Functional Properties of Protein Concentrates from Pressed Jojoba Meal¹

MEGANNE O. WISEMAN^{2,3} and RALPH L. PRICE²

ABSTRACT

Cereal Chem. 64(2):94-97

Seeds from Simmondsia chinensis were commercially pressed and defatted with hexane. Protein concentrates were prepared from the oil-free meal by four methods: extracting protein with water; extracting protein with 0.15M NaCl; after washing the meal with methanol/acetone (3:1), extracting protein with water (SDI); rewashing SDI with a solution of methanol and 1N HCl (98:2) (SDII). These concentrates were evaluated for their functional properties of protein solubility, gelation, water absorption, oil absorption, and foaming. The protein concentrates were more soluble at alkaline pH than at neutral or acidic pH. Gelation required dispersions with at least 6% protein at neutral pH and at least 20% protein at alkaline

pH. Capacities for absorbing oil and water were similar to those of soy protein concentrates. The most stable foams were formed at pH 4. The foaming properties of SDII concentrate were further tested at 4, 37, 55, 80, and 110° C; and in the presence of sucrose, potassium acid tartrate, salt, and both sucrose and potassium acid tartrate. The foam capacity of protein dispersions held for 30 min increased as incubation temperature increased to 110° C. The additives did not affect the expansion capacity or stability of the foams. The capacity and stability of foams from jojoba protein concentrates were similar to those of egg albumin.

Jojoba, Simmondsia chinensis, is a shrub native to the Sonoran Desert. Jojoba seeds contain liquid wax with lubricating properties similar to sperm whale oil. Although the oil is used by the cosmetics industry, the pressed meal is an unused byproduct being kept in storage in hope that a use will be developed. Although the meal is toxic to rats and mice (Booth et al 1974), the protein from the protein-rich pressed meal (up to 25% protein) may be usable in feeds, food products, and industrial products. Recently, the proteins have been isolated and characterized (Cardoso 1980, Samac et al 1980, Wiseman and Price 1987).

Because plant proteins are less expensive sources of protein than animal proteins, they are used to fortify and to produce formulated food products with desirable functional properties. Egg proteins have good functional properties and are frequently used for comparison. In a previous paper (Wiseman and Price 1987), the procedures for preparing jojoba protein concentrates were described. In this paper, protein solubility, gelling, absorption capacities for oil and water, and foaming properties of jojoba protein concentrates are discussed.

MATERIALS AND METHODS

Protein Concentrates

The concentrates from pressed jojoba meal were prepared according to methods described in Wiseman and Price (1987). Four methods were used to extract protein: with water (water), with 0.15M NaCl (salt), with water after washing meal with methanol/acetone (3:1) (SDI), and by rewashing SDI protein concentrate with a solution of methanol and 1N HCl (98:2) (SDII). Egg albumin (crystallized, lyophilized, saltfree) was used for comparison in evaluating functional properties. The moisture content of the protein concentrates was approximately 12% in SDI and SDII and 6% in the water and salt extracts.

Protein Solubility

One-gram samples of the concentrates were individually dispersed in 10-ml phosphate buffers over the range of pH from 3 to 9. The samples were then centrifuged at $1,500 \times g$ in a clinical centrifuge for 10 min. Protein content of the supernatant was quantified using the biuret method (AOAC 1980). The protein content in the concentrate was multiplied by the yield of the concentrate from the pressed meal to standardize the value for protein content based on the meal.

Gelation

Gelling properties were evaluated by heating 10-ml samples of aqueous dispersions (6, 8, 10, and 20% protein), adjusting pH to 4, 7, or 9 with 1N HCl or 1N NaOH, and placing them in a boiling water bath for 10 min and cooling overnight at 4° C. Gel formation was evaluated by inverting centrifuge tubes containing the treated aqueous dispersions. Solidified samples were classified as gelled.

Water Absorption

Water absorption capacity was determined by modifying the method used by Sathe et al (1982a). Concentrates were oven-dried for 24 hr at 110°C. Approximately 0.5 g of dried protein concentrate was placed into 15-ml conical centrifuge tubes and mixed with 4.5 ml of distilled water. After incubating for 30 min at 20° C, the tubes were centrifuged for 30 min at $1,500 \times g$ on a clinical centrifuge, decanted, and reweighed. Water absorption capacity was calculated by dividing the weight of water absorbed by the weight of the protein concentrate.

