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Full Name of Author — Nom complet de l'auteur

Shin YAMAGATA

Date of Birth — Date de naissance

December 28, 1956

Country of Birth — Lieu de naissance

JAPAN

Permanent Address — Residence fixe

S.24, W.11, Chuo-ku
Sapporo, Hokkaido
JAPAN, 064

Title of Thesis — Titre de la these

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Name of Supervisor — Nom du directeur de these

Dimitri HADZIYEV

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Shin Yamagata

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AMINO ACIDS AND PROTEINS OF RAW AND GRANULATED POTATOES

by

Shin Yamagata

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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(SIGNED) ...Shin Yamagata.....

PERMANENT ADDRESS:

S24, W.11

Chuo-ku, Sapporo

Hokkaido, Japan 064

DATED ...Oct. 7,.....

1983

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **AMINO ACIDS AND PROTEINS OF RAW AND GRANULATED POTATOES** submitted by **Shin Yamagata** in partial fulfilment of the requirements for the degree of **Master of Science** in **Food Chemistry**.

.....

Supervisor

.....

Co-Supervisor

.....

.....

Date: September 9, 1983

ABSTRACT

Potato tubers were found to have 1.67% N and 1.799 ppm S on a dry matter basis. The N and S were distributed in all anatomical regions of the tuber. Of the total N content, dialyzable-N was 43% and true protein-N 32.9%. The latter provided 67% albumin, 23% globulin, 1.4% prolamin and 9% glutelins by solubility fractionation. As revealed by SDS-PAG electrophoresis, albumin had two major protein species, at 45 and 20-25 x 10^3 daltons, and globulin one, at 25 x 10^3 daltons. Prolamin and glutelins contained protein bands coinciding with those of albumin and globulin. Amino acid composition of proteins, as assessed by both gas-liquid and ion-exchange chromatography, was similar. In this analysis protein hydrolysis was in 4 N methanesulfonic acid, instead of 6 N HCl. SDS-PAG electrophoresis provided evidence that fractionation based on solubility is an unreliable method for tuber proteins. Total amino acid content and composition of raw and cooked tubers were determined by gas-liquid and ion-exchange column chromatography. Results were similar. Attempts to assess tryptophan levels in proteins (and whole tuber) by colorimetry, using the glyoxylic acid-tryptophan chromophore reaction, gave inflated results. Cystine + cysteine contents in raw and cooked tubers were assessed successfully colorimetrically using Ellman's reagent. Amino acid composition of potatoes processed by a Freeze-Thaw granule technique was accurately assessed only by ion-exchange chromatography.

These results provided evidence that the granule process has little detrimental affect on total amino acids and their composition.

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I. INTRODUCTION

When automatic amino acid analyzers equipped with ion exchange columns are used errors due to destruction of some amino acids during hydrolysis of proteins cannot be avoided. Averages of 52% methionine and 62% cysteine/cystine, and up to 90% tryptophan can be destroyed by conventional potato protein analysis.

The effects of potato flake processing on amino acid destruction have been reported in the literature. The destruction was doubled when the flakes were processed with emulsifiers as additives. However, amino acid losses and the effect of emulsifiers have not been reported for a dehydrated granule process. It has been suggested that dehydrated granules be enriched with L-methionine and some other amino acids in order to improve the nutritional protein value of the end product. Furthermore, the export of dehydrated granules for snack food processing has introduced a demand for a high content of methionine since during deep frying methionine is converted to an aldehyde, methional, via a Strecker degradation mechanism. A methional level of 10^{-6} M can enhance the aroma of extruded French fries, and potato rings, balls and "pringles".

Therefore, flavoring aspects and, partly, nutritional aspects have provided the impetus for the Alberta potato dehydration industry to support the acquiring of knowledge of amino acids, particularly sulfur-containing acids and tryptophan, in Alberta-grown raw and processed granulated

potatoes.

This project involves amino acid determination by gas-liquid chromatography (using the N-heptafluorobutyryl derivative of amino acid isopropanol esters) and by methods of ion exchange chromatography. Determination of protein-bound amino acids includes the protein fractions: albumin, globulin, prolamins and acidic and basic glutelins. Cysteine/cystine was assayed using Ellman's reagent after cystine was reduced by Na-borohydride, and tryptophan colorimetrically using glacial acetic acid + Fe^{+3} and conc. sulfuric acid. The above procedures were found to be easy, reproducible and rapid, hence they will be recommended as standard methods of quality control for the potato dehydration industry.

II. LITERATURE REVIEW

A. Potato Tuber Proteins

Protein and Amino Acid Levels and Composition in Potato Tubers as Affected by Agricultural Practices

A thorough investigation of the effect of N, P and K fertilization on potato tuber free amino acids and proteins was made by Mulder and Bakema (1956). They separated protein from the soluble non-protein (free amino acid) fraction by heat coagulation of potato sap, hydrolyzed the protein with 6 N HCl and separated the amino acids by paper chromatography. The main conclusion of the study was that protein levels are affected by fertilizer treatment, but the amino acid composition of the protein (albumin + globulin) is not affected. However, the composition and level of the non-protein-N fraction were greatly affected by mineral nutrition and climate.

Ample N fertilizer levels provided a high content of non-protein-N in which amides were the predominant constituents (70-75% of the total non-protein-N), with glutamine content being higher than that of asparagine. P- and K-deficiencies in soil brought about an increase in protein content and particularly an increase in soluble non-protein-N. The contribution of amides to the latter fraction was considerably higher than in tubers grown under normal PK supply. With P-deficiency alone asparagine was

dominant, while with K-deficiency asparagine and glutamine contributed equally to the amide-N content.

Fertilizer treatments mostly affected the free amino acids: tyrosine, glutamic and γ-aminobutyric acid (which was absent in the protein fraction) and arginine. K-deficiency increased, while P-deficiency decreased, the content of tyrosine. The opposite was found for glutamic acid. Ample N supply in soil considerably increased the contents of arginine and γ-aminobutyric acid, the latter being too low in P-deficient tubers. In all cases, when amides were excluded, arginine and glutamic and γ-aminobutyric acids accounted for more than 50% of the non-protein-N.

Recent investigations (Eppendorfer *et al.*, 1979; Talley, 1983) essentially confirmed the above findings. Klein *et al.* (1980) re-examined the effect of P-fertilization on the contents of protein and non-protein N. At 50 kg P/ha treatment they found increased contents of N in both fractions when compared to a control (P=0). However, when 112 or 168 kg P/ha were applied, both N fractions decreased and were lower even than at 50 kg P/ha.

Of interest was the finding that free amino acid changes are accompanied by changes of tuber nitrate content at high N-fertilization levels. Augustin *et al.* (1977) proved that nitrate content at high N levels is also highly correlated with irrigation. When optimum irrigation was applied, nitrate-N increased from 82-122 ppm per tuber on a dry matter basis to 192-285 ppm, while in soils with no or

deficient irrigation it was increased to 786 ppm and up to 1200 ppm in some tubers.

In conclusion, all the data reviewed strongly suggest that free amino acid composition of potato tuber or its comparison to other data is rather meaningless unless the fertilizer, climate and cultivar data are also provided.

B. Potato Tuber Protein Fractionation

Solubility of protein is affected by pH, ionic strength, temperature and dielectric constant of solvents. General methods for separation and classification of plant proteins were established by combining different solvent extractions. Proteins soluble in distilled water, dilute salt solution, 70% alcohol, and dilute acid or alkaline solutions are classified as albumin, globulin, prolamin and glutelin fractions, respectively. Similar solvent extraction schemes have been used for isolating plant proteins on a preparative scale (Bietz, 1979).

The first potato protein characterized by solubility was a globulin named "Tuberin" (Osborne and Campbell, 1896). Groot *et al.* (1947) reported another protein, "Tuberinin", which was more hydrophylic than Tuberin and was albumin-like. The ratio of Tuberin to Tuberinin was 3:7.

Albumin and globulin fractions, further studied by various workers, have been somewhat contradictory. Levitt (1951) extracted two-thirds of the potato protein with a dilute salt solution. This crude protein extract was

precipitated with ammonium sulfate, resuspended and dialyzed. As opposed to the data from Groot *et al.* (1947), this procedure gave almost equal quantities of albumin and globulin fractions. Similar work by Nakasone *et al.* (1972) also showed a 1:1 ratio of these two fractions. However, Lindner *et al.* (1960) reported 4% albumins and 78% globulins (further classified in slightly and readily soluble fractions; 1.4 and 76.4%, respectively).

Kapoor *et al.* (1975) compared two fractionation procedures to clarify the uncertainty of fractionation. The author concluded that Tuberin extracted by Lindner's (1960) method was highly contaminated by albumin. It was probable that during preparation some of the albumin was denatured, thus becoming insoluble. Denaturation was also observed by Nakasone *et al.* (1972). They reported that, after salting-out with ammonium sulfate, potato protein extract became partly insoluble in salt solution. The alternative method used by Kapoor *et al.* (1975) did not involve salting-out precipitation.

Seibles (1979) in his study on potato proteins fractionated tuber proteins by exhaustive dialysis of the potato sap against water. This resulted in a water-soluble fraction (albumin) and a water-insoluble fraction, which subsequently proved to be soluble in 5% K_2SO_4. The water-insoluble fraction (globulin) was about one-fourth of total potato sap protein. Using a similar dialysis procedure, Levitt (1951) obtained nearly equal amounts of

globulin and albumin, whereas Kapoor *et al.* (1975) found 46-48% albumin and 26-30% globulin. The latter data are similar to those on outer tuber layer protein extracts of Alberta-grown potatoes (Golan-Goldhirsh, 1979). Hence, Seibles (1979) procedure and others like it should not be assumed to give sharp distinction between solubility classes, but it should be considered useful for preliminary fractionation. It appears that the complexity and extreme instability of potato tuber proteins tend to make distinctions based on solubility unreliable. Therefore, as stated by Seibles (1979), potato tuber albumin and globulin are terms used strictly for convenience.

Preparative density gradient isoelectric focusing in an LKB column with a volume of 110 ml provided further evidence that albumin and globulin fractions separated by solubility are not homogeneous (Seibles, 1979). Electrofocusing within a pH range of 3.5-10 in a linear glycerol density gradient (0-60% w/v) provided evidence that most of the components of albumin and globulin have isoelectric points between pH 4-5.2, i.e., they are acidic in nature. Electrofocusing of globulin fractions in a narrow pH range of 4-6 provided three distinct bands. They had apparent isoelectric points of 4.2, 4.4 and 5.3 and had similar amino acid compositions. Although small amounts of prolamins and glutelins have been reported (Lindner *et al.*, 1960; Kapoor *et al.*, 1975; Golan-Goldhirsh, 1979), albumins and globulins are the major potato proteins.

Two other fractionation methods based on solubility are acid coagulation (Levitt, 1951; Lindner *et al.*, 1980-81) and steam coagulation (Gatfield and Stute, 1980)

The effect of tuber size on protein content was studied by Desborough and Weiser (1974). Tuber weights ranged from 2.6 to 172.9 g and protein (Nx7.5) from 3.2 to 20.1%. The correlation coefficient between tuber fresh weight and protein content was -0.126. Arbitrary division of tubers into classes by protein content and tuber size could not effectively correlate fresh weight with protein content. In their related experiment the average protein contents of the cortical, medullary and pith regions of cv. Norchip were 6.0, 5.3 and 5.8%. These tissues accounted for 95% of the total fresh weight. Hence, the protein distribution within the tuber did not appear to be tissue specific.

C. Potato Tuber Protein Characterization

Plant protein extracts are usually purified and/or characterized by chromatographic techniques (Bietz, 1979). The separation principle of the chromatography can be based on size, charge, partition between liquid phases, adsorption, specific interactions, etc. Gel filtration separates proteins by size, whereas ion-exchange chromatography separates by charge. Electrophoresis can separate by size and/or by charge, depending on the technique employed (Deyl *et al.*, 1979). Because of its importance in the field of protein research, this technique

is reviewed in detail.

Electrophoretic Techniques

Electrophoresis refers to the process of charged macromolecules migrating toward an oppositely charged electrode under the influence of an electric field. Carboxylic (α, β, γ), amino (α, ω), imidazole, sulfhydryl, phenoxy and guanidinium ionic groups contribute to net charge, thus regulating the migration speed (Catsimpoolas, 1980).

As early as 1947, Groot *et al.* separated two potato protein components by Tiselius moving-boundary electrophoresis. They concluded that tuberin and tuberinin were the major components of potato protein. However, the resolution of this technique was not sufficient to reveal the heterogeniety of potato proteins.

Zone electrophoresis, using paper and gel supports, was developed and showed better resolution. In particular, the introduction of polyacrylamide gel electrophoresis (PAGE) extensively improved separation (Raymond and Weintraub, 1959). In this system, the effect of molecular sieving as well as difference in charge density of molecules were related to migration rate, thus improving separation. Polyacrylamide gels were prepared by copolymerization of acrylamide monomer in the prescence of a cross-linking agent, N,N-methylenebisacrylamide monomer (Tanaka, 1981). Ammonium persulfate and N,N,N',N'-tetramethylethylenediamine (TEMED) are usually added to initiate the free radical

polymerization reaction. The concentrations of the two monomers and the degree of polymerization determine the gel porosity. The extent of porosity is adjusted in order to obtain the highest separation of a protein mixture.

Loeschcke and Stegemann (1966) studied potato tuber sap proteins using PAGE. They found up to 25 proteins migrating toward the anode, and 2 to the cathode. Nakasone *et al.* (1972) discovered a total of 28 protein bands from albumin and globulin fractions extracted from tuber sap.

Kapoor *et al.* (1975) used PAGE to examine the protein fractionation procedure of Lindner *et al.* (1960). The tuberin fraction extracted with this method had a similar electrophoretic pattern to the albumin fraction. Therefore, they suspected that the tuberin fraction contained a significant amount of albumins.

Using preparative PAGE, Stegemann (1978) isolated 3 major proteins from the European cultivars Maritta, Voran and Hydra. Amino acid analysis of these protein isolates proved that they were not oligomers of a parent protein. These findings suggested that the composition of potato tuber protein cannot be represented only by tuberin and tuberinin.

Application of electrophoresis for potato cultivar identification was demonstrated by Zwartz (1966). He subjected the soluble tuber proteins to paper electrophoresis and the protein band patterns were used to identify 59 Dutch potato varieties. Using PAGE, similar

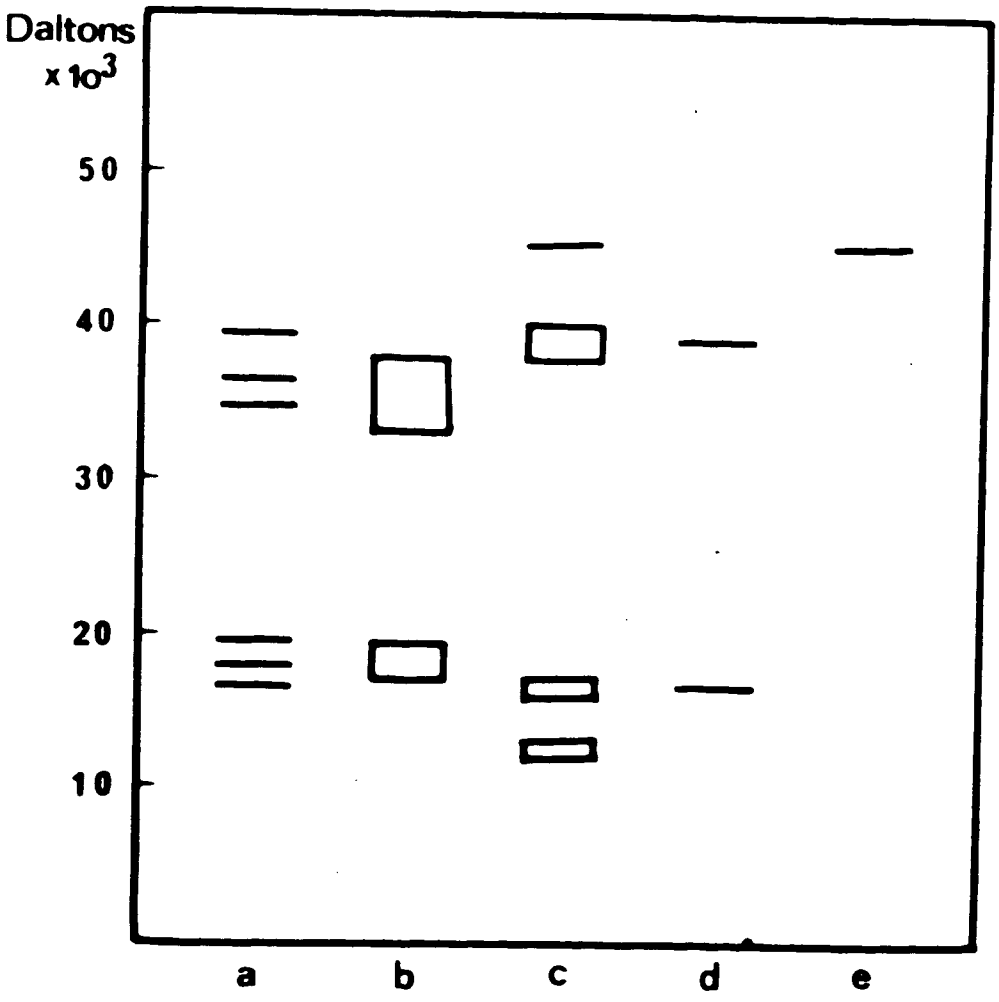
studies were done by others (Loeschcke and Stegemann, 1966; Desborough and Peloquine, 1966). As early as 1971, the identification of potato cultivars by electrophoresis was a routine practice of German testing stations (Stegemann, 1979). Compilation of the data was organized as a potato index book and a systematic identification procedure became available for 530 registered cultivars grown in most European countries (Stegemann and Loeschcke, 1976, 1979). By this procedure, electrophoretic patterns of proteins and esterases serve as fingerprints of a cultivar. Since protein band patterns obtained in the cathodic region with PAGE were relatively simple and similar among different cultivars (Stegemann *et al.*, 1979), the cultivars were classified into 9 groups with respect to the presence and/or absence of each of four major bands in this region. Further classification was achieved by patterns in the anodic region and of esterases.

Despite its useful application, PAGE cannot be considered as the best method for protein characterization. One drawback of PAGE is that it cannot distinguish two molecules of the same mobility which differ in both charge and molecular weight. Two additional electrophoretic methods were thus developed by which proteins can be separated solely by size. The first is the gradient gel, which is prepared by increasing concentration of polyacrylamide, thus decreasing the size of the pores in the direction of electrophoretic mobility (Lambin, 1978; Poduslo, 1980). When

an electric field is applied, protein molecules start moving at a rate determined by their charge. As they migrate, the pore size decreases, thus slowing the migration rate of larger molecules. Ultimately, the protein molecules are immobilized at the point where the pore size prevents further migration.

The second method involves the treatment of protein with sodium dodecyl sulfate (SDS), an anionic detergent, and β-mercaptoethanol (Shapiro *et al.*, 1967). This results in extensive disruption of hydrogen, hydrophobic and disulfide linkages, and unfolds each polypeptide chain to form a long rod-like molecule coated with a layer of SDS (SDS-polypeptide complex). Sulfate groups of SDS are exposed to the aqueous medium and exhibit a net negative charge. The ratio of SDS to protein is usually about 1.4:1 by weight. When single chain protein treated with SDS is applied to a polyacrylamide gel containing SDS, the velocity of the electrophoretic process is determined primarily by the mass of the SDS-polypeptide complex (Weber and Osborn, 1969). A calibration curve is prepared, using standard proteins of known molecular weights, by plotting the logarithms of molecular weights versus electrophoretic mobility. The molecular weight of protein can be determined in this way with an accuracy of better than ±10%.

Stegemann *et al.* (1973) studied molecular weight distribution of dormant tubers by SDS-PAGE (Figure II.1). They found only 3 major protein bands (molecular weights of



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

Figure II.1. Major potato protein bands of SDS-PAGE as reported by several authors.

16,800, 18,000 and 19,500 daltons) when no β-mercaptoethanol treatment was used. There were also 3 minor protein bands, with molecular weights of 34,500, 36,500 and 39,500 daltons, at about one-tenth the concentration of the major bands. When S-S bridges were reduced by β-mercaptoethanol, one of the main subunits split into at least 2 faster migrating zones (13,800 and 10,200 daltons).

Snyder *et al.* (1977) studied the changes in the various protein molecular weight classes during tuber growth. There were two major broad protein bands (molecular weights 33-38,000 and 17-19,000), which were more intensely stained with later harvest and thus increased in concentration during tuber growth.

Nuss and Hadziyev (1980) reported major bands with molecular weights of 12-13,000, 16-17,000, 38-40,000 and 45,000 daltons in tuber sap proteins. On the other hand, Seibles (1979) reported only two major protein bands (16,500 and 29,000 daltons). When molecular weight distributions reported by different authors are compared (Figure II.1), it is conceivable that there are two regions where most of the major bands are located. One is from 12,000-20,000 and the other from 33,000-40,000 daltons. One protein band reported by Nuss and Hadziyev (1980) is slightly away from these regions. The importance of this band was assessed by Racusen and Foote (1980), who stated that proteins with molecular weight of 45,000 daltons (determined by SDS-PAGE) comprised 20% of the soluble proteins in 31 tested potato cultivars.

Since β-mercaptoethanol treatment did not affect the molecular weight, this protein is not an oligomer of subunits. However, when subjected to PAGE, it showed 3-4 zones, indicating that the protein exists in several ionic forms.

According to Stegemann *et al.* (1973), the size distribution of protein from mature tubers, when treated with SDS, is almost independent of cultivar. Nuss and Hadziyev (1980) observed the same SDS-PAGE pattern for 4 Alberta-grown cultivars. Makinen *et al.* (1979) also reported that almost identical SDS-PAGE patterns were observed with most of the European and South American cultivars. Cultivar-specific PAGE patterns of soluble proteins when SDS treatment is omitted are thus attributable to the differences in the net charges of the proteins.

The complexity of tuber proteins has been further studied with more advanced electrophoretic techniques, such as isoelectric focussing (IF) and two-dimensional migrations. IF is based on the principle that a protein migrates in a pH gradient until it reaches the pH corresponding to its isoelectric point (pI), where the net charge of the protein becomes zero. Prior to analysis, a pH gradient is generated in a gel support by using a mixture of carrier ampholytes. Any movement of the protein away from its isoelectric point, once it is reached, produces a regeneration of net charge, thus the protein is pulled back to the pI point. Hence, diffusion is greatly minimized and

resolution is high. Differences in pI as little as 0.01 pH units can be resolved (Bio-Rad, 1982).

Two-dimensional techniques combine two electrophoretic separations, perhaps two different types of electrophoresis or the same type with different analytical conditions such as pH or ionic strength of buffers. The techniques include IF followed by SDS-PAGE, PAGE followed by IF, PAGE followed by SDS-PAGE, etc. (Stegemann *et al.*, 1973). Macko and Stegemann (1969) studied electrophoretic patterns of tuber proteins of 3 cultivars and demonstrated that isoelectric focussing patterns were different and genetically determined. They also demonstrated that each electrophoretic PAGE band consists of several proteins with different pI. In addition, each band from isoelectric focussing consists of several proteins with identical charge but different molecular weights. They applied a two-dimensional technique with IF and PAGE and showed characteristic and reproducible protein maps for each cultivar, irrespective of place of growth, harvest year or agricultural practice (fertilizer treatment and/or irrigation).

Stegemann *et al.* (1973) did not find identical protein maps among 200 potato cultivars in the same physiological state. In order to reconfirm the cultivar dependency of the protein map, the effect of growth regulator, fertilization level and physiological state of tubers was studied. Electrophoretic patterns were not affected by growth regulator or fertilization at different levels. However,

immature tubers contained one characteristic band which was common to all cultivars. During maturation, this protein disintegrated into lower molecular weight proteins (Stegemann, 1979).

Potato protein separation by isoelectric focussing was also applied by Kaiser *et al.* (1974), Nuss and Hadziyev (1980) and Seibles (1979). Kaiser *et al.* (1974) separated 42 protein bands in the range of pH 3-10 from protein extracts of 12 potato cultivars. However, the separation in the alkaline region was not clear. Nuss and Hadziyev (1980) reported 40-45 bands in the same pH range from 4 cultivars. In basic and acidic regions, 13-18 and 9-10 bands were observed, respectively. Between pH 6.2 and 7 there were 7 common protein bands in all 4 cultivars.

According to Seibles (1979), most of the proteins in albumin and globulin fractions appeared in acidic regions (pH 4-5.2). Three bands, of pI 4.2, 4.4 and 5.3, of the globulin fraction were isolated and it was found that their amino acid compositions were very similar. However, neither individual nor total amide contents (asparagine, ASN; glutamine, GLN) were determined. It is probable that the degree of amidation was the primary factor in the differences in protein net charge and isoelectric points of his protein isolates.

The importance of protein-bound amides in protein electrophoretic behavior was demonstrated by Stegemann *et al.* (1973). They found that the cultivar dependent

pattern of IF was not apparent under high pH conditions (pH≥10). Extensive deamidation occurred at pH 10 after heating at 50°C of the free amides (ASN, GLN) as well as dipeptide (GLN-GLN). The rate of GLN deamidation was greater than that of ASN. Similar observations were reported by Nuss and Hadziyev (1980). They found that not only the combination of heating at 100°C and high pH (≥10) but heating alone could induce extensive deamidation.

As originally suggested by Stegemann *et al.* (1973), all these observations confirm that the major differences in IF patterns of potato proteins are due to different degrees of amidation of a parent protein.

D. Potato Tuber Amino Acid Composition

Free Amino Acids

The nitrogen containing compounds are listed in Table II.1. Though some of these results might be questionable, the ratio of protein-N and non-protein-N appears to be accurate. Protein of the potato tuber contains about 50% of the N, the rest being non-protein-N, which includes inorganic and amide-N, glycoalkaloid, vitamin, nucleotide and quaternary N-compound nitrogen, and free amino acid N.

Free amino acids of potato tubers are of great relevance to the problems of potato processing and to the flavor of cooked, fried or baked potato (Self, 1967). Since the free amino acids of tubers may account for up to or even

Table II.1. N-compounds present in potato tuber[1]

N-fraction	% of Total-N
True protein-N	50
Non-protein-N	50
Inorganic	
Nitrate-N	1
Nitrite-N	traces
Amide-N	
Asparagine	13
Glutamine	10
Remaining N	
Free amino acid-N	15
Basic N	8

[1]Schreiber (1961)

more than half of the total amino acid-N, they also have a bearing on the nutritive value of the potato (Burton, 1966).

A systematic study on the variation of free amino acids in potato tubers grown in North America, as related to location of growth, year and cultivar, was carried out by Talley *et al.* (1970). Cultivars grown in the Netherlands were reported by Mulder and Bakema (1956). A survey of published results set out according to potato cultivar was provided by Synge (1977). For Russet Burbank (Netted Gem) grown in Maine, New Brunswick and New Jersey, the composition in Table II.2 was reported.

Free amino acids in 13 cultivars grown in 7 different countries were obtained by ion-exchange columns, except one cultivar for which gas-liquid chromatography was used. In Synge's (1977) review incomplete analyses were also cited, related to approximately 45 additional cultivars.

The results of 91 analyses of the free amino acids of potato tubers of 31 cultivars grown in England and Ireland were reported by Davies (1977). Though a discriminant analysis of these results stongly suggested quantitative differences between cultivars, there was ample evidence of differences due to growth location, climate and year of growth. The means and ranges of free amino acids for the 31 cultivars are given in Table II.3.

As found by Davies (1977), soil and climate variations influenced the levels of a few amino acids more than those of the rest. Those with appreciable variation were:

Table II.2. Free amino acids of potato tuber Russet Burbank[1] (mg amino acid/100 g tuber dry matter)

Dry matter, % fresh weight	25
N as % of dry matter	1.73-2.81
Non-protein-N as % of total N	58-62

Aspartic acid	210-284	Alanine	20-110
Asparagine	---	2-aminobutyric acid	---
Threonine	70-80	Cystine	9
Serine	90-110	Valine	128-350
Glutamine	---	Methionine	25-50
Glutamic acid	260-334	Isoleucine	40-80
Proline	41-60	Leucine	23-40
Glycine	10	Tyrosine	73-170
Tryptophan	14	Phenylalanine	75-130
Histidine	42-80	Lysine	77-180
4-aminobutyric acid	150	Arginine	200-320

+ Ammonia, β-alanine, several unknowns

[1]Synge (1977)

Table II.3. Means and ranges of free amino acids of potato cultivars grown in England and Ireland[1] (mg amino acid/100 g tuber dry matter)

Amino Acid	Mean	Range
Aspartic acid	184	32-376
Asparagine	1487	371-3490
Threonine	48	14-143
Serine	60	15-128
Glutamine	1820	220-9122
Glutamic acid	294	225-371
Proline	88	0-484
Glycine	12	1-35
Alanine	32	6-118
Valine	138	15-370
Methionine	55	9-108
Isoleucine	58	14-165
Leucine	33	8-131
Tyrosine	95	17-316
Phenylalanine	77	0-204
Tryptophan	66	7-174
Lysine	65	9-319
Histidine	117	17-328
Arginine	257	60-730
4-aminobutyric acid	156	15-448
Ornithine	36	2-244

[1]Davies (1977)

glutamine, proline, alanine, valine, tyrosine, histidine and γ-aminobutyric acid. The amino acid which differed the most between tubers grown in England and Ireland was proline, a result similar to earlier location-related American findings (Talley *et al.*, 1970). A correlation of the tyrosine content in tubers with rainfall and humidity was proved by Mapson *et al.* (1963). Davies (1977) reported similar patterns in free amino acid contents averaged by year (1968, 1969, 1970 and 1973). Such consistency was also found in tubers during short periods of storage, up to 5 weeks. Longer storage (3 months) was reflected by a consistent increase in contents of two amides: glutamine and arginine. However, an increase of arginine content during prolonged storage may also indicate a change in the binding affinity or location of arginine in the tuber rather than its "*de novo*" synthesis (Talley *et al.*, 1958).

Most of the above results were obtained from a tuber slurry in 70% ethanol, which was mixed with a filter aid (Celite 545), poured into a glass chromatography column, and percolated overnight with 70% aqueous ethanol, collecting an eluate totalling 1900 ml. An aliquot of this eluate was reduced to about 10 ml using a rotary evaporator at 40°C, then slightly acidified with 0.1 M HCl and applied to an amino acid analyzer operated at 60°C (Boulter, 1966). At this temperature part of glutamine is lost by cyclization to pyrrolidonecarboxylic acid, hence considerable doubt should exist concerning the accuracy of glutamine results.

Moreover, the procedure applied by Boulter (1966) uses Na-citrate buffer, which does not separate glutamine and asparagine. These amides are eluted between threonine and serine, the two amino acids which in potato samples are unresolved from the large amide peak. Hence, the amount of asparagine is in doubt, as are the amounts of threonine and serine, which are based on difference in A_{440nm} over A_{570nm} ratios (Holy, 1966). Asparagine can be resolved from glutamine and glutamine from glutamic acid by applying the Nunn and Vega (1968) method (Davies and Laird, 1976).

S-Containing Amino Acids

The two S-containing amino acids, methionine and half-cystine (cysteine + cystine), belong to the essential amino acids of the potato tuber, along with eight other acids (isoleucine, leucine, lysine, phenylalanine, tyrosine, threonine, tryptophan and valine). It has been reported that methionine and cystine are the limiting amino acids of potato protein (Knorr, 1978).

The free methionine (cystine) contents in mg/100 g tuber dry matter were reported by Synge (1977) to range from 26-87 (2-5) for cv. Bintje, 42-64 (0) for Superior, 46-127 (trace) for Katahdin, 25-50 (9) for Russet Burbank, 64-89 (--) for Kennebec and 17 (--) for Maritta. For some cultivars grown in England and Ireland, Davies (1977) reported the following methionine contents: King Edward 39-95 (mean 37), Record 32-64 (mean 48), Désirée 76-108

(mean 95), Bintje 51-72 and Duke of York 103. For a total of 17 cultivars from England, Davies (1977) found methionine averaging 57 and for 23 cultivars from Ireland 46 mg 100 g tuber dry matter. Its change over three consecutive harvest years was from 43 to 65 mg/100 g tuber dry matter. In his study no results for cystine were provided.

Desborough *et al.* (1974) reported the presence of an additional peak before the aspartic acid peak in an amino acid chromatogram. It has been identified as methionine sulfoxide and was limited to acid hydrolysis product of potato powder. They stated that sulfoxide is not a naturally occurring component of potato tuber.

Some recent methods have enabled separation of methionine, methionine sulfoxide and methionine sulfone obtained under acid hydrolysis of proteins. Thin-layer chromatography (TLC) has been used (Tannenbaum *et al.*, 1968) to separate free methionine (Rf 1.0) from its sulfoxide (Rf 0.26) and sulfone (Rf 0.33). Some separation was also achieved using N-trifluoroacetyl methyl ester (TFAM). Here the sulfone could be separated from methionine but not from sulfoxide. TFAM-methionine could, however, be readily determined by gas chromatography with reproducible results over a wide concentration range. However, TFAM-methionine could not be separated from TFAM-methionine sulfoxide. No peak could be observed for TFAM-methionine sulfone, probably due to direct deoxygenation (Tannenbaum *et al.*, 1968). A satisfactory separation of methionine from its sulfoxide and

sulfone was reported by O'Keefe and Warthesen (1978). Their separation is based on HPLC of the compounds derivatized with dansyl chloride (5-N-dimethylaminonaphthalene sulfo-chloride) and use of a reversed phase Bondapak C_{18}-column and acetonitrile - phosphate buffer pH 7.9 as eluent.

However, in spite of the available methods no attempt has been made so far to verify the presence or absence of methionine sulfoxide or sulfone in raw or processed potatoes.

