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INFLUENCE OF DIFFERENT SOLVENTS ON THE QUALITY OF THE SOLUBILIZED PROTEIN

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ABSTRACT

The proteins of soybean and cottonseed meals were extracted with different solvents, with the aim of examining their effects on the composition, antinutritional factors, toxic ingredients, digestibility and some functional properties of the extracted proteins products. The examined solvents included sodium hydroxide, sodium chloride, sodium carbonate, sodium bicarbonate and sodium carbonate/sodium bicarbonate buffers. Results show that sodium hydroxide 0.05 M was the best . It extracted highest protein from both investigated oilseed meals, it also resulted in highest reduction in the phytate content. Urease content of soybean was lowered to below the permissible level. The protein extracted with the sodium hydroxide possessed the highest digestibility compared to proteins extracted with other solvents. The functional properties of the extracted protein revealed a closeness to those of the original meals. The only disadvantage with 0.05 N sodium hydroxide is that it extracted very little gossypol from cottonseed meal resulting in a protein with 0.235% free gossypol. In this respect the carbonate/bicarbonate buffer pH 10.4 would be the choice solvent since it results in a protein product with 97% less gossypol than the original cottonseed meal.

INTRODUCTION

Plants are the main sources of protein for humans, animals and birds. Oilseed protein ranks high among the plant proteins as a potential unconventional source of protein. Generally, proteins are obtained from oilseeds after the extraction of the oil, leaving behind the meal which contain Ca. 40-50% protein. This meal can be used as such in case it will go to the fodder industry. If more refined protein product are required, then the protein has to be obtained by extraction, solubilization or isolation from the meal or by washing away other constituents in the meal other than protein. Oilseed proteins are mainly globulins Ca. 80% and the rest includes albumins, enzymes, proteinase inhibitors, lipoproteins, etc. (Hettiarachchy and Kalapathy, 1997). Extractability of oilseed proteins is influenced by a variety of factors including: moist heat treatment of the meal, method of oil extraction, particle size, meal age, temperature, solvent to meal ratio, pH and salt concentration (Wolf, 1978).

A large variety of aqueous solvents have been used to extract protein from defatted oilseed meals. Smith *et al*. (1966) reported that of all the solvents used for protein extraction from soybean meal, water, water plus dilute alkali (pH 7-9) and aqueous solutions of sodium chloride (0.5-2.0 M) are among the most efficient. Aqueous extraction using water containing hydrogen peroxide has been used by Lawhon *et al*. (1981), during the preparation of protein products from soybean. Murray *et al*. (1997) patented a salt extraction process to prepare protein isolates using salts of ionic strength

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0.3-0.6 M, pH 5.0-6.0. The use of sodium hydroxide solutions of different normalities have been recommended for sunflower (Taha *et al*., 1981), cottonseed (El-Nockrashy and Frampton, 1967), sesame (Taha *et al*., 1987), peanut (Abbasy *et al*., 1981) and rapeseed (El-Nockrashy *et al*., 1977).

Lasztity and Samei (1992) reported maximum nitrogen extractability from *Phaseolus vulgaris* seeds, in decreasing order, sodium carbonate, disodium phosphate, sodium citrate, magnesium chloride and sodium sulphate. Hojilla-Evangelista *et al*. (1992) extracted protein by 45% ethanol/55% 0.1 M sodium hydroxide from defatted corn by sequential extraction processing. They found that this extraction process can produce high quality protein suitable for food and industrial uses. Swanson (1990) developed wet processes, including alkaline and salt acid solubilization, together with isoelectric precipitation or ultrafiltration for preparation of protein products from pea and lentils. The prepared protein exhibited comparable and complementary functionality to homologous soybean products. Niranjan *et al*. (1998) investigated simultaneous aqueous extraction of oil and protein from soybean, while Rosenthal *et al*. (1997) examined an aqueous and enzymic process for soybean oil and protein production. Wolf *et al*. (1962) used ultracentrifugation while Hill and Breidenbach (1974) used sucrose density gradient centrifugation to fractionate soy protein according to their sedimentation properties. Intact protein bodies of storage protein can be separated from the cellular constituent, by fine milling and density flotation (Kolar *et al*., 1985). Hensley and Lawhon (1979) used ultrafiltration and diafiltration to separate protein after conventional extraction.

