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SOME FUNCTIONAL PROPERTIES OF JOJOBA, LINSEED AND CANOLA SEEDS PROTEINS

El-Badawi, A. A.; M. Ragab; Somaya M. A. Ahmed and A. O. Tolaiba

Dept. of Food Science, Fac. of Agric., Zagazig Univ.

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ABSTRACT: Functional properties of jojoba, linseed and canola defatted meals as well as protein isolates were studied. After oil extraction, the resultant defatted meals comprised 32.13, 35.34 and 43.75% crude protein including 83.54, 87.55 and 94.08% true protein for jojoba, linseed and canola meals, respectively. Simmondsin as antinutritional factor was 2.53% in jojoba defatted meal. Linseed and canola seeds were free from simmondsin. Glucosinolate was 32.10 µmol/gm in canola defatted meal only. Yields of protein isolate were 25.9, 16.0 and 17.0% from jojoba, linseed and canola defatted meals with protein recovery 70.41, 41.27 and 32.31 %, respectively. The concentration of simmondsin in jojoba protein isolate and glucosinolate in canola protein isolate were 2.95% and 4.9 µmol/gm, respectively. The lowest protein solubility of studied defatted meals was observed at pH 3-4, while the maximum extractable protein was found at pH 11. Linseed protein isolate had the highest water holding capacity (6.8 ml/gm), while canola as well as linseed defatted meals had the highest oil holding capacity (2.5 ml/gm). Canola protein isolate acted as a good emulsifying agent. It's emulsifying activity and emulsifying stability were 85.5 % and 82.5 %, respectively, which was higher than those of jojoba and linseed proteins. The foaming capacity and foaming stability of all defatted meals and protein isolates studied specially those of canola, increased with increasing pH value up to pH 7. Strong correlation was observed between pH and foaming stability. The presence of sugar improved the viscosity of all studied protein dispersions, especially linseed defatted meal.

INTRODUCTION

Oilseeds are valuable agriculture crops in the world trade (Shahidi, 1990). After oil extraction, their defatted meals are good sources for crude protein, carbohydrates, crude fibers and ash. Proteins from oilseeds are recognized to be much more than simple sources of nutrients. They have good functional properties such as emulsifying activity and stability, foaming capacity and stability and water and oil holding capacities. Therefore, they possess high functionality in food systems.

Few reports were published for jojoba protein isolate. Wiseman and Price (1987) found that the protein concentrates of jojoba were more soluble at alkaline pH than at neutral or acidic pH. The capacities for absorbing oil and water were similar to those of soy protein concentrates. The capacity and stability of foams from jojoba protein concentrates were similar to those of egg albumin.

Flaxseed (linseed) meal may be considered as a potential source of high- quality plant protein for incorporation into food products (Madhusudhan and Singh, 1983). Functional properties of alkali-extracted linseed proteins were comparable to those of soy protein isolate (Dev and Quensel, 1986). Heat treatment improved water absorption, but reduced fat absorption, nitrogen solubility and foaming as well as emulsifying properties of the isolated linseed proteins (Madhusudhan and Singh, 1985). The high mucilage protein concentrate of linseed had better water absorption and emulsifying properties, higher foaming capacity, but lower nitrogen solubility, oil absorption and foam stability than low mucilage flour and low mucilage protein concentrate of linseed (Dev and Quensel, 1988) . Aqueous slurries of linseed meal behaved as a thixotropic liquid. They had high viscosity when subjected to high shear forces. Moreover, the foaming properties of linseed meal were highly correlated with protein concentration and viscosity of the meal (Oomah and Mazza, 1993). The functional properties and uses of flaxseed protein were previously reviewed by Oomah and Mazza (1995).

Canola meal had low solubility properties but very high fat absorption compared with soybean meal (Naczk et al., 1985). Emulsification activity of canola protein isolate was related to

protein solubility, hydrophobicity, zeta potential and flow behavior of aqueous dispersions of the protein. On the other hand, emulsification stability was affected by protein solubility, zeta potential, apparent viscosity of protein dispersions and difference in density between the aqueous and oil phase (Paulson and Tung, 1988) :Canola proteins had poor solubility between pH 2 and 10. The effects of pH on solubility of canola meal showed minimum protein solubility at pH 4 and maximum solubility between pH 9 to 12. Canola meal and canola protein isolate exhibited a good functionality (El- Morsi et al., 2000 and Aluko and McIntosh, 2001). The canola protein isolate with a protein content greater than or equal to 90 % was described for use as a partial replacement for components providing functionality in food products (Murray et al., 2003).

