Improvement of Functional Properties of Rapeseed (*Brassica campestris* Var. Toria) Preparations by Chemical Modification

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Rapeseed freeze-dried meal and water-soluble fraction were acylated with succinic and acetic anhydride and methylated with formaldehyde at different concentrations of these reagents, and changes in phytic acid, phenols, glucosinolate content, and functional properties were determined. In general, the greater was the extent of acetylation, the lesser was the extractability of phytic acid, phenols, and glucosinolates in both preparations. Water absorption and fat absorption capacities were enhanced by acetylation, but succinylated meal absorbed a maximum amount of oil at a minimum level of modification. Nitrogen solubility, foaming capacity, and viscosity were markedly improved by succinylation. Emulsifying properties were adversely affected by acylation, while methylation proved to be stimulatory. Modified water-soluble fractions had low content of antinutritional constituents, and acylated extracts showed better emulsifying and foaming properties.

Keywords: *Antinutritional factors; chemical modification; functional properties; rapeseed meal; water-soluble fraction*

INTRODUCTION

Rapeseed is an excellent source of protein (45%), and its essential amino acid composition compares favorably with that of FAO/WHO reference protein (World Health Organization, 1973; Thompson and Cho, 1984). However, high phytic acid, glucosinolate, and phenol levels are antinutritional factors limiting the food applications of rapeseed proteins (Jones, 1979). Utilization of rapeseed flour in other types of food products is feasible, if the flour itself or chemically modified flour is found to have low amounts of toxic constituents with high functionality. Chemical modification of food proteins, especially acylation, has been used to achieve this goal. Acylation with acetic or succinic anhydride has been applied to many plant proteins including wheat (Grant, 1973), soybean (Franzen and Kinsella, 1976), cottonseed and peanut (Beuchat, 1977), sunflower (Kabirullah and Wills, 1982), and pea (Johnson and Brekke, 1983). No studies to date are available on the methylation of plant proteins.

Typical functional properties of food proteins include such diverse phenomena as foaming, emulsification, and gelation, and these have been extensively reviewed (Kinsella, 1979). A first investigation of the functional properties of succinylated rapeseed globulin was described by Nitecka and Schwenke (1986). Although rapeseed products are shown to possess good emulsifying and binding properties, it would be fruitful to improve further these and other functional properties, particularly solubility, which is the most desirable character for finding use in foods. This will enhance the value of rapeseed as a food ingredient. The purpose of this study was to obtain rapeseed preparations that are low in antinutritional factors but rich in functional properties by employing simple treatments under ordinary laboratory conditions.

MATERIALS AND METHODS

Pure-line seeds of rapeseed (*Brassica campestris* var. Toria) were obtained from Punjab Agricultural University, Ludhiana, India. All chemicals used were of analytical grade.

Rapeseed meal was acylated by reaction with succinic anhyride and acetic anhydride separately and methylated with formaldehyde by adding different concentrations of these reagents (0.2−1.0 g/g) at pH 8. The slurry was left for 2 h at room temperature; it was then dialyzed against distilled water for 48 h at 4 °C and freeze-dried. Supernatant after centrifugation was separately dialyzed under the same conditions. These two preparations, freeze-dried meal and water-soluble fraction, were taken to study different properties. Controls were treated in the same manner except that no modifying reagents were added.

Analytical Procedures. Extent of chemical modification was determined by calculating reduction in free amino groups using the method of Paik and Kim (1972).

Antinutritional Factors. Phytate was estimated according to the procedure given by Thompson and Erdman (1982) using the difference method. Phenols were measured by the method of Swain and Hillis (1959). Glucosinolates were extracted according to the procedure employed by Craig and Draper (1979). In this method, glucosinolates were enzymatically hydrolyzed to glucose, which was measured by glucose oxidase method (Colowick and Kaplan, 1966).

Functional Properties. Nitrogen solubility was determined using the micro-Kjeldahl method (McKenzie and Wallace, 1954). Water absorption and fat absorption capacities of meals were estimated according to Rahma and Narasinga Rao (1983) and expressed as milliliters of water or groundnut oil bound per 100 g of meal.

Foam capacity and foam stability of meals and water-soluble fractions were assessed according to the methods of Lawhon *et al*. (1972) and Ahmed and Schmidt (1979), respectively, using 1 g of sample in a blender at high speed. Foam volume at 0 min of standing at room temperature was expressed as foam capacity (FC) and the volume over time as foam stability (FS).

