POTATO PROTEINS

THEIR PROPERTIES AND NUTRITIVE VALUE

Dit proefschrift met stellingen van

ALY IBRAHIM LABIB

geboren te Giza/Egypte, 8 april 1929, is goedgekeurd door de promotor Dr. C. den Hartog, hoogleraar in de leer van de voeding en de voedselbereiding.

De Rector Magnificus
der Landbouwhogeschool

W. F. Eijsvoogel.

Wageningen, 11 april 1962.

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(MET EEN SAMENVATTING IN HET NEDERLANDS)

ملخص باللغة العربيـــــة

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS
IR. W. F. EIJSVOOGEL
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE
BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 8 JUNI 1962 TE 16.00 UUR

DOOR

A. I. LABIB

DRUKKERIJ GEBR. VERWEIJ – WAGENINGEN – 1962

THEOREMS

I

In determining the nutritive value of nitrogenous substances of potato tuber, too less attention has been paid to the supplementary effect of the free amino acids.

II

The microbiological assay for the determination of amino acids is preferred to other methods in the routine work.

III

In countries where protein malnutrition (kwashiorkor) is of frequent occurrence among children, animal proteins are usually found to be deficient in the diet. It would be economical to compose balanced diets, the proteins of which can be mainly drawn from the available plant sources.

IV

The term "Nitrogen Retained" as used in the literature indicates:

$$N - (U - E) - (F - Fm)$$

does not give the right meaning of the formula. In this respect it would be suggested to use the term "Nitrogen gained".

N = nitrogen intake.
U = urine nitrogen.
E = endogenous nitrogen.
F = faecal nitrogen.
Fm = metabolic faecal nitrogen.

V

The costs of food spoiled in two years in an Egyptian middleclass house will amount to the price of a small refrigerator.

Thesis, A. I. Labib
Wageningen, The Netherlands,
June 1962

VI

The opinion of Kodicek and coworkers that tortillas - a product which results after treating maize with lime and baking the masa - have a superior effect on rats and pigs to maize, only because the nicotinic acid becomes free, is subject to discussion.

KODICEK, *et al.*, (1959). Brit. J. Nutrition **13**, 363.
—, and WILSON, (1959). Ibid. **13**, 418.

VII

The electrophoretic properties of potato proteins offered the possibility of developing a method for variety identification which is quicker than "forced sprouting by diffused light" method.

VIII

The unavailability of the essential amino acids has to be taken into consideration when the chemical composition of the ingredients is used for making up the formulated feeds for pigs and poultry.

IX

Basic objections can be raised to Böttcher's opinion, that his findings concerning the lipid composition of the arterial plaques in atherosclerosis can be regarded as a warning against the consumption of foods rich in linoleate.

BÖTTCHER, (1961). Voeding **22**, 409.

X

It is thought that if the large sums of money and the huge collective efforts made for building up the atomic destructive weapons, would be devoted to the fight against such fatal diseases as cancer, great and rapid results will be obtained.

POTATO PROTEINS

THEIR PROPERTIES AND NUTRITIVE VALUE

THESIS

IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF
AGRARIAN SCIENCES
AT THE AGRICULTURAL UNIVERSITY OF
WAGENINGEN, THE NETHERLANDS
ON FRIDAY 8th JUNE 1962 AT 16 O'CLOCK

BY

A. I. LABIB
M.Sc., CAIRO UNIVERSITY

FROM THE

NETHERLAND INSTITUTE OF NUTRITION
(Nederlands Instituut voor Volksvoeding)

AND THE

INSTITUTE FOR STORAGE AND PROCESSING OF AGRICULTURAL PRODUCTS
(Instituut voor Bewaring en Verwerking van Landbouwprodukten)

PROMOTOR: PROF. DR. C. DEN HARTOG

To my Parents

ACKNOWLEDGMENT

This investigation is a part of a cooparative study accomplished by the Netherland Institute Of Nutrition, Wageningen, and the Institute For Storage And Processing of Agricultural Products, Wageningen. I shall always remember the agreeable contact between these two institutions as a luminous example of cooperative efficiency.

First of all I wish to express my gratitude to my promotor, Dr. C. den Hartog, Professor of Nutrition at the State Agricultural University and Director of the Netherland Institute Of Nutrition where the present investigation was carried out. The freedom he gave me during my work, his supervision, valuable suggestions, interest and criticisms were a great support to me.

I shall never forget the valuable advice, assistance, and guidance offered to me by Dr. G. Pol, Acting Director of the Netherland Institute Of Nutrition. His enthusiasm and stimulating criticisms were highly appreciated.

The discussions with Professor Dr. E. G. Mulder, State Agricultural University, Wageningen, on some physiological aspects in the potato tuber, and the interest he showed in this respect have been very much appreciated.

I am much obliged to Prof. Dr. H. K. Oosterhuis, of the Amsterdam University for his advice on the paper electrophoresis techniques, as well as to Dr. T. A. J. Payens, Netherland Institute For Dairy Products, Ede, (NIZO), who was so kind as to carry out the experiment of zone-electrophoresis on potato sap discussed in this text.

I want to offer my special obligations to Miss H. Bosveld, and Miss A. Huizinga for their help in some experimental work in the laboratory.

Grateful acknowledgment and thanks are also due to the Department of Home Economics in the State Agricultural University, Wageningen, for providing some technical facilities and for executing the drawings in this text.

Finally I want to thank Mr. A. Spoorenberg for correcting the English text.

CONTENTS

CHAPTER ONE

INTRODUCTION

Proteins are important components in the living material. The simplest form of life exists in viruses which mainly consist of proteins and nucleoproteins. Reproduction and growth are two functions of life in which proteins are of primary importance. Life is also dependent on a complicated system of biochemical reactions catalyzed by enzymes. Enzymes, which are proteins, are specialized to activate specific reactions in the living cell. A number of hormones of the ductless glands are proteins specialized to a definite function. Toxins of bacteria and antibodies are also proteins.

Although the kingdom of proteins comprises a large number of varieties, functions and specifications, it seems that they are built up of no more than 25 amino acids. The arrangement and the pattern of these amino acids in each protein will determine the differences, activities and functions of each one.

Plants have the capacity of utilizing mineral nitrogen to build up amino acids and consequently proteins. Animals lack this power. They must be provided with food protein to satisfy their amino acid requirements. In the animal the proteins provided in the diet are decomposed to their amino acids. The animal body makes use of these amino acids for building up its enormous number of proteins. Many amino acids, in the animal body, can be synthesized from other amino acids. There are, however, some amino acids, called indispensable or essential amino acids, which cannot be synthesized in the body. In nutrition therefore the protein quality is determined by its amino-acid pattern.

It is obvious that the plant is the factory of proteins, and as proteins are essential to animal, studies on plant proteins are of primary importance in nutrition.

Most proteins in plants, however, are found in mixtures and not in a single protein fraction. The evaluation of such protein mixtures will give a general idea and not a specific one. It is possible that one or two proteins in a plant has an inferior nutritional value, which may influence the entire picture and give a low protein quality for the mixture. Protein separation studies, therefore, are of great value because they may help to reveal the quality of each separate protein.

From this it follows that physical and biochemical studies on plant proteins, may serve as a basic start to obtain a better understanding of plant proteins and their value in nutrition.

As early as 200 A. D. the potato, *Solanum tuberosum,* was cultivated in some mountainous regions in Peru, where it served the Indians as food source. By the

16th century potatoes were introduced into European countries, (HAMILTON 1934). In the eighteenth and nineteenth centuries, potatoes were regarded as principal source of food in Europe (TALBURT and SMITH 1959). From the Food Balance Sheets, published by the Food and Agriculture Organization of the United Nations 1949 and 1950, it is shown that the average annual consumption of potatoes per capita in European countries (excluding the Soviet Union) ranged from 628 lbs. in Poland to 372 lbs. in Belgium in the years 1934 to 1938. In the years 1948-1949 the figure of Poland dropped to 528 lbs., while Belgium still had the lowest consumption, viz. 311 lbs. From this it may be concluded that the consumption of potatoes is decreasing. Yet it is still one of the foods most consumed in Europe, notwithstanding the fact that due to the improvement of means of transport many other kinds of food were introduced. After World War II this introduction of new foodstuffs was more pronounced in Europe which may be attributed to a more intensified contact with subtropical and tropical countries. Another cause of the decrease in the consumption may be the considerable improvement in the preservation of potatoes resulting in minimizing the spoilage, which might not be the case in the years 1934-1938. It should not, however, be forgotten that although the consumption of potatoes showed an apparent decrease, the importance of this product in the field of nutrition is still very great.

In many countries the starch industry is dependent on potatoes. Until recently, the washings produced during the extraction process of potato starch were wasted. It has been shown that from 70 to 90 % of the potato proteins are soluble in water. In other words, the washings that had so far been discarded contained a good quantity of proteins. These proteins can be precipitated and used in animal or even in human nutrition.

Many workers have already shown a great interest for potato as a research subject, but most of them have carried out investigations on starch, sugars, vitamine C and the cooking quality.

The study of potato proteins goes back to EINHOF (1805) who compared the chemical composition of the tuber with that of various cereals and pulses. The nitrogenous substances in potato were investigated during the end of the nineteenth century and in the early years of this century. SCHULZE and BARBIERI (1880), SCHULZE and EUGSTER (1882) and SCHULZE (1904), could demonstrate the amino acids, leucine, tyrosine, arginine, lysine and histidine in the potato tuber. OSBORNE and CAMPBELL (1896) gave the protein fraction of potato the name of tuberine. SJOLLEMA and RINKES (1912) investigated the amino acids in potato, while ROSE and COOPER (1917) studied the nutritional value of potato proteins. KEISEL *et al.* (1934) investigated the protein in the potato leaf and that of the tuber. STEWARD after 1940, nearly made a school investigating the nitrogenous substances and its relation to the respiratory system of the potato. GROOT *et al.* (1947), could separate two fractions of the protein complex found in the potato. MULDER and BAKEMA (1956) studied the effect of fertilization on the nitrogenous substances of potato.

As in most of the storage organs of different plants, the potato tuber also shows a high percentage of non protein nitrogen components. The percentage of protein nitrogen in the potato tuber as compared with the total nitrogen, is generally higher than in storage organs of other plants, turnip for instance. The non protein nitrogen, forms about 50 % of the total nitrogen in potato.

The amino acid studies carried out on potatoes, were mostly devoted to the whole tuber, which will give a crude idea of the influence that different circumstances have on the nitrogenous substances in the tuber.

Some studies were carried out on one of the two fractions namely protein and non-protein nitrogen fractions.

THOMPSON and STEWARD (1952) and MULDER and BAKEMA (1956) studied the amino-acid pattern in protein and non-protein fractions in potatoes. Yet what happens to the nitrogenous substances in potato during storage is still a problem of which little is known. Storage is an important aspect in potato production, since potatoes are not immediately eaten after harvesting, but during a period of six months or more after collecting them from the field. It is believed that during and after the dormant period many biochemical reactions are taking place in the tuber, which may change the composition of the potato.

THE SCOPE OF THIS STUDY

As an approach to the study of potato proteins, some of their physical properties were investigated. The migration of potato proteins in an electrical field is of primary importance, because it will give an idea of the number of components the worker is dealing with if a protein separation study would be carried out.

In the second place the ten essential amino acids were studied in protein nitrogen and non-protein nitrogen fractions. This is of importance for a better understanding of the nutritional value of the potato nitrogenous substances.

Further, a study was made on the biological value of the nitrogenous substances, where a microbiological method was employed.

The study included the effects of nitrogen fertilization, and storage time and temperature, on the protein and non-protein fractions, their amino-acid pattern and their nutritional value, as well as these effects on the different protein components of potato tuber exploited during this investigation.

CHAPTER TWO

THE ELECTROPHORETIC PROPERTIES OF POTATO PROTEINS

I. INTRODUCTION

The proteins of potato were believed to be a mixture of two proteins, namely tuberin and tuberinin.

The idea put forward by OSBORNE and CAMPBELL (1896) implied that the protein of potato was mainly a globulin named tuberin. KIESEL *et al.* (1934) studied the protein-amino-acid composition of the potato leaf as well as that of the tuber. He came to the conclusion that the protein present in the tuber was one single protein, although he obtained two fractions, one of which was soluble in water, whereas the other proved to be insoluble. He drew his conclusions on the basis that the two fractions had the same amino-acid-pattern.

Only after more than two decades was this subject approached again by GROOT *et al.* (1947). They reported that when using zone electrophoretic techniques, two peaks representing two different components, could be detected in the potato tuber sap, from which they concluded that in the potato tuber two proteins were present in the proportion 70 : 30, these were given the names tuberin and tuberinin.

CHICK and SLACK (1949), claimed that they could detect at least two proteins in potato sap from which starch had been largely removed. The first component could be separated at pH 4 with gentle warming at 30° C, the second was obtained from the first supernatant by boiling. The proportion of the two proteins was 30 : 70 and became 65 : 35 after the potato was stored for one year.

A year before Groot's discovery of the two proteins, a study on the colloidal properties of the potato proteins had been carried out by JIRGENSONS (1946). The latter succeeded in obtaining three fractions from the tuber protein. He described the first as a casein-like protein, difficult to dissolve, the second as an albumin-like protein and the third as a globulin. In changing the techniques he could obtain other fractions showing different properties. From this he concluded that the different components might be artifacts due to the conditions of fractionation.

HOFSTEE (1949) agreed to Groot's conclusions. In the study he made he could obtain two fractions with the ratio of 70 : 30.

The publications dealing with the subject are scarce and the methods and techniques that were employed in the literature cited above are rather limited as well.

Several methods are available for protein separation. The precipitation of proteins is one of the oldest procedures that up to this day is still successfully used. The precipitating techniques include: salting-out, pH changing, precipitation with a heavy metal, coagulation with heat or organic solvents. If a system of salting-out is applied, repeated precipitations by means of different salt concentrations will be needed. In the same technique other reagents such as alcohol or acetone under special conditions are also used. Any fraction thus obtained may comprise a mixture of proteins or it may entirely consist of only a single protein. For this reason, a special technique has to be applied in order to determine whether the fraction obtained is composed of one or more components. The measuring of isoelectric point or the molecular weight in this case need not to be considered as conclusive.

In the literature cited only GROOT *et al.* (1947) tried a definite technique namely free electrophoresis. In their study they only worked with a pH of 7.2. This pH value is in good agreement with normal conditions, but it is reasonable to assume that it might not represent the optimum requirements for electrophoretical separation. It is, however, of importance to know more details about electrophoretic separation when carried out under optimal conditions.

The method chosen here for the extension of the work of GROOT *et al.* (1947) was paper-electrophoresis. The scientific base of paper-electrophoresis is the same as that of zone-electrophoresis. However, the technique of free-electrophoresis offers the possibility of seeing and photographing the separation directly in the field, whereas in paper electrophoresis, the proteins have to be coloured and the intensity of the colour has to be measured.
The present investigation was planned to give information about:

1. The electrophoretic properties of the potato proteins.
2. The relation between different potato varieties and their different protein-fractions.
3. The effect of storage on the potato-protein-pattern.
4. Potato-protein-pattern as influenced by nitrogen fertilization.

II. MATERIALS AND SAMPLING

A. MATERIALS

Four varieties of potatoes were included in this study, viz., Bintje, Alfa, Eigenheimer and Kwinta, to investigate the effect of nitrogen fertilization and storage on the potato proteins.

1. Nitrogen fertilization:

The nitrogen fertilization experiment was carried out at the experimental farm "Dr. H. J. Lovink Hoeve" at Marknesse, North East Polder, the Netherlands. The variety used was Bintje. In the experiment six nitrogen levels were involved in double blocks. Nitrogen was supplied in the form of calcium ammonium nitrate and the levels per hectare were 0, 40, 80, 120, 160 and 200 kilograms. The nitrogen treatments were arranged in randomized blocks according to the following sketch:

200	120	40	0	160	80
0	80	160	200	40	120

The crop was collected by hand on the seventeenth of September 1960 and was then transported to Wageningen.

2. Storage:

Three varieties - Alfa, Eigenheimer and Kwinta - were included in this investigation. They were planted in Zeeland, a South-Western Province of the Netherlands. Owing to the high rainfall in the months of September, October and November 1960, it was impossible to harvest in time. The crop had to remain in the fields until the fifteenth of November when it was collected by hand.

B. SAMPLING

The sampling was carried out in two stages. Firstly, representative samples had been drawn from the crop, and secondly a homogeneous part was removed from every sample, for chemical analyses.

1. Sampling the crop:

The crop was transported from the fields to the Institute for Storage and Processing of Agricultural Products (Instituut voor Bewaring en Verwerking van Landbouwprodukten I.B.V.L.), Wageningen. The potatoes were washed to remove clay and sand. Then they were sorted according to the following diameter:

> 60 m.m.
55 — 60 m.m.
50 — 55 m.m.
45 — 50 m.m.
40 — 45 m.m.
35 — 40 m.m.
< 35 m.m.

Both the first fraction, (> 60 m.m.) and the last (< 35 m.m.) were excluded, because only small quantities were available. The five other fractions were equally divided (per weight and count) over a certain number of samples. Each sample was kept in a small sack and weighed about 3 kilograms. All the samples were treated in the way mentioned above.

Samples of the fertilizing experiment were stored at 5° C, whereas for studying the effect of storage they were kept at 2, 6, 10 and 15 ° C. The dates of sampling are summarized in table 1.

TABLE 1. The dates of sample analyses for paper electrophoresis.

Treatment	Date
Nitrogen fertilization (kept at 5° C), var. Bintje	6th March 1961
Storage experiment kept at 2, 6, 10 and 15° C, var. Alfa, Eigenheimer, and Kwinta	19th December 1960 13th February 1961 10th April 1961 5th June 1961

2. Sampling in the laboratory:

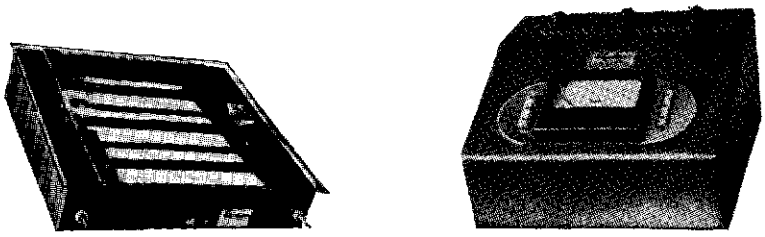
Each tuber in the sack was divided into two parts along the axis of symmetry. A longitudinal section was then removed from the cut parts. All the tubers in the sack were used. The segments collected had an ultimate weight of about 500 g. The slices were cut into small pieces and minced in a blender after adding a few crystals of sodium sulfite to prevent enzymatical colouring. The final samples were drawn from this mixture using a wide opening pipette. For electrophoresis, minced potatoes were forced through an asbestos filter (Carlson K 5) by the application of vacuum. The almost clear potato sap acquired by filtration contained about 1—1.5 % protein, and was directly used for electrophoresis.

III. METHODS

A. APPARATUS

The apparatus used during this study was that of "Elphor Drucktasten Speisegerät" for a closed horizontal system of paper electrophoresis. It is composed of three baths and an electric-feeding unit. Each bath is provided with two electrodes, which can be connected to the feeding unit. Six filter paper strips each measuring 30 x 4 cm, can be hung on three strip holders in each bath. The electric-feeding unit can supply the three baths at one time with 120, 220 or 400 volts. Each

bath contains a tubing system to bring the buffer solution in equal levels into the two chambers of the bath. Because the system used is a closed one, each bath is provided with a slanting glass cover, the slope of which prevents drops of water from falling on the paper. Figure 1 shows a photograph of the different parts of the apparatus.



a b

FIGURE 1. The "Elphor Drucktasten Speisegerät" apparatus: a. an electrophoretic bath, wherein six hanged paper strips can be seen; b. the electric Feeding unit, that can supply the electric current for three baths.

The intensities of the colours of proteins on the strips were measured and recorded by the "Extinction Recorder II" (C. Zeiss, Oberkochen, Würtemberg, Germany) shown in figure 2. The apparatus works on a two-beam compensation system. The filter used was FE 60. Measuring a strip requires about two minutes.

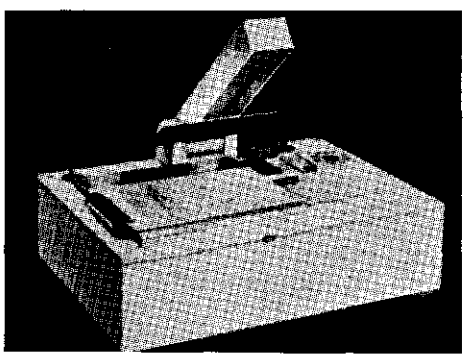


FIGURE 2. The "extinction Recorder II" C. Zeiss.