This work was designed to study some functional properties of jojoba, linseed and canola defatted meal proteins as wall as protein isolates including solubility, water and oil holding capacities, emulsifying activity and emulsifying stability and viscosity.

MATERIALS AND METHODS

Commercial jojoba (Simmondsia *chinensis*, Link) seeds were obtained from Egyptian Natural Oil Co., Cairo, Egypt during 2001/2002. Linseed (*Linum usitatssimum*), variety Sakha 1 and canola (*Brassica napus*) seeds, variety Pactol were purchased from Field Crops Institute, Agricultural Research Canter, Giza, Egypt during 2001/2002.

Preparation of defatted meal:

Jojoba, linseed or canola whole seeds were ground for 3 min using a Moulinex mixer (Type 716, France) at maximum speed and extracted with hexane for 8 hrs as described by Simbaya et al. (1995). After drying, the meals were ground to pass through a 1 mm^2 sieve.

Preparation of protein isolates:

Defatted meals (20 gm) were extracted with distilled water (500 ml) after adjusting pH to 11 with 1 N NaOH. The extraction was carried out by initial homogenization for 5 min followed by shaking for 2 hrs at room temperature (25 ± 1 °C). The pH was maintained at 11 during the extraction period by adding 1 N NaOH. After centrifugation at

4000 r.p.m for 20 min, the protein isolate was obtained from the supernatant after adding 0.5 % sodium metabisulfite (Blaicher et al., 1983) by decreasing its pH to 4 using 1 N HCl. The protein precipitate was collected by centrifugation at 4000 rpm for 20 min. The protein isolate was washed twice with distilled water and then air dried at ambient temperature.

Chemical analysis:

Moisture, crude protein, carbohydrates, ash and SO_2 were determined according to the methods of AOAC (1995). The crude protein was estimated by multiplying nitrogen content by 6.25. All analysis were performed in triplicates and the means were reported.

The non protein nitrogen (NPN) was determined according to the method of Singh and Jambunathan (1981). The true protein content was obtained from the following equation:

True protein (%) = (% total nitrogen - % NPN) $\times$6.25.

Determination of antinutritional factors:

Simmondsin was determined according to the method of Van Boven et al. (1993). Glucosinolate was determined by a rapid screening technique as described by Watter and Youngs (1976).

Determination of functional properties:

Protein solubility was determined using the micro-Kjeldahl method (A.O.A.C., 1995) at pH values ranged between 1 and 12.

The method of Sathe and Salunkhe (1981) was followed to determine the water and oil holding capacities. One gram of the sample was mixed with 10 ml water or corn oil and mixed for 30 sec using a Moulinex mixer (type 716, France) at the maximum speed. The samples were then allowed to stand at room temperature (25 ± 1 °C) for 30 min and centrifuged at 5000 rpm for 30 min. The volume of the supernatant was recorded in a 10 ml graduated cylinder. Results were expressed on dry weight basis.

Emulsifying activity and emulsifying stability were determined by the method of Yasumatsu et al. (1972). The emulsion was prepared with simple dispersion at pH 7.0 at an ultimate sample concentration of 3 % in the emulsion. The sample (1.5 gm)

was dispersed in 20 ml distilled water with a magnetic stirrer. The dispersion was adjusted to pH 7 with 0.5 N NaOH and the volume made up to 25 ml with distilled water. Corn oil (25ml) was added and the mixture was homogenized for 3 min at the highest speed of a Moulinex mixer (type 716, France). The obtained emulsion was centrifuged at 3200 rpm for 5 min. The height of the emulsion layer expressed as percent of the total height of fluid in the tube was recorded as emulsifying activity. Emulsifying stability was determined in a similar manner, but after heating at 80 C° in a water bath for 30 min and cooling under running tap water for 15 min.

Foaming capacity and foaming stability of the samples were studied at different pH values (4, 5, 6 and 7) according to the method of Sathe and Salunkhe (1981). One gram of the sample was whipped with 100 ml of citrate-phosphate buffer at pHs 4, 5, 6 and 7 for 5 min using universal laboratory aid mixer (type 309, Poland) at speed setting "150" and was pound into a 250 ml cylinder. The total volume was recorded at time intervals of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hrs. Volume increase (%) was calculated according to the following equation:

$$\text{Vol. increase \%} = (a - b) / b \times 100$$

Whereas: a = Volume after whipping and b = Volume before whipping.

