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EFFECT OF CONCENTRATIONS AND TYPE OF SALT SOLUTIONS ON THE EXTRACTION OF NITROGENOUS COMPOUNDS FROM PHASEOLUS VULGARIS SEEDS

Radomir LÁSZTITY* and M. B. ABDEL SAMEI**

*Department of Biochemistry and Food Technology
Technical University of Budapest
**Agricultural Chemistry Department
Faculty of Agriculture
El-Minia University, El-Minia, Egypt

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Abstract

Effect of different salts, with varying concentrations on the extractability of nitrogenous compounds from three cultivars of *Phaseolus vulgaris* showed that maximum nitrogen extractability attained by the salts can be arranged on a decreasing order as follows: sodium carbonate, disodium phosphate, sodium citrate, magnesium chloride and sodium sulphate.

The amount of nitrogen extracted can be arranged on decreasing order for each variety as follows: Swissblan, Contender and Giza and this agrees with the amount of total nitroten resp. protein in each variety and found to be 25.1%, 23.8% and 21.45%, respectively (NX 6.25 basis).

Keywords: bean, Phaseolus vulgaris, proteins, nitrogenous compounds, extraction.

Introduction

Among the protein sources for human nutrition the role of legume proteins including different varieties of beans is one of the most important. The relatively high protein content of beans makes them a potential raw material for production of protein concentrates and isolates. The most effective technique of protein extraction needs exact data about solubility and extractability of these proteins. In contrast to soybean proteins, common bean proteins have not been studied extensively. Although research on identification and quantification of the storage proteins of beans started at the end of last century with the pioneering work of Osborne, nevertheless a lack in uniformity of nomenclature of fractions separated by solvents may be observed [1]. It is generally accepted that among the storage proteins globulin and albumin like proteins are dominating. The dependence of solubility on pH is well known and is characterized by solubility profile of proteins resp. protein preparations [2].

Nitrogen dispersibility of legume seeds was studied by numerous investigators [3, 4, 5, 6]. Their results indicated that the highest nitrogen dispersibility occurred at pH 1.0 – 2.0 in the acidic range and above pH 7.0 in the basic range with minimum solubility occuring in the pH range 4.0 – 5.0. Moreover, HANG et al. [6] concluded that the amount of nitrogen extracted from mung beans at alkaline pH was greater than that produced at their neutral or acidic pH values and the minimum point of nitrogen dispersion occurred at pH 4.0.

Many laboratory studies indicated that the ratio 1:10 (meal/solvent) is adequate with or without a second extraction at a 1:15 ratio. A 1:20 or 1:40 ratio may remove a greater amount of the total protein but also results in a more dilute protein solution [7]. Although extraction with high pH alkaline solvents results in the highest yield of extracted protein nevertheless some chemical changes (racemization, lysino-alanine formation) may occur decreasing nutritional value of proteins. Therefore the possibility to use salt solution for extraction may be interesting both from theoretical and practical point of view.

The purpose of this work was to study the effect of six salts in wide ranges of concentrations on nitrogen extractability of three local varieties of Phaseolus vulgaris.

Materials and Methods

The Phaseolus varieties used in this investigation were Contender, Swissblan and Giza. One kg of each variety obtained from plant breeding department Ministry of Agriculture was used, then cleaned by excluding the foreign seeds and materials; the grains were then milled and sieved using prufsieb (0.315 Din 4188) sieve.

Six different salt solutions, sodium chloride, sodium sulfate, trisodium citrate, sodium carbonate, disodium phosphate and magnesium chloride at different concentrations 0.25 and 1.00 M; except sodium phosphate solutions were 0.25, 0.50 and 0.75 M. The defatted seed meals (about four grams) were extracted in duplicate with each of the previous extractants in 250 ml by stirring for two hours at room temperature (30 °C); and then centrifuging for 15 minutes at 2500 r.p.m. The pH of the clear supernatant was measured, and nitrogen was determined in 5 ml of each extract by using the method of GÁBOR [8, 9] adapted to beans.

Results and Discussions

The amount of extracted protein with six different salt solutions and the pH of the clear supernatant are summarized in *Table 1*.

