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Preparation and properties of a white protein fraction recovered at high yield from potato haulms

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Summary

More than 50 % of protein free of chlorophyll has been recovered from potato haulm juice after two successive pH treatments. The white protein coagulum is rich in lysine and in hydrophobic and aromatic amino acids. A low relative proportion of polypeptides of high molecular weight suggests that proteins were partially degraded during fractionation. Protein digestibility and other components of the final preparation were also studied.

Introduction

The haulms (stems and leaves) are unexploited by-products of potato culture (Carruthers & Pirie, 1975; Hanczakowski & Makuch, 1980). Leaves may be important sources of protein and, in particular, the white protein concentrate obtained after the separation of chlorophyll from the protein of the leaf juices, has a potential use for human consumption (Heath, 1977; Pirie, 1980). Mechanical extraction of protein from potato haulms has the advantage of lowering the glycoalkaloid content (Hanczakowski & Makuch, 1980). Recently (Merodio et al., 1983), we found an extraction method, based on successive changes of pH, that greatly improves the recovery of white protein from leaf juices. By using a slight modification of that method, we have prepared a white protein concentrate from potato haulms. Here, we report some important properties of this concentrate such as its amino acid composition and digestibility in vitro. In Spain, potato cultivation occupies ca. 350 000 ha and at 15 tonnes of haulms per hectare with ca. 2 % protein on fresh weight, some 100 000 tonnes of protein are lost. We will also show that a high proportion of this figure may be recovered as white protein.

Materials and methods

Potato, cv. Baraka, was planted during June of 1984 in fields (calcareous fluvisol fertilized with 100 kg N per ha) irrigated by the Henares river in the vicinity of the University of Alcalá de Henares where the climate is mediterranean.

Haulms (cut 5 – 10 cm above ground level) were harvested during September at the same time as tubers, washed with tap water and 500-g batches of pieces 2 to 5 cm long were chopped in a Waring Blendor with 0.5 % ascorbic acid (a cheaper antiox-

Table 1. Composition of white protein concentrate from potato haulms. [1]

	Dry matter (%)	Content (% of dry matter)							Carotenoid (mg/kg)	Recovered protein (kg/ha)
		protein		ash	lipid	soluble sugars	starch	other N-free		
		(Lowry)	(Kjeldahl)							
White	93.4 ± 0.6	59.4 ± 3.2	57.6 ± 1.1	4.2 ± 1	10.6 ± 1.6	0.78 ± 0.01	1.1 ± 0.06	24.8	75.7 ± 8.3	75
Green *	92.4 – 94	37.5 – 45.8		12.2 – 19.9	7.4 – 13.6		25 – 29.9		85 – 335	31 – 155

[1] The indicated values are the means ± SE of 3–4 independent experiments with different samples of white protein preparations. For a comparison, the values reported by Hanczakowski & Makuch (1980) for green, unfractionated, protein concentrates of ten potato varieties are also included (*).

idant such as sodium sulphite may be used). The juice, extracted in a screw press and collected through a nylon cloth, contained about 47 % of the haulm protein.

To obtain the white protein concentrate, the pH of the juice (initially 5.7) was lowered with 1 mol/l HCl to pH 5.0 and, after 5 min at room temperature (24 °C), raised to pH 5.5 with 1 mol/l NaOH. White soluble protein was recovered in the supernatant after centrifugation at 2000 *g* for 10 min. The original juice contained about 11.8 g protein and 210 mg chlorophyll per litre. The final preparation contained about 6.3 g protein per litre and was virtually free of chlorophyll (less than 5 mg/l). White protein coagulum was obtained by heating the preparation for 5 min at 85 – 90 °C and then centrifuging it for 10 min at 2000 *g*. The resultant pellet was dried at room temperature in the presence of CaO.