Viscosity was determined using a Brookfield viscometer (Model DV-I, USA) at room temperature (25± 1°C) with spindle No. 2 at speed of 60 rpm as described by Askar and Treptow (1993) . Three grams of the sample was dispersed in 100 ml of distilled water and adjusted to pH 7 using 0.2 N NaOH . The solution-suspension was whipped for 6 and 8 min using Universal laboratory aid Mixer (type 309, Poland) at speed setting "150" .Seventy five grams of sugar were added to the solution-suspension and were whipped for 6 and 8 min . Viscosity of the resulting whip was measured as mentioned above. The results of studied functional properties are the mean of there determinations

RESULTS AND DISCUSSION

Table (1) represents the proximate composition of jojoba, linseed and canola defatted meals. After oil extraction, the resultant defatted meals comprised 32.13, 35.34 and 43.75 % crude protein

including 83.54, 87.55and 94.08 % true protein for jojoba, linseed and canola meals, respectively . These results are in line with those obtained by Wisniak (1994) and Shani (1996) for jojoba, Robbelen et al. (1989) for linseed and Naczk et al. (1985), Zhou et al. (1990) and El-Morsi et al. (2000) for canola. The crude fibers and ash contents of canola defatted meals were higher than those of linseed and jojoba defatted meals. The lowest hydrolizable carbohydrate content (28.79 %) was shown in case of canola meal.

Simmondsin, as antinutritional factor, was 2.53 % in jojoba defatted meals. Linseed and canola seeds were free from simmondsin. In addition, glucosinolate was 32.10 µmol /gm in case of canola defatted meals only. These results are lower than those reported by Verbiscar and Banigan (1978) and Van Boven et al. (1993). They stated that simmondsin content in jojoba defatted meal ranged between 4.2-7 %. Concerning the glucosinolate content in canola meal, results observed in this study were higher than those reported by Zhou et al. (1990) and El-Morsi et al. (2000).

Table (2) shows the yield, protein recovery and proximate composition of jojoba, linseed and canola protein isolates resulted from defatted meals. Yield of protein isolates were 25.9, 16.0 and 17.0 % from jojoba, linseed and canola defatted meals with protein recovery 70.41, 41.27 and 32.31 %, respectively. The crude protein of resultant protein isolates ranged between 77.65 and 79.89 %. Our obtained data are in agreement with those found by Wanasundara and Shahidi (1996 and 1997) for linseed, and Gellberg and Tornell (1976) for canola. The air dried linseed protein isolate held moisture (12.36 %) higher than jojoba (9.12%) and canola (6.61 %) protein isolates. Canola protein isolate had the highest content of carbohydrate (13.87 %) and the lowest ash content (1.87 %) among the studied samples.

The simmondsin content of jojoba protein isolate and glucosinolate content of canola protein isolate were 2.95 % and 4.9 µmol/gm, respectively. Sosulski and Dabrowski (1984) reported that canola protein isolate contained 1.4 µmol/gm glucose-inolate. On the other hand canola protein isolates were free from glucosinolate (Tzeng et al., 1988; Zhou et al., 1990). The bleaching process during protein isolate preparation using sodium

metabisulfite led to color improvement and produced a protein isolates with SO_2 contents ranged between 166 and 181 ppm. The SO_2 concentration limit is within the range of Egyptian Standards for Foods recommendation which is up to 2000 ppm.

Protein solubility profile:

The results given in Fig. (1) illustrate the protein solubility profile of jojoba, linseed and canola defatted meals. The data indicated that at pH 3, twenty percent of the protein was extracted from linseed defatted meal and at pH 4, twenty two percent and thirty five percent of canola and jojoba proteins were extracted from defatted meals, respectively. The extracting of protein was even better at pH 11, where 80 % or more was recovered. Below pH 10 the extraction reached a minimum as previously mentioned at pH 3 and 4. Therefore, the protein was extracted from defatted meals at the optimum pH (11). The protein solubility profile curves obtained from the studied samples were of the same general trend as those reported by Medina and Trejo-Gonzales (1990) for jojoba, Dev and Quensel (1988) for linseed and Allam et al. (1997) and El-Morsi et al. (2000) for canola defatted meals.