S-amino acids are important in providing secondary flavoring characteristics for cooked (in water), baked or fried potatoes. Degradation of methionine yields methyl mercaptan, while methional, the major flavoring compound of French fries, is formed via a Strecker's degradation pathway. Additional secondary reactions might occur among these sulfur compounds. Thus, the stongly nucleophilic S^{-2} can attack a methoxy group to yield the even more nucleophilic methyl S^{-1}. This could lead to dimethyl sulfide. The latter might give rise to disulfides and polysulfides and some 25 other sulfur containing volatiles from processed potatoes (Gumbmann and Burr, 1964).

Protein Amino Acids

Approximately half of the potato tuber nitrogen is true protein. The average total amino acid composition (free and bound amino acids) of cv. Russet Burbank (USA) and Bintje (Denmark) is given in Table II.4.

Table II.4. Average total amino acid composition of potato tubers cv. Russet Burbank (USA) and Bintje (Denmark) (g amino acid/16 g total-N after hydrolysis)

Amino Acid	Russet Burbank[1]	Bintje[2]
Aspartic acid	28.9	23.7
Threonine	3.6	3.6
Serine	4.3	4.0
Glutamic acid	15.9	13.8
Proline	3.0	3.6
Glycine	2.9	2.9
Alanine	2.3	3.1
Cystine + Cysteine	1.2	---
Valine	5.8	4.6
Methionine	1.6	1.84
Isoleucine	3.9	3.3
Leucine	4.8	5.1
Tyrosine	3.7	2.9
Phenylalanine	3.9	3.7
Lysine	5.3	4.9
Histidine	2.3	1.6
Arginine	4.9	4.0
Tryptophan	1.7	---

[1]Kaldy and Markakis (1972)
[2]Rexen (1976)

The values for methionine and for cystine + cysteine for cv. Russet Burbank were based solely on data obtained by the performic acid method. Averages of 52.3% methionine and 62.2% cystine + cysteine are destroyed during protein acid hydrolysis when performic acid oxidation is omitted. For cv. Bintje methionine was determined microbiologically by using *Streptococcus zymogenes* after the protein was hydrolyzed with hydrochloric acid for one hour. Nevertheless, under such conditions of hydrolysis the extent of methionine destruction is unknown (Rexen, 1976). Hence, unlike the other amino acids, the accuracy of results of S-containing acids are doubtful. These data amply illustrate that the lysine concentration of potato protein is higher than that of most plant proteins, while methionine and cystine are the limiting amino acids. As reviewed by Knorr (1978), both amino acid analysis and bioassay have verified the protein quality of potatoes for humans. It is comparable to that of whole egg and is equal or better than casein. Rexen (1976) reported essential amino acid indices (EAAI) of 33 potato cultivars in relation to whole egg to be between 55 and 84. Similar results were reported by Desborough and Lauer (1977) and Kapoor *et al.* (1975). The latter authors' EAAI values ranged from 72-100.

The influences of potato cultivar and nitrogen fertilizer level on the protein content and quality of potatoes were reported by Rexen (1976). He used 20 cultivars and two N-fertilizer levels 110 (114) and 180 (186) kg N/ha

in each of two growing seasons (levels for the second season in brackets). Based on his data, it appears that fertilizing with high levels of N results in increased crude protein content (Kjeldahl N x 6.25). In this respect significant differences ($P>0.999$) were found between the cultivars. Potato quality, expressed as EAAI, decreased significantly ($P>0.999$) with higher N application. With higher N there was also a significant decrease in essential amino acids: valine, threonine, isoleucine, leucine, phenylalanine and lysine. For some cultivars isoleucine became the limiting amino acid. However, there was no significant effect of N level or cultivar on methionine. For the nonessential amino acids there were significant differences both between N levels and between cultivars, an exception being the lack of influence on proline and arginine. The study of the relationship between amino acids of each tuber and the tuber nitrogen content showed that all amino acids were negatively correlated with tuber N content, except for aspartic acid, threonine and arginine. Regression lines for eleven amino acids versus tuber %N were provided.

The relative amounts of various amino acids in potato protein, obtained from samples dialyzed in order to remove the pool of free amino acids are presented in Table II.5.

A comparison of total amino acids (protein and free amino acids) and dialyzed (protein) samples revealed that after dialysis there is a loss of all amino acids except glycine. The three nonessential amino acids in the free

Table II.5. Mean relative percent of amino acids from tuber protein[1]

Amino Acid	Relative %	Range, 40 samples (mg/g tuber protein)
Asp	13.91	2.80-17.57
Thr	5.20	0.66- 7.39
Ser	5.90	1.56- 7.39
Glu	11.20	1.46-15.01
Pro	5.29	0.94- 6.79
Gly	6.05	1.58- 7.06
Ala	5.32	0.99- 5.75
Val	4.88	0.57- 6.71
Met	1.51	0.19- 2.69
Iso	3.73	0.81- 5.04
Leu	9.70	1.54-13.51
Tyr	5.43	1.29- 5.44
Phe	6.38	1.60- 7.20
His	2.39	0.59- 2.79
Lys	8.18	1.62-10.82
Arg	4.74	0.65- 6.45

[1]Desborough and Weiser (1974)

pool, aspartic, glutamic and tyrosine, comprised up to 30.9% of the total. The two essential amino acids, valine and leucine, comprised only 2.6 and 6.1%, respectively, of the total pool. Hence, it was concluded that the essential amino acids occur mainly as components of tuber proteins (Desborough and Weiser, 1974). This study also found that a conversion factor of 7.5, rather than 6.25, would be more appropriate for calculating potato protein N when using the micro-Kjeldahl method.

Amides

It is well known that some established potato cultivars have high amino acid content in their soluble nitrogen, and an amide moiety which tends to be rich in asparagine. In contrast, some cultivars grown under high levels of nitrogen fertilizer treatment have higher total soluble nitrogen and a higher proportion of amide, predominantly as glutamine. It appears that during cooler nights and longer days potatoes store more asparagine, and warm temperatures and shorter days more glutamine (Steward and Durzan, 1965). Total amide N, as percent of soluble N, averages 60-65%, asparagine 1374 µg/g and glutamine 3020 µg/g fresh weight (Steward and Street, 1946; Thompson and Steward, 1952).

Determination of total amide-N involves hydrolysis in which the released ammonia is recovered by distillation. Glutamine constitutes the "easily hydrolysable" and asparagine the "retardable" fraction. Isolation of

crystalline amides from potato tuber employs lead acetate to clarify and precipitate the protein in potato sap, and mercuric nitrate to precipitate the amides, followed by their fractional crystallization (Steward and Street, 1946).

As stated by Steward and Street (1946), aqueous extraction of fresh potato tissue is preferred over 70% alcohol for amides. Protein is removed by heat coagulation prior to determination of soluble nitrogen fractions.

A similar approach in amide determination was followed by Kaspers (1959). He obtained potato tuber sap using a press, removing starch and cell debris with a filter cloth, then using the aqueous filtrate for paper chromatographic separation of amino-N and amide-N constituents. He obtained 591 µg/g fresh weight for asparagine and 98-246 µg/g for glutamine, i.e. much lower values than those of Thompson and Steward (1952). He also found that, among early, mid- and late ripening cultivars, the amide-N did not differ. Also, storage from November until March did not change the content. N fertilizer treatment from 80-200 kg N/ha did not bring about significant changes, except in amide ratios: there was an increase in glutamine at the expense of free glutamic acid, and a decrease in the content of asparagine. Lastly, he reported that the amount of arginine increases after potato Phytophthora infection. This appears to contrast with X-virus infection of potato plants. As found by Skofenko (1966), the total content of amides in healthy tubers was higher than in tubers infected with virus, the

predominant change being in asparagine. Glutamine remained practically unchanged. This finding is the reverse of that for green potato leaves.

In a study to determine if virus infection was responsible for reduction in size and yield of tubers from diseased plants, McDonald (1977) found that leaf roll virus infection brought about an increase in α-amino-N and glutamine fractions, and a slight decrease in asparagine amide-N throughout the growing season. Lastly, under commercial practice two and three year reproduction of seed potatoes on heavy loams after fertilization by N, P and NPK (45 kg/ha of each element) showed no change in amino acid composition of seed tubers, but there was an increase in the content of total and essential amino acids and amide-N (Ilina, 1975).

Amino acid and amide separation by ion exchange chromatography has also been the subject of several studies. By employing Na-citrate buffer as the column eluent, glutamine and asparagine could not be separated without sacrificing resolution of other amino acids (Talley *et al.*, 1964). In one column systems glutamine was separated from serine, however asparagine was eluted with serine. Therefore, it was necessary to rechromatograph the samples after an aliquot was hydrolyzed, then to determine changes in aspartic and glutamic acid values relative to those of untreated sample. Contrary to this general method, Benson *et al.* (1967) achieved separation of amides without

hydrolysis and without interference from other amino acids on a 55x0.9 cm resin column (Beckman PA-28 and AA-15 Li form) using an Li-citrate buffer with thiodiglycol at a flow rate of 70 ml/hr at 37°C throughout the analysis.

An earlier method involved cyclization of glutamine to pyroglutamic acid in 2.1 M phosphate buffer pH 6.7 at 100°C for 90 min (Talley *et al.*, 1964). Asparagine then was estimated directly from column effluent and the pyroglutamic acid was collected before reaction with ninhydrin, hyrdolyzed with acid and rerun on the amino acid analyzer as glutamic acid.

E. Effect of Processing on Potato Tuber Proteins and Amino Acids

Cooking (Boiling)

The protein and amino acid compositions of peeled potatoes boiled in water (2 ml/g tuber) by the usual household method were reported by Hughes (1958). Protein was separated from non-protein-N by ethanol treatment of peeled and homogenized tubers, while amino acids were determined by the method of Moore and Stein (1954). The peeled raw tuber had a total N content of 1.9% dry matter. Protein-N was only 34% of the total N, indicating that a large portion of protein-N was confined to peel and adjacent cortex. After water cooking the total N loss was 5.3%. Apart from increased amounts of aspartic and glutamic acids, due to

amide hydrolysis, cooking did not substantially change the amino acid composition of the tuber.

Processing into French Fries and Chips

In the commercial production of French fries peeled potatoes are cut into strips, blanched in hot water, partially deep fat fried (par-fried) and then frozen. The customer performs the finish frying. Blanching is done at temperatures from 60-82.2°C (most often at 71°C) for a period of 15 min, followed by par-frying for 1 min at 160-185°C, and finish frying of frozen fries is carried out at 185°C for 3 min. Under these conditions the oil content of the par-fries is close to 3.4% and of the finished fries 8.2%. The weight loss from before blanching to after par-frying and freezing is 18.9% (Nonaka *et al.*, 1977).

The role of blanching in hot water is to inactivate enzymes responsible for browning and off-flavors. In addition during blanching sugars leach from the surface of the cut potato strips, thus reducing the extent of Maillard reactions in the frying step, i.e. avoiding an undesirable dark brown color of the finished product. Finally, blanching firms the texture and decreases the oil uptake of the par-fries.

In tubers selected for French fries there are requirements related to the content of some amino acids. To minimize Maillard reactions and to obtain light-colored fries, tyrosine and proline must be in abundance, whereas

leucine and isoleucine contents must be low (Holm, 1974). Tyrosine, on the other hand, increases the risk of enzymic browning during peeling and strip cutting. As found by Rexen (1976), isoleucine, leucine and tyrosine are highly positively correlated with lysine, another essential amino acid. Hence, potato cultivars aimed for French fries should be characterized by a low essential amino acid index combined with a fairly high proline content.

A three-step blanching for potatoes was suggested by Weaver and Ng (1979). A sequence of steam or water blanching (blanch, dry, blanch) provided a 25-35% decrease in oil uptake and a 15% reduction in weight loss of fries. This process was followed by an innovative two-step blanching, suggested by Ng and Weaver (1979), which was more suitable for both high and low specific gravity tubers. The tubers were subjected to water (or steam) blanching, followed by an air-drying step. control of the blanching temperature provided a texture from very firm to mealy. The process was suggested for potatoes under retorting in cans or pouches. Similarly, Yu and Swanson (1979) introduced the short Dry-Fry method, which combines steam-blanching with partial dehydration of potato strips, primarily to optimize color and texture of French fries. It is obvious that amino acids are also involved in a successful blanching step. However, none of the developers of these new blanching processes provided data to reveal the effect on amino acids.

Changes in amino acids in chips made from fresh, stored and reconditioned Kennebec potatoes were reported by Fitzpatrick and Porter (1966). The potatoes were abrasion peeled, chipped and fried batchwise in a basket-type fryer at 180°C for 4 min until dehydration was completed. The study showed that the ethanol extractable-N of the tubers, containing 24 free amino acids and amides, increased on potato storage and reconditioning, thus reflecting protein degradation. Chip frying caused a general decrease in almost all amino acids and a marked loss of amides. Losses of methionine were partly offset by increases in sulfoxide. An increase in β-alanine was ascribed in part to aspartic acid decarboxylation. In addition several sugar-amine condensation products were found, and after hydrolysis provided larger amounts of glycine, serine, alanine and leucine. An increase of aspartic and glutamic acids and ammonia in chips indicated amide degradation.

However, in chips from stored and reconditioned potatoes most of the amino acids changed to forms not recoverable by acid hydrolysis (Fitzpatrick and Porter, 1966). When the tubers were stored in the cold, reducing sugars accumulated. With such tubers chip frying caused a loss of 88% of the reducing sugars with a matching loss of free amino acid-N. The ratio of N to reducing sugar (as fructose) in such a loss was 1:1.35 µmol/g tuber dry matter. Acid hydrolysis proved that the nature of the loss was an amino acid-sugar interaction. When the reducing sugar

content was low; as in fresh and reconditioned tubers, the ratios were 9:1 and 6.3, respectively. Losses of amino acids were not explained in these cases, though amino acid polymerization was suggested but not proved.

New Brunswick-grown cv. Russet Burbank of low and high specific gravities (LSG and HSG, respectively) was the subject of a study by Jaswal (1973) on the contents of free and bound amino acids as affected by processing into chips, French fries and flakes. Free amino acids from raw and processed samples were extracted with 70% ethanol, while total amino acids (free + bound) were obtained by hydrolysis with 6 N HCl for 24 hr. The available lysine of the raw and processed samples was also determined using the spectrophotometric 1-fluoro-2,4-dinitrobenzene method of Carpenter (1960).

The LSG potatoes contained a total of 108.1 mg amino acids per g dry matter, of which 21.3% was in the free state. Close to one-third of the bound form consisted of essential amino acids, the rest being aspartic and glutamic acids. Processing into chips resulted in a significant loss of both free and bound acids (44.8 and 36.9%, respectively). The decrease in aspartic and glutamic acids, histidine, lysine, phenylalanine, proline and tyrosine accounted for most of the loss of the bound fraction, while the loss of the free amino acid pool involved almost all the individual acids. Here arginine loss was the greatest, while minor losses were found for leucine, proline and glycine. Unlike

chipping, French frying showed no significant loss (only 4.5% of the total amino acid content).

The HSG study revealed considerably lower values for total (90.3), bound (71.3) and free amino acids (19.0 mg/g tuber dry matter). Their changes with processing followed the pattern of LSG tubers, however the extent of destruction was significantly lower. The total amino acid loss was 22.9% with chips but only 5.2% with fries. In both cases the loss was mainly from the free amino acid pool.

The total lysine contents of LSG and HSG potatoes were 5.7 and 4.2 mg/g dry matter, respectively, of which close to 31% was in the free state. Of the total lysine, 80-90% was in the available form in raw tuber, i.e. with its free ϵ-amino group. Processing adversely affected the levels of total and available lysine. Chips showed the greatest loss, 58 and 68%, and fries the least, 14 and 12%, respectively, for LSG and HSG tubers.

Processing into Dehydrated Flakes and Granules

The change of free and bound amino acids in laboratory-made flakes was reported by Jaswal (1973). The flakes were prepared from tubers peeled, washed and cooked in boiling water for 20 min, followed by mashing with 0.2% glyceryl monostearate and drum drying. There was an overall 22.8% amino acid loss which was closely divided between the bound and free amino acid pools. Of interest is the finding that when monoglyceride was omitted the overall loss

decreased to 18.5%. However, when HSG (1.095-1.106) potatoes were processed there was a negligible destruction of amino acids, amounting to only 4.3% for bound and 2.1% for free fractions in the absence and 9.3% and 11% in the presence of monoglyceride.

The fate of free amino acids during commercial processing by drum drying (potato flakes) and extrusion (French fries) was reported by Maga and Sizer (1979). Flakes containing 48% added water were extruded at 70, 100, 130 and 160°C. The authors reported high losses of all free amino acids as a result of dehydrated flakes production. The most affected were methionine, glycine and lysine (losses of 86, 82.7 and 67.7%, respectively). During extrusion the free amino acids phenylalanine, tyrosine, serine and isoleucine were readily destroyed at all temperatures. Other amino acids were more resistant to destruction at lower temperatures but suffered high losses at 160°C. At 160°C destruction loss was 67% for glycine and 98% for tyrosine, with an overall free amino acid content loss of 89%.

The change of total N, as determined by the micro-Kjeldahl method during commercial production of add-back granules and flakes in Idaho and Washington States, was reported by Augustin *et al*. (1979). There was a 91% retention of total N during water blanching, with similar retentions after cooling, steam-cooking, mash-mixing and conditioning steps of the granule process. The retention by unit operations was highest in steam-cooking, mash-mixing

and conditioning (99-100%), and less in water blanching (91%) and dehydration (93%) steps. During flake production, consisting of water blanching, cooling, steam-cooking, mashing and drum dehydration, N retention was 85-87% overall, with the highest unit operation retention occurring with steam-cooking and mashing (99%).

Amino acid changes as affected by an add-back commercial granule process and by a freeze-thaw pilot scale process have not been reported. Some data of Golan-Goldhirsh (1979) emphasized the need for such a study, but provided no answers on the effect of processing parameters on total, bound or free amino acids of dehydrated mashed potato granules.

F. Analytical Procedures

Total Protein Determination

Protein can be quantitated by numerous techniques, including UV spectrophotometry, Kjeldahl's digestion procedure, elemental analysis, dye binding, colorimetric methods, and summation of the amino acid content. Since UV light is absorbed by tyrosine, phenylalanine or tryptophan residues, the concentration of a protein solution can be determined accurately at 280 nm provided that the absorbance coefficient is known and the sample is free of impurities.

Protein assay by nitrogen determination is based on the assumption that a pure protein mixture will contain close to 16% nitrogen. Thus, the nitrogen content determined by Kjeldahl or elemental analysis is multiplied by 6.25 to estimate the amount of potato protein. While this conversion factor is common, other values have been used for particular proteins, depending on the nitrogen content of pure standards, e.g. 5.7 for wheat flour or 6.38 for milk. The factor for potato protein is usually 6.25, regardless of cultivar. However, Desborough and Weiser (1974) reported that 7.5 was a more appropriate conversion factor for a number of cultivars. If the sample is not a pure protein isolate, non-protein nitrogen (NPN) is also included in the nitrogen value. NPN includes free amino acids, ammonia, amides and basic (nucleotide) nitrogen. In the case of elemental analysis, highly oxidized nitrogen (consisting or nitrites and nitrates) is also included in the nitrogen value. Therefore, to obtain pure protein-N, NPN is removed by dialysis prior to nitrogen determination by the Kjeldahl method, which is well established and standardized for this purpose. However, there has been a need for further improvement in the digestion step. Various catalysts have been introduced, such as mercury, copper or selenium ions. Concon and Soltess (1973) recommended the use of hydrogen peroxide and lauric acid to reduce digestion time to 10 min while minimizing foaming of the sample.

Colorimetric methods, such as the Biuret method (Bailey, 1967), Lowry's method (Lowry *et al.*, 1951), Potty's method (Potty, 1969) and various dye binding methods, are simple and reproducible for protein estimation. High correlation (r=+0.9827) of the potato protein contents determined by Orange G and bromophenol blue dye binding and Kjeldahl methods was reported by Kaldy *et al.* (1972). The bromophenol blue method was also found to be in good agreement with micro-Kjeldahl, Potty's method and amino acid analysis (Desborough, 1975). Finally, Heidelbaugh *et al.* (1975) reported that the summation of individual amino acid contents was a reliable and accurate method for protein quantitation.

Protein Hydrolysis

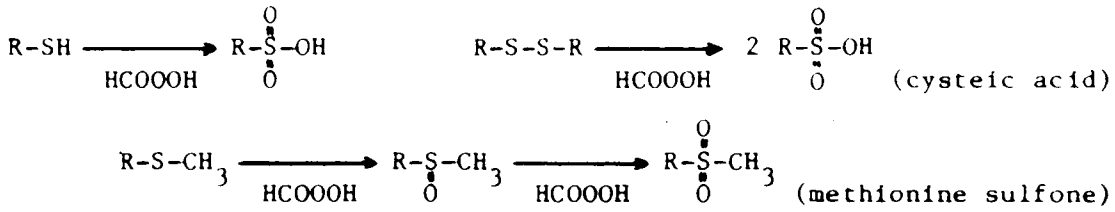
The hydrolysis of a protein or polypeptide is the most critical step in an amino acid analysis. The methods can be classified by the type of catalyst, i.e. acidic, basic and enzymic hydrolyses. By far the most reliable general method is hydrolysis with 6 N HCl *in vacuo* or under nitrogen. Within 20-24 hours the protein sample can be hydrolyzed in a sealed tube at 110°C with minimal decomposition of the amino acids (Blackburn, 1978; Fletcher and Buchanan, 1977). However this method has limitations as incomplete recoveries of some amino acids and their compounds have been reported. Generally, amides cannot be determined since they are hydrolyzed to ammonia and to the corresponding amino acids

(Rees, 1946). Furthermore, serine, threonine (Rees, 1946) and tyrosine (Sanger and Thompson, 1963) and most S-containing amino acids (Meister, 1965) are partially degraded and tryptophan (Friedman and Finley, 1971) is decomposed extensively. In samples which contain large amounts of carbohydrates, such as cereals, destructive losses become even more extensive. In order to circumvent these problems, a variety of protective agents have been used in conjunction with HCl, as exemplified by: thioglycolic acid, for better recovery of half cystine and tryptophan (Matsubara and Sasaki, 1969); phenol for inhibition of oxidative loss of tyrosine (Sanger and Thompson, 1963; Lipton and Bodwell, 1973); mercaptoethanol to prevent interference by trace dimethyl sulfoxide (Bates and Deyoe, 1973); and ion exchange resins to accelerate the release of aspartic and glutamic acids, threonine, serine, valine and isoleucine (Davies and Thomas, 1973).

Since none of these modifications is successful in recovering all 20 amino acids of protein, additional techniques are often required. For example, careful measurement of ammonia released from amides provides a method for total amide content determination (Rees, 1946). However, some of the labile amino acids also decompose and release ammonia.

For recovery of S-containing amino acids, treatment of the sample with performic acid is often carried out before 6 N HCl hydrolysis (Moore, 1963). Performic acid oxidation

converts cysteine and cystine to cysteic acid, and methionine to methionine sulfone:



These derivatives can be recovered after 6 N HCl hydrolysis, even in the presence of up to 94% carbohydrates in a sample (Moore, 1963). However, the oxidation is not fully specific to S-containing amino acids. Over-oxidation should be avoided by removing excess performic acid before hydrolysis. This is usually accomplished by dilution of the samples with water, followed by lyophilization. Alternatively, HBr is added as a reducing agent and the resulting bromine is removed (Hirs, 1967).

In any of the hydrolysis procedures mentioned above, the original concentrations of cysteine and cystine cannot be estimated individually as both are determined as half-cystine, or cysteic acid. Simpson *et al.* (1976) introduced a method which allowed the discrimination of these two amino acids in a single hydrolysate. The sample was first treated with iodoacetic acid to alkylate the cysteine residue. After hydrolysis (using methanesulfonic acid in the presence of 3-[2-aminoethyl]indole), the disulfide linkage was cleaved with DTT (dithiothreitol) and the free sulfhydryl group was then reacted with sodium tetrathionate. Cysteine was determined as S-carboxymethyl cysteine, and cystine as S-sulfocysteine.

The reagents mentioned above (except for methanesulfonic acid) are not successful in protecting tryptophan. Oxygen, cystine, serine and heavy metals may all contribute to the degradation of the indole ring of tryptophan (Davies and Thomas, 1973). Several alternative acid catalysts have been proposed, i.e. 3 N *p*-toluene sulfonic acid containing 0.2% 3-[2-aminoethyl]indole (Liu and Chang, 1971), mercaptoethanesulfonic acid (Penke *et al.*, 1974), or 4 N methanesulfonic acid containing 0.2% 3-[2-aminoethyl]indole (Simpson *et al.*, 1976). The latter method has provided the best recovery of tryptophan from pure protein, however the loss of tryptophan is still appreciable in samples containing more than 20% carbohydrate.

Determination of Free and Bound Amino Acids

Ion-Exchange Chromatography

One of the most reliable methods for amino acid analysis is the use of an automatic analyzer equipped with ion-exchange columns (Blackburn, 1978). A variety of instruments based on one or two column methods have been used in routine practice (Beckman, Jeolco, Beckman-Spinco, Durrum, Technicon). Automation of sample injection, buffer flow control, effluent detection, data processing and other functions have reduced analysis time for a complete set of amino acids to less than 1 hr. It was also essential that suitable ion-exchange columns and specific reactions for effluent detection and measurement be developed. The

development of these principles is briefly reviewed.

The idea of separating amino acids by column chromatography was originally present by Elsden and Synge (1944). However, they used potato starch as packing material, which had its drawbacks: irregular elution peaks and variable recoveries. As found by Stein and Moore (1948), performance was improved after preliminary washing of the starch column with 8-hydroxyquinoline. Symmetrical and well-resolved peaks were then obtained. Such a column was used for analyzing protein hydrolysates (Stein and Moore, 1949; Pierce and du Vigneaud, 1950). However, a complete analysis required two weeks, with other problems encountered such as starch standardization, regeneration and solubility (Jacobs, 1966).

During the 1950's starch was replaced by cation-exchange resins such as Dowex 50-X8, a sulfonated 8% divinylbenzene cross-linked polystyrene (Moore and Stein, 1951). An amino acid mixture was separated on a single, long column (0.9x100 cm) using a step-wise elution technique with two different buffer systems. At this time it was suggested that a successful separation of amino acids cannot be explained solely by ion-exchange processes, since neutral amino acids were also well separated, indicating that the sulfonated polystyrene resin matrix may provide sites for both ionic and non-ionic interactions (Moore and Stein, 1951). However, buffers of pH 8 or higher were necessary to elute the basic amino acids (lysine,

histidine, arginine and tryptophan) When the separation was conducted on a short column (0.9x15 cm), buffers with pH 7 or below could quantitatively resolve these basic amino acids. By combining both long and short columns, all amino acids were separated in 5 days.

Since Dowex 50-X4 was expected to provide better resolution, Moore and Stein (1954) studied a single column method using this resin. They could separate as many as 50 components of a synthetic mixture using a 0.9x150 cm column and gradient elution by increasing the ionic strength from 0.2 N to 1.4 N Na concentration and the pH from 3.1 to 5.1. Although quantitative recovery of amino acids (100±3%) was obtained by this technique, several problems still remained. The Dowex 50-X4 column could not be reused directly due to shrinkage of the resin bed (Moore and Stein, 1954). It was obvious that improved separation with a less cross-linked resin sacrificed the re-usability of the resin. A systematic study showed that Amberlite 120, another sulfonated polystyrene, was superior to Dowex 50 in its uniformity and performance of the column, hence it was applied by Moore and Stein from late 1950 on.

The first laboratory scale automatic amino acid analyzer was constructed in 1958 (Moore *et al.*; Spackman *et al.*) using two columns packed with Amberlite 120. Acidic and neutral amino acids were separated on a long column (0.9x150 cm) with Na-citrate buffers of pH 3.25 and 4.25 as eluents, while basic amino acids were eluted from a short

column (0.9x15 cm) using an Na-citrate buffer of pH 5.28. Regeneration was necessary for the long column, but both columns could be used repeatedly. Analysis time was reduced to 24 hr, with automatic recording. This instrument served as a prototype for commercial auto analyzers (Foreman and Stockwell, 1975), and since then substantial improvements have been achieved by individual manufacturers. One such improvement was manufacture of high quality resins consisting of fine spherical particles of uniform size. Because of this improvement, the size of the column could be reduced without sacrificing amino acid resolution. The diameter was reduced to 6.3 mm (Hamilton, 1963), 2.9 mm (Liao *et al.*, 1973) or 1.75 mm (Blackburn, 1978). Long column lengths were shortened from 150 to 50-60 cm and short columns from 15 to 5 cm (Benson and Patterson, 1965).

For the convenience of analysis, a single column procedure was developed by Piez and Morris (1960) and Hamilton (1958, 1963). The automatic analyzer developed by Hamilton (1963) could separate a mixture of amino acids and other amino compounds on one column. Improvement of the resolution was mainly due to the use of a resin with fine spherical particles (17.5 u diameter) packed in a narrow column (0.636x150 cm). Detection sensitivity was increased to 10^{-8} moles by use of a longer light-path photometer.

Commercial automatic analyzer systems are available with both single and dual column systems, with both systems having the desired performance and flexibility (Robinson,

1978). Generally, single column systems appear to be more convenient for sample application because of decreased pipetting errors. However, the higher amounts of ammonia in the buffers or samples used in single columns could cause extensive baseline shift and peak broadening of basic amino acids. Hence, single column systems require preliminary removal of ammonia from buffers and samples.

Gas-Liquid Chromatography

Cumulated research on the development of gas-liquid chromatography (GLC) now allows amino acid analysis comparable to conventional ion-exchange chromatography (Gehrke *et al.*, 1971; Hall *et al.*, 1980) with apparent advantages such as: (1) simplicity; (2) high sensitivity; (3) low initial cost of instrumentation; (4) greater versatility of the instrument; (5) shorter analysis time; (6) ease of interfacing the gas chromatograph with a mass spectroscope.

In contrast to ion-exchange chromatography, however, preparation of stable volatile derivatives is a prerequisite for amino acid analysis by GLC (MacKenzie, 1981). The main objective of derivatization is to modify protonic groups, such as amino, carboxyl, hyrdoxyl, thiol or quanidino groups, in order to stabilize them and to prevent intermolecular hydrogen bonding. A large number of methods applied in the past are briefly reviewed.

Because of simplicity, several single-step derivatization procedures, such as alkylation or TMS (trimethyl silyl) derivatization, have been developed, but none is suitable for general applications. The most common derivatizations are based on the two-step formation of N(O,S)-acylamino acid alkyl esters (MacKenzie, 1981). One of the widely used acylating reagents is trifluoroacetic anhydride (TFAA). Gehrke *et al.* (1968) used TFAA derivatization methods for macro, semi-micro and micro scale amino acid analysis.

Instability of these derivatives in the presence of water, in contact with a hot metal surface, evaporative loss during elimination of solvent and/or acylating reagent, and poor resolution are potential difficulties (Hušek and Maček, 1975). Moreover, two columns are usually required to achieve separation of all 20 protein amino acids. Moss and Lambert (1971) introduced the formation of heptafluorobutyryl (N-HFB) n-propyl ester derivatives. They were able to separate all 20 amino acids on a single column. Heptafluorobutyric anhydride (HFBA) is a strong acylating reagent. Its derivatives are more stable and less volatile than their TFA counterparts, thus losses during preparation are minimized (Hušek and Maček, 1975). Quantitation on a single column has also been reported with N-HFB-n-propyl, -isopropyl, -isobutyl and -isoamyl esters (Moss and Lambert, 1971; Kirkman, 1974; Kirkman *et al.*, 1980; March, 1975; MacKenzie and Tenaschuk, 1974; Zanetta

and Vincendon, 1973; Golan-Goldhirsh and Wolfe, 1979).

The selection of column and liquid phase are of major importance in GLC. Most studies have been carried out on packed glass columns 1-3 m in length with 2-4 mm I.D. In order to prevent contact of derivatives with metal or all glass components, on-column injection is preferred. Gas Chrom Q and Chromosorb W or G (both HP) have been recommended for their inertness and strong affinity for the liquid phase. Although the resolution improves as the polarity of the liquid phase increases, stability at high temperature is also required for amino acid analysis. Hence, the thermostable silicone base liquid phases (OV or SE types) provide good separation in temperature programming mode (Hušek and Maček, 1975).

A number of major interferences should be considered. The presence of interfering compounds makes it difficult to analyze biological samples prior to a clean-up procedure by GLC (Gehrke *et al.*, 1968). Zumwalt *et al.* (1970) reported that ion-exchange based chromatography (IEC) was suitable for eliminating some of the substance which interfere with the GLC analysis of protein amino acids. Problems exist in the derivatization of histidine, arginine, tryptophan and S-containing amino acids (Stalling and Gehrke, 1966; MacKenzie and Tenaschuk, 1974; Pearce, 1977; Tannenbaum *et al.*, 1968; MacKenzie and Finlayson, 1980). Moodie (1974) attributed the unsuccessful derivatization of histidine to the formation of mono- and diacylated histidines. Traces of

water might affect arginine derivatives, forming a salt which cannot be derivatized. The problem with tryptophan is elution as two peaks rather than one peak (Pearce, 1977). This was initially attributed to the formation of mono- and diacylated derivatives or to inadequate acylation caused by a low reaction temperature. MacKenzie and Finlayson (1980) reported that the N-HFB isobutyl ester of cysteic acid was converted upon injection to its corresponding derivative, 2-amino acrylic acid. Hence, an increase in the injection port temperature was suggested to ensure its quantitative conversion. Finally, Tannenbaum *et al.* (1968) reported that TFAM (trifluoroacetyl methyl ester)-methionine could not be separated from TFAM-methionine sulfoxide, and that a peak could not be observed for TFAM-methionine sulfone. Such findings illustrate and justify the fact that protein hydrolysates, even after oxidation, cannot be used for determination of S-containing amino acids by GLC.