Emulsifying activity (EA) and emulsion stability (ES) were calculated according to the procedure employed by Yasumatsu *et al*. (1972): 100 mL of groundnut oil was added to 10 mL of protein solution (10%) and homogenized for 2 min at 4000 rpm; it was then centrifuged at 7000 rpm for 5 min at 4 °C. EA and ES were expressed as percent emulsion formed. For calculating ES, emulsions were heated for 30 min in a boiling water bath preceding centrifugation.

Viscosity measurements were done using Ostwald's viscometer at room temperature (28 °C). Time of flow and density of protein were determined. Relative and specific viscosities

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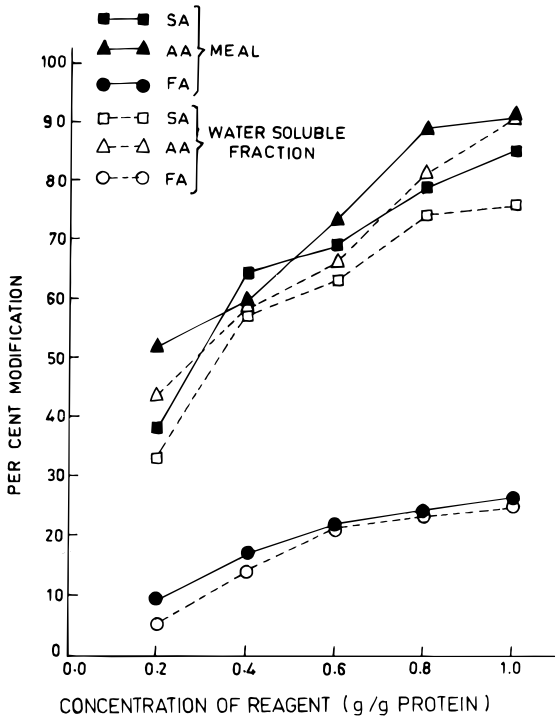

Figure 1. Effect of treatment with different levels of succinic anhydride (□), acetic anhydride (△), and formaldehyde (○) on percent modification of rapeseed meal (—) and water-soluble fraction (- - -).

were calculated by using following equations:

$$\eta_{rel} = \frac{\eta}{\eta_0} = \frac{t}{t_0}\frac{d}{d_0} \quad (1)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (2)$$

η, t, and d represent coefficient of viscosity, time of flow, and density of protein solution, respectively. η_0, t_0, and d_0 are the corresponding values for water.

RESULTS AND DISCUSSION

Degree of Chemical Modification. The level of modification of rapeseed preparations as determined from free amino groups increased with increasing levels of succinylation, acetylation, and methylation in meals and water-soluble fractions (Figure 1). Acetic anhydride was the most reactive and formaldehyde the least. The maximum amounts of modification were 89.7% and 90% in meal and water-soluble fraction, respectively, with acetic anhydride.

Antinutritional Factors. *Phytic Acid.* In the water-soluble fractions, phytic acid was lower as compared to the meals. However, it decreased in all treatments with increasing levels of reagents (Table 1). At the highest level of modification with acetic anhydride (1.0 g/g of protein), phytic acid extractability was lowest (1.38%).

The degree of interaction between phytic acid and protein is affected by the protein charges and salt concentration at a given pH. Acetylation changes an amino group to an amide and completely removes the positive charge of lysine. With succinic anhydride, a two-charge change from the positive charge of amino to the negative charge of carboxyl is achieved. These charges disrupt the protein—mineral—phytic acid ternary complex which exists in extracts of unmodified flour. At the highest level of modifying reagent (1.0 g/g of protein), the phytic acid extractability was lowered to 1.38% (showing reduction of 32%) in the water-soluble fraction, thereby indicating that the formation of protein—mineral—phytate complex was reduced by modification. Methylation causes reductive alkylation of amino groups and has no effect on charge (Sen *et al.*, 1981). The negligible decrease in the phytic acid content in rapeseed preparations with methylation suggests a minimum change in protein conformation.