Water and oil holding capacities:

The results of water and oil holding capacities are given in Fig. (2). In general, water holding capacities of defatted meals and protein isolates were higher than those in case of oil holding capacities. The linseed proteins showed the highest water holding capacity, particularly linseed protein isolate (6.8 ml/gm). Moreover, the defatted meals of studied samples had water absorption less than protein isolates. The same trend was observed for oil holding capacity. Furthermore, the protein isolates absorbed oil less than defatted meals. The water and oil holding capacities may be due to distribution of polar and non-polar groups to be available on the surface of protein molecule and hence would be able to bind more water and / or oil. This is in line with the results of Dev and Quensel (1988), Wanasubdara and Shahidi (1997) for linseed proteins and Thompson et al. (1982) and El-Morsi et al. (2000) for canola proteins.

Emulsifying activity and emulsifying stability :

Data presented in Fig. (3) show the emulsifying activity and emulsifying stability of jojoba, linseed and canola defatted meals and protein isolates. Canola products, especially protein isolate had the highest emulsifying activity (85.5%) and emulsifying stability (82.5%) followed by linseed and jojoba protein isolates. The emulsifying activity and emulsifying stability of defatted meals were less than those of protein isolates for all studied seeds. Generally, the emulsifying stability is usually lower than the emulsifying activity of studied products. The differences exhibited by such products to emulsifying properties may be attributed to differences in the size and shape of the particles and the charges produced on the protein during the preparation process (Oomah and Mazza, 1995). These results agree with those obtained by (Oomah and Mazza, 1995) and Wanasundara and Shahidi (1997) for linseed and Dev and Mukherjee (1986) and El-Morsi et al. (2000) for canola protein products.

Foaming capacity and foaming stability:

Foam capacity properties were determined for defatted meals and protein isolates of jojoba, linseed and canola seeds at different pH values ranging between 4 to 7 as presented in Fig. (4). The foaming capacities of studied defatted meals markedly increased with increasing the pH values reaching maximum value at pH 7. The same trend was observed with respect to protein isolates. However, the foaming capacities of protein isolates were higher than those of defatted meals. Canola products had the highest foaming capacity followed by linseed and jojoba protein products. The canola protein isolates reflected also the highest foaming capacity at different pH values, being 95 % at pH 7. Same trend was observed with respect to protein isolates. However, the foaming capacity of protein isolates was found to be higher than those of defatted meals.

Fig. (5) demonstrate the effect of pH on foaming stability of jojoba, linseed and canola defatted meals. It is obvious that the canola proteins of defatted meals have the highest foaming stability and still keep the foam after 3 hours at the different studied pH values, especially at pH 6. The foam of jojoba defatted meal protein disappeared quickly after 2 hours at pH 4 and at pH 5, and after 1.5 hours at pH 7. The

linseed defatted meal protein lost the foam at pH 4 and at pH 5 after 2.5 hours, but the foam was still found at pH 6 and at pH 7 after 3 hours .

Fig. (6) show the effect of pH on foaming stability of jojoba, linseed and canola protein isolates. Jojoba protein isolate lost the foam rapidly than those of linseed and canola protein isolates, particularly at pH 4 after 3 hours, but the foam volume of jojoba protein isolate was constant at pH 6 after 2 hours. Canola protein isolate had the highest foam volume at zero time. However, the foam was lost rapidly through the first 0.5 - 1 hour at the different pH values. In spite of this fact, canola protein isolate was still able to preserve foam even after 3 hours at pH 6. The behavior of foaming stability for linseed protein isolate was almost similar to that of canola protein isolate. The foam bubbles produced in such products depend on several factors including mixture of gases, subdivided solids, subdivided liquids and multi-component solutions of water, polymers and surfactant. The functionality of proteins in foam capacity depends also upon their abilities to reduce interfacial tension, structures, solubility and physical-chemical conditions (Cherry and McWatters, 1981; Halling, 1981 and Kinsella and Srinivasan, 1981). The obtained results are in agreement with those reported by Oomah and Mazza (1995). They stated that the foaming capacity and foaming stability of linseed proteins increased linearly with protein concentration. This improvement of foaming properties with increasing protein concentration is due to the increase in the proportion of soluble protein. On the other hand, foaming properties improved by increasing the pH values due to the increase in soluble protein. For canola products, the same trend of the effect of pH values on foaming properties of rapeseed proteins was observed by Allam et al. (1997). The present data are lower than those reported by El- Morsi et al. (2000) and Aluko and McIntosh (2001).

