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EFFECT OF EXTRACTION METHODS ON PROTEIN ISOLATE YIELD AND FUNCTIONAL PROPERTIES OF SOME LEGUMES

A. M. KHORSHID, MERVAT A. OSMAN , AND SANIA A. HASSAN

Food Technology Research Institute , Agricultural Research Centre , Giza, Egypt.

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Abstract

Three local samples of unsound (damaged soy - bean), decorticated lentil and broad bean *(Vicia faba)* were used as source of protein in this study. Ca $(OH)_2$ and NaOH methods were applied in protein isolate extraction. Protein extractability and precipitability were determined and results showed that NaOH method gave higher extractability than ca $(OH)_2$ method whil the precipitation value showed a reverse trend. Results also show that broad bean residue after extraction of protein contain a pronounced amount of carbohydrate as strach , while soy - bean and lentil still had a relatively good balance of protein and carbohydrate contents and lentil residues could be used as good and well balanced feed stuff while broad bean might be used as a source of starch production. It could be concluded also that both extraction methods produce protein isolates with a very close purity which determined by the nitrogen content or crude protein of the isolates.

Generally , results show that the method of protein extraction had a little effect on the protein functional properties except that of the coagulated protein percent which increased in case of using NaOH, method . As a conclusion the three damaged and unsound samples could provide a good source of absorption capacity , which suggests potential use of these proteins as a meat binder or extender, and also in doughs and baker products.

INTRODUCTION

There is a constant world demand for less expensive protein with good nutri-

tional and functional properties (Hammonds and Call 1972) Researchers have made many efforts to develop processes using vegetable protein sources other than the soy - bean . Beacuse field peas are already an accepted part of the human diet in many parts of the world , horse beans widely grown in Eutope and Asia, have also been suggested as an alternative source of human food protein (Flink and Christiansen 1973).

Liener (1964), reported the up grading of the nutritional quality of horse-bean protein by removing or destructing the toxic components and indigestible carbohydrates. Also improvement of functional properties by processing may lead to increase the acceptance of these legume proteins (Fan and Sosulski 1974). Toxic factors such as trypsin inhibitor, cyanogenic glucosides. saponins , alkaloids, and hemaglutinin have been identified from legumes and most are water soluble and heat labile. For this reason method of processing and protein isolation from our local legume must be chosen carefully in order to obtain a better yield and better functional properties.

Patel and Johnson (1974), used $Ca(OH)_2$ solution (0.01, 0.02, 0.03 M) with 1:5 flour solvent ratio in isolation of horse beans protein. Preparing protein isolate from soy , peanut , and safflower has been well documented . Kramer and kwee (1977), developed a simple , inexpensive method for extracting a protein concentrate from tomato wastes by raising the pH to 8 - 10 with Na OH(0.1 N)

The protein isolate functional properties will largely determine its acceptability as an ingredient in prepared foods. Therefore the aim of this investigation was to evalute the efferct of using NaOH and $Ca(OH)_2$ solution in the extraction of the protein isolates from soy bean, lentil and broad bean on its extractability, precipetability and its functional properties.

METERIALS AND METHODS

Materials

Three local waste material samples of soy bean , lentil and broad bean (vicia faba) were obtained from , field crops Research Institute Agric . Res. Center . Giza, Egypt.

Methods

Samples were milled to flour (130 u) , and the ground samples were defatted

by refluxing with hexane in a soxhelt apparatus for 14 to 16 hr. The samples were air dried at room temperature 20° C and stored in plastic bags at 5 - 10° C until used .

The proteins were extracted by using Na(OH) and Ca $(OH)_2$ methods as follows:-

A - Ca $(OH)_2$ extracting method

Defatted samples were mixed with Ca $(OH)_2$ solvent 0, 02M at 1:10 ratio (W:V) . Then continous stirriing was carried out for 6 hr . The mixture was filterated and the residue was extracted twice again by the same manner mentioned above.

