced to 1.0% in the SDII concentrate. The trypsin inhibitor activity is not a crucial variable since the inhibitor is easily heat denatured, but since it is more soluble in water than salt, it was monitored throughout the extraction. Trypsin inhibitor activity was initially high in the albumin fraction, and was successfully reduced by the solvent extractions.

Since the polyphenolic and simmondsin content, and the trypsin inhibitor activity of the SDII protein concentrate were reduced while simultaneously increasing the protein content, the SDII protein concentrate was used in the next chapter for optimizing conditions for investigating the functional properties of jojoba meal proteins.

FUNCTIONAL PROPERTIES OF PROTEIN CONCENTRATES OF JOJOBA PRESSED MEAL

Introduction

Proteins are isolated from plant sources partially for their nutritional value and partially for their functional value in food products. Egg proteins are frequently used as the standard for comparison of functional properties. The functions of eggs in food products, according to Baldwin (1977), are coagulation, foaming, emulsification, and nutrition. The functional properties investigated in this chapter were gelation, oil absorption, emulsification, water absorption, and foaming. The nitrogen solubility was discussed in the previous chapter, since it characterizes both protein structure and function.

Experimental

Samples

The concentrates from jojoba pressed meal obtained by methods outlined in the previous chapter were used, i.e. albumin, globulin, SDI, SDII, and GOX treated. The protein used for comparison was egg albumin (No. A-5503,

crystallized, lyophilized, salt free, Grade V, Sigma Chemical Co., St. Louis, Mo.)

Gelation

Gelating ability was tested by heating 10 mL samples of aqueous dispersions (6%, 8%, 10%, and 20% protein) adjusted with 1N HCl or 1N NaOH to pH 4, 7, and 9, for 10 minutes in a boiling water bath and cooled overnight at 4C. A gel by definition is the simultaneous loss of fluidity and loss of liquidity. This was tested by inverting the centrifuge tubes.

Oil Absorption

Oil absorption ability was determined using modifications of the procedure of Lin, Humbert and Sosulski (1974). A 0.5 gm sample was placed in a 15 mL conical centrifuge tube with 3 mL corn oil and dispersed for 15 seconds. After 30 minutes at 20C, the tubes were centrifuged in a clinical centrifuge at fast speed for 20 minutes. The oil was decanted into a 10 mL graduated cylinder and the volume was read. The volumes were multiplied by 2. The concentrates were not dried prior to this experiment, but had approximately 12% moisture in the SDI and SDII, and 6% moisture in the albumiin, globulin, and GOX-treated.

Emulsification

Emulsifying capacity was determined using modifications of the method of Huffman, Lee, and Burns (1975). Two 20 mL samples of 2%, 6%, and 10% dispersions of the protein concentrates in citrate-phosphate buffer at pH 5 and pH 7, and 0.05M Tris (pH 9) were stirred on a magnetic stirrer while corn oil was added from a burette at 1 mL/min. The capacity was determined by a drop in viscosity (monitored by the need to decrease the rate of mixing). The emulsions were incubated at 80C for 30 minutes, depending on the amount of separation, the emulsion stability was classified as completely separated (unstable), partially stable and stable.

Water Absorption

Water absorption was determined by a modification of the method used by Sathe and Salunkhe (1981a). Concentrates were oven dried for 24 hours at 110C prior to weighing 0.5 gm into 15 mL conical centrifuge tubes. The centrifuge tubes were weighed, then samples were mixed with 4.5 mL distilled water, 5% NaCl, saturated NaCl, or saturated $CaCl_2$. After incubation for 30 minutes at 20C, the tubes were centrifuged for 30 minutes at high speed on a clinical centrifuge, decanted, and reweighed. Water

absorption ability of the protein concentrates was calculated by dividing the grams of water absorbed (i.e. the difference in the weight of the centrifuge tube) by the weight of the protein concentrate, and multipling by 100 for percentages.

Foaming

The methods of Eldridge, Hall, and Wolf (1962) were followed. The first experiment blended two 20 mL samples of 1% dispersions of SDII concentrate in pH 4, pH 7, and pH 9 buffers, and water at 20C for 9 minutes. The volume of foam was recorded initially as expansion in mL, and after standing 30 minutes and 120 minutes at room temperature as stability data.

