Detoxified and Debittered Jojoba Meal: Biological Evaluation and Physical-Chemical Characterization

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ABSTRACT

A new methodology was established to remove the toxic compounds present in jojoba (*Simmondsia chinensis*) seed and meal. Jojoba meal and seed without testa were defatted with hexane and detoxified with 70% aqueous isopropanol, which removed 86% of the total phenolic compounds and 99% of the simmondsins (simmondsin and simmondsin 2-ferulate) originally present. It appears that simmondsin-2-ferulate is the major bitter principle and that the conjugation of simmondsin with ferulic acid results in a combined bitter and toxic response. Protein from detoxified, debittered jojoba flour was comprised of three fractions: water-soluble (61.8%), salt-soluble (23.6%), and alkaline-soluble fractions (14.6%). The nitrogen solubility curves showed isoelectric points of pH 3.0 for the water- and salt-soluble fractions and pH 4.5–5.0 for the alkaline-soluble fraction. All fractions had maximum solubilities at pH 7.0. The detoxified and debittered jojoba meal and flour showed improved palatability and caused no deaths of experimental rats. Net protein ratio values obtained for diets containing the detoxified and debittered jojoba products were not significantly different from the casein control. The methodology reported here offers a viable solution for the elimination of toxic and bitter simmondsins and phenolics from jojoba meal or seeds.

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The jojoba (*Simmondsia chinensis* [Link] Schneider), native to the arid zones of the United States and northern Mexico, produces a seed that contains a liquid wax (jojoba oil) useful in cosmetics, pharmaceutical products, and industrial lubricants (Yermanos 1974). The meal remaining after oil extraction contains about 25% protein and could be of interest in animal feed formulations (Wells 1955).

The presence of a family of cyanoglucosides, the simmondsins, which are highly toxic to rats, mice, chickens, rabbits, and sheep, has prevented direct utilization of the meal (Booth et al 1974, Weber and Reid 1975). The simmondsins have an LD_{50} for rats of 4 g/kg (Booth et al 1974), but the toxicity of the simmondsin conjugate simmondsin-2-ferulate has not been determined. It has been hypothesized that the death and weight loss of rats fed jojoba meal is not due solely to the simmondsins. Phenolic compounds, a trypsin inhibitor, and phytic acid are some of the compounds that have been proposed to be toxic constituents (Storey et al 1983, Wiseman 1983). It has also been reported that animals discriminate against the rations containing jojoba meal (Ngoupayou et al 1982, Ngoupayou et al 1985, Verbiscar et al 1980, Verbiscar et al 1981), and the presence of a bitter principle has been postulated (Verbiscar and Banigan 1978, Verbiscar et al 1981).

Phenolic compounds, which may impart bitter and astringent tastes, have poor solubility in either pure water or solvents, whereas aqueous mixtures are efficient extraction media (Joslyn and Goldstein 1964, Garcia-Prado and Trejo-Gonzalez, *unpublished*). We report the elimination of toxic and bitter components of jojoba flour and meal with aqueous isopropanol. The physical-chemical characterization and biological evaluation of the detoxified and debittered products are presented.

MATERIALS AND METHODS

Defatting Jojoba Products

Jojoba seeds were obtained from a commercial plantation on the Coast of Hermosillo, Mexico. Both seeds and press cake were used in this study. Seed testa were removed manually after a vapor heat treatment, and the seeds were dried at 45°C. Seeds without testa were ground in a Waring Blendor with hexane (1:6 w/v), extracted for 24 hr in a Soxhlet apparatus, and air-dried for solvent removal. The resulting defatted product is called "jojoba flour". The press cakes were obtained from commercially screw-pressed jojoba seeds and contained 10% residual oil. They were defatted in the same way as the seeds without testa and called "jojoba meal."