Viscosity:

Table (3) show the viscosity of jojoba, linseed and canola defatted meals and protein isolates as affected by addition of sugar and whipping time. Generally, jojoba and canola proteins improved the viscosity after whipping for 6 or 8 min. On the other hand, the presence of sugar with defatted linseed meals and protein isolates clearly increased

the viscosity after whipping for 6 and 8 min. This characteristic can be utilized in manufacturing of toppings, chiffon mixes and confectionary products. These results are in agreement with those obtained by Oomah and Mazza (1993). They observed that aqueous slurries of linseed meal behaved as a thixotropic liquid which have high viscosity. The influence of sugar addition on viscosity was previously stated by Khalil et al. (1985) who reported that the viscosity of canola, soybean, sunflower, safflower, peanut, glandless cottonseed and sesame protein isolates were increased in presence of sugar.

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Table (1): Proximate composition of jojoba, linseed and canola defatted meals (on dry weight basis).

Component (%)	Jojoba	Linseed	Canola
Moisture (%)	5.88	8.67	8.88
Crude protein (CP, %)	32.13	35.34	43.75
True protein (% of CP)	83.54	87.55	94.08
Untrue protein (% of CP)	16.46	12.45	5.92
Hydrolysable carbohydrate (%):	46.95	42.99	28.79
Soluble carbohydrate	11.61	9.21	7.2
Non-soluble carbohydrate	35.34	33.78	21.59
Crude fibers (%)	13.2	13.7	18.2
Ash (%)	4.96	5.79	6.65
Ant nutritional factors :			
Simmondsin (%)	2.53	0	0
Glucosinolate (μmol/gm)	0	0	32.1

(0) = (free)

Table (2): Yield, protein recovery and proximate composition of jojoba, linseed and canola protein isolates (on wet weight basis).

Parameters	Jojoba	Linseed	Canola
Moisture (%)	9.12	12.36	6.61
Yield (gm/100gm)	25.9	16.0	17.0
Protein recovery (%)	70.41	41.27	32.31
Crude protein (%)	79.83	79.89	77.65
Total carbohydrate (%) (by difference)	5.5	3.08	13.87
Ash (%)	2.6	4.67	1.87
Antinutritional factors:			
Simmondsin (%)	2.95	0	0
Glucosinolate (μ mol/gm)	0	0	4.9
SO_2 (ppm)	170	166	181

(0) = (free)

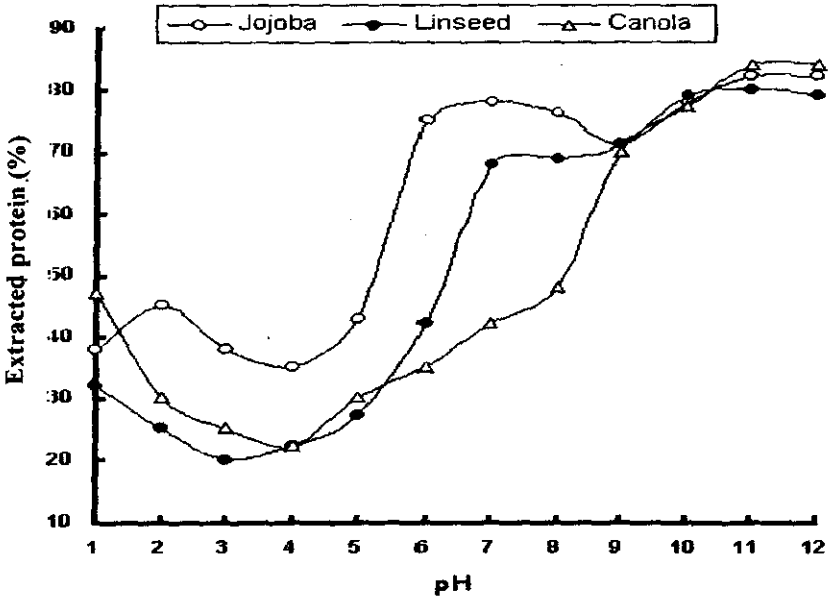

Fig. (1): Protein solubility profile of jojoba, linseed and canola defatted meals.