In the second experiment, two 20 mL of 1% dispersions of SDII concentrate in water and pH 4 buffer were incubated at 4C, 20C, 37C, 55C, 80C, and 110C for at least 30 minutes, then blended for 9 minutes, and the volume of the foam is recorded initially, after 30 minutes and after 120 minutes.

Using the optimum pH and temperature determined by the above two experiments, two 20 mL samples of 1%, 3%, 6%, and 10% dispersions of SDII concentrate were blended for 9

minutes. The volumes initially, after 30 minutes, and after 120 minutes were measured.

Two 20 mL samples of 3% dispersions of all concentrates in water were blended for 12 minutes at 20C and 55C, the volume was measured initially, after 30 minutes, and after 120 minutes.

The last experiment tested the effect of some additives on the expansion and stability of foams. Chemically pure, reagent grade sucrose (5.0 gm/0.5 gm concentrate), salt (0.5gm/0.5gm concentrate), potassium acid tartrate (KHT) (0.13gm/0.5gm concentrate), and sucrose and KHT combined were added to duplicate 20 mL samples of 3% SDII concentrate dispersions in water, incubated at 20C or at 55C for 30 minutes, blended for 12 minutes, decanted into graduated cylinder and recorded the volume of foam initially, after 30 minutes, and after 120 minutes.

Results and Discussion

Gelation

Gelation is the thickening of a product due to the loss of fluidity (Baldwin, 1977). It is dependent on the balance of anions and cations, as well as pH. Too low a salt concentration can prevent gelation. The results show that at low pH, more protein was required for gelation

(Table 7). Generally gelation required a higher concentration of protein at alkaline pH than at neutral pH. However, the SDII concentrate was an exception. A 6% dispersion was sufficient for gelation at neutral pH.

The effect of pH was tested with 0.05M citrate-phosphate buffer, or 0.05M Tris, The use of buffers lowered the amount of protein required for gelation. This was evident after comparing the data of Cardoso (1980) to this data. Cardoso (1980) reported that 8% dispersion of albumin and 10% dispersion of globulin were required for gelation. The firmness of the gel varied between the concentrates presumably due to the interactions of proteins, carbohydrates, and buffer components with cations and anions (the ratio of cations to anions is important). Cardoso (1980) reported that the albumins had soft gels at 10%, and firm gels at 14%, while the globulins had soft gels and hard gels at 7%, and 8% respectively. The gels from the globulins, in this study, were softer than the other gels.

Sathe, Deshpande, and Salunkhe (1982a) reported 14% and 8% dispersions were required for gelation of lupin seed flour, and protein concentrate respectively. Sathe and Salunkhe (1981a) reported that 10% and 8% dispersions for the gelation of Great Northern bean flour, and protein

Table 7. Minimum Percentage of Protein Concentrate Required for Gelation at Various pH Values.

Sample	pH 4	pH 7	pH 9
GOX treated	10	6	8
SDI	-	6	10
SDII	20	8	6
Albumin	-	6	6

1. Concentrations used (6%, 8%, 10%, 20%)

concentrate respectively. Comparison of data indicate that gelation in the jojoba pressed meal protein concentrates is comparable to other plant proteins and jojoba seed concentrates.

Oil Absorption

Oil absorption is an important function in sausage products and doughnut baking. In general, each purification step added to the protein extraction procedure removes more of the lipophilic moieties and therefore, decreases the amount of oil that the protein concentrate can absorb (Table 8). The crude albumin and globulin samples absorb more oil than the purifications of these fractions. The values in this study were lower than those reported by Cardoso (1980) for the jojoba seed albumin (3.5) and the globulin (4.0), presumably due to differences in sample preparation including the use of pressed meal instead of freshly ground seeds.

The ability of albumin and globulin concentrates from pressed jojoba meal to absorb oil is similar to the ability of protein concentrates from the Great Northern bean (Table 9). The ability to absorb oil of the other protein concentrates from pressed jojoba meal is greater

Table 8. Oil Absorption Property of Protein Concentrates

Sample	mL corn oil absorbed/gm concentrate
GOX treated	2.30
SDI	1.66
SDII	1.86
Albumin	3.18
Globulin	3.04

Table 9. Summary of Oil Absorption Data on Plant Proteins.

