ACTA CHEMICA SCANDINAVICA 25 (1971) 2225–2231

Preparation and Chemical Analyses of a Lipoprotein Concentrate From Niger Seed (*Guizotia abyssinica* Cass.)*

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A lipoprotein concentrate was prepared from niger seeds (*Guizotia abyssinica* Cass.) by a simple extraction procedure, using a water-ethanol-salt solution as solvent. The extraction was followed by a heat treatment step. The freeze-dried product contained about 46 % protein, 20 % fat and 4 % moisture. The amino acid content was estimated. Available lysine, amounting to 86 % of total lysine, was the first limiting amino acid, and the chemical score was 59.

In trials to find new methods for processing vegetable protein sources into non-toxic products suitable for human consumption, methods involving different types of water extraction constitute an interesting approach.

The production of lipoprotein isolates from peanuts and coconuts by impulse rendering in alkaline water solutions has been described by Smith.[1] Martinez [2] has reported a two-step wet procedure for the production of cottonseed protein concentrates from glandless material. Löfqvist and Munck [3] have isolated protein concentrates from certain legumes by a fractionation procedure, involving extraction with water at different pH above 7.5.

Niger seed (*Guizotia abyssinica* Cass.), more commonly known by its local name nug, is the main oil seed crop in Ethiopia.[4] The preparation of a protein concentrate from nug seeds by extraction with a 3 % NaCl-solution was recently described by Eklund and Ågren.[4] A degradation of extracted protein took place during this preparation, probably due to simultaneous extraction of proteolytic enzymes.[4] Only about 50 % of the extracted protein was recovered in the protein concentrate after the dialysis and freeze-drying procedure, *i.e.* about 1/3 of the total protein content of the seeds.[4] This complication and the low nutritional value found for the nug protein concentrate

* CNU Report No. 46.

(the PER * estimated on growing male rats was only 1.5 [4]) indicates the need of other preparation methods, in which the degree of autoproteolysis is lower.

The present paper describes a simple and rapid preparation procedure, involving extraction of the seeds with an alcoholic salt solution, and giving a fairly high protein recovery. Chemical analyses for proximate composition of the product were carried out, as well as a determination of the amino acid content.

EXPERIMENTAL

Materials. Nug seeds were obtained from the Ethiopian Nutrition Institute (ENI),** Addis Ababa. They were cleaned by sieving and ground in a Turmix before being used for preparations.

Solution *S* was prepared by dissolving 3000 ml of 96 % ethanol and 500 g of NaCl in distilled water to a final volume of 10 l.

Preparation of the nug lipoprotein concentrate (NLPC). 2 kg of ground nug seeds were extracted with 10 l of solution *S* by gradually increasing the temperature from 22°C to 60°C during a period of 30 min under continuous stirring. The extract, which consisted of both dissolved and undissolved finely dispersed material, was then separated from the coarse seed residue by two successive sievings. Metal-sieves with the mesh numbers 12 and 16 were used.

The seed residue was extracted once more with 5 l of solution *S*. The ethanol was then distilled from the two pooled extracts by heating them to (and above) the boiling point of ethanol (79°C), so that protein coagulation should occur simultaneously. After the evaporation of ethanol, when a temperature of 98°C was reached, the heated liquid was cooled and centrifuged at 4°C and at 3000 *g* in a Stock centrifuge, taking 7.5 l.

By this procedure, three fractions were obtained which could easily be separated from each other: a top phase consisting of nug oil, a water phase and a protein fraction at the bottom of the tube. The protein fraction was washed four times with distilled water and freeze-dried. The protein material obtained in this way was the NLPC. Fig. 1 illustrates the preparative procedure in a consecutive scheme and the approximate time demand for each separate step of the process.

Analytical methods. Moisture was determined as described previously.[8] The fat content was determined by a Soxhlet-extraction with diethyleter.[8] Total nitrogen content of the material containing more than 30 % of protein was analysed by a micro-Kjeldahl method.[9] The nitrogen content of the other material was analysed by a macro-Kjeldahl method.[8]

Amino acid analyses were performed according to the method of Spackman *et al.*[10] The samples were hydrolysed with 6 M HCl for 20 and 70 h at 110°C.[11] Cystine was determined by the method of Moore,[12] and tryptophan using a method described by Miller.[13]

Available lysine was analysed by the method of Rao *et al.*,[14] modified according to Blom *et al.*,[15] so that 1.00 g of 2,4-dinitrophenol was added just prior to hydrolysis of

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* Abbreviations and definitions used in this report:

NLPC: Nug Lipoprotein Concentrate.