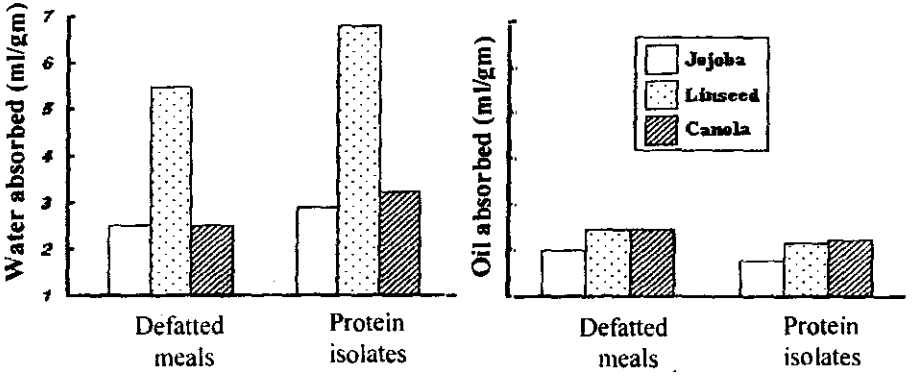

Fig. (2): Water and oil holding capacities of jojoba, linseed and canola defatted meals and protein isolates.

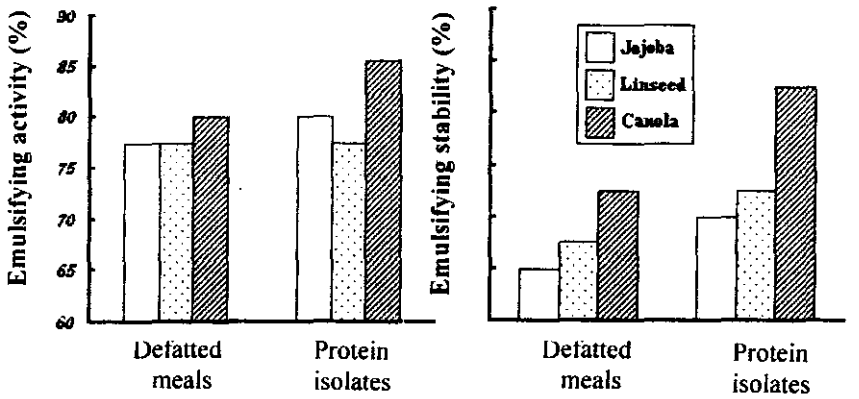

Fig. (3): Emulsifying activity and emulsifying stability of jojoba, linseed and canola defatted meals and protein isolates.

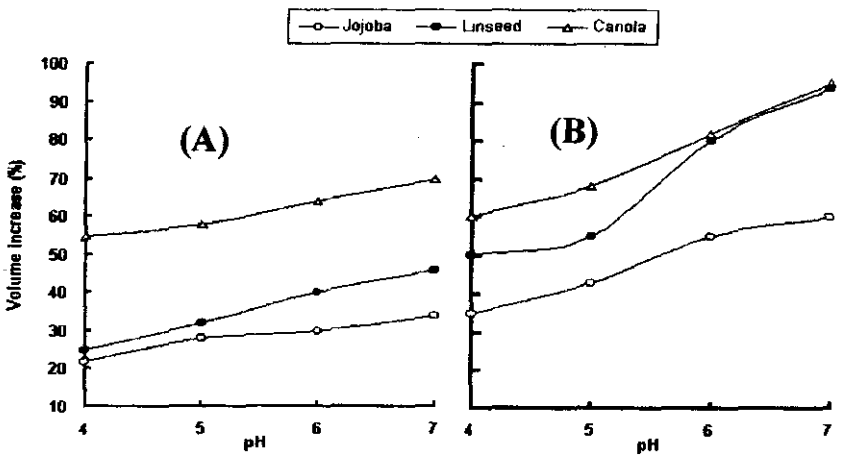

Fig. (4): Effect of pH on foaming capacity of jojoba, linseed and canola defatted meals (A) and protein isolates (B).