B. SOLUTIONS

After preliminary studies, which will be treated under "IV. Results and Discussion", the following solutions were chosen.

1. The Buffer

The buffer used was veronal at pH 8.6, with an ionic strength of 0.05. It was composed of:

1.84 g barbital
10.30 g sodium barbital,
made up to one litre with distilled water. The pH of such a solution ranged between 8.55 and 8.65 (BLACK, DURRUM and ZWEIG, 1958).

2. Dyeing solution

Use was made of a saturated solution of amido black 10 in a solution of methanol and acetic acid 90 : 10 (GRASSMANN and HANNIG, 1950).

3. Washing solutions

Methanol acetic acid 90 : 10 v/v (GRASSMANN and HANNIG, 1950).
One percent acetic acid solution in water (OOSTERHUIS, 1954).

C. PROCEDURE

The following procedure is the result of what has been developed after some preliminary investigations (IV. Results and Discussion page 10).

1. Strips of filter paper (Whatman no 1, in 4 cm rolls Grade no 1, W. & R. Balston Ltd., England) measuring 30 x 4 cm, were cut from the supplied rolls. The strips were marked with a pencil at 11 cm from one of the ends. This mark indicated the point of introduction of the protein solution.

2. The strips were wetted with the buffer solution and blotted between filter paper.

3. They were then fixed to the two edges of the strip holders.

4. By means of a 2.5 cm glass slide, quantities of 0.02—0.03 ml were brought exactly between the two pencil points on the strip.

5. The strip holders were brought into the baths, (where the liquid level of the buffer chambers in the whole bath had been brought into equilibrium). The system was then closed with the glass covers and the electric current was allowed to pass. In this study the use of 400 volts for 5 hrs was required in order to get a sharp separation. The rate of flow of electric current was 15—17 mA/bath, 2.5—3.0 mA/strip or 0.6—0.7 mA/cm paper.

6. After five hours the strips, still hanging on the paper holders, were taken out and allowed to dry overnight at room temperature in an undisturbed corner in the laboratory (OOSTERHUIS, 1954).

7. The strips were cut, 2 cm above, and 10 cm below the point at which the protein was introduced.

8. The dyeing took place in polyethylene baths. Eight strips could be dyed at one time for 12 minutes.

9. The dyed strips were washed, first in methanol-acetic acid baths, till their background became pale blue, afterwards in a two liter beaker filled with 1 % acetic acid in water at 70° C, the solution being continuously stirred. Usually the strips required from 3 to 4 washings in the second step to obtain a rather white background.

10. The strips were measured when wet, by recording their extinction on a paper using "the Extinction Recorder" described before, and were then dried between filter paper and kept for reference.

IV. Results And Discussion

A. THE ELECTROPHORETIC PROPERTIES OF POTATO PROTEINS IN GENERAL

At the beginning of the research in question, the application of a system which would be greatly in agreement with that of Groot *et al.* (1947) was considered. A phosphate buffer has, therefore, been used. In addition barbital and borate buffers were applied. Part of the work was carried out as preliminary experiments and will be briefly discussed.

Phosphate buffer was used, in the studying of the electrophoretic properties of potato protein, at three different pHs, viz. 4.3, 7.2 and 8.6. At a pH of 4.3 the proteins were positively charged, and travelled towards the negatively charged electrode. At this pH, there was a fraction that did not move and another that moved towards the negative pole. At pH 7.2, however, the proteins were negatively charged and travelled for a short distance. Two fractions could be seen. At a pH of 8.6, the phosphate buffer gave no fractionation. Working with phosphate buffers, different voltages and times were applied.

At a pH of 8.6 the potato proteins, using borate buffer did not show any fractionation.

Table 2. The number of bands obtained under different conditions using bartital buffers.

mA/strip	pH 8.0			pH 8.6					pH 9.3			
	Time of operating hrs.			Time of operating hrs.					Time of operating hrs.			
	1.5	3	5	3	5	7	8	12	3	5	7	12
2	3			5								
3		4			6	4	3	NO	3			
3.5			5-6		6							
4.0					6							NO
7.5										3		
5.5											2	

Barbital buffer was used at three different pHs, viz. 8.0, 8.6 and 9.3, the ionic strength of which was adjusted to 0.05. Moreover different amperages and varying operating times were included in the investigation. Table 2 shows a summary of the work.

In the phosphate buffer the two fractions that appeared were not sharp. The general conclusion after experimenting with phosphate buffers was that they were inefficient for this study. With respect to the borate buffer no fractions could be seen. When using the veronal buffer, however, the optimum conditions required for obtaining sharp bands proved to be as follows:

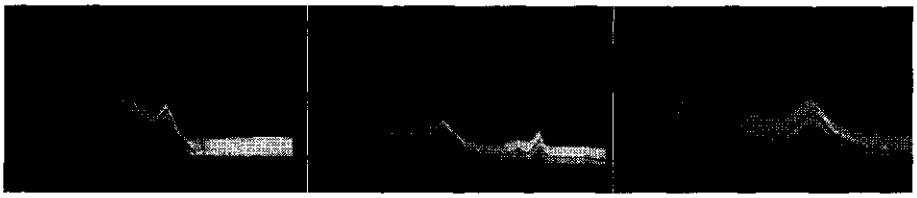
ionic strength 0.05 , pH 8.6
operating time 5 hr and 400 volts.

A description of the method ultimately selected was given under III. Methods page 9.

In order to ensure that the results obtained using this method are not artifacts, the following experiment was carried out. Five ml of the potato sap and 5 ml of the buffer were put in "a kollodiumhülse", a dialysing sac in which liquid samples can be concentrated, (Membranfiltergesellschaft, Göttingen, Germany). The tube was hung in a conical flask, connected with vacuum, filled with the barbital buffer. After 10 hours, the volume was reduced to about 2 ml. The electropherograms of the concentrated dialysed sap showed the same pattern as that of the original sample. The conclusion was then drawn, that the other components which were dialysed out during this procedure had no influence on the separation of the protein into different zones. Also the concentration of the proteins did not influence the pattern. Figure 3 shows a paper strip of a typical separation and its corresponding diagram. It is noteworthy to mention that the system is composed of six fractions. As to the sixth component, it appears that it is a mixture of at least three components.

FIGURE 3. A dyed electrophoretic paper strip and its corresponding diagram showing the six potato protein fractions.

To check the reliability of this conclusion, zone electrophoresis was applied. With the same buffer, it was found that the potato protein, from the dialysed sap, is not a mixture of two components, but rather a mixture of eight. Photographs, showing the migration of the different fractions, were taken at 40, 80 and 120 minutes. The photographs are shown in figure 4, and may be described as follows:



a b c

FIGURE 4. Three photographs taken during the operation time of zone electrophoresis; a. after 40 min., b. after 80 min., and c. after 120 min.

Fig. 4a; is a photograph of the ascending electrophorese taken after 40 min. Three components could be seen.
Fig. 4b; shows the situation after 80 min. The first high peak corresponds to the salts present. This peak is followed by three smaller leading peaks, after which a somewhat higher peak followed by three other peaks can be distinguished.
Fig. 4c; the photograph shows the rising boundary after 120 min. The first four peaks of the previous picture have moved out of the optical system. A fourth peak appeared in between the three flat fractions shown in fig. 4b.

It may be stated that there are at least eight components in potato proteins with different electrophoretic properties. Five of these eight components form sharp and pronounced peaks, while the first three peaks are rather small.
The results obtained by the zone electrophoresis gave a good confirmation to the conclusions of the paper electrophoresis. Both methods gave five sharp components. The 6th fraction in the paper-electrophoresis (the faster travelling components) appeared to consist of three peaks. This is in agreement with the results of the free electrophoresis, where the leading migrants consisted of three small, but well differentiated components.

From the pertinent literature no extension for the preliminary work of GROOT *et al.* (1947) could be traced. Groot's results determine the present knowledge of the tuber protein fractions. When trying to find a sensitive method for separation with paper electrophoresis the conditions used by Groot were also applied in the present work. Although the separation obtained was not sharp, the results of Groot could generally be confirmed. On changing the operating conditions, an absolute proof was found that the potato proteins consisted of more than two fractions.

After carrying out the work published here and drawing the conclusions resulting from it, the writer came through the two following communications. In a short communication SCHWARZE (1953) stated that he could obtain five bands from the potato proteins when using paper electrophoresis. The first four fractions were sharp but the fifth was vague. So far no original article has been published supplying information on the method used by Schwarze nor are there any, dealing with his results. Furthermore, YASUDA, PAYNE and FAULTS (1955) examined the effect of 2.4 dichlorophenoxyacetic acid and malic hydrazid on potato proteins pattern using the paper electrophoresis technique. They stated that "Six probable protein fractions appeared in the electropherograms". They did not illustrate their publication with any photographs of the paper strips, but gave some electropherograms, one of which is presented in figure 5. It is, however, very difficult to distinguish in their obtained electropherograms the six fractions to which they referred.

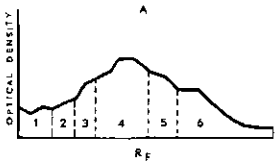


FIGURE 5. Electropherogram obtained by YASUDA *et al.* (1955).

B. THE DIFFERENTIATION BETWEEN THE POTATO VARIETIES

In the course of this study, during which the separated fractions of potato protein were successfully obtained, evidence was found that there existed constant differences between the potato varieties studied.
This was found to hold true even when the potatoes received different storage or nitrogen fertilizer treatments. Although the variations are clearly visible to the eye, an objective method of measuring them is appropriate.

1. Measuring of the graphs

Dificulties were encountered in finding a method suitable for comparing the graphs. Considering that the total graphs are enormous in number and each one is composed of six fractions, a simple and reliable method had to be applied. Some of the methods described in the pertinent literature are:

a. The surface of each component is cut out of the electropherogram and weighed. The weights obtained in this way can be compared (ROULET, OWEN and STEWART, 1956).

b. A comparison between the areas resulting from dividing the graphs by vertical lines at the deepest points between two peaks (WUNDERLY, 1961).

c. The area of each peak can be determined by means of the equation (quoted from WUNDERLY, 1961).

$$J = k.c.a.$$

where J = the area,
k = constant for the bell shaped curve and equals 1.064,
c = the height of the peak from the base line,
a = the width of the peak at half peak height.
For practical purposes, the area can be measured by a. and c. for each peak.

The first two methods were found to be time consuming and lacking in accuracy. The interaction of the peaks is not known and with these two methods it is assumed that all the components of the graph are intercrossing in equal parts. Yet this assumption can only be true when each individual component is found in the same quantity and practice shows that this is more the exception than the rule. The third method however is reported to be an accurate and practical one, yet it has been cited after another method had already been adopted.

From the observations made during this study, it was found that the heights of the peaks in the electropherograms were in constant proportion to each other and characteristic of each sample studied. The results showed a high reproducibility when about the same quantity of protein was brought on the paper. Even without this restriction and when different quantities of proteins are employed, the ratios of the peaks will still hold true.

The nearest shape to the bell curves, is an isosceles triangle, the area of which is determined by:

$$1/2\ b.h$$

where b = the base
and h = perpendicular height.

It is noteworthy that in a triangle the middle width is the same as half the base. So the formula of the triangle will equalize the simplified form of the bell shaped curve described above. Moreover if the base in a series of triangles is supposed to be constant, the only variable will be their heights. Therefore, it is important to know whether the bases of the peaks remain constant for different electropherograms. As the different peaks overlap each other, it is difficult to measure the base of each peak in the grahps obtained, however there are some facts that may lead to the solution. It has been found that:

a. The base of the whole figure is rather constant in all the graphs obtained comprising those of different varieties and treatments.
The average base was 5.13 cm with a standard deviation of 0.1.

b. The tops of the peaks are well localized, it can be noticed in table 3 that the different components migrated over a constant range.

TABLE 3. The range of migration of the first four peaks.

	mm	S.D.
The distance from the origin (starting point of the graph) to the top of peak one	11.6	0.6
The distance between the top of peak one and that of peak two	7.0	0.5
The distance between the top of peak two and that of peak three	5.4	0.6
The distance between the top of peak three and that of peak four	4.1	0.5

S.D.: standard deviation.

When the base of the whole graph is constant and moreover the tops of the peaks are always localized in the same place, it would be irrational to conclude that the bases of each peak in different electropherograms are not constant.

The previous discussion, which is supported by the empirical data, has led to the employment of the peaks' height as a parameter for the interpretation of the different electropherograms. To enable a better comparison the heights of the different peaks will not be expressed as absolute figures, but rather as figures relative to the height of peak number two, which has been taken as 10.

2. The influence of variety on protein pattern, and variety identification from the corresponding electropherograms

The chemical composition of a potato tuber varies from variety to variety as well as within any single variety. Identification of a certain variety from chemical composition would therefore be difficult. Even if the differences between varieties were large, a comparison based on chemical composition would still be poor.

Different varieties of potatoes can be identified by botanical methods. A morphological examination will not be satisfactory. Usually the tuber has to be forced to sprout with special methods, e.g., exposure to light. The variety can be identified by the colour, the length and other properties of the sprouts. This method will take from 5 to 6 weeks and in many cases 8 weeks.

During the course of this study, it has been observed that the protein pattern may furnish a trustworthy and rapid method for identification of potato varieties. To what extent this is true, will be the subject of discussion in the coming paragraphs. The differences between the protein patterns of the four varieties are quite apparent. If, however, a great number of varieties are to be considered, a suitable method for treating the data should be selected. Obviously it will not suffice to state that peak A is higher in variety I than in variety II. This phenomenon will only indicate the differences in varieties. The different systems adopted here will be discussed. If storage or fertilization will affect the specification of a variety, it is impossible to draw any conclusion as to the variety in question. In this study, however, both a storage experiment with different temperatures and storage time, and a nitrogen fertilization experiment were included. Therefore, notwithstanding the different treatments of the four potato

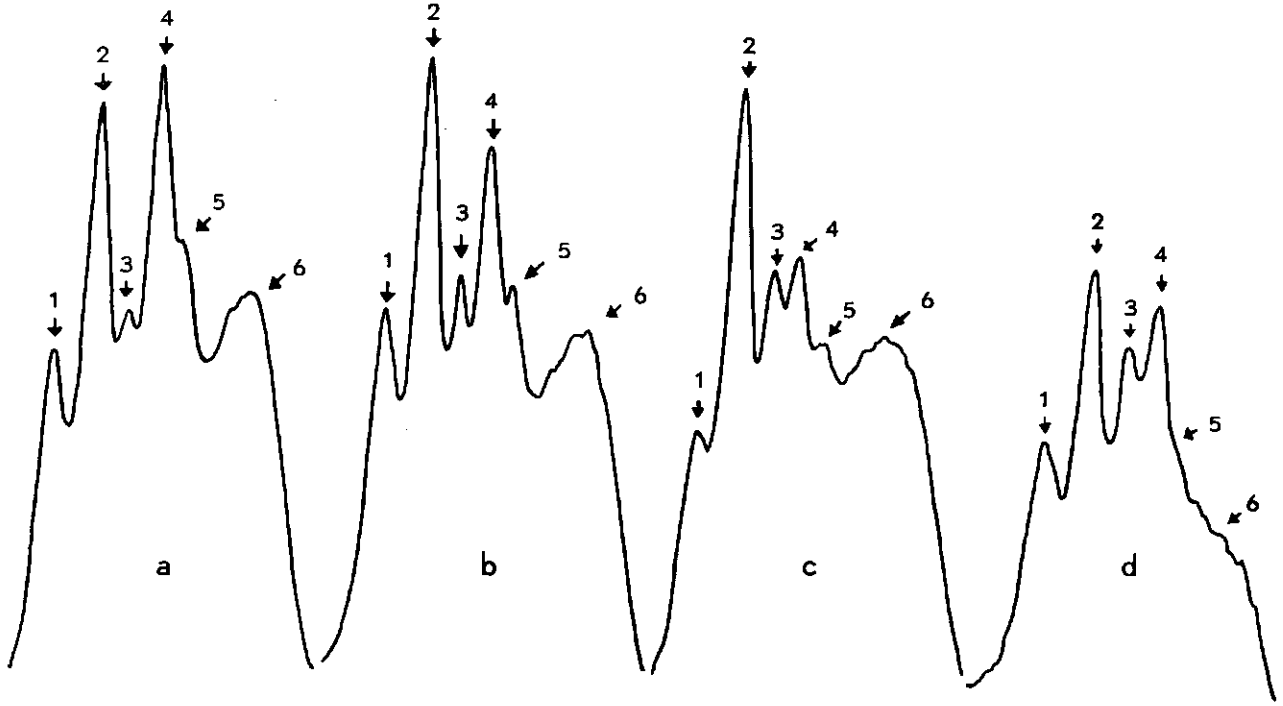


FIGURE 6. The electropherograms of the four varieties studied; a. Alfa, b. Eigenheimer, c. Kwinta, d. Bintje.

varieties, which may present some difficulties, all the data of each variety were taken into consideration.

In other words, other factors than the variety were not eliminated, so that the study will give a true picture under different circumstances. This way of dealing with the data was esteemed to be of great importance especially in a study concerning the identification of varieties. Four ways to treat the data that can be used separately or together for the identification of the four potato varieties are now taken in consideration.

a. The protein pattern:

For an experienced observer it will not be difficult to identify the different varieties. If the four varieties included here are to be distinguished it can be noticed that:

ALFA, figure 6a: In contradiction to all the other varieties the second peak is lower than the fourth.

EIGENHEIMER, figure 6b: The second peak is higher than the fourth, which eliminates Alfa but on the other hand can also be Kwinta or Bintje. The third peak is very low as compared with the fourth, which is not the case with the other two varieties.

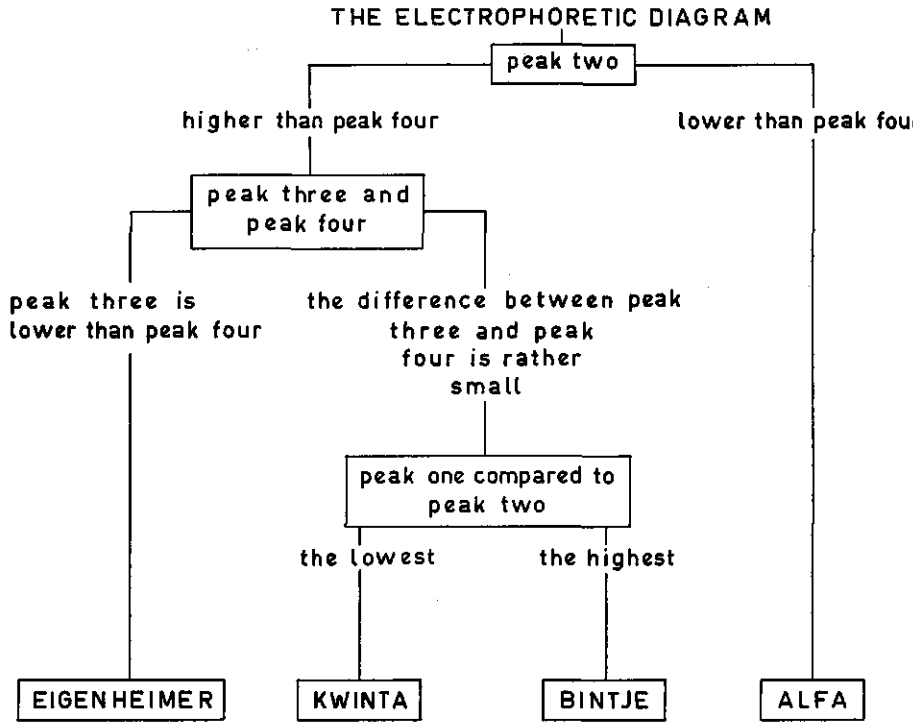


FIGURE 7. A schedule showing a system for identifying the studied potato varieties from their electrophoretic protein patterns.

KWINTA, figure 6c: The second peak is higher than the fourth, this is in contradiction to Alfa. The difference between the third and the fourth peak is rather small, this will discard the variety Eigenheimer. The first peak as compared to the second peak is very low, it is the lowest of the four varieties, while in Bintje it is not the case.

BINTJE, figure 6d: The second peak is higher than the fourth, so it can not be Alfa. The differences between the third and the fourth peak is small, anyhow, it cannot be Eigenheimer. The difference between peak one and peak two is far smaller than in the case of Kwinta.

