76. The Nitrogen of the Potato

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The value of the potato as a source of dietary nitrogen has been examined by several authors, but the interpretation of their results is made difficult, since in nearly all these experiments the total nitrogen only is recorded. The potato tuber contains besides protein a large variety of low-molecular nitrogenous substances, some of little or no nutritive value, and their relative proportions may vary greatly in different samples. Feeding experiments without chemical analysis of the material used will therefore give little information of a useful character.

The data obtained in different countries have recently been summarized [Lampitt & Goldenberg, 1940]; little is known about the nitrogen distribution of domestic varieties. In this paper methods used to characterize the different nitrogenous fractions are described and data for a number of varieties are presented showing fairly wide variations in total nitrogen and in the relative proportions of the different fractions. The distribution of the various fractions over the different morphological layers has also been investigated.

PART I

METHODS

Potatoes were stored at 8–10° and usually worked up within one month after lifting. The tubers were cut into slices and quickly frozen and dried at −15°. Chemical changes affecting the distribution of N are unlikely to occur during the drying process since results obtained by this method agree well with those obtained on press-juice. The dried material was then finely powdered and stored.

Most of the total and also of the protein N can be extracted by water or dilute salt solution (Table 1), although 2% NaCl is more efficacious than water for extraction of coagulable N. Increase of the concentration of electrolyte offers no advantage; 2% NaCl was therefore used to extract the total soluble N. Two successive extractions with this solvent supply a representative sample as shown by the following experiment: 140 g. of the dried powder of the variety Dunbar Rover were shaken with 700 ml. of 2% NaCl and the mixture centrifuged. Total and coagulable N were estimated on aliquots of the supernatant fluid. The extractions were repeated eight times using 280 ml. portions of solvent. The relative proportions of the coagulable N in the first and second extractions were 39·8 and 39·9%, whilst in the third and fourth extracts which contained only small amounts of total N the corresponding figures were 31 and 28% respectively. The definite decrease in the percentage of coagulable N in the later extracts may possibly be due to the fact that prolonged extraction removes fairly insoluble low-molecular substances such as purines and tyrosine which are known to occur in potatoes.

Insoluble protein

It appears from Table 1 that a certain amount of N cannot be extracted by salt solutions, acid or alkali. In later experiments it was found that it could not be rendered soluble by alcohol, ether, alcoholic alkali or urea, neither is its extraction facilitated by more thorough grinding. As will be shown later this insoluble N is almost entirely concentrated in the outer layers of the tuber and is probably therefore not formed by de-

naturation of protein during extraction. The residue obtained after repeated extraction with aqueous solvents contained usually between 0·11 and 0·14% N corresponding to about 8–10% of the total N originally present, and gave positive colour reactions with the

Table 1. *Estimation of soluble nitrogen in the potato*

Solvent	mg. N extracted	% of total	mg. coagulable N
Water	175·8	77	56
2% NaCl	23·35	10·4	12
10% NaCl	5·1	2·2	2·3
10% NaCl (2nd extract)	1·1	0·5	1·0
0·2*N* Na_2CO_3	3·5	1·5	—
Residue	19·6	8·5	—

The potato powder was successively extracted with the solvents.

Millon, Sakaguchi and Hopkins-Cole reagents, the latter being very weak. No positive biuret reaction could be obtained. Digestion with pepsin at *p*H 1·5 rendered about half of the residual N soluble. It may therefore be assumed that at least a large part of the insoluble material is protein. The presence of other substances of unknown chemical character forming part of the cuticular structure cannot however be excluded and the inclusion of the insoluble N in the total protein fraction which is adopted here for the sake of convenience is admittedly somewhat unsatisfactory.

Total protein

The total protein can be determined in two ways: (*a*) the sample is exhaustively extracted and the protein-N in the extracts and residue is estimated; (*b*) a sample is extracted with hot water and N is estimated in an aliquot of the solution after protein has been removed. The second method which is much less laborious gives usually slightly higher results. We have not been able to explain this discrepancy. The values recorded in Table 4 were obtained by the second method.

