ELECTROPHORETIC SEPARATION OF PROTEINS AND THEIR AMINO ACID COMPOSITION IN RAW AND PROCESSED POTATOES

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ABSTRACT

Potato tubers had 1.67% N/dry matter. Nitrogen was distributed in all anatomical regions of the tuber. Of the total N content, 43% was dialyzable-N and 32.9% true protein-N. The protein, by solubility fractionation, provided 67% albumin, 23% globulin, 1.4% prolamin and 9% glutelins. As revealed by sodium dodecyl sulfate electrophoresis (SDS-PAGE), the albumin had two major protein species, one of 45 × 10^3, the second of 12–25 × 10^3 daltons. Prolamin and glutelins contained protein bands coinciding in molecular weight with those of albumin and globulin.

Protein hydrolysis with 4 N methanesulfonic acid was superior to that of 6 N HCl. Amino acid composition of proteins was determined by ion-exchange column chromatography. Some minor losses in protein composition of potatoes occurred during processing.

INTRODUCTION

Potatoes are a good source of amino acids (Talley *et al.* 1983). Approximately half of the potato tuber nitrogen is protein. Potatoes supply proteins which are rated higher in quality than those of soybeans by some nutritionists (Eppendorfer *et al.* 1979; Rhoades 1982). The average total amino acid composition (free and bound amino acids) of cv. Russet Burbank (USA) and others was studied by Kaldy and Markaris (1972) and Rexen (1976). Osborne and Campbell (1896), Levitt (1951), Seibles (1979) and others found 46–48% albumin and 26–30% globulin. These data are similar to those for outer tuber layer protein extracts of

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Alberta-grown potatoes (Golan-Goldhirsh 1979). The Seibles (1979) fractionation procedure, and other similar procedures, should not be assumed to give sharp distinction between solubility classes but they should be considered useful for preliminary fractionation. The complexity and instability of potato tuber proteins tend to make distinctions based on solubility unreliable, because albumin and globulin fractions separated by solubility were not homogeneous (Seibles 1979). Although small amounts of prolamins and glutelins have been reported (Lindner *et al.* 1980–1981; Kapoor *et al.* 1975; Golan-Goldhirsh 1979), albumins and globulins are the major potato proteins. The molecular weight distribution of dormant tuber proteins by electrophoresis was studied by Smith (1986); Stegemann *et al.* (1973, 1979); Snyder *et al.* (1977); Seibles (1979); Racusen and Foote (1980); Nuss and Hadziyev (1980); Poduslo and Rodbard (1980) and Gatfield and Stute (1980). The major tuber protein "tuberin" was isolated, purified, N-terminal sequenced and antibodies made against it for quantitation (ELISA)(Mignery *et al.* 1984; Park *et al.* 1983).

The effect of processing on the amino acid composition of potatoes was studied recently by Golan-Goldhirsh (1986), Talley *et al.* (1983) and Maga and Sizer (1979). Nutrient assessment during processing is part of the study to develop a "Food Process Simulator" (Kozempel *et al.* 1982; Sullivan *et al.* 1985).

This paper reports on the fractionation and characterization of proteins from raw and processed potatoes and amino acid determination by ion-exchange chromatography and the differences of proteins in potatoes after processing.

MATERIALS AND METHODS

Medium size potato tubers (*Solanum tuberosum*, cv. Russet Burbank) grown in Alberta and obtained from I & S Produce Ltd. (Edmonton) were peeled and sliced. After soaking in 500 ppm $NaHSO_3$ solution to prevent enzymatic browning, slices were freeze-dried (model FFD-42-WS). Slices from the same batch were also subjected to two different cooking treatments: (1) precooking at 70 °C for 20 min, cooling in tap water for 20 min, and boiling for 30 min; (2) steaming for 30 min, followed by cooling in an air-blast freezer.

In each batch, one medium-size tuber was washed, peeled and diced. The cubes were wrapped in canvas, and squeezed in a hydraulic press at 24,000 psi. Tuber sap was collected.