Detoxification

Defatted flours and meal were extracted three times with aqueous solutions of isopropanol (10–90%, v/v). The suspensions were stirred for different periods (2–24 hr) at room temperature, after which the supernatants and residues were analyzed for simmondsin and total phenolic substances, and the residues were analyzed for macrocomponents. Simmondsin and simmondsin-ferulate were determined semiquantitatively by thin-layer chromatography with detection of spots under ultraviolet light at 254 nm as described by Verbiscar and Banigan (1978). This method has a detection limit of 0.5 μg simmondsin-ferulate. Total phenolics in the isopropanol extracts were determined by the Folin-Denis colorimetric method using tannic acid as a standard (Joslyn and Goldstein 1964).

Macrocomposition

Analyses of lipid, nitrogen, ash, crude fiber, and nonprotein extract were carried out according to the methods of the AOAC (1984). Protein was calculated with a 5.30 conversion factor, an appropriate factor for oil seeds (Southgate 1984). Carbohydrates were determined by difference. Gross energy content was determined in a Parr adiabatic calorimeter.

Protein and Carbohydrate Fractions

The isolation of protein and carbohydrate fractions according to solubility was carried out by suspending defatted flour in 70% isopropanol (1:30, w/v) at 20°C with agitation during 24 hr. The suspension was subsequently dialyzed at 4°C against three changes of deionized water during 24 hr to eliminate phenolic compounds that would otherwise interfere in the colorimetric determination of jojoba protein (Medina-Juarez 1986, Wiseman and Price 1987). The suspension was centrifuged at 6,000 rpm for 10 min, and the albuminlike fraction was determined in this supernatant. The globulinlike fraction was extracted from the residue with sodium phosphate buffer (pH 7) containing 0.1*M* NaCl in a volume 20 times the weight of the suspension at 20°C for 30 min. After centrifugation, the glutelin-like fraction was extracted from the residue with 50 m*M* borate buffer (pH 10), and the resultant residue was finally extracted with 18% NaOH to liberate the hemicellulosic fraction. Protein and carbohydrate content in each extract were determined by a modified Biuret (Chaykin 1966) and phenol-sulfuric (Dubois et al 1956) colorimetric method, respectively.

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TABLE I
Composition of Jojoba Products Before and After Detoxification with 70% Isopropanol

Component[a]	Meal		Flour		
	Untreated	Defatted Detoxifed	Defatted Untreated	Defatted Detoxified	Testa[b]
Protein, % (N × 5.30)	24.6 ± 0.7	27.5 ± 1.0	33.1 ± 1.0	42.4 ± 1.2	6.0 ± 0.5
Water, %	4.0 ± 0.5	4.0 ± 0.7	42. ± 0.5	2.5 ± 0.3	10.0 ± 1.0
Oil, %	10.0 ± 0.2	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
Crude fiber, %	9.5 ± 1.0	11.0 ± 1.2	8.0 ± 0.9	9.0 ± 1.2	63.3 ± 1.2
Ash, %	3.7 ± 0.5	4.0 ± 0.8	3.9 ± 0.6	4.1 ± 0.7	4.4 ± 1.0
Carbohydrates, %[c]	48.8	53.0	50.3	41.5	15.6
Energy, kcal/g[d]	...	...	4.6 ± 0.1	4.9 ± 0.1	...
Simmondsin, %[e]	4.6 ± 0.7	ND[g]	6.2 ± 0.7	ND[g]	0.3 ± 0.1
Simmondsin-2-ferulate, %[e]	1.8 ± 0.3	ND[g]	0.9 ± 0.3	ND[g]	0.1 ± 0.1
Phenolics, %[f]	...	0.14 ± 0.04	1.12 ± 0.12	0.16 ± 0.03	6.50 ± 0.01
Bitterness[h]	+	−	+	−	+

[a] Dry weight basis unless otherwise specified; values are means of triplicate samples ± standard deviation.
[b] Analysis of testa alone; constitutes 15% of seed by weight.
[c] By difference.
[d] Parr adiabatic calorimeter.
[e] Thin-layer chromatography (TLC) method.
[f] Folin-Denis method.
[g] Not detected by TLC; limit of detection 0.5 μg.
[h] Presence or absence of bitterness detected by tasting a few milligrams of the product.