Phenols. Phenol content decreased with increasing modification, and maximum reduction occurred with acetic anhydride in meal and water-soluble fraction (Table 1). It has been proposed by Loomis (1974) that during production of flours and protein concentrates, quinone oxidation products of polyphenols may bind covalently with sulfhydryl groups of cysteine and the ϵ-amino group of lysine as well as the ϵ-terminal amino groups of proteins.

It could be hypothesized that the increase in net negative charges due to succinylation and introduction of bulky side groups due to acetylation and methylation affects the degree of protein—phenol interaction and hence decreases the phenolic content of modified rapeseed meals and water-soluble fractions. With methylation, the degree of modification was minimum and, thereby, the decrease in phenols was also minimum.

Glucosinolates. Data listed in Table 1 indicate that glucosinolate content declined with increasing concentrations of modifying reagents and this reduction was more apparent with acetic anhydride in both meal and water-soluble fraction. No literature is available regarding the effect of chemical modification on the changes in glucosinolate content. Glucosinolates can form complexes with proteins under certain conditions. Chemical treatment may result in an increase in the number of functional groups available for reaction on the protein surface to bind the glucosinolates. Another possibility is that amino groups of enzyme myrosinase are affected by chemical modification, thereby inactivating the enzyme. In conventional rapeseed processing for commercial purposes, glucosinolates in seed are preserved and retained in meal by thermal destruction of myrosinase. Chemical modification adopted in the present work also seems to be equally effective in inhibiting the enzyme myrosinase.

Functional Properties. *Nitrogen Solubility.* Both meal and water-soluble fraction showed essentially similar patterns of changes of nitrogen solubility in each of the treatments (Figure 2). However, water-soluble fractions had low N_2 solubility as compared to the meals. Succinylation increased markedly the nitrogen solubility of rapeseed preparations. Acetylation improved solubility at some concentrations of acetic anhydride (0.6 and 0.8 g/g of protein), but this increase was not as pronounced as with succinylated samples. Alkylation reduced the nitrogen solubility in both preparations up to 20% modification.

The better N_2 solubility of succinylated rapeseed preparations than of acetylated ones can be explained by the facts that succinylation introduces longer side chains compared with acetylation, produces more electrostatic repulsions in the protein, and produces greater change in the conformation, which results in better protein—water interactions.

The increase in nitrogen solubility of succinylated and acetylated proteins is in agreement with reported values of soy protein (Franzen and Kinsella, 1976), fish protein concentrate (Chen *et al.*, 1975), and cottonseed flour (Rahma and Narasinga Rao, 1983). The decrease in

Table 1. Effect of Modification by Succinic Anhydride (SA), Acetic Anhydride (AA), and Formaldehyde (FA) on Antinutritional Constituents (Phenols, Glucosinolates, and Phytic Acid) in Rapeseed

	water-soluble fraction									meal								
concn of reagent	phenols (%)			phytic acid (%)			glucosinolates (mg/g)			phenols (%)			phytic acid (%)			glucosinolates (mg/g)		
(g/g of protein)	SA	AA	FA	SA	AA	FA	SA	AA	FA	SA	AA	FA	SA	AA	FA	SA	AA	FA
0.0	0.495			2.03			3.15			0.535			2.56			3.45		
0.2	0.440	0.420	0.454	2.19	2.08	2.22	2.80	2.21	2.90	0.500	0.480	0.515	2.44	2.39	2.49	2.94	2.75	3.00
0.4	0.415	0.390	0.435	1.80	1.61	2.03	2.05	1.85	2.11	0.485	0.440	0.495	2.33	2.30	2.47	2.28	2.60	2.75
0.6	0.390	0.364	0.399	1.73	1.57	1.92	1.36	1.21	1.64	0.442	0.410	0.460	2.26	2.27	2.42	2.00	2.40	2.05
0.8	0.375	0.329	0.380	1.59	1.41	1.71	1.11	1.16	1.51	0.392	0.380	0.410	2.24	2.21	2.37	1.64	2.00	1.80
1.0	0.352	0.315	0.362	1.54	1.38	1.59	1.09	1.06	1.40	0.370	0.340	0.380	2.19	2.14	2.83	1.54	1.51	1.64

