EXTRACTION AND ISOLATION OF PROTEIN FROM LUPINE (Lupinus termis L.) SEEDS

M. KHALIL OSMAN* and L. SIMON—SARKADI

Department of Biochemistry and Food Technology
Technical University, H-1521, Budapest

Received May 14, 1990
Presented by Prof. Dr. R. Lásztity

Abstract

Extraction and isolation of protein from lupine seeds by using distilled water, salt solution, and alkaline solution and precipitation with 0.1 N hydrochloric acid at pH 4.00, followed by centrifugation and freeze drying was studied. Extraction with water yielded 28.5%, with 5% sodium chloride solution 43.5% and with 0.1 N sodium hydroxide solution 79.7% protein. Alkaline solution was found most satisfactory for maximal extraction of protein from lupine seeds. The effect of various factors on the protein extraction, concentration of extractant, time of extraction and relative amount of solvent to dry seeds were also investigated. The digestibility of isolated protein—measured with *in vitro* enzymatic method—was high (90%).

Introduction

The deficiency of food, especially proteins is one of the important problems of the world, about 500 million people suffer from severe protein-calorie malnutrition. The rapid increase of human population has caused many serious problems (LÁSZTITY et al., 1983).

Legumes are important components of the Egyptian diet because of their cheap prices, and the wide variations of the meals prepared from them. Legumes were considered as "poor man's meat". Legumes have a high protein content ranging from 17 to 25% in the dry form. The protein content of the edible portion of legume seeds is double that of cereals and is slightly higher than that of meat, fish, and eggs (WATT and MERILL, 1963). Legumes contribute 8—10% of the world protein supplies. Legume proteins have low biological values compared to animal proteins because of their deficiency in sulphur containing amino acids. Any improvement in amino acid balance will significantly improve the contribution of legumes to world nutrition (Protein Advisory Group, 1973).

* University of Mansoura, Mansoura EGYPT

The growing need to produce and use protein isolates and concentrates in food and feed industry stimulated and stimulates research work connected with the extraction and isolation of seed proteins (BERK, 1970, OWEN and CHICHESTER, 1971, TECSON et al., 1971, CONCON, 1973, NIELSEN et al., 1973, PATEL and JOHNSON, 1974, WU and SEXON, 1975, WU, 1978, WU and SEXON, 1979, and MANDAL et al., 1986).

The aim of this investigation is to study the optimum conditions for isolating protein from lupine seeds as well as to study the *in vitro* digestibility of the protein so as to evaluate its role in human nutrition.

Materials and methods

Lupinus termis seeds preparation

Lupinus termis seeds were used in this investigation from the local market of Egypt. They were cleaned and ground in an electric mill, followed by packaging in polyethylene pouches and stored at 4 °C to be used during the course of study.

Methods adapted for the isolation of protein

Lupine seeds were used as raw material which contains 42.09% of crude protein on wet weight basis, <44.89% of crude protein on dry weight basis (N × 6.25). Lupine seeds were extracted with sodium hydroxide in five concentrations and for five periods ranging between 10 and 50 minutes, and five different ratios of extractant to sample, namely 10 : 1, 50 : 1, 100 : 1, 150 : 1 and 200 : 1.

Protein was precipitated from the extract by adding 0.1 N hydrochloric acid till reaching pH 4.00, followed by washing with water, the protein was finally collected by centrifugation and then freeze dried.

In vitro digestibility of protein concentrates

Protein isolated from *Lupinus termis* seeds was subjected to trypsin and pancreatin digestion, according to the method of SALGÓ et al. (1985). The method was used as pH-stat method. Each sample should contain 200 mg of protein, using the automatic titrator (Radiometer, Copenhagen) at pH value of 8.00, sodium hydroxide (0.05 N) was used as titrant. The quantity (ml) of the alkali consumed was measured from the moment of injection for 10 minutes and the true digestibility was calculated according to the formula:

$$\text{True digestibility} = 52.00 + 0.0223\ x$$

where x: The quantity of the alkali consumed.

Analytical methods

Moisture content: The moisture content of the samples was determined by heating the sample at 100 °C using vacuum oven method as described in the A.O.A.C. Protein determination: Protein content of all the samples was determined with Kjeltec Auto 1030 Systems.

Results and discussion

Results

Extraction and isolation of lupine protein

The influence of various factors on the extracted protein were investigated. The extractant concentration, time of extraction and ratio of solvent to dry material, in order to study the most suitable conditions for extraction.

The results of experiments were as follows
Type of extractant

Three extractants were used for solubilizing the protein in Lupine seeds.

Distilled water, 5% sodium chloride and 0.1 N sodium hydroxide were used.

The results are summarized in Table 1. It can be seen that alkaline solution results the highest protein extraction from seeds. These findings are in agreement with the data given by SMITH et al. (1959).

I. Concentration of extractant

According to the above results, sodium hydroxide solution was used in five concentrations. The highest solubility of protein occurred at 0.1 N and 0.5 N concentration as shown in Table 2.

The results obtained showed that 0.1 N sodium hydroxide extracted the largest proportion of protein from *Lupinus termis* seeds.

Seeds to Extractant ratio

Seeds to extractant ratio affects the solubility of proteins. However, economic considerations may play a limiting role in that respect, since the cost of extracting solution has to be taken into consideration. The results obtained are summarized in Table 3.