Proteins are least soluble at their isoelectric regions and solubility sharply increase above and below this pH. Isoelectric region of oilseed protein lies within the region of pH 4.0-5.0. Commercial extraction of proteins from oilseeds is accomplished by solubilization of the meal protein in mild alkali solutions, followed by precipitation of the protein at its isoelectric point. Henupi *et al*. (1999) investigated factors affecting the industrial process of protein separation and purification.

Although this large variety of aqueous solvents, and different extraction and separation procedures have been studied, yet most of the work was concerned with the percentage of the total meal protein extracted, and only few characterization studies are available on the protein extracted with different solvents.

This work aimed was to investigate different solvents on extraction of protein from soybean and cottonseed meals . The solvents used were sodium hydroxide, sodium chloride, sodium carbonate, sodium bicarbonate, and sodium carbonate/sodium bicarbonate buffers .The effect of this solvents on the chemical composition,and the nutritional quality such as digestibility, antinutritional factors,and toxic ingredients of the extracted proteins were investigated. The functional properties included flowability, bulk density, wettability, protein dispersibility, water absorption, oil holding capacity and emulsifying capacity were also studied .

MATERIALS AND METHODS

Soybean (*Glycine max*) and cottonseed (*Gossypium barbadence*) were the crop of the year 1999 and were supplied by the Ministry of Agriculture.

Preparation of the meals

Dehulled soybeans and cottonseeds were ground using a Wiley mill and extracted in a Soxhelt extractor with n-hexane for 48 hrs. The meal was reground and re-extracted for further 24 hrs. The defatted meal was then spread to dry at room temperature, ground to pass on 80 mesh screen and saved-for-further work.

Extracting solvents

Aqueous extracting solvents used included 0.02 M-0.07 N sodium hydroxide, 1.0 M, 0.5 M sodium chloride, 0.5 M, 0.2 M sodium carbonate, 0.5 M, 0.2 M sodium bicarbonate and sodium carbonate/sodium bicarbonate buffers pHs 10.0-10.6 which were prepared by mixing different volumes of 0.2 M of each according to
(Delory and King) 1945 .

Solubilization procedure

10 g meal were extracted at a solvent : meal ratio of 100:1 for 1 hr. using an Ultra-Turrax stirrer at room temperature, the protein solution was then centrifuged at 5000 g and the supernatant was spray dried.

Method of analysis

Moisture, protein, ash and fiber were determined according to A.O.C.A standard method (1998), soluble nitrogen estimation was carried out as described by Lyman *et al*. (1953). Digestibility was determined according to Hsu *et al*. (1977). Urease activity was determined according to A.O.C.S (1998), total and free gossypol as described by Pons *et al*. (1958). Flowability (FL), bulk density (BD) and wettability (WA) by Lucas (1982). Protein dispersibility index (PDI), water absorption capacity (WAC) were determined as described by Sosulski (1962), oil holding capacity (OHC) was determined by Childs and Forte method (1976) and emulsifying capacity (EC)was achived by Swift *et al*. method (1961).

RESULTS AND DISCUSSION

Previous reports on the use of different solvents for the extraction of plant proteins concentrated mainly on the amount of protein were solubilized by different solvents together with the various factors that may influence the amount of protein extracted (Smith *et al*., 1966; Lasztity and Samei, 1992; Swanson, 1990). A few investigators were concerned with the quality of the protein extracted. It seemed worthwhile to investigate the effect of some aqueous extracting solvents on some of the protein properties that determine its quality.

