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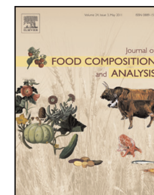

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2 Original Research Article

3 Amino acid composition and nutritional value of four cultivated 4 South American potato species

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A R T I C L E I N F O

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L-Cysteine
L-Lysine

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A B S T R A C T

Protein content, amino acid composition, nutritional quality and patatin characteristics were determined in tubers of four South American cultivated potato species (*Solanum andigenum*, *Solanum goniocalyx*, *Solanum phureja*, *Solanum stenotomum*) and a cultivar of the commonly cultivated cultivar Desirée of *Solanum tuberosum.* Protein content (on dry matter basis) of *S. andigenum* and *S. stenotomum* was highest at 7.9% and 8.0%, respectively, and the relative quantities of patatin for both species were 41.7% and 34.0%, respectively. The nutritional value of patatin, in terms of essential amino acid index with respect to a reference protein of FAO/WHO ($EAAI_{adult}$) ranged from 93.0% (*S. phureja*) to 112.5% (*S. goniocalyx*). In case *S. goniocalyx*, the patatin fraction was a nutritionally better protein fraction than a protein concentrate or tuber dry matter, which had $EAAI_{adult}$ values of 97.6% and 82.9%, respectively. This suggests that this species may have potential in potato breeding programmes and in human nutrition.
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11 ## 1. Introduction

12 Q3 Potato (*Solanum tuberosum* L.) is the fourth major crop 13 worldwide. It is grown both for food and as an important source 14 of high-quality starch. Moreover, potato cultivation also produces 15 a larger amount of dry matter and protein per hectare than cereals (Galdón et al., 2010). Nevertheless, it is important to improve the 16 quality of potato protein in order to improve its contribution to 17 human nutrition. Protein content ($N \times 6.25$) in potato tubers on a 18 dry-weight basis can be about 10%, which is comparable with 19 wheat and is higher than rice or maize (Lachman et al., 2005; Bárta 20 and Bártová, 2008; Bártová et al., 2009; Galdón et al., 2010). About 21 50% of potato nitrogen in potato tubers is derived from proteins; 22 free amino acids, amides, nucleic acids, inorganic nitrogen and 23 alkaloid nitrogen make up the remaining nitrogenous constituents 24 (Bártová et al., 2009). On the basis of amino acid composition, the 25 calculated quality of potato protein is about 70% that of whole egg 26 protein, and potatoes provide a good source of lysine, but only low 27

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levels of sulphur-containing amino acids (methionine and cysteine).

Results of human feeding experiments suggest that potato proteins are of very high quality, probably higher than indicated by their amino acid composition (Kaldy and Markakis, 1972; Eriksen, 1981; Galdón et al., 2010). The chemical score of potato proteins is probably influenced primarily by genotype. Galdón et al. (2010) reported a wide range of potato protein chemical scores between 26.2% and 66.5% for the traditional *S. tuberosum* cultivars. The reasons for such genotypic variability are still not clear. One possible reason may be variability in the quantity and composition of patatin proteins (39–40 kDa). Patatin was identified as the most nutritiously improvable component of *S. tuberosum* having an essential amino acid index (EAAI) value of 86.1% (Bártová and Bárta, 2009). Furthermore, this protein group has properties such as foaming, emulsifying and enzymatic activities of significance in many biotechnological applications (Ralet and Gueguen, 2001; Van Koningsveld et al., 2006).

There are two reasons for the production of potatoes with higher protein content and quality. In developing countries there is general lack of protein, and in some of these countries, potatoes are an important dietary constituent. Thus, breeding for increased protein quantity and quality is an important objective. Potatoes are also used as a source of industrial starch. During processing, potato protein can be recovered as a by-product and used as a valuable feed supplement (Bradshaw and Mackay, 1994) or more recently, as a novel food ingredient. The biochemical properties and quantitative characteristics of tuber proteins in general, and patatin proteins specifically, from *S. tuberosum* L. have been studied extensively (Pouvreau et al., 2001; Tonón et al., 2001; Bárta and Bártová, 2008; Bártová and Bárta, 2009). However, little or no information is available concerning the characteristics of total tuber protein and the nutritional value of the other cultivated South American species, despite the fact that they could play an important role in human nutrition and in *S. tuberosum* breeding programmes.

The aim of this study was to determine and evaluate the quantitative and nutritional characteristics, and amino acid compositions of total and patatin proteins present in tubers of four cultivated non-traditional South American cultivated potato species.