Tuber protein was fractionated for amino acid analysis and electrophoretic studies by Golan-Goldhirsh's procedure (1979). Tuber sap was dialyzed against distilled deionized water at 4 °C. Nondialyzable and insoluble materials were centrifuged at 14,000 × g for 10 min at 4 °C. A few drops of toluene were added as a preservative to the supernatant (albumin fraction), while the precipitate was

resuspended in 0.02 M phosphate buffer (containing 5% NaCl) and stirred for 3 h. After centrifuging at 14,000 × g, the supernatant (globulin fraction) was dialyzed against distilled deionized water. The cell debris residue recovered from the canvas was extracted twice with 100 mL each of 70% ethanol, 0.1 M acetic acid, and 0.2% NaOH. The supernatants separated by centrifugation at each extraction step were designated as prolamin fraction, and acidic and basic glutelin fractions. The prolamin fraction was concentrated at 30 °C on a rotary evaporator. After dialysis against distilled deionized water, all five protein fractions obtained were freeze-dried, then kept at −20 °C.

In the albumin and globulin extraction procedure the cooked samples were freeze-dried and powdered, then suspended in a 0.02 M phosphate buffer, pH 7.5, containing 5% NaCl, stirred and centrifuged to recover the albumin and globulin fractions. These proteins were then dialyzed against distilled water at 4 °C, during which time the albumin remained soluble, while the globulin precipitated. In the latter procedure no SDS or mercaptoethanol was used and the temperature did not exceed 4 °C.

In order to track the recovery of nitrogen, aliquots in triplicate were withdrawn from each extraction step, and were subjected to the standard micro-Kjeldahl method (AOAC 1980). A conversion factor of 7.5, rather than 6.25, was more appropriate for calculating potato protein nitrogen (Desborough and Weiser 1974).

In addition to the extraction procedure based on the solubility of proteins (Golan-Goldhirsh 1979; Yamagata 1983) total proteins were extracted from freeze-dried powders of raw, steamed, precooked and boiled potatoes. Approximately 1 g of each sample was suspended in 5 mL of Tris-borate buffer (0.01 M Tris, 0.2 M boric acid; pH 7.1). After adding 250 mg SDS and 62 mg mercaptoethanol, the mixture was boiled for 6 min, then centrifuged at 14,000 × g for 20 min (Beckman J-21B). Sucrose was added (10% w/v) into the supernatant to make a dense solution, and the solution was stored at 4 °C. Similarly, 4 mg of protein sample, obtained by solubility fractionation, was treated with 0.5 mL of 2 mM $NaNO_3$ solution containing 2.5 mM EDTA and 2.5% SDS, and 0.1 mL of 2 M Tris-glycine buffer. The mixture was then heated in a boiling water bath for 5 min. Immediately after removal from the bath, 0.4 mL of 60% glycerol was added, the solution vortexed thoroughly, cooled to room temperature, and stored at 4 °C (Yamagata 1983).

Extracted potato proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Appropriate amounts of samples (75–500 μg) were applied to the top of polyacrylamide gel tubes. Electrophoresis was performed in an electrode buffer consisting of 0.2 M Tris-glycine buffer, pH 8.8, containing 0.1% SDS. Quantitation of bands resolved by SDS-PAGE was done by scanning the gel rods at 640 nm on a Beckman model 2400 spectrophotometer equipped with a scanning attachment. Myofibrillar proteins (myosin, M-protein,

α-actinin, actin, tropomyosin and RNase A) and ribonuclease A were used as protein markers (Yamagata 1983).

Albumin, globulin and basic glutelins were subjected to amino acid analysis (Golan-Goldhirsh and Wolfe 1979; Golan-Goldhirsh *et al.* 1982). In order to follow the extent of destruction of labile amino acids (half-cystine, methionine, tryptophan and tyrosine), three different hydrolytic procedures were used for comparison studies for each protein sample. Protein hydrolysis methods were:

(1) hydrolysis with 6 N HCl in the presence of 0.1% phenol to protect tyrosine;
(2) protein oxidation by performic acid prior to hydrolysis as in (1); and
(3) hydrolysis with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.

In method 2, half-cystine was determined as cysteic acid, and methionine as methionine sulfone.