PER: Protein Efficiency Ratio [5] $= \frac{\text{weight gain (g)}}{\text{protein intake (g)}}$.

TEAA: Total Essential Amino Acids.

A/E-ratios [7] (valid for essential amino acids): mg of each essential amino acid calculated per g of TEAA.

Chemical score according to Block and Mitchell[6] is defined as:

$$\frac{\text{Amount of the first limiting amino acid (mg/g of total nitrogen)} \times 100}{\text{Amount of the same amino acid in the reference protein (mg/g of total nitrogen)}}$$

Chemical score based on A/E-ratios [7] is defined as:

$$\frac{\text{Amount of the first limiting amino acid (mg/g of TEAA)} \times 100}{\text{Amount of the same amino acid in the reference protein (mg/g of TEAA)}}$$

** Formerly named the Children's Nutrition Unit (CNU).

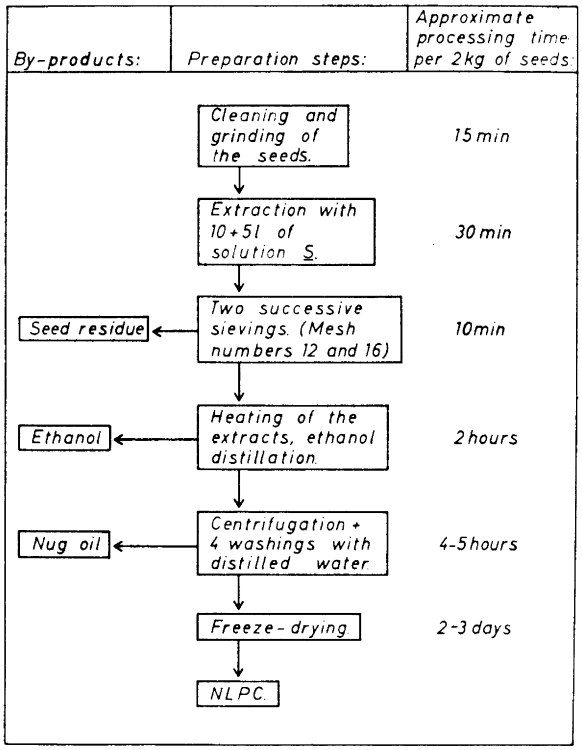

Fig. 1. Diagram showing the preparation of NLPC.

the DNFB-treated sample. Parallel determinations of available lysine were performed by the method of Kakade and Liener.[16] From the amino acid analyses, a true nitrogen to protein conversion factor for NLPC was obtained in the following way. The analytical figures, expressed as mg amino acid per g of total nitrogen, were recalculated as amino acid residues, present in the same unit weight. From the total weights of all amino acid residues, including ammonia, in relation to total nitrogen, a more accurate nitrogen to protein conversion factor was obtained.[17] A conversion factor of 5.50 was used for whole nug seeds.[17] Total amino acid nitrogen as per cent of total nitrogen was calculated by the following procedure. The molar concentration of all amino acids (tryptophan and lysine added twice, histidine three times, and arginine four times) was added, and the sum was multiplied by 14.01. The obtained value was then expressed as per cent of the amount of Kjeldahl nitrogen, present in the same unit weight.

For ash determinations, 2.5 g of the protein material was ashed at 550°C in a Gallencamp furnace.[8] Soil contaminants present in the material were estimated as the insoluble residue obtained after extraction of the ash with 4 M HCl.[8] Crude fibre was determined as previously described.[17] Calcium and iron were analysed according to Horwitz,[18] and phoshorus by the method of Fiske and Subbarow.[19]

Carbohydrate was indirectly estimated as "total carbohydrate by difference"[17] by subtracting the sum of the weight figures (g/100 g of material) for moisture, protein, fat and ash from 100. By subtraction of the crude fibre value from the "total carbohydrate", a value for "soluble carbohydrate" could be obtained.[17] Calorie factors for calculation of food energy have been given by Ågren and Gibson.[17]

RESULTS

After processing 2 kg of nug seeds with 10 + 5 l of solution *S*, about 350 g of NLPC was obtained. The NLPC had a slight brownish color. When tasting the preparation, there was no specific flavour or smell (for chemical composition of NLPC, see Table 1). The moisture content was 4 %, and the fat content 20 %.