It is quite clear from the results shown in *Table 1* that maximum nitrogen extractabilities attained by the salt extractants, except sodium chloride, can be arranged on a decreasing order as follows: sodium carbonate, disodium phosphate, sodium citrate, magnesium chloride and sodium sulfate.

Interestingly, if arranged similarly, the pH of the protein extracts with these extractants will have the same decreasing order for the sodium salt, e. g. 11.05, 8.9, 7.35 and 7.1, respectively. Therefore, salt concentrations producing the most alkaline final pH extract most of the nitrogen, The differences in protein solubilizing capacity between and within the extractants, observed, are not surprising. It is well known that the solubility of proteins is a function of several parameters.

In different Na_2SO_4 concentrations the solubility rise to maxima at 0.5 M and increasing the concentration of this salt beyond the previously mentioned value results in a sharp decrease in its dispersing power. Dilute solutions of $MgCl_2$ lower the extraction of nitrogenous constituents.

The solubilities of the nitrogenous constituents in three basic salts, Na_2HPO_4, $Na_3C_6H_6O_7$ and Na_2CO_3, indicated that increasing of the quantity of these salts causes a gradual increase in the amount of nitrogen extracted from the beans until the solubilities reach maxima and then drop slightly.

In case of NaCl solutions maximum nitrogen extractabilities were at 0.5 M [NaCl]. It is noted that the amount of nitrogen extracted can be arranged on decreasing order for each variety as follows, Swissblan, Contender and Giza and this agreed with the amount of total nitrogen resp. protein and found to be 25.1 %, 23.8 % and 21.45 %, respectively (NX 6.25 basis).

Our results about protein extraction by different salt solutions showed that alkaline salts were found to be fairly effective dispersing agents for *Phaseolus proteins*; this agreed with the results published by HANG et al. [6], PADHYE et al. [10] observed that lower concentration of polyphosphate solubilized more proteins than the higher one in case of black gram (*Phaseolus mungo*) proteins. This fact may be elucidated taking in mind that the solubility of proteins depends upon hydration. Increasing the ionic strength of the solvent the hydration increases also. But after reaching an optimum a decrease in hydration resp. solubility may occur. Degree of solubility of mostly globular bean proteins depends also on the surface hydrophobicity resp. hydrophility.

Table 1

Extraction of Phaseolus seed proteins by salt solution at different concentrations (averages of five replications)

Salt	Seed	0.25 M		0.50 M		0.75 M	
		mg N/100 ml extract	Resultant pH of extract	g N/100 ml extract	Resultant pH of extract	mg N/100 ml extract	Resultant pH of extract
NaCl	1	35.56	6.1	47.04	5.2	53.76	5.0
	2	40.6	4.1	50.68	5.9	57.4	5.7
	3	32.62	5.3	40.8	5.9	47.32	5.8
$MgCl_2$	1	45.92	5.4	44.52	5.3	43.68	4.75
	2	51.52	5.4	47.04	5.3	49.00	4.7
	3	38.64	4.9	36.12	5.3	40.88	4.95
Na_2SO_4	1	38.08	7.1	41.03	7.1	35.98	7.45
	2	41.44	6.9	45.08	7.2	43.82	7.7
	3	32.06	7.1	35.98	7.35	32.48	7.7
Na_2CO_3	1	52.92	11.05	55.16	11.35	45.36	11.4
	2	55.72	11.15	58.8	11.3	54.88	11.5
	3	21.24	11.2	51.8	11.35	39.76	11.6
Na_2HPO_4	1	44.8	8.9	49.84	8.85	36.12	8.95
	2	49.0	8.85	60.48	8.85	47.04	8.95
	3	41.44	9.0	44.8	8.9	31.64	8.95
$Na_3C_6H_5O_7$	1	46.2	7.35	48.72	7.4	50.68	7.65
	2	52.8	7.35	52.64	7.45	54.32	7.7
	3	41.44	7.35	43.12	7.6	45.8	7.8

Variety of seeds: 1 — Contender, 2 — Swissblan, 3 — Giza

Denaturation of globular proteins would theoretically increases the access to polar residues for hydration which in turn would result in better hydration (and then solubilization) of globular proteins (SATHE et al., [7]), but protein–protein interactions may compensate this action.

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