3.1. Solubilization of the protein

Table (1) gives the % protein solubilized from soybean and cottonseed meals when using different extracting solvents. It is clear from the results in Table (1) that sodium hydroxide 0.05 N resulted in highest solubilization of the protein from both soybean and cottonseed meals, 92.07 and 90.23%, respectively. Sodium hydroxide solutions with normalities less or greater than 0.05-N resulted in less solubilization of protein from both investigated meals. Sodium chloride, sodium carbonate, sodium bicarbonate and carbonate/bicarbonate buffers showed inferior solubility when extracting protein from soybean meal. As for extracting cottonseed meal protein, sodium chloride and sodium bicarbonate also showed inferiority but the efficiency sodium carbonate and sodium carbonate/bicarbonate buffer approached that of sodium hydroxide, in extracting the cottonseed meal protein.

Table 1: % solubilized protein from soybean and cottonseed meals using different extracting solvents.

Solvent	Concentration	Solubilized soybean meal protein (%)	Solubilized cottonseed meal protein (%)
NaOH	0.02 N	62.05	28.07
	0.03 N	62.05	40.11
	0.04 N	86.90	72.25
	0.05 N	92.07	90.23
	0.06 N	82.81	76.22
	0.07 N	41.44	77.28
NaCl	1.0 M	16.62	33.13
	0.5 M	49.74	48.15
Na_2CO_3	0.5 M	37.33	72.23
	0.2 M	41.42	80.21
$NaHCO_3$	0.5 M	24.81	48.23
	0.2 M	20.74	48.23
CO_3/HCO_3 buffer	pH 10.0	23.65	76.62
	pH 10.2	40.33	84.68
	pH 10.4	35.47	88.77
	pH 10.6	43.36	80.65

Calculations made on the assumption of full recovery of total volume.

The efficiency of sodium hydroxide as an extracting solvent for protein was elucidated by several investigators (El-Nockrashy and Frampton, 1967; El-Nockrashy *et al*., 1977; Taha *et al*., 1981, 1987). Their results are in agreement with the above results. Although sodium chloride in this work was not among the recommended solvents for extraction of protein, yet Smith *et al.* (1978) reported 0.5 M-2 M sodium chloride to be among efficient solvents for extraction of soybean protein.

3.2. Chemical analysis of extracted proteins

The protein extracted by the different solvents was spray dried and saved and subjected to further studies.

Table (2) gives the chemical analysis of dried protein products prepared from soybean and cottonseed meals. Results in Table (2) agree with results in Table (1) proving that the protein products resulting from the extraction with sodium hydroxide had the highest protein content, for soybean 42.5% compared to 47% in the soybean meal, and for cottonseed 41.5% compared to 46% in the cottonseed meal. The buffer also resulted in a cottonseed protein product containing 40.6% protein. Although both sodium hydroxide and the carbonate/bicarbonate buffer resulted in extracting a product from cottonseed meal with high protein content, yet it was noticed that these two protein products had the dark color of cottonseed meal. The carbonate and bicarbonate resulted in extracted protein product with much lighter colors.

Table 2: Chemical composition of protein product resulting from solubilization with different extracting solvents.

Extracting solvents	Protein (%)	Ash (%)	Crude fiber (%)	Nitrogen free extract (%)
Soybean				
0.05 N NaOH	42.5	1.1	0.2	56.2
0.5 M NaCl	17.5	0.9	0.14	81.5
0.2 M Na_2CO_3	16.0	1.2	0.4	82.4
0.5 M $NaHCO_3$	10.0	0.8	0.5	89.2
Na_2CO_3/$NaHCO_3$ buffer pH 10.6	22.0	1.0	0.41	76.6
Cottonseed				
0.05 N NaOH	41.5	1.5	0.1	56.9
0.5 M NaCl	9.3	0.9	0.8	89.0
0.2 M Na_2CO_3	16.2	0.9	0.1	82.8
0.5 M $NaHCO_3$	10.7	1.3	0.2	87.8
Na_2CO_3/$NaHCO_3$ buffer pH 10.2	40.6	1.4	0.33	57.7

All values are calculated on moisture free basis.
Soybean meal analyzed 47%protein, 5.5% fiber, 6.0% ash and 41.5% NFF.
Cottonseed meal analyzed 46%protein, 10.0% fiber, 7.0% ash and 37% NFF.