The protein hydrolysates produced with HCl were analyzed on a Durrum D-500 analyzer (single column operation, DC-6A cation-exchange resin), while those hydrolysates with methanesulfonic acid were run on a Beckman 121MB analyzer (single column operation, AA-10 cation-exchange resin (Hugli and Moore 1972; Golan-Goldhirsh and Wolfe 1979; Yamagata 1983). Mean values of 8 determinations are reported in this study. Analysis of variance was done on the original data for each table. In this study all statistical data were determined by Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

Total nitrogen content is presented in Table 1. Statistical analysis showed that the whole tuber contained 1.67% N. High amounts of N were found in the cortex. The medulla was the next highest in N. The perimedulla N-content was lower, while the lowest content was in the vascular bundle.

Data for potato tuber protein fractionation according to solubility are given in Table 2. When fresh tuber was peeled and diced and, in the presence of phosphate buffer, subjected to high pressure using a laboratory hydraulic press, the sap collected contained 76.1% of the total nitrogen of the peeled tuber. This amount of total nitrogen may contain soluble free amino acid pool, amides, nucleotides, inorganic forms of nitrogen and a large proportion of protein bound nitrogen (albumin and globulin). The dialyzable nitrogen (all forms but protein nitrogen) was 43.2% of the total nitrogen of the tuber, while the nondialyzable protein nitrogen was 32.9% (i.e., 24.5% was water-soluble albumin and 8.4% salt-soluble globulin). The composition of the protein nitrogen was: 67% albumin, 23% globulin, 1.4% prolamin and 9% glutelins. The cake remaining after sap extraction retained 23.9% of the tuber total nitrogen. Treatment of the remaining cake was done by water and saline water (albumins and globulins).

TABLE 1.
TOTAL NITROGEN CONTENT OF THE MAJOR ANATOMICAL REGIONS OF THE POTATO TUBER CV. RUSSET BURBANK (ALBERTA)

	Weight of the whole tuber (%)	Dry matter (%)	Nitrogen[a] (%)
Whole tuber	100	24.25	1.67
Anatomical region:			
Cortex[b]	19.15	22.20	2.10
Vascular bundle + outer phloem	24.62	26.89	1.22
Perimedulla (inner phloem)	46.10	24.39	1.55
Medulla (pith)	10.07	20.72	1.92

[a]Determined by a Perkin-Elmer Model 240 B elemental analyzer.
[b]The cortex included the peel and the adjacent 5 mm thick layer of the tuber.
Average coefficient of variation (CV) = ±0.75

Nitrogen was not recovered in these fractions. Treatment of the cake with 70% ethanol gave 0.5% of the total nitrogen as prolamin. Treatment of the cake with 0.1% acetic acid followed by 0.2 N sodium hydroxide, gave 0.3% as acidic and 3.0% as basic glutelins, respectively. The residual nitrogen of the cake was 23% of the total nitrogen of the tuber. Treatment of the cake with water and saline water (albumins and globulins) did not recover nitrogen.

The electrophoretograms of potato tuber proteins and the corresponding scanning diagrams are given in Fig. 1. Freshly prepared myofibrillar proteins with added ribonuclease A (14×10^3 daltons) were used as standard reference proteins. The separation indicated little change in the protein patterns for raw, steamed or cooked tubers (Fig. 1). In all cases there were about 13 bands (Fig. 1). The band at 45×10^3 daltons was the major band in the higher molecular weight (MW) region for all samples (Table 3), confirming that it is the major protein of most potato cultivars (Racusen and Foote 1980). The lower MW

TABLE 2.
SEPARATION OF POTATO TUBER PROTEINS BY SOLUBILITY DIFFERENCES
(cv. Russet Burbank, Alberta)

Fraction	Total Nitrogen[a] (%)
Total tuber N	100.0
Tuber sap N	76.1
Dialyzable N	43.2
Nondialyzable N	
Albumin fraction	24.5
Globulin fraction	8.4
Tuber cake, pellet, residual N	23.9
70% Ethanol-soluble N	0.5
0.1% Acetic acid-soluble N	0.3
0.2% NaOH-soluble N	3.0

[a]Average values of two batches of potatoes supplied by I & S Produce, Edmonton.

region (12–25 × 10^3 daltons) was the dominant region, and its intensity was not altered by steam cooking or cooking in water (Table 3). This is in agreement with the findings of Nuss and Hadziyev (1980).