To determine the pH of protein maximum percipition, 50 ml aliquots of protein extract were adjusted to pH value between 6.5 - 3.0 by using 1 N HCl . During the ddjustement the solution was stirred continously with a magentic stirrer. The obtained crude protein was allowed to form and settle for 2 hr then centrifugation was carried out at 10.000 r. p. m. for 15 min .

Wet crude protein was washed twice by resuspending it in a volume of tap water equal to the whey volume . Crude protein was separated from the washing water by centrifugation, then freeze drying was carried out. The efficiency of protein , extraction was expressed as percentage of total sample protien.

B- NaOH extraction method

The defatted samples were extracted with NaoH according to the method described by MA., CY 1983

The legumes were mixed with the solvent at 1:10 legumes : Solvent ratio and stired at room temperture for 1 hr. The slurry was directly centrifuged and supernatant was neutralized with 2 N HCl and freeze-dried to yield the protein concentrate.

Chemical analysis

Crude protein, fat , ash , crude fiber and moistrue were determined in the samples according to the A.O. A. C. (1970) methods. Total hydrolizable carbohydrates was estimated according to Smith *et al.* (1955).

Test of functional properties

The method described by Kramer and Kwee (1977), were conducted to determine functional properties.

Heat coagulation test

0.2g of protein isolate was dissolved in 10ml citrate phosphate buffer pH 7 shaken for 5 min then centrifuged for 15 min at 3500 r. p. m. To 2 ml of supernatant, 8 ml Biuret reagent was added , then hold in the dark for 30 min, and the % transmitance at 540 nm was measured The remaining supernatant was heated for 15 min in a 100 ^{o}C waterbath, and after cooling the procedure was repeated . Percent transmittance readings were converted to absorbance and % coagulated proteins was caluclated.

$$\% \text{ Coagulated Protein} = \frac{\text{absorbance before heating - absorbance after heating}}{\text{absorbance before heating}} \times 100$$

Solubility and water binding tests

A standard curve was made for the solubility test using gelatin solution as the standard protein , ranging from 1 mg / ml to 20 mg/ ml. Gelatin was dissolved in pH 7 buffer and heated for 15 min at 60 ^{o}C 2ml aliquots were placed in test tubes and 8 ml Biuret reagent were added , then held in the dark for 30 min. Percent transmittance reading s were made at a wavelength of 540 nm and plotted on a semi. - log graph paper with the percent of transmittance on the log scale.

Solubility test

The same procedure was followed as for the heat coagulation test except transmittance readings were made on samples for 15 min in 60 ^{o}C water bath . The % transmittance readings were checked against the standard curve to obtain its equivalent gelatin concentration to give the % soluble protein.

Water binding test

Precipitates from the solubility tests were drained and then weighed. The weighed wet samples were placed in an air oven for 24 hr at 110^{o}C . The loss in weight is the amount of bound water. Results were expressed as concentration of bound water (ml water/ 9 protein concentrate). Corrections were also made for salt precipitated from buffer solution.

Water hydration capacity (WHC) was determined according to the method of Quinn and Paton 1979.

The method for fat - binding capacity (FBC) was that described by Lin *et al.* (1974).

RESULTS AND DISCUSSION

The chemical composition of soy - bean , lentil , broad bean defatted samples are presented in Table 1. Results show that soy - bean contains the highest crude protein 45.9% while lentil and broad bean (vicia faba) contain comperable crude protein precentages of 28.74 and 28.53 % respectively . Results also revealed that the total hydrolizable carbohydrate content of the defatted soy - bean sample was the lowest 21.0 % while those of lentil and horse bean were 56.0 % and 55.0 % respectively .

Table 1. Chemical composition of soy - bean , Broad bean and lentil samples.

