Food Chemistry 44 (1992) 113–118

A study of soluble proteins from four potato varieties used in the Swedish starch industry

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(Received 5 June 1991; revised version received and accepted 3 July 1991)

The purpose of this study was to characterize proteins from four different varieties of potato used in the Swedish starch industry. Proteins in potato fruit juices prepared from Prevalent, Senator, Saturna and Producent potatoes were analysed by gel filtration chromatography on a Sephacryl S–200 column, polyacrylamide gel electrophoresis (PAGE) in sodium dodecylsulphate (SDS) and isoelectric focusing (IEF). The major concentrations of proteins appeared at molecular weights of approximately 44000, 20000 and 14000 without exception for the potato varieties characterized. A more complex pattern of proteins was revealed by isoelectric focusing than by SDS–PAGE. The pattern of proteins from isoelectric focusing revealed both acidic and basic proteins, some of them unique for a given potato variety.

INTRODUCTION

A large amount of potato fruit juice is obtained as a by-product in the potato starch industry. In Sweden the potato fruit juice, with a total solids content of 5·2%, contains approximately 2·5% protein (Nilsson *et al.*, 1988). At present potato fruit juice in Sweden is distributed on cultivated fields as a fertilizer. It is of interest to recover the potato proteins from potato fruit juice, not only because of their contribution to environmental problems, but also because potato proteins are considered to be amongst the most valuable vegetable proteins (Nuss & Hadziyev, 1980; Lindner *et al.*, 1981). The recovery of proteins from the effluents of potato starch mills is commonly carried out on an industrial scale by heat coagulation (Oosten, 1976; de Boer & Hiddink, 1977; Knorr *et al.*, 1977). Although this protein concentrate has a favourable amino acid content, it can only be used as animal feed since the proteins are denatured by the heat coagulation process. New techniques, however, such as membrane separation, make it possible to produce an undenatured protein concentrate of high quality (Eriksson & Sivik, 1976; Wojnowska *et al.*, 1982).

At the Lyckeby Stärkelson potato starch mill at Kristianstad, Sweden, efforts have been made to produce a native protein concentrate for human consumption from potato fruit juice using ultrafiltration. The purpose has also been to use a separation technique which, in the future, will make it possible to obtain proteins with unique qualities. This study, the characterization of proteins in potato fruit juice, is part of these efforts to produce a native protein concentrate. It was also of interest to ascertain whether there were variations in potato varieties grown at different times.

Potato fruit juices were prepared on a laboratory scale from four potato varieties: Prevalent, Senator, Saturna and Producent. The potato varieties prepared were from seed potatoes intended for the production of potatoes for the commercial manufacturing of potato starch.

The characterization of potato proteins was performed with the following separation techniques: gel filtration chromatography, sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and isoelectric focusing (IEF). Earlier results (of the authors, unpublished) showed that the molecular weights of the proteins, from the four varieties, varied between 150000 and 10000, which gave the separation range for the gel used during the first separation step, gel filtration chromatography.

MATERIALS AND METHODS

Raw material

The four potato varieties; Prevalent, Senator, Saturna and Producent were grown in 1987 and 1988. After

Food Chemistry 0308-8146/92/$05.00

harvest the potato tubers grown in 1987 were stored for between 1 and 5 months and potato tubers grown in 1988 were stored for 1–2 weeks at +7°C before the preparation of potato juice.

Preparation of potato juice

The potato tubers were washed, peeled and pureed in a food processor together with 25% MilliQ filtrated water and 1·0 g $NaHSO_3$/kg tubers. The slurry was strained through a filtering cloth and the juice was centrifuged for 20 min at 8000*g* to remove remaining starch granules and other coarse particles (Shomer *et al.*, 1982). The supernatant was lyophilized and stored at −20°C. The preparation of potato juices was performed at +7°C.