3.3. Effect of extracting solvents on the urease activity present in soybean meal

The urease enzyme present in soybean meal acts on urea to liberate ammonia and carbon dioxide, while the trypsin inhibitor inhibits the action of trypsin in the small intestine. Usually, urease activity and trypsin inhibitor are inactivated at about the same rate during heat processing. Usually urease activity is used as indicator of the trypsin inhibitor activity (Albrecht *et al*., 1966).

Table (3) indicated the effect of extracting solvent on the urease activity of the extracted proteins from soybean meal, it is clear that all extracting solvents resulted in protein products with reduced urease activity when compared to the meal prepared thereform. Soybean meal has a urease activity of 2.3 units while protein extracted with the different solvents possess

0.1 units of urease activity. Only sodium chloride resulted in protein with high levels of urease activity 0.8 units. Permissible level for using soy protein for feeding was 0.2 units of urease (Wright, 1968).

Table 3: Effect of different extracting solvents on the urease activity of soybean extracted meal protein.

Extracting solvents	Urease activity (units)
0.05 N NaOH	0.1
0.5 M NaCl	0.8
0.2 M Na_2CO_3	0.1
0.5 M $NaHCO_3$	0.1
CO_3/HCO_3 buffer pH 10.6	0.1
Soybean meal	2.3

3.4. Effect of extracting solvents on the phytate content present in soybean and cottonseed meal

Phytate or inositol hexaphospate is considered as an antinutritional factor because it affects the bioavailability of minerals and proteins. Phytates are known to chelate with minerals such as Ca, Mg, Zn and Fe rendering then unavailable for the body. They also form protein-phytate complexes which interferes with the proteolytic digestion of the protein (Erdman, 1981; O'Dell and Boland, 1976).

Table (4) shows the effect of extracting soybean and cottonseed meals with different solvents on the phytate content of the resulting extracted protein product. Soybean meal and cottonseed meal contain 4.49 and 8.53 mg phytate P/g meal, respectively. Results in the table show that the protein extracted with 0.05 M sodium hydroxide and 0.2 M sodium carbonate resulted in products with reduced phytate content. When soybean was extracted with sodium hydroxide and sodium carbonate the resulting protein product contained 0.33 and 0.56 mg phytate P/g product, respectively, which means that 92.6 and 87.5% phytates were removed, respectively.

Table 4: Effect of different extracting solvents on the phytate content of the extracted meal protein.

Extracting solvents	pH	Total phytate (mg phytate P/g sample)	
		Cottonseed	soybean
0.05 N NaOH	12.1	0.63	0.33
0.5 M NaCl	9.12	2.3	3.7
0.2 M Na_2CO_3	11.2	0.95	0.56
0.5 M $NaHCO_3$	8.58	2.4	3.5
CO_3/HCO_3 buffer pH 10.6, 10.4	10.6 10.4	2.6	3.6

Cottonseed meal contains 8.53 mg phytate P/g meal.
Soybean meal contains 4.99 mg phytate P/g meal.

Similarly, cottonseed meal extracted with sodium hydroxide and sodium carbonate resulted in protein products where 92.6 and 88.9% of the phytates present in the meal were removed, respectively. This can be

explained by the fact that at high alkaline pH values, the protein phytate complex is dissociated and the phytate is precipitated (Chergan, 1980), and since in this work centrifugation was carried after the solubilization of the protein, and only the supernatant was spray dried, then probably the precipitated phytates were discarded with the residue. DeRham and Jost (1979) also working in the solubility of soybean protein and phytates, found that the phytates were about 80% soluble until pH 11.3 where a sudden drop in the solubility of the phytates took place and at pH 12 only 5% of the phytate was soluble.