Table 1

Effect of extractant type on the solubility of lupine protein

Type of extractant	% Lupinus termis protein
Distilled water	28.46
5% sodium chloride	43.54
0.1% sodium hydroxide	79.72

Seeds to extractant ratio 1 : 100
Extraction time 30 min

Table 2

The effect of sodium hydroxide concentration on the solubility of *Lupinus termis* protein

Concentration of sodium hydroxide	% protein isolated
0.025 N	59.49
0.05 N	62.98
0.1 N	79.72
0.6 N	79.80
1.0 N	75.32

Seeds to extractant ratio 1 : 100
Extraction time 30 min

Table 3

The effect of the quantity of extractant on the solubility of *Lupinus termis* seed protein

Seeds to extractant ratio	% protein isolated
1 : 10	56.47
1 : 50	70.75
1 : 100	80.89
1 : 150	80.90
1 : 200	80.91

Extraction time 30 min
Sodium hydroxide 0.1 N

Table 4

Effect of extraction time on the solubility of *Lupinus termis* protein

Extraction time	% protein isolated
10 min	60.55
20 min	71.54
30 min	79.69
40 min	79.65
50 min	79.70

Sodium hydroxide solution 0.1 N
Seeds to extractant ratio 1 : 100

The results showed that the protein extracted increased with increasing extractant to seeds ratio, and 100 : 1 solvent to seeds ratio was found most satisfactory.

The extraction time

The time of extraction has an effect on the solubility of *Lupinus termis* protein.

The results shown in Table 4 indicated that an extraction time of 30 min is most favourable.

Digestibility of protein concentrates

The protein isolated from *Lupinus termis* seeds was nutritionally evaluated. The true digestibility was measured by the pH-stat method. The true digestibility of lupine protein concentrate was high (90.53%).

References

A.O.A.C. (1975): Official methods of analysis. Washington, D. C.

BERK, Z. (1970): Soy protein concentrates and isolates; In Food Sci. Technol. Proc. Int. Congr., 3rd (1970).

CONCON, J. M. (1973): Rapid and simple method for the quantitative extraction of corn endosperm proteins. *Analytical Biochem., 55*: 563—573.

HSU, H. W., VOWAK, L. D., SATTERLEE, L. D. and MILLER, G. A. (1977): A multienzyme technique for estimating protein digestibility. *J. Food. Sci., 42*: 1269.

KOHLER, G. C., SAUNDERS, R., KUZMICKY, D. D. and ENOCHIAN, R. V. (1970): Biological availability of nutrients and computer evaluation of millfeeds, Feed stuffs, *42*: 410.

LÁSZTITY, R., A. E. EL-MORSI, M. B. ABDEL-SAMEI and M. E. RAMADAN (1983): Solubility of sunflower proteins and gel filtration chromatography of their water soluble fractions. *Periodica Polytech.*, 27: 55—62.

LUPANO, C. E. and ANON, M. C. (1986): Denaturation of wheat germ proteins during drying. *Cereal. Chem.*, *63* (3), 259—262.

MANDAL, B., GHOSH MAJUMDAAR, S. and MAITY, C. P. (1986): *Acta Alimentaria*, *15* (1): 69—77.

NIELSEN, A. C., INGLETT, G. E., WALL, J. S. and DONALDSON, C. L. (1973): Corn germ protein isolate, preliminary studies on preparation and properties. *Cereal Chem.*, *50*: 435.

OWEN, D. F. and CHICHESTER, C. O. (1971): A process for producing nontoxic raps seed protein isolate and an acceptable feed by-product, *Cereal Chem.*, *50*: 92.

PATEL, K. M. and JOHNSON, J. A. (1974): Horsebean as a protein supplement in bread making. I. Isolation of horsebean protein and its amino acid composition. *Cereal Chem.*, *24*: 435.

Protein Advisory Group of United Nations System, New York (1973): Uprising Human Nutrition through Improvement of Food Legume, 22.

SALGÓ, A.—GANZLER, K., and JÉCSAI, J. (1985): Simple enzymic methods for prediction of plant protein digestibility. Proc. Int. Assoc. Cereal Chem. Sym. Amino Acid Comp. and Biol. Value of Cereal Proteins, 311—324. R. Lásztity and M. Hidvégi, Eds., Reidel Publ. Co., Dordrecht

SAUNDERS, R. M. and KOHLER, G. D. (1972): In vitro determination of protein digestibility in wheat mill feeds for monogastric animals, *Cereal Chem.*, *49*: 98.

SMITH, C. R., ERULE, E. R. and WOLFF, I. A. (1959): Comparison of solubility characteristics of selected seed proteins. *J. Agric. Food Chemistry*, 1(2).

TECSON, E. S., ESMOMA, B. V., LONTOK, L. and JULIANO, B. O. (1971): Studies on the extraction and composition of rice endosperm glutelin and prolamin. *Cereal Chem.*, *43*: 169.

WATT, B. K. and MERILL, A. L. (1973) : Composition of Food and Agricultural Handbook, 8. United State of Agriculture Washington D. C.

WU, Y. V. (1978): Protein concentrate from normal and high lysine sorghum: preparation, composition, and properties. *J. Agric. Food Chem.*, *26*: 216.

WU, Y. V. and SEXSON, K. R. (1975): Composition and properties of protein concentrates from normal and high protein wheats. *J. Agric. Food Chem.*, *23*: 906.

WU, Y. V. and SEXSON, K. R. (1979): Protein concentrate from normal and high-lysine corns by alkaline extraction, preparation. *J. Food Sci.*, *41*: 509.

M. KHALIL OSMAN University of Mansoura, EGYPT
Livia SIMON—SARKADI H-1521, Budapest