The findings related to these two major bands (12–25 and 45 × 10^3 daltons) strongly suggest that during steam cooking in water there is no appreciable protein leaching and other interactions between proteins and other compounds (Kozempel *et al.* 1982; Sullivan *et al.* 1985) from peeled and sliced tubers.

Densitometric scan of the electrophoretically separated protein fractions is shown in Fig. 2. The albumin fraction had 10 distinct bands, the four major ones being at 45, 12, 20 and 25 × 10^3 daltons. It is clear that 12, 25 and 45 × 10^3 dalton bands coincide with the major constituents detected among the tuber total proteins. Intermediate bands were at 29, 35, 52 and 100 × 10^3 daltons (Stegemann *et al.* 1973; Snyder *et al.* 1977). A few additional minor bands were present in the region above 140 × 10^3 daltons.

The globulin fraction of the tuber showed a major band in the low MW range at 25 × 10^3 daltons, which coincided with that of albumin. An additional major band at 22 × 10^3 daltons and five distinct intermediate bands at 12, 26, 30, 45 and 55 × 10^3 daltons were present, along with a few minor bands above 83 × 10^3 daltons.

The prolamin pattern contained similar bands in molecular weight to globulin. Therefore, proteins with different solubility had similar molecular weights. There appear to be only trace levels of prolamin in the tuber.

Acidic and basic glutelins (Fig. 2) contained the major low MW protein of 25 × 10^3 daltons, while the basic glutelins had a distinct component at 150 × 10^3 daltons. The acidic glutelins retained the 12 × 10^3 daltons. Both glutelins had intermediate or minor bands at 32 and 45 × 10^3 daltons.

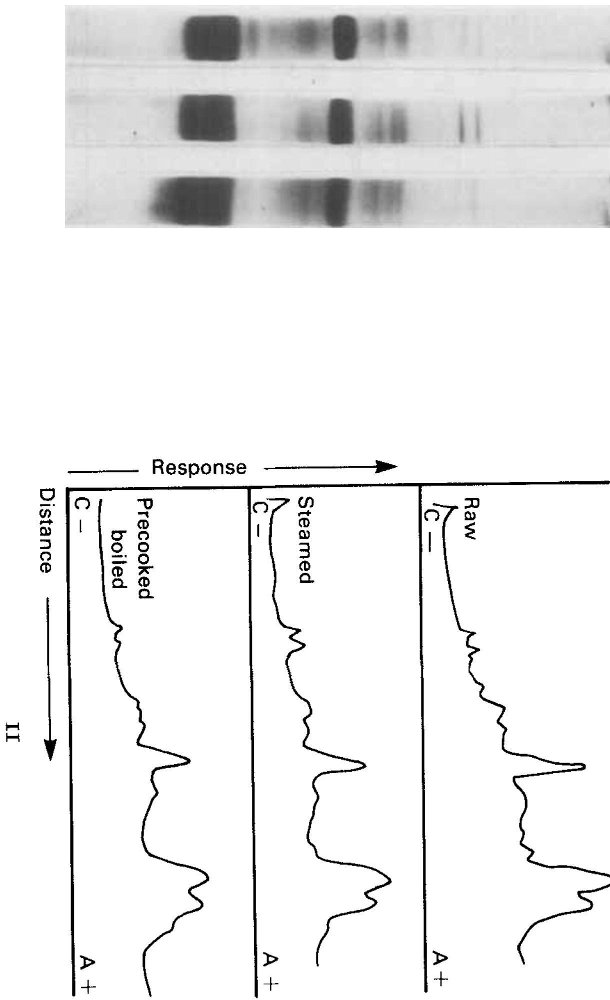

FIG. 1. SEPARATION OF PROTEINS IN RAW(a), STEAMED(b), AND PRECOOKED-BOILED(c) POTATO TUBER
(I)-electrophoretograms; (II)-scanning diagrams of the electrophoretograms.