Source	Oil Absorption
Lin, Humbert and Sosulski (1974)	
Wheat Flour	0.84 mL/gm
SoyFlour	0.84 mL/gm
Soy Conc./Isolate	0.94 to 1.54 mL/gm
Sunflower flour	2.08 mL/gm
Sunflower Conc./Isolate	2.26 to 2.57 mL/gm
Sathe, Deshpande, and Salunkhe (1982a)	
Lupin Seed Flour	1.67 gm/gm
Lupin Seed Concentrate	2.86 gm/gm
Lupin Seed Defatted Concentrate	3.89 gm/gm
Sathe, Deshpande and Salunkhe (1982b)	
Winged Bean Flour	2.03 gm/gm
Winged Bean Concentrate	4.01 gm/gm
Sathe and Salunkhe (1981a)	
Great Northern Bean Flour	1.00 gm/gm
Great Northern Bean Concentrate	4.12 gm/gm
Great Northern Bean Isolate	1.57 gm/gm
Great Northern Bean Albumin	3.29 gm/gm
Great Northern Bean Globulin	3.23 gm/gm

than the ability of soybean proteins, but less than that of sunflower protein concentrates.

Lin, Humbert, and Sosulski (1974) suggest that the amount of nonpolar sidechains in the protein, and their position in the molecule play a major role in determining the lipophilic nature of proteins. From the data in Table 4 of the previous chapter, 25% or less branched chain amino acids are present in the concentrates, and therefore branch chain amino acid content does not appear to be a major factor affecting the lipophilic nature of the concentrates. Another possibility for the decreased lipophilic nature of the protein concentrates might be due to denaturation and removal of lipophilic moieties in the many methanol, acetone, and acidified methanol washes used for removal of simmondsin and polyphenolic compounds. As a consequence, the oil absorption ability decreased during the purification of the SDI and SDII concentrates in comparison to the albumin and globulin concentrates.

Emulsification

Emulsification can be of two types, oil in water, and water in oil. The bridge which keeps the two liquids intermingled is the protein. Emulsification capacity is the amount of oil that can be added per gram of protein

before the viscosity decreases indicating a separation of phases. Huffman, Lee, and Burns (1976) found that the emulsifying capacity depended on the rate of addition of oil, the amount of protein, the mixing speed, and pH.

Wu, Wisakowsky, and Burns (1976) found that the emulsifying capacity decreased as solvents extracted the oil from soybean meal. Since different organic solvents are used to extract the oil, they quantitated the amount of oil extracted by each of the organic chemical systems and found no correlation at the 95% level of confidence between the amount of oil removed, and the ability of the defatted meal to emulsify and stabilize emulsions. Lipophilic proteins extracted were not quantitated. Presumably the stabilizing effect of proteins in emulsions occurs because the protein has both polar and nonpolar groups which can interact with the aqueous and lipid layers respectively. Since solvent extraction removes both the oil and lipophilic proteins, it was expected that the more oil removed would correlate negatively with the emulsion stability. Since this was not the case, other structural and conformational properties must be important in emulsification.

Cardoso (1980) used the method of Swift, et al (1961) for determining emulsifying capacity, which adds oil

from a burette until there is an inversion. Capacity determined by inversion is usually much higher than that found using the method reported here, because the amount of oil added must be enough to make the emulsified layer less dense than the oil. Cardoso (1980) reported the capacity at 325 mL/gm and 370 mL/gm for globulin and albumin respectively.

Huffman, Lee and Burns (1975) reported a range of 60 to 200 mL/gm for sunflower meal depending on the variables mentioned before. The maximum capacity was at neutral pH, independent of the other variables. The faster the addition of oil, the higher the capacity. Stirring at 4500 rpm had greater capacity than stirring at faster rates.