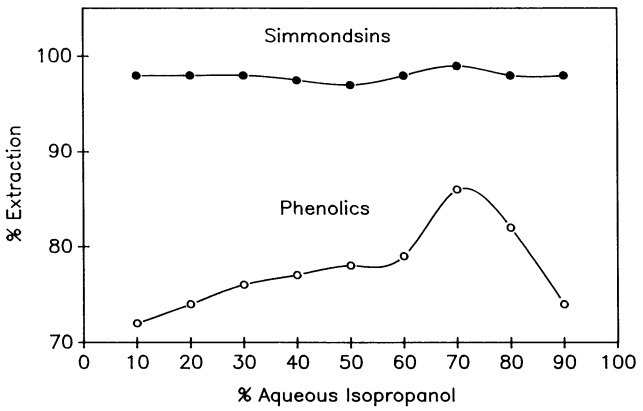

Fig. 1. Extraction of simmondsins and phenolics from defatted jojoba meal treated with isopropanol-water mixtures.

Phenolic Compounds

Separation of phenolic compounds by column chromatography was carried out using insoluble polyvinylpyrrilidone (PVP) as described by Vargas-Estrada (1976). After column preparation by washing with water and 0.1*M* glycine-HCl, pH 2.5 buffer, the phenolics in the isopropanol extract were eluted with the following sequence buffers in 10% methanol: 0.1*M* glycine-HCl, pH 2.5; 0.2*M* acetate, pH 3.5; 0.2*M* acetate, pH 5.0; and Na citrate-HCl, pH 6.0. Fractions of 10 ml were collected, combined, and the absorption spectra of each peak were determined from 230 to 450 nm (Harborne 1984).

Biological Evaluations

Biological evaluations of flour and meal were conducted with 70 rats (Sprague Dawley), 25 days old with initial weights between 35 and 45 g, placed in individual stainless steel cages under controlled conditions of 22–23°C, 55–56% rh with 12 hr each of light and darkness. Fresh rations and water were provided ad libitum and the rats were weighed every other day.

The basal diet consisted of 8.0% cottonseed oil, 1.0% Premix Vitamin, and 5.0% Premix Minerals (BioServ, Inc., New Jersey), 1.0% cellulose, 5.0% water, and 0.2% chromate. Protein was provided by casein or jojoba products (meal or flour) as 10% by weight, and the diet was completed to 100% by addition of starch and dextrose. Oil, mineral, cellulose, and water were adjusted by addition of these components with the jojoba flour or meal according to the official protocol (AOAC 1984). The diets conformed to the nutrient requirements as published by the National Research Council committee (1978).

Net protein ratio (NPR) was determined by the method of Bender and Doell (1957) and included a group of animals that received the basal diets and a group with an N-free diet.

The biological evaluations were carried out in a completely randomized design with 10 replicates per treatment. The results were analyzed by one-way analysis of variance, and means were separated by the Student-Newman-Keuls multiple range test (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Simmondsin and Phenolic Extraction

Most detoxification studies on jojoba have focused on the extraction or transformation of simmondsin as the principal toxic constituent. Other components may contribute to the toxicity and unpalatability of jojoba seed and meal. Phenolic compounds may impart astringency and bitterness (Ozawa et al 1987), and Wiseman and Price (1987) reported an 8% polyphenolic content in defatted jojoba meal. In the present study, the testa contained 6.5% phenolics, whereas the seed without testa contained only 1.1% (Table I). Elimination of the testa therefore substantially reduced the phenolic content of resultant jojoba products.