Chemical analyses

The nitrogen content, *N*, was determined according to Kjeldahl (AOAC, 1975) and the protein content was calculated as $N \times 6{\cdot}25$. The total solids content of the lyophilized potato juices was determined by the reduced weight after drying at 105°C until constant weight was achieved (AACC, 1962).

Pretreatment of potato juices

A 0·05 mol/litre phosphate buffer, pH 7·3, containing 0·2 mol/litre NaCl and 0·02% sodium azide (to avoid microbiological growth), was used to dissolve the lyophilized potato juices and the lyophilized potato protein fractions and as an elution buffer during gel filtration chromatography (Nakasone *et al.*, 1972). The lyophilized potato juices were dissolved in the buffer to a concentration of about 7·5 mg protein/ml. After slow stirring overnight at +7°C the samples were centrifuged at 5000*g* for 15 min and then filtered through Acrodisc 0·2 μm (Gelman Sciences, Michigan, USA) before gel filtration chromatography was performed.

Gel filtration chromatography

Gel filtration chromatography was performed on a 1·6 × 90 cm column of Sephacryl S-200 Superfine (Pharmacia LKB Fine Chemicals, Uppsala, Sweden). All protein separations took place at +7°C. The different protein fractions were collected with a fraction collector (Fraction Collector Frac-100, Pharmacia LKB, Uppsala, Sweden) which was controlled by a UV detection unit (280 nm). Samples were applied to the column in 4·0 ml portions and the elution was carried out at a flow rate of 18 ml/h. Protein fractions from all four potato varieties were lyophilized and stored at −20°C before further separation.

SDS–PAGE and isoelectric focusing

Polyacrylamide gel electrophoresis SDS and IEF were performed in Pharmacia's Phast System and Phast System Development Unit (a commercially available instrument system from Pharmacia LKB). The gels used were SDS PAA 8–25% gradient gel for SDS–PAGE and Phast Gel IEF, pH 5–8 for IEF.

To investigate whether the potato proteins existed as subunits or not, SDS–PAGE was performed both with and without the addition of β-mercaptoethanol. (The addition of β-mercaptoethanol breaks the intermolecular bonds of a protein).

Standards used during SDS–PAGE were bovine serum albumin (MW ≈ 66000), ovalbumin (MW ≈ 45000), β-lactoglobulin (MW ≈ 18400) and lysozyme (MW ≈ 14300) (Sigma Chemical Company, St Louis, USA). The marker protein β-lactoglobulin showed two peaks after separation on a Superose 12 HR 10/30 column (prepacked from Pharmacia LKB), which showed that it had formed a dimer with a molecular weight of 36800. The authors' previous results (unpublished) showed that dialysis of the protein fractions before separation on SDS–PAGE and IEF was not necessary.

RESULTS AND DISCUSSION

The proteins present in potato fruit juice, the byproduct from the potato starch process, ought to be the soluble groups of proteins albumin (soluble in water) and globulin (soluble in salt solution). This was observed during the laboratory preparation of potato fruit juice in this study. These soluble proteins are usually considered to be the largest groups of proteins present in potato tuber, constituting approximately 80% of the total protein (Seibles, 1979).

The lyophilized potato juices prepared from the four varieties, Prevalent, Senator, Saturna and Producent (grown in 1987 and 1988) varied in protein content from 35% to 49% and in total solids content from 89% to 95%.

Gel filtration chromatography

One fraction of the potato juice, constituting (approximately) less than 5% of the original sample was not redissolved. This fraction was not characterized.