This system of identification is illustrated in a graphic schedule shown in figure 7.

b. The relative heights of the peaks:

The difference between this method and the one discussed before is that the differences between the varieties were estimated exactly and the data obtained were treated statistically. In this way the discrimination was strengthened. On the other hand, the system of identification will be the same as that described under method one. Table 4 gives the relative values of peaks nos. 1, 2, 3, 4, 5 and 6. It will be noticed that peak 2 is taken as 10. Table 5, shows the significance of the differences between the relative heights as influenced by the variety. The significance of the differences throughout this study has been measured by the WILCOXON test (1945), described by WABEKE and EEDEN (1955).

TABLE 4. The relative heights of the six peaks of the potato protein pattern in the four

Storage		Alfa						Eigenheimer					
Period	temp.	Peak No						Peak No					
		1	2	3	4	5	6	1	2	3	4	5	6
8 weeks	2° C	6.8	10.0	8.0	11.1	9.2	6.6	7.0	10.0	6.4	8.2	6.4	5.5
	6° C	6.3	10.0	6.8	11.6	7.8	6.1	7.5	10.0	6.8	9.7	7.8	5.6
	10° C	7.5	10.0	8.3	11.8	9.7	7.3	8.0	10.0	6.7	9.7	8.0	5.6
	15° C	7.4	10.0	8.0	13.0	9.4	7.4	8.5	10.0	6.3	9.2	7.0	5.9
16 weeks	2° C	8.2	10.0	7.3	10.4	7.8	5.4	8.2	10.0	5.9	7.9	5.1	4.9
	6° C	8.0	10.0	6.6	10.1	7.5	8.3	6.7	10.0	6.5	8.1	6.2	6.1
	10° C	7.9	10.0	7.2	10.1	7.7	6.7	6.1	10.0	7.3	9.4	6.8	6.5
	15° C	6.0	10.0	7,2	10.8	8.2	6.7	6.0	10.0	6.5	8.4	6.3	6.1
24 weeks	2° C	6.6	10.0	8.6	10.5	—	—	6.4	10.0	7.2	8.8	6.0	5.3
	6° C	5.7	10.0	8.2	11.3	8.4	6.0	6.0	10.0	7.2	8.8	5.4	4.3
	10° C	3.7	10.0	8.3	12.6	8.4	4.7	3.9	10.0	7.6	9.7	5.4	3.7
	15° C	4.2	10.0	9.0	12.0	9.1	4.6	3.6	10.0	8.4	9.5	7.8	5.6

From the tables 4 and 5 it can be noticed that the differences between the four varieties are:

ALFA and EIGENHEIMER:

Peak 3 is higher in Alfa than in Eigenheimer, 2 P $<$ 0.05 (at two-sided test).

Peak 5 is lower in Eigenheimer than in Alfa, 2 P $<$ 0.02.

ALFA and KWINTA:

Peak 1 is higher in Alfa than in Kwinta, 2 P $<$ 0.05.

Peak 4 is higher in Alfa and highly significant, 2 P $<$ 0.02.

ALFA and BINTJE:

Peak 1 is higher in Bintje than in Alfa and the difference is significant, 2 P $<$ 0.05.

Peak 3 is higher in Bintje than in Alfa. This difference is highly *significant*, 2 P $<$ 0.05.

Peak 4 is significantly higher in Alfa, 2 P $<$ 0.02.

Peak 5 is higher in Alfa than in Bintje, 2 P $<$ 0.02.

EIGENHEIMER and KWINTA:

Peak 1 is higher in Eigenheimer, 2 P $<$ 0.05.

Peak 3 is higher in Kwinta than in Eigenheimer, 2 P $<$ 0.02.

EIGENHEIMER and BINTJE:

Peak 1 is higher in Eigenheimer than in Bintje, 2 P $<$ 0.05.

Peak 3 is highly significant higher in Bintje than in Eigenheimer, 2 P $<$ 0.02.

KWINTA and BINTJE:

Peak 1 is higher in Bintje than in Kwinta, 2 P $<$ 0.02.

varieties: Alfa, Eigenheimer, Kwinta and Bintje.

Kwinta						Fertili-zation	Bintje					
Peak No							Peak No					
1	2	3	4	5	6	kg/ha	1	2	3	4	5	6
6.4	10.0	7.8	9.4	8.3	6.6	0	7.4	10.0	8.5	8.7	7.3	6.4
6.3	10.0	8.6	10.2	9.4	7.9	0	7.6	10.0	8.7	8.9	7.6	6.5
5.6	10.0	7.6	9.5	8.6	6.9	40	7.7	10.0	8.7	9.6	7.8	6.8
5.9	10.0	7.8	10.5	8.5	6.9	40	7.6	10.0	8.5	9.3	7.4	6.4
6.3	10.0	7.6	8.0	4.9	3.1	80	8.1	10.0	8.9	9.4	7.5	6.6
4.8	10.0	6.6	6.9	5.4	5.9	80	7.4	10.0	7.9	8.3	6.9	5.9
3.7	10.0	8.5	9.9	7.9	7.4	120	8.2	10.0	9.4	10.1	7.2	5.7
—	—	—	—	—	—	120	7.4	10.0	8.7	9.5	7.4	6.3
5.2	10.0	8.4	8.7	6.5	5.4	160	7.8	10.0	8.8	9.6	7.2	6.1
4.7	10.0	9.1	9.5	7.4	4.7	160	6.9	10.0	8.4	9.0	6.7	5.1
3.8	10.0	11.8	11.9	9.0	6.6	200	7.0	10.0	8.3	9.3	5.8	4.5
—	—	—	—	—	—	200	6.8	10.0	8.8	9.5	6.2	4.3

TABLE 5. The significant differences between the relative heights of the peaks in the four potato varieties.

	Peak 1		Peak 3		Peak 4		Peak 5	
	Lower	Higher	Lower	Higher	Lower	Higher	Lower	Higher
Alfa and Eigenheimer				××		×××		×××
Alfa and Kwinta		×				×××		
Alfa and Bintje	×		××			×××		×××
Eigenheimer and Kwinta		×	×××					
Eigenheimer and Bintje	×		×××					
Kwinta and Bintje	×××							

× significant at 5 %.
×× significant at 2 %.
××× significant at less than 2 %.

c. The differences between the peaks.

In the second method the relative heights have been taken into consideration. However, it was noticed that the differences between the relative heights of the different peaks can give sharper results. In the method described here, the differences between the heights will be the subject of discussion. The results and their significances are summerized in table 6. It can be seen from the table that many differences between the four varieties exist.

d. Ranking method.

Finally it was thought that a ranking system would give the easiest way for application. In this system the differences between the peaks were ranked. The differences were arranged in such a way that a descending sequence was obtained. Each value had been provided with a rank number, so that the highest value was indicated by the lowest rank number and the highest rank number by the lowest value. In table 7 six ranking systems are given. In the first three groups the ranking system can only eliminate Alfa. In the fourth Alfa as well as Kwinta can be distinguished from Bintje and Eigenheimer. In system 5 Alfa as well as Eigenheimer were eliminated, while Bintje and Kwinta have the same pattern. Ranking system nr 6 gives a complete and single system to identify the four varieties.

It is shown that with the aid of even less than the six ranking systems selected, it is possible to distinguish definitely between the four varieties.

TABLE 6. A comparison between the four potato varieties using the difference between the relative heights of the peaks.

diff.[1] between peaks no	Comparison between the varieties											
	A versus E		A versus K		A versus B		E versus K		E versus B		K versus B	
	higher [2]	lower [2]	higher [2]	lower [2]	higher [2]	lower [2]	higher [2]	lower [2]	higher [2]	lower [2]	higher [2]	lower [2]
1 — 2	—	+	++	—	+	—	++	—	—	+	—	++++
1 — 3	—	+	+++	—	+	—	+++	—	+	—	—	++++
1 — 4	—	++	—	+	—	++++	+++	—	—	+	—	++++
1 — 5	—	+++	—	+	—	++	+++	—	—	+	—	++++
2 — 3	—	+++	+	—	++	—	++++	—	++++	—	+++	—
2 — 4	—	++++	—	++++	—	++++	++++	—	+++	—	—	+++
2 — 5	—	++++	—	++	—	++++	+	—	+	—	—	+
3 — 4	—	++++	—	++++	—	++++	+++	—	—	+++	—	+
3 — 5	—	+++	—	+	+	++++	—	+	—	++	—	+
4 — 5	+	—	+	—	+	—	—	+	+	—	—	+

A = Alfa
E = Eigenheimer
K = Kwinta
B = Bintje

\+ not significant (higher or lower, but not significant at 5 % level of probability).
++ significant at 5 % level of probability.
+++ significant at 2 % level of probability.
++++ significant at less than 2 % level of probability.

[1]) differences: the difference between peak 1 and peak 2 and so on.

[2]) higher and lower here do not mean that the differences are bigger or smaller in absolute sense, but do refer to the values with their signs taken into account.

Applying one of the four methods mentioned can be used to differentiate between the four varieties studied. It is, however, believed that the fourth method will give more sharp and exact conclusions. Obviously, it would be advisable to use the four methods together, to obtain a definite conclusive opinion about the variety in question.

TABLE 7. Ranking of the differences between the peaks for the four varieties.

Ranking Systems	Ranking of differences					variety
system one	1 — 2 *)	1 — 3 *)	1 — 4 *)	2 — 5 *)	— *)	
order 1	3	2	4	1	—	Alfa
order 2	4	2	3	1	—	Eig.[1]) - Kw.[2]) - Bintje
system two	1 — 2	1 — 3	1 — 4	1 — 5	2 — 3	
order 1	4	2	5	3	1	Alfa
order 2	5	3	4	2	1	Eig.[1]) - Kw.[2]) - Bintje
system three	2 — 5	3 — 4	3 — 5	4 — 5	—	
order 1	2	4	3	1	—	Alfa
order 2	1	4	3	2	—	Eig.[1]) - Kw.[2]) - Bintje
system four	1 — 5	2 — 4	2 — 5	3 — 4	4 — 5	
order 1	4	3	2	5	1	Alfa
order 2	5	3	1	4	2	Kwinta[2])
order 3	4	3	1	5	2	Eig.[1]) - Bintje
system five	1 — 3	1 — 4	2 — 5	3 — 4	4 — 5	
order 1	3	5	2	4	1	Alfa
order 2	3	4	1	5	2	Eigenheimer
order 3	4	5	1	3	2	Kw.[2]) - Bintje
system six	1 — 4	1 — 5	2 — 5	3 — 4	4 — 5	
order 1	5	3	2	4	1	Alfa
order 2	4	3	1	5	2	Eigenheimer
order 3	5	4	1	3	2	Kwinta
order 4	5	3	1	4	2	Bintje

*) The difference between the relative height of peak 1 and that of peak 2 and so on.
[1]) Eig. = Eigenheimer.
[2]) Kw. = Kwinta.

C. EFFECT OF STORAGE ON POTATO PROTEIN'S PATTERN

Two factors were investigated here. The first dealt with the storage temperature and the second with the storage period. The temperatures involved were 2, 6, 10 and 15° C, and the periods used were 0, 8, 16 and 24 weeks. The study included the varieties Alfa, Eigenheimer and Kwinta.

1. The effect of the storage temperature

To eliminate the effect of the storage period the average of the relative heights of the different peaks for each temperature in the three periods will be used in this discussion. In other words, if peak 1 was 7.2 at 2° C with a storage period of 8 weeks, 7.7 at 2° C with a 16 weeks' period and 7.0 at 2° C with a 24 weeks' period, the average would be 7.3 at 2° C for the periods together.

Table 8 shows the relative heights of the peaks as influenced by the storage temperature for the three varieties, table 9 the relative heights of the peaks at the onset experiment and figure 8 the relation between the relative heights of the peaks and the storage temperature. It can be noticed that:

a. Peak one: showed a declining curve in the three varieties, the trend of which was nearly the same.

b. Peak three: with increasing temperature, the relative heights of the three varieties decreased and after a minimum value was reached, they increased together with temperature. Eigenheimer and Kwinta showed the drop at 2° C, with Alfa the minimum was reached at 6° C. The rise at higher temperatures was more pronounced in Kwinta than in Alfa and Eigenheimer.

c. Peak four: with the 2° C treatment a sharp drop was noticed which was generally followed by a rise. Except for Eigenheimer this rise continued up to the 15° C treatment. In Eigenheimer, however, the fourth peak decreased at 15° C.

TABLE 8. The effect of storage temperature on the relative heights of the different potato protein peaks.

peak no.	Alfa				Eigenheimer				Kwinta			
	2°C	6°C	10°C	15°C	2°C	6°C	10°C	15°C	2°C	6°C	10°C	15°C
1	7.2	6.7	6.4	5.9	7.2	6.7	6.0	6.0	6.0	5.3	4.4	—
3	8.0	7.2	7.9	8.1	6.5	6.8	7.2	7.1	7.9	8.1	9.3	—
4	10.7	11.0	11.5	11.9	8.3	8.9	9.6	9.0	7.8	8.9	10.4	—
5	8.5	7.9	8.6	8.9	5.8	6.5	6.8	7.0	6.6	7.4	8.3	—
6	6.0	6.8	6.2	6.2	5.2	5.3	5.3	5.9	5.0	6.2	7.0	—

TABLE 9. The relative heights of different potato protein peaks at onset time.

variety	peak 1	peak 3	peak 4	peak 5	peak 6
Alfa	8.3	8.1	12.9	11.0	8.5
Eigenheimer	8.2	7.5	9.7	8.5	4.8
Kwinta	6.1	8.5	10.6	8.8	7.9

d. Peak five: it can be noticed from figure 8 that in the three varieties at 2° C storage temperature, a drop followed by a slight increase occurred.

e. Peak six: again a sharp decline occurred in Alfa and Kwinta at 2° C whereas Eigenheimer showed a rise. A rather sharp increase then took place in Kwinta. With the other two varieties no important alterations were observed.

From figure 8 and the preceding discussion a constant drop in peak height was observed at 2° C, and this was then followed by an increase in height except for peak one which decreased constantly. This holds good for the three varieties. The increase of the four peaks at higher temperatures can be explained in different ways. The incline in relative heights of the peaks does not necessarily imply that the quantities of proteins actually increased, but it can also be the result of unequal breakdown of the different components. If breakdown of protein occurs, it should be more pronounced in the protein represented by peak two than in the other components at 6, 10 and 15° C.

Peak two which was considered as the reference peak, was taken as constant which may not be the case in reality, logically a drop in this peak will cause a rise in the relative heights of the other peaks.

As to peak one, it can be easily noticed that its breakdown is so strong that its relative height is sloping down, even at higher temperatures.

Nevertheless there is a possibility of some proteins being synthesized during the storage specially at higher temperatures. Taking into account the results obtained in the amino acids analyses, which will be discussed in Chapter Three, it is therefore hard to make out whether synthesis or breakdown plays the dominating part.

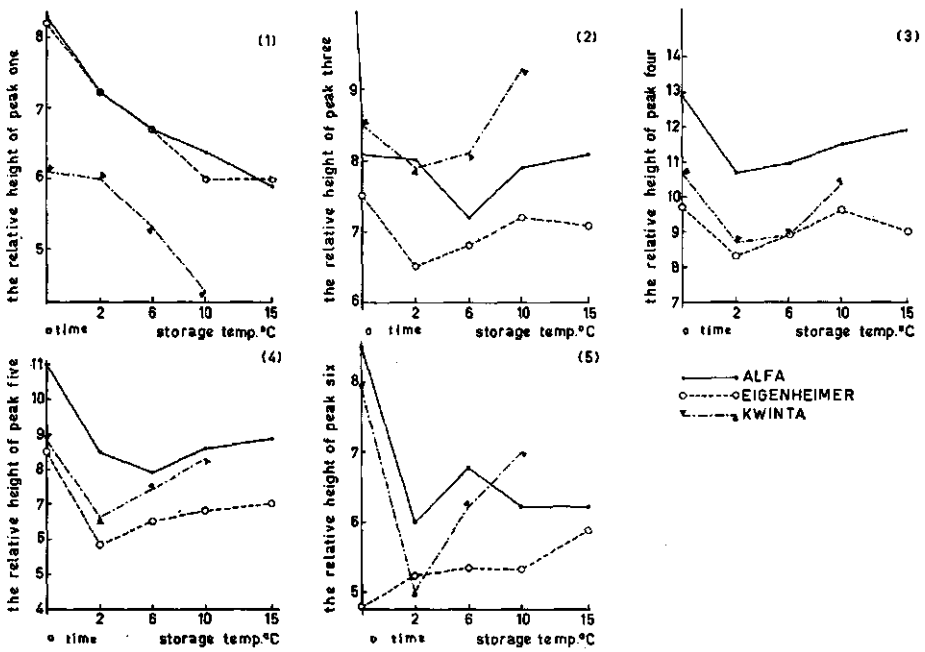


FIGURE 8. The effect of storage temperature on the relative heights of the peaks.

2. The effect of storage period

Figure 9 and table 10 show how the relative heights of the peaks can be influenced by the storage time. The temperature has been eliminated by averaging all the temperature treatments of each period.

TABLE 10. The effect of storage time on the relative heights of the potato protein peaks.

period	Alfa					Eigenheimer					Kwinta				
	Peak no.:					Peak no.:					Peak no.:				
	1	3	4	5	6	1	3	4	5	6	1	3	4	5	6
8 weeks	7.0	7.8	11.9	9.0	6.9	7.8	6.6	9.2	7.3	5.7	6.1	8.0	9.9	8.7	7.1
16 weeks	7.5	7.1	10.4	7.8	6.8	6.8	6.6	8.5	6.1	5.9	4.9	7.6	8.3	6.1	5.5
24 weeks	5.1	8.5	11.6	8.6	5.1	5.0	7.6	9.2	6.2	4.7	4.6	9.8	10.0	7.6	5.6

a. Peak one: With the three varieties a steady decline of peak one occurred.
b. Peak three: The decline in the three varieties advanced until the 16th week followed by an increase that may be called sharp.
c. Peak four: The three varieties showed a decreasing tendency until the 16th week after which they began to rise.
d. Peak five: The decreasing tendency may be noticed to occur in the three varieties until the 16th week. In the 24th weeks' period a rise occurred.
e. Peak six: With Alfa and Kwinta a steady decrease could be noticed. With Kwinta a slight rise occurred in the last storage period. Eigenheimer showed a constantly rising tendency followed by a decreasing one in the 24 weeks' period.

It can be seen that in all the varieties and with the five variable peaks a declining tendency generally occurred till the 16th week, after which a rather sharp increase was noticed in peaks 3, 4 and 5. Peak 1 and 6 were declining till the end. The only exception was Eigenheimer in peak six, where the peak was rising and then dropping only in the last period.

From the foregoing discussion it can be noticed that the variable relative heights of the peaks decreased, while the second peak was kept constant. It seems, however, that the whole system was decomposing or breaking down. It is likely that the protein corresponding to peak two is undergoing a breakdown which is less pronounced than in the other proteins. As a result of this, some peaks will appear to be higher. Moreover, the speed of such a breaking-down may differ from one protein to another. It seems, however, that the protein system is breaking down owing to some activities (germination for instance) which may vary in different varieties of potatoes. When, on the other hand, the curves in the second phase began to increase, this might be attributed to the unbalance of the protein breakdown of peak 2 and the other peaks.

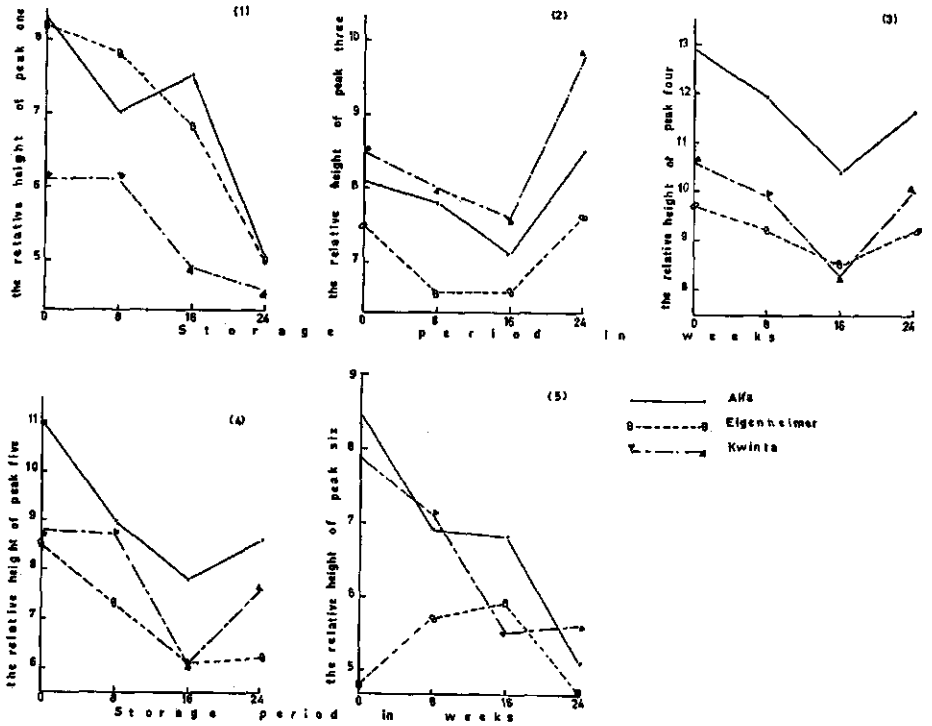


FIGURE 9. The effect of the storage period on the relative heights of the peaks in the potato varieties Alfa, Eigenheimer, and Kwinta.