Sample	Moisture	Protein %	Fiber %	Ash %	Oil %	Total hydrolyzable carbohydrate %
Soy - bean	8.6	45.9	4.89	8.17	1.315	21.0
Broad bean	10.8	28.53	4.6	3.48	0.71	55.0
Lentil	10.0	28.74	3.6	2.42	1.52	58.0

Protein isolates were prepared by extraction with two alkali solutions NaOH 0.1 N and Ca $(OH)_2$ 0.01 N . The total extracted protein and precipitation value are shown in Table 2 . It is clear that protein solubility in all samples was better in NaOH than in Ca $(OH)_2$ while the precipitation value gave a contrast results. Results also show that soy - bean gave the lowest protein extractability while broad bean was the highest either in Na OH or in Ca $(OH)_2$ methods.

Generally the protein isolate yield % of soy - bean , broad bean and lentil samples were (28.57, 23.39 and 22.22) and (25.89 , 21.53 and 20.24) for the NaOH and Ca $(OH)_2$ methods respectively.

These differences in the protein isolate yield % may be due to the little variation in the pH of protein maximum precipitation between the two alkali solutions, and also between samples.

Table 2. Protein extractability of soy - bean, broad bean and lentil samples extracted by Ca $(OH)_2$ of NaOH soultions.

Sample	Ca $(OH)_2$ 0.02		Na $(OH)_2$ 0.1N	
	Protein Solublity %	Protein extractability %	Protein Solublity %	Protein extractability %
Soy - bean	56.4	84.94	62.24	79.19
Broad bean	75.46	76.91	81.98	68.20
Lentil	70.42	71.54	77.31	65.26

The Chemical composition of the protein isolates from soy bean broad bean and lentil extracted by both Na OH and Ca $(OH)_2$ solutions are represented in Tables 3. and 4.

Results show that total crude protein of all isolates determined as nitrogen by kjldahl method are very close in case of NaOH or Ca $(OH)_2$ methods , and the little variation may be due to the experimental errors . Results also show that the crude protein contents of the three isolates are in a good agreement to those found by Patel and Johnson (1974).

Table 3. Chemical composition of protein isolates of soy - bean , broad bean and lentil extracted by Ca $(OH)_2$ 0.02N.

Sample	Moisture	Protein %	Oil %	Total hydrolyzable carbohydrate %
Soy - bean	11.56	77.24	0.43	8.0
Broad bean	12.9	78.64	0.442	5.5
Lentil	10.4	79.73	0.44	8.0

Table 4. Chemical composition of protein isolates of soy-bean, broad bean, and lentil extracted by Na OH O.1 N.

Sample	Moisture	Protein %	Oil %	Total hydrolyzable carbohydrate %	Fiber %
Soy - bean	10	2.15	79.11	5.5	0.44
Broad bean	10.3	2.27	11.76	6.0	0.78
Lentil	11.3	2.76	77.27	7.0	0.40

The residue after protein isolation was analysed for crude protein , fat , ash, crude fiber and total hydrolizable carbohydrate and the results are reported in Tables 5 and 6. It is clear from these results that soy - bean residue in case of $Ca(OH)_2$ and Na OH method contains about 20.0 - 17.33 % crude protein and 27% - 28.5 % total hydrolizable carbohydrates in addition to its ash and crude fiber contents , so it could be used as feed stable material .

Table 5. Chemical composition of protein isolates of soy-bean, broad bean, and lentil extracted by $Ca(OH)_2$ 0.02 M.

Sample	Moisture	Protein %	Oil %	Total hydrolyzable carbohydrate %
Soy - bean	6.885	20.0	1.9	27.0
Broad bean	9.9	7.18	0.26	64.0
Lentil	13.8	8.677	1.16	62.0

Table 6. Chemical composition of protein isolates of soy - bean , broad bean and lentil extracted by NaOH 0.1 N.

Sample	Moisture	Protein %	Oil %	Total hydrolyzable carbohydrate %
Soy - bean	3.6	17.33	0.97	82.5
Broad bean	9.46	5.4	0.485	69.0
Lentil	8.17	6.5	1.04	77.0

While in case of broad bean and lentil results show that their residue contained a pronounced amount of total hydrolizable carbohydrates 69.0 % and 77.0 % - 62.0% and 64 % in case of CaOH and NaOH methods respectively . Therefore the broad bean and lentil residues could be used as a source of starch isolation in order to economize the protein isolation process.