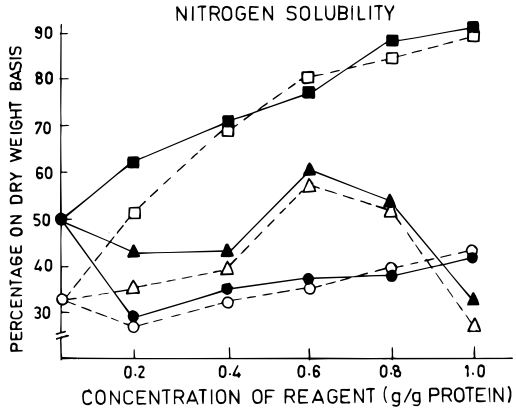

Figure 2. Effect of various concentrations of succinic anhydride (□), acetic anhydride (△), and formaldehyde (○) on nitrogen solubility of rapeseed meal (—) and water-soluble fraction (- - -).

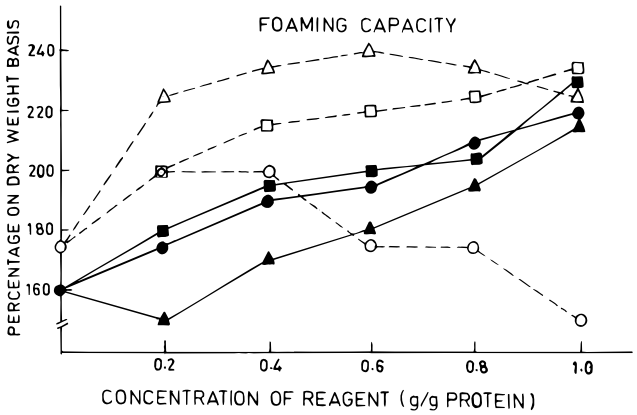

Figure 3. Effect of various concentrations of succinic anhydride (□), acetic anhydride (△), and formaldehyde (○) on foaming capacity of rapeseed meal (—) and water-soluble fraction (- - -).

Table 2. Effect of Modification by Succinic Anhydride (SA), Acetic Anhydride (AA), and Formaldehyde (FA) on Water Absorption Capacity and Fat Absorption Capacity in Rapeseed

concn of reagent (g/g of protein)	SA	AA	FA
	Fat Absorption Capacity		
0.0	120.0	120.0	120.0
0.2	340.0	140.0	180.0
0.4	300.0	160.0	220.0
0.6	240.0	160.0	240.0
0.8	220.0	180.0	220.0
1.0	200.0	180.0	280.0
	Water Absorption Capacity		
0.0	232.5	232.5	232.5
0.2	285.9	269.2	241.8
0.4	286.6	289.0	247.0
0.6	284.7	292.0	252.0
0.8	248.7	293.8	259.0
1.0	179.4	297.0	259.5

nitrogen solubility of rapeseed preparations with methylation is due to introduction of hydrophobic groups which favor protein interactions in aqueous solution.

Water Absorption Capacity (WAC). Acetylation caused maximum increase in WAC, whereas with succinylation, WAC increased at low concentrations (0.2−0.6 g/g of protein) and started decreasing at higher concentrations. Methylation caused a small increase in WAC (Table 2).

The increase in WAC due to acylation may be due to both physical and chemical changes in protein. Chemical modification may cuase proteins to denature, unfold, or dissociate (Appu Rao and Narasinga Rao, 1979). Unfolded protein would have a greater number of water binding sites. The combined effect could be to increase water absorption capacity. The increase in WAC by acylation has been reported in the case of other oilseeds such as peanut flour (Beuchat, 1977) and glandless cottonseed (Choi *et al.*, 1981).

Decreased WAC at high concentrations of succinic anhydride may be due to high solubility of protein. It has been reported that highly soluble protein exhibits poor water absorption (Hermansson, 1973).

Fat Absorption Capacity (FAC). Data listed in Table 2 indicate that FAC of meals increased with increasing modification. Succinylation caused maximum increase (183.3% over control) at the level of 0.2 g/g of protein, whereas acetylation resulted in minimum increase (16.7% over control).