Despite the previously mentioned drawbacks, some highly encouraging comparative results have been reported for IEC and GLC analysis of amino acids (Gehrke *et al.*, 1971; Tajima *et al.*, 1978; Golan-Goldhirsh and Wolfe, 1979). Based on these findings, free and bound amino acids of potato have been determined by GLC (Hoff *et al.*, 1971, 1972; March, 1975; Tajima *et al.*, 1978). Furthermore, using the same GLC technique, coupled with mass spectrometry, Golan-Goldhirsh *et al.* (1982) analyzed γ-aminobutyric acid and ornithine in the free amino acid pool of the potato

tubers as well as changes in the composition of free amino acids during production of dehydrated mashed potatoes.

High Pressure Liquid Chromatography

During the past decade high pressure liquid chromatography (HPLC) instrumentation has been developed, based on the Spackman, Stein and Moore (1958) methodology for amino acid analyzer systems. The HPLC system appears to have the advantage that it can provide separation of amino acids using a linear rather than a step gradient elution system. In addition HPLC can be used to separate protein and peptide mixtures.

The HPLC system involves a cation exchange resin (Na^+ form) using Na-citrate buffers, followed by an on-line, post-column derivatization step. The derivatization is necessary to permit detection either spectrophotometrically (proline and amino acids with secondary amino groups, at 440 nm, and amino acids with only α-amino groups, at 570 nm) or fluorimetrically.

Traditionally, three-buffer step gradients have been used in conjunction with a temperature change to effect separation. The purpose of the temperature change is to enhance the separation between the threonine/serine and tyrosine/phenylalanine pairs of peaks. With current columns (3 mm x 250 mm Pickering 10 micron cation exchange) equally good resolution can be obtained using a constant temperature in the range of 45-55°C. A distinct advantage of an automated linear gradient HPLC system versus a

classical amino acid analyzer system is that the time of analysis can be shortened significantly without sacrificing resolution. In this case, the total analysis time is close to 85 min at a flow rate of 0.2 ml/min, as compared to 110 min for a step gradient analysis under the same conditions. At a flow rate of 0.3 ml/min the analysis time can be reduced to less than 55 min.

A typical HPLC buffer system for amino acid separation consists of (A) Na-citrate buffer pH 3.28, 0.2 M; (B) Na-citrate buffer pH 7.45, 1.0 M; and (C) a column regenerant. The run at a flow rate of 0.2 ml/min is 100% A for the initial 17 min, followed by a linear gradient from 100% A to 100% B over 43 min. For column regeneration 100% C is used for 5 min. This is followed by 15 min equilibration with 100% A prior to the next injection.

Use of a linear rather than a step gradient has additional benefits. It provides column bed stability since ion-exchange packing is not subjected to sudden changes in eluant composition, thus avoiding rapid swelling or contracting of resin beads.

In HPLC the post-column reaction system consists of a heated stainless steel reaction coil with a temperature control between 100 and 135°C. With a thermally stable ninhydrin reagent reaction at 135°C is preferred, allowing a shorter reaction coil, thereby resulting in sharper peaks and improved resolution and sensitivity. An alternative approach is the use of either fluorescamine or

o-phthalaldehyde to derivatize amino acids to fluorescent compounds and the use of a fluorescence detector. This approach appears to have advantages of higher sensitivity and a fast derivatization step which does not require elevated temperature. A mixing and a post-column reaction coil long enough to provide a residence time of 15 min is sufficient. However, proline cannot be determined by this approach as its secondary amino group does not react with the reagent.

Determination of Total S and S-Containing Amino Acids and Tryptophan

Total Sulfur

Total sulfur content in potato tuber ranges from 100-200 mg per 100 g dry matter. Many methods have been suggested for total S analysis of plant materials, but none has proved to be a satisfactory combination of speed, accuracy and precision. To determine total S in potato it is necessary to quantitatively convert the various forms of sulfur to a single form which can be determined accurately. This is best accomplished by methods in which total sulfur is oxidized to sulfate by acid or ignition techniques and the sulfate is then estimated turbidimetrically, gravimetrically or by reduction to hydrogen sulfide. The turbidimetric procedure, initially developed by Tabatabai and Bremner (1970a) appears to be simple and reliable. A sample of 250 mg of plant material is digested in a

micro-Kjeldahl flask with conc. nitric and perchloric acids. The SO_4 ion is then precipitated by barium chloride. After stirring for 10 min, the transmittance of the turbid solution is read at 420 nm and compared with a reagent grade K-sulfate calibration curve (linearity region 0-700 µg/ml of sulfate).

This procedure was automated by Mottershead (1971), who employed the standard modules of a Technicon Auto-analyzer, using clear tubing and a filter of 623 nm. This wavelength was selected for sensitivity to barium sulfate, while avoiding absorption by dichromate or metavanadate ions in the digest. Mottershead (1971) estimated the standard error to be 0.001 for plant material dry matter (estimated sulfur content 0.271%), and 4 for methionine, 4 for L-cystine and 8 for sulfate. Variations in estimated sulfur content due to different sample sizes were negligible. Using ^{35}S-sulfate and following recovery with a scintillation counter, he found no evidence of loss of sulfate sulfur during digestion for 3 hr at 190°C in a mixture of conc. nitric and perchloric acids containing U-dichromate and NH_4-metavanadate.

However, the use of acid antioxidants risks the gaseous loss of sulfur as H_2S, SO_2 or SO_3. Therefore, Tabatabai and Bremner (1970b) suggested wet oxidation of sulfur compounds to sulfate under alkaline conditions, followed by analysis of the entire oxidation residue for sulfate by the Johnson-Nishita (1952) procedure, which

involves reduction of sulfate to H_2S and its colorimetric determination as methylene blue. In this approach the sulfur-containing sample is heated with alkaline sodium hypobromite solution in a flask that can be connected directly to a modified Johnson-Nishita digestion-distillation apparatus. The sulfate ion is reduced to H_2S at 115°C using a mixture of hydriodic, formic and hypophosphorous acids. Quantitative results were obtained with pure sulfur amino acids, sulfonic acids, sulfoxides and inorganic sulfur compounds such as sulfates, sulfites, sulfides and elemental sulfur.

A pyrolytic method at 1200°C has also been used for total S determination in plant meals (Bhatty *et al.*, 1977). Organic and inorganic sulfur is converted to sulfur dioxide, which then bleaches the blue color of a starch-iodide solution. The volume of K-iodate solution required to maintain the blue color gives an estimate of the S-content of the sample.

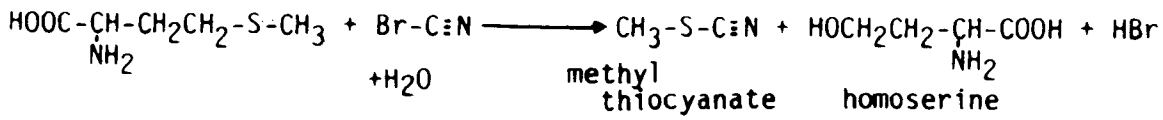
S-Containing Amino Acids

Methionine

Methionine is present in small amounts in proteins. Its determination usually involves acid hydrolysis followed by amino acid analysis. However, methionine-S is easily oxidized during hydrolysis, providing a mixture of methionine, methionine sulfoxide and the sulfone [VI] (Finlayson and MacKenzie, 1976). Sulfur oxidation to its VI state prior to analysis overcomes this difficulty, but it

is not readily applicable to potato tubers due to their high content of carbohydrate (Finlayson, 1969). Protein acid hydrolysis in the presence of carbohydrate leads to hydrolytic losses of methionine.

Proteins react at methionyl residues with cyanogen bromide, yielding homoserine and methyl thiocyanate (Gross and Witkop, 1961):



As indicated by Finlayson and MacKenzie (1976), this approach presents an alternate method for the determination of methionine in plant material that avoids acid hydrolysis. The method uses a 10% solution of cyanogen bromide in formic acid, which is then added to an aqueous solution or suspension of protein or plant meal. The protein samples dissolve readily in formic acid solutions, while the meals remain in suspension. After 3-4 hr at room temperature, with occasional shaking, aliquots of supernatant are taken and injected into a gas chromatograph equipped with dual flame ionization detectors. Ethylene glycol adipate at 100°C was found to be a suitable stationary phase, with N_2 as carrier gas. The reaction mixture is injected directly onto the column and the use of 50% aqueous formic acid in the analysis provides a much cleaner separation than the use of absolute formic acid.

This method had not been applied to potato meal, however, as shown by Finlayson and MacKenzie (1976) methionine recoveries from pea globulins or canola meal were always higher than from the seed protein hydrolysates. This again substantiates the conclusion that protein acid hydrolysis causes methionine losses.

Cystine + Cysteine -- Spectrophotometric Methods

Colorimetric determination of cystine and cysteine in proteins (Cavallini *et al.*, 1966) involves several steps: protein unfolding with guanidine HCl (urea); reduction of disulfide bridges into sulfhydryl groups with Na-borohydride; followed by destruction of excess hydride and color development with Ellman's (1959) reagent [5,5'-dithio-bis(2-nitrobenzoic acid)]. The dianion readings are taken at 410 nm, using a molar absorption coefficient of 13,600 M^{-1} cm^{-1} for calculation (Figure II.2).

Felker and Waines (1978) adopted the above assay for cystine plus cysteine in legume seed meals (field beans, peas, lentils). They incubated the seed meals for 1 hr at 38°C with Na-borohydride in 8 M urea and the thiols produced were determined with Ellman's reagent. A comparison of values from this procedure and from performic acid oxidation ion-exchange chromatography of 33 legume seed meals showed good correlation for peas and lentils but lower than expected values for field beans and fava beans. Since disulfide bonds in a few proteins are resistant to

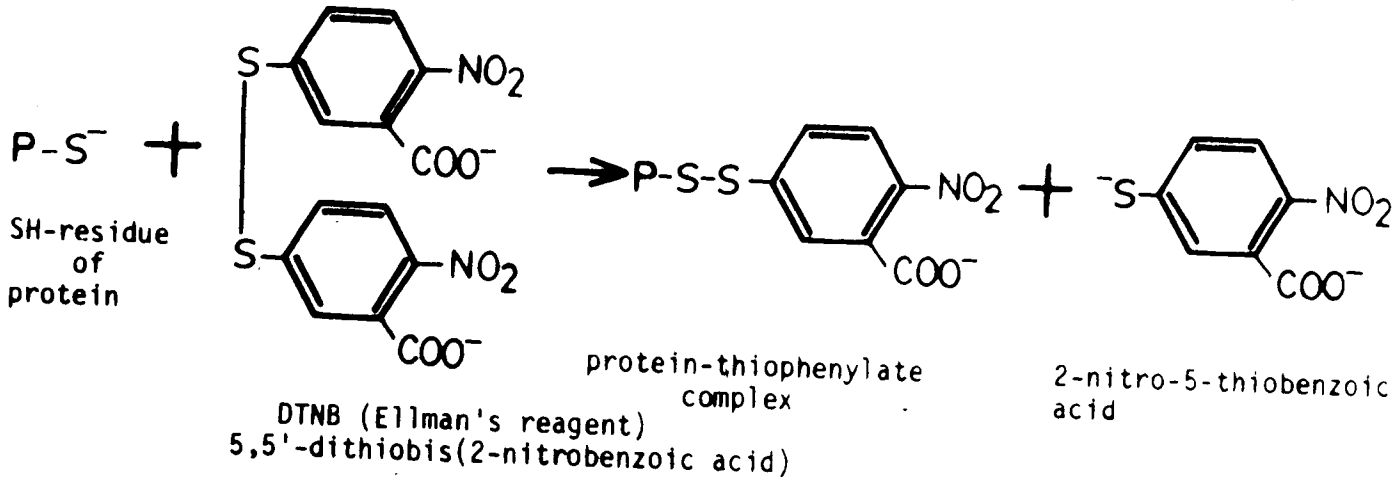


Figure II.2. Reaction between SH (sulfhydryl) residue of protein and DTNB (Ellman's reagent)

reduction in 8 M urea, but easily reduced in 6 M quanidine HCl, Felker and Waines (1978) repeated the determination in the latter unfolding agent. The results obtained were low and were comparable to the urea approach. When the experiment was repeated at 60°C, even lower values were obtained than at 38°C (6 M quanidine HCl takes 3 weeks to completely denature soybean trypsin inhibitor at 25°C, but only 15 min at 70°C).

Ellman's value for molar absorption coefficient has been routinely used in protein analysis. However, several reports have provided $E_{1\%}^{1cm}$ values ranging from 11,400±600 to 14,140±170 M^{-1} cm^{-1} (Silverstein, 1975). Riddles *et al.* (1979) reexamined the use of Ellman's reagent $E_{1\%}^{1cm}$ value and found that accurate determination of thiol groups is limited by uncertainty of the molar absorption coefficient of the dianion 2-nitro-5-thiobenzoic acid (TNB). They purified Ellman's reagent, prepared by reoxidation of purified TNB, followed by gel chromatography and crystallization, and found a molar absorption coefficient of 14,150 at 412 nm in dilute aqueous salt solutions. This value was then confirmed independently by reduction of purified Ellman's reagent with cysteine.

The absorption spectrum of TNB was also critically examined (Riddles *et al.*, 1979). Though the absorption maximum is at 409.5 nm, the molar absorption coefficients were reported for 412 nm since this wavelength has been used by virtually all workers in accord with Ellman's

original paper (Ellman, 1959). In the presence of 6 M guanidine HCl at 412 nm a decrease in molar absorption coefficient to 13,700 was found.

As stated by Cavallini *et al.* (1966), the success of disulfide groups determination by Ellman's reagent depends greatly on removal of the excess of Na-borohydride. A 3 ml protein assay volume usually contains 1 ml of 2.5% Na-borohydride and a drop of octanol as an antifoaming agent. After disulfide reduction 0.5 ml of a solution of 1 M KH_2PO_4 containing 0.2 M HCl is added. After 5 min the destruction of Na-borohydride is completed by addition of 2 ml of acetone. With these precautions, the absorbance of blanks is usually low (0.03-0.05).

Cystine + Cysteine -- Ion-Exchange Chromatography

Ion-exchange resin based automated amino acid analyzers are not suitable for direct determination of protein cystine + cysteine content. Cysteine is subject to oxidation during acid hydrolysis and chromatography and the cystine peak of a protein hydrolysate is not symmetrical since there is racemization of L-cystine into meso and DL forms. Also, major losses of cysteine and cystine occur during protein hydrolysis as a result of reaction with liberated tryptophan and hydrolyzed poly- and oligo-saccharides. Therefore, methods have been introduced to quantitatively change cystine + cysteine residues of proteins to derivatives stable to hydrolysis with 6 N HCl at 100°C for 24 hr. The derivatives can then be eluted from

resin columns as discrete peaks and be determined along with other amino acids by the usual colorimetric detection systems.

Such a method was introduced by Schram *et al.* (1954). It is based on protein oxidation with performic acid, during which cysteine and cystine residues are quantitatively transformed to cysteic acid residues which, after acid hydrolysis, are determined as cysteic acid.

After the preliminary oxidation step, the excess performic acid must be destroyed. Moore (1963) showed that addition of HBr as a reducing agent provides a 94±2% recovery of cysteic acid and a 100±2% recovery of methionine sulfone. Hence, this modification might provide simultaneous results for protein (half)-cystine and methionine contents. However, Friedman (1973) reported that applying this method to cereals and seed meals seldom provides accuracy and reproducibility. A promising alternative method was reported by Spencer and Wold (1969). Performic acid treatment prior to acid hydrolysis was replaced by hydrolysis using 6 N HCl containing 0.2-0.3 M dimethyl sulfoxide. This led to quantitative oxidation of both cystine and cysteine to cysteic acid. The disadvantage of this method was the need for a parallel hydrolysate for other amino acids obtained in the absence of dimethyl sulfoxide.

The procedure of Schram *et al.* (1954) was applied on potato tuber samples by Kaldy and Markakis (1972). Freeze-dried samples of 600 mg were mixed with 10 ml of performic acid, chilled to 0°C and left for 16 hr. When oxidation was complete, 20 ml ice cold water were added and the mixture again freeze-dried. These samples were then hydrolyzed in sealed ampules with 6 N HCl at 110°C for 22 hr. Loss of cystine + cysteine content in unoxidized versus oxidized samples of six potato cultivars ranged from 50.0 to 71.4%, with an average loss of 62.2%. The methione content was determined simultaneously in both samples, with losses of 28.6-70.6% and an average of 52.3% for unoxidized samples.

Tryptophan

Tryptophan -- Methods Based on Enzymic and other Hydrolytic Procedures

Tryptophan constitutes about 1% of the amino acid residues in most proteins (Smith *et al.*, 1965). Acid hydrolysis followed by ion-exchange chromatography commonly results in extensive destruction of tryptophan, yielding ammonia as the only recognized product. Many methods have been tried for determining tryptophan in the presence of other amino acids. They include acidic, alkaline or enzymatic hydrolysis, followed by spectrophotometric or spectrofluorometric assay. These methods exploit the reaction of the indole ring to produce colored derivatives in acidic solutions. Later methods included reaction with

K-nitrite and an aliphatic or aromatic aldehyde. Among these procedures the most widely used is p-dimethyl-amino-benzaldehyde in sulfuric acid (reaction I), and then oxidation of the product with Na-nitrite (reaction II). Nevertheless, even this procedure is limited in accuracy and tends to be tedious. In potato tuber analysis by enzymic hydrolysis phenolase activity inhibition is required since the brown phenol reaction products interact with tuber proteins, resulting in complexes resistant to enzymatic digestion. Defatting of freeze-dried potato powder, which removes phenols, is a recommended preliminary procedure.

Spies (1967, 1968) introduced two hydrolytic methods for the determination of tryptophan in plant proteins. In one method protein is hydrolyzed in oxygen-free NaOH in the presence of basic lead acetate and histidine, the latter two preventing the destruction of tryptophan by serine and cystine. Interference in spectrophotometric analysis of alkaline hydrolysates by sulfur-containing compounds such as sulfides or bisulfites was eliminated by using silver sulfate.

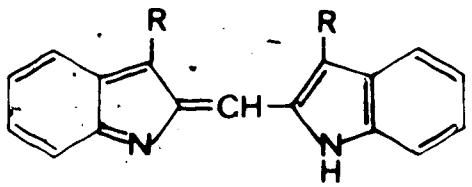
In the other method protein is hydrolyzed with the enzyme pronase. This is the so-called Spies "procedure W" (Spies, 1967). It involves a solution of 10 mg pronase/ml 0.1 M phosphate buffer pH 7.5, of which 100 µl are added to 1-6 mg of pulverized protein (10-100 µg tryptophan). Incubation is at 40°C for 24 hr. Then Reaction I

(dimethyaminobenzaldehyde is carried out at 25°C for 1 hr followed by reaction with sodium nitrite for 1 min after which the absorbance is read at 544 nm.

As reviewed by Friedman and Finley (1971), protein-bound tryptophan gives a greater color yield with p-dimethylaminobenzaldehyde than free tryptophan. This observation indicates that recovery of added free tryptophan may not be a valid test of the method. In alkaline media free tryptophan is subjected to racemization more extensively than tryptophan residues in peptides, which result in different color yield. They cited data by Spies and Chambers (1949). In 5 N NaOH after 18 hr at 100°C racemization of free tryptophan was only 11%, of N-acetyl-tryptophan amide 65% and of protein-bound tryptophan 100%. Therefore, a need still exists for a fast, accurate and generally applicable method for tryptophan determination.

Tryptophan -- Methods Based on Hopkins-Cole Reaction

These methods are based on the reactivity of the indole-ring to develop a colored derivative with glyoxylic acid:



R= $-CH_2-\underset{NH_2}{CH}COOH$

Chromophore
(A_{545nm})

The reaction is greatly enhanced with copper sulfate [illegible] when the protein is dissolved in [illegible] % NaOH or 85% formic acid before the glyoxylic acid is added. Glacial acetic acid in the presence of iron and some sulfuric acid might be converted to glyoxylic acid and thus used in the Hopkins-Cole reaction (Oplenska Blauth et al. [illegible]). The latter method is sensitive over a wide range of concentrations of tryptophan (2-40 µg). It is extremely simple and rapid and the colored chromophore is stable for more than 10 hr. As proved by Oplenska-Blauth et al. (1963) with [illegible] globulins, their method can be applied equally well to free tryptophan and that bound in proteins soluble in water. Chlorides, phosphates and some biological reducing compounds do not influence the results. However, the method, like all others based on the color reaction between an indole ring and aldehydes in acidic medium, is not specific for tryptophan but is applicable to all indole compounds.

The above procedure has been successfully applied as a rapid and simple method for the determination of tryptophan in cereal grains (Concon, 1975). Alkaline extracts of cereal proteins were treated in the prescribed sequence: glacial acetic acid-ferric chloride and 25.8 N sulfuric acid solutions. Specified amounts of these solutions and the sample were added in order to adjust the required concentration of acid. The reaction mixture was then heated at 60°C for 45 min and the absorbance of the resulting

reddish violet colored solution read at 545 nm. As claimed by [illegible] the results for cereal proteins agreed quite favorably with those of the Spies [illegible] method using papain enzyme hydrolysates. When the Opienska-Blauth *et al.* [illegible] method is applied to unhydrolyzed cereals as prescribed by these authors serious browning reactions occur.

Concon [illegible] set up conditions which avoid the browning and other interfering color reactions. Firstly, Concon's method depigments the carotenoid-enriched grain samples with n-butanol, followed by successive treatments with anhydrous ether-absolute ethanol mixture and hexane or pet. ether. To avoid browning reactions, the sequence of reagent addition was also changed. First, glacial acetic acid-ferric chloride solutions are added to the sample and only then the 25.8 N sulfuric acid. The test solutions are then heated at 60°C for 45 min while shielded from direct light. Thus, proper choice of reaction conditions and butanol extraction of sample minimize, if not eliminate, the interference of grain pigments that posed serious difficulties with the original Opienska-Blauth *et al.* (1963) method.

In cereals the protein extraction efficiency with 0.075 N NaOH is 90% or greater. For freshly ground cereals it is 98% or even higher. The tryptophan chromophore has a violet color, but occasionally, as with rye, barley and sometimes wheat, it is red-violet without the λmax being

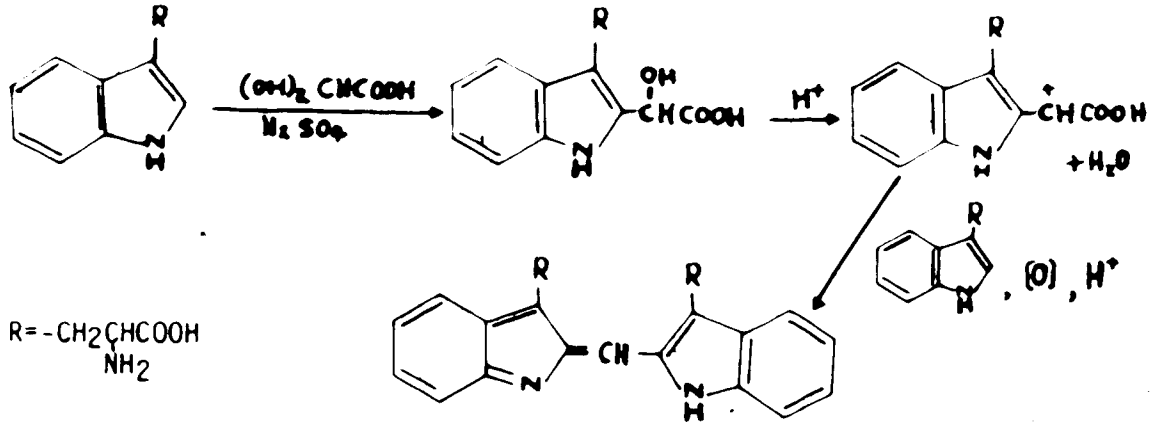
affected. It is stable at 60°C even after 1 hr; however, above 70°C the chromophore is gradually destroyed at different rates, while at 100°C 80% or more is destroyed in the concentrated acid.

Sulfuric acid functions in the above reaction both as a proton donor and a dehydrating agent (see reaction 1) in glyoxylate formation from acetic acid:

$$CH_3COOH \xrightarrow[[O],H^+]{Fe^{++}} OHC{-}CHCOOH \xrightarrow{H_2SO_4} O{=}CHCOOH$$

glyoxylic acid

and also in formation of the tryptophan chromophore:



As established by Concon (1975), color development with cereal proteins, expressed as percent of the maximum color under the conditions of his method, was a direct and inverse function of the sulfuric acid and water concentrations, respectively. There was a 17% increase in color intensity for each 1 N increment in acid concentration. Experimentally this was valid only up to 10

N sulfuric acid, since above this normality significant destruction of tryptophan occurred, accompanied by increased browning.

Another simple method for determination of tryptophan in food was developed by Oste *et al.* (1976). In this method the food proteins are completely solubilized by partial hydrolysis with 8 M urea and the fluorescence and the fluorescence of tryptophan is measured directly at 348 nm during excitation at 288 nm. The quenching effects of food constituents of a mixed school lunch, canned baby foods, haddock or ham were negligible after the insoluble food residues (not hydrolyzed by papain) were removed by centrifugation. As found by the same authors, the tryptophan fluorescence of these food samples is much less influenced by changing pH than is the fluorescence of free tryptophan. A pH range of 7-9 was suggested to acquire correct values when tryptophan is used as the internal standard.

The ability of Sephadex G-25 columns to adsorb aromatic compounds to a greater extent than aliphatic compounds has also been used in tryptophan analysis (Slump and Schreuder, 1969). In this method deaerated barium hydroxide was applied to hydrolyze the food protein at 120°C for 8 hr. The hydrolysate was then adjusted to pH 3-4 with conc. HCl, diluted and an aliquot put on a Sephadex G-25 column, connected to an automatic amino acid analyzer. After the barium ions were eluted from the columns, a

ninhydrin pump was switched on, and the elution chromatogram of the amino acids ended with the tryptophan peak well separated from other acids. The authors did not elaborate on the extent of tryptophan destruction during alkaline hydrolysis of proteins by peptide-linked cystine, serine and threonine. The high recovery of tryptophan in their method could be ascribed to a great extent to omission of removal of barium ions by precipitation as sulfate or carbonate. As found by Robel (1967), tryptophan was occluded during precipitation as barium carbonate.

Removal of barium ions from the alkaline hydrolysate with sulfuric acid, followed by tryptophan determination directly in the hydrolysate by colorimetry at 590 nm with p-dimethylaminobenzaldehyde and sodium nitrite, was a method for cereal and legume food samples introduced by Matheson (1974) and applied by Piombo and Lozano (1980).

As found by many authors, carbohydrates resulting from acid hydrolysis of starch into smaller polysaccharides and glucose may be primarily responsible for the partial destruction of tryptophan. Protective agents such as p-toluenesulfonic acid (Liu and Chang, 1971), thioglycolic acid (Matsubara and Sasaki, 1969) and 3,3-indoylpropionic acid and other reagents (Penke *et al.*, 1974) have been offered to attenuate tryptophan degradation during classical protein acid hydrolysis. Nevertheless, the addition of such components still did not yield quantitative recovery of tryptophan. When starch carbohydrates were

reduced *in situ* into sorbitol, quantitative recovery of sorbitol was restored (Finley *et al.*, 1975). The use of barium hydroxide instead of acids has been reported not to cause destruction of tryptophan during hydrolysis in the presence of starch (Miller, 1967). It appears that 4 N lithium hydroxide might be superior in this respect (Lucas and Sotelo, 1980).

Finally, a novel method for tryptophan determination was introduced by Messineo and Musarra (1972). It is based on a green chromophore condensation product obtained from furfural derivatives of ketohexoses and cysteine hydrouloride (maximum at 415 nm) which, upon the addition of tryptophan, forms a pink chromophore (maximum at 518 nm). In this method, whenever the indole derivatives are not present, tryptophan can be determined quantitatively. The reaction requires fructose in excess to which sulfuric acid and cysteine HCl are added. The mixture is heated at 45°C for 10 min. The green chromophore is indicative of reaction completion. Then tryptophan (protein) is added at room temperature to the reaction mixture, the presence of tryptophan being confirmed by the appearance of the pink chromophore. After 1 hr, the absorbance of the sample is read at 518 nm. Under the conditions of this method, when bound tryptophan is present in the sample (protein), prior hydrolysis is not required. The method was successfully applied to some purified proteins (egg albumin, gelatin hydrolysate, wheat gluten gliadin), but was not tested with

vegetable proteins, either purified or in the presence of starches.

III. MATERIALS AND METHODS

A. Sample Preparation

Potatoes -- Raw, Cooked, and Dehydrated Granules

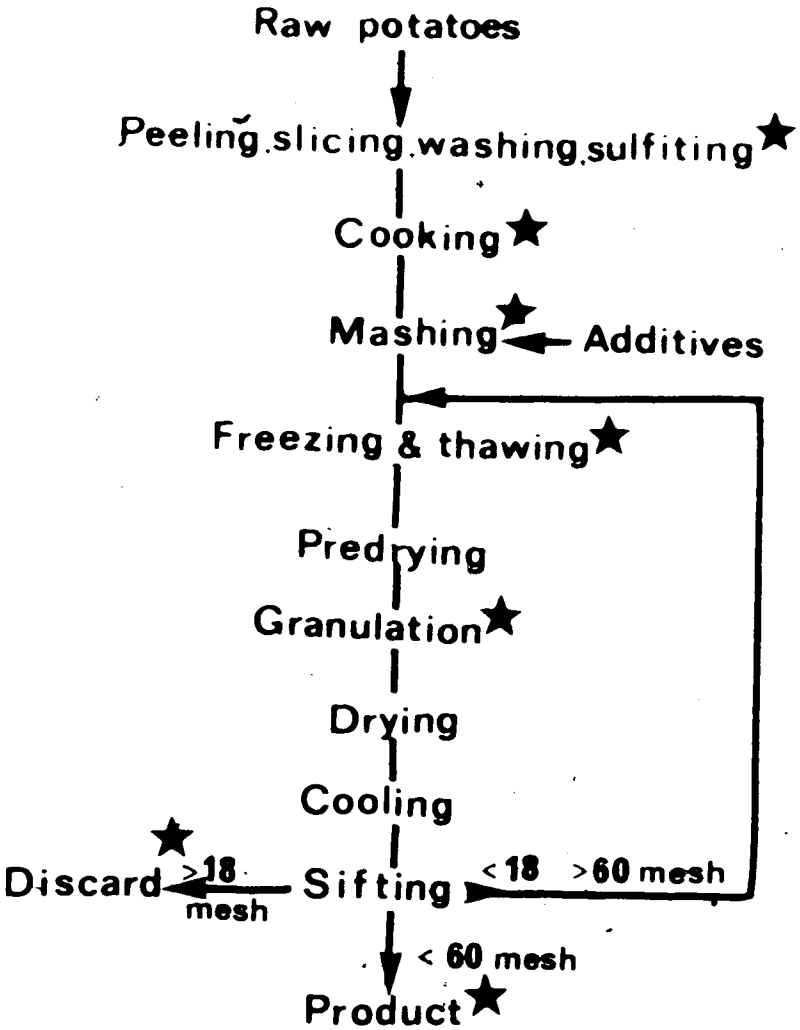
Potato tubers (*Solanum tuberosum*, cv. Russet Burbank) grown in Alberta were obtained from I & S Produce Ltd. (Edmonton) and were used throughout all experiments.

Medium-size tubers were peeled and sliced. After soaking in 500 ppm $NaHSO_3$ solution, slices were freeze-dried (model FFD-42-WS). Slices from the same batch were also subjected to two different cooking methods: (i) precooking at 70°C for 20 min, cooling in tap water for 20 min, and boiling for 30 min; (ii) steaming for 30 min, followed by cooling in an air-blast freezer.

The boiled and steamed samples were also freeze-dried. All samples were powdered in a Waring blender, and stored in brown bottles at -20°C.

Granule preparation by the Freeze-Thaw (F-T) process developed by Ooraikul (1977) was used for the preparation of dehydrated potato granules (Fig. III.1).

Approximately 4.3 kg of potato tubers were washed, peeled and sliced. After soaking in 500 ppm $NaHSO_3$ solution for 5 min, the slices were steamed for 35 min. The cooked potato was mixed with Myvatex (0.25%), BHA (100 ppm) and sodium acid pyrophosphate (10 ppm) in a Kitchen Aid mixer at an agitation speed of approx. 400 rpm. The mashed potato was



Flow chart
Freeze-Thaw Granule Process

Figure III.1. Flow Chart of the Freeze-Thaw Process

frozen in an air-blast freezer at -28°C, thawed at room temperature to 0-5°C and kept at 4°C for 3 hr.

In the predrying step, the thawed mash was placed in the air dryer at 70°C, and then drying air (93°C, 115 m/min) was blown in, with the stirrer spinning at 20 rpm. In the granulation step, the stirrer speed was increased to about 490 rpm, and the air temperature and velocity were reduced to 52°C and 15 m/min, respectively.

On the completion of final drying, stirring was terminated and the air temperature and velocity were increased to 85°C and 90 m/min, respectively. The dehydrated potato granules were then sifted. Particles <60 mesh were collected as "End Product", while coarse particles (>80 mesh) were discarded.

Samples were withdrawn from each stage indicated by an asterisk in Fig. III.1. Each sample was frozen immediately in liquid nitrogen and then freeze-dried. After homogenization in a Waring blender, the powdered samples were stored at -20°C.

Chemicals

Reagent grade water, obtained from reverse osmosis water which had been passed through a Milli-Q system (Millipore, Mississauga, Ont.), was used unless otherwise specified.