Aqueous mixtures of isopropanol (10–90%) were tested for extraction of phenolics as well as simmondsins. The most effective mixture for removing phenolics was 70% isopropanol (Fig. 1). This same concentration was found effective in removing 92% of the cinnamic acid derivatives of sunflower meal (Garcia-Prado and Trejo-Gonzalez, *unpublished*). The simmondsins were extracted at levels of 97–99% with the range (10–90%) of isopropanol concentrations tested. The most effective extraction conditions with 70% isopropanol were achieved with a meal-to-solvent ratio of 1:30 over 4 hr. Extraction ratios of 1:20 and 1:10 removed 94 and 90% of the simmondsins, respectively.

The simmondsin content of jojoba meal treated with various solvents and procedures is compared in Table II. Simmondsins are most effectively extracted with the more polar solvents, exemplified by the differences in extractability with pure and 70% isopropanol. Isopropanol is less deleterious to protein denaturation than methanol (Garcia-Prado and Trejo-Gonzalez, *unpublished*). Although water quite effectively extracts simmondsins, it is not efficient in removing phenolic acids.

PVP chromatography corroborated the phenolic nature of the compounds extracted in aqueous isopropanol (Fig. 2). Ultraviolet spectra before chromatography showed broad absorption maxima at 220, 263, 271, 300, and 322 nm. Chromatography resulted in better resolution of peaks and the appearance of two new

ones at 314 and 325 nm, the former probably due to ferulic acid. All phenolic peaks were eluted between pH 2.0 and 3.5.

Composition of Jojoba Products

Weight loss of the flour treated with 70% isopropanol was 36.5%. The macrocomponents of untreated and detoxified meal and flour are shown in Table I. For both products, detoxification with 70% isopropanol removed all detectable simmondsin and simmondsin-2-ferulate. A large proportion (86%) of the phenolic compounds were also removed in the detoxified flour with this treatment. The testa constituted 15% of total seed weight and contained 6.5% phenolic compounds, a major portion being anthocyanins (data not shown). The manual removal of the testa significantly reduced the initial phenolic content of the flour. The simmondsin content of the testa was low in comparison to that of the meal and flour (Table I).

Protein and Carbohydrate Solubility

Phenolic compounds interfere with the determination of protein by colorimetric methods. The removal of the major portion of phenolics by 70% isopropanol permitted the determination of jojoba proteins according to their solubility profiles (Fig. 3). The isoelectric points of the three protein fractions were well defined and occurred at pH 3.0 for the water- and salt-soluble fractions, and at pH 4.8 for the alkali-soluble fraction. The region of maximum protein solubility was between pH 6 and 8. The quantitative distribution of protein and carbohydrates showed that the highest percentage was solubilized in the water fraction (Table III). Extraction of the residue with 18% NaOH resulted in a high amount of carbohydrate, presumably hemicellulosic in nature. No starch was detected in the jojoba flour.

Biological Evaluations

The effects of different jojoba diets on food consumption, weight change, and rat mortality are shown in Table IV. Both the untreated meal and flour were consumed in low amounts and were highly toxic, resulting in 100% mortality by four and nine days, respectively. Both detoxified products resulted in high food consumption and weight gains by 12 and 14 days. In the case of partially detoxified flour, in which less than 5% of the original simmondsin was present, mortality was 50% after 12 days, and weight gain was less than one-fourth that of completely detoxified flour (Table IV). Food consumption was also significantly less than for completely detoxified flour.

The unconjugated simmondsins are tasteless (Verbiscar et al 1981). During the first hours of the feeding trial it was observed that the rats stopped eating the diets containing defatted jojoba meal and flour, suggesting that they were unpalatable. The rejection of jojoba-supplemented diets was observed in previous studies with calves, sheep, and rats, in which the animals separated out and avoided consuming jojoba particles (Verbiscar et al 1981).