Figure 1 shows the chromatogram of protein fractions from the prepared potato juice of Prevalent (grown in 1988). The chromatograms of Prevalent (grown in 1987), Senator (88) and Senator (87) were identical to the chromatogram shown in Fig. 1. Figure 2 shows the chromatogram of protein fractions from the prepared potato juice of Saturna (88). The chromatograms of Saturna (87), Producent (88) and

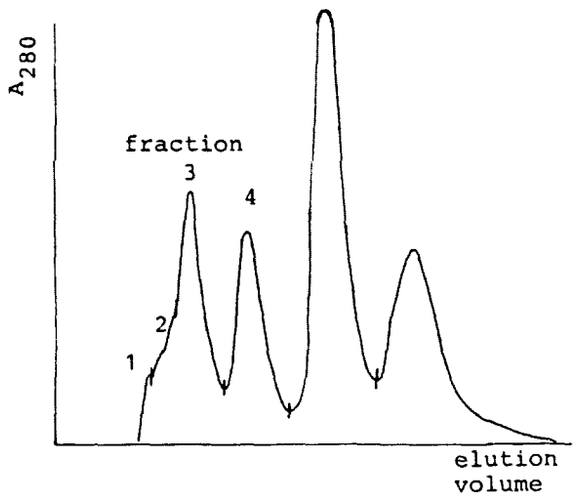

Fig. 1. Chromatogram of protein fractions from the prepared potato juice of Prevalent (88).

Producent (87) were identical to this chromatogram. Only the first two protein fraction peaks shown in the chromatograms (Figs 1 & 2) were of interest for collection for further separation using SDS–PAGE and IEF, while the last two peaks consisted of proteins with molecular weights of less than 10000, and of no interest regarding further separation in the present case. The first peak in the chromatograms was collected as three protein fractions (in the chromatograms shown as fractions 1, 2 and 3), while the second peak in the chromatogram was collected as one peak, fraction 4.

SDS–PAGE

Figures 3–6 show the SDS–PAGE patterns of the protein fractions prepared from the four potato varieties. Protein standards and their molecular weights are shown in Figs. 3–6. Figure 3(b)–(e) shows the patterns of the protein fractions 1–4 from Prevalent (88) when β-mercaptoethanol was added to the fractions before separation using SDS–PAGE. Protein fraction 3 (Fig. 3(d)) has three major bands corresponding to apparent molecular weights of 44 000 (dominant), 55000 and 66000, respectively. These three major bands were visible in protein fraction 2 (Fig. 3(c)) which also has a protein zone with a molecular weight of approximately 100000. Protein fraction 1 (Fig. 3(b)) shows similar zones of protein at molecular weights of 55000 and 66000. Furthermore, fraction 3 has a distinct protein pattern at a molecular weight of 10000. Protein fraction 4 (Fig. 3(e)) exhibits two major bands with molecular weights close to 20000 and one very distinct band at a molecular weight of 14000. Here too the zones of proteins at molecular weights of 55000 and 66000 are visible.

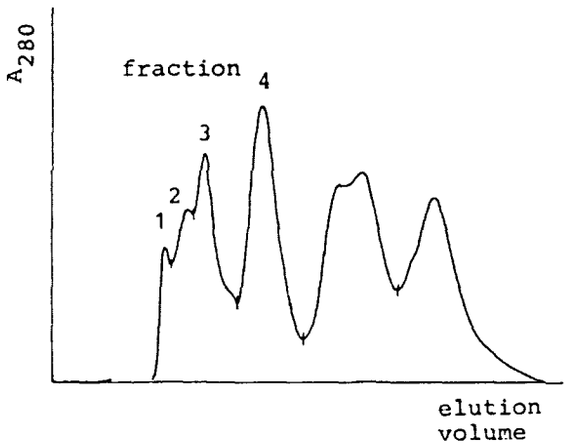

Fig. 2. Chromatogram of protein fractions from the prepared potato juice of Saturna (88).

Figure 4(b)–(e) shows the patterns of protein fractions 1–4 from Senator (88); Fig. 5(b)–(e) and Fig. 6 (b)–(e) show the patterns of protein fractions 1–4 from Saturna (88) and Producent (88), respectively. (All protein fractions contained β-mercaptoethanol.) These three potato varieties exhibit almost identical SDS–PAGE patterns of protein fractions 1–4 to those of Prevalent (88). One exception, however, is protein fraction 4 for both Senator (88) and Producent (88), where one more band was visible at a molecular weight close to 20000.