Ethanol, glacial acetic acid and sodium chloride were obtained from Fisher Scientific Co. Ltd. (Fairlawn, NJ); sodium hydroxide from American Scientific & Chemicals (Portland, OR); EDTA(Na_2) and sodium bisulfite from J.T. Baker Chemical Co. (Phillipsburg, NJ); mono- and disodium phosphates (used to prepare 0.02 M phosphate buffer, pH 7.5) from Matheson, Coleman & Bell Manufacturing Chemists (Norwood, OH).

Apparatus

Dialysis tubing; flat width 4.4 cm (Fisher Scientific Co., Fairlawn, NJ).

Hydraulic press; Carver model B (F.S. Carver Inc., Summit, NJ).

Centrifuge; Beckman model J-21B (Beckmann Instruments, Inc., Palo Alto, CA).

Rotary evaporator; Büchi/Brinkmann R/A (Brinkmann Instruments Ltd., Ont.).

Air-blast freezer, NOVA (Bally Refrigerator of Canada, Ltd., Brodeville, Ont.).

Freeze driers; model 10-102 Macro Unitrap, and model FFD-42-WS (Virtis Co., Inc., Gardiner, NJ).

Blender; Waring-type, Osterizer (Oster Corp., Milwaukee, WI).

Kitchen Aid mixer (Hobart Mfg., Co., Ltd., Troy, OH).

Modified Manesty Petrie fluid bed dryer, model MP.10.E (Manesty Machines, Ltd., Speke, Liverpool, UK). Modified as

described by Ooraikul (1978).

Speedomax 12-point temperature recorder (Leeds and Northrup, Canada, Ltd., Toronto, Ont.).

Canadian Standard Sieve series and portable sieve shaker (W.S. Tyler, Co. of Canada, Ltd., St. Catharines, Ont.).

Potato Protein Extraction and Fractionation

Based on Golan-Goldhirsh's procedure (1979), tuber protein was fractionated for amino acid analysis and electrophoretic studies.

Tuber Sap Extraction

In each batch, one medium-size tuber was washed, peeled and diced. The cubes were wrapped in canvas, immersed in 100 ml 5% NaCl containing 500 ppm $NaHSO_3$, and squeezed in a hydraulic press at 24,000 psi. Tuber sap was collected in a beaker containing 10 mg of EDTA(Na_2); while the residue was mixed with 50 ml of the above NaCl solution and homogenized in a Waring blender for 30 seconds. The homogenate was decanted onto the canvas and squeezed again. The effluent was combined with the previous sap, while the residue was recovered from the canvas for further extraction. The addition of $NaHSO_3$ and EDTA was aimed at prevention of enzymatic browning induced by polyphenoloxidase.

Albumin and Globulin Fractions

The tuber sap was transferred to dialysis tubing and dialyzed against distilled deionized water (DDW) at 4°C for 3 days, with 6 changes of water. Nondialyzable material was centrifuged at 14,000 x 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 hr. After centrifuging at 14,000 x g, the supernatant (Globulin Fraction) was dialyzed against DDW. The precipitate was resuspended in water, stirred, dialyzed, and centrifuged. This supernatant was mixed with the previous Albumin Fraction. The precipitate was designated as globulin.

Prolamin Fraction; Acidic and Basic Glutelin Fract

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 dialyzing against DDW, all five protein fractions obtained were freeze-dried, then kept at -20°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).

Potato Tuber Sampling for Nitrogen, Sulfur and Cysteine Determination

Large potato tubers (approx. 300 g) were washed, weighed and sliced without peeling. Each slice was divided into four major anatomical zones: cortex (including periderm), vascular bundle (including outer phloem), perimedulla (including inner phloem), and medulla (pith). Each class was weighed before and after freeze drying (model FFD-42-WS). The dried samples were first mashed into coarse powder in a Waring blender at low speed, and then into fine powder with a mortar and pestle.

B. Elemental Analysis

Nitrogen Determination

Unless otherwise stated, nitrogen content was determined by the standard micro-Kjeldahl method (AOAC, 1980).

The nitrogen content of the protein fraction was determined by a Perkin Elmer Model 240B elemental analyzer (Perkin Elmer Corp., Norwalk, CT) in the Microanalytical Laboratory of the Department of Chemistry, University of Alberta. A 1-3 mg sample in a platinum boat was combusted with pure oxygen at about 950°C. The resultant gas was passed through a reduction tube (650-700°C), and sent to a mixing volume where sample gas was mixed for 90 sec with helium at 1500 mm Hg. The mixed gas was passed through a

water trap (magnesium perchlorate column) and then a carbon dioxide trap (magnesium perchlorate and colorcarb column) by helium carrier gas at 75±0.1°C. A thermal conductivity detector was used to measure the amount of water and carbon dioxide trapped, as well as the amount of nitrogen gas remaining after these trapping steps. The contents of nitrogen, hydrogen and carbon present in the original sample were calculated from the amount of N_2, H_2O and CO_2 detected.

Sulfur Determination

Total sulfur content of the protein fraction was determined by an oxygen flask combustion method (AOAC, 1980) in the Microanalytical Laboratory. A 2-6 mg sample folded in a paper carrier was inserted into an oxygen flask, and was combusted in the presence of pure oxygen and 10 ml of 6% H_2O_2 solution. After shaking for 30 sec, the stopper and the platinum sample basket were washed with ethanol. The solution was decanted into an Erlenmeyer flask, made up to 100 ml with ethanol, and titrated with 0.01 N barium perchlorate standard solution.

Total sulfur contents of the samples obtained from different anatomical zones were determined by an alkaline oxidation method (Tabatabai and Bremner, 1970b) in the Research Laboratory, Soil Science Department, University of Alberta. A 30 mg sample was oxidized by heating with alkaline sodium hydrobromite solution. The sulfate thus formed was reduced to H_2S, and then reacted with

p-aminodimethylaniline to produce methylene blue. The amount of methylene blue formed was determined colorimetrically. A calibration curve was obtained using potassium sulfate standard solution, and the amount of total sulfur was calculated from it.

C. Electrophoretic Separation of Potato Proteins

Extracted potato proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Chemicals and Gel Preparation

Acrylamide, bisacrylamide and N,N,N',N'-tetramethylethylenediamine (TEMED), all electrophoresis purity grade, were from Bio-Rad Laboratories (Richmond, CA). Tris(hydroxymethyl)aminomethane and bromophenol blue were obtained from Matheson, Coleman and Bell Manufacturing Chemists (Norwood, OH) and Sigma Chemical Co. (St. Louis, MO), respectively. As molecular weight markers, chicken myofibril proteins were kindly prepared by Dr. P. Nagainis of this Department; while ribonuclease A, a marker protein with low molecular weight, was obtained from Pharmacia Fine Chemicals (Pitscataway, NJ). Other chemicals were obtained from Fisher Scientific, Ltd. (Fairlawn, NJ).

Gel casting solution composition was:

36 ml	acrylamide solution (25 g acrylamide and 0.25 g bisacrylamide in 100 ml)
18 ml	Tris-glycine buffer (0.5 M Tris, 1.5 M glycine, pH 8.8)
9 ml	50% glycerol
3.6 ml	2.5% SDS, 2.5 mM EDTA
3.6 ml	1% TEMED

3.6 ml ammonium persulfate (prepared daily)
16.2 ml distilled deionized water

90.0 ml total

After gentle mixing, 2.3 ml of the solution were rapidly pipetted into a 6 mm (i.d.) x 10 cm glass tube with a serum (vacutainer) cap at the bottom. To exclude interference in polymerization by oxygen from the air, each gel was overlayed with 75 µl of the following solution:

800 µl Tris-glycine buffer
160 µl 1% TEMED
160 µl 2.5% SDS, 2.5 mM EDTA
16 µl 1% ammonium persulfate (prepared daily)
2864 µl distilled deionized water

4.0 ml total

Gels were polymerized in the dark for 2 hr, overlaying solution was removed, and the surface of the gel was rinsed twice and relayered with the following solution:

400 µl Tris-glycine buffer
200 µl 50% glycerol
100 µl 2% SDS
1.30 ml distilled deionized water

2.00 ml total

Preliminary Sample Treatment

Prior to electrophoresis 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-boric acid buffer (0.01 M Tris, 0.2 M boric acid; pH 7.1). After adding 250 mg SDS and 62 mg mercapto-ethanol, the mixture was boiled for 6 min, then centrifuged at 14,000 x 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 pure protein sample, obtained by solubility fractionation, were treated with 0.5 ml of 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 and the solution was vortexed thoroughly, cooled to room temperature, and stored at 4°C.

Electrophoretic Protein Separation

Appropriate amounts of samples (75-500 μg) were applied on the surface of polyacrylamide gels. Electrophoresis was performed in an electrode buffer consisting of 0.2 M Tris-glycine buffer pH 8.8 and 0.1% SDS. An initial current of 0.25 mA per tube, applied for 30 min, was followed by 1 mA constant current per tube for 7 hr. The gels were removed by running water, stained overnight in 0.2% Coomassie Brilliant Blue (in a solvent consisting of methanol, acetic acid, water, 100:20:100), and destained electrophoretically for 12 min in aqueous 10% isopropanol-10% acetic acid. Gels were then washed and stored in 7% acetic acid.

Quantitation of bands resolved by SDS-PAGE was done by scanning of the gel rods at 640 nm on a Beckman model 2400 spectrophotometer equipped with a scanning attachment.

D. Hydrolysis of Protein and Determination of its Amino Acid Composition

Chemicals and Apparatus

Isopropanol, formic acid, sodium cyanide, sodium acetate, and ethylene glycol monoethyl ether (methyl cellosolve) were obtained from Fisher Scientific Co. Ltd.; hydrogen peroxide (30%) and phenol from J.T. Baker, Co.; reagent grade 37% hydrochloric acid from American Scientific and Chemicals; and 47% hydrobromic acid from Koch-Light Laboratories, Ltd. (Colnbrook Bucks, UK).

Methanesulfonic acid (95%) and 3-(2-aminoethyl)indole hydrochloride were from Eastman Kodak Company (Rochester, NY); ammonium hydroxide from Canadian Industries Ltd.; and cation exchange resin, Bio-Rad AG50W-X4 (H^+ form, 100/200 mesh, exchange capacity 1.2 meq/ml resin bed) from Bio-Rad Laboratories (Mississauga, Ont.). Before use, the resin was regenerated with 1 N HCl, and washed extensively with Milli-Q water.

L-pipecolic acid and L-α-amino-β-guanidinopropionic acid (AGPA) were from Aldrich Chemical, Co., Inc. (Milwaukee, WI) and Calbiochem-Behring Corp. (La Jolla, CA), respectively. In order to eliminate trace moisture, these amino acid standards were dried overnight over P_2O_5 under reduced pressure.

Amino Acid Standards H (2.5 μM/ml of each amino acid, except for cystine, 1.25 μM in 0.1 N HCl) were obtained from Pierce Chemical Co. (Rockford, IL), and ninhydrin from Sigma Chemical Co. (St. Louis, MO).

Culture tubes (16 mm o.d. x 125 mm) used for hydrolysis were either Kimble 45066 (Kimax brand) or Corning 9825 (Pyrex brand). Pasteur pipettes (Dispo pipettes; ASTM designation E438-71) were from Scientific Products (McGraw Park, IL); 1 ml reacti-vials with Teflon-lined discs (Pierce #13221) were from Pierce Chemical Co. All glassware was cleaned by heating for a few hours in a mixed acid (conc. HNO_3-conc. H_2SO_4, 1:3 v/v), followed by extensive rinsing with Milli-Q water.

Millipore depth prefilter, type AP25025-00 (25 mm diameter), and HP-Millipore filter, type HAWP 02500 (pore size 0.45 μm, 25 mm diameter) were obtained from Millipore Corp. (Bedford, MA).

Hydrolysis of Raw and Cooked Potato Tuber and of Freeze-Thaw Processed Samples

Two types of hydrolysates, namely "oxidized" and "nonoxidized", were prepared in powder form from each of the following: raw and cooked potato tubers, and samples obtained after various steps of the F-T potato granule process.

Oxidation Step

Oxidizing reagent (performic acid) was prepared 1 hr before use by mixing 9 volumes of 99% formic acid and 1 volume of 30% hydrogen peroxide in an ice bath. Approximately 25 mg of each potato sample were weighed in a culture tube, mixed with 1 ml of the oxidizing reagent, and kept in an ice bath, with occasional mixing, for 16 hr. Excess performic acid was then reduced by adding 0.15 ml of 47% hydrobromic acid, with swirling of the reaction tube in an ice bath. The tubes were then attached to a rotary evaporator coupled to a high vacuum line. Solvent was evaporated to dryness at 40°C, and the oxidized sample was subjected to hydrolysis.

Hydrolysis

Approximately 25 mg of each sample were weighed in a culture tube and hydrolyzed as with the nonoxidized sample. A solution of 5 ml 6 N HCl containing 0.1% phenol was added to both oxidized and nonoxidized samples. After freezing in a dry ice-acetone bath for a few minutes, the sample tube was connected to a high vacuum line and the content was thawed slowly. Evacuation was continued for 5 min after the termination of bubbling. Nitrogen gas was then slowly introduced as the stopcock in the vacuum line was closed. Immediately after removing the tube from the nitrogen stream, it was tightly closed with a Teflon-lined screw cap. After heating in an oven at 110°C for 22 hr, the tube was cooled and stored at -20°C.

Filtration and Solvent Evaporation

AGPA (1.25 μM) and L-pipecolic acid (5 μM), as dilute aqueous HCl solutions, were added to the oxidized and nonoxidized hydrolysates. After vortexing for 1 min, the content was washed with 4 x 10 ml water into a filtration unit consisting of double layers of Millipore filters (prefilter and membrane filter). The filtrate collected in a 125 ml flat bottom flask was evaporated on a rotary evaporator at 50°C under aspirator suction. The dried residue was redissolved in 1 ml of Milli-Q water, then dried. This step was repeated four times to eliminate residual HCl. Finally, the residue was dissolved in 10 ml 0.01N HCl, divided into ten 1 ml aliquots, and each was stored in a small test tube at -20°C.

Cation Exchange Chromatography

Filtered protein hydrolysate was purified by cation exchange chromatography.

Preparation of the Cation Exchange Column

A plug of acid-washed glass wool was inserted well down into the stem of a Pasteur pipette, and the cation exchange resin was washed with water into the pipette and allowed to settle. The height of the resin bed was adjusted to 1.2 cm, which was equivalent to 0.24 ml wet resin volume (0.286 meq). The resin surface was covered with water, and the column stored at -4°C.

Hydrolysate Purification

To the prepared column, 1 ml of filtered hydrolysate in 0.01 N HCl was slowly applied. The sample test tube was washed with 200 µl of water, which was added immediately to the column. The column was washed twice with 500 µl water, and then the amino acids were eluted into a test tube with 2 ml 5 N ammonium hydroxide. Evaporation of the eluant to reduce volume was done on a rotary evaporator at 50°C under aspirator suction. Eluates for ion exchange chromatography were dried in the freeze drier; while those for gas-liquid chromatography were washed into 1 ml reacti-vials using a small amount of water, then freeze-dried.

Determination of the Recovery of Amino Acids

The performance of the cation exchange resin was tested for a standard amino acid mixture and for raw potato tuber hydrolysate. A standard amino acid mixture equivalent to 3 µM/ml total amino acids (excluding NH_3) was prepared by diluting Amino Acid Standard H with 0.1 N HCl.

A 1 ml aliquot of the standard and 1 ml of filtered raw tuber hydrolysate were applied to the cation exchange column. The initial effluent (1st eluate) and the eluate after using 2 ml of 5 N NH_4OH (2nd eluate) were collected separately. An additional 2 ml NH_4OH was used to elute any trace of amino acids retained on the column (3rd eluate). All the eluates were dried on a rotary evaporator at 50°C. Dried residues were redissolved in 0.5 ml water and evaporated, repeating four times to ensure removal of

ammonia. Finally, all samples were dissolved in 1 ml water. The amino acid content of each eluate was determined by a modified ninhydrin reaction (Rosen, 1957), and then the recovery of amino acids in each eluate was calculated.

Modified Amino Acid-Ninhydrin Reaction Method

Rosen's method (1957) was used, with modifications. Reagents were prepared as follows:

1. Stock NaCN solution: 0.01 M sodium cyanide
2. Acetate buffer: 108.51 g sodium acetate, 133.33 ml H_2O and 33.33 ml glacial acetic acid were made up to 500 ml with H_2O; pH 5.3
3. Acetate-cyanide solution: 20 ml of stock NaCN solution was made up to 1 l with acetate buffer
4. Ninhydrin solution (3%): 3% ninhydrin in methyl cellosolve; freshly prepared
5. Diluent: isopropanol-water, 1:1 (v/v)

To a 1 ml sample were added 0.5 ml acetate-cyanide solution and 0.5 ml ninhydrin solution. The mixture was vortexed for 30 sec and then placed in a boiling water bath for 15 min. Immediately after removal from the bath, 5 ml of diluent were added rapidly, followed by vortexing for 30 sec and cooling to room temperature. The absorbance was measured at 570 nm.

Two sets of blanks were prepared (reagent and chromatographic blanks). The reagent blank was prepared by replacing sample solution with 1 ml water. Chromatographic blanks were necessary to compensate for enhanced absorption

readings due to residual ammonia derived from the eluant (5 N NH_4OH). Chromatographic blanks were prepared in triplicate by:

1. Applying 1 ml water to the cation exchange column;
2. Collecting eluates 1, 2 and 3 separately;
3. Eliminating ammonia as under recovery of amino acids;
4. Dissolving each residue in 1 ml water.

Recoveries of amino acids were calculated as follows:

$$Cos = Aos - Arb$$

$$Cs_n = As_n - Acb_n$$

where A represents the absorbance reading, and the subscripts are defined as follows:

os = original sample solution

rb = reagent blank solution

s_n = sample eluate, n = 1, 2, or 3

cb_n = chromatographic blank eluate; n = 1, 2, 3

The % amino acid recovery for each eluate would then be calculated by: $100 \times Cs_n/Cos$

Hydrolysis of Individual Protein Fractions

Protein fractions were hydrolyzed by:

(a) HCl, with a preliminary oxidation step;

(b) HCl, without preliminary oxidation;

(c) Methanesulfonic acid.

Hydrolysis by HCl

Approximately 2-3 mg of Albumin, Globulin and Basic Glutelin Fractions were weighed into test tubes (18 mm x 150 mm). Sample oxidation was as in section D, except that the reaction time was reduced to 3 hr. Both oxidized and nonoxidized samples were mixed with 2.5 ml 6 N HCl containing 0.1% phenol. The neck of the tube was narrowed with the aid of a gas burner. After freezing the sample in a dry ice-acetone bath, the test tube was connected to a high vacuum line and the content was thawed. After termination of bubbling, gas evacuation was continued for 5 min, then the tube was sealed, heated in an oven at 110°C for 22 hr, cooled, and stored at -20°C.

Hydrolysis by Methanesulfonic Acid

This procedure followed that of Simpson *et al.* (1976).

Concentrated methanesulfonic acid was diluted to 4 N, then flushed with nitrogen. Using the procedure reported by Liu and Chang (1971), 3-(2-aminoethyl)indole was prepared from its hydrochloride, and then dissolved in methanesulfonic acid to achieve a final concentration of 0.2%.

Approximately 2 mg of protein sample were placed in a test tube, mixed with 1 ml of the above solution, and sealed under vacuum and heated as described in the previous section.

E. Amino Acid Separation by Ion-Exchange Chromatography

This analysis was performed with two models of automatic amino acid analyzers. The protein fraction hydrolysates produced by reaction with HCl were analyzed on a Durrum D-500 analyzer (single column operation, DC-6A cation exchange resin), while those from reaction with methanesulfonic acid were run on a Beckman 121 MB analyzer (single column, AA-10 cation exchange resin).

F. Amino Acid Analysis by Gas-Liquid Chromatography

Chemicals and Column Preparation

N-heptafluorobutyric acid anhydride (HFBA), Silyl-8 and amino acid standard H were obtained from Pierce Chemical Co. (Rockford, IL). Valine, leucine, phenylalanine, tyrosine and arginine were from Nutritional Biochemical Corp. (Cleveland, OH); glycine, lysine, cysteine, cystine and AGPA from Calbiochem-Behring Corp.; β-alanine from J.T. Baker Chemical Co.; D-allo-isoleucine from Shwarz/Mann (Mississauga, Ont.); pipecotic acid from Aldrich Chemicals; and the other individual amino acids from Sigma Chemical Co. The column packing material, 3% SE-30 on Chromosorb WHP (80/100 mesh) was obtained from Chromatographic Specialities (Brockville, Ont.). Isopropyl alcohol was distilled over sodium sulfate and stored under nitrogen. The esterification reagent was prepared by slowly pipetting 0.2 ml of acetyl chloride (Fisher Scientific Co.) into 1.0 ml of isopropanol at 0°C.

In column preparation a spiral-shaped glass column, 2.6 m x 1/8 in (i.d.), was heated with conc. HCl for 3 hr, then cooled to room temperature. The column was washed with water repeatedly until the wash was neutral, then was successively rinsed with methanol, acetone and n-hexane. The column was dried in a stream of nitrogen, then packed with the 3% SE-30 (see above) and conditioned as recommended by the manufacturer. Silylation of the column was conducted at 200°C by several injections of 10-20 µl of Silyl-8 reagent.

Instruments and Operating Conditions

A Varian series 3700 gas chromatograph equipped with a flame ionization detector (FID) was coupled to a Hewlett Packard 3388 computing integrator. Flow rates, in ml/min, were: carrier gas, 24; air, 300; hydrogen, 16. Injector and detector temperatures were 250 and 280°C, respectively. The column was temperature programmed: 100°C for 1 min, followed by a 10 C°/min to 250°C and holding at that temperature for 20 min.

Derivatization

All reactions were carried out in a reacti-vial with a Teflon-lined disc (Pierce Chemical Co.). Aluminum blocks were preheated to 80±0.5°C and 110±2°C, respectively, in a Dri-Block DB-8 (Techne, Inc., Princeton, NJ) and a forced-draft Isotemp Oven (Fisher Scientific Co.).

Standard amino acid mixtures and sample eluates from the cation exchange purification step, which were equivalent to 50-500 nmoles amino acids, were dried in reacti-vials as described in section D. Trace water was removed azeotropically with dichloromethane. The dried residue was dissolved in 200 µl of the esterification reagent, flushed with nitrogen, and vortexed for 1 min. The vials were then heated in an aluminum block at 80°C for 2 hr. After cooling, a stream of nitrogen was introduced to evaporate solvent at room temperature. The residue was redissolved in 200 µl of HFBA and 100 µl of dichloromethane, flushed with nitrogen, and vortexed for 1 min. Then the vials were heated at 110°C for 10 min. After cooling to room temperature and evaporation of reagents, 50-100 µl of dichloromethane were added, and the samples were stored at -20°C.

Analytical Conditions

Depending on the sample, 1-10 µl were injected, with and without coinjection with HFBA. In the case of coinjection, equal volumes of sample and HFBA were mixed in the syringe prior to injection. In order to maintain column effectiveness, occasional reconditioning and Silyl-8 injection were necessary.

G. Colorimetric Determination of Total Half-Cystine Content

The freeze dried samples of raw and cooked tubers and from various steps of the F-T process and different anatomical regions of the tuber were subjected to colorimetric cysteine determination using DTNB, 5,5'-dithio-bis(2-nitrobenzoic acid) (Felker and Weine, 1978).

Chemicals and Apparatus

Chemicals and suppliers were: *n*-octanol and KH_2PO_4, Fisher Scientific Co.; urea, Mann Research Labs. (Orangeburg, NY); $NaBH_4$, Sigma Chemical Co. (St. Louis, MO); acetone, Terochem Labs. (Edmonton); DTNB, Aldrich Chemical Co., Inc. (Milwaukee, WI); and EDTA(Na_2), J.T. Baker Chemical, Co. (Philipsburg, NJ).

Acidic phosphate solution consisting of 1 M KH_2PO_4 in 0.2 N HCl, and 0.1 M EDTA solution were prepared and stored at 4°C. DTNB solution (4 mg/ml acetone) and aqueous $NaBH_4$ solution (2.5%) were prepared just before use.

Computing spectrophotometer, UV-VIS, Beckman DU-8 (Beckman Instruments, Inc., Palo Alto, CA).

Automatic superspeed refrigerated centrifuge, Servall Type RC-2 (Ivan Sorvall, Inc., Norwalk, CT).

Metabolyte water bath shaker (New Brunswick Scientific Co., New Brunswick, NJ).

Multimanifold unit (made in our laboratory).

Procedure

Approximately 10 mg of freeze dried sample and 0.576 g of urea were weighed into 17 x 119 mm polypropylene centrifuge tubes and mixed with 5 µl octanol, 0.04 ml 0.1 M EDTA, and 0.4 g of 2.5% $NaBH_4$. It was necessary to add the latter by weight as bubbling of the $NaBH_4$ solution prevented precise addition by volume. After vortexing for 1 min, tubes were placed in the water bath shaker at 38°C for 1 hr. During this period, they were vortexed at 20 min intervals and gently rotated to cause large particles to settle. After incubation, destruction of excess $NaBH_4$ was begun by addition of 0.2 ml acidic phosphate solution (1 M KH_2PO_4 in 0.2 N HCl), followed by vigorous vortex mixing. After 5 min, 0.8 ml of acetone was added and the tubes were sealed with a piece of Parafilm and vigorously inverted. Nitrogen was bubbled into eight tubes simultaneously through a multimanifold unit.

Addition of 0.2 ml DTNB solution to each tube occurred after 5 min of bubbling. Water was then added to bring the total volume up to 2.4 ml. After an additional 3 min, the tubes were sealed with Parafilm. Care was taken to fill the head space as thoroughly as possible with nitrogen. A buret dust cap was placed on each tube to support the Parafilm during centrifugation (10,000 x g for 10 min at 4°C). A 1 ml aliquot of supernatant was withdrawn from each tube, and the absorption was measured in a 1 ml quartz cuvette at 412 nm.

Two sets of blanks were prepared:

1. Sample blank, containing sample but no DTNB. Used for subtracting absorbance due to turbidity and the original color of the sample.
2. Reagent blank, containing DTNB but no sample. Used to correct for absorbance due to residual $NaBH_4$, which could reduce the DTNB reagent to give an anionic chromophore.

In each batch, a standard solution containing 0.1 μM of L-cysteine was also subjected to the same procedure to minimize error due to variation in reaction conditions. Quadruplicate samples, two reagent blanks, and two standards were analyzed in each batch. The absorbances of all sample blanks were measured in advance, for the readings were low and constant throughout the analysis. The cysteine content was calculated using the molar absorptivity of 12,700, as obtained in a calibration run.

H. Colorimetric Tryptophan Determination

Tryptophan content in protein extracts obtained without preliminary hydrolysis was determined by the procedure reported by Concon (1975).

Chemicals and Reagent Preparation

The chemicals used and suppliers were: L-tryptophan, Sigma Chemical Co.; sulfuric acid, American Scientific Chemical; ferrichloride hexahydrate ($FeCl_3 \cdot 6H_2O$), BDH

Chemicals; tris(hydroxymethyl)aminomethane and glacial acetic acid, Fisher Scientific Co.

25.8 N Sulfuric Acid: Concentrated H_2SO_4 was diluted in a 1 l volumetric flask to make a final concentration of 25.8 N. The normality was measured by titration using 2 N tris(hydroxymethyl)aminomethane solution.

Glacial acetic acid-ferric chloride solution (Reagent A): $FeCl_3 \cdot 6H_2O$ (5.4 g) was dissolved in 10 ml DDW containing 3 drops of glacial acetic acid. A 0.5 ml aliquot of this solution was made up to 1 l with glacial acetic acid, and stored in a brown bottle. In order to generate glyoxylic acid, it was necessary to keep the solution standing at room temperature for at least one week before use.

Extracting solvent: Four extraction solvents were prepared:

1. 70% ethanol;
2. 0.075 N NaOH;
3. 70% ethanol:0.075 N NaOH, 1:4 (v/v);
4. 70% ethanol:0.5 M NaCl:0.075 N NaOH, 2:3:5 (v/v/v).

Analytical Procedure

Each freeze dried sample (1 g) obtained from raw and cooked potatoes was weighed into a polypropylene centrifuge tube and mixed with 20 ml of extraction solvent. After vortexing 3 min, samples were centrifuged at 10,000 rpm for 10 min, and the supernatant was decanted and passed through

Whatman No. 4 filter paper on a Büchner funnel. The filtrate was washed into a 10 ml volumetric flask, made up to mark with extracting solution, and vigorously mixed. Two 1 ml aliquots were subjected to micro-Kjeldahl nitrogen analysis, while the remaining solution was used for tryptophan determination.

A 1 ml aliquot was added into a culture tube (10 mm i.d. x 125 mm), and mixed with 3 ml of reagent A, then 2 ml of the sulfuric acid. All the solutions and mixtures were kept in an ice bath. Each tube was thoroughly vortexed, and then incubated in the dark at 60°C in a Lo-Temptrol Model 154 water bath. After the reaction time, which ranged from 10-130 min, at 10 min time intervals, each tube was cooled in an ice bath. Standard tryptophan solution was treated in the same way as sample solution, but the reaction time was maintained at 110 min, as recommended by Concon (1975).

Two types of blanks were prepared:

1. *Reagent blank*. In order to detect any change in the performance of reagent, 1 ml of 0.075 N NaOH solution was subjected to the same reaction conditions as the sample.
2. *Sample blank*: In order to subtract the absorption value due to the original sample color, 1 ml of sample protein extract was mixed with reagents. Incubation was omitted; instead, the solution was deaerated under aspirator suction to eliminate bubbles.

The percentage of protein extracted was calculated using the crude nitrogen content determined for the protein extract aliquots.

Tryptophan content was calculated at 545 nm using the following equation:

Tryptophan (g/100 g crude protein) = ((As-Asb)xF)/N where F = (Tstd/Astd)x16. "A" stands for absorbance; "s" for sample; "sb" for sample blank; "std" for tryptophan standard solution; "Tstd" for mg(s) tryptophan in 1 ml standard solution; and "N" for mg(s) nitrogen in 1 ml protein extract.

IV. RESULTS AND DISCUSSION

A. Total Nitrogen and Sulfur of the Potato Tuber

Total nitrogen and sulfur contents present in the major anatomical regions of the potato tuber cv. Russet Burbank (Alberta) are presented in Table IV.1. Nitrogen content of the whole tuber was 1.67±0.09%. A high level of N was found in the periderm (peel) and the adjacent cortex region (total thickness 0.5 mm) plus 4 mm of the storage parenchyma cells, the three regions being designated as "cortex" (see Figure IV.1). The medulla (pith) also had a high N content. The perimedulla N-content (1.55±0.12%) was lower, while the lowest content (1.22%) was in the xylem ring (vascular bundle region) plus the adjacent layer (outer phloem) attached to the ring.

The richest region in S (1,799±30 ppm) was the periderm (peel) plus the adjacent cortex and 4 mm of the storage parenchyma cells, the combined region again being designated "cortex". The inner regions of the tuber contained close to 1,100 ppm S, while the content in the medulla was higher (1,583±30 ppm S).

Nitrogen distribution within the tuber regions and total solids distribution (or specific gravity variations) between different tissues and tuber zones have been the subject of many reports, most of them highly contradictory. The most reliable have been those by Reeve *et al.* (1970, 1971). N-content correlation (negative) with tuber solids

Table IV.1. Total nitrogen and sulfur contents present in the major anatomical regions of the potato tuber cv. Russet Burbank (Alberta).

	Weight percent of the whole tuber	Dry matter (%)	Total Nitrogen[1], %	Sulfur[2], ppm
Whole Tuber	100	24.25 ± 0.84	1.67 ± 0.09	---
Anatomical Region:				
Cortex[3]	19.15 ± 2.31	22.20 ± 1.00	2.10 ± 0.24	1,799 ± 30.1
Vascular bundle + outer phloem	24.62 ± 2.48	26.89 ± 0.35	1.22 ± 0.09	1,031 ± 27.0
Perimedulla (inner phloem)	46.10 ± 4.07	24.39 ± 1.26	1.55 ± 0.12	1,178 ± 43.4
Medulla (pith)	10.07 ± 2.36	20.72 ± 0.94	1.92 ± 0.06	1,583 ± 30.8

[1]Determined by a Perkin-Elmer Model 240 B elemental analyzer.

[2]Determined by an alkaline oxidation method of Tabatabai and Bremner (1970).

[3]The cortex included the peel and the adjacent 5 mm thick layer of the tuber.