At the same time, the possibilities of protein synthesis are established especially in the beginning of the storage period. This assumption should not be neglected.

It should be borne in mind that the alteration in protein pattern as influenced by storage temperature and time tend to be almost the same in the different varieties studied here. Although the storage as it has been shown, influences the protein pattern of potatoes, the interference is so small that it may hardly play a part in the identification of potato varieties after storage.

D. EFFECT OF NITROGEN FERTILIZATION ON THE PROTEIN PATTERN

Table 11 and figure 10, show the influence of nitrogen fertilization on the protein pattern of potatoes. No influence could be observed in all the peaks except in the 5th and the 6th peak, which tend to decrease at the 160 and 200 kg N/ha level. The standard deviation of each peak is shown in the bottom of table 11. It may be noticed that the variation from the mean is rather small. The results of the experiment did not show a real influence when nitrogen fertilization

was used. The variations seen, however, may be considered to be of the same magnitude as those of the sampling error. The standard deviation was calculated following the method described by SNEDECOR (1959).

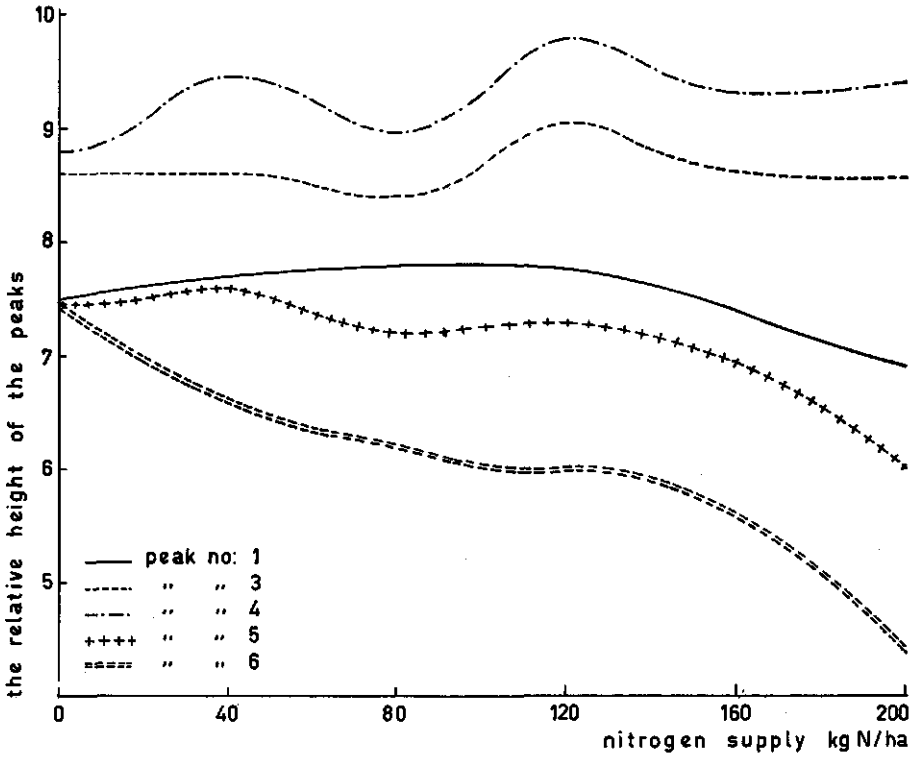


FIGURE 10. The effect of nitrogen fertilization on the relative heights of the peaks in the variety Bintje.

TABLE 11. The influence of the nitrogen fertilization on the relative heights of the different potato-protein peaks, and the standard deviation, in the variety Bintje.

Kg N/ha	Peak 1	Peak 3	Peak 4	Peak 5	Peak 6
0	7.5	8.6	8.8	7.5	6.5
40	7.7	8.6	9.5	7.6	6.6
80	7.8	8.4	8.9	7.2	6.3
120	7.8	9.1	9.8	7.3	6.0
160	7.4	8.6	9.3	7.0	5.6
200	6.9	8.6	9.4	6.0	4.4
Mean	7.5	8.6	9.3	7.1	5.9
S.D.	0.35	0.24	0.36	0.60	0.82

S.D.: standard deviation.

The figures mentioned in this table are the average of two field experiments. Peak nr 2 is always constant and is considered as equal to 10.

V. Summary And Conclusions

This work aims at obtaining information about some characters of the proteins present in the potato tuber. The electrophoretical properties of the potato proteins were studied. The study involved four varieties namely Alfa, Eigenheimer, Kwinta and Bintje. The first three were studied for the effect of storage while Bintje was studied for the nitrogen fertilization effect.

A refined method for potato proteins separation, in which paper-electrophoresis was used, has been developed. From the results obtained it may be concluded that the potato proteins are a mixture of no less than six components. Moreover, the sixth peak is thought to be a mixture of at least three components. These findings were confirmed by zone electrophoresis.

A simple and reliable method for measuring the electropherograms has been worked out, the base of which is to make use of the heights of the peaks.

The results obtained by the electrophoresis, showed that identification of potato varieties can be carried out by using the protein patterns. Four methods for identification have been discussed, one of which is a ranking system. The characteristic patterns of the different varieties hold true under nitrogen fertilization and storage.

Furthermore, the breakdown or synthesis of proteins due to storage time and temperature were discussed. Their influence, however, on the characteristic properties of the protein pattern is of minor importance.

A nitrogen fertilization experiment was also studied. No obvious influence on potato protein pattern could be attributed to low or high nitrogen fertilization.

CHAPTER THREE

THE NITROGEN AND THE AMINO ACID DISTRIBUTION IN THE POTATO

I. Introduction

It is not the protein but its amino acids that are used by the body. The quality of a protein is judged from its amino acid content. The amino acids supply the animal with the principal needs for protein synthesis. A protein is a combination of many amino acids. Due to this character, the importance of protein in nutrition is a basic one.

The nitrogen fraction of the potato tuber comprises about 1—1.5 % on dry basis. If expressed as crude protein, it will amount to 6.25—9.50 % of dry material. It is really astonishing that although cooking quality, starch and sugars have been investigated thoroughly, yet the nitrogenous substances did not receive such attention. The importance of the potato is attributed to two factors, viz. its high consumption and its nutritional value. At first sight it seems that the potato contains only small amounts of proteins. But if it is considered that the moisture content amounts to about 75 %, the crude protein content on dry basis will appear to be nearly the same as that of the cereal products. In view of the great quantities consumed, the potato supplies more than 10 % of the daily protein requirements in a great part of Europe. It is therefore of major importance, from the nutritional standpoint to investigate the protein of the potato in detail. The potato is not only consumed immediately after harvesting but also after storage periods which may exceed six months. The effect of storage on the nitrogen components important for nutrition, has not yet been fully investigated. The effect fertilization, irrigation and other tillage operations on the nitrogenous substances is also far from being well known.

This chapter comprises a study of the nitrogen distribution between protein and non-protein fractions, and the amino acid content of these fractions. The investigation also includes a study of the effects of nitrogen fertilization and of storage on the nitrogenous substances of the potato tuber. Four varieties of potatoes were selected to serve as experimental material.

II. Materials And Sampling

A. MATERIALS

The materials used in the present investigation were the same as those described in Chapter Two page 5.

B. SAMPLING

1. Sampling the crop

The sampling of the crop was the same as described in Chapter Two page 6 except that the dates of sampling were as follows:

TABLE 12. The dates of sample analyses.

treatment	date
nitrogen fertilization experiment, kept at 5° C, var. Bintje.	14th Nov. 1960 9th Jan. 1961 1st May 1961
storage experiment, kept at 2, 6, 10 and 15° C, var. Alfa, Eigenheimer and Kwinta.	19th Dec. 1960 13th Feb. 1961 5th June 1961

2. Sampling in the laboratory

The sampling in the laboratory was carried out in the same way as described in Chapter Two, page 7. The final samples were drawn with the aid of a wide opening pipet, from the mixture obtained. For the different determinations the following techniques were employed:

a. Total solids: a sample weighing ± 15 g was pipetted out of the mixture obtained into a metal weighing dish with cover. The plate was covered and weighed to the nearest one tenth of a milligram.

b. Total nitrogen: a sample weighing ± 3 g was quickly pipetted into a weighed kjeldahl flask with a stopper. The kjeldahl flask was then weighed with its contents.

c. Soluble nitrogen: a sample of about 150 g was pipetted out of the potato mixture delivered into a beakerglass, and weighed to the nearest 0.01 g. The sample was then washed several times with water in a Büchner funnel with asbestos filter (Carlson K 5) to obtain a final volume of about 1500 ml filtrate. The volume was measured with a measuring cylinder. The soluble nitrogen was determined using 20 ml of the filtrate.

III. METHODS

A. MOISTURE CONTENT

The dishes with the weighed potato mixture were placed in an oven at 100° C and dried overnight to constant weight. The total solids were weighed and the moisture content was estimated.

B. NITROGEN DETERMINATION

The method used was that of the microkjeldahl. The principle and summary of the method will be discussed below. Organic matter was destroyed with concentrated sulphuric acid catalized with seleen catalyzator composed of 1.5 g cupper sulfate, 2 g seleen and 95 g anhydrous sodium sulphate. The mixture was heated on an electric heater until a clear blue-green solution was obtained. From this moment the solution was heated for another hour for final digestion. The resulting fluid was then transferred to a Parnas Wagner apparatus. To the digest in the distillation flask 15—20 ml of a 40 % sodium hydroxide solution was added. The added alkaline solution also contained 4 % sodium thiosulfate. The distilled ammonia was received in a 250 ml conical flask containing 50 ml boric acid pH 4.6 and 2 drops of an indicator composed of 2 : 1 methyl red and methylene blue both in 0.2 % solution of 95 % alcohol. From the moment the indicator turned green the distillation was continued for another six minutes. The boric acid-ammonia solution was then titrated against 0.05 N HCl.

C. FRACTIONATION OF THE NITROGEN COMPONENTS IN POTATO

It is known that the nitrogenous substances in the potato are readily soluble in water. This solubility ranges from 70 to 90 %. In the present study the nitrogenous substances were divided into the following three categories:

1. Total nitrogen (T.N.):

Total nitrogen was determined directly in the minced potato.

2. Soluble nitrogen (S.N.):

Soluble nitrogen was determined in 20 ml of the filtrate obtained from washing the potato mixture (see sampling page 30).

3. Non-protein nitrogen (N.P.N.):

Non-protein nitrogen fraction was determined in the filtrate (mentioned under 2) after precipitating the proteins in the following way:
One litre was used to precipitate the soluble potato proteins, according to the method described by MULDER and BAKEMA (1956), i.e., the solution was heated

in a boiling water bath, after adding a few drops of acetic acid and a knife-point of sodium chloride. The precipitated proteins were filtered after cooling, and the filtrate was collected in a one litre flask. The protein was washed 3 times with 2 % acetic acid until the filtrate volume reached one litre. The filtrate contained the N.P.N.-fraction. The nitrogen content was determined in 20 ml volume.

4. Protein nitrogen (P.N.):

Protein nitrogen was determined by taking the difference between T.N. and N.P.N.

The soluble protein precipitated as described under **3**, was then washed twice with alcohol and once with ether. To dry the precipitate thus obtained, it was kept in a descicator under vacuum with sulphuric acid for 48 hrs. The nitrogen content of the dried protein precipitate was determined in 50 mg. The nitrogen content was found to range from 13.5 to 14.5 %.

D. AMINO ACID DETERMINATIONS

1. Methods for the amino acid determination used in the current research work:

Although most of the naturally occurring amino acids were isolated before the forties, the development of accurate methods for their determination took place throughout the last two decades only. Many methods for amino acid determinations are available nowadays. It is beyond the scope of this study to review all the methods described, yet a short account of the methods mostly used will be given.

NEUBERGER (1938) observed that, when acetylated neutral amino acids were distributed between water and nonaqueous solvents, it was found that different amino acids had different partition coefficients. Later on, this fact was used to develop a chromatographic method with silica gel by MARTIN and SYNGE (1941). This technique was followed by the application of paper chromatography for qualitative tests. Afterwards the elution of the amino acid spots on the filter paper offered a means for quantitative estimations, although a fault of 10 % or more should be taken into account. A full description of paper chromatography was published by BLOCK, DURRUM and ZWEIG (1958).

In recent years, however, the application of column chromatography as developed by MOOR and STEIN (1948, 1949, 1951 and 1954), has gained ground. The very outline of the method is the use of starch or of a polystyrene resin (such as amberlite or dowex 50) as an adsorbent or ion exchanger and the elution of the amino acids with solvents of gradually increasing polarity or with solutions of gradually increasing acidity. The elutions are then collected in small tubes in equal volumes. Ninhydrin is used as a colouring reagent and the densities of colours developed can be read in a spectrophotometer. The different amino

acids can be recognized from the ease with which they are eluted i.e. from the number of the tubes in which they are collected. With the aid of standard curves obtained by colouring distinct quantities of amino acids with ninhydrin the amount of eluted and coloured amino acids can also be calculated. In recent publications it has been claimed that the average error is rather low, being of the order of 3 to 5 %.

Some amino acids, however, can be directly determined spectophotometrically due to their absorption of light in the ultra violet region, as in the case of tryptophan and tyrosine, HOLIDAY and OGSTON (1938).

Biological methods with rats or chicks, have also been used to determine amino acids. OUSTERHOUT, GRAU and LUNDHOLM (1959) used chicks assay to determine the amino acids, they stated: the method was succesful for lysine, methionine, cystine, tryptophan, phenylalanine, histidine and threonine. Less for arginine and unsatisfactorily for glycine, isoleucine, leucine and valine. These methods require a lot of animals and much time, besides they are not accurate. Other methods are therefore preferable.

WOOD, GEIGER and WERKMAN (1940) suggested to measure the amino acids quantitatively by microorganisms. Before that time some investigators found that many microorganisms need amino acids in their media for growth. SNELL, STRONG and PETERSON (1937) found that tryptophan is an essential amino acid for *Lactobacillus helveticus*. From that time on many workers began to investigate the microorganisms requirements for amino acids. SNELL and WRIGHT (1941) developed a method for the determination of nicotinic acid using *Lactobacillus arabinosus*. Using a medium with vitamin free casein, they could observe that tryptophan and cystine were essential for the growth of this organism. KUIKEN *et al.* (1943) came to the conclusion that valine, leucine and isoleucine can be determined with accuracy from the growth of *L. arabinosus*. HIER and BERGEIM (1945) found that this organism could also be employed for the determination of threonine while STOKES *et al.* (1945) found that *Streptococcus faecalis* could be used for accurate quantitative determinations of the following amino acids: valine, isoleucine, leucine, threonine, methionine, arginine, lysine, histidine and tryptophan. The amino acid requirements of *Leuconostoc mesenteroides* and *Leuconostoc citrovorum* were investigated by STEEL *et al.* (1949). They found that the organisms could be used to determine eighteen different amino acids with accuracy.

In biological assays the error usually amounts to 10 %. This high limit is due to the individual variations when a small number of animals is used. In the early days of vitamin B complex and amino acid assays with the aid of microorganisms, it was thought that an error of 10 % could not be avoided. It is, however, irrational to treat the data obtained with microorganisms in the same way as those obtained with higher animals. In animal experiments an investigator would usually limit himself to a number of animals not far in excess of twenty. In microbiological methods, millions of organisms are involved, a fact which tends to minimize the individual variations. In this respect SCHWEIGERT and SNELL (1946-1947) presented values of amino acids in protein obtained by micro-

biological methods in different laboratories, using different media, different samples and different microorganisms. They stated: "Only in a few cases where several values are available, does the variation amount to as much as ± 10 %". VAN DER LINDEN (1949) working on the microbiological methods for determining amino acids, studied the accuracy of the method. He used phenylalanine as the experimental amino acid. The standard deviation from the mean was found to be 3.1 % in nine different hydrolysates analysed on the same day. When, however, new hydrolyzates were analyzed at intervals of two weeks the variation was greater and amounted to 5.2 %. He concluded that such higher variations were due to differences in the lag phase of growth in different assays as well as to differences in the lag phase of growth seen in "sample tubes" and that of the "standard ones". He added that the inter-day variations may be considerably diminished by growing the inoculum in the basal medium, to which the missing factor has been added. POSTEL (1956) found that, when microbiological methods for amino acids were employed, the error in three determinations was found to be in phenylalanine, threonine, histidine, lysine and tryptophan ± 2 %, in valine, leucine and arginine ± 3 %, and in methionine and isoleucine ± 4 %. REIF (1958) came to a similar conclusion and noted that the experimental error of microbiological determinations of amino acids did not exceed 4 % when growth was evaluated by titration.

2. The method employed in this study:

Considering the fact that microbiological determinations lend themselves extremely well to routine work and taking into account the good reproducibility mentioned in the foregoing discussion, it was decided to use the microbiological method in the determination of all amino acids except in that of tryptophan in the soluble protein, where a spectrophotometric method was used. A description of the methods used are summarized below.

a. The microorganisms:

Three microorganisms were used, namely: *Leuconostoc mesenteroides* P.60 (ATCC 8042), *Lactobacillus arabinosus* 17/5 (ATCC 8014) and *Streptococcus faecalis* (*Lactis* Rogers) (ATCC 9790). The cultures were obtained from the collection of the Delft University, Microbiological Department.

Histidine, lysine, phenylalanine, methionine and tryptophan were determined with the aid of *L. mesenteroides;*

Leucine, isoleucine and valine with the aid of *L. arabinosus;*

Arginine and threonine with the aid of *S. faecalis.*

The media used for the three microorganisms are shown in table 13. The medium used for *L. mesenteroides,* was described by STEEL *et al.* (1949). The other two media were quoted from BARTON-WRIGHT (1952).

The three microorganisms were kept on the "Micro Assay Culture Agar Difco" (Difco Manual of Dehydrated Culture Media Reagents 1953), the composition of which was:

TABLE 13. Basal Media for the organisms *L. mesenteroides*, *L. arabinosus* and *S. faecalis* (two times strength).