Fat absorption is physical entrapment of oil by a protein matrix (Kinsella, 1976). The amount bound is affected by the method used, the protein content, the surface area, the charge and topography, the hydrophobicity, and the liquidity of the oil. Therefore, fat absorption capacities of different flours and meals differ markedly. Acylation of glandless cottonseed flour increased the oil absorption capacity (Choi *et al.*, 1981) while it did not change up to 73% acetylation in cottonseed flour (Rahma and Narasinga Rao, 1983). Beuchat (1977) showed that no marked changes in oil absorption capacity of peanut flour due to succinylation were found, but in cottonseed flour, FAC increased up to 35% succinylation (Rahma and Narasinga Rao, 1983). The changes in the FAC of rapeseed preparations in the present study are indicative of involvement of several factors.

Foaming Properties. In meal, foam capacity (FC) increased with the modification and was maximum with succinic anhydride and minimum with acetic anhydride. In water-soluble fraction, however, FC increased with succinylation and acetylation, while with methylation it started decreasing after the concentration of 0.4 g/g of protein (Figure 3). FS decreased constantly with time in all treatments (Table 3). In water-soluble fraction FS (after 60 min) decreased with increase in modification and was maximum with methylation at all

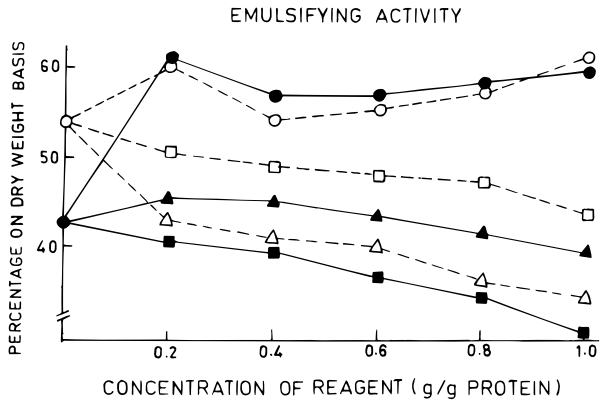

Figure 4. Emulsifying activity of rapeseed meal (—) and water-soluble fraction (- - -) as affected by various levels of succinic anhydride (□), acetic anhydride (△), and formaldehyde (○).

Table 3. Foam Stability at Different Time Intervals Using Different Concentrations of Succinic Anhydride (SA), Acetic Anhydride (AA), and Formaldehyde (FA) in Rapeseed Meal and Water-Soluble (WS) Fraction

concn of reagent (g/g of protein)	time interval							
	30 s		5 min		30 min		60 min	
	WS	meal	WS	meal	WS	meal	WS	meal
unmodified	80.0	36.0	64.0	26.0	60.0	20.0	50.00	0.0
SA								
0.2	64.0	30.0	60.3	24.0	52.0	22.0	50.0	12.0
0.4	56.8	32.7	51.7	21.8	50.0	18.2	48.0	4.5
0.6	68.4	36.4	42.4	20.0	22.0	12.7	15.4	10.9
0.8	55.0	38.3	33.3	15.0	15.0	11.7	9.0	10.0
1.0	57.3	40.0	24.6	13.3	11.0	10.0	1.6	8.3
AA								
0.2	71.6	93.7	58.3	83.3	50.0	68.7	35.0	62.5
0.4	63.9	92.0	54.1	80.0	47.5	74.0	27.9	70.0
0.6	57.1	90.2	47.6	84.3	31.7	57.1	23.8	49.2
0.8	61.9	96.2	39.7	92.3	23.8	53.8	15.9	40.0
1.0	48.4	90.9	24.2	81.8	16.1	47.3	14.5	40.0
FA								
0.2	72.9	87.5	64.6	64.6	52.0	60.4	51.7	41.7
0.4	66.7	83.3	58.3	58.3	50.0	54.2	41.7	37.5
0.6	75.0	82.6	62.5	50.0	50.0	46.2	37.5	27.3
0.8	73.8	76.4	59.5	52.7	47.6	43.6	35.7	27.3
1.0	87.5	75.0	75.0	50.0	45.0	39.3	31.5	26.3

concentrations. In meal, it was maximum (40%) with acetylation.

Good foaming agents must have a mixture of hydrophilic and hydrophobic groups in the molecule. Succinylation introduces the maximum number of hydrophilic groups. Foam stability is reduced with succinylation because of negative charges imparted during modification causing the protein molecule to unfold. Increased net charge density may prevent protein−protein interaction in the foam lamellae, causing foam destabilization and poor stability (Cheftel *et al*., 1985).