The SDS–PAGE patterns of protein fractions from the potato varieties grown during 1987 are not significantly different from the potato varieties grown during 1988, except for differences in concentrations of proteins in the various zones.

The SDS–PAGE protein patterns have a more complex appearance when β-mercaptoethanol was added to the samples before separation. This shows that the characterized proteins also exist, not only as subunits, but as polypeptides intermolecularly crosslinked by disulphide bonds (the addition of β-mercaptoethanol breaks the intermolecular disulphide bonds). The major bands with molecular weights of 55000 and 66000, present in all samples of protein fractions from the four potato varieties when β-mercaptoethanol was added, were not visible in the SDS–PAGE patterns when no β-mercaptoethanol was added. Furthermore, the major zones of proteins close to the molecular weight of 20000 showed one more band of proteins for protein fraction 4 from the four potato varieties when no β-mercaptoethanol was added. One possible explanation of this (apart from the fact that the proteins close to this molecular weight not only exist as subunits), could be that a short polypeptide was bound to one of the proteins and became hydrolysed when β-mercaptoethanol was added. Another explanation may be that this band of proteins consists of aggregated subunits no longer visible in this molecular weight range.

The results of the separation of potato proteins from the four potato varieties using SDS–PAGE show that the first peak in the chromatograms, (collected and separated further as fractions 1, 2 and 3), can be considered and treated as one protein fraction. The

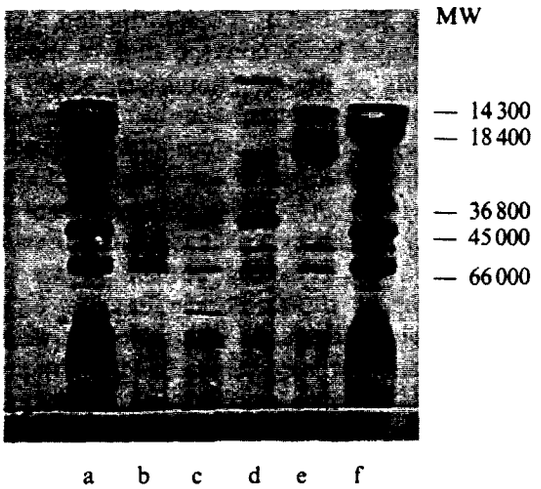

Fig. 3. SDS–PAGE Electrophores of proteins fractions from Prevalent (88): (a) and (f) standard proteins; (b) protein fraction 1; (c) protein fraction 2; (d) protein fraction 3; (e) protein fraction 4.

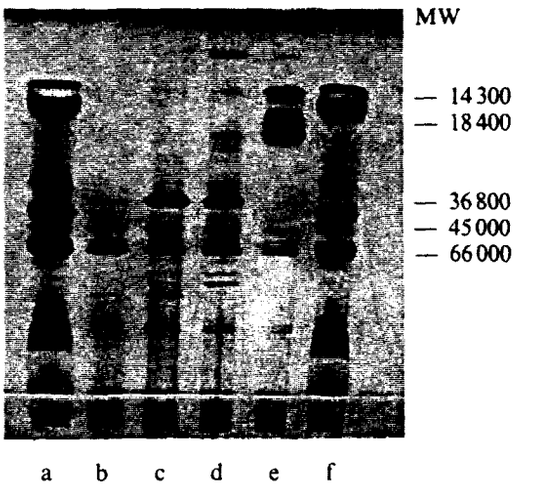

Fig. 5. SDS–PAGE Electrophoresis of protein fractions from Saturna (88). For (a)–(f) see Fig 3 caption.

only noticeable difference between the protein patterns for these fractions after separation with SDS–PAGE is that fraction 3 shows a band of proteins of approximate molecular weight 10000. One possible explanation of this may be differences in protein concentrations, leading to this zone not being visible in fractions 1 and 2.