Ingredients	*L. mesenteroides*	*L. arabinosus*	*S. faecalis*
Dl-*a*-Alanine	200 mg	100 mg	100 mg
L-Arginine	242 mg	50 mg	50 mg
L-Asparagine	400 mg	—	400 mg
L-Aspartic Acid	100 mg	800 mg	—
L-Cysteine	50 mg	—	—
L-Cystine	—	100 mg	200 mg
L-Glutamic Acid	300 mg	400 mg	400 mg
Glycine	100 mg	20 mg	20 mg
L-Histidine HCl	62 mg	50 mg	50 mg
DL-Isoleucine	250 mg	200 mg	200 mg
DL-Leucine	250 mg	200 mg	200 mg
L-Lysine mono-HCl	250 mg	200 mg	200 mg
DL-Methionine	100 mg	100 mg	100 mg
DL-Penylalanine	100 mg	100 mg	100 mg
L-Proline	100 mg	—	—
DL-Serine	50 mg	80 mg	50 mg
DL-Threonine	200 mg	200 mg	100 mg
DL-Tryptophan	40 mg	80 mg	200 mg
L-Tyrosine	100 mg	40 mg	80 mg
DL-Valine	250 mg	200 mg	200 mg
Glucose	25.0 g	20.0 g	20.0 g
Sodium acetate	20.0 g	33.0 g	—
Sodium citrate 2 Aq.	—	—	25.0 g
Xylose	—	1.0 g	—
Ammonium chloride	3.0 g	—	—
Ammonium Sulphate	—	3.0 g	—
Mono-potassium phosphate	600 mg	500 mg	—
Di-potassium phosphate	600 mg	500 mg	500 mg
Magnesium sulphate 7 Aq.	200 mg	200 mg	200 mg
Ferric chloride (anhydrous)	—	2 mg	2 mg
Ferro sulphate 4 Aq.	10 mg	—	—
Manganese sulphate 4 Aq.	20 mg	10 mg	10 mg
Sodium chloride	10 mg	500 mg	500 mg
Adenine	10 mg	10 mg	10 mg
Guanine	10 mg	10 mg	10 mg
Uracile	10 mg	10 mg	10 mg
Xanthine	10 mg	10 mg	10 mg
Aneurin-HCl	500 μg	200 μg	200 μg
Pyridoxine-HCl	1000 μg	200 μg	1200 μg
Pyridoxamine-HCl	300 μg	—	—
Pyridoxal-HCl	300 μg	—	—
Calcium d-pantothenate	500 μg	200 μg	400 μg
Riboflavin	500 μg	200 μg	200 μg
Nicotinic Acid	1000 μg	600 μg	600 μg
p-Aminobenzoic Acid	100 μg	100 μg	—
Biotin	1 μg	0.5 μg	0.5 μg
Folic Acid	10 μg	—	2 μg
Water to	500 ml	500 ml	500 ml

Bacto-Yeast extract	20.0 g
Proteose Peptone Nr 3 Difco	5.0 g
Bacto-Dextrose	10.0 g
Monopotassium phosphate	2.0 g
Sorbitan Monooleate complex	0.1 g
Bacto-Agar	10.0 g

Streptococcus faecalis and *L. mesenteroides* were grown on a slope culture, while *L. arabinosus,* on a stab culture. Every third or fourth week the organisms were sub-cultured on new agar culture medium. This period was found to be adequate to keep the microorganism in a good active form. To assure purity, each microorganism was cultured in 4 tubes, viz. A, B, C and D. A and B were usually kept as stock cultures, whereas C and D were used for the running work. In subculturing, culture A served to inoculate the new A and B, while old culture B was used to inoculate the new tubes of C and D.

To prepare the medium from the "micro assay culture agar", 48.5 g was dissolved in water, boiled at 100° C for three minutes, made up to one litre and distributed in 10 ml volumes to tubes having a diameter of 16—20 mm. The tubes were plugged with cotton wool and sterilized at 15 lb for 15 min. They were allowed to cool either in an upright position or at a sloping position, according to whether a stab or a slope culture was required. The tubes, after solidification, were kept in the refrigerator at 5—7° C. When subcultures were prepared, the tubes were kept at 30° C for 16—20 hours after inoculation. If good growth appeared, they were stored at ± 5° C.

The inoculums were, when required, prepared from cultures C and D. They were grown on a full "Riboflavine Assay Medium", Difco, which was composed of:

Photolyzed peptone	22.0 g
Yeast supplement	2.0 g
Bacto-Dextrose	20.0 g
Sodium Acetate	1.8 g
L. Cystine, Difco	0.2 g
Dipotassium Phosphate	1.0 g
Magnesium sulphate	0.4 g
Sodium chloride	20.0 mg
Ferrous sulphate	20.0 mg
Manganese sulphate	20.0 mg

48 g of this medium was dissolved in 1000 ml, and boiled for 3 minutes. To prepare the full medium, 5 ml volumes of the hydrated medium were distributed to a number of test tubes, 2.5 ml of standard solution containing 100 mμg vitamin B_2/ml, and 2.5 ml water were added to each tube. The tubes were plugged with cotton wool and sterilized at 10 lb pressure for 10 min. After being allowed to cool the tubes were inoculated from the stab or slope culture, for which a sterile platinum needle was used. The inoculated tubes

were incubated at 30° C for 16—20 hrs. After incubation the tubes were centrifuged asceptically, the supernatents were discarded and the precipitated microorganisms were dispersed in sterilized saline water (0.9 % NaCl) and centrifuged again. These washings were repeated three times. Finally the precipitates were dispersed in sterilized saline water, mixed thoroughly and diluted to suitable turbidity. A sterilized pipette was used for inoculation of the solutions to be analyzed.

b. Preparation of the sample for the amino acid analyses with microorganisms:

Protein: From the precipitated and dried proteins ± 50 mg was weighed in a 100 ml conical flask, 25 ml 6N HCl was added, the flask was then covered with a beaker glass and the contents hydrolysed for 12 hr at 120° C in an autoclave. The resulting solution was directly brought up to pH 4.5, completed to a suitable volume and filtered. A volume of the filterate was used for the microbiological determinations after suitable dilutions and adjusting the pH to 6.8.

Non protein fraction: The non-protein nitrogen fraction was used directly, after adjusting the pH of a volume to 6.8 and diluting to the required concentration.

c. Set up of the assay:

Table 14 shows the ranges of standard curves for the studied amino acids in $\mu g/2$ ml.

TABLE 14. The ranges of standard curves for the studied amino acids in $\mu g/2$ ml.

Amino Acid	Range	Amino Acid	Range
Arginine	0—15	Methionine	0—15
Histidine	0—15	Phenylalanine	0—15
Isoleucine	0—15	Threonine	0—15
Leucine	0—15	Tryptophan	0— 4
Lysine	0—40	Valine	0—15

For each amino acid assay the media mentioned in table 13 were used, with the omission of the amino acid to be analyzed. The media, samples and standards were adjusted to pH 6.8. The determinations were carried out on 2 ml final volume (STEEL *et al.* 1949) in tubes having a diameter of ± 10 mm × ± 8.0 cm.

To determine an amino acid, three different volumes, each in triplicate of the sample solution were pipetted in the tubes. (In many cases, however, four or five different volumes were used). The three levels (usually 0.4, 0.7 and 1.0 ml) were made up to 1 ml using distilled water. The standard curves were composed of eight levels, viz. 0.0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and

1.0 ml of the standard solutions prepared before. Each level of the standard curve (being in triplicate) was completed to 1 ml with distilled water. With "Cornwall" automatic blowing pipet, 1.0 ml of the corresponding basal medium was then added to the tubes. Next, the tubes were plugged with cotton wool, sterilized at 10 lb for 10 minutes. After the tubes were cooled, they were inoculated with one drop of the prepared inoculum and incubated at 30° C in the case of *L. arabinosus* and *S. faecalis* assays and at 37° C in the case of *L. mesenteroides*. After an incubation period of 72 hours the produced lactic acid in the tubes was titrated against sodium hydroxide solution ± 0.1 normal. The acid production is a measure for the bacterial growth and thus for the amount of amino acids present. The standard curve was drawn plotting the titration figures against the corresponding quantities of the amino acid in question. The bacterial growth in the sample tubes can be evaluated and expressed in amounts of amino acids with the aid of the standard curve. The amount present in 1 ml sample solution can then be calculated. The nine values thus obtained were averaged. The results showed that the within sample variation was less than 5 %. The error ranged from 1.4 to 4.9 %. A recovery test was carried out with a mixture of amino acids (L. form). The mixture of the amino acids was added to the protein and all the operations from the start of hydrolysing, up to the end of the assay were carried out. The recovery percentages of the ten essential amino acids will be found in table 15. They ranged from 95.0 % with methionine to 104.0 % with lysine.

TABLE 15. The recovery of the essential amino acids.

Amino Acid	Recovery %	Amino Acid	Recovery %
Arginine	96.5	Methionine	95.0
Histidine	97.7	Phenylalanine	103.3
Isoleucine	98.5	Threonine	102.0
Leucine	99.2	Tryptophan	98.2
Lysine	104.0	Valine	96.3

d. The determination of tryptophan in the potato proteins: Tryptophan was determined spectophotometrically. Tryptophan and tyrosine have the property to absorp light in the ultraviolet region. It is known that phenylalanine, tyrosine and tryptophan are responsible for the observed absorption in protein solutions. Phenylalanine shows weak absorption and the maximum occurs at 258 mμ. The absorption exerted by tyrosine and tryptophan is much greater. The method used followed the work of LEMBKE, KAUFMANN and SCHMIDT (1952), GOODWIN and MORTON (1946) and HOLLIDAY (1936).

Tryptophan could be determined by measuring the obtical density of the protein solution in 0.1 N NaOH at the two wave lengths of 280 mμ and 305 mμ. By using the following equation the tryptophan content of the protein could be estimated:

$$\text{M Tryptophan} = \frac{E_{305} \cdot E_{Ty280} - E_{280} \cdot E_{Ty305}}{E_{Ty280} \cdot E_{Tr305} - E_{Tr280} \cdot E_{Ty305}} \qquad (1)$$

where M = molar concentration.
E_{280}, E_{305} = the extinction coefficient of the protein solution at the wave lengths 280 and 305 mμ.
E_{Ty280}, E_{Ty305} = the molecular extinction coefficient of tyrosine at the wave lengths 280 and 305 mμ.
E_{Tr280}, E_{Tr305} = the molecular extinction coefficient of tryptophan at the wave lengths 280 and 305 mμ.

The molecular coefficient can be estimated from the equation:

$$E = \frac{mW \cdot Ac}{C} \qquad (2)$$

where mW = molecular weight.
Ac = absorption coefficient.
C = concentration g/litre.

Equation (1) can be simplified by the substitution of the molecular coefficients of tryptophan and tyrosine in 0.1 N NaOH solutions. LEMBKE, KAUFMANN and SCHMIDT (1952) gave the following equation:

molar concentration of tryptophan = $(0.216\ E_{280} - 0.215\ E_{305})\ 10^{-3}$.

PANTLISCHKO (1952) gave:

molar concentration of tryptophan = $(0.204\ E_{280} - 0.239\ E_{305})\ 10^{-3}$.

HOLLIDAY (1936) gave:

molar concentration of tryptophan = $(0.210\ E_{280} - 0.288\ E_{305})\ 10^{-3}$.

In the present study the following equation was found:

molar concentration of tryptophan = $(0.207\ E_{280} - 0.273\ E_{305})\ 10^{-3}$.

To prepare the required protein solutions ± 40 mg of the potato protein were accurately weighed and dissolved in 0.1 N NaOH solution, made up to a suitable volume with the alkali solution. A Bekmann spectrophotometer was used for measuring the absorption coefficient. Two sorts of recovery test were carried out. The first recovery was made by mixing different known quantities of tryptophan solutions with different known quantities of tyrosine solutions. The second recovery was carried out by adding known quantities of tryptophan and tyrosine to the protein. The results in case of tryptophan in the two methods were 98.0 to 102.6 % recovery.

IV. Results And Discussion

A. NITROGEN DISTRIBUTION

In plants the nitrogenous substances are found as proteins, free amino acids and amides. In the vegetative storage organs such as potato tuber, turnips and onions, a high percentage of non-protein nitrogenous substances is present. It seems that the potato tuber contains a higher proportion of its nitrogen as protein than turnips and onions. These proportions seem to change under different treatments and circumstances and are not the same in different varieties. In this respect, Mulder and Bakema (1956) found that the ratio P.N. : N.P.N. in potato tuber varied very much in different varieties, e.g. in Noordeling, it was found to be 1.20, in Voran 0.92 and in Eersteling 0.76. This reflects the differences between varieties that may occur, apart from the environmental conditions that may also contribute to change the proportions of P.N. and N.P.N. fractions.

Zaleski (1898, 1901, 1913) studying the effect of storage on the nitrogenous substances in bulbs, found that during storage protein synthesis occurred disturbing the proportions of P.N. and N.P.N. to that of T.N. In one experiment (1898), 32 % of the T.N. was P.N. at the beginning of the storage period, after germination the P.N. increased to represent 50 % of T.N. He could also confirm that even at lower temperatures protein synthesis was a normally occurring process.

From these results it can be seen that the proportions of P.N. : T.N. may vary very much in the storage organs of plants during storage. Beside the effect of storage and environmental conditions, treatments such as watering and fertilization, may influence the picture too. In view of these facts it is really difficult to draw any conclusion as to storage effects without studying the other factors.

The nitrogenous substances of the potato tuber did not receive much attention as compared with other components, starch for instance. Stuart and Appleman (1935) found that no important changes in the nitrogenous substances occurred when the tubers were stored at 35.6 to 37.4° F. At room temperature on the other hand, the changes were more pronounced. Shallenberger (1955), however, reported the highest T.N. in potato tubers stored at room temperature and the lowest at 32°—40° F. Tagawa and Okazawa (1955) found no changes in the various nitrogen fractions of the potato after a 60 days' storage. One hundred days after harvesting they found that the soluble N.P.N. fraction increased. This increase was accompanied by a decrease in the protein nitrogen. In the following 30 days, P.N. as well as N.P.N. increased in the terminal bud, in the other parts of the tuber P.N. decreased.

In this connection it is also worthwhile to mention that the respiration of the tuber is more related to protein metabolism than to carbohydrate metabolism. Gregory and Sen (1937) developed a theory stating that the products of protein breakdown are deaminated and the carbon skeletons respired. The free ammonia

is taken up again in the nitrogen metabolism. Their main conclusion is that most of the carbon dioxide respired is derived from those carbohydrate residues which were previously taken up in the nitrogen metabolism. In an experiment with C^{14}-glucose VITTORIO, KROTKOV and REED (1955) could obtain radioactive CO_2 from respiration in wheat plants, but only after a rather long period. This means that the carbohydrate carbon is not directly involved in respiration.
STEWARD, BIDWELL and YEMM (1956) suggested that the CO_2 resulting from the respiration is mostly drawn from proteins. Free amino acids are not directly used for protein synthesis. The protein synthesis might take place by a block-condensation of products which are built in some locations of carbohydrate metabolites and nitrogen groupes. These intermediate amino acids are not free and could not mix up with free amino acids in the tuber.
STEWARD and PRESTON (1940-1941) as well as STEWARD and STREET (1946) concluded from their experiments that the following steps seemed to be involved in the mechanism of protein synthesis:

Free amino acids → Ammonia → unstable amide
↓
protein

MCKEE (1958) stated: "Protein synthesis in the potato tuber appears thus to be in general associated with enhanced metabolic activity, and may be a response to increased energy production rather than its cause". He further stated:
"In general the effect of external conditions on respiration and on protein synthesis is in the same direction".

From this short review of the literature it can be seen that variations in circumstances and conditions of storage will to a large extend affect the proportions of the nitrogenous substances in the potato tuber.

1. General Results.

The average values for T.N., P.N. and N.P.N. are plotted in figure 11. Kwinta had the highest T.N. content and Bintje the lowest. Figure 12 shows the P.N. and the N.P.N. content of the tuber expressed as a percentage of T.N., a range of 42.3 to 47.3 % for N.P.N. and of 52.7 to 57.9 % for P.N. was found.

2. Effect of nitrogen fertilization and storage at 5° C with regard to the nitrogen distribution in potato variety Bintje.

a. Total nitrogen:

SCHUPHAN (1959), KÜRTEN (1957), MULDER and BAKEMA (1956), and others showed that the T.N. content of the tuber increased ultimately with increasing nitrogen supply. POL (1960) found that T.N. increased with the increase of nitrogen supply. He recorded a negative correlation between T.N.

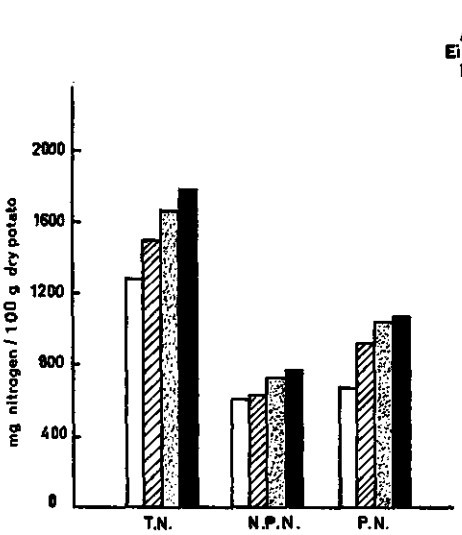


FIGURE 11. The average values of the total nitrogen, protein nitrogen, and non-protein nitrogen, expressed as mg nitrogen per 100 g dry patato.

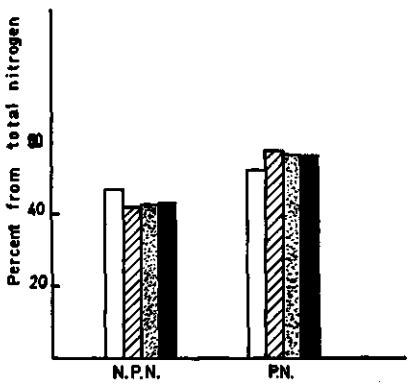


FIGURE 12. The average protein nitrogen and non-protein nitrogen as a percentage of the total nitrogen.

and starch content of the tuber. His results showed more T.N. as percentage of the dry matter, after storage than at harvesting. Moreover, POL's (1960) results indicated that the highest increase in T.N. during storage occurred in those potatoes which were most heavily dressed with nitrogen. The starch content on the other hand gradually dropped during the storage period. STREET, KENYON and WATSON (1946) found that with prolonged storage time a loss in the nitrogen content could be observed. They noted that in sprouting potatoes the tubers retained only about 20 % of their initial nitrogen content.

In the present investigation it was found that the T.N. content increased with increasing nitrogen supply. In figure 13 three curves are shown, obtained by plotting T.N. against nitrogen fertilization after different storage periods. The results showed that T.N. tended to increase in all the periods studied, with the increase of nitrogen supply.

On comparing the results obtained in the three periods, it is evident that after 8 weeks' storage the T.N. content as percentage of dry matter in potatoes dressed with 80 kg N dropped as compared with the starting curve. At the highest levels of nitrogen supply the curve representing the 8 weeks' period showed an apparent increase in T.N. The T.N. content of the tubers after 24 weeks' storage at 5° C, was generally lower than that of 0 and 8 weeks' storage. At 200 kg nitrogen supply, however, the T.N. content was somewhat higher than at 0 time. In broad lines this picture can be described in the following way: there are principally two periods, in the first the T.N.,

expressed as percentage of dry matter, increases and in the second this percentage decreases.

As will be discussed afterwards, germination of potatoes needs nitrogen to build up the protein in the sprouts. So it may be expected that the T.N. content of the tuber will be lower after storage than at 0 time. Nevertheless, this was not the case when potatoes of the highest nitrogen supply were considered. The factor that can cause the T.N. content of the tuber to rise, is a loss of dry matter. In this respect the respiration of potatoes is expected to be involved. POL (1960) showed that after storage the starch content dropped. In his experiments potatoes were stored for about 8 months at 4° C. Notwithstanding that in the course of storage some starch is converted into glucose, POL (1960) found that after storage the loss of total carbohydrates ranged from 0.5 % at 0 kg nitrogen supply to 4.2 % at 100 kg N/ha supply. He came to the conclusion that the higher the nitrogen dressing the higher the rate of respiration was during storage, and consequently the higher the losses of carbohydrates.

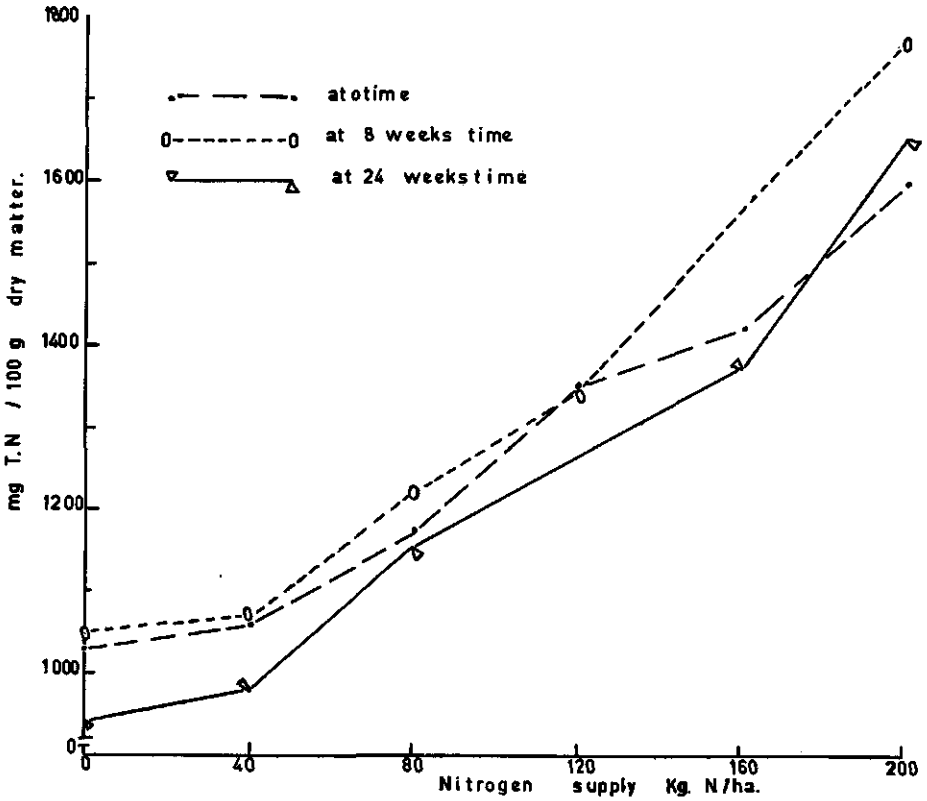


FIGURE 13. The total nitrogen content of the potato tuber in three storage periods, 0 time, 8 weeks, and 24 weeks, as affected by the nitrogen fertilization in the variety Bintje.