TABLE 3.
MOLECULAR WEIGHTS FOR PROTEIN FRACTIONS OF POTATO TUBERS

Sample	Major	Intermediate (dalton × 10^3)	Minor
Tuber total proteins[a]	12;25;45	32;28;58;60;105;120	30;36;93;100
Raw tuber protein fractions			
Albumin	12;25;28;45;	29;35;52;100;	140;150
Globulin	22;25	12;26;30;45;55	83,90;115;140;150
Prolamin	12;25	45	35;67;140;150
Glutelins			
basic	25;150	32;45;	12;28;40;85;100
acidic	25	12;20	32;45;150

[a]The same values were obtained for the proteins from raw, steamed or precooked and boiled potatoes

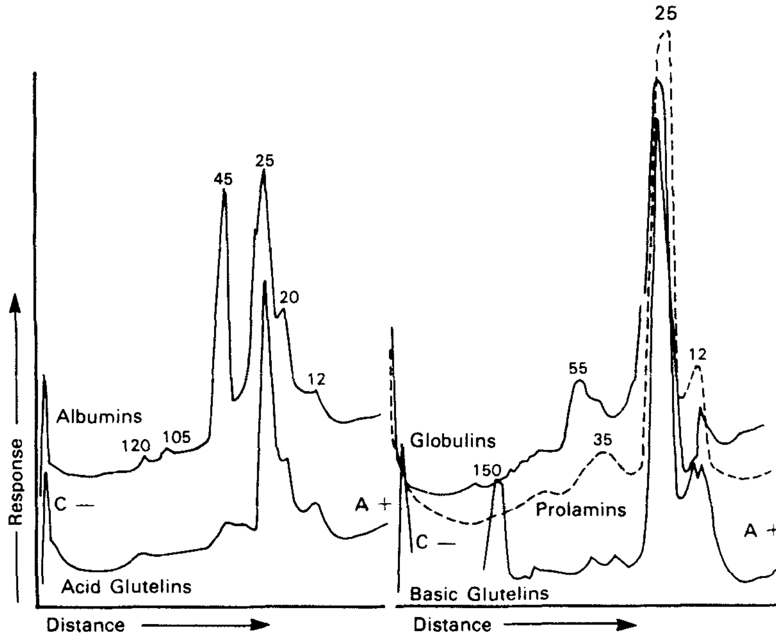

FIG. 2. DENSITOMETRIC SCAN OF ELECTROPHORETICALLY SEPARATED PROTEIN FRACTIONS

The lower MW albumin comprised 47% of the total albumins and the 45 × 10^3 daltons protein close to 30%. The lower MW globulin component was at least 60% of the total globulin proteins. This lower MW component also made up 60% of the total prolamin and 45 and 56%, respectively, of the total proteins of acidic and basic glutelins.

When MW distributions reported by different authors are compared (Fig. 3), it is apparent that there are two regions where most of the major bands are located. One is from 12–20 × 10^3 and the other is from 33–45 × 10^3 daltons.

The amino acid composition of the albumins, globulins and basic glutelins by different hydrolytic procedures are available, but are not given in this report. Only data obtained using the 6 N HCl, phenol hydrolysates and the amount of cystine, methionine and tryptophan by different hydrolytic procedures are