Results in Table (4) also reveal that 0.5 M sodium chloride, 0.5 M sodium bicarbonate and carbonate/bicarbonate buffer extracted less phytate with the protein. The resulting protein products contained higher amounts of phytates than these protein products resulting from the extraction with sodium hydroxide and sodium bicarbonate. Soybean protein products and cottonseed protein products resulting from extraction of the meal with sodium chloride, sodium bicarbonate, sodium carbonate/bicarbonate buffer contained 3.7, 3.5 and 3.6 mg phytate P/g product and 2.3, 2.4 and 2.6 mg phytate P/g product, respectively. Champagne *et al*. (1985) reported that soybean phytate and protein were highly soluble at pH range 6-10. This is in agreement with present results since the extracted phytate remained soluble in the supernatant which when spray dried yielded the protein product with relatively high phytate content.

The same authors also reported cottonseed phytate to be less soluble at pH 6-10, which explains why cottonseed protein products extracted at this pH range contained less phytates than the corresponding soy protein products Fontaine *et al*. (1946) reported the same findings.

3.5. Effect of extracting solvents on the gossypol content present in cottonseed meal

Gossypol is a bright yellow pigment which is characteristic of cottonseed. It is a polyhydroxyphenolic compound having two carbonyl groups. Gossypol toxicity to monogastric animals is well documented. The permissible level of gossypol for the safe feeding of monogastric animals is 0.06% (Pons *et al*., 1959). Figure (1) represents the effect of the extracting solvents used on the gossypol content of cottonseed meal. Cottonseed meal investigated contains 1.2% total gossypol, 0.25% free gossypol and 0.941% bound gossypol. It is the free gossypol that is related to the toxicity. It can also bind with the epsilon amino group of lysine, rendering it unavailable for the body. Data in Fig. (1) shows that sodium hydroxide extracts almost all of the free gossypol with the protein giving rise to a protein product containing 0.235% free gossypol. On the other hand, the other solvents used seemed to extract less of the free gossypol resulting in protein products with low gossypol content. The ability to solubilize free gossypol was in the following decreasing order sodium carbonate/bicarbonate buffer > sodium bicarbonate > sodium carbonate > sodium chloride > sodium hydroxide, resulting in protein products with 0.007, 0.019, 0.033, 0.06 and 0.235% free gossypol, respectively. These results show that when using carbonate/bicarbonate buffer, sodium bicarbonate, sodium carbonate and sodium chloride 97.3,

92.7, 87.25 and 76.8% of the free gossypol in the meal was not extracted, respectively. All solvents used except sodium hydroxide extract protein products with safe levels of free gossypol for feeding.

3.6. Effect of different extracting solvents on the digestibility of the extracted proteins

The nutritive value of plant proteins depend not only on the quantity of the protein, but also on the quality of the protein. The availability of amino acids as well as the digestibility are two main factors that influence the performance of the protein. Intrinsic factors such as level of antinutritional factors, protein structure and external factors such as heat treatment, purification processes, all affect protein digestibility (Hettiarchcy and Kalapthy, 1997).

Figure (2) is a diagrammatic representation of the effect of different extracting solvents on the digestibility of the extracted protein. Results indicated that the different extracting solvents used all affected the digestibility of the resulting protein. Extracting both soybean and cottonseed meal with 0.5 M sodium chloride solution resulted in protein with improved digestibility of 98.82 and 90.33%, respectively, compared to 90.28 and 87.63% for the original meals, respectively. Extracting the meals with 0.05 N sodium hydroxide and 0.2 M sodium bicarbonate resulted in proteins with digestibilities close to the meals prepared therefrom. Using sodium hydroxide extracted proteins with digestibility values of 88.66 and 84.63% for soybean and cottonseed, respectively, while sodium carbonate resulted in 88.07 and 86.56% digestibility for soybean and cottonseed, respectively. Extracting the meal protein with both sodium bicarbonate and carbonate/bicarbonate buffer decreased the digestibility of the resulting protein to 79.99 and 78.45%, respectively, for soybean products, and to 70.99 and 69.88%, respectively for cottonseed products. The results showing that protein products extracted with sodium hydroxide and sodium carbonate possessed higher digestibilities than the other products, could be explained by the fact that during the course of this work extracting the meals with sodium hydroxide and sodium carbonate proved to give products with reduced phytate content. That sodium hydroxide extracted a protein product with high gossypol content cannot be judged with the in vitro protein digestibility procedure carried in this study, but needs some feeding trials. Digestibility of soybean meal was reported to be 84.90% (Hettiarachchy and Kalapathy, 1997) and cottonseed meal to 87% (Wolf, 1978).