Chlorophyll and trichloroacetic precipitable protein were determined as described previously (Merodio et al., 1983) following the procedures of Arnon (1949) and Lowry et al. (1951) respectively. Protein was also determined by measuring nitrogen content by conventional Kjeldahl method and applying a factor of ×6.25. Moisture content was determined after drying at 100 °C for 5 h. Ash and total lipids were determined by standard methods (AOAC, 1975). Carotenoid was determined after ethanolic extraction according to Davies (1976). Soluble sugars (McCready et al., 1950) were determined according to Somogyi (1952). Starch was determined by measuring sugar by Somogyi's method (1952) after acid hydrolysis (AOAC, 1975) and subtraction of soluble sugars. Polyacrylamide gel electrophoresis of protein in the presence of sodium dodecyl sulphate was carried out in a vertical slab in a linear gradient (10 – 22 %) of total acrylamide according to García et al. (1983). Amino acid composition was determined in a Beckman 121 MB analyzer.

Protein digestion was measured both by decrease in protein precipitable with trichloroacetic acid and by increase in absorption at 280 nm due to material soluble in trichloroacetic acid after incubation with pepsin (Rick, 1962), trypsin (Laskowski, 1955) or pancreatic extract (Laskowski, 1955).

Results and discussion

Analysis of the white protein preparation

When compared with green, unfractionated, protein concentrates from potato (Hanczakowski & Makuch, 1980), our white preparation contains a high percentage of protein and a low percentage of ash (Table 1). Seven of the ten green protein concentrates prepared by Hanczakowski & Makuch (1980) contained higher percentages of lipid than our white preparation. These comparisons agree with results reported for other plants (Subba Rau et al., 1972; Hanczakowski, 1983) according to which white protein preparations contain lower percentages of ash and lipid than green preparations. Despite recovering only protein free of chlorophyll, the estimated figure (75 kg/ha) is high. For the ten green preparations of Hanczakowski & Makuch (1980) only clones PB 2080, 56/72/027 and cv. Mercury produced higher yields (155, 79 and 84 kg/ha respectively). It must be pointed that our procedure yields an additional green protein concentrate residue estimated at 67 kg/ha.

Polyacrylamide gel electrophoresis of the white protein preparation in the presence of sodium dodecyl sulphate (Fig. 1) shows that it is rich in polypeptides of low molecular weight. A prominent peptide of 14 000 D is, probably, the small subunit of ribulose-1,5-bisphosphate carboxylase (Wildman, 1979). A polypeptide of

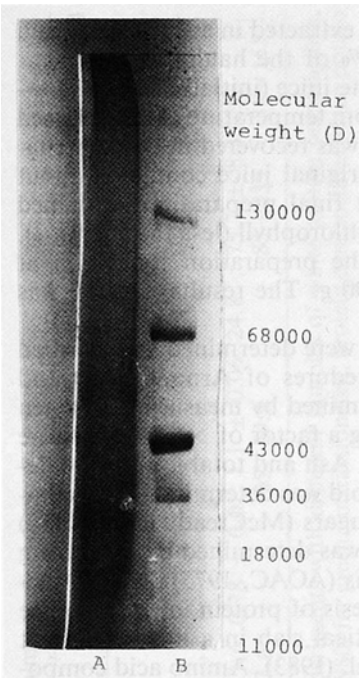

Fig. 1. Polyacrylamide gel electrophoresis of white protein concentrate from potato haulms in the presence of sodium dodecyl sulphate. Lane A, white protein concentrate. Lane B, molecular weight markers: β-galactosidase (130 000 D), bovine albumin (68 000 D), egg albumin (43 000 D), lactate dehydrogenase (36 000 D), β-lactoglobulin (18 000 D) and cytochrome c (11 000 D), (bottom end, not shown).

55 000 D, probably the large subunit of ribulose-1,5-bisphosphate carboxylase, was detected at a low concentration. These results suggest that proteins are partially degraded during the preparation of the concentrate. In contrast with the white protein of lucerne (Free & Saterlee, 1975), most of the polypeptides recovered as white protein of potato haulms have molecular weights lower than 60 000.