Table 1. Chemical composition of NLPC.

Constituent	Number of analyses	Gram/100 g of material	
		Mean value	Range
Moisture	10	4	2 – 5
Ash	4	12	11 – 12
Soil contaminants	4	2	2 – 3
Protein (N × 5.90)	10	46	44 – 48
Fat	10	20	17 – 21
Crude fibre	2	7	7
"Soluble carbohydrate" [a]	–	11 [a]	–
Calcium	3	0.8	0.6 – 0.9
Phosphorus	2	2.2	2.0 – 2.4
Iron	2	0.008	0.006 – 0.010

[a] Calculated value.

From the amino acid analyses (Table 3), a nitrogen to protein conversion factor for NLPC of 5.90 was estimated. Using this factor, the true protein content of NLPC was 46 % (48 % if calculated on dry weight). As the ash amounted to 12 %, and the crude fibre content was 7 %, "soluble carbohydrate" amounted to 11 %. These figures were rather constant from one preparation to another.

Phosphorus, calcium, iron, and soil contaminants constituted about 42 % of the rather high value found for ash. The character of the remaining ash is still unclear.

As seen in Table 2, NLPC contains 47 % of the protein originally present in the nug material. 31 % of the protein material still remained in the seed residue. Apparently about 20 % of the seed protein was lost during the prep-

Table 2. Recoveries of protein and fat during the preparation of NLPC from 2000 g of nug seeds.

Fraction	Amount g	Protein content %	Protein content g	Recovery of protein %	Fat content %	Fat content g	Recovery of fat %
Whole seeds	2000	17	340	–	36	720	–
NLPC	350	46	161	47	20	70	10
Nug oil	330	0	0	0	100	330	46
Seed residue	1050	10	105	31	26	273	38

aration. Some of this might represent a protein fraction which cannot be heat coagulated. A study of the solubilization of proteins from nug seeds during extraction with solution *S* will be published separately.

About 94 % of nug seed fat was recovered, either as free oil (46 %), present in NLPC (10 %), or in the seed residue (38 %).

Amino acid analyses. The results of the amino acid analyses are presented in Table 3. For comparison, the amino acid content in whole egg (hen's) is given.[7,20] Chemical scores were calculated, with reference to the values published for whole egg by the FAO/WHO Expert Group.[7]

Table 3. Amino acid content of whole egg (hen's) and NLPC.

Amino acid	Whole egg [7]		Whole egg [20]	NLPC		
	mg/g of total nitrogen	mg/g of TEAA [a]	mg/g of total nitrogen	mg/g of total nitrogen	mg/100 g of NLPC [b]	mg/g of TEAA [a]
Isoleucine	415	129	393	341	2658	126
Leucine	553	172	551	505	3945	187
Lysine	403	125	436	279 [c]	2183	103
Methionine	197	61	210	125	978	46
Cystine	149	46	152	97	762	36
Phenylalanine	365	114	358	385	3008	142
Tyrosine	262	81	260	225	1756	83
Threonine	317	99	320	263	2049	97
Tryptophan	100	31	93	85	662	31
Valine	454	141	428	397	3099	147
Arginine			382	627	4899	
Histidine			152	192	1498	
Alanine			370	290	2272	
Aspartic acid			601	673	5260	
Glutamic acid			796	1357	10602	
Glycine			207	357	2788	
Proline			260	270	2106	
Serine			478	390	3046	
Ammonia				132	1048	
TEAA	3215		3201	2702		

[a] A/E-ratios. [b] Calculated with dry weight. [c] Available lysine was only 239 mg/g of total nitrogen.