Lin, Humbert and Sosulski (1974) used a method developed by Inklaar and Fortuin (1969), which if adapted to the available equipment, would have given less subjective data. The method used a 10% dispersion (100 mL) and added 50, 100 or 150 mL of oil while stirring at 1400 rpm. After a predetermined period of time, the volume of emulsified layer was recorded. Emulsion stability was determined by the volume of the emulsified layer after incubation at 80C for 30 minutes. The advantage of this

method over the inversion method is that it is more useful in formulating salad dressings, gravies, and ice cream.

Sathe, Deshpande, and Salunkhe (1982a,b) used the same method as reported here, except that 5 mL of oil was added at a time to a blendor for 30 seconds at high speed. Stability was measured by the leakage of water at room temperature over time (Table 10).

Compared to the bean protein emulsions, the jojoba protein emulsions (Table 11) were not stable. Capacity of jojoba protein concentrates to emulsify decreased with increased protein concentration. Emulsion stability was greatest at pH 4 or neutral pH, but none of the jojoba protein samples were stable at pH 9. The capacity and stability of the globulins were lowest, in agreement with Cardoso (1980). The SDI concentrate had the best emulsification capacity and stability of the concentrates tested. The order of capacity is:

SDI > SDII > GOX > Albumin > Globulin.

The capacity and stability of the emulsions with pressed jojoba meal concentrates appeared to be comparable to other plant proteins. Since the methods for determining emulsifying capacity and stability for soy protein were different from those methods used with jojoba proteins, a direct comparison cannot be made. However, since soy

Table 10. Emusification Capacity and Stability of Plant Proteins in the Literature.

Source	Capacity	Stability
Lin, Humbert and Sosulski (1974)		
Sunflower Isolate	2.5 gm/gm	
Sunflower Concentrate	1.0 gm/gm	
Soy Isolates	2.3 gm/gm	
Sathe, Deshpande, and Salunkhe (1982b)		
Winged Bean Flour	71 gm/gm	0.5 hrs.
Winged Bean Concentrate	222 gm/gm	4 hrs.
Sathe and Salunkhe (1981a)		
Great Northern Bean Flour	40 gm/gm	0.5 hrs
Great Northern Bean Concentrate	73 gm/gm	5 days
Great Northern Bean Isolate	64 gm/gm	62%/35hrs.
Great Northern Bean Albumin	64 gm/gm	5 days
Great Northern Bean Globulin	44 gm/gm	15%/35hrs.

Table 11. Stability and Emulsifying Capacity of Protein Concentrates from Pressed Jojoba Meal

Sample	Dispersion (5 mL)	pH	ml corn oil emulsified	capacity mL/gm	stability for 30 min. at 80C
GOX-treated	6%	5	24	80	stable
		7	22	73	separation
		9	24	80	separation
	10%	5	24	48	stable
		7	25	50	stable
		9	28	56	separation
SDI	2%	5	19	190	partial
		7	18	180	
		9	23	230	partial
	6%	5	34	113	stable
		7	34	113	stable
		9	34	113	separation
	10%	5	24	48	stable
		7	22	44	stable
		9	25	50	partial
SDII	2%	5	17	170	stable
		7	19	190	partial
		9	17	170	partial
	6%	5	28	93	stable
		7	28	93	stable
		9	28	93	stable
	10%	5	24	48	partial
		7	22	44	stable
		9	25	50	partial

1. Stable means separation was not visible at 30 minutes, separation means that greater than 2 mL of liquid was visible, and partial means that less than 2 mL liquid was visible.

Table 11. Continued. Stability and Emulsifying Capacity of Protein Concentrates from Pressed Jojoba Meal.

Sample	Dispersion (5 mL)	pH	mL corn oil emulsified	capacity mL/gm	stability for 30 min. at 80C
Albumin	6%	5	20	67	stable
		7	20	67	stable
		9	27	90	separation
	10%	5	26	52	stable
		7	23	46	stable
		9	27	54	separation
Globulin	10%	5	23	46	stable
		7	17	34	stable
		9	24	48	partial
Ovalbumin	2%	5	16	160	stable
		7	17	170	stable
		9	20	200	separation

protein is useful in stabilizing emulsions in meat analogs, the protein concentrates from pressed jojoba meal, with higher capacity and stability data than the soy protein, ought to be useful too.