Estimation of soluble protein (Table 2). The amount of N coagulated by heating at 100° for 1 min. in 2% NaCl and at the slightly acid reaction of the extract is taken as

Table 2. *Comparison of different methods of estimating protein in the aqueous extract*

Precipitant	% of soluble N precipitated	Precipitant	% of soluble N precipitated
Trichloroacetic acid at 0°	40·4	Barium hydroxide and 45% ethyl alcohol	36·9
Trichloroacetic acid at 37°	37·8		
Heating at 80 for 5 min.	37·2	20% lead acetate	42·7
50% ethyl alcohol	—	Copper hydroxide	43·1

measure of the soluble protein-N. Typical protein precipitants remove a similar proportion of the total soluble N; the filtrate from the heat coagulation does not give a precipitate with trichloroacetic acid at 0°. Lead acetate precipitates more N than the other reagents, probably because it forms insoluble complexes with low-molecular nitrogenous substances present in the extract, and the same applies to cupric hydroxide. The small amount of N precipitated by 50% alcohol is noteworthy. A sample of the extract was also filtered through membranes standardized and prepared by Elford's method and put at our disposal through the courtesy of Dr K. Smith. A membrane of the average pore size of 7 mμ retained 41·5% of the total N, whereas one of porosity 13 mμ retained only 35·1%. The filtrate from the first experiment gave no precipitate with trichloroacetic acid, whilst that from the second experiment produced a slight but definite cloudiness. It can be concluded therefore that the same nitrogenous material is precipitated by heat and by trichloroacetic acid and is retained by the 7 mμ membrane; this material is called soluble protein.

Non-protein nitrogen

The total non-protein N was obtained by direct estimation after removal of the protein by heat coagulation. Total amides were estimated by measuring in the Parnas apparatus the ammonia formed on hydrolysis by *N* HCl for 3 hr. at 100°.

Glutamine. Glutamine was estimated according to Vickery, Pucher, Clark, Chibnall & Westall [1935]. This method, which is based on the liberation of ammonia from glutamine at neutral reaction at 100°, is probably not quite specific, since, as pointed out by the authors themselves, other substances such as urea and allantoin may also split off ammonia under these conditions. The method of Pucher & Vickery [1940] which appeared to be very specific did not yield satisfactory results in our hands. A very convenient and in our opinion fairly specific method for the estimation of glutamine was then developed; it is based on experiments, summarized in Table 3. α-Amino acids react quantitatively with ninhydrin to give 1 mol. of CO_2 [Van Slyke & Dillon, 1938]. Aspartic acid is an exception in yielding 2 mol. of CO_2, but in asparagine this anomaly disappears. Glutamine also gives nearly 100 % of the theoretical value, i.e. reaction with ninhydrin is very much faster than cyclization. Pyrrolidone carboxylic acid, as might be expected, does not yield any CO_2. If glutamine is heated first for 2–3 hr. at *p*H 6·8 the reaction with ninhydrin disappears nearly completely, whilst asparagine is unaffected; the small amount of CO_2 found consistently in heated glutamine solutions may be due to the formation of glutamic acid by a side reaction.

Glutamine can therefore be estimated by determining the CO_2 developed by ninhydrin treatment both before and after heating at *p*H 6·8. Interference by unstable peptides which might be split under these conditions is a remote possibility which cannot be entirely excluded. Another possible source of error might be cyclization of glutamic acid leading to a loss of reactive carboxyl groups; it appears, however, from the results of Wilson & Cannan [1937] that the rate of such a reaction at neutrality is too low to cause any appreciable error.