Many phenolic compounds have bitter and astringent tastes (Van Sumere et al 1975). We hypothesize that the elimination of phenolic compounds and simmondsins with 70% isopropanol improved the acceptability of the products as well as detoxifying them. We sampled the defatted and defatted detoxified jojoba products and noted a lack of bitterness in the latter (Table I). We also tasted the pure compounds simmondsin, simmondsin-ferulate, and ferulic acid, alone and in combination, confirming the tasteless character of simmondsin and the bitter flavor imparted by the simmondsin-ferulate and ferulic acid. In addition, an enhanced bitterness was detected when ferulic acid was added to simmondsin or simmondsin-ferulate.

Our results corroborated previous suggestions (Verbiscar and Banigan 1978, Verbiscar et al 1981) that the mortality of rats fed jojoba diets was due not only to the toxic simmondsins, but also to the presence of bitter principles, probably phenolic compounds and conjugates. These make the diets unpalatable, resulting ultimately in starvation and death.

The NPR methodology permits the evaluation of low-quality

TABLE II
Residual Content of Simmondsins in Jojoba Products Detoxified by Various Methods

Treatment	Simmondsin (%)	Simmondsin-2-Ferulate (%)	Protein, % (N × 6.25)
Methanol[a]	0.05	0.01	28.2
Acetone[a]	0.63	0.05	31.3
Isopropanol[a]	2.90	0.40	32.2
Water, pH 3.2[a]	Trace	0.43	22.5
Water[b]	0.10	0.08	29.0
Heat, 135°C, 15 hr[a]	0.27	0.18	26.7
Ammonia[a]	0.19	0.03	40.9
H_2O_2-Ammonia[a]	Trace	0.03	32.4
Fermentation[c]	0.13	0.24	29.5
Isopropanol (70%)[d]	ND	ND	31.7[e]

[a] High-performance liquid chromatography (HPLC), Verbiscar et al 1980.
[b] Thin-layer chromatography (TLC), Medina and Yeomans 1982.
[c] HPLC, Verbiscar et al 1981.
[d] Not detected (ND) by TLC; limit of detection = 0.5 μg.
[e] Calculated as N × 5.30.

TABLE III
Distribution of Proteins and Carbohydrates of Detoxified Jojoba Flour According to Solubility Properties[a]

Fraction	Protein (g)		Carbohydrate (g)	
Soluble in water	1.57	(61.8%)	0.73	(38.3%)
Soluble in 0.5*M* NaCl, pH 7.0	0.60	(23.6%)	0.12	(6.2%)
Soluble in borate buffer, pH 10.0	0.37	(14.6%)	0.35	(18.0%)
Soluble in 18% NaOH	···	···	0.72	(37.4%)
Total	2.54	···	1.92	···

[a] Based on a 10.0-g sample of defatted jojoba flour in which protein (N × 5.30) = 33%.

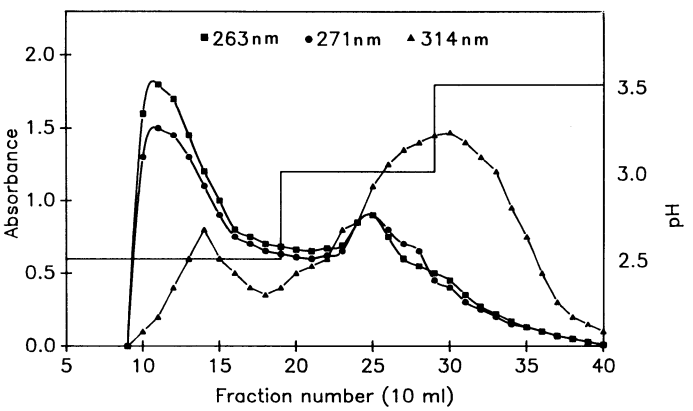

Fig. 2. Polyvinylpyrrilidone column chromatography of the 70% aqueous isopropanol extract of jojoba meal.