2. Materials and methods

2.1. Plant material preparation

Cultivated potatoes (*Solanum andigenum*, *Solanum goniocalyx*, *Solanum phureja*, *Solanum stenotomum*, *S. tuberosum* cv. Desirée) were obtained as *in vitro* plants from the gene bank of the Potato Research Institute Ltd., Havlíčkův Brod, Czech Republic. Reproduction of plants *in vitro* was carried out on Murashige and Skoog (MS) agar medium containing 30 g L^{-1} of sucrose without growth hormones (Murashige and Skoog, 1962). Rooted *in vitro* plants were transferred to *in vivo* conditions of a greenhouse, and tiny tubers that were obtained were stored at 5 °C for dormancy and then used as the mother tubers for further reproduction. Tubers obtained under greenhouse conditions were manually harvested, washed thoroughly, weighed and cut into slices. Tuber material was freeze dried (freeze-dryer Alpha 1–4, Martin Christ, Osterode am Harz, Germany) to constant weight for gravimetrical determination of dry matter and the dried material was homogenised to a powder for subsequent analyses.

2.2. Analysis of nitrogen and protein content

Total nitrogen on dry matter basis was determined in duplicate by the elemental analyser FLASH EA 1112 (ThermoQuest, Milan, Italy). Crude protein content was estimated as nitrogen content multiplied by a factor 6.25. A BCA Protein Assay Kit (Pierce, Rockford, IL, USA) was used for determination of protein content. Protein was extracted from potato dry matter using SDS-extraction buffer (0.065 M Tris–HCl, pH 6.8, 2% (w/v) SDS) and protein content was measured as absorbance at a wavelength 405 nm, using bovine serum albumin (BSA) as a standard. Every sample was analysed four times.

2.3. Analysis of glycoalkaloid content

Freeze-dried tuber flours were extracted with 50% CH_3OH for 30 min at room temperature. The homogenates were then filtered through a Büchner funnel. An aliquot of this tuber extract was subsequently filtered through a PTFE membrane (5 μm) and analysed using a UHPLC system coupled with a QTrap 5500 tandem mass spectrometer equipped with Turbo VTM ion source. The UHPLC analyses were performed using an Acquity UltraPerformance LC System equipped with an HILIC Atlantis® Silica column (100 mm × 3 mm i.d., 3 μm particle size, Waters, Milford, MA, USA) maintained at 30 °C. The mobile phase consisted of acetonitril (A) and 0.005 M ammonium acetate in Mili-Q water (B).

2.4. Preparation of protein concentrate

A sample of harvested tubers from each of the tested potato species was used to prepare potato fruit juice. Tubers were washed thoroughly and cut into large pieces that were then crushed in a household juice extractor (AEG, Electrolux, Stockholm, Sweden) and a 2% (w/v) solution of $NaHSO_3$ was added to juice at 50 mL kg^{-1}, to prevent enzymatic browning. The resulting liquid was centrifuged (15 min, 9000 × g, 4 °C) and the supernatant was filtered through a paper filter (KA 1, ThermnoFisher, Waltham, MA, USA). Tuber proteins were precipitated from the clear filtrate using 90% saturated of ammonium sulphate (2 h, 4 °C). Precipitates were subsequently washed twice by suspending in 90% ammonium sulphate and they were finally desalted on a Sephadex G-25 gel filtration column (PD-10 desalting columns, GE Healthcare, Fairfield, CT, USA). Desalted precipitates were subjected to amino acids analysis.

2.5. Purification of patatin proteins

Homogenised tuber dry matter was extracted with 0.0625 M Tris-HCl buffer, pH 6.8 (4 °C, 30 min). Extracts were centrifuged (15 min, 9000× g, 4 °C) and filtered. After adjusting the pH to 7.4, extracts were loaded onto an equilibrated (25 mM Tris-HCl buffer, pH 7.4) anion exchange column– diethylaminoethyl (DEAE) 52-Cellulose SERVACEL® (Serva, Heidelberg, Germany). The bound protein fraction was eluted with 25 mM Tris-HCl buffer (pH 7.4) containing 0.5 M NaCl and was subsequently loaded onto a Concanavalin A (ConA) Sepharose 4B affinity column (Pharmacia Biotech, GE Healthcare, Uppsala, Sweden). Equilibration and washing steps were performed with 25 mM Tris –HCl buffer, pH 7.4 + 0.5 M NaCl; regeneration of the column was with 50 mM Na –acetate buffer pH 4.5 containing 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$ and 50 mM Tris –HCl buffer pH 8.5 containing 0.5 M NaCl, 1 mM $MnCl_2$ and 1 mM $CaCl_2$. A bound protein fraction (patatin) was eluted using 25 mM Tris – HCl buffer (pH 7.4) + 0.5 M NaCl + 100 mM α-methyl-D-glucoside and was desalted on a Sephadex G-25 gel filtration column (PD-10 desalting columns; GE Healthcare, Fairfield, CT, USA).

2.6. Determination of amino acid composition

Amino acids were determined after hydrolysis of potato freeze-dried flour, potato protein concentrates and patatin samples using

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