Table 3. *Reaction of ninhydrin with amides*

	α-Amino-nitrogen			α-Amino-nitrogen	
	Found %	Calculated %		Found %	Calculated %
Glutamine before heating	9·2	9·55	Asparagine before heating	9·15	9·27
Glutamine after heating at *p*H 6·8	0·37	0	Asparagine after heating	9·25	9·27
Pyrrolidone carboxylic acid	0	0			

The new method was compared with that of Vickery *et al.* [1935*a*] on a sample of potato juice freed from protein by heat coagulation. The ammonia method gave a value of 0·142 of glutamine amide-N per ml., whilst the ninhydrin method gave 0·138 mg. The agreement between these two methods together with the isolation of glutamine, although in poor yield, makes it appear certain that both methods give an accurate estimate of the glutamine content of the potato.

Asparagine. This compound has been estimated as the difference between total amide and glutamine amide. Such a method is somewhat unsatisfactory and it was attempted therefore to utilize the different behaviours of asparagine and aspartic acid with ninhydrin for estimation of the amide. It was assumed that aspartic acid would react with ninhydrin to form in the first place malonic semialdehyde which would then decompose into acetaldehyde and CO_2; in fact no volatile aldehyde could be detected, although Virtanen & Laine [1938] found that a small amount of acetaldehyde was formed in this reaction.

It can actually be assumed that the Sachsse hydrolysis in potato extracts gives approximately correct results for the following reasons. As will be shown later, asparagine has

been isolated in yields up to 80% of that to be expected. Furthermore, other substances which are known to yield ammonia on acid hydrolysis such as urea, ureides and allantoin were shown to be absent or to be present in such small amounts as not to affect the accuracy of the estimation. The most serious objection to this method of estimation is the possibility that amides present in peptide linkage would be estimated as asparagine.

Amino-acid fraction. The total carboxyl carbon or its equivalent of α-amino-N obtained by the ninhydrin method derives from amides and other amino-acids. The difference between total α-amino-N and total amide-N is termed for convenience α-amino-acid-N. The estimations were carried out in the earlier experiments according to Van Slyke & Dillon [1938]; in the later experiments the improved technique of Van Slyke, Dillon, MacFadyen & Hamilton [1941] was used.

Free amino-acids in plant extracts have hitherto been estimated usually by the nitrous acid method of Van Slyke. Experiments were therefore carried out on four samples to compare results obtained by the nitrous acid and the ninhydrin methods. It was shown that the nitrous acid method gave results which were about 15–25% too high, even if allowance were made for the excess N evolved by glutamine [Chibnall & Westall, 1932]. It may be inferred that substances other than α-amino-acids containing amino groups which can react with nitrous acid were present. This error becomes of considerable magnitude if amino-acids are determined in the presence of large amounts of amides and are estimated by difference. Apparent values for amino-acid-N might be obtained which are too high by more than 100%.

Basic N. The N precipitated by phosphotungstic acid at *p*H 1 after keeping overnight at 0° is termed basic N. It is well known that phosphotungstic acid may precipitate non-basic amino-acids and the values for basic N may therefore be too high. Moreover, basic amino-acids are partly estimated both under α-amino-acid-N and under basic N and these two facts are probably the reasons why the fractions add up in most samples to about 104%.

PART II

Isolation of compounds

The most comprehensive work on low-molecular nitrogenous substances of the potato tuber was done by Schulze and his collaborators more than 50 years ago. Very little work involving actual isolation has been done since, the only notable exception being the isolation of glutathione from sprouting potatoes by Guthrie [1932]. Schulze & Barbieri [1877] showed that asparagine accounted for most of the amide; no glutamine could be found, nor could glutamic acid be isolated after acid hydrolysis. In later papers [Schulze & Eugster, 1882; and Schulze, 1904] the presence of amino-acids was demonstrated; arginine was isolated in moderately large amounts, whilst lysine and histidine could be obtained in traces only; tyrosine and leucine were also isolated. Schulze [1883; 1904] also found the bases hypoxanthine, choline and possibly trigonelline in potato extracts. Our isolations can add little to the classical work of Schulze but our justification for recording our results is that modern methods have enabled us to obtain higher yields of substances already found by Schulze and to isolate a few other compounds for which no good methods were available in Schulze's time.