Separation with SDS–PAGE shows that the proteins from the four potato varieties migrate to only a few molecular weight ranges which are quite similar for the different potato varieties studied. The major concentrations of proteins appeared at molecular weights of approximately 44000, 20000 and 14000 without exception for the potato varieties and year of harvesting characterized here. Also, pretreatment with β-mercaptoethanol showed no effect on these bands of proteins which means that these proteins probably exist as subunits.

IEF

Figure 7 shows the electrophoretic patterns of the four potato variety protein fractions on a pH gradient (pH 5–8). Based on the results from the second separation step, SDS–PAGE, protein fractions 1 and 2, from the four different potato varieties, were not analysed with IEF.

Protein fraction 3 from Prevalent (88) shows a number of protein bands with isoelectric points (pI) in the acidic range between pH 5·0 and pH 5·5 (Fig. 7(a)). The other three potato varieties (grown in 1988) show similar IEF patterns for protein fraction 3.

The IEF of protein fraction 4 from the various potato varieties (grown in 1988) shows a different IEF pattern from that of protein fraction 3. Protein fraction 4 from Prevalent (88) has major components, separated isoelectrically at pH values of approximately 6·0, 6·8, 7·2 and 7·8 (Fig. 7(b)). A number of minor components which are hardly visible with pIs between pH 7·0 and

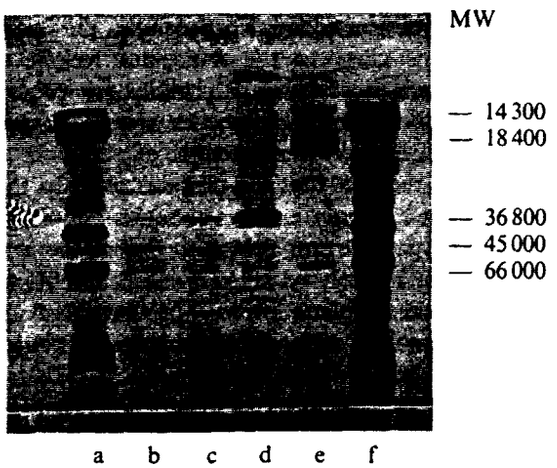

Fig. 4. SDS–PAGE Electrophoresis of protein fractions from Senator (88). For (a)–(f) see Fig. 3 caption.

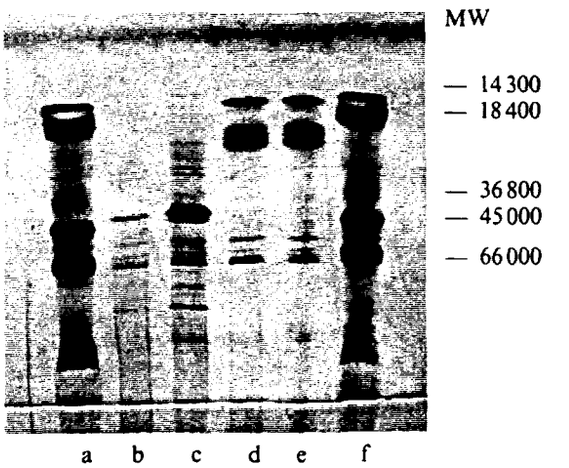

Fig. 6. SDS–PAGE electrophoresis of protein fractions from Producent (88). For (a)–(f) Fig. 3 caption.