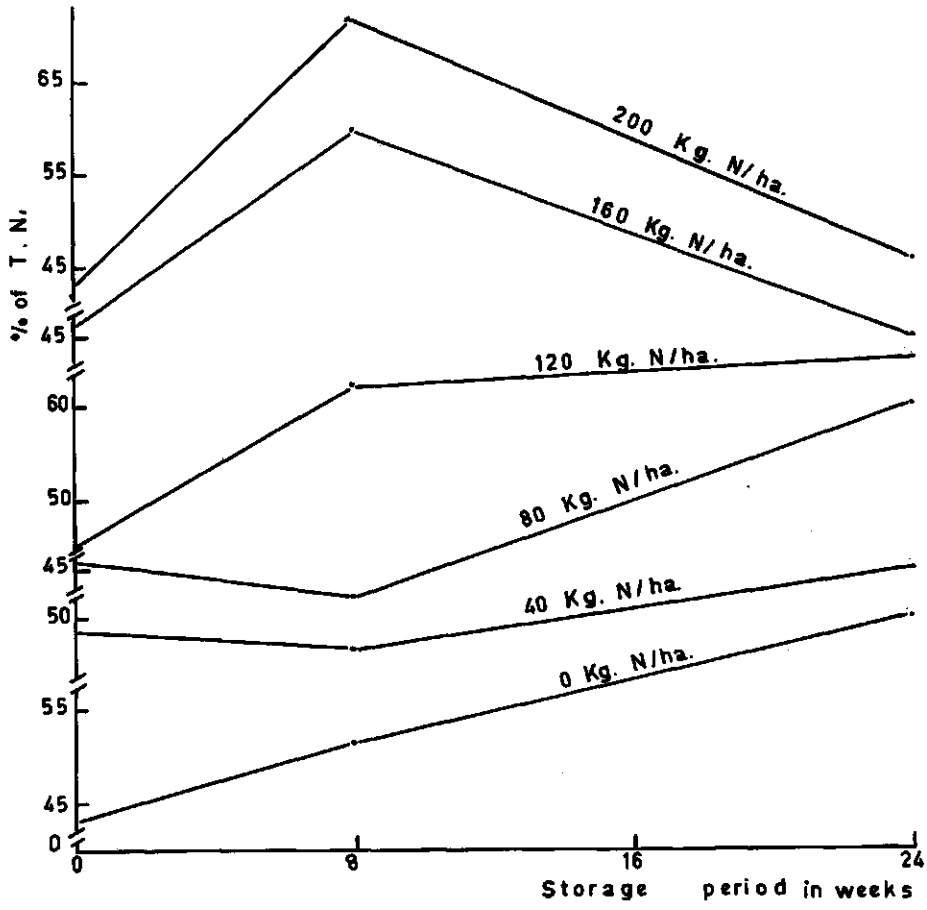


FIGURE 14. The effect of storage period on the protein nitrogen, as a percentage of total nitrogen, of six different nitrogen fertilization levels, in the variety Bintje.

From this it can be concluded that if the loss in T.N. due to germination is less than the loss in carbohydrate due to respiration, a rise in T.N. % will be recorded. On comparing results after 8 weeks with those at the beginning of the experiment, an increase in the T.N. content of the potatoes with high nitrogen dressing could be observed. This may be attributed to the loss of carbohydrates due to respiration, which consequently must have been higher in the potato tubers with the higher T.N. contents. If STREET, KENYON and WATSON (1946) observed so great a loss of nitrogen that the tuber retained only 20 % of the initial nitrogen, it may be assumed that the samples were in an advanced stage of germination. In the present work germination surely had its effect on T.N. at advanced storage time, especially at the lower nitrogen supply of the 24 weeks' samples. On the other hand SHALLENBERGER (1955) found a higher T.N. content in potatoes stored at room temperature than in potatoes stored at 32—40° F. This could be explained by the increase in the respiration rate at higher temperatures.

b. Protein and non protein nitrogen:

MULDER and BAKEMA (1956) found that with high nitrogen fertilization P.N. as a percentage of T.N. was lower, than with low nitrogen fertilization. SCHUPHAN (1959) confirmed the results obtained by MULDER and BAKEMA.

In the present study six levels of nitrogen supply ranging from 0 kg N/ha up to 200 kg N/ha, were included. Figure 14 shows the behaviour of P.N. as a percentage of T.N. during the storage period. The effect of each fertilization level is shown separately. From this figure it is evident that the behavior of P.N. is influenced both by the storage period and by the nitrogen fertilization. It can be noticed that at 0, 40 and 80 kg N/ha levels the protein proportion increased at the end of the storage period. This rise was gradual at the 0 kg N/ha supply, whereas at the other two levels the increase was only apparent in the last part of the storage period. At 120 kg N/ha the picture changed, in the first 8 weeks the rise was markedly followed by a steady flat level after 24 weeks. At 160 and 200 kg N/ha, the protein nitrogen showed a sharp rise in the first 8 weeks followed by a drop after 24 weeks. With the N.P.N. the reverse was true.

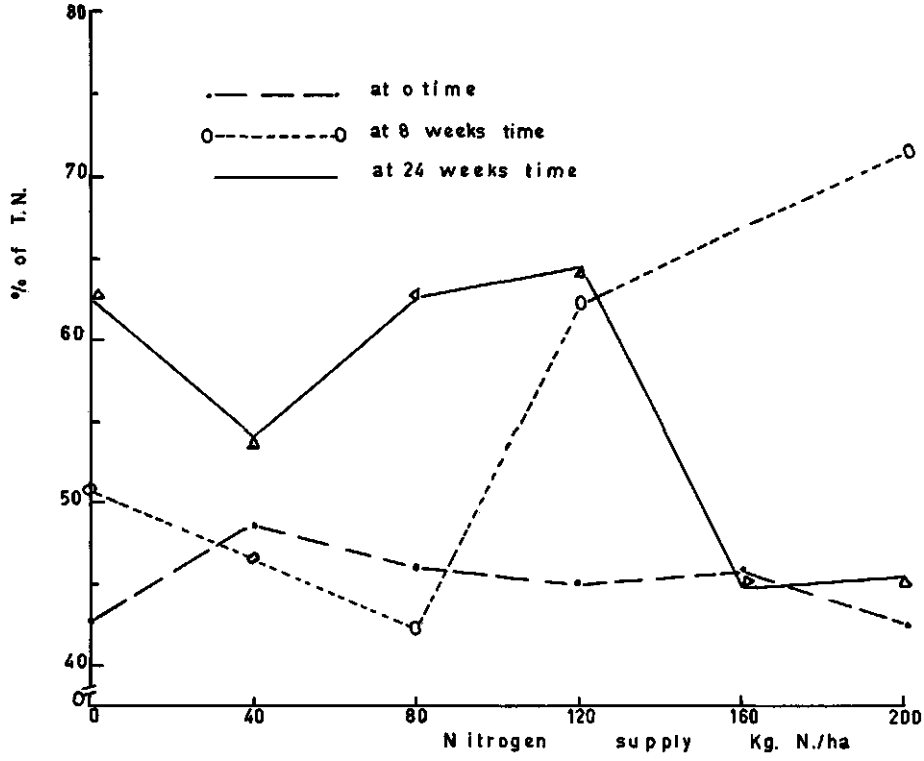


FIGURE 15. The effect of nitrogen fertilization on the protein nitrogen as a percentage of total nitrogen, shown in three storage periods, viz, 0 time, 8 and 24 weeks, in the variety Bintje.

In figure 15 the values of the P.N. relative to T.N. are plotted against the nitrogen supply at three different times of storage. It can be noticed that at the start of the experiment the highest P.N. : T.N. ratio was obtained at the 40 kg N/ha level, and the lowest value at 200 kg N/ha level. Dealing with the P.N. : T.N. ratio after 8 weeks, it can be seen that the lowest value was recorded at 80 kg N/ha, while the highest was encountered at 200 kg N/ha supply. After a 24 weeks' storage period at 5° C, the highest protein portion was noticed at the level of 120 kg N/ha, whereas at the levels of 160 and 200 kg N/ha the lowest portions of P.N. were obtained. In this respect MULDER and BAKEMA (1956) found that high nitrogen fertilization decreases the P.N. as a percentage of the T.N. MULDER (1949) studied the influence of mineral nutrition on protein and non-protein nitrogen fractions. Table 16 was estimated from his results.

TABLE 16. The effect of nitrogen fertilization on the P.N. expressed as percentage of T.N., (estimated from MULDER's (1949) results).

Date of sampling	experiment nr 589 (1946) fertilization system				experiment nr 907 (1946) fertilization system				the corresponding time
	K_2O N	300 O	K_2O N	300 300	P_2O_5 N	500 O	P_2O_5 N	500 300	
July	64.6		55.2		66.0		59.0		at harvest
October	70.5		—		69.0		60.0		at harvest
March	65.7		52.5		67.0		58.0		stored at 10° C for 5 months

From these figures given in the table it appears that under the circumstances of the experiment the influence of storage was not at all apparent in the proportion of P.N. : T.N. The ratio was rather stable, from which it might be concluded that the nitrogenous substances did not undergo any change during storage.

On the other hand, POL (1960) suggested from his results that the P.N. as a percentage of T.N. decreased with increasing T.N. content of the tuber, the greatest losses in P.N. during storage seemed to occur in tubers rich in T.N. after about 5 months storage. His figures showed that in samples at harvest time a rather stable level or, in one experiment, even an increase in P.N. on increasing the nitrogen supply could be found.

From the foregoing discussion the results obtained in the present study confirm that nitrogen fertilization affects slightly the P.N. : T.N. ratio, i.e., at 40 kg N/ha the P.N. as a percentage of the T.N. was 49 %, at 200 kg N/ha it decreased to 42.5 %. On the other hand the changes in the protein portion during the storage seemed to be much higher than those resulting from the nitrogen fertilization. It must not be neglected that during storage, where respiration and germination had altered the composition of the tubers owing to catabolic and anabolic reactions, the nitrogenous substances are

involved for an important part. It can be seen from figure 14, that a sample taken after 8 weeks' storage which received a nitrogen supply of 40 kg N/ha, contained 46 % of its T.N. as P.N., whereas a sample which received a higher nitrogen dressing of 160 kg N/ha, had a content of 60 % of its T.N. as P.N. The variations after 24 weeks of storage were remarkable. A sample of the 40 kg N/ha level showed a P.N. content of 54 % of T.N., whereas a sample of 160 kg N/ha supply contained only 45 % P.N. as percentage of T.N. In the former case the conclusion could be drawn that P.N. was increasing with nitrogen supply. In the latter case the reverse conclusion is not less true. Considering these findings, it is evident that the date of sampling is of great importance. MULDER and BAKEMA (1956) analyzed samples after 4 months' storage, and MULDER (1949) used samples at harvest time and after 5 months' storage. Their conclusions in this respect can be compared with the results obtained in the present study after a 24 weeks' storage period.

3. Effect of storage time and temperature on the nitrogen distribution in the potato varieties Alfa, Eigenheimer and Kwinta.

a. Total nitrogen:

Table 17 shows the effect of the storage period and temperature on the T.N. content of the tuber. The general trend is not the same throughout the whole storage period. After 8 weeks' storage there was a tendency for T.N. to decrease with increasing temperature. This was the case up to 10° C, after which a rise was noticed at 15° C. After a 24 weeks' storage period T.N. content decreased ultimately, with increasing temperature.

TABLE 17. Total nitrogen content of the potato tuber as influenced by the storage period and temperature.

storage period in weeks	T.N. mg/100 g dry matter													
	Alfa					Eigenheimer					Kwinta			
		Storage Temperature					Storage Temperature					Storage Temperature		
		2° C	6° C	10° C	15° C		2° C	6° C	10° C	15° C		2° C	6° C	10° C	15° C
start	1650					1690					1805				
8		1617	1429	1132	1309		1547	1653	1574	1579		1804	1861	1433	1793
24		1693	1461	1528	1251		1780	1720	1723	1631		1763	1891	1796	—

Losses of nitrogen may be attributed to germination and the increase in nitrogen content may be the result of carbohydrate losses during respiration. This was fully discussed when the experiment of the effect of nitrogen fertilization was treated.

b. Protein nitrogen:

The protein nitrogen will be considered as the percentage of the T.N. Since P.N. and N.P.N., expressed as percentages of T.N., change at each others expense, only one of them will be discussed. In the following discussion the P.N. will therefore be treated. The results obtained of the effect of the storage period and temperature on the three varieties studied are plotted in figure 16. The idea of eliminating one of the two factors, viz. period and temperature of storage, in order to obtain discussable averages was rejected. The reason for this will be clear when the trends of the curves obtained are observed. Each temperature and each period had a special trend, which would give a misleading tendency in case of the values being averaged.

During the storage of potatoes the changes taking place in the nitrogenous substances, seemed to be due to the interaction of the following factors:

1. variety
2. storage period
3. storage temperature
4. germination

1. Variety:

Some varieties are known to have good storage qualities, while others have not. It is believed that the reactions that are taking place in the potato tuber will be more or less the same in all varieties, with some differences in the reaction rates. Such differences in reaction rates may be responsible for the apparent changes between varieties.

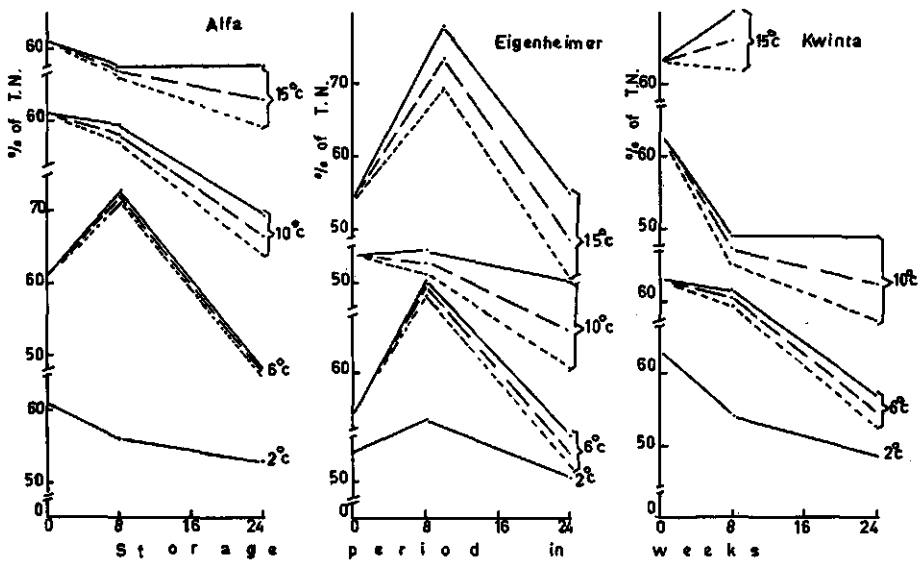


FIGURE 16. The effect of storage period and temperature on the protein nitrogen as a percentage of total nitrogen, in the varieties Alfa, Eigenheimer, and Kwinta. ———, The determined ratio, — — —, the ratio if the nitrogen content of the sprouts was one time as that of the tuber, - - - - - - - -, the ratio if the nitrogen content of the sprouts was two times as that of the tuber.

2. Storage period:
   Protein synthesis is likely to take place during the dormant phase of the tuber, especially at the beginning of the storage period when no signs of germination appear. If the potato tuber is old, the power to synthesize proteins is diminished or overlapped by the increased breakdown. In this connection, MULDER (1955 and 1956) working with potato slices, showed that the respiration rate is more pronounced in new potatoes than in old ones.
   In the introduction of this part, the relation between respiration and protein synthesis was briefly discussed. There, the power of protein synthesis could be shown to be slower in old potatoes than in new ones.
3. Storage temperature:
   Higher temperatures will enhance the reactions occurring in the tuber, in other words, they will enhance the processes of synthesis as well as breakdown of proteins. Likewise, germination will start earlier at higher temperatures than at lower temperatures.
4. Germination:
   The new sprouts will need nitrogen that can be supplied by the tuber, to build up their special proteins. The nitrogen content of sprouts is also expected to be higher than that of the tuber. These losses in nitrogen will explain the loss of N.P.N. from the tuber. However, the situation remains complicated owing to other reactions which are taking place at the same time.

If these four factors are assumed to react the complicated picture obtained can be analyzed in the following way:
First, it has to be mentioned that the 0 time of the storage experiment in the present study does not reveal the composition of the tuber at harvesting time, which actually took place 8 weeks earlier, so it would be expected that at 0 time of the experiment, the protein content of the tuber was already higher than that at harvest time. The 8 weeks' sample of the experiment corresponded with the 16 weeks' period from harvest time. It is therefore believed that at this time the ability of the protein synthesis was weaker than before.
At 2° C the activity of protein synthesis as well as that of protein breakdown was depressed. It is probable that the bulk of protein synthesis occurred during the period of 8 weeks before storage when the temperature was ranging between 8 and 10° C. At 2° C the potency of the tubers to synthesize proteins decreased. Moreover it seems that cooling at 2° C depresses the synthesis more than the breakdown of protein. In Eigenheimer a small increase was observed after 8 weeks followed by a decrease after 24 weeks. With Alfa and Kwinta the curves showed a declining tendency throughout the whole period, which became rather flat at the end. No germination appeared at this temperature.
At 6° C the reaction rate was expected to increase, in accordance with which protein synthesis was apparent after 8 weeks. At this time the weights of

the sprouts were 0.4 %, 1.1 % and 1.9 % of the tuber weight for Alfa, Eigenheimer and Kwinta respectively.

Alfa and Eigenheimer showed a rise in the ratio of P.N. : T.N. In Kwinta the protein level was rather stable. This does not mean that no synthesis occurred in Kwinta, but it does indicate that the rate of breakdown was somewhat higher in Kwinta than in the other two varieties. In all the three varieties a decrease occurred after 24 weeks, which might be attributed to the activity of the breakdown of proteins.

At 10° C synthesis as well as breakdown were enhanced. The percentages of the sprouts after an eight weeks' period were 2.2, 3.6 and 4.4 % of the potato weight for Alfa, Eigenheimer and Kwinta respectively. In Alfa and Eigenheimer the graphs showed declining curves, but the decrease was not very pronounced during the first period. Later on excessive sprouting appeared which required more nitrogen. This was supplied by the N.P.N. fraction which in turn was supplied by the breakdown of the proteins. In Alfa the rate of consuming the nitrogen from the tuber (N.P.N.) seemed to be less great than that of the protein breakdown. The speed of sprouting seemed to be more advanced in Eigenheimer than in Alfa, so the curve was decreasing but more flatty than that of Alfa. This will explain that in this case the rate of depleting the N.P.N. of the tuber is higher than in Alfa. It is clearly demonstrated in the graph that the breakdown of proteins proceeded more rapidly in Kwinta. A sharp drop in the P.N. portion was noticed in the first period. Afterwards the level of P.N. seemed to be rather stable, which did not mean that the metabolic processes in the tuber stopped but merely indicated that the rate of breakdown was compensated by the transfer of nitrogen to the sprouts from the N.P.N. fraction.

At 15° C Alfa showed more or less, a similar equilibrium as that noticed at the final period of Kwinta at 10° C. With Eigenheimer a rapid germination could already be observed in the first 8 weeks of the storage period. The loss of the N.P.N. due to sprouting was more pronounced than with Alfa. This loss of N.P.N. had undoubtedly given too high figures for P.N. : T.N. ratio. Nevertheless, on eliminating this factor, by assuming a nitrogen content of the sprouts one or two times as high as the nitrogen content of the tuber, a clear synthesis of protein during storage could be observed. After 24 weeks the P.N. : T.N. ratios underwent a sharp decrease, which might be attributed to a dominating protein breakdown. Kwinta also showed the same trend as Eigenheimer after 8 weeks' storage. After 24 weeks, the sprouting had proceeded so far that no chemical analyses on the tuber could be carried out.