Water Absorption

Water absorption is important property of proteins in bakery products and in sausage and meat analog products. It is a measure of the amount of water that a concentrate can absorb. The water absorption is a function of the humidity, the temperature, and the concentration. The higher the temperature, the more moisture the protein molecules in the concentrates can hold. Other factors affecting water absorption (Sathe and Salunkhe, 1981a) are size, shape and conformation of the protein; steric factors; hydrophilic/hydrophobic balance of amino acids; lipid and carbohydrate moieties on the proteins; bonding energies; interfacial tension; pH; ionic strength; presence of surfactants; and protein solubility.

The samples prior to analysis were dried for 24 hours at 110C. The absorbed moisture after centrifugation included both bound and free entrapped water. The absorption and desorption curves are different, the water

absorption data is routinely reported, and the desorption data is reported only in comprehensive studies.

The water absorption of the protein concentrates from pressed jojoba meal increased as temperature was increased, however, there were several discrepancies (Table 12). At room temperature, the water absorption of protein concentrates from pressed jojoba meal were much lower than those reported for the protein concentrates of the winged bean and Great Northern bean (Table 13). The ability of the jojoba proteins to absorb water was closer to the ability of soy protein. The ability of jojoba albumins and globulins to absorb water were lower (1.0 and 0.6 gm/gm respectively) than for other plant protein concentrates. However, the other jojoba concentrates were able to absorb water similarly to the sunflower and soybean protein concentrates, 1.0 to 2.2 gm/gm. The glucose oxidase treated jojoba concentrate was by far the most hygroscopic (2.6 gm/gm).

Other data shows the effect of using 5% NaCl instead of water on absorption. Water absorption was independent of temperature at 5C and 20C. The water and 5% NaCl w/v had the same water absorption with slight variations due to the concentrate and sample (Table 12). The water absorption property is important in bakery goods;

Table 12. Water Absorption of the Protein Concentrates from Pressed Jojoba Meal, grams water absorbed/gram concentrate.

Sample	Conditions	Held at 5C for 30 min.	Held at 20C for 30 min.
GOX treated	water	1.8	2.2
	5% NaCl	2.0	2.0
SDI	water	1.4	1.0
	5% NaCl	1.8	1.6
SDII	water	1.4	1.4
	5% NaCl	1.6	1.2
Albumin	water	ND	1.0
	5% NaCl	ND	1.2
Globulin	Water	1.0	0.6
	5% NaCl	ND	0.7

1. ND means not determined.

Table 13. Comparison of Water Absorption Data for Plant Proteins in the Literature.

Source	Water Absorption
Sathe and Salunkhe (1981a)	
Great Northern Bean Flour	1.67 gm/gm
Great Northern Bean Concentrate	5.93 gm/gm
Great Northern Bean Isolate	2.73 gm/gm
Great Northern Bean Albumin	3.18 gm/gm
Great Northern Bean Globulin	2.77 gm/gm
Sathe, Deshpande and Salunkhe (1982b)	
Winged Bean Flour	2.28 gm/gm
Winged Bean Concentrate	3.52 gm/gm
Sathe, Deshpande and Salunkhe (1982a)	
Lupin Seed Flour	1.20 gm/gm
Lupin Seed Concentrate	1.37 gm/gm
Lupin Seed Defatted Concentrate	1.55 gm/gm
Rahma and Narasinga Rao (1981)	
Sunflower Meal	2.1 gm/gm
Water Extracted	3.6 gm/gm
0.002N HCl Washed	3.6 gm/gm
70% Ethanol Washed	3.4 gm/gm
Acidified Butanol	2.6 gm/gm
All sequentially	5.1 gm/gm
Lin, Humbert and Sosulski (1974)	
Sunflower Flour	1.07 gm/gm
Sunflower Concentrate	1.38-2.03 gm/gm
Sunflower Isolate	1.55 gm/gm
Wheat Flour	0.60 gm/gm
Soy Flour	1.30 gm/gm
Soy Concentrates	1.96-2.27 gm/gm
Soy Isolates	4.17-4.48 gm/gm

formulated dry foods, which will be later reconstituted; the intermediate moisture foods; and meat and sausage products.