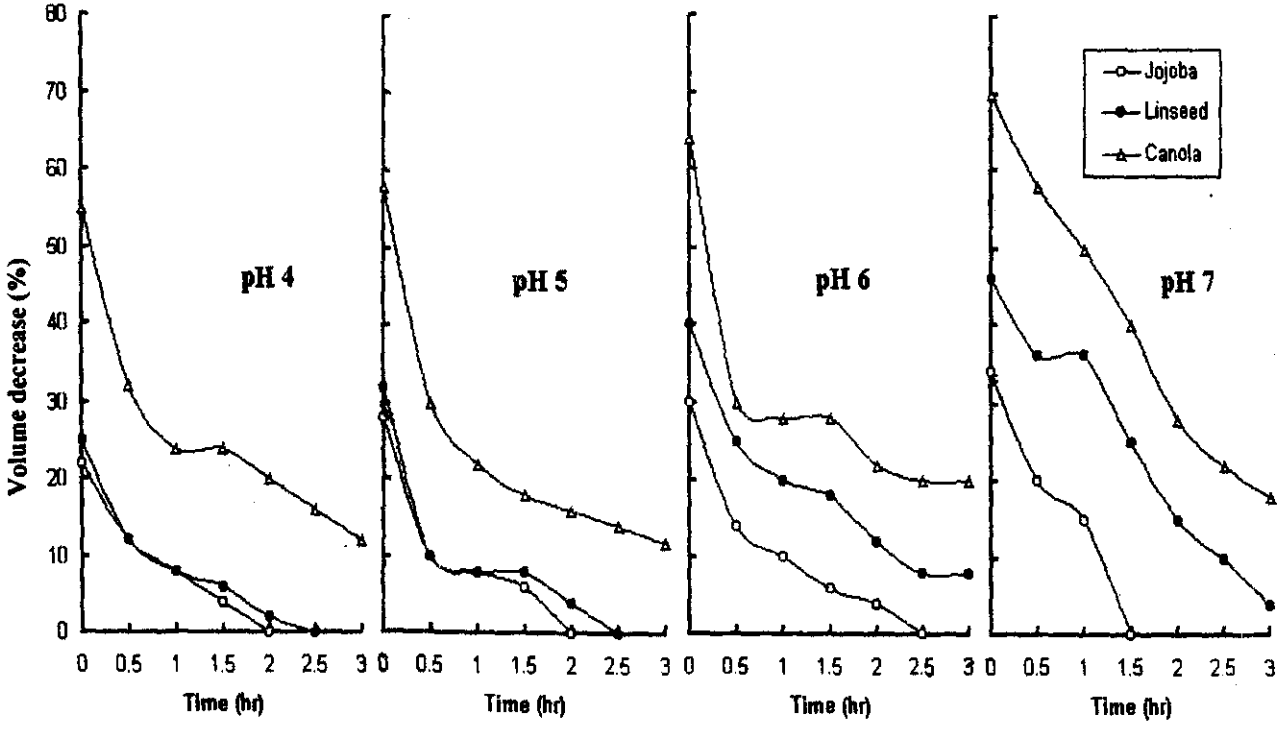

Fig. (5): Effect of pH on foaming stability of jojoba, linseed and canola defatted meals.

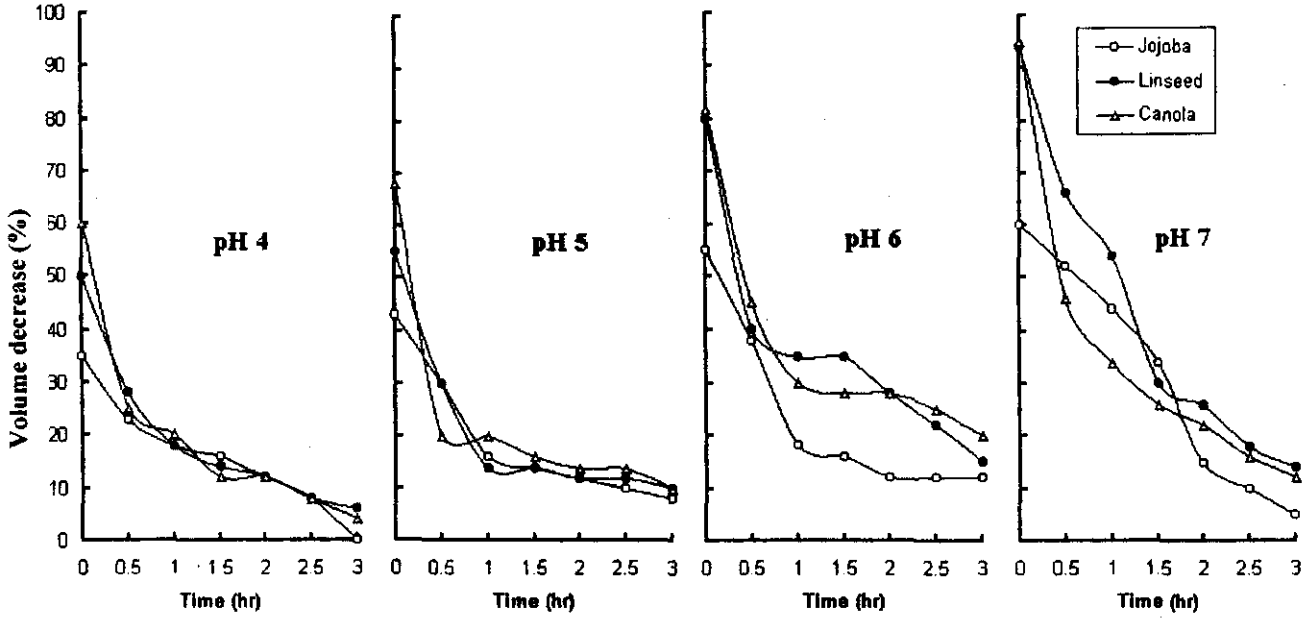

Fig. (6): Effect of pH on foaming stability of jojoba, linseed and canola protein isolates.