From the foregoing discussion, it can be observed that the processes of protein synthesis and breakdown take place together but with different rates. The loss of the nitrogen from the N.P.N. fraction of the tuber in favour of the sprouts, will theoretically result in a decrease of the N.P.N. fraction and consequently, an increase in the P.N. : T.N. ratio.

Yet this phenomenon was not the case due to the other catabolic reactions taking place such as protein breakdown, which will not only decrease the

amount of P.N., but also will supply the N.P.N. with the protein breakdown products, amino acids, which will alternatively increase the N.P.N. portion. These processes as has been mentioned before, are believed to participate in all the varieties, but with different rates.

B. THE AMINO ACID DISTRIBUTION

Only a small number of investigators have so far studied the amino acid content of the potato tuber. Most studies were carried out on the whole tuber without a differentiation between the free amino acids and the amino acids of the proteins. MULDER and BAKEMA (1956) as well as THOMPSON and STEWARD (1952) studied the amino acid contents of the two fractions. Previously some other workers such as SLACK (1948), GROOT (1947) and SJOLLEMA and RINKES (1912) confined themselves to a study of the amino acid pattern of the potato tuber protein. The amino acid composition of the N.P.N. was also studied. DENT, STEPKA and STEWARD (1947) could identify cystine, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, γ-amino butyric acid, histidine, arginine, lysine, proline, valine, methionine, isoleucine, phenylalanine, tryptophan and tyrosine in the N.P.N. fraction. In their study on the browning reaction of potato, HABIB and BROWN (1957) determined some amino acids in the N.P.N. fraction. SZALAI (1959) studied the amino acid content of the N.P.N. of the potato as influenced by sprouting.

The aim of the present study was to investigate the influence of some factors on the ten essential amino acids present in the protein and the non-protein nitrogen fractions of the potato tuber. The composition of the potato after harvesting changes during storage. During storage the germination of the tubers starts after different periods of time depending on the storage temperature, the humidity of the atmosphere and the variety. The germination will surely cause many changes in the composition. The sprouts will need nitrogen to build up their special proteins. This nitrogen will be removed from the free amino acids in the potato tuber. The depleted N.P.N., will be replenished by the products of the protein breakdown. Due to this and to other processes, the composition of the tuber may alter considerably. A study therefore was carried out on the effect of different conditions of storage. Since mineral nitrogen is the source of nitrogen for plants, it was also of interest to know whether different levels of nitrogen supply have any influence on the distribution of the amino acids.

1. General pattern of the amino acids.

The average values of the ten essential amino acids in P.N. and N.P.N. fractions (expressed as gram amino acid per 16 gram nitrogen) of the four potato varieties studied, are shown in figure 17.

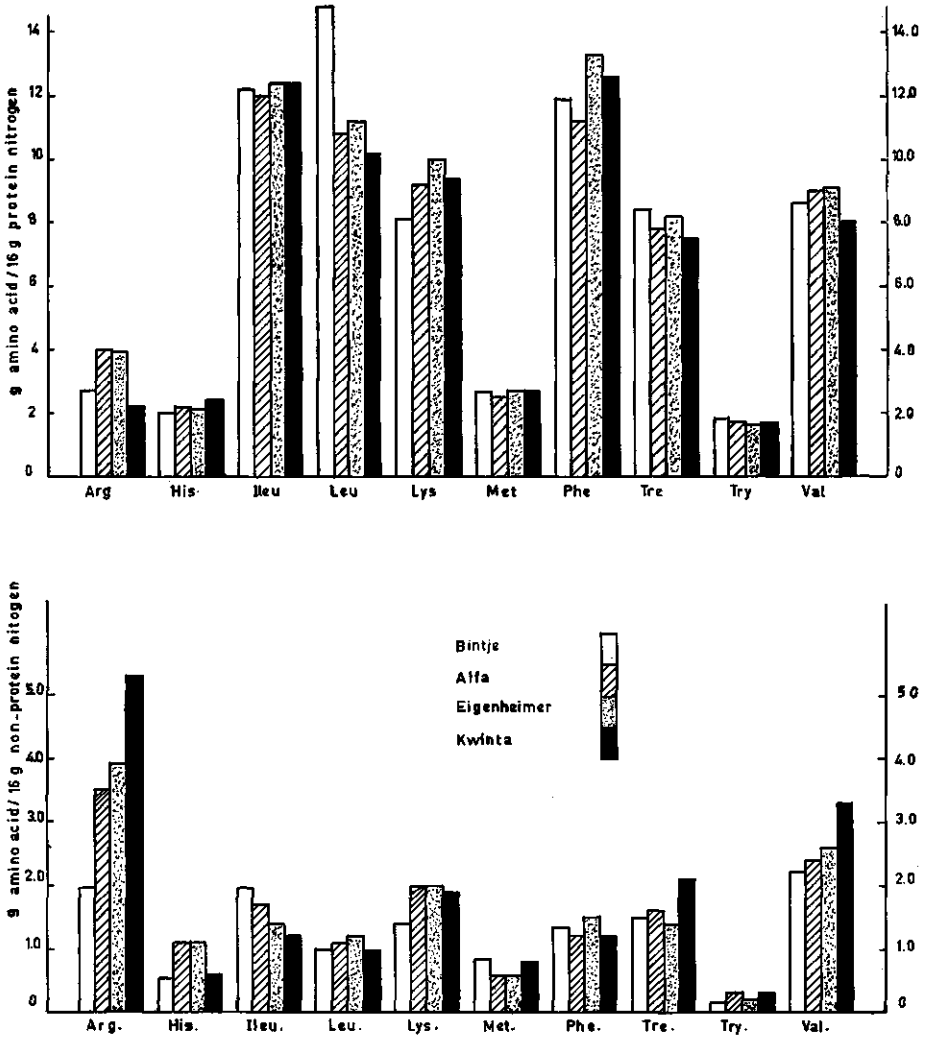


FIGURE 17. The average values of the ten essential amino acids determined in the protein and non-protein nitrogen fractions in the varities Alfa, Eigenheimer, Kwinta and Bintje.

a. The protein amino acid:

The differences between the protein-amino acid pattern of the four varieties is apparent.

This is in agreement with the fact that the different varieties contained variable quantities of different protein fractions obtained by electrophoresis. Thus it is quite rational to assume that the amino acid composition of these different proteins is not the same.

It is difficult to compare the results obtained, with those found by other investigators, because the materials and treatments are different. A general idea, however, with regard to the variations could be ascertained on comparing the results available. Table 18 shows the range of values obtained in the present study and the values obtained by other investigators, all of which are expressed as gram amino acid per 16 gram of protein nitrogen.

TABLE 18. The amino acid content of the potato protein as determined by different investigators, expressed as gram amino acid per 16 gram protein nitrogen.

Amino acid	present study				MULDER & BAKEMA (1956) Range of		THOMPSON and STEWARD (1952)	GROOT (1947) Noordeling	NAGASE (1957)	SJOLLEMA and RINKES (1912)	CHICK and SLACK (1949)
	Kwinta	Alfa	Bintje	Eigenheimer	Noordling	Crosses [1])					
Agr.	4.0	2.7	3.9	2.2	3.2— 5.8	3.6— 4.0	6.0	4.8	6.6	4.2	6.0
His.	2.2	2.0	2.1	2.4	2.4— 3.1	—	—	2.2	3.7	2.3	2.2
Ileu.	12.0	12.2	12.4	12.4	15.2—17.9	17.4—20.2	17.6	14.6	5.4	12.2	17.5
Leu.	10.8	14.8	11.2	10.2					7.5		
Lys.	9.2	8.1	10.0	9.4	3.7— 5.0	3.2— 3.9	8.0	3.6	8.3	3.3	7.7
Met.	2.5	2.7	2.7	2.7	1.9— 3.1	1.8— 1.9	1.5	2.7	3.4	—	2.3
Phe.	11.5	11.9	13.3	12.6	5.0— 5.5	—	12.7	5.9	7.4	3.9	6.6
Thr.	7.8	8.4	8.2	7.5	5.1— 6.7	3.3— 4.3	3.2	6.9	5.4	—	5.9
Try.	1.7	1.8	1.6	1.7	1.8— 2.0	—	—	2.3	2.0	—	1.6
Val.	9.0	8.6	9.1	8.0	5.9— 7.6	7.8— 8.9	7.7	7.6	5.4	1.1	6.1

[1]) Crosses between different *salanum* especies and *salanum tuberosum.*

The values reported by different workers of each amino acid showed great differences. Arginine varied from 3.2 to 6.6 g/16 g P.N. In the present study, the range of arginine was between 2.2 and 4.0 g/16 g P.N. Histidine, methionine and tryptophan values did not differ much. Isoleucine and leucine figures were higher in this study than those reported by other investigators. MULDER and BAKEMA (1956), GROOT (1947) and SJOLLEMA and RINKES (1912) gave rather low values for lysine, whereas CHICK and SLACK (1949), THOMPSON and STEWARD (1952) and NAGASE (1957) found a higher lysine content which is in agreement with the findings of the present work. THOMPSON and STEWARD (1952) gave high results for phenylalanine as was found also in the present study.

b. The N.P.N. amino acids:

The free amino acids are closely related to the production of browning of potato, in chips and fried potatoes. HABIB and BROWN (1957) found a correlation coefficient of —0.95 for the relation between basic amino acids present and the colour obtained. They stated: "This would indicate that basic amino acids are more effective in colour formation than neutral or acidic amino acids". MULDER (1956) found that the free amino acid tyrosine plays an important part in the blackening of the raw potato tissues, yet it is not the sole determining factor.

MULDER and BAKEMA (1956) found that the variations in the N.P.N. amino acids were higher than in those of the protein fraction. SZALAI (1959) studied the behaviour of the free amino acids in the potato tuber as influenced by forced germination. He divided the tuber into four sections, viz. "opical, girdle, basal and pith". He concluded that in various parts of the tuber the rate of change in the amount of each particular amino acid during germination was dependant on the variety and the age of the tuber.

The results of the present study are plotted in figure 17, four varieties were included. For the sake of comparison the results obtained in this investigation, those of MULDER and BAKEMA (1956), and those of THOMPSON and STEWARD (1952) are shown together in table 19. It must be emphasized that a comparison of average values only gives a general idea of the variations that may occur owing to different varieties and conditions.

TABLE 19. The average values of the ten essential amino acids in the N.P.N. fraction obtained for the four potato varieties investigated, as compared with the results of MULDER and BAKEMA (1956) and THOMPSON and STEWARD (1952), expressed as gram amino acid per 16 gram N.P.N.

Animo Acid	The present study				Mulder and Bakema (1956)		Thompson & Steward (1952)
	VARIETY						
	Alfa	Bintje	Eigen-heimer	Kwinta	Noorde-ling	Voran	Sebago
Arg.	3.5	2.0	3.9	5.3	3.3	4.1	4.6
His.	1.1	0.5	1.1	0.6	1.5	1.4	—
Ileu.	1.7	1.6	1.4	1.2	1.8	2.2	1.3
Leu.	1.1	1.0	1.2	1.0			
Lys.	2.0	1.4	2.0	1.9	tr.	tr.	0.8
Met.	0.6	0.9	0.6	0.8	tr.	tr.	1.1
Phe.	1.2	1.3	1.5	1.2	1.0	1.3	1.8
Thr.	1.6	1.5	1.4	2.1	0.5	0.5	1.3
Try.	0.3	0.2	0.2	0.3	—	—	—
Val.	2.4	2.2	2.6	3.3	2.3	3.0	3.2

From the above results, it can be noticed that there are many variations in the quantities of the different amino acids in the N.P.N. fraction in the different varieties. The results of MULDER and BAKEMA (1956) also showed many variations between the two varieties studied. SZALAI's (1959) results could not be compared with the data in table 19. In the first place he worked with fractions of potatoes and secondly the figures he gave were related to the quantities of solution he used in analysing, no figures being given for amino acids per unit of nitrogen. Nevertheless, the graphs given by him showed that the variation due to germination was also unique to each variety. For example, he found a drop in the content of aspartic-acid-glycine, glutamic acid-glutathione, tryptophan - valine - γ amino-butyric acid and tyrosine after 8 days' treatment with "Rindite" in the variety Kivarda Rozso. With the variety Ella, however, the first three groups showed an increasing tendency. This was explained as the effect of variety differences.

2. The effect of nitrogen fertilization and storage at 5° C with regard to the amino acid content of the potato tuber, in the variety Bintje.

a. Protein amino acids:

MULDER and BAKEMA (1956) investigated the effect of mineral nutrition on the amino acid content of the potato proteins of the variety Noordeling. They stated: "The main conclusion of the present investigation is that the amino-acid composition of potato protein is independant of the mineral nutrition of the plants. This was found to be true of nitrogen, phosphorous and potassium supply."

Figure 18 shows the results obtained in the present study. It can be noticed that the effect of the storage period on the amino acid content of potatoes supplied with different levels of nitrogen is obvious. Table 20 shows the average values together with the coefficient of variation (SNEDECOR, 1959) of the ten essential amino acids as influenced by nitrogen fertilization in the three storage periods. At first examination the coefficient of variation ranged from 7.6 % for phenylalanine to 23.6 % for leucine. The variation in the second period after 8 weeks ranged from 2.7 to 13.3, if arginine content was excluded, it showed a variation of 57.8 %. After 24 weeks the variation ranged from 8.2 % to 19.3 %. This variation, which may be considered as a response to nitrogen fertilization, is not great if compared with the effect of the storage. It can be noticed in figure 18 that arginine content after 24 weeks showed a tendency to increase, which was remarkable at the level of nitrogen supply of 160 and 200 kg N/ha, whereas at 80 and 120 nitrogen levels a weak trend to decrease was observed. Histidine, however, showed a rather constant picture with the same trend for all the different levels of nitrogen supply, i.e. a small rise after 8 weeks followed by a small decrease after 24 weeks. With isoleucine a rising tendency could be noticed with the advance of the storage period, whereas leucine showed a tendency to decrease.

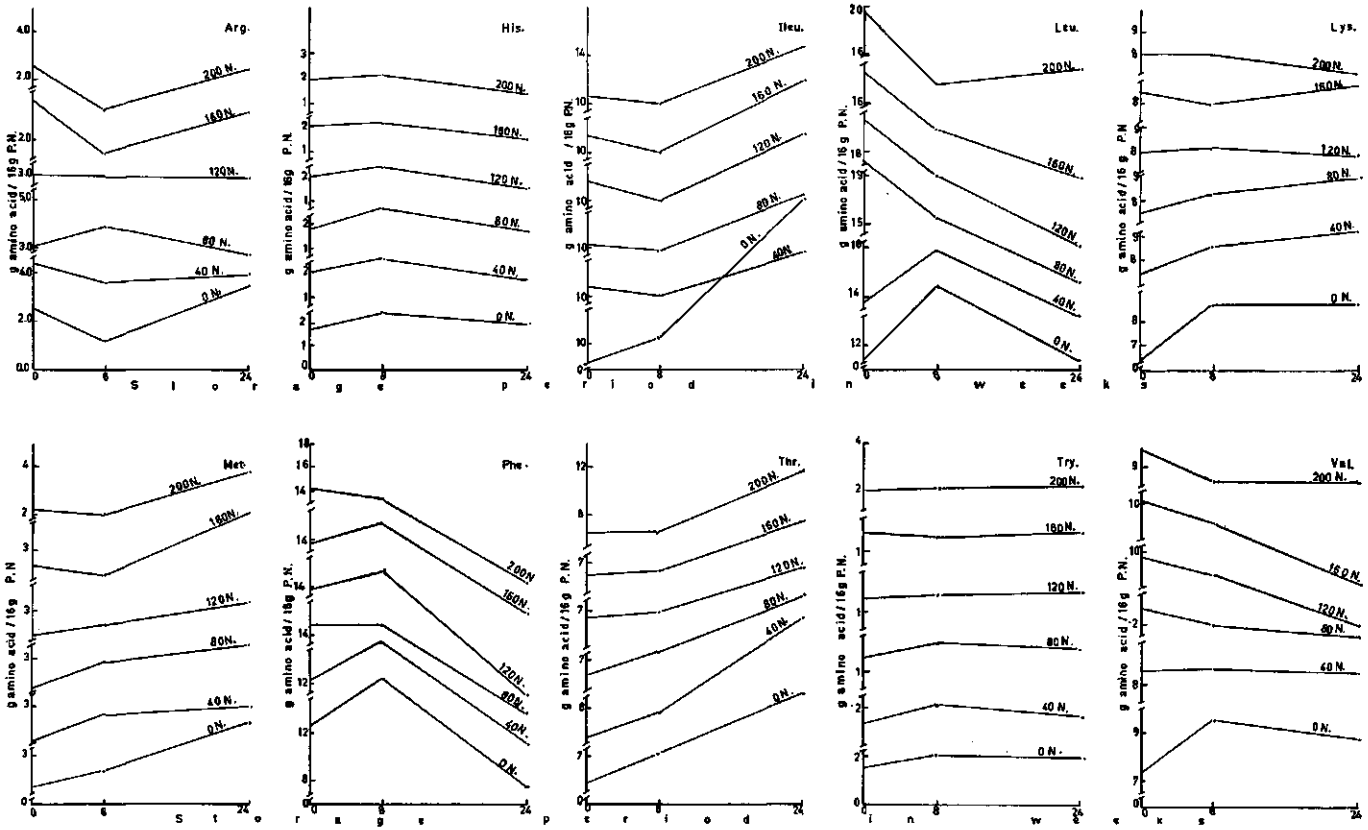


FIGURE 18. The effect of storage on the amino acid content of the protein nitrogen of potatoes treated with different nitrogen fertilization levels in the variety Bintje.

TABLE 20. The average values and the coefficient of variations (C.V. %) of the protein amino acids in the variety Bintje as influenced by fertilization during three storage periods.

Animo Acid	0 time		8 weeks		24 weeks	
	average	C.V. %	average	C.V. %	average	C.V. %
	g/16 g P.N.		g/16 g P.N.		g/16 g P.N.	
Arg.	2.9	17.2	2.3	57.8	3.1	17.5
His.	1.9	8.2	2.4	8.3	1.6	11.6
Ileu.	10.6	10.4	10.0	2.7	16.1	19.3
Leu.	17.4	23.6	15.7	9.6	11.4	16.8
Lys.	7.7	9.5	8.3	3.6	8.3	8.2
Met.	2.0	14.6	2.4	13.2	3.8	13.2
Phe.	13.6	7.6	15.3	6.1	6.8	13.8
Thr.	5.9	11.4	7.1	7.3	11.9	9.5
Try.	1.7	11.4	2.0	13.5	1.9	9.7
Val.	9.1	11.1	8.9	5.6	7.8	10.9

The changes in lysine are not so pronounced as those in isoleucine and leucine. After 8 weeks all the levels showed a rise in lysine content except the 160 and 200 kg N/ha levels, which showed a small decrease. After 24 weeks the lysine values did not differ much from those of the 8 weeks' samples. With methionine, however a rising tendency was recorded with the advance of the storage period. A small increase in phenylalanine occurred after 8 weeks with the nitrogen supply levels of 0, 40, 120 and 160 kg, whereas with 80 kg N/ha a stable curve and with 200 kg a declining one was observed. After 24 weeks all the levels showed sharply declining curves. Threonine showed a tendency to increase with the advance of the storage period. Tryptophan altered only slightly. Valine, however, showed declining curves all over the storage period and with all the nitrogen levels except with the 0 kg N/ha, where a preliminary rise was noticed.

Summarizing these results it can be said that on the whole arginine, isoleucine, methionine, lysine, threonine and tryptophan tended to rise during storage, whereas the curves of histidine leucine, phenylalanine and valine showed a tendency to decline. The magnitudes of these changes were such that after 24 weeks of storage the average isoleucine increased by 60 %, methionine by 90 %, threonine by 100 %, and tryptophan by 12 %. On the other hand, the mean content of histidine decreased by 15 %, leucine by 35 %, phenylalanine by 50 % and valine by 14 % after 24 weeks storage. The remaining amino acids changed no more than 10 %. MULDER and BAKEMA (1956) studied the effect of nitrogen supply on the potato protein composition. No important variations were found. They analyzed two samples, one with poor nitrogen supply (40 kg N/ha), the other with ample nitrogen (160 kg N/ha). The results of MULDER and BAKEMA are summarized in table 21.