Oil Absorption

Oil absorption capacity was determined using a modified procedure of Lin et al (1974). A 0.5-g dried protein concentrate sample was placed in a 15-ml conical centrifuge tube with 3 ml of corn oil, dispersed using a glass stirring rod for 15 sec, incubated for 30 min at 20° C, and centrifuged in a clinical centrifuge at 1,500 \times g for 20 min. Free oil was decanted into a 10 ml-graduated cylinder and the volume of oil was measured. The capacity was calculated by dividing the milliliters of oil absorbed by the weight of the protein concentrate.

Foaming

All experiments were done in duplicate using 20-ml samples of 1% protein dispersions (w/v) unless otherwise specified. The dispersions were blended on a Sorvall Omni mixer at medium speed and decanted into a graduated cylinder. The volumes of foam were recorded immediately after blending for 9 min (except in the experiment with the additives, which was blended for 12 min), and after standing at room temperature for 30 min and 120 min (Eldridge et al 1962).

This procedure was repeated for each of the following conditions. Protein from SDII was dispersed in phosphate buffers at pH 4, 7, and 9 and in water. Dispersions of SDII protein concentrate in water and in pH 4 phosphate buffer were incubated for 20 min or 16 hr at 4, 20, 37, 55, 80, and 110°C. Protein dispersions of SDII concentrate (1, 3, 6, and 10%) were incubated for 30 min at 20°C or at 55°C. Three percent protein dispersions of each concentrate were incubated for 30 min at 20 or 55°C and blended for 12 min. Reagent grade sucrose (5.0 g/0.5 g SDII concentrate), solt (0.5 g/0.5 g SDII concentrate), and sucrose and

Arizona Agricultual Experiment Station, Journal Article no. 3931.

 ²Department of Nutrition and Food Science, University of Arizona, Tucson 85721.
 ³Present address: Louisiana State University, Food Science Department, Baton Rouge 70803.

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KHT together were added to 3% protein dispersions in water, incubated for 30 min at 20 or 55° C, and blended for 12 min.

RESULTS AND DISCUSSION

The functional properties were investigated most thoroughly with the SDII concentrate because this concentrate had the lowest concentration of simmondsin, a toxic compound in jojoba, and polyphenolic compounds (Wiseman and Price 1987). The concentration of simmondsin must be reduced before jojoba meal or protein concentrates can be used in food or feed products.

Protein Solubility

The protein was more soluble at alkaline pH than at acidic or neutral pH for all the concentrates (Table I). Minimum solubility for all protein concentrates was in the acidic pH range except for the water extract. The protein solubility for the water extract was less at pH 7 but relatively constant over the acid and neutral pH range. The protein was more soluble in water than salt. Therefore, the water extract was further purified by using solvent washes. The SDI concentrate, which was washed with methanol and acetone, had the lowest solubility of all of the concentrates at acidic and neutral pH and good solubility in the alkaline pH region. The SDII concentrate, which was SDI concentrate rewashed with methanol/HCl, was the least soluble concentrate at neutral and alkaline pH.

Gelation

Gelling of protein depends on protein concentration, pH, balance of cations and anions, and the absence or presence of interfering substances. The results in Table II show that less protein was required for gelation at neutral and alkaline pH than at acidic pH. One possible explanation is that because the protein solubility is lowest in the acidic pH region, there may be insufficient dispersion of the protein for a network of bonds to form. The inability of the salt extract to gel may result from the presence of too many ions, which interfere with the formation of protein-protein bonds. The gelling ability of the concentrates was similar to those reported by Sathe and Salunkhe (1981) for the Great Northern bean.

	TABLE I	
Protein Solubility Profile	of the Jojoba Protein Concentr	ates ^a

Sample ^b	pH 3	рН 4	pH 5	рН 6	рН 7	pH 8	pH 9
Meal	0.140	0.132	0.126	0.195	0.216	0.236	0.240
Salt	0.090	0.098	0.114	0.125	0.141	0.177	0.205
Water	0.192	0.195	0.198	0.192	0.183	0.300	0.315
SDI	0.009	0.058	0.058	0.102	0.175	0.292	0.219
SDII	0.083	0.112	0.138	0.172	0.171	0.174	0.172

^a Results expressed as grams of protein per gram of meal soluble in 0.10*M* buffers at the specified pH.

^bSalt = 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.

TABLE II
Minimum Percentage of Protein Concentrate
Required for Gelation at pH 4, 7, and 9

	% Protein Concentrate				
Sample ^a	рН 4	рН 7	рН 9		
Salt	>20	>20	>20		
Water	> 20	6	6		
SDI	>20	6	10		
SDII	20	8	6		

^aSalt = 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.

Water Absorption

As shown in Table III, absorption of water by the protein concentrates generally increased as temperature increased from 20 to 55° C. At room temperature, water absorption values were much lower than those reported for the protein concentrates from winged bean (Sathe et al 1982b) and Great Northern bean (Sathe and Salunkhe 1981) but similar to sunflower and soybean protein concentrates (Lin et al 1974, Huffman et al 1975).