Foaming

Eldridge, Hall, and Wolf (1962) have reported extremely stable foams produced by unhydrolyzed aqueous dispersions of soybean protein. Frequently soybean proteins were hydrolyzed by pepsin at pH 2 at 45C until 40% was soluble at pH 5. The optimum conditions for stable foams of alkali leached soybean protein washed with alcohol and precipitated with acid, were found to be at room temperature (4C, 60C, 70C, 80C, and 90C were tried), 1% or 2% dispersions (expansion was 8 to 9 times, and 11 to 12 times, respectively), and pH 5.1. After optimization, stability and expansion were compared with the addition of common additives found in toppings. A 1% dispersion of soybean protein with salt expanded 9.6 times (37% leakage), with potassium acid tartrate (KHT) expanded 8.1 times (57% leakage), with sucrose and KHT expanded 3.6 times (no leakage), and with no additives expanded 9.3 times (24% leakage). Eldridge and associates concluded by saying that the foams were usable in toppings. Leakage decreased with

increased temperature, but increased temperature was not necessary for stable foams.

Sathe, et al. (1982a) reported the maximum foam expansion with 0.6% to 1.0% NaCl in lupin seed concentrates. The foaming capacity of several plant proteins including the lupin seed protein are given in the literature (Table 14). In addition the maximum expansion was at pH 2, but stability at pH 4 was the greatest (very little leakage at 48 hours, and at pH 10 the foam lasted only 15 minutes before separating. This seems to agree with the data for jojoba protein concentrates (Table 15). The addition of various carbohydrates decreased the stability, although sucrose, amylose, amylopectin vand potato starch added at 0.25 gm/gm increased the expansion.

Huffman, et al reported the most stable foams from sunflower protein dispersions were at pH 9 and the greatest volume at pH 4. The expansion decreased with increased temperature up to 55C from a maximum at 15C. A concentration greater than 8% or whipping time longer than 12 minutes also decreased expansion. Sucrose and potassium acid tartrate decreased both the expansion and stability, 5% NaCl increased the expansion, but not the stability, while sucrose and potassium acid tartrate together increased both the stability and expansion.

Table 14. Comparison of Foaming Capacity of Plant Proteins in the Literature.

Source	Foaming Capacity
Sathe, Deshpande and Salunkhe (1982a)	
2% Lupin Seed Concentrate	50%
2% Lupin Seed Defatted Concentrate	58%
10% Lupin Seed Concentrate	92%
Sathe, Deshpande and Salunkhe (1982b)	
2% Winged Bean Concentrate	36%
4% Winged Bean Concentrate	80%
10% Winged Bean Concentrate	92%
2%, pH2, Winged Bean Concentrate	96%
2%, pH4, Winged Bean Concentrate	60%
2%, pH6, Winged Bean Concentrate	52%
2%, 0.6% to 2.0% NaCl, Winged Bean Concentrate	94% to 104%
Lin, Humbert and Sosulski (1974)	
3% Egg Albumin	250%
Soy Flour	70%
Soy Concentrate	135-170%
Soy Isolate	230-235%
Sunflower Flour, Concentrate, Isolate	220-230%

Table 15. Effect of pH on Expansion and Stability of Foams. (from 20 mL 1% Dispersions of Jojoba Protein Concentrate SDII at Room Temperature and Blended for 9 Minutes)

Buffer pH	Expansion mL	mL Foam at 30 min.	mL Foam at 120 min.
4.0	8.0	4.0	3.1
7.0	15.5	4.0	0.0
9.0	9.5	5.0	0.0
Water	6.0	2.5	1.5

Rahma and Narasinga Rao (1981) reported the foaming ability of solvent-extracted sunflower meal. A 1% dispersion at room temperature of both water and 0.002N HCl washed meal decreased expansion and stability but the meals extracted with 70% ethanol or 2% NaCl had an increased stability of a less expanded foam compared to the control. However, the meal washed with acidic butanol, and the meal washed sequentially with water, dilute acid and acidic butanol had increased both stability and expansion in comparison to the control.