Table (3): Viscosity of jojoba, linseed and canola defatted meals and protein isolates.

Seeds	Protein product	Viscosity (mpas)			
		No sugar added		Sugar added	
		6 min	8 min	6 min	8 min
Jojoba	Defatted meal	1.51	1.45	1.56	1.56
	Protein isolate	0.63	1.56	1.38	1.50
Linseed	Defatted meal	1.88	4.00	15.10	6.31
	Protein isolate	1.38	2.20	3.20	2.80
Canola	Defatted meal	1.03	1.90	2.31	2.44
	Protein isolate	0.92	0.94	2.31	1.94

بعض الخواص الوظيفية لبروتينات بذور الجوجوبا والكتان والكانولا

احمد عادل البدوي – محمد رجب عبد المجيد –
سومية محمد عبد المنعم أحمد – عباس عمر طليبة

قسم علوم الأغذية – كلية الزراعة – جامعة الزقازيق – الزقازيق.

تم دراسة الخواص الوظيفية للمطحون منزوع الزيت وكذلك المعزول البروتيني لبذور كل من الجوجوبا والكتان والكانولا ، فبعد استخلاص الزيت بلغت نسبة البروتين الخام فى المطحون الناتج ٣٢,١٣ ، ٣٥,٣٤ ، ٤٣,٧٥ % وان نسبة البروتين الحقيقى بها ٨٣,٥٤ ، ٨٧,٥٥ ، ٩٤,٠٨ % لكل من الجوجوبا والكتان والكانولا على التوالى. وسجلت نسبة السيمندزين (من العوامل المضادة للتغذية) ٢,٥٣ % فى مطحون الجوجوبا منزوع الزيت أما بذور الكتان والكانولا فكانت خالية من السيمندزين. وبلغت نسبة الجلوكوسينولات ٣٢,١ ميكرومول / جم فى مطحون الكانولا منزوع الزيت واتضح من الدراسة أن ما تحصل عليه من معزول البروتين ٢٥,٩ ، ١٧،١٦ % فى حالة الجوجوبا والكتان والكانولا وان نسبة البروتين المستخلصة ٧٠,٤١ ، ٤١,٢٧ ، ٣٢,٣١ % على التوالى. وجد أيضاً أن محتوى السيمندزين فى معزول بروتين الجوجوبا ٢,٩٥ % ومحتوى الجلوكوسينولات فى معزول بروتين الكانولا ٤,٩ ميكرومول / جم. وكانت اقل ذائبية للبروتين فى المطحون منزوع الزيت من البذور الثلاثة عند رقم حموضة ٣ – ٤، بينما كانت أعلى ذائبية عند رقم حموضة ١١. وسجل مطحون الكتان منزوع الزيت أعلى قدرة على ربط الماء (٦,٨ مل / جم) بينما كانت أعلى قدرة على ربط الزيت فى حالة المطحون منزوع الزيت لكل من الكانولا والكتان على حد سواء (٢,٥ مل / جم). ويعتبر معزول بروتين الكانولا عامل استحلاب قوى ، حيث أن نشاط الاستحلاب وكذلك ثبات المستحلب له كانا ٨٥,٥% ، ٨٢,٥% على التوالى وهى أعلى من بروتينات الجوجوبا والكتان. وتؤدى زيادة رقم الحموضة إلى زيادة فى السعة الرغوية وثبات الرغوة لمطحون الكانولا منزوع الزيت وخاصة المعزول البروتينى له وصولا لأقصى قيمة عند رقم حموضة ٧ وذلك مقارنة ببروتينات الجوجوبا والكتان. وقد لوحظ وجود علاقة قوية بين رقم الحموضة وثبات الرغوة. وأدى وجود السكر الى تحسين لزوجة كل العينات محل الدراسة خاصة مع مطحون الكتان منزوع الزيت.