Oil Absorption

As emulsifiers, proteins must be able to associate with both water and oil. Table III compares the abilities of jojoba protein concentrates to absorb oil. The amount of oil absorbed by the protein decreased with increased purification. The water and salt extracts absorbed more oil than the SDI and SDII protein concentrates. The methanol and acetone washes used to remove simmondsin and polyphenolic compounds may be responsible for denaturing protein and changing the conformation and steric factors important for water and oil absorption. The seed protein concentrates reported by Cardoso (1980) absorbed more oil than the pressed meal protein concentrates in this study. Oil absorption capacities of jojoba protein concentrates were less than those for sunflower protein concentrates (Huffman et al 1975) but greater than those of soybean protein concentrates (Lin et al 1974).

Foaming

In preliminary experiments, the capacity of foams made from protein dispersions in the range of 1-6 g/100 ml varied directly with concentration. However, the variation in foam expansion among the protein concentrates was enhanced using 1-3% dispersions. Maximum foam expansion and stability of jojoba protein concentrates occurred between 9 and 15 min of whipping. Longer whipping times decreased the stability of the foam.

Foaming capacity and stability of protein depended on the environment of the protein (Table IV). Foams prepared from protein concentrates obtained from pressed jojoba meal were more stable at pH 4 but had greatest capacity in pH 7 buffer. Jojoba protein foams were less stable in water (pH 6.7) than in neutral pH

	ml Oil Absorbed/	g Water Absorbed/g of Concentra		
Sample ^a	g of Concentrate	at 5° C	at 20° C	
Salt	3.04	1.0	0.6	
Water	3.18		1.0	
SDI	1.66	1.4	1.0	
SDII	1.86	1.4	1.4	

 TABLE III

 Maximum Oil and Water Absorption of Jojoba Protein Concentrates

^aSalt = 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.

TABLE IV	
Comparison of Foaming Properties of Jojoba Protein Concentrates	

Temperature		Capacity	% Capacity Remaining After		
(° <u>C)</u>	Sample ^a	(%)	30 min	120 min	
20	Water	220	27.3	22.7	
	Salt	200	32.5	30.0	
	SDI	170	32.4	29.4	
	SDII	225	31.1	26.7	
	Ovalbumin	135	16.6	5.6	
55	Water	220	9.1	9.1	
	Salt	195	35.9	30.8	
	SDI	140	17.9	14.3	
	SDII	235	42.6	38.3	
	Ovalbumin	130	19.3	1.9	

^aSalt = 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. buffer, which was less stable than in pH 4 buffer. This agrees with the studies reported by Cherry and McWatters (1981) which indicated that foam capacities and stabilities of protein dispersions in the presence of ions (buffers) are increased compared to those of protein dispersions in unbuffered water. Whereas the capacity of foams may increase in the presence of ions, their stability may be decreased by aggregation or coagulation of protein. This may explain the instability of the foams in pH 7 and pH 9 buffers.

The effect of temperature on foaming capacity was tested in water. The foams from the SDII and salt extract were more stable at 55° C than at 20° C (Table IV). Protein concentrate dispersions incubated for only 30 min expanded more and were more stable as temperature increased from 4 to 80° C. After 2 hr, 14% of the capacity of the SDII protein concentrate foam incubated at 55° C remained compared to 6% or less of the capacity of dispersions incubated at 20° C (Table V). The increased stability at higher incubation temperatures may have resulted from increased protein solubility. However, prolonged incubation at elevated temperatures may cause denaturation of the protein, which can result in aggregation of the protein molecules obstructing the necessary water protein interactions needed for foaming.

Optimum foaming properties vary with the source of protein, method of isolation, temperature, pH, mixing time, and protein concentration. For example, Eldridge et al (1962) reported stable foams from alkali-leached soy protein concentrate at 20° C, but temperature for maximum foaming capacity depended on the method of isolation. In addition, these foams of soy protein concentrates have maximum stability at pH 5 and maximum foaming capacity in pH 7 buffer. Sathe et al (1982a) reported that maximum foam capacity for lupin protein concentrates is at pH 2 and maximum foam stability is at pH 4. Maximum foaming capacity for sunflower seed proteins is at pH 4 and 15° C, but

TABLE V Effect of pH and Temperature on Capacity and Stability of Foams from SDII Jojoba Protein Concentrate^a

	Capacity	% Capacity Remaining After		
Condition	(%)	30 min	120 min	
pH 4.0	140	14	11	
7.0	180	11	0	
9.0	150	17	0	
Water	130	10	6	
Temperature (°C)				
4	130	8	0	
20	130	10	6	
37	130	12	6	
55	143	16	14	
80	160	25	17	
110	175	3	3	

^a SDII concentrate prepared by washing meal with with methanol/acetone and rewashing the water-extractable protein obtained with methanol/HCl.