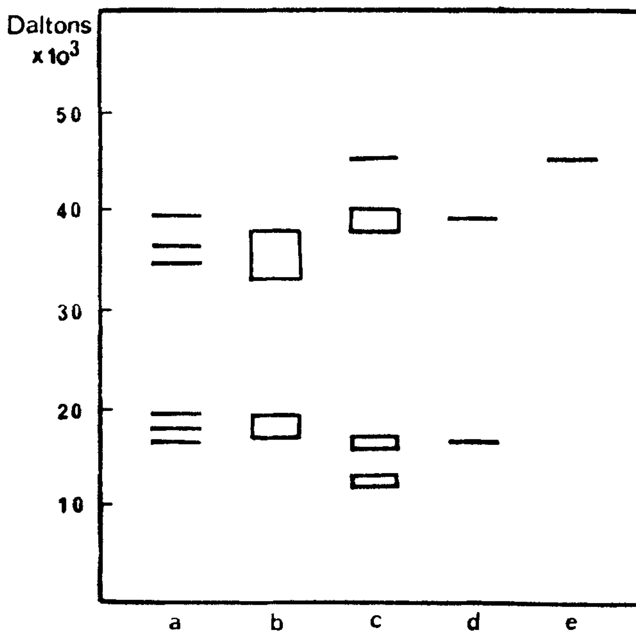

FIG. 3. MAJOR POTATO PROTEIN BANDS FOR SDS-PAGE AS REPORTED BY SEVERAL AUTHORS

a. Stegemann *et al.* 1973
b. Snyder *et al.* 1977
c. Nuss and Hadziyev 1980
d. Seibles 1979
e. Racusen and Foote 1980

shown in Table 4. The individual amino acid values obtained using method 2 were significantly ($p < 0.05$) lower than corresponding values using methods 1 or 3. This is in agreement with some other researchers (Golan-Goldhirsh *et al.* 1982; Golan-Goldhirsh and Wolfe 1979; Sullivan *et al.* 1985) that several of the 18 amino acids analyzed, such as proline, were extensively degraded, and tyrosine and tryptophan were completely destroyed. However, sulfur-containing amino acids were recovered in good yields as cysteic acid and methionine sulfone.

TABLE 4.
AMINO ACID COMPOSITION OF POTATO ALBUMINS, GLOBULINS AND BASIC GLUTELINS FROM RAW TUBERS OF CV. RUSSET BURBANK (ALBERTA). THE RESULTS ARE IN g/16 g CRUDE NITROGEN. (MEAN AVERAGE 8 RUNS)

	Protein Fractions:					
	Experimental data[c]			Literature Data[b]		
Amino Acid[a]	Albumins	Globulins	Basic Glutelins	Albumins	Globulins	Basic Glutelins
Asx	11.21	12.86	12.86	13.94	13.44	13.71
Thr	5.63	4.75	5.38	4.08	4.67	4.39
Ser	5.40	5.70	6.74	5.89	4.67	5.29
Glx	12.03	8.73	12.58	10.56	11.95	12.55
Pro	4.60	6.24	3.83	4.81	4.59	4.55
Gly	4.67	5.96	6.06	6.57	4.56	5.17
Ala	5.29	3.00	6.07	5.74	5.24	5.94
½ Cys	1.72	2.60	0.00	-----	-----	-----
½ Cys[d]	2.73	4.82	2.62	-----	-----	-----
½ Cys[e]	2.03	4.83	1.01	-----	-----	-----
Val	5.87	8.39	7.14	4.39	5.08	4.66
Met	1.63	1.08	0.00	2.01	2.42	2.07
Met[d]	1.63	1.19	2.02	-----	-----	-----
Met[e]	1.05	0.67	0.25	-----	-----	-----
Ile	4.87	5.84	5.82	4.45	4.40	4.10
Leu	9.76	7.78	11.84	7.66	7.24	8.53
Tyr	4.57	4.80	0.00	7.91	6.25	4.94
Phe	5.10	6.16	5.23	5.34	5.02	4.94
His	2.13	2.24	3.07	1.94	2.07	2.17
Lys	7.99	7.09	7.41	7.34	7.86	7.09
Trp	0.00	0.00	0.00	1.40	1.60	1.29
Trp[d]	0.00	0.00	0.00	-----	-----	-----
Trp[e]	0.59	0.40	0.57	-----	-----	-----
Arg	5.84	5.09	4.69	4.46	4.36	3.97

[a]Amino acids are listed according to their elution sequence on ion-exchange columns of a single-column automatic amino acid analyzer.
Half-cystine and methionine were determined as cysteic acid and methionine sulfone, respectively.
[b]Kapoor *et al.* (1975)
[c]6 N HCl, 0.1% phenol.
[d]6 N HCl, 0.1% phenol, performic acid.
[e]4 N methanesulfonic acid + 0.2% 3-(2-aminoethyl)indole
Average coefficient of variation (CV) = ±0.93

When hydrolysis was conducted by method 3, the values for amino acid contents were similar to those of method 1. Exceptions were in S-containing amino acids, tyrosine and tryptophan which were significantly ($p < 0.005$) higher in content using method 3 than in method 1. However, recoveries of cystine by method 3 were comparable ($p < 0.05$) to those obtained by oxidation method 2. Small peaks corresponding to cysteic acid (first peak, with the lowest retention time) were recorded, suggesting that even use of methanesulfonic acid cannot avoid oxidative losses of the amino acids present in proteins. Tryptophan, regardless of protein fraction, was separated, between ammonia and arginine peaks, but much lower than those found by the Spies and Chambers (1949) method as applied by Kapoor *et al.* (1975).