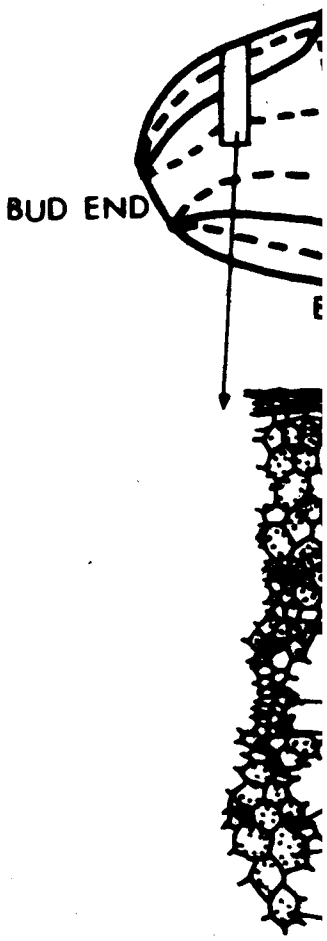
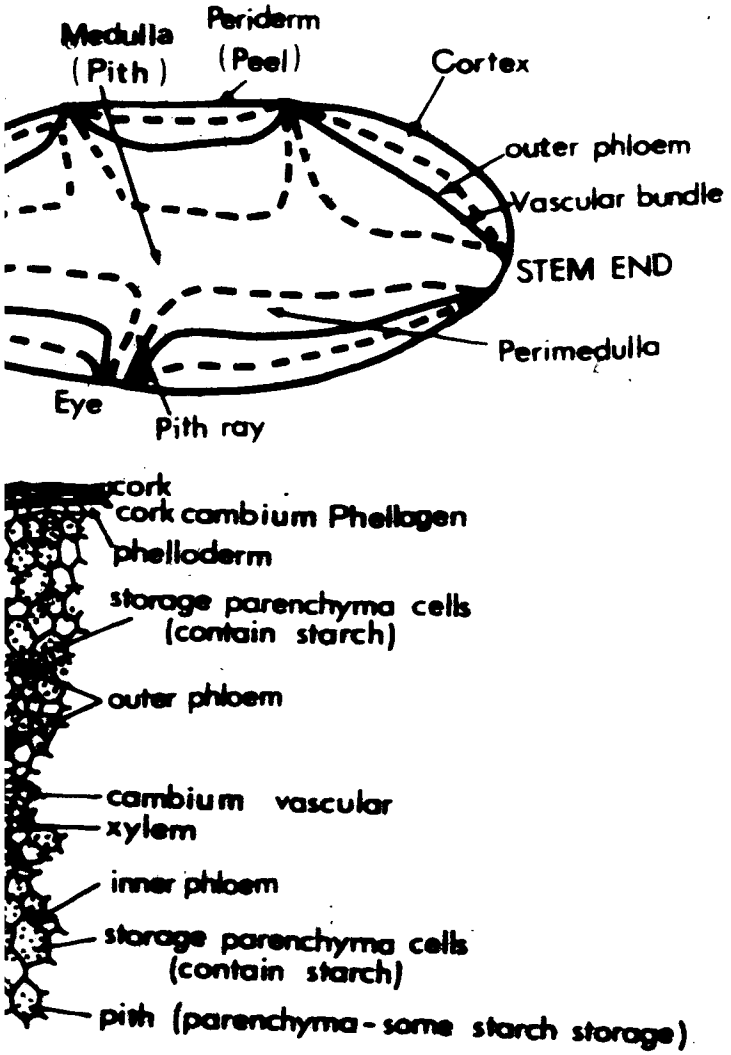


Figure IV.1 Ana



Anatomical regions of the potato tuber

and N-distribution in bud, core and stem sections of six potato cultivars were the subjects of a recent report by Weaver *et al.* (1978).

Sulfur data for potatoes have only been reported for volatile constituents of cooked or fried potatoes (Self, 1967), but not for the raw tuber.

B. N-fractions of the Tuber

The data for potato tuber protein fractionation according to solubility are given in Table IV. 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-N of the peeled tuber. This suggested that the majority of the total-N is made up of the readily soluble free amino acid pool, amides, nucleotides, inorganic forms of N and a large proportion of protein bound-N (albumin and globulin). When the sap was dialyzed against distilled water, the dialyzable-N (all forms but protein-N) was 43.2% of the total-N of the tuber, while the non-dialyzable, protein-N was 32.9% (i.e., 24.5% was water-soluble albumin and 8.4%, salt-soluble globulin). The composition of the pure protein-N was: 67% albumin, 23% globulin, 1.4% prolamine and 9% glutelins.

The cake remaining after sap extraction retained 23.9% of the tuber total-N. When the cake was treated with 70% ethanol, 0.5% of the total-N was prolamine, and when it was

Table IV.2. Recovery of potato tuber proteins according to solubility (cv. Russet Burbank, Alberta)

N-fraction	% of Total-N[1]
Total tuber N	100.0
Tuber sap-N	76.1
Dialyzable-N	43.2
Non-dialyzable-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

[1]Average values of two potato tubers supplied by I&S Produce, Edmonton

treated with 0.1% acetic acid followed by 0.2 N sodium hydroxide, the acidic (0.3%) and basic (3.0%) glutelins were collected. The residual-N retained by the cake was 23% of the total-N of the tuber.

These results differed from those reported by Levitt (1951), who extracted powdered freeze-dried tuber with 0.02 N KH_2PO_4 pH 6.9-7.1 and found only 10% of the total-N of the powder was non-extractable. This residual-N could not be extracted with water, 1 M NaCl or other protein solvents. The proteins extracted were at least two-thirds of the total-N of the powder. Fractionation of proteins by dialysis against water provided little protein sedimentation, i.e. globulin. When the albumin collected was freeze-dried, it failed to solubilize in distilled water but was readily dissolved in dilute salt solutions. Moreover, when Levitt (1951) analyzed two halves of the tuber, one kept at 0-5°C and the other at 25°C for 10 days, the albumin fraction of the latter increased significantly while the amount of globulin decreased. This may indicate a facile *in vivo* conversion of the latter fraction to the former.

The methodology applied by Kapoor *et al.* (1975) can be used to illustrate that results might be affected by the fractionation procedure. They homogenized the tuber in 1% K_2SO_4, strained the slurry through cheese cloth, centrifuged the filtrate and recovered albumin and tuberin from the supernatant. The pellet contained the other proteins. The tuberin was precipitated overnight at 4°C by 40% $(NH_4)_2SO_4$

and the albumin was precipitated from the supernatant the following day by 80% $(NH_4)_2SO_4$. Tuberin was solubilized with 1% K_2SO_4. The so-called globulin fraction was recovered from the pellet by 5% K_2SO_4, prolamine by 55% isopropanol and glutelins by 0.2% NaOH. This method of fractionation yielded tuberin as the main protein (71.3%), while only 1.7% prolamine was found. There was close to 7% of each of albumin and glutelin, while globulin was 3% of the total protein-N. Residual-N in the pellet provided another 10%.

However, when Kapoor *et al.* (1975) applied the conventional solubility method of protein fractionation, as done in this study, and used the same batches of potatoes (cv. Red Pontiac), the water-soluble albumin became the largest fraction (49%) while globulin was 25% of the total pure protein-N. Prolamine and glutelin were 4.3 and 8.8%, respectively, of the total protein-N. These results were in close agreement with those of this study. The residual-N in the pellet was as in their previous method, i.e. about half that found in this study using cv. Russet Burbank.

A conventional solubility fractionation method applied by Seibles (1979) also supported the results of this study. He subjected clarified potato sap to dialysis against water and obtained albumin and globulin fractions soluble in 5% K_2SO_4. The latter was about 25% of the total potato sap protein. The author noted that the procedure, though found useful for preliminary fractionation, does not provide a sharp distinction between protein solubility classes. The

results of this study led to the same conclusion. Moreover, the extreme instability of the tuber proteins tends to make the results of individual classes vary with conditions of tuber storage over short periods of time, hence all data based on solubility are somewhat unreliable and not readily comparable.

C. Protein Separation by SDS-PAG Electrophoresis

Protein separation by molecular weight requires protein markers. In this study muscle fiber proteins were used as marker proteins. As seen from Figure IV.2, freshly prepared myofibrillar proteins exhibited several major protein bands. In order of increasing mobility in the direction of cathode (-) to anode (+) they were: myosin, M-protein, α-actinin, actin, tropomyosin and LC_2 and TN-C proteins. The bands covered a range of 20 to 200 x 10^3 daltons. Their intensities were revealed by scanning the gel rods at 560 nm. In order to extend the molecular weight range to 13 x 10^3 daltons, RNAase A was included as an additional protein marker (see Figure IV.2).

The migration distances of these protein markers and their corresponding molecular weights, when plotted on a semilogarithmic scale, provided a linear calibration curve which followed the equation:

$$LOG\,mol.wt. = -0.02459X + 5.61958$$

(where X is the migration distance in mm)

The separation of the marker proteins and the calibration

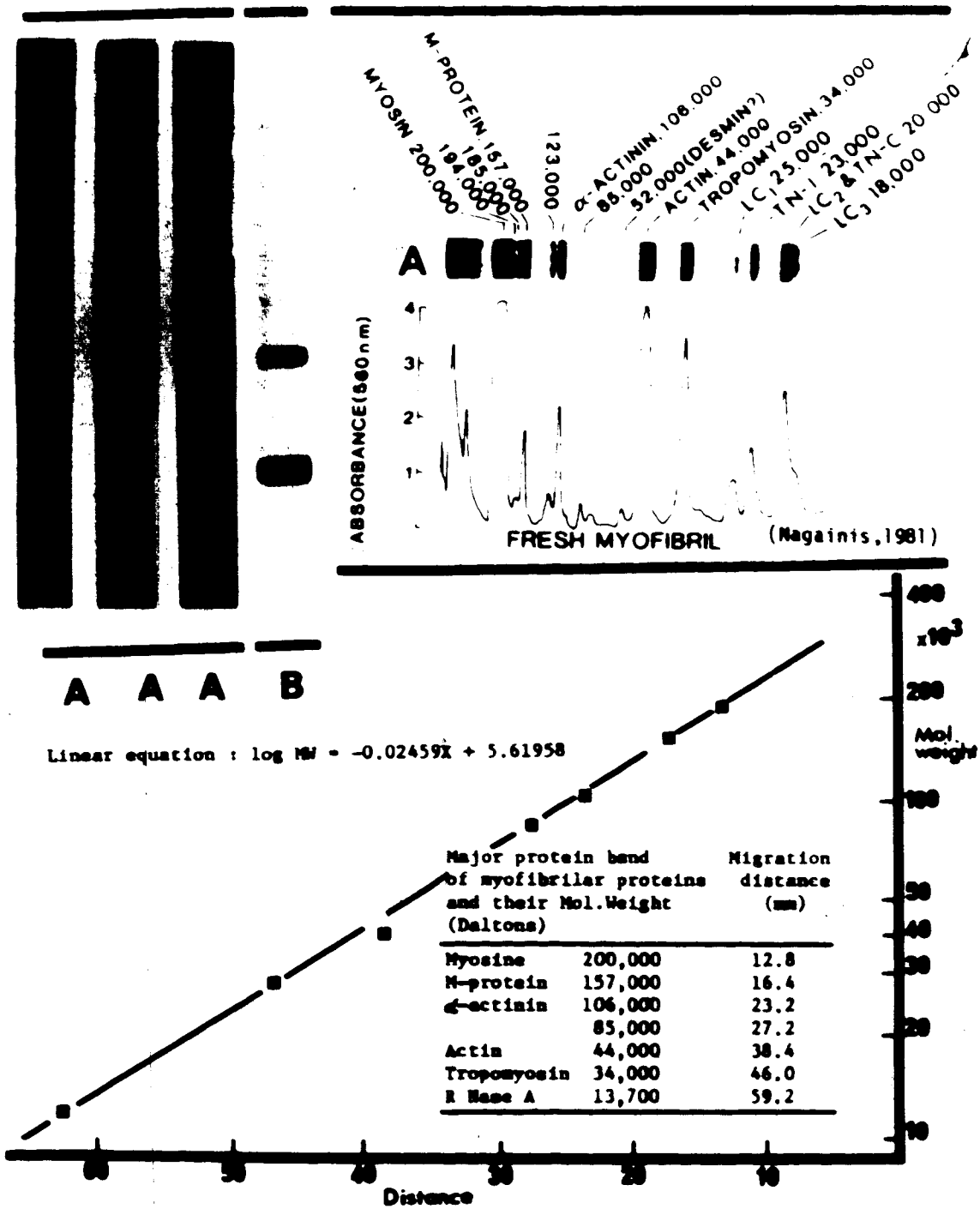


Figure IV.2 Electrophoretic patterns of myofibrillar proteins and Ribonuclease A

curve were verified throughout the study, and the separation pattern of the freshly prepared myofibrillar proteins was reproducible and accurate regardless of the PAGE run.

The electrophoretograms of potato tuber total proteins and the corresponding scanning diagrams are given in Figures IV.3 and IV.4, respectively, for the following potato samples:

(a) raw,

(b) steam-cooked, and

(c) precooked at 70°C for 20 min, cooled for 20 min in tap water, then cooked in water for 30 min.

The separations provided practically unchanged protein patterns for raw or cooked tubers. In all cases there were about 13 bands. Major bands were at 12, 25 and 45 x 10^3 daltons; intermediate bands close to 32, 38, 58, 60, 105 and 120 x 10^3 daltons; and distinct minor bands at 30, 36, 93 and 100 x 10^3 daltons. The band at 45 x 10^3 daltons was the major band in the higher molecular weight region for all samples. This band corresponds to the 45 kD glycoprotein reported as the major soluble protein of most potato cultivars by Racusen and Foote (1980). The lower molecular weight region (12-25 x 10^3 daltons) was the dominant region, and its intensity was not altered by steam-cooking or cooking in water. This is in agreement with the findings of Nuss and Hadziyev (1980).

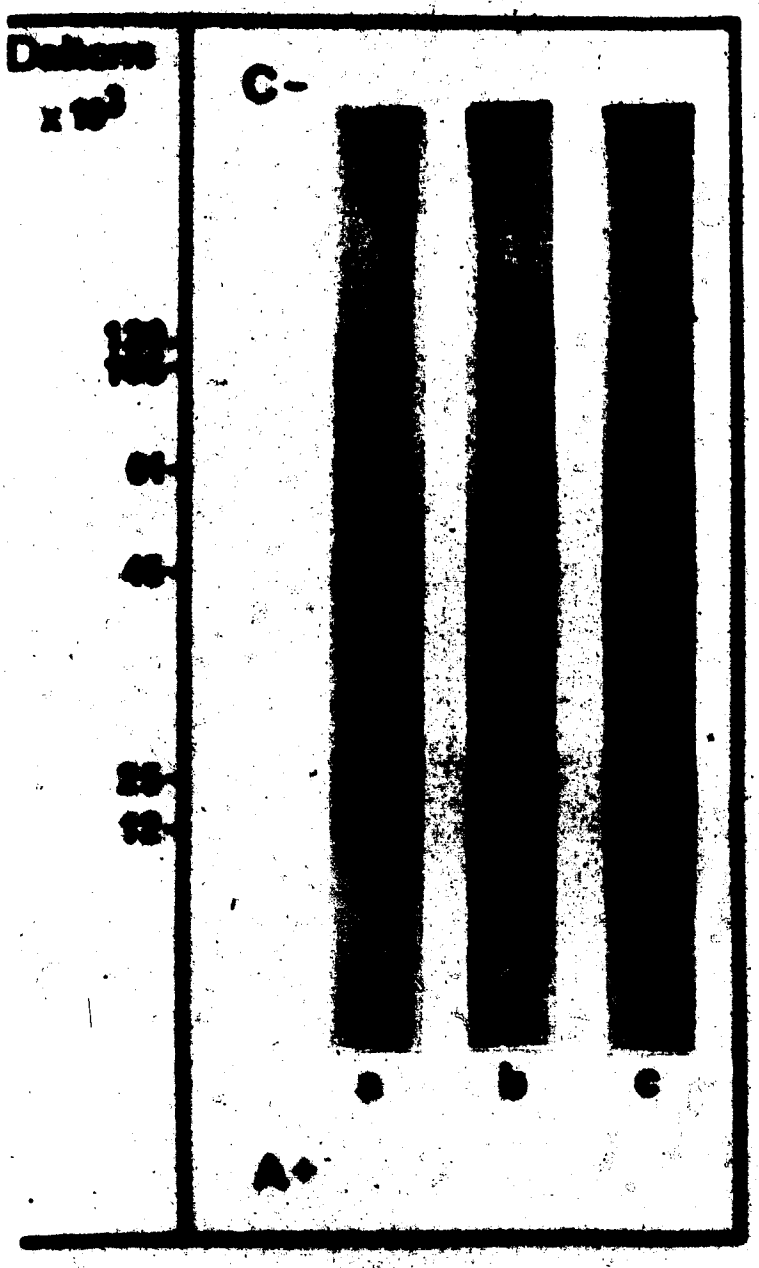


Figure IV.3 Electrophoretogram of raw, steamed and pressurized-boiled potato tuber total proteins

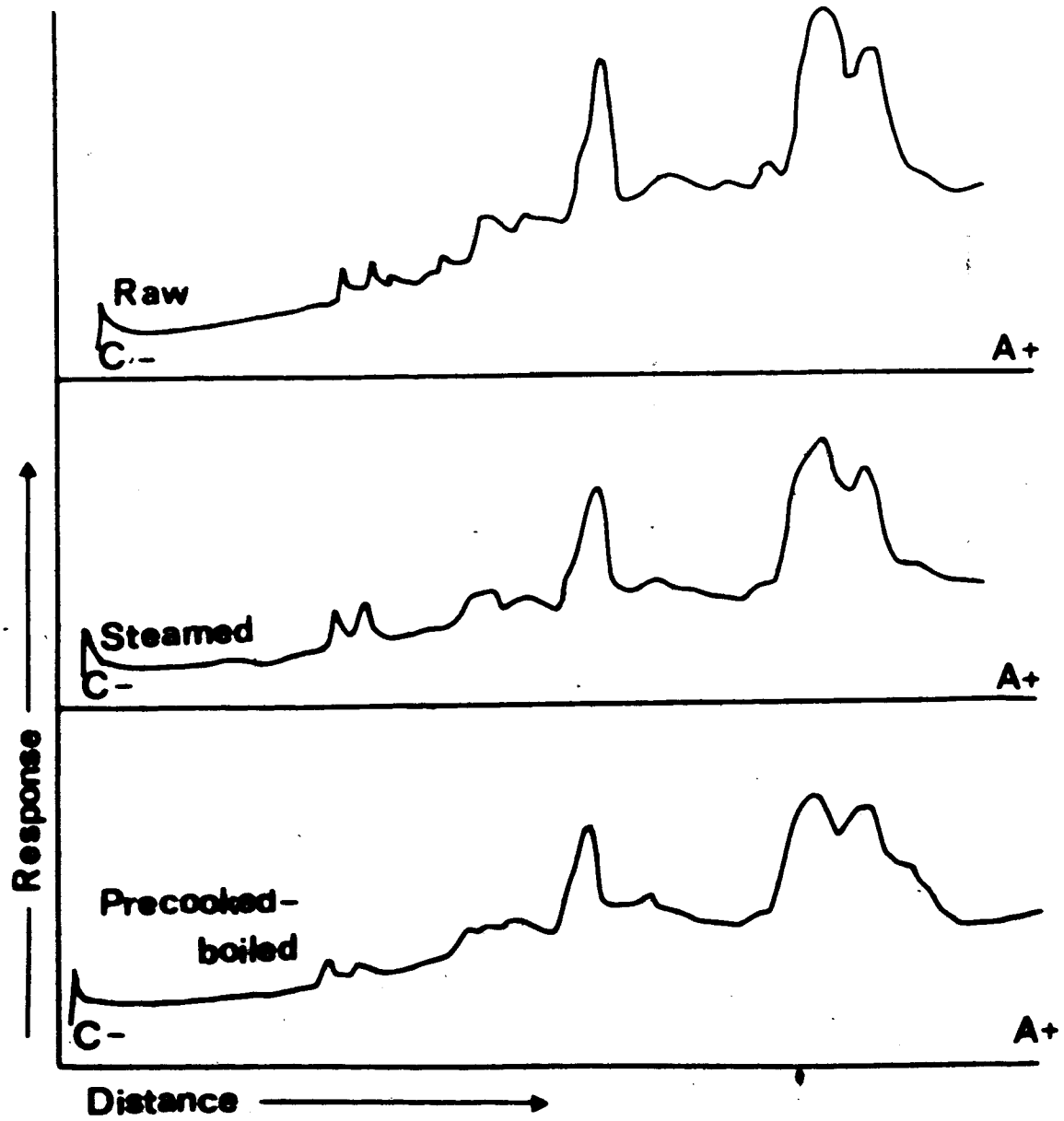


Figure IV.4 Scanning diagrams of the electrophoretograms of raw, steamed and precooked-boiled potato tuber total proteins

The findings related to these two major bands (12-25 and 45 x 10^3 daltons) strongly suggest that during steam-cooking or cooking in water there is no appreciable protein leaching from peeled and sliced tubers. Protein hydrolysis was not evident, since an enrichment in the lower molecular weight region was not observed.

SDS-PAGE separations of purified protein fractions are illustrated in Figure IV.5. The albumin fraction (a) had 10 distinct bands, the four major ones being at 45, 12, 20 and 25 x 10^3 daltons. It is clear that the 12, 25 and 45 x 10^3 dalton bands coincide with the major constituents detected among the tuber total proteins. Intermediate bands were at 29, 35, 52 and 100 x 10^3 daltons. A few additional minor bands were present in the region above 140 x 10^3 daltons.

The globulin fraction of the tuber (b) showed a major band in the low molecular weight range at 25 x 10^3 daltons, which coincided with that of albumin, while the high band at 45 x 10^3 daltons was not a major band. An additional major band at 22 x 10^3 and five distinct intermediate bands at 12, 26, 30, 45 and 55 x 10^3 daltons were present, along with a few minor bands above 83 x 10^3 daltons.

The 70% ethanol extractable prolamin pattern (see separation c) contained similar bands to globulin. This strongly suggested that some of the globulin resisted extraction during protein fractionation (5% NaCl in phosphate buffer pH 7.5 for globulins) and was later solubilized with ethanol. The finding might also suggest at

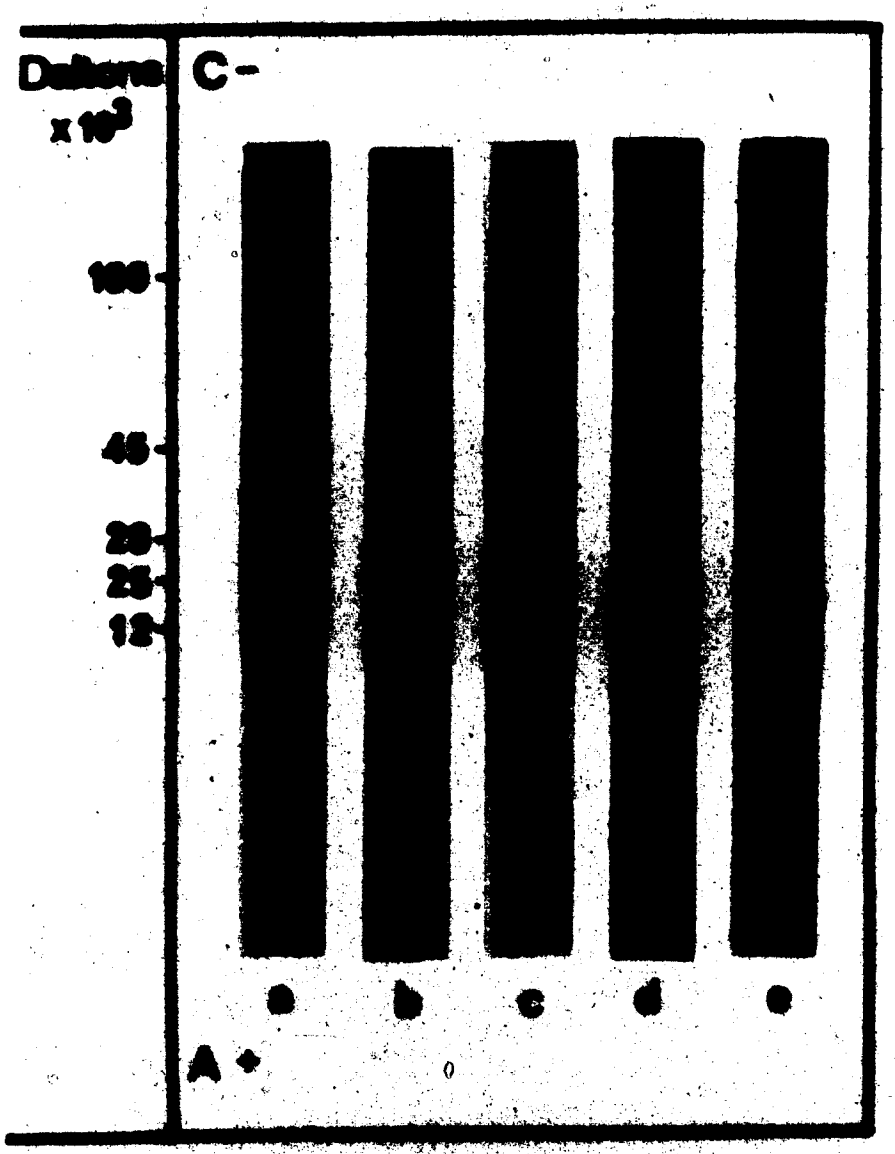


Figure IV.5 SDS-PAGE separation of purified protein fractions

best trace levels of prolamin in the tuber.

The separations of protein fractions of the peeled potato tuber solubilized in acidic or basic solvents are presented in electrophoretograms d and e. These are the acidic and basic glutelins. These proteins still contained the major low molecular weight protein at 25 x 10^3 daltons previously assigned as the major bands of albumins and globulins. The glutelins retained the 12 x 10^3 dalton component, while the basic glutelins had a distinct component at 150 x 10^3 daltons. Both glutelins had intermediate or minor bands at 32 and 45 x 10^3 daltons.

Semiquantitative data for fractionated protein were obtained by scanning the stained gel rods (see Figure IV.6). The lower molecular weight albumin comprised 47% of the total albumins and the 45 x 10^3 dalton protein close to 30%. The lower molecular weight globulin component was at least 60% of the total globulin proteins. This lower component also made up 60% of the total prolamin and 45 and 56%, respectively, of the total proteins of acidic and basic glutelins.

The electrophoretograms shown in Figure IV.7 were obtained from albumin and globulin fractions isolated from stored tubers which had subsequently been diced, freeze-dried and powdered. Albumin was represented by two major bands coinciding with those of fresh potato sap isolate, and globulin by a major band at 25 x 10^3 daltons. When the peeled tuber was steam-cooked or cooked in water

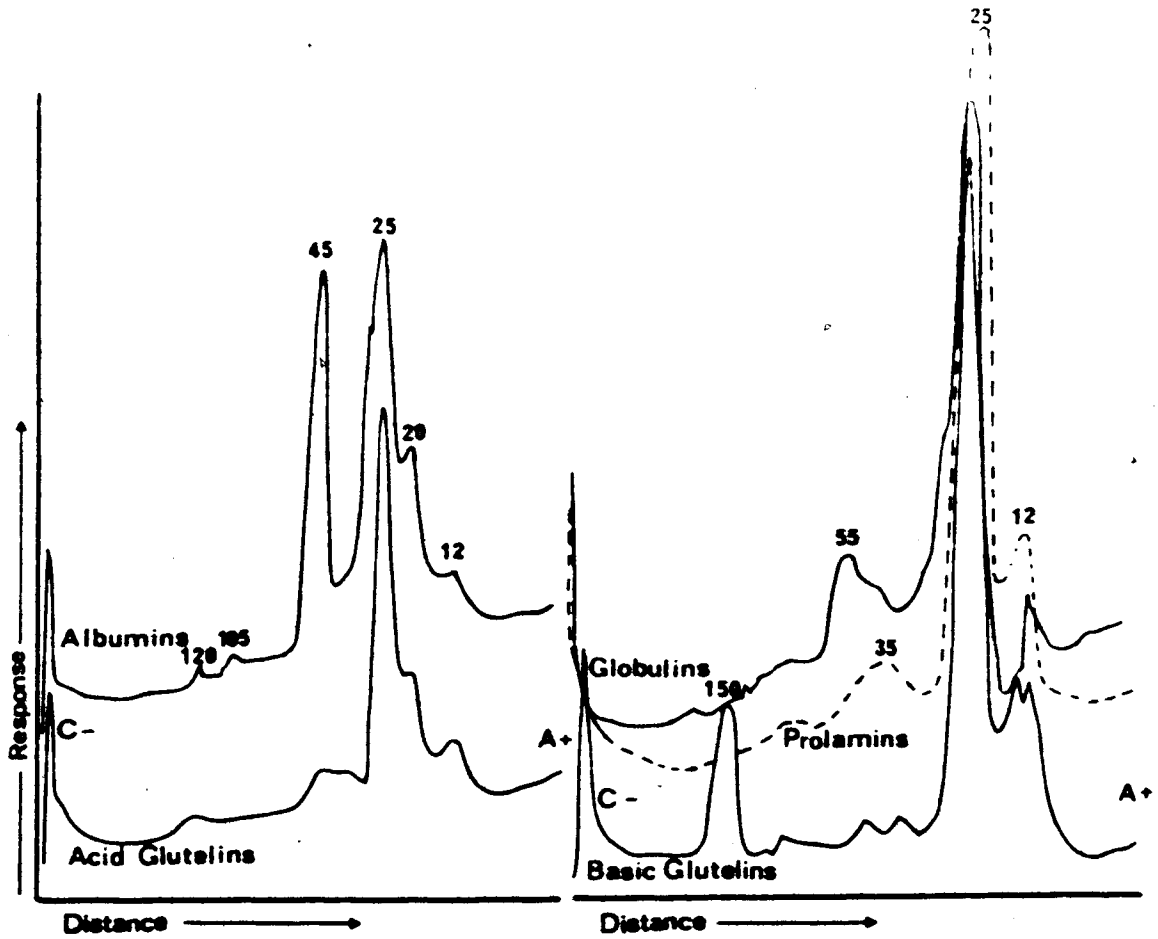


Figure IV.6 Scanning diagrams of the electrophoretograms of purified protein fractions

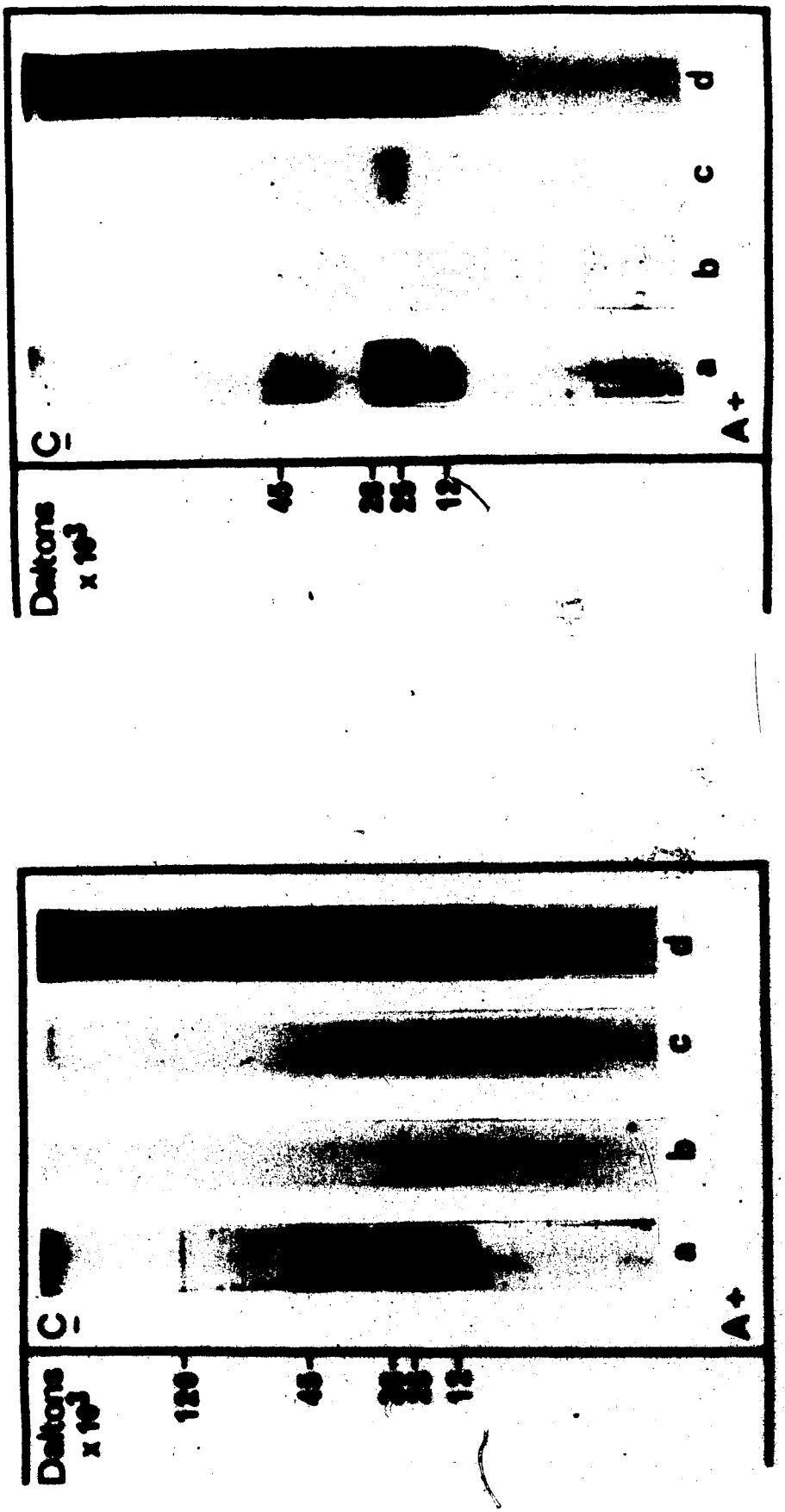


Figure IV.7 Electrophoretograms of albumin and globulin fractions of raw, steamed and precooked-boiled potato tuber

and then freeze-dried and powdered, isolation of albumin and globulin was not successful, as illustrated by electrophoretograms b and c in Figure IV.7 [Total protein from steam-cooked or boiled samples was readily isolated using Tris-borate buffer pH 8.2 in a sample to buffer ratio of 1:1, followed by incubation at 100°C in the presence of 4% SDS and 1% mercaptoethanol. In the albumin and globulin extraction procedure the cooked samples were freeze-dried and powdered, then suspended in a 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 albumin remained solubilized, while globulin precipitated. Hence, in the latter procedure no SDS or mercaptoethanol was applied and the temperature did not exceed 4°C]. Therefore, it appears that, after cooking, the two protein fractions are well trapped within the matrix of gelatinized starch and are released only when detergent and mercaptoethanol are applied at higher temperatures (electrophoretogram d in Figure IV.7).