The functional properties of protein isolates extracted by Ca $(OH)_2$ NaOH were estimated and results are shown in Table 7. Which show that protein isolates extracted by Na OH gave higher percent of coagulated protein than that extracted by Ca $(OH)_2$.

Soluble protein (mg/ml buffer pH_2) show a contrast trend , the protein isolates extracted by NaOH contain soluble protein in buffer 7.0 lower than that of protein extracted by Ca $(OH)_2$.

Results also show that protein water holding capacity % and amount of bound water (ml / mg protein) in all protein isolates were Comparable and it seems that Ca $(OH)_2$ method Slightly increase the ability of the protein isolate to hold more water. Data in Table7 also show that protein fat binding ability did not vary as much as by the method of protein extraction.

Table 7. Some functional properties of soy-bean, broad bean and lentil protein isolates.

Property	Soy-bean		Broad bean		Lentil	
	$Ca(OH)_2$	NaOH	$Ca(OH)_2$	NaOH	$Ca(OH)_2$	NaOH
Coagualted protein %	12.5	17	6.6	14.2	5.8	16.6
Fat binding Ml oil /100 sample	271.3	277.7	227	246.6	312	249.4
Soulble protein mg/ml buffer pH7	5.0	3.87	2.51	2.30	3.87	1.50
Amount of bound water (ml /g)	5.7	5.44	5.19	6.236	4.3	3.8
Water hydration capacity (WHC) %	315.2	330.6	359.9	325	359.9	326

* Calculated as % from soluble protein

As a general conclusion damaged and badly stored samples of soy - bean , broad bean and lentil could be used as source of good quality protein at a relatively low cost and using NaOH in extraction is recommended . The relatively high water and fat absorption capacity of the protein isolates suggests potential use as a meat binder or extender , or used in doughs and bakery products as fortification material (D'Appolonia and Youngs 1978).

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تأثير طرق الأستخلاص علي كمية وصفات البروتين المفصول من البقول

أحمـــد خورشيد ، مرفت أحمد عثمان
سنيـة عبـد اللطـيف

معهد بحوث تكنولوجيا الأغذية، مركز البحوث الزراعية – الجيزة – مصر

في هذه الدراسة تم استخدام كسر فول الصويا والعدس والفول المقشورين كمصدر للبروتين. ولقد استخدم كل من ايدروكسيد الكالسيوم وايدروكسيد الصوديوم في فصل واستخلاص البروتين . ولقد تم تقدير نسبه الاستخلاص والترسيب ووجد أن استخدام طريقة ايدروكسيد الصوديوم تعطي نسبه استخلاص اعلي من طريقه ايدروكسيد الكالسيوم في حين أن نسبه البروتين المترسب كانت نتائجة عكس ذلك .

تدل النتائج أيضا علي أن المتخلف من الفول البلدي بعد استخلاص البروتين يحتوي علي كميه واضحه من الكربوهيدرات في صورة نشا . بينما في حالة فول الصويا والعدس يمكن استخدامها في علف متوازن القيمه الغذائيه للحيوان بينما في حالة الفول البلدي يمكن استخدام المتخلف كمصدر لانتاج النشا. وقد اظهرت النتائج أن طريقتي الاستخلاص تنتج بروتين عالي النقاوة عند تقدير المحتوي البروتيني في البروتين المفصول .

عموما توضح النتائج أن طريقة الاستخلاص لها تأثير بسيط علي صفات البروتين فيما عدا درجة تجليد البروتين حيث تزداد في حالة استخدام طريقة ايدروكسيد الصوديوم ملخص القول أن العينات الثلاثه تعتبر مصدر جيد لفصل البروتين وبالرجوع لقدراتها العاليه علي امتصاص الماء والدهن فأنه يمكن استخدامهم كمادة رابطة او معدلة عند خلطهم باللحوم وكذلك في تدعيم القيمه الغذائية للعجائن ومنتجات المخابز.