3.7. Effect of different extracting solvents on some functional properties of the extracted proteins

The functional properties of protein governs the performance of protein in food systems. Thus, in the course of this work it was necessary to examine the effect of the different extracting solvents on some of the functional properties of the extracted proteins.

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Table (5) gives the values of the examined functional properties for the extracted protein from both soybean and cottonseed meals when using different extracting solvents. Flowability (FL), bulk density (BD), wettability (WA) and protein dispersibility index (PDI) are important properties which should be determined when the protein is to be used in instant products and beverages. The less the time of flowability and wettability the better for instant product, and the smaller the bulk density the better for a smaller packaging unit. Flowability values of all products ranges between 10.0 seconds to 5.3 seconds for cottonseed and soybean protein products and wettability takes between 16.0 to 6.5 seconds for all cottonseed and soybean protein products to become completely wet. These values are appropriate for instant products and beverage. Values for bulk density are 0.625 to 0.33 g/cm^3 indicating small packaging units.

Protein dispirsibility index (PDI) is a very important criteria in all food systems. Protein dispirsibility index is a method to measure the amount of solubilized protein. Comparing the PDI of soybean meal protein with that of the extracted protein of soybean meal with different solvents (Table 5), it can be seen that extraction of soybean protein with sodium hydroxide and sodium carbonate/bicarbonate buffer does not affect the PDI of the extracted protein, while extracting the soy protein with sodium chloride, sodium carbonate and sodium bicarbonate resulted in decreased PDI of the extracted protein products. The same above findings for PDI of soybean meal extracted proteins are also true for the extracted cottonseed meal protein.

Water absorption capacity (WAC) is the ability of a product to absorb water or swell. This property is important in the manufacture of bakery products, pastas, doughnuts and others. All extracted protein products from both soybean and cottonseed meal using different extracting solvents possess lower WAC than the meals prepared therefrom.

Oil holding capacity (OHC) is a measure of a protein ability to bind oil and is an important criteria in the meat industry, such as sausages, hamburgers and also doughnuts. OHC of the soy protein products extracted with different solvents are slightly lower than the OHC of the soybean meal protein prepared therefrom. Only the soy protein product extracted with sodium chloride possessed 42% OHC lower than the soy meal protein. As for extracted cottonseed meal products, results show that their OHC was 50% less than the original cottonseed meal. Only sodium bicarbonate extracted cottonseed meal protein which had an OHC 37% less than that of the original cottonseed meal.

Emulsifying capacity (EC) is essential for a protein to perform well in meat systems. Also, a protein's stability to form emulsion is critical to their application in mayonnaise, salad dressing, milks and frozen deserts. EC of extracted soybean meal protein with different solvents showed between 10.5-31% reduction when compared to the original soy meal. Extraction of cottonseed meal protein with different solvents resulted in protein products less affected than the soy protein products. % reduction in the emulsifying capacity ranged between 3.8-23% over the original cottonseed meal.

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Results of functional properties show that the PDI, WAC, OHC and EC were all negatively affected when extracting soybean and cottonseed meal protein with the different solvents investigated whereas the properties necessary for instant products were improved.