Table 2 shows the amino acid composition (except for cysteine and tryptophan) of the white protein preparation. Potato white protein is rich in aromatic and hydrophobic amino acids. The percentage of lysine (6.1 %) is also important. The percentage of methionine (2.7 %), although not high, is important when compared with either

Table 2. Amino acid composition of white protein concentrate from potato haulms (g per 16 g N).

Amino acid	Content	Amino acid	Content
Phe	6.7	Asp + AspN	10.2
Tyr	5.4	Glu + GluN	5.2
Leu	10.4	Ala	7.4
Ileu	6.5	Arg	6.8
Val	7.6	Gly	5.4
Thre	5.5	His	2.4
Lys	6.1	Pro	5.7
Met	2.7	Ser	3.9

potato green protein (1.81–2.43 %) or soja bean meal (1.6 %) (Hanczakowski & Makuch, 1980). Lysine and sulphur amino acids are usually scarce in protein obtained from cereal grain and legume. Because we did not measure cysteine and tryptophan and the latter was not measured in green protein (Hanczakowski & Makuch, 1980), there is some uncertainty about the estimation of the total chemical composition. However, the comparison of the amino acids analyzed suggests that our white protein preparation has a higher biological value than that of the green protein.

In vitro digestibility

Pepsin, trypsin and pancreatin all show good activity for the hydrolysis of white protein preparation of potato (Table 3) when compared with their activity on albumin. However, as shown for pepsin, activity quickly diminished (Fig. 2). After 2 h in the assay conditions, almost all albumin has been degraded while 40 % of white protein from potato remains undegraded. Further washing or other conditions of drying of the white protein preparation would probably improve its in vitro digestibility. Hanczakowski & Makuch (1980) found a low digestibility in vivo of the green proteins prepared from potato haulms. The lower ash content in the white protein preparation probably improves its net utilization (Heath, 1977).

Table 3. Initial rate of protein hydrolysis by pepsin, trypsin and pancreatin.

Substrate	Activity (in absorption at 280 nm/10 min) with:		
	pepsin	trypsin	pancreatin
Potato white protein	0.236	0.118	0.194
Bovine albumin	0.514	0.168	0.009

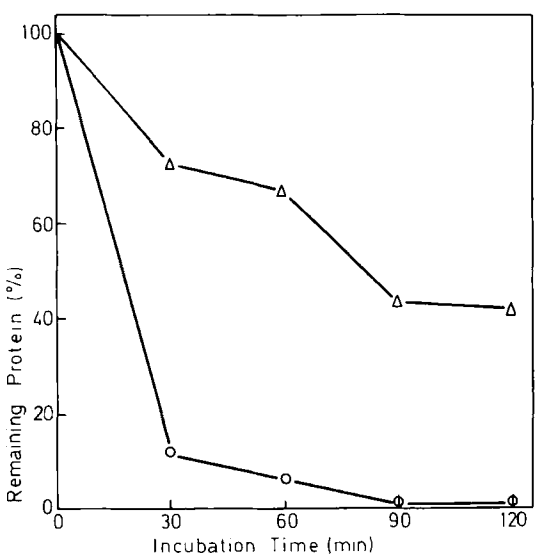

Fig. 2. Time course of the degradation of bovine albumin and white protein concentrate by pepsin. Protein precipitable by 10 % trichloroacetic acid was measured by Lowry method in 0.2-ml aliquots taken at the indicated times from 2-ml incubation mixtures for pepsin assay (Rick, 1962) with bovine albumin (o) or with white protein concentrate from potato haulms (Δ).

As far as we know, the white protein preparation reported here is the first described and analysed from potato haulms. The results obtained suggest that this high yield of white protein preparation from potato is similar to the white protein obtained but with low yields from other plants. It must be noted that the preparation procedure described here, required no more than appropriate control of pH and low centrifugal force.

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