Characterization of Protein Concentrates of Jojoba (*Simondsia chinensis*) Meal[1]

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ABSTRACT

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Protein was extracted from mechanically pressed and hexane-extracted jojoba seed meal (*Simmondsia chinensis*), using four different procedures. These methods involved extracting protein with water, extracting protein with 0.15*M* NaCl, washing the meal with methanol/acetone (3:1) before extracting protein with water, or then rewashing this concentrate with methanol/1N HCl (98:2). The soluble protein content of the defatted meal was 20%. The above procedures recovered 18–65% of the total protein in the meal. Trypsin inhibitor activity and phytic acid concentrations were reduced to acceptable levels. Concentrations of simmondsin and polyphenolics were also significantly reduced.

Although oil from the jojoba seed, *Simmondsia chinensis*, is presently being used in cosmetics and lubricants, the pressed meal is of little value (Yermanos and Duncan 1976). The meal, which contains 25–30% protein, has toxic or antinutritive compounds that make it unsuitable for livestock feed (Yermanos and Duncan 1976, Cotgageorge et al 1978, Verbiscar and Banigan 1978). The toxicities of jojoba meal and of simmondsin, a cyanoglucoside, have previously been discussed (Booth et al 1974, Verbiscar et al 1980, Williams and Price 1980). Williams and Price (1980) indicated that simmondsin by itself may not be responsible for the deaths of laboratory mice fed the meal. Compounds other than simmondsin, including polyphenolics, phytic acid, and trypsin inhibitors, may be contributing to the observed weight loss (Glick and Joslyn 1970, Wheeler and Ferrel 1971, Liener 1977, Anderson et al 1979, Elias et al 1979). Because of these toxicities, it may be desirable to concentrate jojoba protein free from simmondsin, polyphenolics, and other potentially toxic compounds. The purpose of this investigation was to characterize properties of various protein concentrates prepared from the jojoba meal.

MATERIALS AND METHODS

Preparation of Protein Concentrates

A pressed cake (meal) was obtained from a commercial jojoba screw press operation and extracted with hexane until the solvent was no longer colored. The defatted meal was spread out in trays to dry overnight at 20° C. The defatted meal was subsequently ground with a hammer mill through a U.S. no. 60 mesh screen. Protein concentrates were obtained by extracting the defatted meal with solvents (1:20, w/v) at 20° C as shown in Figure 1. The water concentrate was water-extractable protein; the salt concentrate was protein extracted with 0.15*M* NaCl; the concentrate termed SDI (spray-dried concentrate I) was water-extractable protein obtained after washing the meal with methanol/acetone (3:1); and the concentrate termed SDII was the SDI concentrate rewashed with a solution of methanol and 1*N* HCl (98:2). The extractions were either lyophilized or spray dried (inlet temperature 200° C and outlet temperature 80° C) in a Niro mobile minor atomizer (Niro, Copenhagen, Denmark). Extractions were initially done in triplicate using 100-g samples and later with 1,000-g samples of defatted meal. All concentrates from the same extraction procedure were combined. All solvents were ACS certified or reagent grade, and all chemicals were reagent grade.

Protein Analysis

The protein content of the protein concentrates was determined using biuret method (AOAC 1980) with bovine serum albumin as a standard. All concentrates were analyzed in triplicate using 10-mg samples. Absorbance was read at 540 and 650 nm using a Spectronic 20 spectrometer after the reagent and protein reacted for 30 min.

Amino Acid Analysis

Amino acid content of the concentrates was determined using a Beckman model 121 amino acid analyzer. Samples of 100 mg protein concentrates combined with 100 mg thioglycollate were digested with 25 ml of 5.6*N* HCl in a 250-ml round bottom flask, and autoclaved for 17 hr at 250° C, 20 psi. The digests were evaporated to dryness on a rotary evaporator, reconstituted to exactly 10 ml with 0.02*M* citrate buffer (pH 2.2), and filtered through Whatman No. 50 paper.

Simmondsin Analysis

Simmondsin was quantified by high-performance liquid chromatography using a μ-Porasil C18 column (Whatman, 300 × 4.6 mm) eluting with methanol/water (25:75) at a flow rate of 1.5 ml/min and detected at 217 nm. The meal and protein concentrates were extracted directly with water, filtered through sintered glass fiber filters, concentrated on a rotary evaporator, and 10 μl applied to the column. Standards using simmondsin (0.1–5.0 mg/ml) purified by Williams and Price (1980) were run before and after