The isolation of asparagine by the method of Vickery, Pucher & Clark [1935] presented no difficulties. Glutamine on the other hand was difficult to obtain although its presence was indicated in varying amounts by the indirect methods discussed above; Steward & Preston [1940] had also obtained evidence that an unstable amide was associated with protein synthesis in potato discs. It seemed therefore quite important to ascertain whether this substance is in fact glutamine. It was found eventually that glutamine

could be isolated from potato extracts if its concentration were at least equal to that of asparagine; if asparagine were in great excess, mercuric nitrate precipitated most of the asparagine and some amino-acids, but practically no glutamine.

The concentration of free amino-acids other than amides in potato extracts is small and their isolation is considerably more difficult than from a protein hydrolysate. No attempt at quantitative isolation was therefore made and only a fraction of the total amino-acids has been accounted for. Tyrosine has been isolated in a yield slightly higher than that obtained by Isherwood [1937; 1938]. Arginine was found in fairly large amounts, whilst lysine and histidine seem to be absent. Qualitative tests for cystine (nitroprusside after reduction and labile sulphur) and for tryptophan were negative on concentrated potato extracts freed from protein. Leucine and phenylalanine, which are known to occur in plant extracts, have been isolated. Volatile bases are absent apart from minute traces of ammonia which were presumably formed by decomposition of glutamine. We have been unable to obtain any hypoxanthine in the purine fraction, but found instead adenine. The phosphotungstic acid precipitate yielded trigonelline and choline. A more convenient and more quantitative method for estimation of choline was precipitation by Reinecke acid. All basic substances give reineckates which are insoluble at acid, or sometimes even at neutral or mildly alkaline reaction, but reineckates of amino-acids, of primary, secondary and tertiary amines and of betaines dissolve at *p*H 12–13 [cf. also Strack & Schwaneberg, 1937]; only quaternary bases without a carboxyl group such as choline and methyl pyridinium chloride give reineckates which are insoluble at such an alkaline reaction. The explanation presumably is that the cationic form of a base forms an insoluble reineckate and quaternary ammonium compounds are so strongly basic that they are ionized even at *p*H 13 and cannot be rendered soluble, as in the case of betaines, by the possession of a carboxyl group which can form a salt. Choline was therefore precipitated with reinecke acid at *p*H 13 and the salt was dissolved in aqueous acetone. This method is probably not quite specific since other quaternary bases such as tetramethyl ammonium compounds may also be precipitated. The fact however that on decomposition of the reineckate choline chloroplatinate is obtained in good yield indicates that the bulk of the reineckate is the choline derivative.

Experimental

Preparation of asparagine. 1·5 kg. of potatoes (Majestic) were ground in a meat chopper and the pulp was mixed with sand and pressed in a hydraulic press which was kindly put at our disposal by Prof. D. Keilin. The residue was again pressed and washed twice with water. The extract containing 1·5 g. of total N and 0·28 g. of asparagine amide-N was then treated with basic lead acetate. The lead precipitate was washed repeatedly on the centrifuge; 400 ml. of the mercuric nitrate reagent [Vickery *et al.* 1935*b*] were then added to the supernatant and the precipitate which formed was decomposed. The yield of asparagine was 2·4 g. which is about 80% of the quantity found by indirect analysis. The recrystallized sample had M.P. 182° (uncorr.). (Found: N, 18·5; amide-N, 9·3; water of crystallization 12·1%; asparagine requires N, 18·7; amide 9·35; water of crystallization 12·0%.) No glutamine could be isolated from the mother liquors, although indirect analysis indicated the presence of 1·2 g. of glutamine in the press-juice. In a later experiment on King Edward potatoes an appreciable quantity of tyrosine was obtained from the mercury precipitate.

Preparation of glutamine. 1000 ml. of press-juice of the medullary parts of King Edward potatoes, which are rich in glutamine, were treated with lead acetate and mercuric nitrate as described above. Asparagine and glutamine were separated by fractional crystallization. Unfortunately, owing to an accident about half of the glutamine solution was lost; 90 mg. of glutamine were, however, obtained which had the following properties: M.P. 182° (uncorr.). (N found 18·65; amide 9·4%; glutamine requires N, 19·2; amide 9·6%.)