Cardoso (1980)reported an 80% increase in volume for both the globulin and albumin fractions of jojoba seeds using a 1% dispersion at room temperature. Both fractions were 75% stable at 2 hours.

Preliminary foamability tests using SDII concentrate from pressed jojoba meal determined that the optimum blending time was between 9 and 12 minutes, since stability increased with time, but expansion decreased after 12 minutes. Eldridge, Hall and Wolf (1962) reported similar data. The first experiment (Table 14) used 1% dispersions of the SDII concentrate. Expansion in pH 4, pH 7 and pH 9 buffers, and in water was 40%, 78%, 48%, and 30% respectively. The foams at pH 7 and pH 9 were unstable. Twenty-five percent of the foam in the water dispersion and

39% of the foam in the pH 4 dispersion remained after 2 hours at room temperature. Expansion of the protein dispersions increased with temperature even at 110C if incubated for only 30 minutes (Table 16).

Data which shows the effect of protein concentration on expansion, seems to contradict this trend of increased expansion with increased temperature (Table 17). However, the protein samples were held at the incubation temperature overnight. Apparently the proteins were denatured and no longer were flexible enough to reduce the surface tension. However, the expansions at 20C and 55C were still stable. In the same table, the effect of concentration is seen. The 3%, and 6% dispersions were more effective than the 1% dispersions in expansion and stability. Due to limited amount of samples, 10% dispersions were not done on all samples. The samples held at 55C had greater stability than those held at 20C, i.e. up to 150% expansion of the protein concentrates held at 55C, compared to only 25% expansion of the protein concentrates held at room temperature was remaining 2 hours later at room temperature.

The foaming expansion and stability of jojoba protein concentrates all exceeded that of the egg albumin under the conditions of the study (Table 18). The SDII

Table 16. Effect of Temperature on Expansion and Stability of Foams.
(20 mL of 1% SDII dispersion was blended for 9 minutes after incubation for 30 minutes.)

Temperature	Expansion of Foam, mL	mL Foam at 30 min.	mL Foam at 120 min.
4C	6.0	2.0	0
20C	6.0	2.5	1.5
37C	6.0	3.0	1.5
55C	8.5	4.5	4.0
80C	12.0	8.0	5.5
110C	15.0	1.0	1.0

Table 17. Effect of Dispersion Concentration on Expansion and Stability of Foams from Jojoba Protein Concentrate SDII at Varying Temperatures and Buffers.

Dispersion %	Temp. C	Solvent	Expansion mL	mL Foam at 30 min.	mL Foam at 120 min.
1	20	water	9.0	3.5	0.5
3			15.0	10.5	10.0
6			13.0	8.0	8.0
1		pH 4.0	11.0	3.5	1.0
1	55	water	20.0	9.5	4.0
3			15.0	9.0	9.0
6			30.0	19.0	17.0
1		pH 4.0	17.0	11.5	10.0
1	80	water	1.0	0	0
3			12.0	6.0	1.0
6			22.0	2.0	0
10			20.0	3.5	0
1		pH 4.0	1.0	0	0

Table 18. Comparison of the Expansion and Stability of Foams from Different Jojoba Protein Concentrates. (20 mL 3% protein dispersions were blended for 12 Minutes)

Temp. C	Sample	Expansion mL	mL Foam at 30 min.	mL Foam at 120 min.
20	GOX treated	9	7	6
	SDI	14	11	10
	SDII	25	14	12
	Albumin	24	12	10
	Ovalbumin	7	4.5	1.5
55	GOX treated	14	3	2
	SDI	8	5	4
	SDII	27	20	18
	Albumin	24	4	4
	Globulin	19	14	12
	Ovalbumin	6	5	0.5

concentrate and albumin fraction had the greatest expansion, 125% and 120% respectively at room temperature, with 50% of the foam remaining after 2 hours. The GOX treated and the SDI had less expansion, 45% and 70% respectively at room temperature, but two hours later 70% of the foam remained. At 55C, the SDII and globulin fractions were the most stable, 135% and 90% expansion with retention of 67% and 63% of the foam after 2 hours. The GOX treated concentrate was less stable at 55C than at 20C. The acidic methanol (SDII) increased stability and expansion more than methanol and acetone (SDI) washed concentrates. This is in agreement with the results of Rahma and Narasinga Rao (1981) obtained with solvent-extracted sunflower meals.