The results of Kapoor *et al.* (1975) for albumin, globulin and glutelin tuber proteins which are shown in Table 4, provided similar amino acid profiles. This was especially true for the essential amino acids (methionine, threonine, valine, lysine, isoleucine, phenylalanine, histidine and arginine and for nonessential amino acids (alanine, aspartic and glutamic acids, valine and serine). These authors did not determine half-cystine contents.

In this study glutamic acid and half-cystine contents differed in two proteins. In albumin, glutamic acid was 13.0 (g/16 g protein-N) and only 9.0 in globulin. The half-cystine content in albumin (2.03) was significantly less ($p < 0.005$) than half that found in globulin (4.83). Significant differences ($p < 0.005$) were also found in alanine contents (5.6 in albumin and 3.44 in globulin). A reverse trend was found ($p < 0.005$) with valine (5.32 in albumin and 7.43 in globulin). These differences might be expected, since, as mentioned earlier in the SDS-PAGE section, there was a distinct major protein species at 45×10^3 daltons in albumin, but not a major protein species in globulin. The similarity of basic glutelin amino acid composition to that of albumin, with the exception of half-cystine and methionine, was not expected. Glutelin SDS-PAGE electrophoretograms contained a major protein species at 150×10^3 daltons and an intermediate protein at 45×10^3 daltons. Yet to be clarified is whether or not the similarity in amino acid composition is merely a reflection of the aggregation of the 45×10^3 dalton species into the 150×10^3 dalton species.

REFERENCES

AOAC. 1980. *Official Methods of Analysis.* Association of Official Agricultural Chemists, Washington, D.C.

DESBOROUGH, S. and WEISER, C.J. 1974. Improving potato protein. I. Evaluation of selection techniques. Am. Potato J. *51*, 185.

DUNCAN, D.B. 1955. Multiple range and multiple F. tests. Biometrics *11*, 1.

EPPENDORFER, W.H., EGGUM, B.O. and BILLE, S.W. 1979. Nutritive value of potato crude protein as influenced by manuring and amino acid composition. J. Sci. Food Agric. *30*, 361.

GATFIELD, I.L. and STUTE, R. 1980. Studies on the noncoagulable nitrogenous material present in potato juice. Presence of peptides and glucopeptides. Z. Lebensm. -Unters. Forsch. *170*, 429.

GOLAN-GOLDHIRSH, A. 1979. Ph.D. Thesis. Department of Food Science, University of Alberta, Edmonton, Alberta.

GOLAN-GOLDHIRSH, A. 1986. Effect of the add-back process on the free amino acid pool of potatoes. Z. Lebensm. -Unters. Forsch. *182*(1), 29.

GOLAN-GOLDHIRSH, A., HOGG, A.M. and WOLFE, F.H. 1982. Gas chromatographic analysis of the free amino acid pool of the potato and gas chromatography mass-spectrometry identification of γ-aminobutyric acid and ornithine. J. Agric. Food Chem. *30*, 320.

GOLAN-GOLDHIRSH, A. and WOLFE, F.H. 1979. Gas chromatographic analysis of protein amino acid N-heptafluorobutyryl isopropyl esters. Can. Inst. Food Sci. Technol. J. *12*, 123.

HUGLI, T.E. and MOORE, S. 1972. Determination of the tryptophan content of proteins by ion-exchange chromatography of alkaline hydrolysates. J. Biol. Chem. *247*, 2828.

KALDY, M.S. and MARKAKIS, P. 1972. Amino acid composition of selected potato varieties. J. Food Sci. *37*, 375.

KAPOOR, A.C., DESBOROUGH, S.L. and LI, P.H. 1975. Potato tuber proteins and their nutritional quality. Potato Res. *18*, 469.