Preparation of amino-acids. 5000 ml. of potato extract were precipitated with lead acetate, acetic acid being added to keep the *p*H near 4. The precipitate was then decomposed with H_2S, the H_2S was removed and the solution again precipitated with lead acetate. The combined solutions were concentrated to about 1000 ml. to remove most of the acetic acid, made up to 2000 ml. and adjusted to *p*H 9. The precipitate formed was decomposed with H_2S after addition of HCl; the resulting solution after concentration and adjustment to *p*H 5 deposited 535 mg. of tyrosine. (Found: N, 7·6 %; $C_9H_{11}O_3N$ requires N, 7·73 %.) Colorimetric analysis by Lugg's [1937] method gave a value of 0·0198 % for the original extract which corresponds to 980 mg. in the whole sample; the recovery of crystalline tyrosine was therefore about 55 %. The alkaline mother liquor from the tyrosine precipitation was then acidified, excess lead was removed and the solution was again concentrated. Successive crops of crystalline material were obtained which gave after several recrystallizations altogether 10·5 g. of asparagine. The mother liquors from the asparagine crystallizations were combined with the main solution which was now treated with mercuric acetate, sodium carbonate and alcohol [Neuberg & Kerb, 1912]. This precipitate gave after decomposition some more asparagine and a few mg. of tyrosine. The solution after saturation with sodium chloride [Hill & Robson, 1934] yielded a mixture of amino-acids which was free from sulphur, thus indicating the absence of methionine from the precipitate. Further fractionation according to Baptist & Robson [1940] yielded 90 mg. of a picrolonate and 320 mg. of crude leucine. The picrolonate after decomposition yielded 30·5 mg. of phenylalanine. Found: N, 8·35 %; $C_{11}H_{11}O_2N$ requires N, 8·48 %; $[\alpha]_D = -33·5°$ (Baptist & Robson [1940] give $[\alpha]_D = -35°$). The crude leucine fractions were combined and recrystallized: (Found: N, 10·5 %. $C_6H_{13}O_2N$ requires N, 10·7 %. $[\alpha]_D$ in 5 % HCl $= +15·2°$.) The value of the optical rotation indicates that *iso*leucine was almost absent.

Basic fraction. Potato extract (4500 ml.) was treated with lead acetate as above described. Lead was removed in the usual manner and the solution after concentration to 1500 ml. was treated with *d*-tartaric acid to remove potassium. The potassium hydrogen tartrate precipitate contained practically no N. Excess of phosphotungstic acid was added after the solution had been concentrated. After 2 days at 0° the precipitate was filtered off and decomposed with baryta. Precipitation with phosphotungstic acid and decomposition with baryta were repeated in order to remove non-basic amino-acids. Silver sulphate was added at *p*H 3 and the precipitate was centrifuged and decomposed by 0·1 *N* HCl in 2 % NaCl. This fraction, containing purines and no amino-acids (negative ninhydrin reaction), was worked up as described by Hoppe-Seyler & Thierfelder [1924]. Only adenine (47 mg.) was obtained in the form of its picrate of M.P. 282°; this was converted into the hydrochloride and the free base which was analysed. (Found: N, 51·3 %. $C_5H_5N_5$ requires N, 51·85 %.) The main solution was freed from silver and arginine was estimated by the method of Vickery [1940]; 1·32 g. of arginine monoflavianate were obtained corresponding to 0·471 g. of arginine; colorimetric estimation by the method of Jorpes & Thorén [1932] gave a value of 0·571 g. from which it appears likely that small amounts of other monosubstituted guanidines may have been present. The mother liquors from the arginine flavianate preparation were combined and flavianic acid was removed. Precipitation with silver and baryta yielded a small precipitate which, on decomposition, gave no Pauly reaction and contained very little N. Another experiment where the preliminary removal of arginine by flavianic acid was omitted and the Kossel-Vickery procedure was followed also gave no tests for histidine. Silver and baryta were now removed. A very weak ninhydrin reaction indicated that very little or no lysine was present. Further fractionation according to Schulze yielded 0·20 g. of trigonelline and 0·4 g. of choline. We have been unable to isolate cadaverine which Yoshimura [1934] claimed to have isolated from potatoes.