An overview of the protein bands and their corresponding molecular weights for raw and cooked potatoes and purified protein fractions is given in Table IV.3. This overview indicates that tuber protein fractionation based on potato tuber protein solubility is an unreliable procedure. As will be seen later, this view is strongly supported by amino acid data on the so-called "purified" proteins.

Table IV.3. Molecular weights (dalton x 10^3) for protein fractions of potato tubers and tuber proteins

Sample	Major	Intermediate	Minor
Tuber Total Proteins			
Raw or Steamed or Precooked & boiled	12 25 45	32 38 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
Basic glutelin	25 150	32 45	12 28 40 85 100
Acidic glutelin	25	12 20	32 45 150

D. Protein Amino Acid Composition obtained by Ion-Exchange Chromatography

Among the five protein fractions recovered, albumin, globulin and basic glutelins were subjected to amino acid analysis. 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:

(a) hydrolysis with 6 N HCl in the presence of 0.1% phenol to protect tyrosine;

(b) protein oxidation by acetic acid prior to hydrolysis as in (a); and

(c) hydrolysis with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.

By applying method (b), half-cystine was determined as cysteic acid, and methionine as methionine sulfone. As shown in Tables IV.4-IV.6, individual amino acid values obtained using method (b) were lower than corresponding values using (a) or (c). This indicated that many 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.

These results strongly suggest that, when applying the protein hydrolysis method using 6 N HCl, two sets of analyses are necessary -- one without and a second with

Table IV.4. Amino acid compositions of potato albumins from raw tubers of cv. Russet Burbank (Alberta) as affected by protein hydrolysis. The results are in g/16 g crude nitrogen.

Amino Acid[1]	Hydrolysis Method[2] (a)	(b)	(c)
Aspartic Acid	11.21	11.79	12.42
Threonine	5.63	3.22	6.10
Serine	5.40	3.83	5.90
Glutamic Acid	12.03	7.32	13.00
Proline	4.60	0	4.69
Glycine	4.67	2.95	5.14
Alanine	5.29	3.41	5.57
Half-cystine	1.72	2.73	2.03
Valine	5.87	3.61	5.32
Methionine	1.63	1.63	1.05
Isoleucine	4.87	2.68	4.17
Leucine	9.76	6.04	9.24
Tyrosine	4.57	0	5.24
Phenylalanine	5.10	2.54	5.22
Histidine	2.13	1.04	2.09
Lysine	7.99	5.29	7.77
Tryptophan	0	0	0.59
Arginine	5.84	3.13	4.47

[1] In this and following tables:
-- 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

[2] (a) 6 N HCl, 0.1% phenol;
(b) 6 N HCl, 0.1% phenol, HOAc;
(c) 4 N methanesulfonic acid + 0.2% 3-(2-aminoethyl)indole

Table IV.5. Amino acid compositions of potato globulins from raw tubers of cv. Russet Burbank (Alberta) as affected by protein hydrolysis. The results are in g/16 g crude nitrogen.

Amino Acid	Hydrolysis Method[1]		
	(a)	(b)	(c)
Aspartic Acid	12.86	9.03	13.58
Threonine	4.75	3.21	5.04
Serine	5.70	3.99	5.74
Glutamic Acid	8.73	5.96	9.04
Proline	6.24	3.17	5.75
Glycine	5.96	4.16	5.96
Alanine	3.00	2.06	3.44
Half-cystine	2.60	4.82	4.83
Valine	8.39	5.96	7.43
Methionine	1.08	1.19	0.67
Isoleucine	5.84	3.51	4.99
Leucine	7.78	6.92	9.56
Tyrosine	4.80	0	5.26
Phenylalanine	6.16	3.64	6.09
Histidine	2.24	2.32	1.98
Lysine	7.09	4.89	6.40
Tryptophan	0	0	0.40
Arginine	5.09	3.36	3.83

[1](a) 6 N HCl, 0.1% phenol;
(b) 6 N HCl, 0.1% phenol, HOAc;
(c) 4 N methanesulfonic acid + 0.2% 3-(2-aminoethyl)indole

Table IV.6. Amino acid compositions of potato basic glutelins from raw tubers of cv. Russet Burbank (Alberta) as affected by protein hydrolysis. The results are in g/16 g crude nitrogen.

Amino Acid	Hydrolysis Method[1]		
	(a)	(b)	(c)
Aspartic Acid	12.86	7.71	13.84
Threonine	5.38	3.67	5.71
Serine	6.74	3.82	6.25
Glutamic Acid	12.58	8.20	12.84
Proline	3.83	3.14	5.20
Glycine	6.06	3.70	5.72
Alanine	6.07	3.65	5.32
Half-cystine	0	2.62	1.01
Valine	7.14	3.92	5.42
Methionine	0	2.02	0.25
Isoleucine	5.82	4.13	4.87
Leucine	11.84	6.99	10.12
Tyrosine	0	0	4.03
Phenylalanine	5.23	3.24	5.78
Histidine	3.07	2.36	2.79
Lysine	7.41	5.02	6.76
Tryptophan	0	0	0.50
Arginine	4.69	3.07	4.10

[1](a) 6 N HCl, 0.1% phenol;
(b) 6 N HCl, 0.1% phenol, HOAc;
(c) 4 N methanesulfonic acid + 0.2% 3-(2-aminoethyl)indole

performic acid oxidation.

When hydrolysis was conducted with 4 N methanesulfonic acid [method (c)], the values for amino acid contents were similar to those of method (a). Exceptions were S-containing amino acids, tyrosine and tryptophan, which were higher in content using method (c). However, recoveries of S-containing amino acids by method (c) were comparable to those obtained by oxidation method (b). The most important finding for method (c) was the presence of tryptophan in all hydrolysates. Figures IV.8-IV.10 illustrate the ion-exchange chromatograms of the three protein fractions (albumin, globulin, basic glutelins) hydrolyzed with method (c) and chromatographed on a single-column amino acid analyzer. In these chromatograms, surprisingly, 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 contents, regardless of protein fraction, were separated, but were much lower than those found by the Spies and Chambers (1949) method as applied by Kapoor *et al.* (1975).

Taking into account its methodological superiority, method (c), which had the fewest disadvantages of the methods studied, is recommended as a single hydrolytic procedure for amino acid analysis of fractionated and purified potato tuber proteins.

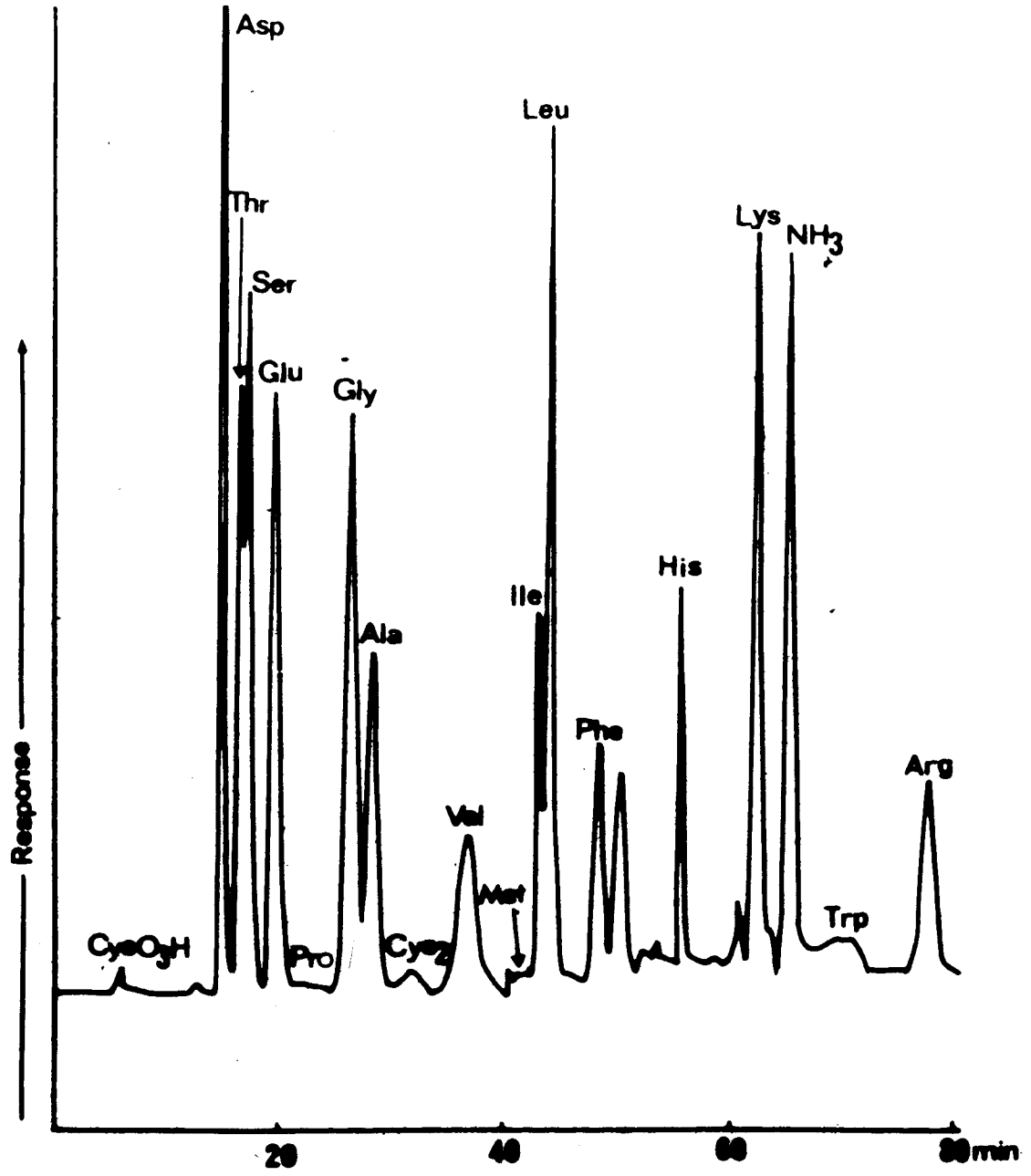


Figure IV.8 Ion-exchange chromatogram of amino acids of potato tuber albumin

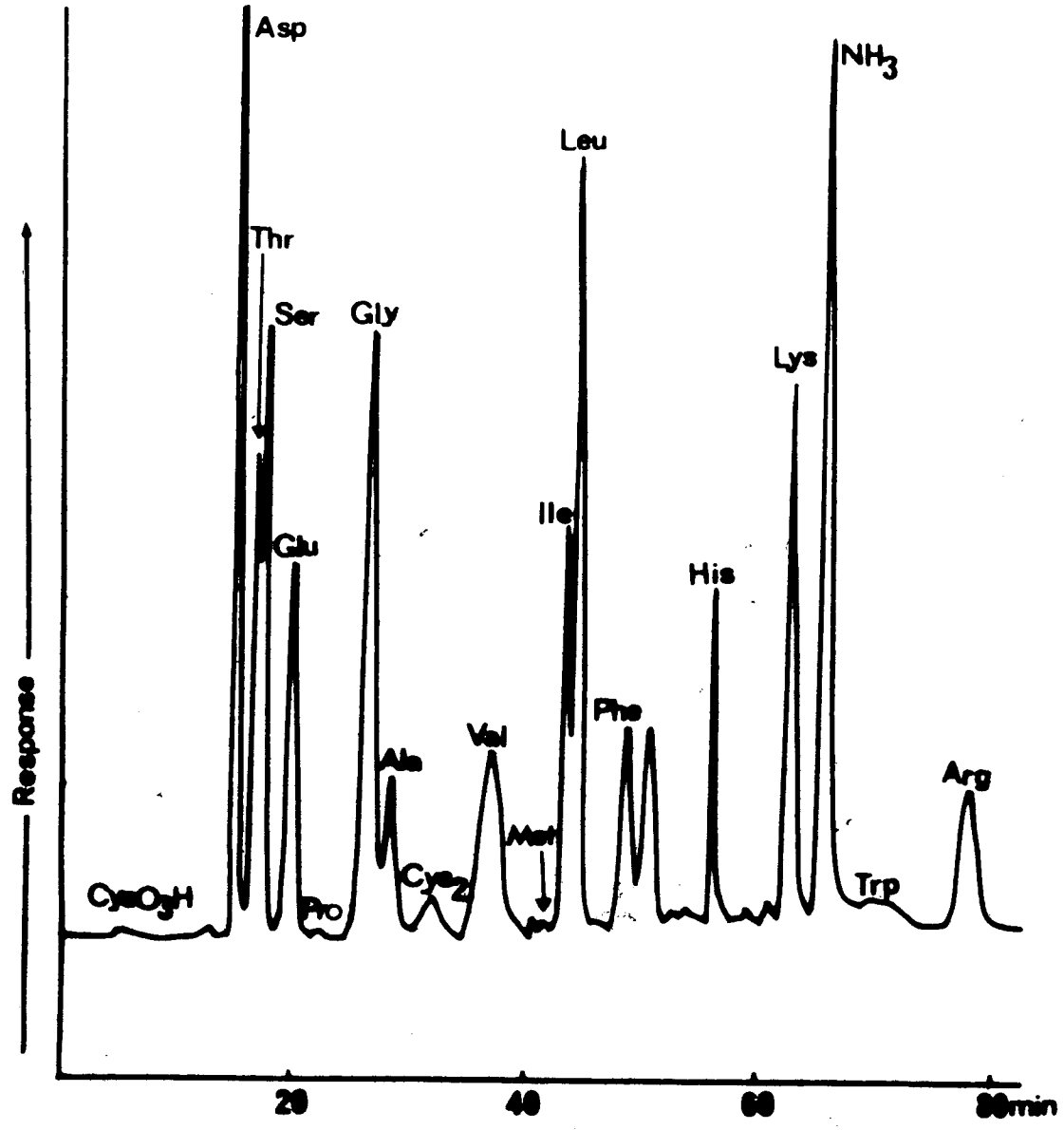


Figure IV.9 Ion-exchange chromatogram of amino acids of potato tuber globulin

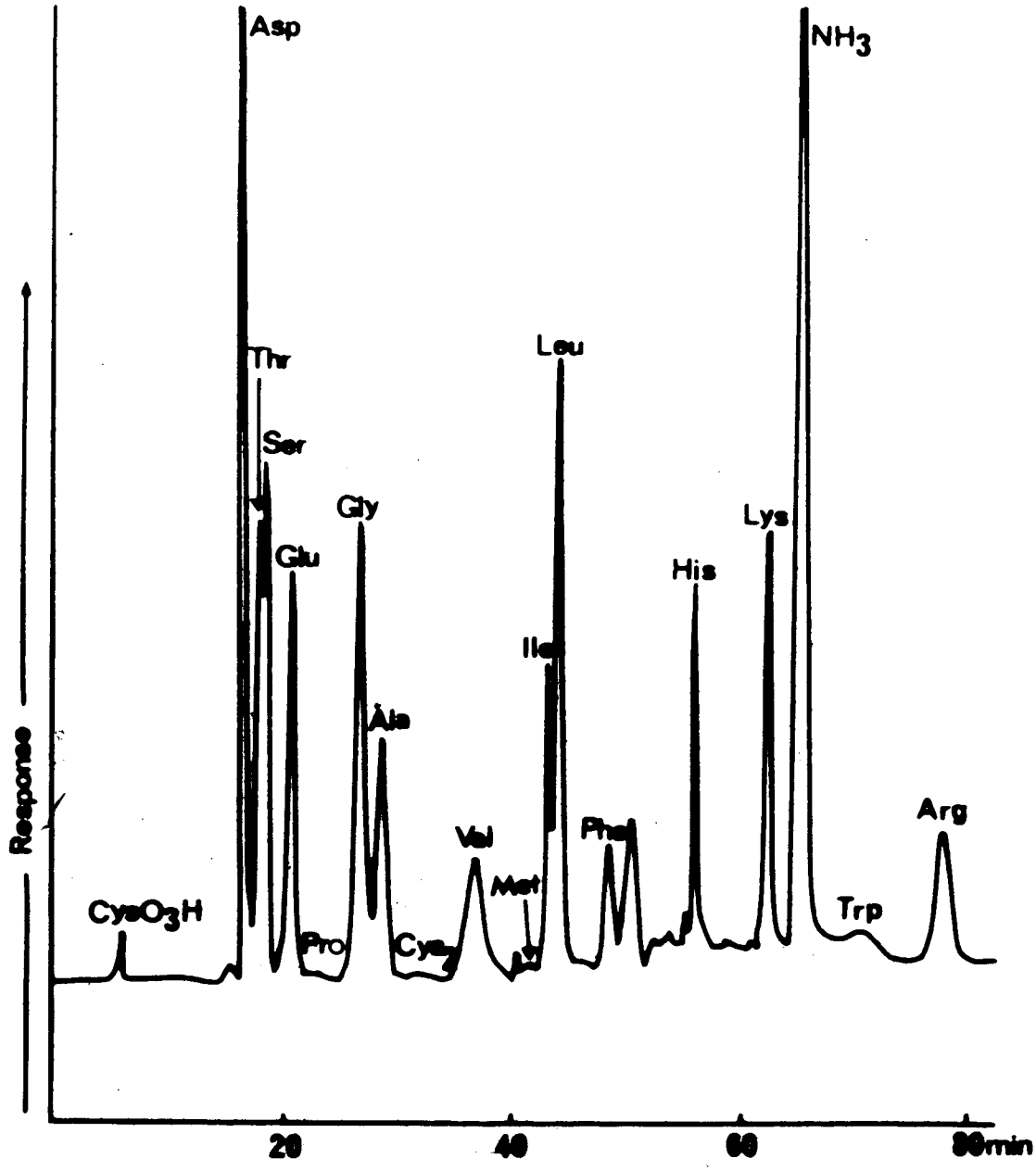


Figure IV.10 Ion-exchange chromatogram of amino acids of potato tuber basic glutelins

The results of Kapoor *et al.* (1975) for albumin, tuberin-globulin and glutelin tuber proteins provided similar characteristics in amino acid profiles. This was especially true for the essential amino acids (methionine, threonine, valine, lysine, isoleucine, phenylalanine, histidine and arginine) and for non-essential 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 less than half that found in globulin (4.83). Differences were also found in alanine contents (5.6 in albumin and 3.44 in globulin). A reverse trend was found 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 x 10^3 daltons in albumin but not a major protein species in globulin.

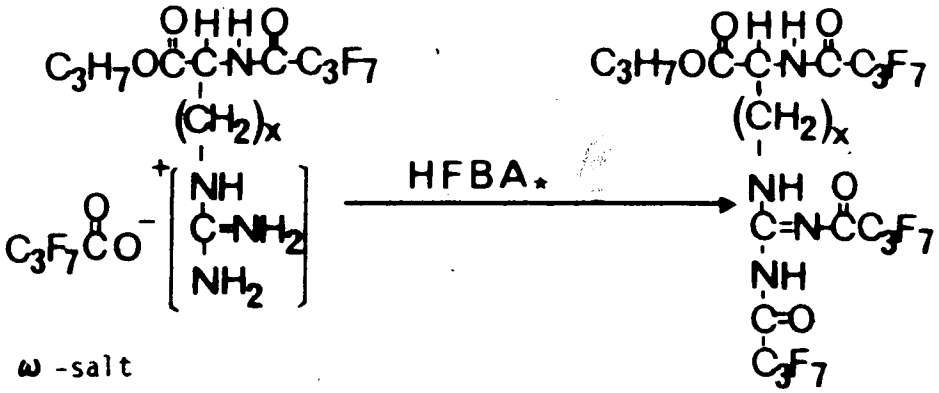
The similarity of basic glutelin amino acid composition to that of albumin, with the exception of half-cystine and methionine, was not expected. Glutelin SDS-PAGE electrophoretograms contained a major protein species at 150 x 10^3 daltons and an intermediate protein 45 x 10^3 daltons. Yet to be clarified is whether or not the similarity in amino acid composition is merely a reflection of the aggregation of the 45 x 10^3 dalton species into the 150 x 10^3 dalton species.

Stegemann *et al.* (1973) had suggested such a possibility.

E. Total Amino Acids of the Tuber as Determined by Gas-Liquid Chromatography

In this study n-heptafluorobutyryl (n-HFB)-isopropyl ester derivatives of amino acids were separated by gas-liquid chromatography (GLC). Glass columns packed with 3% SE-30 silicone impregnated Chromosorb W HP were used and 250-500 μmoles of amino acid derivatives were injected directly onto the column to avoid contact of the vapor with the metal-lined injection port. Injection of a mixture of standard amino acid derivatives was performed with and without co-injection of heptafluorobutyric anhydride (HFBA). As seen on Figure IV.11, α-amino-β-quanidinopropionic acid (AGPA) or arginine esters, when acylated, might exist in their ω-salt forms. These salts are not volatile and are predominant in the presence of even traces of water (Stalling and Gehrke, 1966). To avoid salt formation the injection into the column was done in the presence of excess HFBA, which then induced on-column derivatization, forming volatile derivatives (with imino group hydrogen acylated with the HFB group).

Internal standards were also required in GLC separation to avoid errors during sample preparation and injection. Ornithine and norleucine have often been used as internal standards, however they could not be applied in this study. Ornithine is one of the free amino acid constituents of



ω -salt

* On-column injection with HFBA(n-heptafluorobutyric acid anhydride) at 225°C.

X=1 α-amino β-guanidinopropionic acid (AGPA)

Y=3 arginine

Figure IV.11 On-column derivatization of ω-salts

potato tuber, while norleucine "spiked" (had the same retention time as) the peak of γ-aminobutyric acid, the second unusual acid within the free amino acid pool of the tuber.

Pure compounds tested as internal standards are listed in Table IV.7. AGPA was not suitable since it often was incompletely derivatized with HFBA, giving irreproducible peak heights. Taurin could not be successfully derivatized, while the hydroxyproline derivatized peak coincided with that of aspartic acid. The only internal standard with a stable, reproducible and well-separated peak was pipecolinic acid. Its peak was eluted between proline and aspartic acid and could be easily measured. However, AGPA also added as an internal standard for ion-exchange chromatography since it provided an intense color in the ninhydrin reaction.

The choice of pipecolinic acid as a GLC internal standard was not without a potential drawback. The compound had been detected in tubers by McDonald (1974), but it was present only when potato plants were infected with leaf-roll virus. McDonald (1974) suggested, and this study confirmed, that the acid was not present in crude protein hydrolysates from healthy raw tubers.

The relative retention times of amino acid derivatives are listed in Table IV.8. Mean values and standard deviations are listed in order of chromatographic elution sequence, the first compound eluted being alanine (RT=2.185) and the last arginine (RT=11.273) when coinjected with HFBA

Table IV.7. List of tested internal standards for amino acid separation by gas-liquid chromatography

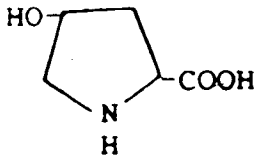
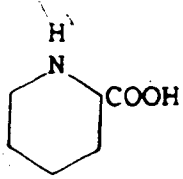
Compound	Structure	Suitability
α-amino-β-guanidino propionic acid (AGPA)	$H_2NC(NH)NHCH_2CH(NH_2)COOH$	-
Hydroxyproline (hydroxypyrrolidine carboxylic acid)		-
Norleucine	$CH_3CH_2CH_2CH_2CH(NH_2)COOH$	-
Ornithine	$H_2NCH_2CH_2CH_2CH(NH_2)COOH$	-
Pipecolinic acid (2-piperidine carboxylic acid)		+
Taurin (2-aminoethane sulfonic acid)	$H_2NCH_2CH_2SO_3H$	-

Table IV.8. Gas-liquid chromatography retention times (RT) of n-HFB-isopropyl ester derivatives of amino acids

Amino Acid	HFBA[1] Coinjected			HFBA not coinjected		
	Mean RT (min)	±SD	%CV	Mean RT (min)	±SD	%CV
Alanine	2.185	0.031	1.42	2.150	0.019	0.87
Glycine	2.350	0.033	1.43	2.320	0.037	1.61
Valine	3.338	0.032	0.96	3.308	0.026	0.78
Threonine	3.815	0.031	0.81	3.824	0.021	0.54
Leucine + Serine	4.060	0.027	0.67	4.076	0.023	0.56
Isoleucine	4.268	0.039	0.93	4.274	0.021	0.49
γ-aminobutyric acid	4.943	0.019	0.38	4.748	0.033	0.70
Proline	5.390	0.016	0.30	5.246	0.021	0.40
[2]IS 1	5.885	0.017	0.29	5.888	0.023	0.39
Aspartic acid	6.683	0.009	0.14	6.622	0.019	0.29
Methionine	7.013	0.009	0.14	7.040	0.017	0.25
Glutamic acid	8.117	0.015	0.19	8.062	0.019	0.24
Phenylalanine	8.255	0.006	0.07	8.320	0.019	0.22
Ornithine	8.885	0.017	0.19	8.794	0.020	0.24
[3]IS 2	9.165	0.006	0.06	---	---	---
Lysine	10.093	0.015	0.15	10.034	0.025	0.25
Tyrosine	10.253	0.005	0.05	10.308	0.019	0.18
Arginine	11.273	0.010	0.09	---	---	---

[1]Heptafluorobutyric acid anhydride
[2]Pipecolinic acid used as an internal standard for GLC
[3]α-amino-β-guanidinopropionic acid used as an internal standard for automatic amino acid analysis

(see Figure IV.12). Separation of leucine and serine was not achieved, nor were peaks obtained for cysteine or cystine, probably due to their insolubility in the esterification mixture. Histidine and tryptophan could not be readily derivatized. When derivatized separately, tryptophan gave two elution peaks (the development of a pinkish color was also observed, which implied a condensation reaction that produced the colored species), however corresponding peaks were not observed in the derivatization of amino acid mixtures. The % coefficient of variance (100 x standard deviation / mean) was less when amino acid derivatives were coinjected with HFBA. Exceptions were the initially eluted amino acids, up to isoleucine, the retention times of which appeared to be slightly shifted by the excess HFBA reagent. Elution of a mixture of 17 amino acids and two standards was achieved after 15 min. However, an additional 10-15 min were required with tuber total protein hydrolysate to elute the residual minor constituents (all unknowns) and thus to clean the column for the next separation.

Relative molar responses (RMR) of n-HFB-isopropyl ester derivatives of amino acids, which were used for their quantitative determination by GLC, are listed in Table IV.9. There were two sets of values, i.e. with and without coinjection of HFBA. Values in the first set, with the exception of ornithine and phenylalanine, were lower. In both sets pipecolinic acid, the internal standard, was assigned a value of 1.000. All calculations followed the

Table IV.9. Relative molar responses (RMR) of n-HFB-isopropyl ester derivatives of amino acids used for their quantitative determination by gas-liquid chromatography

	HFBA[1] Coinjected			HFBA not coinjected		
Amino Acid	Mean RMR	±SD	%CV	Mean RMR	±SD	%CV
Alanine	0.664	0.037	5.59	0.767	0.044	5.78
Glycine	0.645	0.045	7.01	0.523	0.090	17.27
Valine	0.558	0.032	5.78	0.783	0.049	6.26
Threonine	0.948	0.054	5.73	1.442	0.130	9.05
Leucine + Serine	2.299	0.136	5.90	3.026	0.436	14.42
Isoleucine	0.525	0.017	3.14	0.907	0.030	3.29
γ-aminobutyric acid	0.838	0.048	5.76	1.404	0.145	10.34
Proline	1.073	0.006	0.60	1.864	0.022	1.18
[2]IS 1	1.000	---	---	1.000	---	---
Aspartic acid	1.044	0.073	6.97	2.057	0.161	7.82
Methionine	1.037	0.048	4.59	1.776	0.125	7.01
Glutamic acid	1.124	0.083	7.42	2.026	0.212	10.44
Phenylalanine	2.534	0.237	9.36	3.472	0.164	4.72
Ornithine	1.155	0.132	11.42	2.326	0.140	6.00
[2]IS 2	1.588	0.064	4.01	---	---	---
Lysine	1.939	0.035	1.80	2.599	0.263	10.12
Tyrosine	2.462	0.121	4.90	3.815	0.255	6.68
Arginine	1.432	0.130	9.04	---	---	---

[1]Heptafluorobutyric acid anhydride
[2]Pipecolinic acid used as an internal standard for GLC
[3]α-amino-β-guanidinopropionic acid used as an internal standard for automatic amino acid analysis

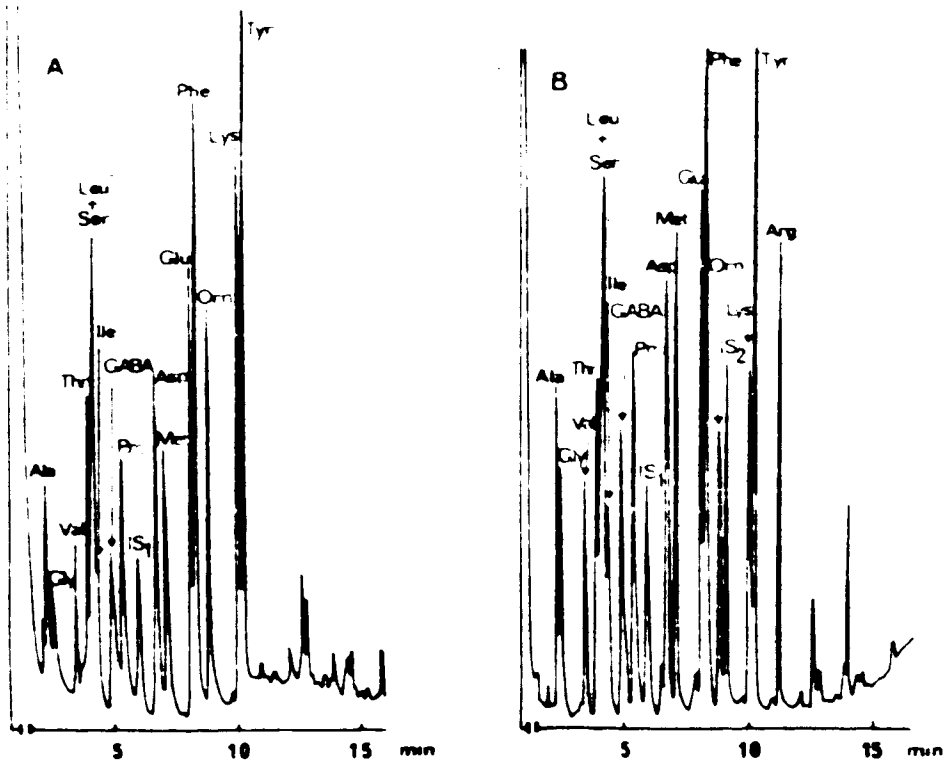


Figure IV.12 Gas-chromatograms of n-HFB-isopropyl ester derivatives of standard amino acid mixture:
A= without co-injection
B= with co-injection of HFBA.
Pipecolinic acid(IS1) and AGPA(IS2) as internal standards.
Temperature program: 100°C 1 min, then temperature programming at 10°C/min to 250°C and hold at 250°C.

equation:

$$RMR = \frac{\text{Amino acid molar response}}{\text{Pipecolinic molar response}}$$

The results listed in Table IV.9 were obtained by injection of an equimolar mixture of amino acids. In amounts of 5-50 nmole, but usually 25 nmole/acid, they were first derivatized in a reaction vial in a total solvent reagent volume of 100 µl. Then, using a microsyringe, 10 µl were removed, followed by 10 µl HFBA reagent, and the combined 20 µl volume injected on-column (25 nmole of each amino acid). As found in this study and by Golan-Goldhirsh (1979), each amino acid analyzed in the range of 1-50 nmoles provided a linear response of the flame ionization detector.

The use of RMR is illustrated by a sample analysis. Freeze-dried potato powder, 25 mg (corresponding roughly to 2.5 mg crude protein or 0.4 mg crude protein-N), was hydrolyzed, and 5 µmole of pipecolinic acid and 1.25 µmole of AGPA were added. After cleaning and evaporation steps, the sample was transferred to a vial, derivatized and coinjected with HFBA. Each GLC peak was integrated and its value (Σ) was divided by the integration value (Σ) of pipecolinic acid. This value was then divide by the RMR of the given amino acid to obtain the amount of amino acid in nmoles:

$$\text{nmoles Amino Acid} = \frac{\Sigma\text{amino acid}}{RMR \times \Sigma\text{pipecolinic acid}}$$

Each nanomole value was then multiplied by the molarity of the internal stadard added, and by the molecular weight of the amino acid to obtain its content in nanograms (or mg) in the protein-N fraction or in the tuber (dry weight basis).

F. Cystine + Cysteine Contents in Raw and Processed Potato Tubers

Cystine + cysteine (or half-cystine) content was determined in tubers spectrophotometrically using Ellman's reagent after peptide chains were unfolded by urea and disulfide bridges reduced by Na-borohydride.

In the reaction mixture containing the Ellman reagent (0.833 mMoles), equimolar contents of cysteine were expected to react with reagent. However, this was only partly true. As shown in Figure IV.13, there was a linear relationship between cysteine and A_{412} up to 0.25 mMoles cysteine/L, after which there was a plateau of readings which clearly commenced at 0.8 mMoles cysteine/L. There was a linear relationship with cysteine concentration in the range of 0.417-83.33 μmoles/L, and this range was used as a calibration curve throughout the study (Figure IV.14).