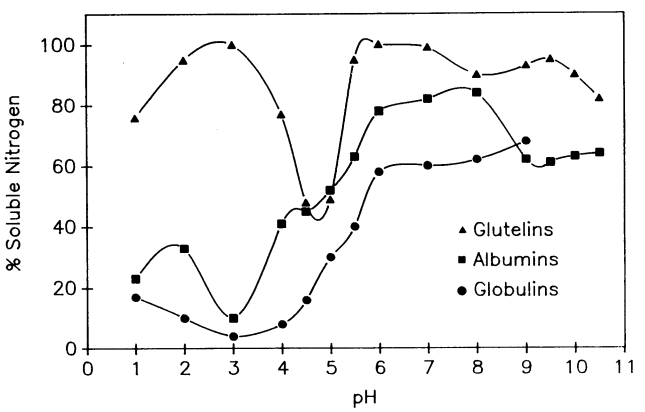

Fig. 3. Solubility of proteins from detoxified jojoba flour as a function of pH.

TABLE IV
The Growth and Mortality of Rats Fed Diets Containing Defatted and Detoxified Jojoba Meal and Flour[a]

Product in Diet/ Days	Toxic Compounds[b] (%)	Weight Change (g)	Food Consumed (g)	Mortality (%)
Defatted meal[c]				
1	2.40	...	...	20
4	2.40	−10.1 ± 1.2	11.5 ± 2.0	100
Defatted flour[d]				
4	1.82	−5.0 ± 0.9	14.6 ± 3.2	20
9	1.82	−7.0 ± 1.7	26.3 ± 3.0	100
Partially detoxified flour[e]				
9	0.10	−0.1 ± 0.7	39.0 ± 3.2	30
12	0.10	3.5 ± 1.0	59.0 ± 5.5	50
Detoxified meal[f]				
12	ND[g]	10.8 ± 3.4	82.1 ± 10.2	0
Detoxified flour[f]				
14	ND[g]	16.8 ± 3.0	97.3 ± 2.7	0

[a]Jojoba products prepared from a seed lot different from that analyzed in Table I.
[b]Simmondsin and simmondsin-2-ferulate.
[c]Defatted jojoba meal from expeller.
[d]Hexane-defatted jojoba flour.
[e]Hexane-defatted and detoxified in solvent/flour of 20:1.
[f]Hexane-defatted and detoxified in solvent/meal or flour of 30:1.
[g]Not detected by thin-layer chromatography; limit of detection of 0.5 μg.

TABLE V
Net Protein Ratio (NPR) and Relative NPR of Flour and Meal of Jojoba

Diet	NPR[a]	Relative NPR
Casein[b]	3.40 ± 0.70	100.0
Detoxified jojoba meal[c]	2.74 ± 0.23	80.6
Detoxified jojoba flour[c]	2.83 ± 0.50	83.2

[a]There were no significant differences between diets at the 5% level.
[b]NPR calculated at 12 days according to Bender and Doell 1957.
[c]NPR calculated at 14 days according to Bender and Doell 1957.

protein sources such as jojoba, which lacks sulfur-containing aminoacids (Bender and Doell 1957). The NPR was calculated from the average weight loss (12.6 g) of the rats fed a diet free of nitrogen at 14 days. The results (Table V) show no significant differences between values for detoxified flour and meal and the casein control diet. The relative NPR values for meal and flour were also similar.

CONCLUSIONS

A new method is described to detoxify and debitter jojoba seed and meal by simultaneously eliminating the compounds responsible, simmondsins and phenolics. The removal of the testa from the jojoba seed eliminates a major source of phenolic compounds. The remaining phenolics can be effectively extracted using the 70% aqueous isopropanol found to be very efficient in the extraction of the toxic simmondsins. The resultant products have improved acceptability and nutritive value. Removal of phenolics permitted the demonstration of characteristic solubility patterns of the protein fractions. The elimination of the simmondsins and phenolic compounds now makes possible the utilization of the protein in the jojoba press cake as a concentrate or isolate to supplement feed rations.

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