Isolation of choline. Freshly prepared ammonium reineckate (2 g.) was added to

1000 ml. of potato extract which was freed from protein and had been brought to *p*H 13·0. The precipitate was left overnight in the dark at 0° and then collected, dissolved in the funnel with 50% acetone and recrystallized from water. The precipitate which now had the typical lustre of choline reineckate weighed 395 mg. corresponding to 97 mg. of choline. The reineckate was decomposed with silver sulphate and baryta, and the solution was evaporated to dryness after acidification with HCl. The residue was extracted with alcohol and excess chloroplatinic acid was added. The chloroplatinate was recrystallized from aqueous alcohol and weighed 160 mg. It had M.P. 241° and analysed correctly for choline chloroplatinate. (Found: N, 4·5%. $C_{10}H_{28}O_2N_2Cl_6Pt$ requires N, 4·54%.)

PART III

NITROGEN DISTRIBUTION IN DIFFERENT VARIETIES AND IN DIFFERENT LAYERS OF THE POTATO TUBER

Typical results for eleven samples of potatoes obtained through the generous assistance of Mrs McDermott of the Midland Agricultural College are recorded in Tables 4 and 5. The potatoes were grown on river gravel (medium loam) and manured with farmyard manure and I.C.I. fertilizer no. 5. The samples consisted of 15–20 tubers of different sizes which had been carefully cleaned but not peeled. The differences in total N on a dry weight basis are very considerable, but are less marked if calculated in terms of fresh weight or fresh weight minus starch and fibre. The relative proportions of insoluble N

Table 4. *Dry matter, total N and protein-N in different varieties of potato*

Variety	Dry matter %	Total N % of dry weight	Total N % fresh weight	Protein-N % of total N	Protein % of fresh weight
Majestic 1	17·83	1·695	0·307	39·7	0·738
King Edward 1	19·67	1·825	0·359	44·5	0·992
Golden Wonder	21·96	1·387	0·3045	63·7	1·20
Doone Star	21·5	1·51	0·339	50·7	1·07
Great Scot	18·9	1·613	0·303	48	0·899
Redskin	16·37	1·949	0·319	37·5	0·694
Kerr's Pink	16·82	1·479	0·238	58	0·856
British Queen	19·15	1·59	0·304	44·5	0·837
Dunbar Standard	20·37	1·159	0·236	44·9	0·657
Arran Banner	18·99	1·652	0·314	37	0·719

Table 5. *Distribution of soluble N in different varieties of potato*

Variety	Protein-N	Asparagine-N	Glutamine-N	$\frac{\text{Glutamine}}{\text{Asparagine}}$ ratio	α-Amino-acid-N	Basic N
Majestic 1	30·2	25·5	14·5	0·51	9·4	23·0
Majestic 2	28·5	27·0	14·8	0·55	11·5	21·0
Majestic 3	29·5	26·0	15·1	0·58	10·8	22·1
King Edward 1	34·5	16·0	17·5	1·1	12·8	23·9
King Edward 2	35·5	15·8	18	1·16	12·7	22·1
King Edward 3	36·0	18	16·8	0·93	—	—
Golden Wonder	51·2	11·8	7·6	0·64	7·3	23·7
Doone Star	39·7	17·7	12·6	0·71	6·4	26·7
Great Scot	37·1	19·0	12·4	0·65	10·5	27·7
Redskin	28·0	22·4	19·7	0·86	—	—
Kerr's Pink	46·7	13·9	8·7	0·63	—	—
British Queen	43·8	19·6	13·3	0·68	—	—
Dunbar Standard	34·7	20·4	12·8	0·60	9·5	20·4
Arran Banner	32·2	21·5	14·7	0·68	—	—

The figures represent % of total N.