Though the analytical procedure used was based on that of Felker and Waines (1978), their recommended molar absorptivity of 12,000 M^{-1} cm^{-1} was not used. The accuracy of this and other molar absorptivities used in the past was questioned by Riddles *et al.* (1978). Therefore, the molar

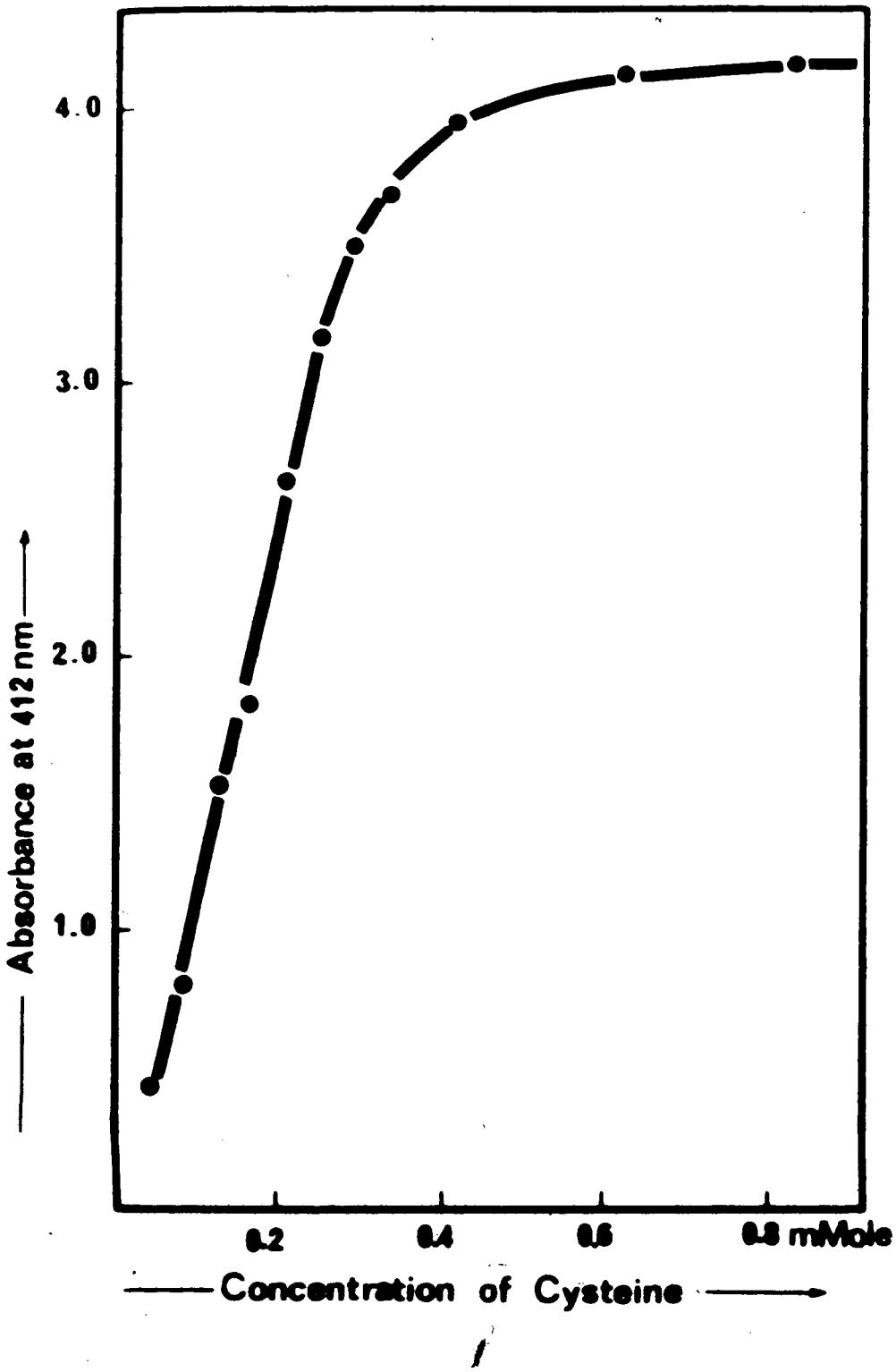


Figure IV.13 Color development of standard cysteine solution with Ellman's reagent

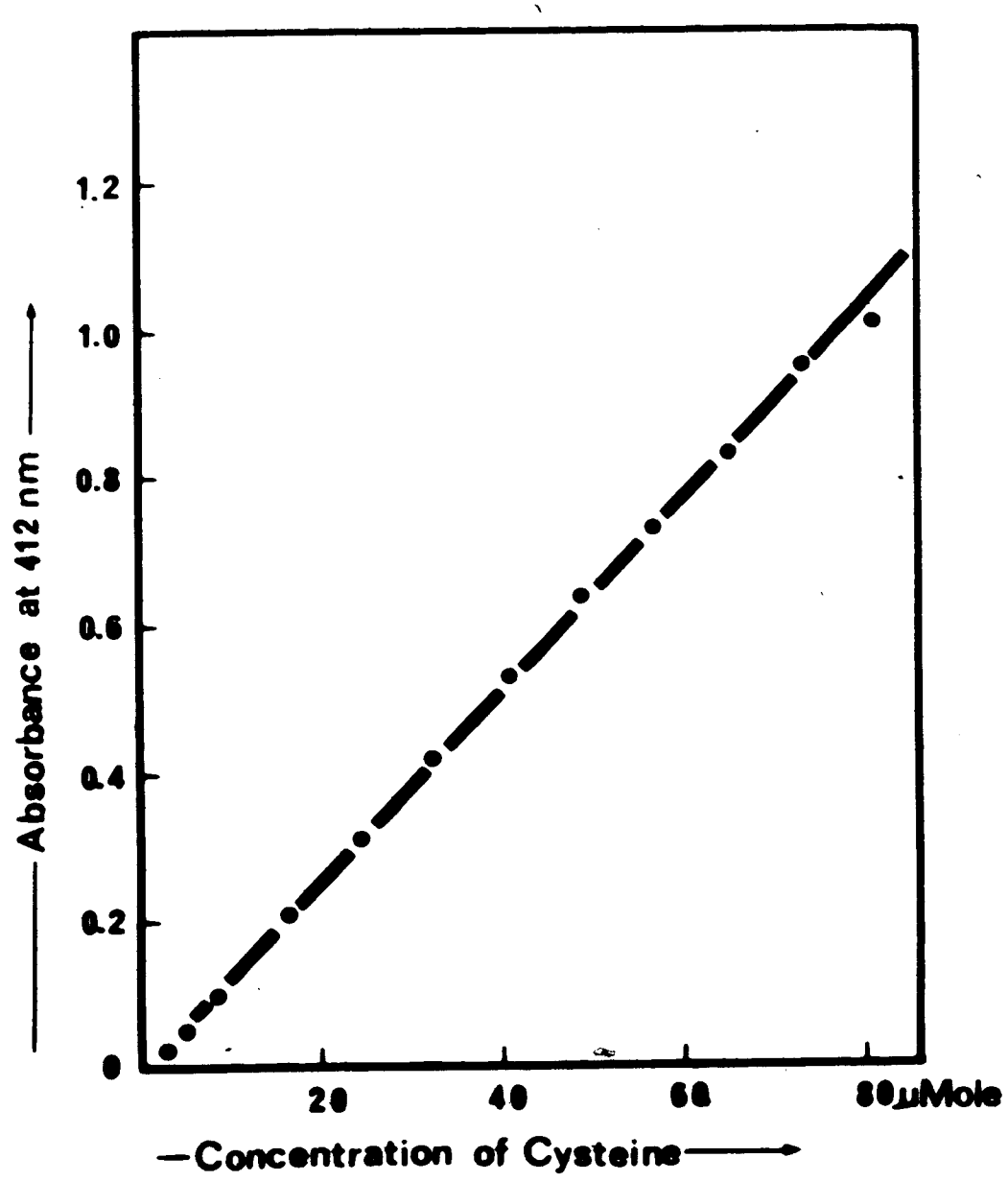


Figure IV.14 Color development of standard cysteine solution with Ellman's reagent

absorptivity was determined in this study (Table IV.10). The values were quite consistent from 16.667-83.333 μmoles of cysteine, giving an average of 12,687.5, a value 5.7% higher than that used by Felker and Waines (1978). The molar absorptivity did change at lower cysteine concentrations, varying from 11,151-11,769 below 16.667 μmoles. In this study all tuber samples were analyzed in the range of 16.6-83.3 μmoles half-cystine using a molar absorptivity value of 12,687.5.

As shown in Table IV.11, raw potato tuber total crude protein contained 1.22% half-cystine, agreeing with the content reported for tubers grown in Michigan by Kaldy and Markakis (1972), who used a method employing performic acid oxidation prior to protein hydrolysis by 6 N HCl. Also given in Table IV.11 are total half-cystine contents of the major anatomical regions of the tuber. The medulla (pith) contained the lowest and the vascular bundle (xylem ring) plus outer phloem the highest levels of total half-cystine, again expressed as percent of the total-N (Kjeldahl-N x 6.25) of the region.

Steam-cooking of tuber and precooking followed by cooking in water resulted in a significant reduction of half-cystine content. As seen in Table IV.11, steaming brought about 11% destruction of half-cystine, and precooking and boiling 24%. A similar study by Hughes (1958) showed as much as 60% destruction of half-cystine after cooking in water under household conditions of the cv.

Table IV.10. Molar absorptivity of cysteine as affected by cysteine concentration in the reaction mixture of the applied procedure

Cysteine Concentration in Final Solution (C; µMole/L)	Molar Absorptivity[1] ϵ (M^{-1} cm^{-1})	
0.417	11151 ± 730	
2.083	11164 ± 805	
4.167	11645 ± 223	
8.333	11769 ± 148	
16.667	12762 ± 167	
25.000	12720 ± 163	
33.333	12606 ± 115	Average
41.662	12720 ± 134	12687.5
50.000	12802 ± 144	
58.333	12528 ± 439	SD
66.667	12472 ± 245	± 265.9
75.000	12721 ± 348	
83.333	12854 ± 495	

[1] ϵ was calculated from the equation:

$$\epsilon = A_{\lambda 12} / C \cdot D; \text{ with } D=1 \text{ cm}$$

Table IV.11. Total half-cystine content in raw and cooked potato tubers and in major anatomical regions of the raw tuber of cv. Russet Burbank (Alta)

Potato Sample	Total Half-cystine Content (% of crude protein)
Whole Tuber	
Raw	1.22 ± 0.23
Steamed	1.08 ± 0.06
Precooked and Boiled	0.93 ± 0.23
Major Anatomical Regions	
Cortex	1.21 ± 0.06
Vascular Bundle + Outer Phloem	1.30 ± 0.05
Perimedulla (Inner Phloem)	1.18 ± 0.02
Medulla (pith)	1.11 ± 0.03

In this and the following table total half-cystine was determined by a spectrophotometric method using Ellman's reagent after cystine was reduced by Na-borohydride (see Materials and Methods)

Epicure. The major decomposition pathways of cysteine and cystine, as reviewed by Carpenter (1974), appear to involve a desulhydration reaction which is catalysed by both heat and water. Hence, the higher destruction in precooked and boiled tuber samples was somewhat expected.

Destruction of half-cystine was also followed for various steps of a Freeze-Thaw granule process. As seen in Table IV.12, the most significant reduction was found during steam-cooking, tuber hot mashing and freeze-thawing steps of the process. In subsequent dehydration steps no significant reduction was found, clearly emphasizing the need for involvement of water.

G. Tryptophan Determination

Tryptophan determination requires, as a first step, total-N (free and protein-bound amino acid-N) solubilization with a suitable solvent. Two solvents appear to be the most suitable for raw and cooked and freeze-dried and powdered potato samples. The first, dilute NaOH (0.075 N), solubilized up to 88% of the raw tuber total-N. However, it had a low efficiency in solubilizing total-N of steam-cooked potatoes (≤33.5%) and of precooked and boiled samples (39.9%). The second solvent system, consisting of 70% ethanol and dilute NaOH in a ratio of 1:4 v/v, solubilized 91% of the available total-N of the raw tuber, while its efficiency was 58.2% for steam-cooked samples and 59.2% for precooked and boiled samples. When 0.5 M NaCl was included

Table IV.12. Total half-cystine content in potato tuber cv. Russet Burbank (Alberta) during its Freeze-Thaw processing into dehydrated granules

After Processing Step of	Total Half-cystine Content (% crude protein)
Slicing	1.20 ± 0.08
Steam-Cooking	1.02 ± 0.05
Hot Mashing	0.92 ± 0.11
Freeze-Thawing	0.74 ± 0.01
Granulation	0.78 ± 0.05
End Product	0.78 ± 0.03
Discard Portion	0.74 ± 0.10

into the solvent system (0.5 M NaCl:70% ethanol:0.075 N NaOH, 2:3:5 v/v), there was improved total-N solubilization (93.7% for raw, 80.1% for steam-cooked and 69.2% for precooked and boiled tubers).

When the binary system of ethanol and NaOH was applied for raw tuber analysis, an increase in the proportion of NaOH provided an increase in protein solubilization, the maximum being reached at a volume proportion of 1:4. However, this was not the case for cooked samples, where an increase in NaOH caused a decrease in solubilization. Improvement could only be obtained by increasing the proportion of ethanol. For cooked samples the optimum was an ethanol:dilute NaOH ratio of 2:1 v/v.

When the potato tuber extracts were checked by iodine reaction for the presence of starch contaminants, the lowest amount of starch was detected with an ethanol:NaOH ratio of 1:4 v/v, while other ratios provided extracts with considerable starch content.

Concon (1975) found that for cereals (wheat and rice) the maximum color development in tryptophan detection occurred after 20 min reaction time. In potato extracts the color development time was found to be much longer. As seen in Figure IV.15, in extracts obtained with 70% ethanol the maximum color intensity was reached after 80 min for raw and boiled potatoes and after 70 min for steam-cooked samples. When dilute NaOH was used instead of ethanol, the time required was 110 min for raw and precooked and boiled

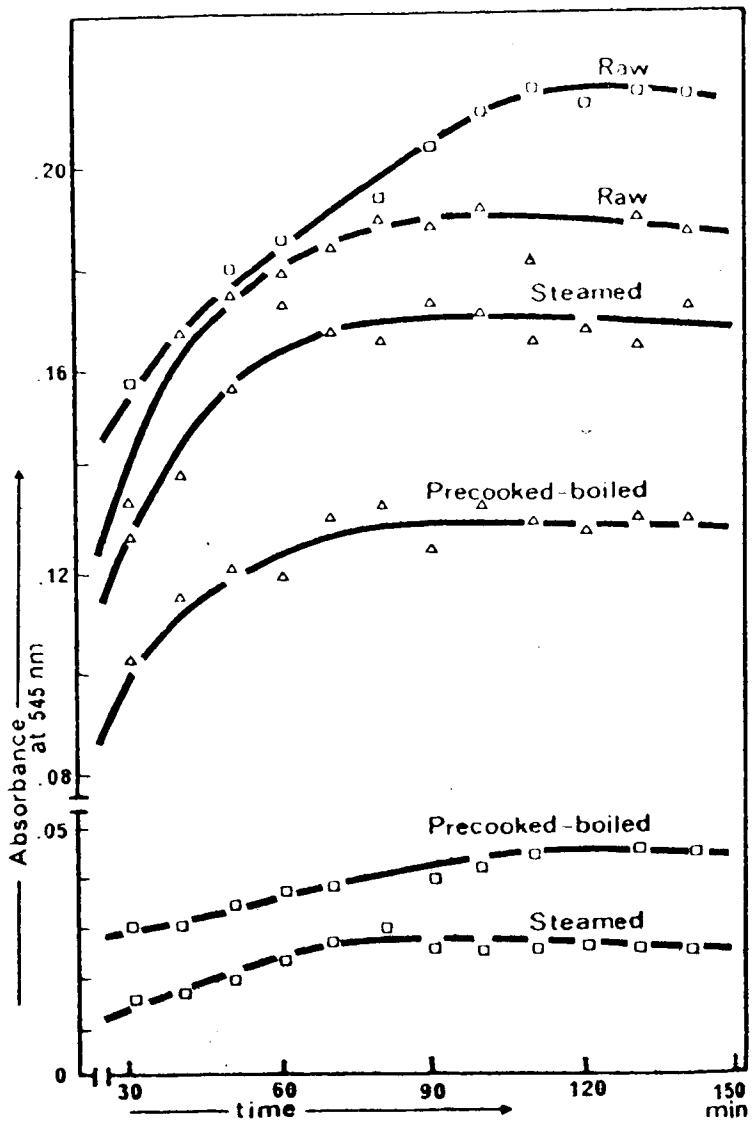


Figure IV.15 Color development of protein extracts by Concon's (1975) method. Extracts were obtained by using 70% ethanol(△) and 0.075N sodium hydroxyde(□).

samples, and 80 min for steam-cooked samples. Similar results were obtained when solvent mixtures were applied (see Figure IV.16). Ethanol:dilute NaOH (4 v:v) provided maximum color after 110 min for all samples while extractant containing NaCl required times in excess of 120 min.

The calibration curve for tryptophan determination that was used in this study is shown in Figure IV.17. The detection limit was close to 20 µg ml and there was a linear relationship between absorbance and tryptophan concentration in a range of 80-200 µg tryptophan ml at preselected reaction times of 70, 90, 110 or 130 min. The absorbance increased with increasing reaction time. The A_{590} increment for a 20 min time difference (for reaction times of 70-90 or 110-130 min) was 0.030 and was independent from tryptophan concentration in the 80-200 µg ml range. The increment readings between 90-110 min varied from 0.020-0.070 and were higher as tryptophan concentration increased.

The maximum color, developed at 110 min, gave rather inconsistent results, depending on the solvent used. Since the variation was unreasonably high, tryptophan content was determined by the Spies (1967) "W" method (M.S. Kaldy, unpublished data) in tubers of cv. Russet Burbank grown in several Alberta locations (those which supply tubers to the dehydration industry). The Spies "W" method was selected since the literature review suggested that it would be the most accurate and reliable method for comparison study. The

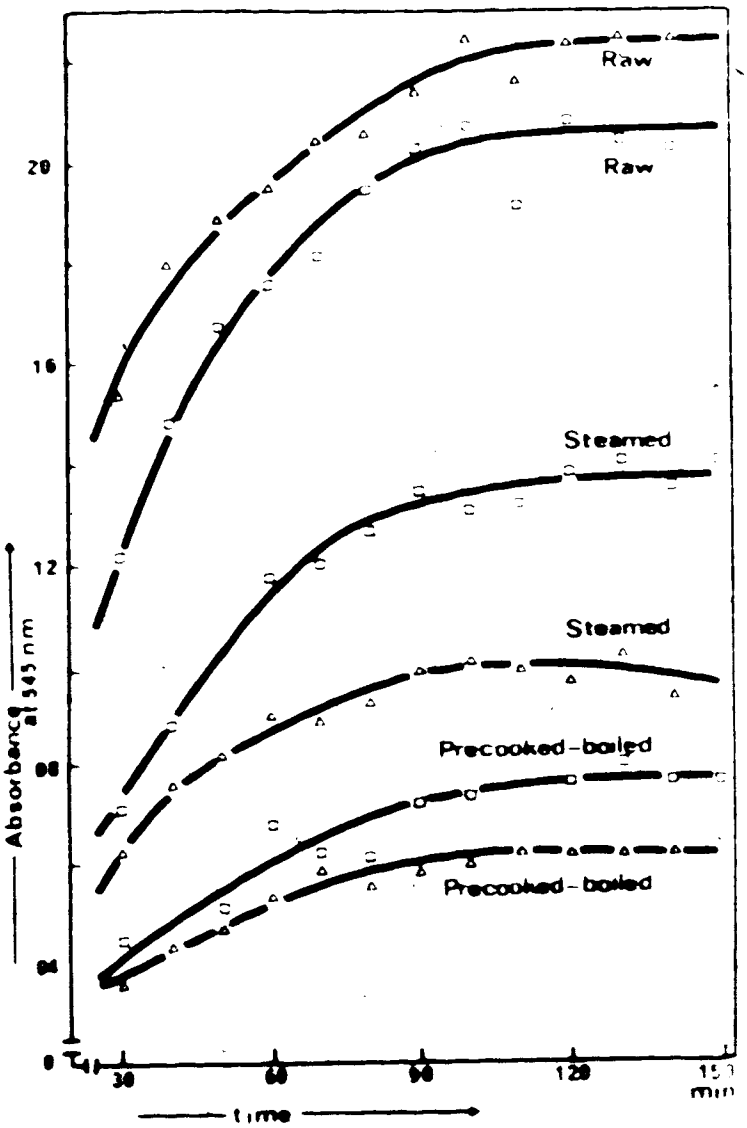


Figure IV.16 Color development of protein extracts by Concon's(1975) method. Extracts were obtained by using a mixture of 70% ethanol, 0.075N sodium hydroxyde in 1:4 ratio(Δ), and a mixture of 0.5M sodium chloride, 70% ethanol and 0.075N sodium hydroxyde in 2:3:5 ratio(□).

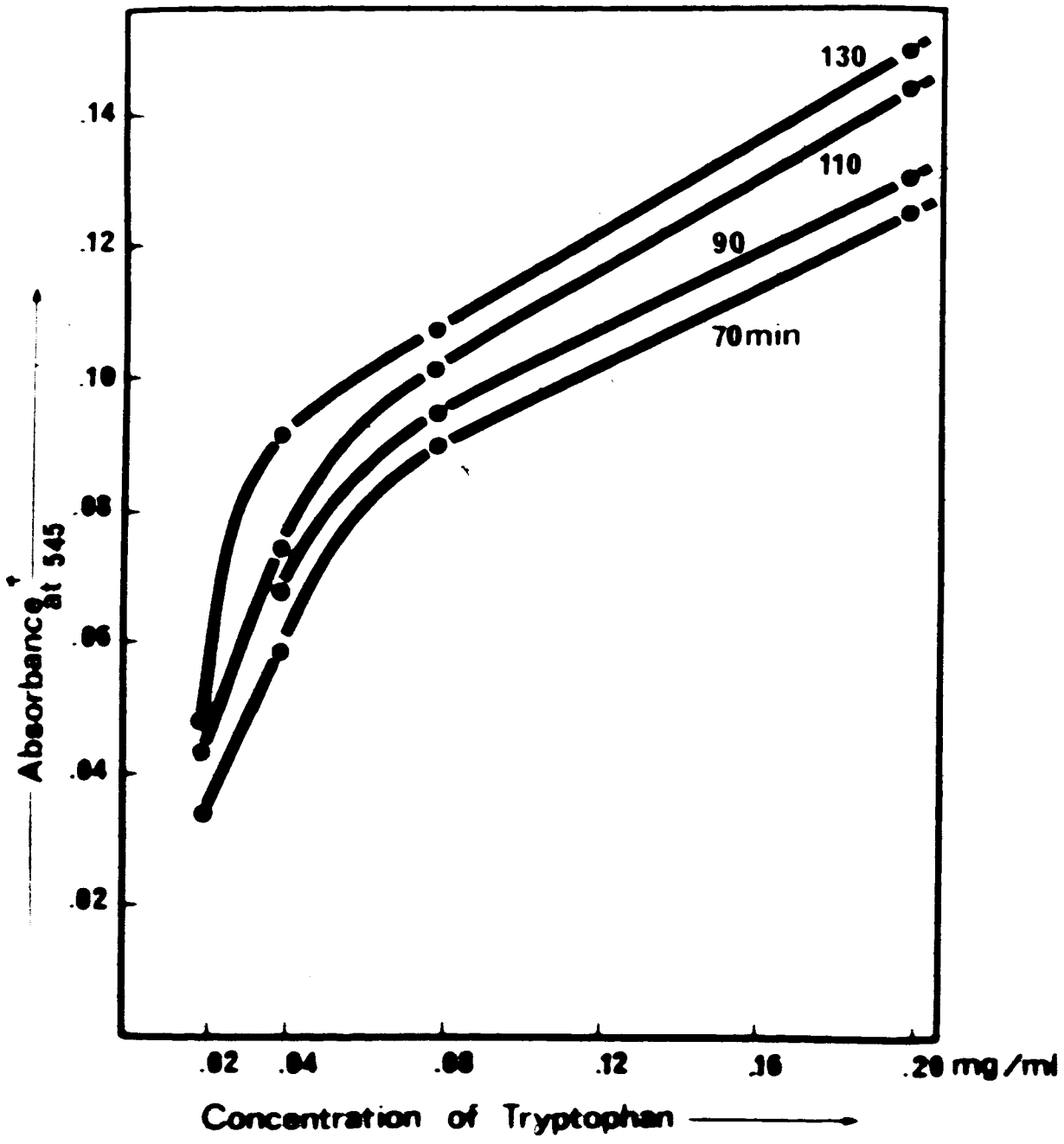


Figure IV.17 Standard calibration curve of Concon's (1975) method for determination of tryptophan

results are presented in Table IV.13. Tryptophan content averaged 1.6% of the tuber total crude-N, with slight differences found for tuber samplings (except for the Edmonton area) and growth location.

The Spies "W" results, when compared to those of colorimetric tryptophan determination (Table IV.14), revealed that colorimetric data are inflated for cooked samples and low for raw tuber when readings were taken after 20 min reaction time. This pattern held regardless of protein extractant, except when NaCl was included, when the reverse trend was true (close to the correct tryptophan value with raw tuber and too low with cooked samples).

Tryptophan color reaction readings taken after 110 min, when the absorbance had leveled off, gave highly inflated tryptophan values, expressed as % of the available tuber proteins. As seen in Table IV. for raw tuber, this inflation was 26-31% for raw tubers and over 80% for cooked tubers.

The source of error which contributed to inflated results was investigated. Reaction of other indoles in a glyoxylate mediated reaction was ruled out since no significant amounts of indoles other than tryptophan have ever been reported in potatoes. Unlike in the Spies and Chambers method (1949), ethanol used in this study should not interfere. However, the possible interference of potato starch was assessed. Potato amylose was solubilized in 0.075 N NaOH (1 mg/ml) and the tryptophan color developed (0.5 ml

Table IV.13. Tryptophan content of potato tubers cv. Russet Burbank grown in four locations in Alberta[1]

Sample Number	Location							
	Edmonton		Lacombe		Brooks		Vauxhall	
	mg/g	%[3]	mg/g	%	mg/g	%	mg/g	%
1	1.27[2]	1.8[3]	1.10	1.2	1.15	1.8	1.54	1.7
2	1.16	1.2	1.26	1.6	1.19	1.5	1.34	1.5
3	1.46	1.9	1.35	1.6	1.19	1.6	1.46	1.8
4	1.18	2.0	1.36	1.8	0.98	1.3	1.08	1.5
Average:	1.27	1.7	1.27	1.6	1.22	1.6	1.36	1.6

[1]Analyses performed by Dr. M. Kaldy (Experimental Research Station, Agriculture Canada, Lethbridge)
[2]mg of tryptophan/g of potato dry matter
[3]Tryptophan percentage of the total protein

Table IV.14. Tryptophan contents in raw and cooked tubers, determined by the colorimetric method of Concon (1975) using the suggested development time of 20 min

Tuber Sample	Extractant EtOH/NaOH ratio	Protein Extracted (%)	Tryptophan (% of protein)	Δ_{110}/Δ_{20} Q-value[1]
Raw	1:4	90.3 ± 0	1.096 ± 0.059	1.652
Raw	0:1	84.2 ± 5.9	1.142 ± 0.028	1.621
Steamed	2:1	51.7 ± 4.9	1.775 ± 0.152	---
Steamed	1:0	39.7 ± 1.40	2.270 ± 0.171	1.600
Precooked and Boiled	2:1	48.1 ± 1.03	1.699 ± 0.018	---
Precooked and Boiled	1:0	28.5 ± 0.91	2.562 ± 0.089	1.635
	Extractant NaCl/EtOH/NaOH Ratio			
Raw	2:3:5	93.69 ± 3.8	1.500 ± 0.16	2.033
Steamed	2:3:5	80.10 ± 2.6	1.352 ± 0.14	2.165
Precooked and Boiled	2:3:5	69.18 ± 4.8	0.972 ± 0.17	2.358

[1]Q-value = ratio of tryptophan color intensity reading at $A_{\lambda_{max}}$ after 110 min when color development levelled off and after 20 min, as used for cereals (Concon, 1975)

amylose solution + 0.5 ml tryptophan [180 µg/ml] + 3 ml reagent and 2 ml sulfuric acid). The A_{595} after 110 min was 0.134. When starch was omitted from the reaction mixture (blank), the reading was 0.122. This difference accounted for an inflation in apparent tryptophan content of 0.04 µg/ml, i.e. only a 2.2% error. Therefore, starch contamination was ruled out as a major source of error. Finally, the water molarity of the reaction mixture was investigated as a possible source of error.

As outlined by Concon (1975), the tryptophan determination reaction mixture consists of 1 ml aqueous sample solution, 2 ml of 25.8 N sulfuric acid and 3 ml of glacial acetic acid. In such a mixture the concentration of sulfuric acid was 8.6 N, and that of water 15.04 M. However, in this study the water content in the solubilized samples, as well as in the final reaction mixture, was the variable component (as shown in Table IV.15). The water molarity varied from 8.55-15.04, a molarity change which closely followed the sigmoid curve for cereals, as established by Concon (1975), i.e.

$$\text{Log } A = b \cdot S - C, \text{ with } b<0$$

where b is the slope of the curve (3.5), S is the sample reading and C is the blank. Hence, just changing the water molarity even within narrow limits would provide A_{595} readings inflated by 40% or more (last column of Table IV.15). Taking into account such corrections, tryptophan contents in raw and cooked potatoes could be adjusted to

Table IV.15. Water molarity in reaction mixture and calculated A_{560} in tryptophan color development for raw and cooked potato tubers

Protein Extractant	70% EtOH	0.075N NaOH	0.5M NaCl	Water in Extract (µl)	Molarity of Water Final Rxn. Mixture[1]	% of max. A_{560} Expected
Ethanol Dominant	1	0		300	8.55	>140
	2	1		533	10.71	>138
	3	1		475	10.18	140
	4	1		440	9.85	141
	5	1		417	9.64	142
Sodium Hydroxide Dominant	0	1		1000	15.04	50-90
	1	2		766	12.87	122
	1	3		825	13.42	115
	1	4		860	13.74	110
	1	5		883	13.95	110
Sodium Chloride Containing	1	5	3	922	14.31	100
	2	5	3	860	13.74	110

[1]Includes 624 µl water content from 2 ml of added 25.8 N sulfuric acid

nearer true values. However, the question would still remain, "Is the partially extracted N of cooked tubers representative of the average tryptophan content of the tubers?".

H. Total Amino Acid Composition of Raw and Processed Potatoes

Raw and processed potatoes were analyzed for their amino acids by (a) ion-exchange column chromatography and, simultaneously, by gas-liquid chromatography (GLC). Sample hydrolysis with 6 N HCl in the presence of 0.1% phenol was the procedure of choice. The advantage of this method was that, despite large amounts of tuber starch, amino acid recovery was satisfactory except for S-containing acids and tryptophan. The oxidation step with performic acid prior to hydrolysis was omitted since it would provide results for only methionine and cysteine + cystine. Since the latter was determined in a separate sample by a colorimetric method using Ellman's reagent, methionine results alone did not warrant such an approach. Moreover, since cysteic acid and methionine sulfone are not detectable by GLC, performic acid oxidation would be inapplicable. Lastly, methanesulfonic acid, though a superior acid for purified protein hydrolysis, was not applied as its application is limited to samples containing less starch than present in potato tubers.

Results obtained by ion-exchange chromatography

The total amino acids and their composition in peeled, steam-cooked, precooked and boiled potatoes analyzed by ion-exchange column chromatography are given in Table IV.16. The raw tuber major acids were aspartic and glutamic acids, the combination accounting for 49% of the total acids present (based on mg/g tuber dry matter).

The essential amino acids comprised 39% of the total, a value close to that of cv. Russet Burbank grown in New Brunswick (low specific gravity, 1.065-1.075). The value was much higher than the 32% for the same cultivar, but high specific gravity (Jaswal, 1973). The non-essential amino acids, excluding aspartic and glutamic acids, comprised close to 12% of the total acids of the tuber. In agreement with many earlier reports, some essential amino acids were present in significant proportions, particularly leucine, lysine, phenylalanine and valine.

Steam-cooking brought about an 11% destruction of the total amino acids of the raw tuber. There was a 7.3% decrease in the sum of aspartic plus glutamic acids, and a slight decrease in the content of total essential amino acids (with lysine, methionine, tyrosine and arginine being among the least affected) and a 1.2% decrease of the non-essential amino acids. Regardless of such destruction, the general amino acid profile, when compared to raw tuber, was practically unchanged.

Table IV.16. Total amino acid composition of raw and cooked peeled potato tubers cv. Russet Burbank.

Amino Acid	Amino Acid Content (mg/g dry weight of potato)[1]		
	Raw	Steamed	Precooked and boiled
Aspartic acid	28.55	23.01	14.14
Threonine[2]	3.11	3.02	2.59
Serine	3.41	3.17	2.73
Glutamic acid	20.27	18.54	11.45
Proline	2.55	2.14	2.09
Glycine	2.93	2.64	2.41
Alanine	2.74	2.59	2.26
1/2 Cystine[2]	0.81	0.72	0.66
Valine[2]	4.98	4.76	3.67
Methionine[2]	1.47	1.43	1.19
Isoleucine[2]	3.54	3.15	2.69
Leucine[2]	4.98	4.46	4.20
Tyrosine[2]	3.64	3.92	3.09
Phenylalanine[2]	4.02	3.64	3.11
Lysine[2]	5.50	5.01	4.62
Histidine[2]	2.09	1.87	1.38
Arginine[2]	4.99	4.91	4.00
Tryptophan[2]	----	----	----

[1]The composition was determined by using an automatic amino acid analyzer with non-oxidized tuber samples. See Materials and Methods.
[2]Essential amino acids.

Precooking followed by cooking in water provided further losses. The destruction was 34% of the originally present amino acids of the tuber. When compared to steam-cooking, the detrimental effect of boiling was obvious: an additional 23.4% destruction. Again, the most affected were aspartic and glutamic acids (23% loss). Total loss of essential amino acid was 8% and of non-essential amino acids 3%, when compared to their initial values in raw tuber. These data strongly suggest that tuber precooking, followed by boiling in water, provides thermal as well as leaching losses of the tuber amino acids.