With exception of lysine and the sulfur-containing amino acids, the concentration of each essential amino acid was about 80 % of that found in egg protein. The first limiting amino acid in NLPC was methionine, with a chemical score of 63 calculated according to the classical method of Block and Mitchell.[6]

However, the mean value found for available lysine was 239 mg/g of total nitrogen, *i.e.* 86 % of the value found for total lysine.* Assuming available

* This should be compared with 237 mg of available lysine per g of total nitrogen present in the raw nug seeds used for the preparations of NLPC. The total lysine content in the same batch of seeds was estimated to 290 mg/g of total nitrogen, and thus available lysine corresponded to 82 % of the total lysine. Consequently, the availability of lysine was not unfavourably influenced by the present preparation method.

lysine in egg protein to be equal to total lysine, this amino acid could possibly be considered as the first limiting one in NLPC, since then the chemical score is only 59. The availability of other amino acids has not been determined.

The A/E-ratios were almost exactly the same as in whole egg, except for lysine, the sulfur-containing amino acids, and phenylalanine. Using the same argument as above, lysine would be the first limiting amino acid with a chemical score (based on A/E ratios) of 72.

Total essential amino acids (TEAA) amounted to 2662 mg/g of total nitrogen, if available lysine was substituted for total lysine. This is 83 % of the amount of TEAA found in whole egg.[7] Total amino acid nitrogen amounted to 100 % of total nitrogen, thus the NLPC did not contain any non-protein nitrogen.

DISCUSSION

The present method for preparing a protein concentrate is not too complicated or time-consuming. On a laboratory scale, the most time-consuming steps are the centrifugations and washings of the lipoprotein precipitate, and particularly the final freeze-drying of the product (Fig. 1). The time required for washing the material, giving 350 g of NLPC, was about 4 – 5 h. The time required for the freeze-drying of the corresponding amount of protein material was 2 – 3 days with the equipment now used. Using a three-way centrifuge and a more rapid drying procedure, *e.g.* spray-drying or drying in a vacuum oven, the total processing time could be considerably reduced. Small amounts of NLPC have been dried in a vacuum oven at a temperature of 80°C without loss in available lysine, compared with materials analysed after freeze-drying.[21]

Since it is generally considered in Ethiopia that nug oil is the most valuable product from the seeds, it is important that the protein preparation is performed without reducing the oil yield. In the present procedure, almost 50 % of the fat content of the seeds was separated as oil (Table 2).

The seed residue (Table 2) after the protein extraction still contains a reasonable amount of protein (10 % by weight) and fat (26 %), together with the nug shells. This residue might be used in different ways.

The choice of extraction solution was based on a series of experiments with different NaCl and ethanol concentrations in the solution.[21] The system finally selected gave a high protein recovery and simultaneously a good separation of nug oil. The high fat content found in the product was of considerable interest. If the product is to be used as a component in a protein-rich weaning food for infants, a high calorie content is desirable, to diminish the use of protein for energy purposes. If the fat content is too high, it might be difficult to prepare the product in the form of a dry flour.

When the extraction solution contained 30 % by volume of ethanol and 50 g of NaCl per l, the fat content of the protein concentrate was 20 %, and a dry flour could be prepared. This solvent was also suitable for an effective separation of the oil phase during centrifuging. The quality of the lipid constituents in NLPC, as well as the stability at storage (shelf life) of the preparation, remain to be established.

The energy content of NLPC was calculated to be 400 kcal/100 g (420 kcal/100 g of dry material). This figure should be compared with about 340 kcal/100 g of edible portion found in some commercial vegetable mixtures.[17]

With exception of lysine and the sulfur-containing amino acids, the pattern of essential amino acids in NLPC was well balanced.

According to Cresta *et al.*,[22] the best procedure for estimating protein score as a measure of the ability of a protein, to meet the needs of young children, and expectant and nursing mothers, is to use the classical Block and Mitchell method [6] with egg protein, recommended by the FAO/WHO Expert Group [7] as reference protein. Calculated in this way, the chemical score of NLPC was 59. This value is within the range found for some cereal proteins, but higher than that found for most pulse proteins.[20]

Results from the biological and toxicological evaluation of NLPC will be published separately.

Acknowledgements. This investigation was supported by grants from the *Tri-Centennial Fund of the Bank of Sweden* to Professor G. Ågren, and from the *Medical Faculty of the University of Uppsala.*

I wish to express my gratitude to Professor G. Ågren for valuable discussions throughout the work. The technical assistance of Miss B. Kruse, Miss G. Brink, and Mr. A. Berglund is gratefully acknowledged.

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Received October 29, 1970.