The samples of varieties Majestic and King Edward 2 and 3 were grown near Cambridge, whilst all others were grown at Sutton Bonnington.

varied between about 8 and 12·5 % of the total N and averaged about 0·33 g./1000 g. of fresh potatoes. In the soluble fraction the relative proportions of protein varied approximately between 25 and 50 %; samples having a high soluble protein content also seem to have a high content of insoluble N. The total protein content in Table 4 is calculated on the assumption that the insoluble nitrogenous material is protein and that the average N content of the protein is 16·1 %. Samples having a high relative protein content are low in amides and *vice versa* so that the sum of protein and amides is fairly constant, i.e. about 70 %. N due to α-amino-acids other than amides was found in only small amounts; it varied between 6 and 12 %.

It cannot be claimed that the values found for these samples necessarily represent entirely varietal characteristics. The potatoes, although grown under the same environmental conditions, may have been lifted at different stages of maturity; moreover the experiment was not sufficiently controlled for definite conclusions to be drawn. It does seem however that variety is an important factor in determining the N distribution of the tuber. Thus samples of varieties Majestic and King Edward grown in the fens, near Cambridge, and at Sutton Bonnington gave analyses which differed but little; all Majestic had much smaller relative protein values than any King Edwards examined. More striking however is the difference in the glutamine-asparagine ratios, which fell in variety Majestic between 0·55 and 0·65, whilst in variety King Edward it was always either close to or slightly above 1. It appears probable therefore that certain chemical estimations such as the estimation of the relative content of soluble protein and the determination of the glutamine-asparagine ratio can be used to characterize varieties. It may also be possible to select certain varieties having a high protein content for particular purposes such as animal feeding.

N distribution in the different layers of the potato

When potatoes are peeled and cooked a fairly large proportion of the outer layers is discarded. It was therefore considered interesting to examine how the different nitrogenous fractions are distributed over the tuber. If a potato is cut longitudinally three different parts can clearly be distinguished. Apart from the pigmented skin the cortex is divided from the pith or medulla by the bundle zone, which reaches the skin near the eyes. It has been known for some time that the water content is highest in the skin, decreases sharply in the cortex and reaches a second maximum in the inner medulla, whilst starch shows the reverse behaviour, i.e. it is highest in the inner cortex or outer medulla [Glynne & Jackson, 1919]. Not very much is known about the distribution of the different nitrogenous fractions; Rathsack [1935] states that soluble N and amino-N behave similarly to total N, whilst Cook [1921] found more insoluble N in the skin than in other parts of the tuber.

Two potato varieties, Majestic and King Edward, were used for these experiments (Tables 6 and 7). Ten tubers of different size were carefully washed and scraped and dissected into three or four parts respectively, which were weighed separately for each tuber. The skin amounted to 1·5–5 % of the total fresh weight, according to the size of the tuber. The cortex was about 35–45 % of the fresh weight. The division between outer and inner medullas was arbitrary. Samples of each layer were pooled, dried and ground as described in Part I. Table 6 shows that the distribution of the different nitrogenous fractions varies considerably in the different layers. Total N is high in the peel, decreases sharply in the cortex and rises again towards the pith, thus confirming the earlier work. The high N content of the peel is due to its high contents of insoluble nitrogenous matter and of asparagine. The insoluble N is mainly concentrated in the outer layers and falls nearly to zero towards the pith. The soluble protein, amino-acid-N and basic N are not quite evenly distributed throughout the tuber but the differences seem insignificant, particularly since the distribution of soluble protein varies in the two samples investigated.

In the skin the concentrations of all these constituents appear to be low. Very striking is the unequal distribution of glutamine and asparagine over the tuber. The cortex and peel store most of their amide in the form of asparagine, whilst glutamine is mainly found in the medulla. This unequal distribution may be interpreted as an indication of local differences in biological function of the various parts of the tuber and may be accompanied by similar differences in the distribution of sugars and organic acids.