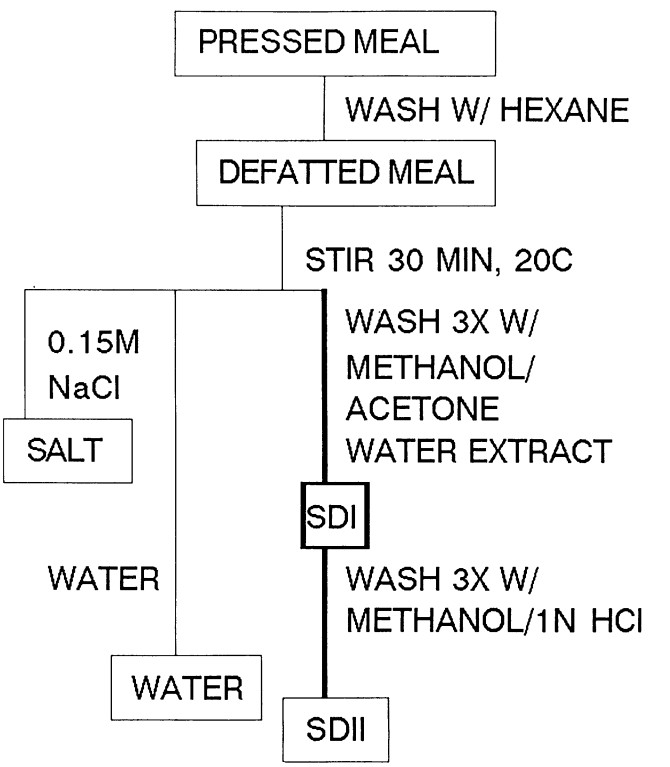

Fig. 1. Outline of the methods used for isolating protein from jojoba pressed meal.

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each sample. Alternatively, the samples were run using a thin layer chromatography procedure (Cotgageorge 1978).

Polyphenolic Compound Analysis

Polyphenolics were quantified by the method of Price and Butler (1977) modified by using 20-mg samples in 3 ml of methanol, and 1 ml of each 0.1*M* ferric chloride in 0.1*N* HCl and 0.008*M* potassium ferricyanate. Absorbance at 720 nm was read using a Spectronic 20 after 10 min of reaction. A standard curve using 10–1,000 μg of gallotannic acid was used.

Trypsin Inhibitor Analysis

Trypsin inhibitors were quantified using the method of Kakade et al (1974) as modified by Chang and Tsen (1979), using *N*α-benzoyl-DL-arginine-*p*-nitroanilide-HCl as the substrate.

Phytic Acid Analysis

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

Chromatography

The jojoba protein concentrates were applied to a Ultrogel A6 gel filtration column (90 × 2.6 cm i.d.). The column was eluted with 0.05*M* sodium phosphate buffer (pH 8) and monitored at 280 nm.

RESULTS AND DISCUSSION

Protein Content

The purpose of this study was to develop a method of increasing the concentration of jojoba protein while decreasing the concentration of simmondsin and polyphenolics. Preliminary work indicated that maximum extractable protein from defatted pressed jojoba meal was obtained in a pH 8.5 solution with a low ionic strength (< 0.2*M* KCl or NaCl), and the minimum solubility was in a solution near pH 4 with high ionic strength (> 0.5*M* KCl or NaCl). However, the traditional isolation procedures of first solubilizing the protein at alkaline pH and then precipitating the protein using 1*N* HCl to adjust the pH to 4–5 or using isopropanol were not useful because the simmondsin and polyphenolic compounds were coextracted.

The protein was extracted at a neutral pH because hydrolyzable tannins, if present, would be less strongly bound to protein (Harborne and Van Sumere, 1975). First, the proteins were extracted with either water or 0.15*M* NaCl. As the protein content was higher with water extraction than with 0.15*M* NaCl, the water-extracted protein concentrate was treated further. Table I shows the protein content of the concentrate and the amount of protein extracted from the meal using both the biuret and sum of the amino acids from the profile.

TABLE I
Protein Content and Yield for Jojoba Protein Concentrates

Sample[a]	Protein/ Concentrate (g/g)	Concentrate/ Meal[b] (g/g)	Protein/ Meal[c] (g/g)
Meal	0.195[d]	1.00	0.195
Salt	0.376[d]	0.57	0.214
	0.223[e]	0.57	0.127
Water	0.596[d]	0.70	0.417
	0.275[e]	0.70	0.193
SDI	0.559[d]	0.47	0.263
	0.372[e]	0.47	0.175
SDII	0.220[d]	0.45	0.099
	0.385[e]	0.45	0.173

[a] Salt = protein extracted with NaCl, water = water-extractable protein, SDI = water-extractable protein obtained after washing meal with a methanol/acetone solution, SDII = SDI concentrate after washing with a methanol/HCl solution.
[b] Mean of duplicate samples.
[c] Determined mathematically using means.
[d] Mean of triplicate samples using the biuret assay.
[e] Mean of duplicate samples. Sum of all amino acids determined by the amino acid analyzer.

Polyphenolic Compounds

Tannins are the polyphenolic compounds of interest because they bind and precipitate proteins; however, it is much easier to quantify the total content of polyphenolic compounds. Using the Prussian blue assay (Price and Butler 1977), the levels of polyphenolic compounds were lowest in the protein concentrates washed with the methanol and acetone solution or with methanol and 1*N* HCl (Table II).