The findings of Narayana and Narasinga Rao (1984) indicate that FC of acetylated winged bean flour increased by 38% at 90% acetylation. Franzen and Kinsella (1976) also reported an increase of FC in succinylated soy protein and leaf proteins.

Emulsification Properties. Methylation resulted in an increase in EA and ES, whereas succinylation and acetylation caused a decrease (Figures 4 and 5).

A direct relationship between nitrogen solubility and emulsifying properties of proteins has been demonstrated in many cases (Kinsella, 1979; Narayana and Narasinga Rao, 1984). Although acylated rapeseed meal and water-soluble fraction had excellent nitrogen solubility, they showed poor emulsification properties;

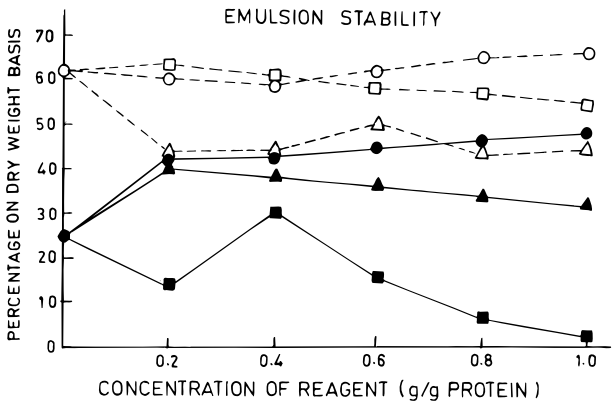

Figure 5. Emulsion stability of rapeseed meal (—) and water-soluble fraction (- - -) as affected by various levels of succinic anhydride (□), acetic anhydride (△), and formaldehyde (○).

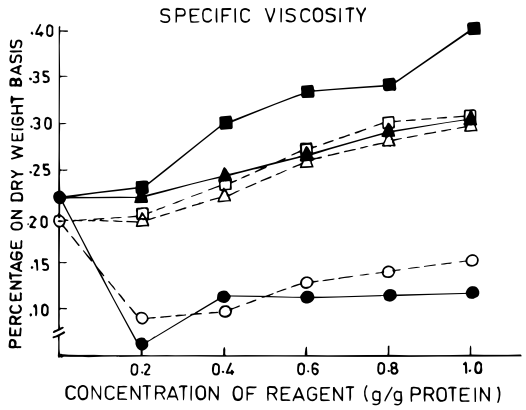

Figure 6. Effect of various concentrations of succinic anhydride (□), acetic anhydride (△), and formaldehyde (○) on specific viscosity of rapeseed meal (—) and water-soluble fraction (- - -).

therefore, emulsifying properties ultimately depend upon a suitable balance between the hydrophile and lipophile and do not necessarily increase as protein becomes more soluble. Schwenke *et al*. (1990) have also reported a decrease in emulsifying capacity of napin after modification in the order native > acetylated > succinylated. The net surface hydrophobicity of protein influences emulsion formation and stability (Nakai *et al*., 1980). Protein with a high relative hydrophobicity tends to be surface active. Pearce and Kinsella (1978) have also demonstrated a significant correlation between emulsification properties and surface hydrophobicity of proteins. Since methylation introduces the maximum number of hydrophobic groups in the protein, it may be the major factor responsible for increased EA in both rapeseed meal and water-soluble fractions.

Viscosity. Results indicate that succinylated rapeseed meal and water-soluble fraction had the greatest viscosity followed by acetylated preparations. Methylation caused a decline in viscosity, and this decrease was more pronounced at low concentrations (Figure 6).

An increase in intrinsic viscosity of legumin at high levels of succinylation has been attributed to the unfolding of proteins (Schwenke *et al*., 1990). A small increase in viscosity at the transition from native state to a level of 10% succinylation of legumin points to changes in protein molecules which may be caused by swelling or small conformational transitions.

Because of the heterogeneous composition of the rapeseed preparations, the present data can be interpreted as indicating an increase in volume of a significant number of the polymer molecules with succinyla-

tion, while conformational changes in protein along with hydrophobicity are responsible for decreased viscosity with methylation.

To conclude, the present data illustrate that the modified, specifically acylated, meal has good N_2 solubility, water absorption, fat absorption, viscosity, foaming stability, and emulsifying activity, whereas water-soluble fractions have better emulsion stability and foaming capacity.

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