 TABLE VI

 Effect of Additives on the Foaming Properties of SDII Protein Concentrate^a

Temperature	Additive	Capacity	% Capacity Remaining After		
(° C)		(%)	30 min	120 min	
20	None	225	31.1	26.7	
	Salt	225	26.7	24.4	
	Sucrose	225	31.1	26.7	
	КНТ⁵	225	33.3	26.7	
	Sucrose				
	+ KHT	210	26.2	23.8	
55	None	235	42.5	38.3	
	Salt	235	31.9	29.8	
	Sucrose	260	38.5	34.6	
	KHT	210	35.7	35.7	
	Sucrose				
	+ KHT	230	34.8	30.4	

^a SDII concentrate prepared by washing meal with methanol/acetone and rewashing the water-extractable protein obtained with methanol/HCl. ^b KHT = Potassium acid tartrate. maximum stability is at pH 9 (Huffman et al 1975).

Foams of jojoba proteins washed with acidic methanol (SDII) had greater capacity and stability than SDI foams made from concentrates washed with methanol and acetone alone (Table IV). Rahma and Narasinga Rao (1981) reported this same increase in stability and capacity for sunflower seed protein concentrates. They found that alcohol decreases capacity but increases stability, and acid by itself decreases both stability and capacity. A possible explanation is that acid and alcohol together cause permanent unfolding of the protein permitting more protein-water interactions than when either denaturant is used by itself.

Because food foams are rarely made without other ingredients, the protein solution was modified by the addition of additives frequently found in whipped products. KHT keeps the protein molecules unfolded, which stabilizes the foam by increasing the number of possible air/protein interactions. Sucrose stabilizes foams by reducing water activity. Low concentrations of salt can increase protein solubility, potentially increasing the expansion of the foam. However, large concentrations of salt can unfold the protein. The combination of the three additives should improve both capacity and stability of foams.

KHT decreased foaming stability of SDII protein concentrate at both 20 and 55° C and capacity at 55° C; sucrose maintained both the capacity and stability (Table VI). All other additives decreased either stability, capacity, or both, but these effects would not prevent the use of pressed jojoba meal concentrates from use as foaming agents.

Similar results are reported in the literature. Two studies used salt as an additive. Eldridge et al (1962) reported that stability of foams from soy protein concentrates was decreased in the presence of salt, but capacity was unchanged. Huffman et al (1975) also reported decreased stability, although capacity increased for foams made from sunflower protein concentrates.

Decreased expansion and stability of the SDII protein concentrate with both KHT and sucrose indicated that other factors were involved. The KHT needs to interact with the protein before the addition of sucrose because sucrose will decrease the available water needed for KHT to act as a surfactant and for protein-water interactions in foams. If KHT and sucrose are added simultaneously the capacity and stability would be expected to decrease, as was observed.

In contrast, Eldridge et al (1962) reported that protein foams increase in the presence of sugar and KHT, but decrease in the presence of either one alone. Huffman et al (1975) also reported that sugar and KHT by themselves decrease the expansion and stability of sunflower seed proteins, but together increase both expansion and stability.

CONCLUSIONS

Protein concentrates obtained from pressed jojoba meal are able to function in food products requiring gelling, oil absorption, water absorption, or foaming properties. Lower amounts of protein are required to gel at neutral or alkaline pH (6–10%) than at acidic pH (at least 20%). The oil absorption capacities of water and salt extracts were higher (3 ml/g) than for the SDI and SDII (1.66–1.86 ml/g). The SDI and SDII had higher water capacities than the water and salt extracts. The water absorption capacities for all the concentrates were similar to those for sunflower and soy protein concentrates, while the oil absorption capacity was between that of the sunflower and soy protein concentrates.

Foams of jojoba protein concentrates had greatest capacity at pH 7 and greatest stability at pH 4. Foaming capacity and stability increased as incubation temperature increased from 20 to 80° C. Additives had little affect on foaming capacity and stability at 20° C, except that the capacity and stability decreased in the presence of both potassium acid tartrate and sucrose. At 55° C the presence of sucrose decreased the capacity, potassium acid tartrate increased the capacity, and both together cancelled the effect. Foam stability decreased 17% when sucrose and potassium acid tartrate were added to the protein dispersion.

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[Received March 10, 1986. Revision received October 20, 1986. Accepted October 21 1986.]