The elution profiles of an ion-exchange column separation are presented in Figures IV.18-IV.20. The apparent increase found for ammonia peaks in cooked tubers does not necessarily reflect the extent of amino acid degradation during cooking since the clean-up and evaporation steps in sample preparation involve ammonia (see Materials and Methods). The AGPA used as an internal standard was eluted before the last peak (arginine) with good resolution. The presence of γ-aminobutyric acid was always beyond doubt. Figures IV.18-IV.20 also show that a portion of methionine was oxidized during hydrolysis and eluted as methionine sulfone, with the lowest retention time of all the acids. The peak of half-cystine was detectable, but was minor due to destruction during hydrolysis. Elution monitoring was at 560 nm, hence the profiles do not show a proline peak (it was quantitated at 440 nm). Proline was

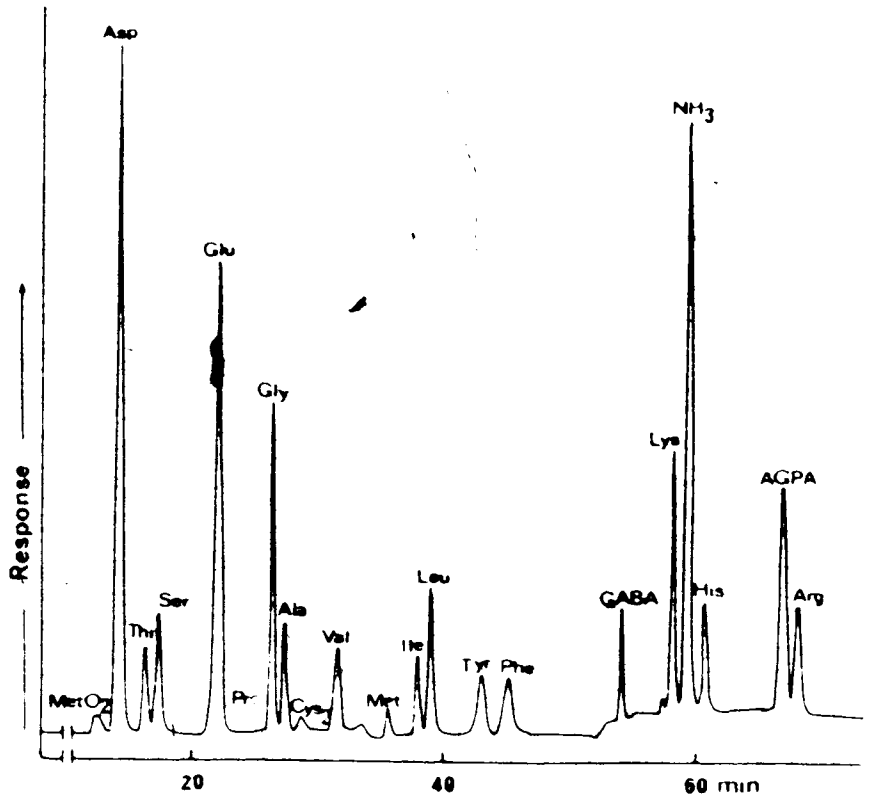


Figure IV.18 Chromatogram of amino acid analysis of raw peeled potato tubers cv. Russet Burbank(Alberta)

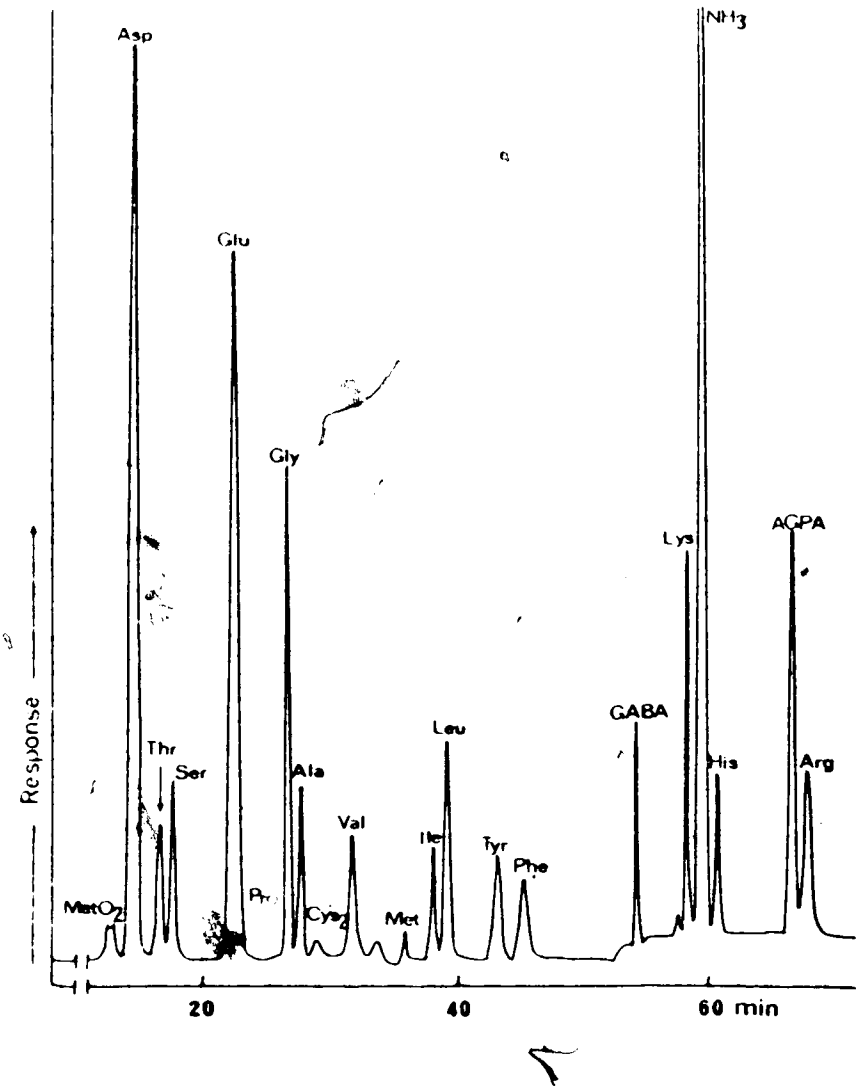


Figure IV. 19 Chromatogram of amino acid analysis of steamed peeled potato tubers cv.Russet Burbank(Alberta)

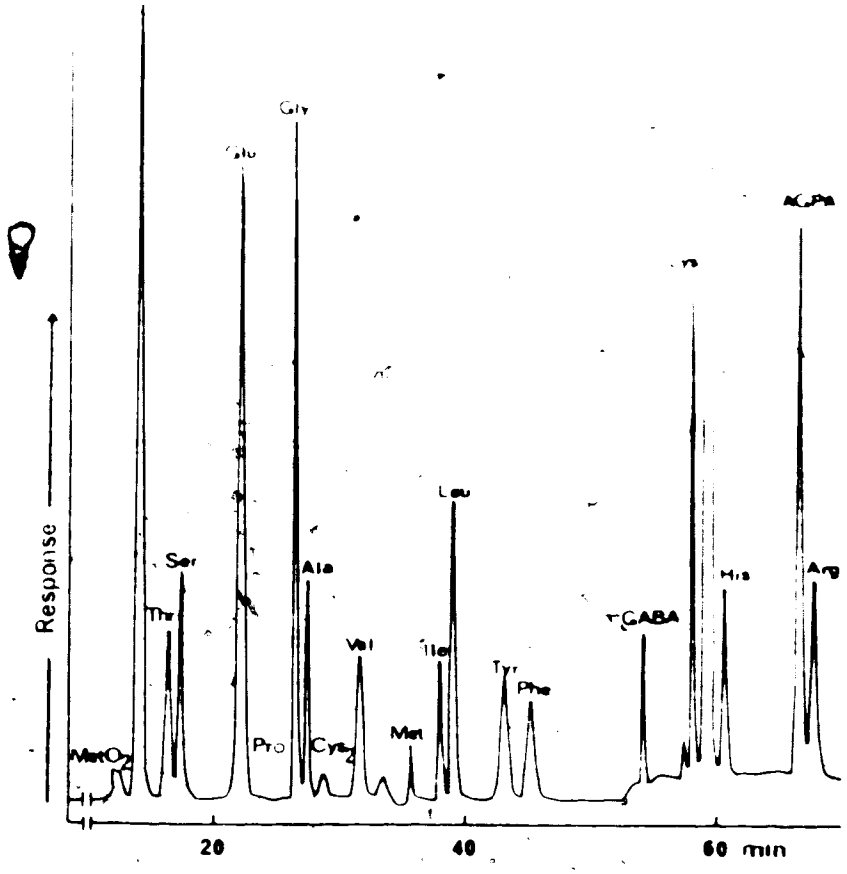


Figure IV.20 Chromatogram of amino acid analysis of Precooked-boiled peeled potato tubers cv.Russet Burbank(Alberta)

well resolved [illegible] between the peaks of glutamic acid and [illegible] ion-exchange chromatography [illegible] reveal the presence [illegible] the number was [illegible] the determination [illegible]

Results obtained by gas-liquid chromatography

The total amino acid contents and compositions of raw and cooked peeled potatoes, as determined by GLC, are given in Tables IV.1[illegible] and IV.18. In the former table data are listed by GC elution sequence (by retention times), while in the latter the elution sequence matches that of ion-exchange column chromatography.

As seen from the GLC elution profiles in Figure IV.2[illegible], the first emerging peak, shouldering the solvent peak, was alanine. The last identified peak was arginine, which was followed by several unknown major and minor peaks eluting over a 10-15 min period. Two internal standards (peak 9, pipecolinic acid and peak 15, AGPA) were readily detected along with the 16 other amino acids. Peak 4 (threonine) was well resolved, but peak 5 (leucine + serine) and peak 6 (isoleucine) overlapped, preventing accurate quantitation.

When the column was operated isothermally rather than with temperature programming, the separation of amino acids was unsuccessful at high temperatures (≥200°C), while the retention time was extremely long at lower temperatures (≤100°C). At an intermediate range, resolution of most of the amino acids was poor. A programmed rate of 10 C°/min was

Table [illegible] Total amino acid composition of peeled potato tubers of Russet Burbank, Alberta, determined by gas liquid chromatography method.

Amino Acid	Amino Acid Content (mg/100 g fresh weight of potato)		
	Raw	Steamed	Pressure Fried and Boiled
Alanine	[illegible]	[illegible]	[illegible]
Glycine	[illegible]	[illegible]	[illegible]
Valine[1]	4.75	4.44	[illegible]
Threonine[1]	3.40	2.48	3.25
γ-aminobutyric acid	3.23	3.[illegible]2	2.2[illegible]
Proline	2.33	2.24	2.85
Aspartic acid	27.43	23.[illegible]	24.9[illegible]
Methionine[1]	[illegible].2[illegible]	[illegible].63	----
Glutamic acid	18.74	16.0[illegible]	13.50
Phenylalanine[1]	4.57	3.8[illegible]	6.9[illegible]
Ornithine	2.46	[illegible].98	2.26
Lysine[1]	3.[illegible]8	3.04	2.62
Tyrosine[2]	5.[illegible]7	4.[illegible]2	5.[illegible]0
Arginine[2]	4.65	4.49	5.90

[1]Determination for non-oxidized sample.
[2]Essential amino acids.

Table [illegible] 8. Total amino acid composition of peeled potato tubers cv. Russet Burbank Alberta determined by a gas liquid chromatography method.

Amino Acid	Amino Acid Content mg g dry weight of potato		
	Raw	Steamed	Precooked and boiled
Aspartic acid	[illegible]	[illegible]	24.9
Threonine[1]	3.40	2.48	3.25
Serine	----	----	----
Glutamic acid	18.74	16.31	13.50
Proline	2.33	2.24	2.85
Glycine	2.28	[illegible]	1.80
Alanine	2.54	1.91	1.02
1/2 Cystine[1]	----	----	----
Valine[1]	4.75	4.44	2.92
Methionine[1]	1.21	1.63	----
Isoleucine[1]	----	----	----
Leucine[1]	----	----	----
Tyrosine[1]	5.17	4.12	5.10
Phenylalanine[1]	4.57	3.81	6.91
Lysine[1]	3.18	3.04	2.62
Histidine[1]	----	----	----
Arginine[1]	4.65	4.49	5.90
Tryptophan[1,2]	----	----	----
γ-aminobutyric acid	3.23	3.72	2.21
Ornithine	2.46	1.98	2.26

[1]Determination for non-oxidized sample.
[2]Essential amino acids.

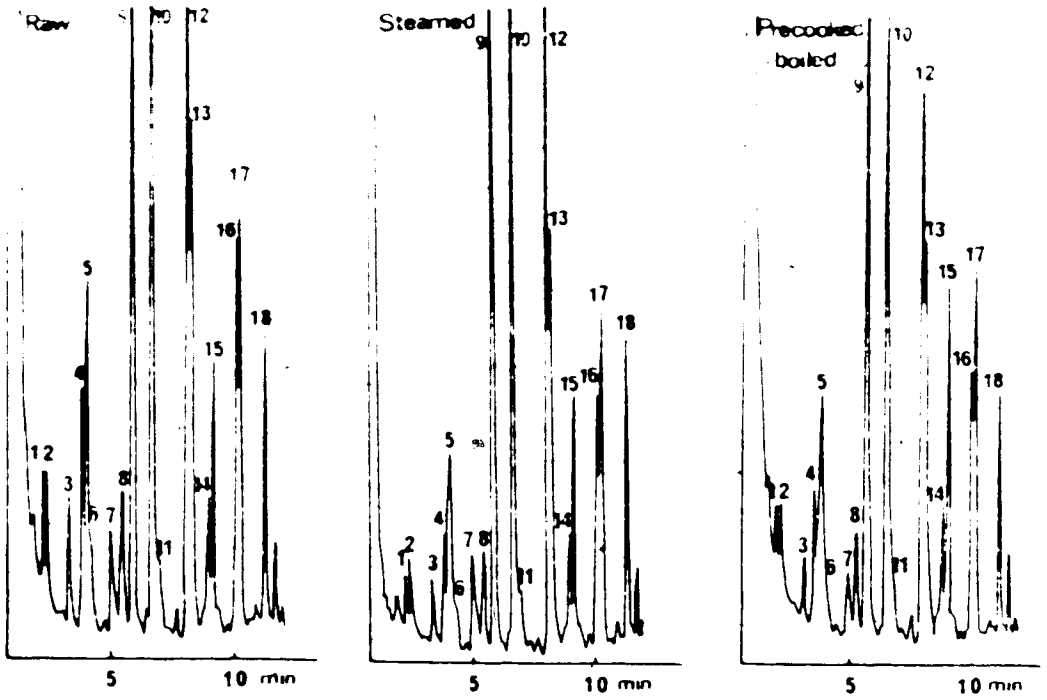


Figure IV.21 Gas-chromatogram of n-HFB-isopropyl ester derivatives of Raw, Steamed, and Precooked-boiled potato tubers cv.Russet Burbank(Alberta).
Pipecolic acid and AGPA as internal standards.
Temperature program: 100°C 1 min, then temperature programming at 10°C/min to 250°C and hold at 250°C.
All samples were co-injected with HFBA.

found to be superior to 6 C° min, the former giving a theoretical plate number for proline of 12,769 and the latter only 7,132. The GLC method, unlike ion-exchange chromatography, did reveal the presence of ornithine (peak 14), which was well resolved from the adjacent doublet (peak 12-13) and from the internal standard (AGPA; peak 15). γ-aminobutyric acid (peak 7) was also well resolved and quantitated. Lastly, the double peaks 12-13 and 16-17 were less well separated than in runs using pure amino acid standard mixtures, but they could be quantitated nevertheless.

The total amino acid contents and compositions for potato tubers determined by GLC were similar to those obtained by ion-exchange chromatography, with two exceptions: GLC always gave higher tyrosine and lower lysine contents in raw and cooked samples. As might be expected, small discrepancies in contents of aspartic acid, proline, alanine, phenylalanine and arginine were found with GLC. For example, potential sources of error were increased apparent phenylalanine content due to its joint elution with glutamic acid, while arginine derivatization was inconsistent due to formation of a salt. Alanine reproducibility could be questionable due to its shouldering on the solvent peak.

Amino Acid Composition of Granulated Potatoes

The amino acid composition of dehydrated potato granules obtained by a semi-pilot scale Freeze-Thaw process

is given in Tables IV.19 and IV.20. The data were obtained by applying gas-liquid and ion exchange chromatography methods and are arranged by the unit operations of the process.

As revealed by GLC (Figures IV.22-IV.24), the peeled tuber contained 90 mg of apparent amino acids/g tuber (dry matter). However, when the amino acids half-cystine, serine, isoleucine, leucine and histidine were included (from results of a parallel determination by ion-exchange chromatography), the total amino acid content was raised to 104.5 mg/g tuber (dry matter). This decreased to 90.1 after steam-cooking, 75.7 after mashing, 74.2 after freeze-thawing and 73.7 after granulation. The end product content was 58.3 and the discard 71.6.

When ion-exchange chromatography data were analyzed, sliced samples provided 101.1 mg/g potato (dry matter). This decreased to 92.6 after steam-cooking, 91.6 after mashing, 90.7 after freeze-thawing and 85.6 after granulation. The end product content was 84.5.

Total amino acid contents as found by GLC were either too low, or the results obtained by ion-exchange chromatography were inflated (even though in the latter case the contents of γ-aminobutyric acid and ornithine were not included). The discrepancy between the two methods was tolerable only in slicing and steam-cooking steps, but was too high in other steps (15.9 mg amino acid in mashing, 16.5 in freeze-thawing, 11.9 in granulation and 26.2 in end

Table IV;19. Total amino acid composition of peeled potato tubers cv. Russet Burbank (Alberta) processed into dehydrated Freeze-Thaw granules.

Amino Acid	Amino Acid Content (mg/g dry weight)[1] Freeze-Thaw Processing Step						
	Slicing	Steaming	Hot Mashing	Freezing/ Thawing	Granulation	End Product	Discard Portion
Aspartic Acid	28,32	26,14	26,39	27,31	22,28	21,79	24,26
Threonine[2]	3,49	3,23	3,18	3,07	3,05	3,01	2,79
Serine	3,49	3,42	3,15	3,31	2,99	2,95	2,75
Glutamic Acid	19,79	16,42	17,41	16,47	16,61	16,46	15,56
Proline	3,03	2,56	2,72	2,25	2,61	2,56	2,06
Glycine	3,07	2,77	2,94	2,86	2,47	2,49	2,19
Alanine	3,13	2,90	2,65	2,69	2,62	2,55	2,41
1/2 Cystine[2]	1,08	0,86	0,92	0,43	1,03	1,08	0,81
Valine[2]	5,18	4,91	4,79	4,66	4,58	4,52	4,32
Methionine[2]	1,06	1,29	0,96	1,30	1,19	1,21	1,27
Isoleucine[2]	3,32	3,08	2,98	2,99	2,82	2,79	2,69
Leucine[2]	5,16	4,78	4,53	4,42	4,36	4,31	4,02
Tyrosine[2]	4,12	4,13	3,87	4,01	3,94	3,88	3,62
Phenylalanine[2]	4,02	3,64	3,51	3,59	3,40	3,33	3,16
Lysine[2]	5,57	5,51	5,08	4,78	4,92	4,99	4,40
Histidine[2]	1,93	1,92	1,77	1,68	1,71	1,68	1,49
Arginine[2]	5,[illegible]	5,02	4,75	4,86	5,05	4,89	4,55
Tryptophan[2]	----	----	----	----	----	----	----
% Crude Nitrogen	1,580	1,507	1,484	1,476	1,394	1,376	1,340

[1]Determined by automatic amino acid analyzer, omitting the oxidation step prior to acid hydrolysis.
[2]Essential amino acids.

Table IV.20. Total amino acid composition of peeled potato tubers cv. Russet Burbank (Alberta) processed into dehydrated Freeze-Thaw granules.

Amino Acid	Amino Acid Content (mg/g dry weight)[1] Freeze-Thaw Processing Step						
	Slicing	Steaming	Hot Mashing	Freezing/ Thawing	Granulation	End Product	Discard Portion
Aspartic Acid	28.19	20.07	19.06	18.87	18.82	17.99	19.26
Threonine[2]	2.98	2.80	2.56	2.54	0.90	2.16	2.21
Serine	----	----	----	----	----	----	----
Glutamic Acid	17.34	18.18	14.21	14.07	14.28	14.00	14.52
Proline	3.13	3.10	1.84	1.82	2.38	1.53	1.70
Glycine	3.21	2.88	2.80	2.77	1.66	1.66	2.13
Alanine	3.23	2.74	2.56	2.55	1.98	1.97	2.23
1/2 Cystine[2]	----	----	----	----	----	----	----
Valine[2]	3.79	4.49	4.29	4.25	1.86	2.84	4.12
Methionine[2]	4.69	0.66	0.73	0.72	1.36	0.88	0.91
Isoleucine[2]	----	----	----	----	----	----	----
Leucine[2]	----	----	----	----	----	----	----
Tyrosine[2]	4.88	4.99	3.44	2.97	4.67	3.25	3.17
Phenylalanine[2]	4.89	2.75	2.06	2.04	4.44	2.22	2.11
Lysine[2]	4.36	2.82	3.09	3.06	2.01	2.49	2.79
Histidine[2]	----	----	----	----	----	----	----
Arginine[2]	4.06	7.05	3.30	3.27	2.35	1.29	1.84
Tryptophan[2]	----	----	----	----	----	----	----
γ-aminobutyric Acid	3.88	2.41	1.65	1.63	2.13	1.63	1.59
Ornithine	1.37	0.98	0.81	0.80	1.58	1.58	1.18

[1]Composition determined for non-oxidized samples using a gas-liquid chromatography method.
[2]Essential amino acids.

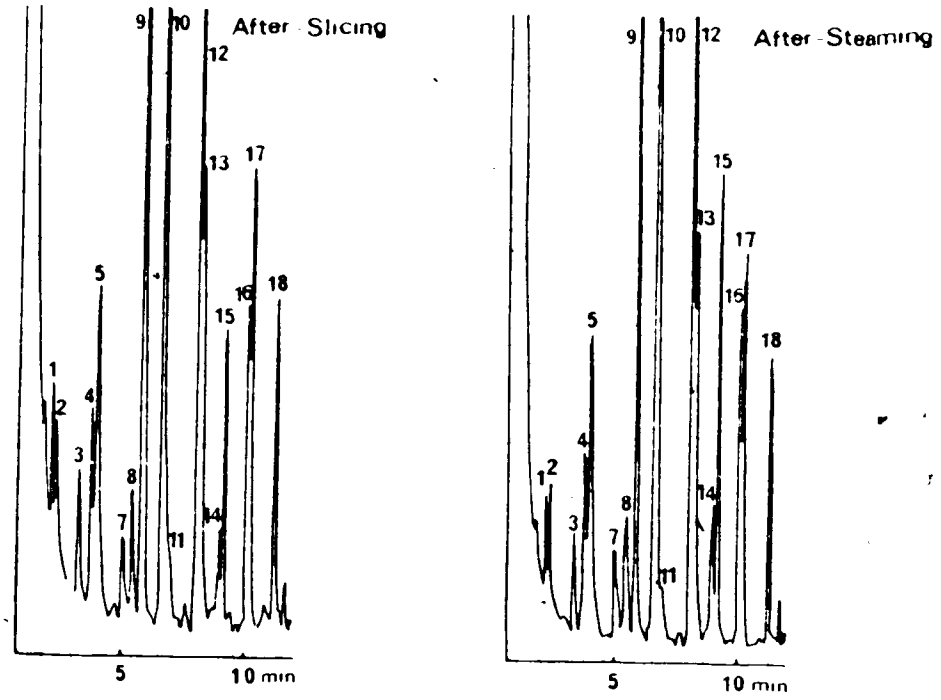


Figure IV.22 Gas chromatogram of n-HFB-isopropyl ester derivatives of samples obtained from a Freeze-Thaw granule process(after: slicing; steaming). Pipecolinic acid and AGPA as internal standards. Temperature program: 100°c 1 min, then temperature programming at 10c°/min to 250°c and hold at 250°c. All samples were co-injected with HFBA.

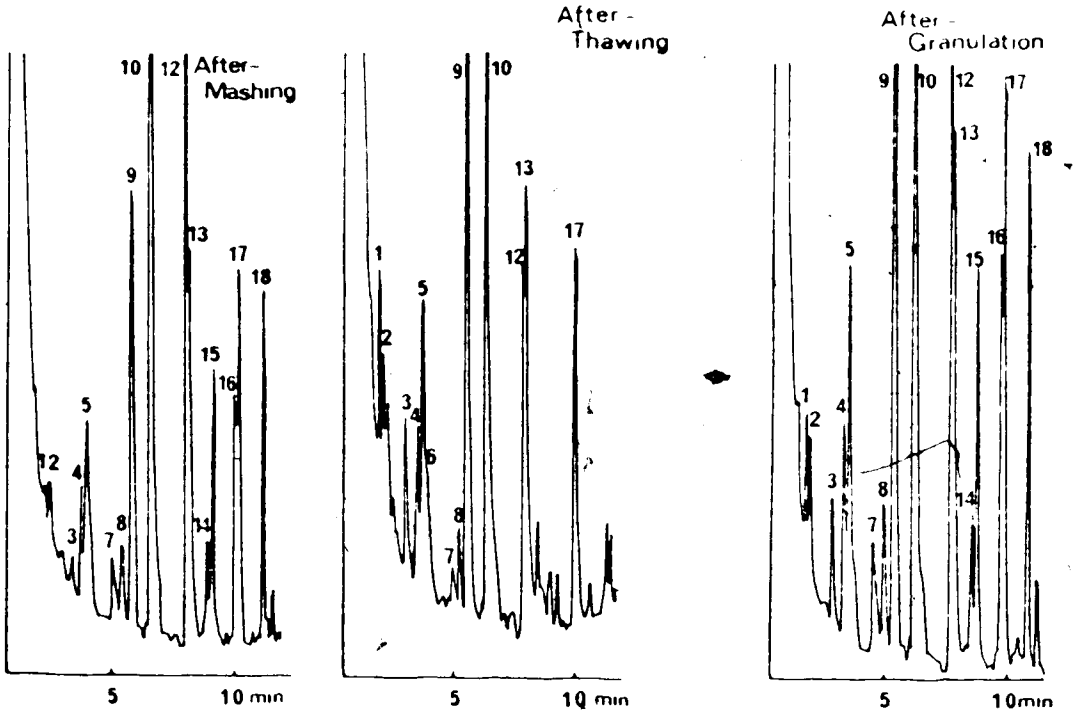


Figure IV.23 Gas chromatograms of n-HFB-isopropyl ester derivatives of samples obtained from a Freeze-Thaw granule process (after:mashing; thawing; granulation). Pipecolinic acid and AGPA as internal standards.
Temperature program: 100°c 1 min, then temperature programming at 10°c/min to 250°c and hold at 250°c.
All samples were co-injected with HFBA.

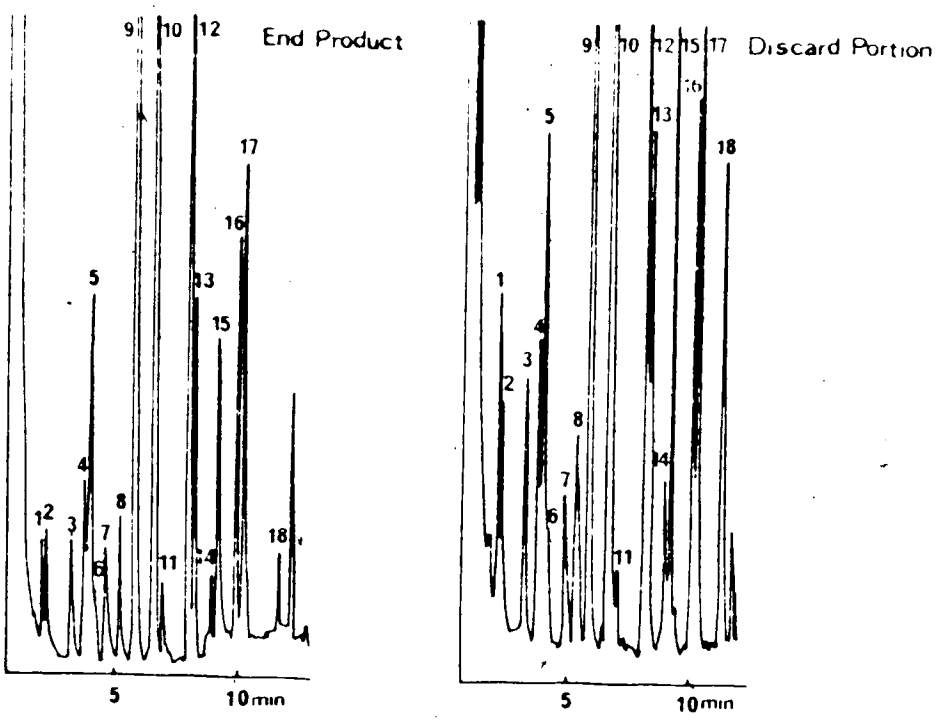


Figure IV.24 Gas chromatograms of n-HFB-isopropyl ester derivatives of samples obtained from a Freeze-Thaw granule process (end product and discard portion). Pipecolinic acid and AGPA as internal standards.
Temperatu program: 100°c 1 min, then temperature programming at 10°c/min to 250°c and hold at 250°c.
All samples were co-injected with HFBA.

program

product).

In order to resolve the discrepancy, the total crude protein-N content was followed during the granule process. The data are listed in Table IV.20. The results revealed a steam-cook-induced loss of 4.6%, the loss increasing by 1.5% in mashing, 0.5% in freeze-thawing and 5.18% in granulation, giving a total loss of 11.78% total crude protein-N. Further loss in the end product was only 1.1%. These results supported the ion-exchange chromatography data but strongly disagreed with those obtained by GLC. Therefore, further consideration of the data involved only those listed in Table IV.20.

As shown by ion-exchange chromatography, potato tuber processing into dehydrated granules by a Freeze-Thaw process brings about only small losses in total amino acid content and little change in their composition. There was only a 4.5% loss of total essential amino acids and a 2.1% loss in non-essential amino acids, excluding aspartic and glutamic acids, which comprised 48% of peeled and sliced potatoes. In the granule process their content decreased to 37.9%, which was the only significant loss recorded by the granule process.

When the granule results are compared to potato flakes made by laboratory drum drying (Jaswal, 1973), the granule process appears much superior. The granule process essential amino acid destruction is less than half that for flakes, while the non-essential amino acid losses are similar and

involve primarily losses of aspartic and glutamic acids.

A previous study in our laboratory on granules obtained by an Add-Back process (Golan-Goldhirsh, 1979) revealed only the change of the free amino acid pool during processing. Peeled raw potato, after reaching the end-product state, decreased its free amino acid pool by 40%. The major loss (27.3%) occurred in the precooking step. Slightly more than half of the losses were assigned to essential amino acids. No other data are available in the existing literature on a granule process.

V. CONCLUSIONS

Both flavoring and nutritional aspects partly provided the major impetus by the Alberta potato dehydration industry to support this study. The total amino acids and their composition, particularly those of sulfur containing acids and tryptophan in Alberta grown potatoes (cv. Russet Burbank) and their stability or destruction during tuber processing into dehydrated granules were of interest.

Although the Freeze-Thaw process was applied and the Add-Back process was not, general conclusions and results obtained were pertinent to both.

The granule process had little affect on the total amino acid content and their composition. Only 4.5% destruction of essential amino acids (which, among others, contained cysteine + cystine, methionine and tryptophan) and a total loss of 12.1% non-essential amino acids classify the process as the least detrimental for commercial potato processing.

Additional objectives of the study to find rapid and reproducible methods suitable for a quality control lab were not completely fulfilled. Although amino acid composition of the pure proteins and/or raw, sliced and steam-cooked potatoes could be determined by the fast GLC approach, the method failed in reliability and accuracy when the moist mash was subjected to dehydration steps, and was unreliable even with the end product. Here, the expensive ion-exchange chromatography appears to be irreplaceable. Tryptophan

determination in processed samples provided highly inflated results. Hence, the slow and tedious Spies W method had to be retained. However, cystine + cysteine determination by a simple colorimetric procedure using $NaBH_4$ and Ellman's reagent was successful for both raw and cooked potatoes.

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CURRICULUM VITAE

Shin Yamagata was born in 1956 in Sapporo, the capital of the prefecture of Hokkaido, the northernmost island of Japan. After completing his elementary and secondary education in Sapporo, he enrolled in the Department of Dairy Agriculture, The College of Dairying (Rakuno Gakuen Daigaku) in the city of Ebetsu. He completed his B.Sc. program in 1979 under the supervision of Professor K. Sakata. His graduation diploma work was related to chemical constituents of dent corn. Part of this work was published under the title "Volatile and non-volatile constituents of dent corn (Zea mays)" in the journal Nippon Nogeikagaku Kaishi in 1980.

During his stay at the College, Shin participated in the College Student English Speaking Society, a society aimed at improving and mastering conversational English. In 1976 he was awarded the gold medal at the intercollegiate recitation contest held by the All-Hokkaido English Language Speaking League.

After graduating from the College with distinction, Shin came to Edmonton as an exchangee of the Alberta-Hokkaido Dairy Science and Technology Exchange Program, a program supported by the Extension Division of Alberta Agriculture. At that time he joined the Department of Food Science at the University of Alberta to pursue an M.Sc. research thesis degree. He was the recipient of a graduate research fellowship granted to him by Dr. D.

Hadziyev, Professor of Food Chemistry, and served as a teaching assistant for the cereal and oil seed technology course (FdSc 506). Also, on many occasions Shin acted as interpreter for the Alberta-Hokkaido Exchange Program Committee of Alberta Agriculture.