The level of tannins in the diet that is toxic has not yet been determined. Sorghum, which contains 30 mg/g catechin equivalents using the vanillin test, is consumed by animals on a regular basis, so this level is assumed to be safe for consumption (Tan et al 1983). Because the level of polyphenolics in jojoba protein concentrates is much lower than 30 mg/g, they are probably of little nutritional importance.

In addition to quantification of polyphenolics, tannins are also quantified. Tannins were separated and purified from methanol and acetone extracts of the defatted pressed meal using the method of Hagerman and Butler (1978). The results indicated that only trace amounts of tannins were present.

Simmondsin

The higher concentration of simmondsin in the water-extracted concentrate than in the pressed meal (Table II) indicated that the simmondsin was coextracted with the protein. Since simmondsin is also soluble in methanol and acetone, they both removed simmondsin. Simmondsin comprised approximately 85% of the methanol-extracted solids and 90% of the acetone-extracted solids. Twenty four times as much simmondsin was extracted with methanol as with acetone. This confirmed the solubility studies done by Cotgageorge et al (1978), who found that water and methanol removed simmondsin more effectively than other solvents.

Trypsin Inhibitor Content

The third factor investigated was trypsin inhibitor. Both Samac et al (1980) and NgouNgoupayou (1982) reported trypsin inhibitor (TI) activity in jojoba. Samac et al (1980) found trypsin, chymotrypsin, and pepsin inhibitors in germinating seeds and in the albumin fraction of commercially pressed jojoba meal, although trypsin inhibitor activity decreased after germination. The activity, measured in trypsin inhibitor units (TIU), for the meal used in this study (Table II) agrees with NgouNgoupayou (4.5 TIU/g average, 3–6 TIU/g untreated deoiled meal, and 0.12–0.72 TIU/g in the albumin fraction).

The water extraction procedure at ambient temperature extracts components with TI activity, however, if extraction is at 80° C, then the TI activity in the extract is reduced to below detectable levels. Since TI activity was reduced in the SDII concentrate, the methanol and 1*N* HCl washes either removed or inactivated the trypsin inhibitor.

Phytic Acid Analysis

The amounts of phytate in the concentrates were below

TABLE II
Concentration of Simmondsin, Polyphenolics, and Trypsin Inhibitors in Jojoba Pressed Meal and Protein Concentrates

Samples[a]	Simmondsin[b] (%)	Polyphenolics[c] (%)	Trypsin Inhibitor[c] (TIU/g)
Meal	5.0[d]	8.07 ± 0.076	4.5 ± 0.4
Salt	2.5[d]	2.13 ± 0.032	8.4 ± 1.0
Water	3.9[e]	1.51 ± 0.027	22.3 ± 0.8
SDI	1.7[e]	0.66 ± 0.022	22.5 ± 5.2
SDII	1.0[e]	0.20 ± 0.148	16.3 ± 4.9

[a] Salt = protein extracted with NaCl, Water = water-extractable protein, SDI = water-extractable protein obtained after washing meal with a methanol/acetone solution, and SDII = SDI concentrate after washing with a methanol/HCl solution.
[b] Mean of duplicate samples.
[c] Mean of triplicate samples ± standard deviation.
[d] Thin-layer chromatographic method.
[e] High-performance liquid chromatographic method.

detectable levels. Tan et al (1983) found 6.1–7.5 mg of phytate phosphate per gram of winged beans, a level comparable to that in soybeans which is not considered to be harmful. The decorticated meal and seed had lower levels, 0.07 and 0.10 mg/g, respectively. Because the phytate content of the jojoba protein concentrates appears to be very low, phytate should not decrease the availability of other absorbed nutrients.

Chromatography

The pressed jojoba meal proteins were probably denatured by the processing methods. Results not shown indicate that the major proteins separated by gel filtration at 20,000 and 18,000 daltons. Shah and Stegman (1983) reported that native jojoba seed proteins applied to sodium dodecyl sulfate gels ranged from 35.5 to 145 kilodaltons. This reported higher range indicates that the pressed meal proteins are denatured.

CONCLUSIONS

The objective of this study was to concentrate the protein in the pressed meal of jojoba seeds while removing simmondsin, polyphenolics, and trypsin inhibitors. The polyphenolic content was reduced in all of the concentrates, but most in the SDII concentrate (from 81 mg/g meal to 2 mg/g SDII concentrate). Simmondsin was reduced from 5% in the meal to 1.0% in the SDII concentrate. The trypsin inhibitor was initially high in the albumin fraction, but was successfully reduced by the solvent extraction.

Chromatography indicated that all of the protein concentrates were composed of low molecular weight protein molecules. Although the solvent treatments are known to denature protein, the heat and pressure treatments during commercial screw pressing of the meal may also be responsible for initially denaturing the proteins and therefore for the observed low molecular weight proteins.

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