KOZEMPEL, M.F., SULLIVAN, J.F., DELLA MONICA, E.S., EGOVILLE, M.J., TALLEY, E.A., JONES, W.J. and CRAIG, JR., J.C. 1982. Application of leaching model to describe potato nutrient losses in hot water blanching. J. Food Sci. *47*(5), 1519.

LEVITT, J. 1951. The isolation and preliminary fractionation of proteins from dormant and growing potato tubers. Plant Physiol. *26*, 59.

LINDNER, P., KAPLAN, B., WEILER, E. and BEN-GERA, I. 1980–81. Fractionation of potato juice proteins into acid-soluble and acid-coagulable fractions. Food Chem. *6*, 323.

MAGA, J.A. and SIZER, C.E., 1979. The fate of free amino acids during the extrusion of potato flakes. Lebensm. Wiss. U. Technol. *12*(1), 13.

MIGNERY, G.A., PIKAARD, C.S., HANNAPEL, D.J. and PARK, W.D. 1984. Isolation and sequence analysis of cDNAs for the major potato tuber protein, patatin. Nucleic Acids Res. *12*(21), 7987.

NUSS, J. and HADZIYEV, D. 1980. Effect of heat on molecular weight and charge of potato sap proteins. Can. Inst. Food. Sci. Technol. J. *13*, 80.

OSBORNE, T.B. and CAMPBELL, G.E. 1896. The proteids of potato. J. Am. Chem. Soc. *18*, 575.

PARK, W.D., BLACKWOOD, C., MIGNERY, G.A., HERMODSON, M.A. and LISTER, R.M. 1983. Analysis of the heterogeneity of the 40,000 molecular weight tuber glycoprotein of potatoes by immunological methods and by NH_2-terminal sequence analysis. Plant Physiol. *71*, 156.

PODUSLO, J.F. and RODBARD, D. 1980. Molecular weight estimation using sodium dodecyl sulfate-pore gradient electrophoresis. Anal. Biochem. *10*, 394.

RACUSEN, D. and FOOTE, M. 1980. A major soluble glycoprotein of potato tubers. J. Food Biochem. *4*, 43.

REXEN, B. 1976. Studies of protein of potatoes. Potato Res. *19*, 189.

RHOADES, R.E. 1982. The incredible potato. National Geographic, *161*, 668.

SEIBLES, T.S. 1979. Studies on potato proteins. Am. Potato J. *56*, 415.

SMITH, D.B. 1986. Variation in the electrophoretic band pattern of tuber proteins from somaclones of potato. J. Agric. Sci. *106*(2), 427.

SNYDER, J., DESBOROUGH, S. and HOLM, D. 1977. Accumulation of protein, non-protein nitrogen and starch during tuber growth of three potato cultivars. Am. Potato J. *54*, 545.

SPIES, J.R. and CHAMBERS, D.C. 1949. Chemical determination of tryptophan in proteins. Anal. Chem. *21*, 1249.

SULLIVAN, J.F., KOZEMPEL, M.F., EGOVILLE, M.J. and TALLEY, E.A. 1985. Loss of amino acids and water soluble vitamins during potato processing. J. Food Sci. *50*, 1249.

STEGEMANN, H. 1979. Characterization of proteins from potatoes, and the "Index of European Varieties". In *The Biology of Taxonomy of Solonaceae*, (J.G. Hawkes, R.N. Lester and A.D. Skelding, eds.) Linnean Soc. Symp. Ser. No. 7, 279.

STEGEMANN, H., FRANCKSEN, H. and MACKO, V. 1973. Potato Proteins: genetic and physiologic changes, evaluated by one- and two-dimensional polyacrylamide gel-technique. Z. Naturforsch. *28c*, 722.

TALLEY, E.A., TOMA, R.B. and ORR, P.H. 1983. Composition of raw and cooked potato peel and flesh: amino acid content. J. Food Sci. *48*, 1360.

YAMAGATA, S. 1983. M.Sc. Thesis. Amino acids and proteins of raw and granulated potatoes. Dept. of Food Science, University of Alberta, Edmonton, Canada.