The expansion was greater with water extract at 20C and salt extract at 55C. Since albumins have been reported to precipitate when denatured by heat applied in an aqueous environment, there may be some denaturation at 55C causing the foamability to decline in comparison to the globulin concentrate In previous studies 80C was needed for precipitation of albumin, but apparently longer time at lower temperatures are also effective. The globulins increase solubility at higher temperatures, since their denaturation temperature is higher.

The last experiment repeated the tests of Huffman, Lee and Burns (1975) with the SDII concentrate, at both 20C and 55C. The expansions were all 125% except for those of sucrose and tartrate, which were only 110%, and the retention was 44.5% to 48% of the foam volume at 20C (Table 19). At 55C, the expansion ranged from 110% to 160%, with retention at 2 hours of 52% to 68% of the foam volume. The potassium acid tartrate (KHT) decreased the expansion while retaining stability, while sucrose increased expansion and decreased stability.

From the results of these experiments, it appears that jojoba protein concentrates can be used for food applications requiring a stable foam. The environment of foam preparation dictates the concentrate and optimization conditions for successful use of the foams in whipped toppings, and desserts. Although the foaming properties are not as good as those reported for beans by Sathe and associates, they are comparable to egg albumin and soybean proteins, which are already being successfully used.

Conclusions

Jojoba pressed meal was a rich source of protein. Five concentrates were investigated for their functional properties. The results indicated that all but the

Table 19. Effect of Additives on Expansion and Stability of Foams From Jojoba Protein Concentrate SDII.

Temp. C	Additive	Expansion mL	mL Foam at 30 min.	mL Foam at 120 min.
20	1 gm salt	25	12	11
	0.26 gm KHT	25	15	12
	10 gm sucrose	25	14	12
	sucrose & KHT	22	11	10
55	none	27	20	18
	1 gm salt	27	15	14
	0.26 gm KHT	22	15	15
	10 gm sucrose	32	20	18
	sucrose & KHT	26	16	14

globulin had good gelation properties. The concentrates gelled preferentially at lower or neutral pH with 6% dispersions for SDI, GOX treated, and albumin, and with 8% dispersion for SDII. These results were comparable to those reported for other plant proteins.

Oil absorption ability was comparable to other plant proteins. The absorption ability of jojoba concentrates was less than that of sunflower proteins, better than that of soybean proteins, and similar to that of the Great Northern bean.

Water absorption / 5% NaCl absorption were independent of temperature (5C and 20C). For GOX treated concentrate the absorption was 2.0 to 2.2 gm/gm; for SDI, SDII, and albumin, between 1.0 and 1.6 gm/gm; and for globulins 0.6 to 0.7 gm/gm. The ability of the jojoba protein concentrates to absorb water, except for the globulins, were similar or slightly lower than other plant proteins, therefore, the ability to absorb water is not especially good.

Emulsification stability was greater at pH 4 and pH 7 than at pH 9. The stability and capacity were comparable to soy and sunflower concentrates. The order of emulsification capacity was:

SDI > SDII > GOX >albumin > globulin.

Foaming ability of jojoba protein concentrates was also comparable to that for other plant protein concentrates. The expansion was only 30% at room temperature with a 1% dispersion, with 25% of the foam remaining after 2 hours at room temperature. At 55C, and/or using 3% to 6% dispersions, the expansion could reach 150% with 70% of the foam remaining after 2 hours. The concentration was the single most important factor for foaming. The expansion and stability were greater at 20C for albumins, and at 55C for globulins. Addition of potassium acid tartrate, salt, and sugar and potassium acid tartrate to the dispersion either decreased slightly or had no effect on the expansion and/or stability, but sucrose slightly increased the expansion without affecting the stability at room temperature. Since the changes were not very large, jojoba pressed meal concentrates could be used in whipped food products.

From the data presented here, if the toxicity of the pressed meal could be eliminated from the protein concentrates, they have the potential of being utilized in formulated food products, since the functional properties are similar to those of soybean protein.

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