The results show clearly that the N distribution varies considerably in different varieties and it is therefore very likely that their nutritive values will vary accordingly. An accurate assessment of the biological values of the different fractions on the basis of purely chemical data is however impossible; for instance nothing is known about the availability of the insoluble nitrogenous material to animals. Amides too, although not related to essential amino-acids, may be of some value in supplementing the N of the protein. It may also be mentioned that in cooked vegetables practically all the glutamine will be converted into pyrrolidone carboxylic acid which, if ingested in large amounts, may be excreted unchanged [Bethke & Steenbock, 1923]. The experiments on local distribution of N in the tuber indicate that a fairly large proportion of the total N is lost on peeling, although its biological value is uncertain.

Table 6. *N distribution in different layers of variety Majestic*

	Peel	Cortex	Outer medullary	Inner medullary
Fresh weight of each layer as % of total fresh weight	5·88	33·01	35·39	25·67
Dry weight of layer as % of its fresh weight	15·0	21·7	22·2	17·2
Total N	0·531	0·33	0·373	0·380
Soluble N	0·405	0·297	0·351	0·3705
Insoluble N	0·126	0·033	0·022	0·0095
Soluble protein-N	0·0626	0·096	0·104	0·083
Total protein-N	0·1886	0·122	0·126	0·107
Soluble non-protein-N	0·343	0·205	0·244	0·286
Total amide-N	0·237	0·101	0·137	0·179
Asparagine-N	0·1786	0·0699	0·085	0·1092
Glutamine-N	0·0587	0·027	0·0533	0·060
α-Amino-acid-N (less amides)	0·0182	0·040	0·0449	0·0396
Basic N	0·0634	0·0734	0·0832	0·0670
$\frac{\text{Glutamine}}{\text{Asparagine}}$ ratio	0·326	0·376	0·639	0·641

The N values are expressed as % of the total fresh weight of the layer.

Table 7. *Distribution of soluble N in King Edward potatoes*

	Peel	Cortex	Medulla
Protein-N	39 (15·4)	29 (32·4)	47 (26·7)
Asparagine-N	31·9 (44·1)	21 (24·2)	16·5 (26·5)
Glutamine-N	16·6 (14·5)	10·8 (9·1)	20·5 (16·3)
$\frac{\text{Glutamine}}{\text{Asparagine}}$ ratio	0·52 (0·326)	0·51 (0·376)	1·24 (0·640)
Total N as % of fresh weight	0·465 (0·531)	0·298 (0·33)	0·384 (0·375)

The values for soluble N are expressed as % of total soluble N.
The figures in brackets represent the corresponding figures for Majestic potatoes.

Summary

1. Methods are described for the estimation of different types of nitrogenous substances in plant extracts. It is shown particularly that the ninhydrin method is more reliable than the nitrous acid method for the estimation of α-amino-acid-N. A new method for the estimation of glutamine is proposed depending on the loss of carboxyl groups reacting with ninhydrin on heating.

2. A number of nitrogenous compounds have been isolated from potatoes such as asparagine, glutamine and arginine; these were shown to be present in fairly large amounts. Histidine, lysine and cystine appear to be absent. Choline was isolated and estimated by precipitation as reineckate in strongly alkaline solution.

3. Analyses are presented of the N contents and distributions in a number of domestic varieties and it is shown that varieties differ considerably in their protein and amide contents.

Thus the figures for total N were between 0·238 and 0·359% of the fresh weights and the relative proportion of protein varied even more. While Majestic potatoes contained about 29·5% of their total N in the form of protein and about 40% as amides, Golden Wonder potatoes had 51·2% of protein and 19·4% of amides.

4. It is also demonstrated that the nitrogenous compounds are not equally distributed over the tuber. Thus the insoluble nitrogenous material is mainly present in the skin and cortex. Glutamine is mainly stored in the inner layers, whilst the outer layers are richer in asparagine. The bearing of these results on the biological value of the potato N is briefly discussed.

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