In conclusion, it is difficult to recommend a certain solvent for extracting meal proteins, each extracting solvent results in some advantages as well as disadvantages. Carbonate/bicarbonate buffer results in high reduction of free gossypol, but also results in 20% reduction in protein digestibility. Sodium hydroxide while extracting very high protein from the meal and causing high reduction in the phytate content and having very little effect on digestibility, yet, it results only in 9% reduction in the gossypol content of the cottonseed extracted protein. As for soybean protein products, the choice solvent would be 0.05 M sodium hydroxide, since it gave a protein product with very low phytate content and urease activity, with very high protein content and the protein digestibility was only 2% less than original meal. As for the functional properties, with sodium hydroxide and the buffer resulted in protein product with functional properties close to the original meal.

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تأثير استخدام المذيبات المختلفه

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قسم الزيوت والدهون – المركز القومى للبحوث

فى هذا البحث تم إستخلاص البروتين من كسب كل من فول الصويا والقطن بإستخدام عدة مذيبات بهدف دراسة تأثير هذه المذيبات على المنتج البروتينى المستخلص من حيث المكونات ، العوامل غير الغذائية ، العناصر السامة والهضم مع دراسة تأثيرها على بعض الخواص الفيزيقية الهامة للمنتج وقد أستخدم لهذه الدراسة المذيبات الآتية :

أ- هيدروكسيد الصوديوم ب- كلوريد الصوديوم

جـ- كربونات الصوديوم د – بيكربونات الصوديوم

هـ- كربونات الصوديوم/بيكربونات الصوديوم (Buffer)

وقد ثبت من هذه الدراسة أن المنتج البروتينى المستخلص بأستخدام هيدروكسيد الصوديوم 0.05 عيارى بالنسبة لكلا الكسبين من حيث إرتفاع نسبة البروتين ، الإنخفاض الملحوظ فى محتوى الفيتات وإنخفاض النشاط الأنزيمى لأنزيم اليوريز فى كسب فو الصويا . كما أنه يمتلك قدرة عالية على الهضم مقارنة بالأخرين بالإضافة إلى أن الخواص الفيزيقية اقرب ما يكون من الكسب الأصلى . الشىء الوحيد الذى يفضل فيه عنه المنتج البروتينى المستخلص باستخدام كربونات/بيكربونات (Buffer) هو نسبة الجوسيبول الحر حيث أنه أعطى سبة جوسيبول حر 0.235% وهو أعلى من السموح به بينما المنتج الثانى أحدث إنخفاض فى نسبة الجوسيبول الحر بلغت حوالى 97% عن الكسب الأصلى بالنسبة لكسب القطن.

Table 5: Effect of different extracting solvents on the functional properties of the extracted meal protein.

Functional Properties	Soy-bean meal	Cotton-seed meal	0.05 N NaOH		0.5 M NaCl		0.2 M Na_2CO_3		0.5 M $NaHCO_3$		CO_3/HCO_3 buffer pH 10.6 pH 10.4	
			Soy-bean	Cotton-seed	Soy-bean	Cotton-seed	Soy-bean	Cotton-seed	Soy-bean	Cotton-seed	Soy-bean	Cotton-seed
Flowability (Sec.)	10.0	6.9	3.2	9.7	5.3	7.2	5.8	8.4	6.1	8.8	6.8	8.9
Bulk density (g/cm^2)	0.41	0.625	0.48	0.38	0.66	0.41	0.46	0.33	0.51	0.58	0.49	0.51
Wettability (Sec.)	16.0	10.0	10.4	12.0	6.5	13.31	10.2	11.4	11.0	9.6	10.8	11.4
Protein dispersibility index (PDI)	13.39	16.25	15.64	12.77	13.8	10.62	12.2	9.90	11.9	10.0	15.20	12.92
Water absorption capacity (%)	320	300	280	290	200	240	240	310	160	290	210	210
Oil holding capacity (%)	1.67	2.69	2.174	0.875	1.559	0.607	2.012	0.819	2.276	1.053	2.223	1.143
Emulsifying capacity (ml oil/100 g sample)	20.8	21.8	19.5	18.0	16.0	17.0	16.0	16.0	15.5	19.0	15.0	20.0