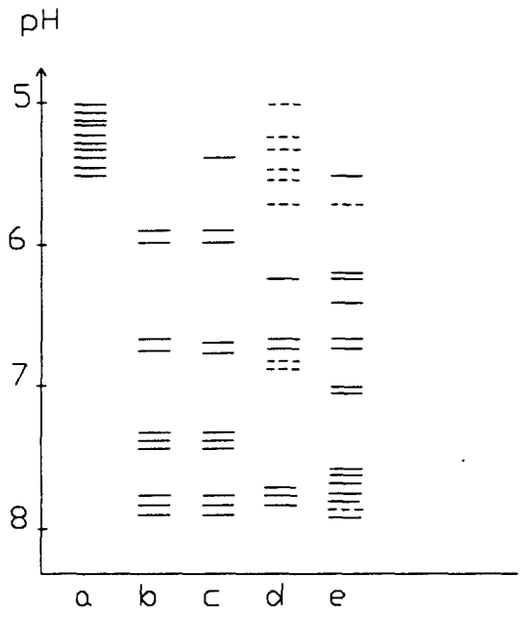

Fig. 7. Isoelectric focusing of protein fractions: (a) Prevalent (88), protein fraction 3; (b) Prevalent (88), protein fraction 4; (c) Senator (88), protein fraction 4; (d) Saturna (88), protein fraction 4; (e) Producent (88), protein fraction 4. (----) Weak band of protein; (———) strong band of protein.

8·0 can be seen. These were more obvious in the IEF pattern of protein fraction 4 from the potato variety Prevalent grown in 1987. Prevalent grown during 1987 shows a similar IEF pattern to Prevalent grown during 1988. Figure 7(c) shows the IEF pattern of protein fraction 4 from Senator (88). Except for a protein zone, visible at approximately pH 5·3, the major bands of protein fraction 4 from Senator (88) have similar pIs to Prevalent (88).

Senator grown during 1987 shows a number of minor bands with pIs in the pH range 7·0 to 8·0. It has an IEF pattern rather similar to that of Senator grown during 1988. The protein fraction 4 from Saturna (88) has major bands of proteins covering the whole pH range (pH 5·0–8·0), i.e. both acidic and basic proteins (Fig. 7(d)). However, there are more dominant bands with pIs at approximately pH 6·2, 6·7 and 7·6. The IEF pattern for the protein fraction 4 of Saturna, grown during 1987, shows a similar pattern of protein bands to Saturna, grown during 1988. Finally, protein fraction 4 of the potato variety Producent (88) has a number of major protein bands in the range of pH 5·5–8·0, most of them appearing, however, in the basic pH range (Fig. 7(e)). Protein fraction 4 from Producent grown during 1987, has a similar IEF pattern to the protein fraction 4 of Producent (88).

Conclusions

It seems likely that the zone of proteins at a molecular weight of 44000 may belong to the same family of proteins that Racusen and Foote (1980) referred to as 'patatin' and Kosier (1983) as 'tuberin'. Other evidence of this can be found in the results from the separation of fraction 3 from the various potato varieties using IEF, where a number of protein bands showed zones with isoionic points ranging from pH 5·0 to pH 5·5. These results are almost identical to the results from similar studies carried out by the authors mentioned above.

No major differences were observed between the IEF patterns from protein fraction 4 from all four potato varieties, both acidic and basic protein zones being visible. However, some differences do exist. Only the potato variety Senator showed a single band at approximately pH 5·3 and only the variety Saturna showed a population of acidic proteins. The potato variety Producent showed a distinct zone of protein at pH 5·8, which is not visible in the IEF patterns of the other three potato varieties.

Although the results of this study show that the four potato varieties Prevalent, Senator, Saturna and Producent contain similar groups of proteins when the soluble tuber proteins are separated on the basis of size by SDS–PAGE, the patterns of proteins, when the soluble tuber proteins are separated on the basis of charge by IEF, are more complex and differ for the varieties examined. This difference may be used for the identification of potato varieties (Kaiser *et al.*, 1974; Stegemann, 1980).

ACKNOWLEDGEMENTS

The authors wish to acknowledge Lyckeby Stärkelsen for financial support and Eva Thulin, Physical Chemistry 2, Lund University, for technical assistance and advice concerning the SDS–PAGE and IEF analyses.

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