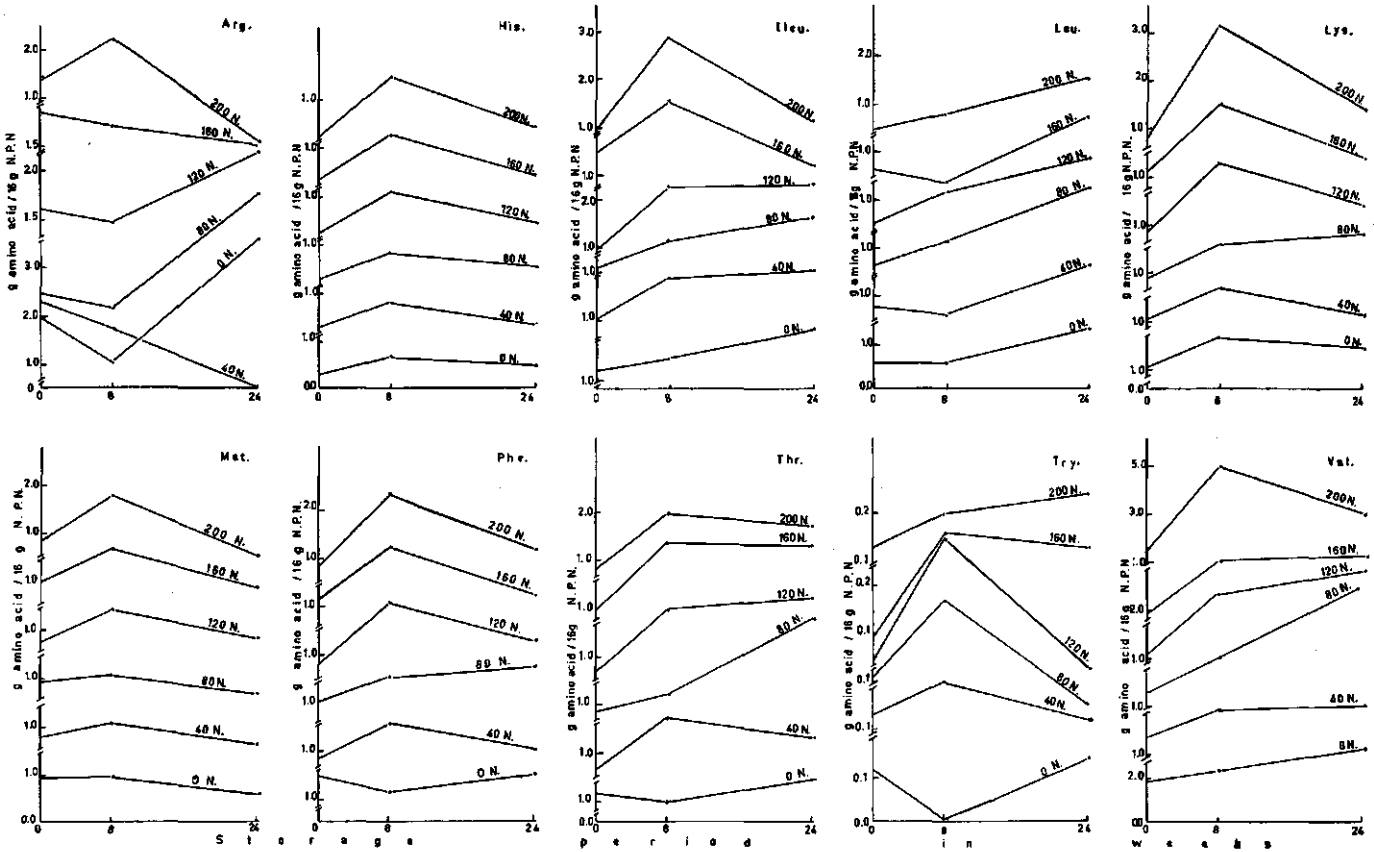


FIGURE 19. The effect of storage time on the N.P.N. amino acids content in potatoes receiving different quantities of nitrogen supply, in the variety Bintje.

for arginine, histidine, isoleucine, metheonine and phenylalanine, whereas leucine, lysine and valine showed a rather stable content. Threonine and tryptophan increased together with nitrogen supply at 24 weeks.

The effect of storage is demonstrated in figure 19. Arginine tended to increase with the advance of the storage period at 0, 80 and 120 kg N/ha, whereas it decreased after 24 weeks at 40, 160 and 200 kg N/ha. For histidine, the same picture was found with all the levels used; i.e. an increase after 8 weeks was followed by a small decrease after 24 weeks of storage. The 8 weeks' samples showed an increase in isoleucine content, which later on became rather stable in the levels 0, 40, 80 and 120 and changed into a decrease at the levels 160 and 200 kg N/ha. The leucine content of the N.P.N. fraction with all the levels of nitrogen supply was higher after 24 weeks than at 0 time. Lysine, in all the nitrogen fertilization levels, showed an increase after 8 weeks followed by a decrease after 24 weeks, except for the treatment of 80 kg N/ha where a small increase occurred. The trend in methionine content was the same with all the nitrogen levels, i.e. a small increase after 8 weeks was followed by a small decrease for the 24 weeks' samples. For phenylalanine an increase was recorded after 8 weeks with all the nitrogen levels, except with the 0 kg N/ha level, where a decrease was observed. The 24 weeks' samples showed a decline, except for the 0 and the 80 kg N/ha level samples, which increased slightly. With all the nitrogen supply levels the threonine content tended to rise after 8 weeks storage, except with 0 kg N/ha, where a small drop took place. This was followed by a small increase with the 0 and 120 kg N/ha levels, a rather sharp one with the 80 kg N/ha level and a slight drop with 40, 160 and 200 kg N/ha levels. The tryptophan content of the unfertilized sample decreased after 8 weeks and increased afterwards. The samples of the other five nitrogen levels showed an increase after 8 weeks, which continued with the 200 kg N/ha level, but with the four remaining levels a drop was observed. With the first five nitrogen levels, valine showed a tendency to rise as the storage period progressed. With the 200 kg N/ha sample the preliminary increase recorded after 8 weeks was followed by a decrease in the content of valine after 24 weeks' period.

The variations in the N.P.N. amino acids are rather high. MULDER and BAKEMA (1956) recorded similar variations due to nitrogen fertilization. They found that tubers from plants with a liberal nitrogen supply, which generally had a considerably higher content of N.P.N., contained a major proportion of this fraction in the form of amides. The amide content was smaller in plants with a poor or a moderate nitrogen supply.

The present work being carried out to study the composition of the potato in relation to nutrition rather than to plant physiology, it is beyond the scope of this publication to enter into details about all the physiological reactions occurring in the potato during storage.

It may be suggested, however, that the lower content of the free amino acids found at 0 time in those samples which were dressed excessively with nitrogen, should be attributed to the increased amide content which produces a higher

T.N. In the second period, after 8 weeks, the amino acids started to increase. It may be assumed that, in this period with the onset of sprouting a big quantity of amides may be used as the nitrogen source for the sprouts' proteins. This will cause the content of the other amino acids to increase. Sprouting is, however, far more advanced at the highest levels of nitrogen supply than at the lowest. Moreover, if figure 15 is consulted it can be seen that at low nitrogen supply the P.N. : T.N. ratio was higher than that at 200 kg N/ha. This implies that some proteolysis of the proteins already occurred and, besides, increased the amount of the free amino acids. The third period, which was rather irregular with regard to the amino acid content, might be a result of the many processes which took place simultaneously in this advanced period of storage.

3. The effect of storage time and temperature on the amino acid pattern of the potato tuber in the varieties of Alfa, Eigenheimer and Kwinta.

a. The amino acids of the protein:

The values of the ten essential amino acids determined are given in figures 20 and 21. It can be noticed that for each amino acid the shapes of the curves were nearly the same in the three varieties. At lower temperatures arginine dropped at first, afterwhich a rise was recorded, at higher temperatures a drop was noticed with the advance of the storage period. Methionine, lysine, and threonine showed a tendency to rise with the progress of the storage period. Leucine, phenylalanine and valine were decreasing. Histidine and tryptophan were rather constant. These results are in full agreement with the results of the fertilization and storage experiment discussed before. It may also be mentioned that the effect of the high temperatures did not alter so much the composition of the protein amino acid except that of arginine.

b. The N.P.N. amino acids:

Figures 22 and 23 illustrate the behaviour of the amino acid content of the N.P.N. It can be noticed that all the amino acids of the N.P.N. fraction increased after 8 weeks except arginine which decreased. In this respect MULDER and BAKEMA (1956) concluded that arginine, like the amides, is apparently associated with ammonia storage. If this is true, it should be expected that arginine behaves as the amides towards protein synthesis and the supplying of new sprouts with nitrogen. Consequently, the fluctuations due to different temperatures that occurred in the three varieties, would reflect the changes in the reaction of various processes, i.e. synthesis and protoelysis of proteins. After a 24 weeks' storage the amino acid levels in the N.P.N. fraction were higher than at the beginning.

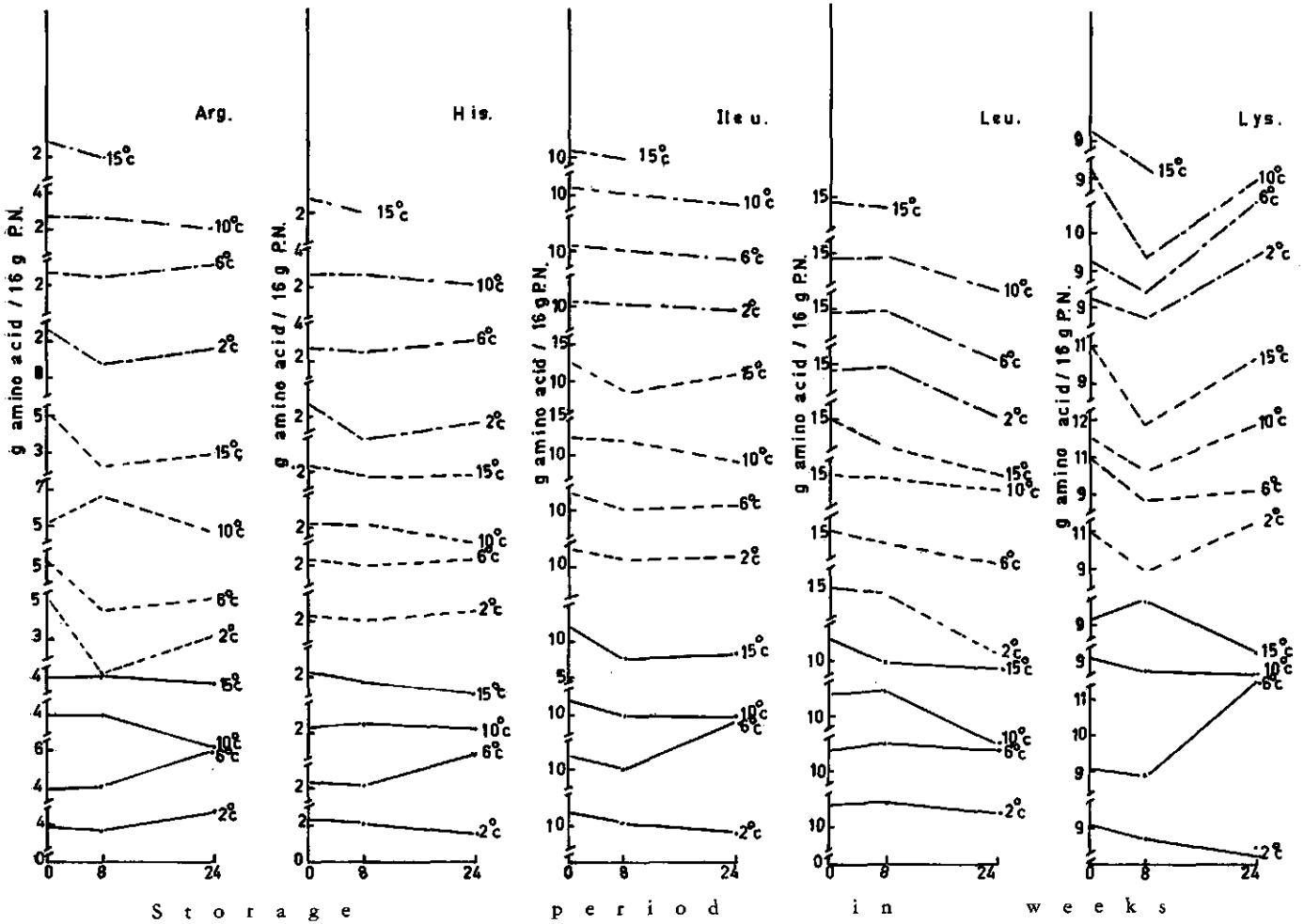


FIGURE 20. The effect of the storage period on the protein amino acids, arginine, histidine, isoleucine, leucine and lysine in the potato varities Alfa ————, Eigenheimer ------, and Kwinta —·—·—·—, stored at 2°, 6°, 10° and 15° C.

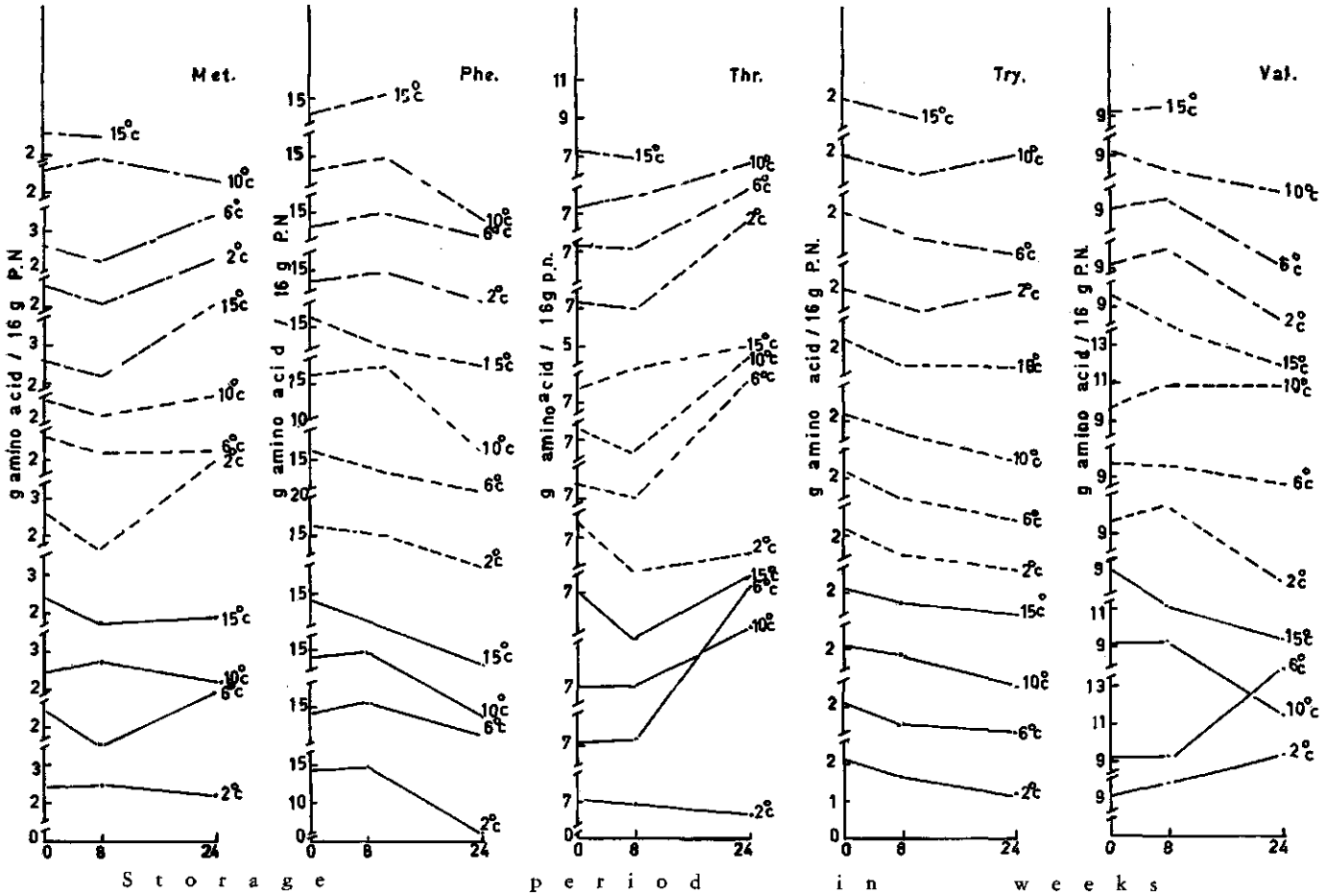


FIGURE 21. The effect of the storage period on the protein amino acids methionine, phenylalanine, threonine, tryptophan and valine in the potato varieties Alfa ———, Eigenheimer ------, and Kwinta —·—·—·—, stored at 2°, 6°, 10° and 15° C.

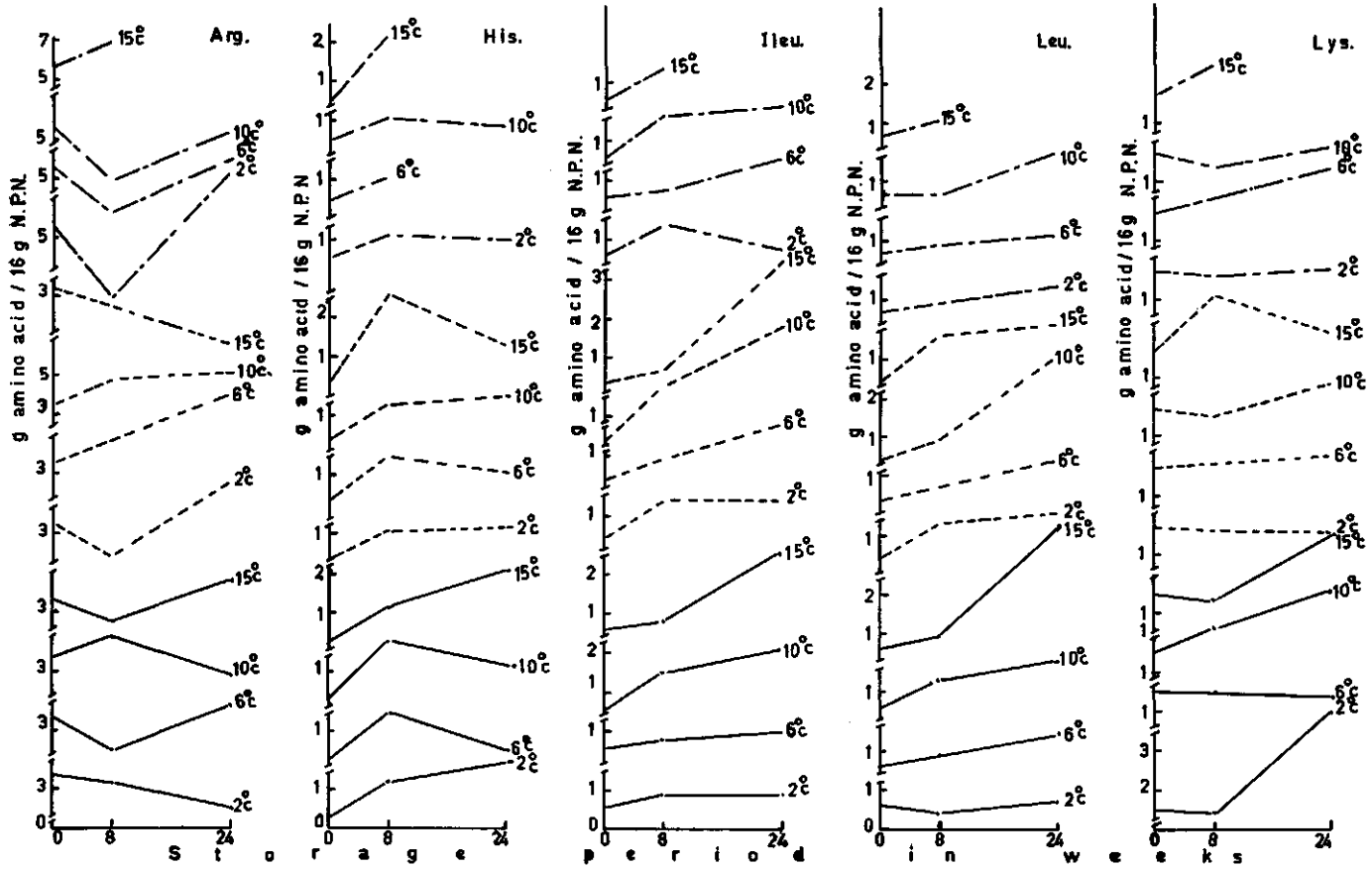


FIGURE 22. The effect of storage period on the non-protein amino acids, arginine, histidine, leucine, isoleucine and lysine in the varieties Alfa ———, Eigenheimer - - - - -, and Kwinta — · — · —, stored at 2°, 6°, 10° and 15° C.

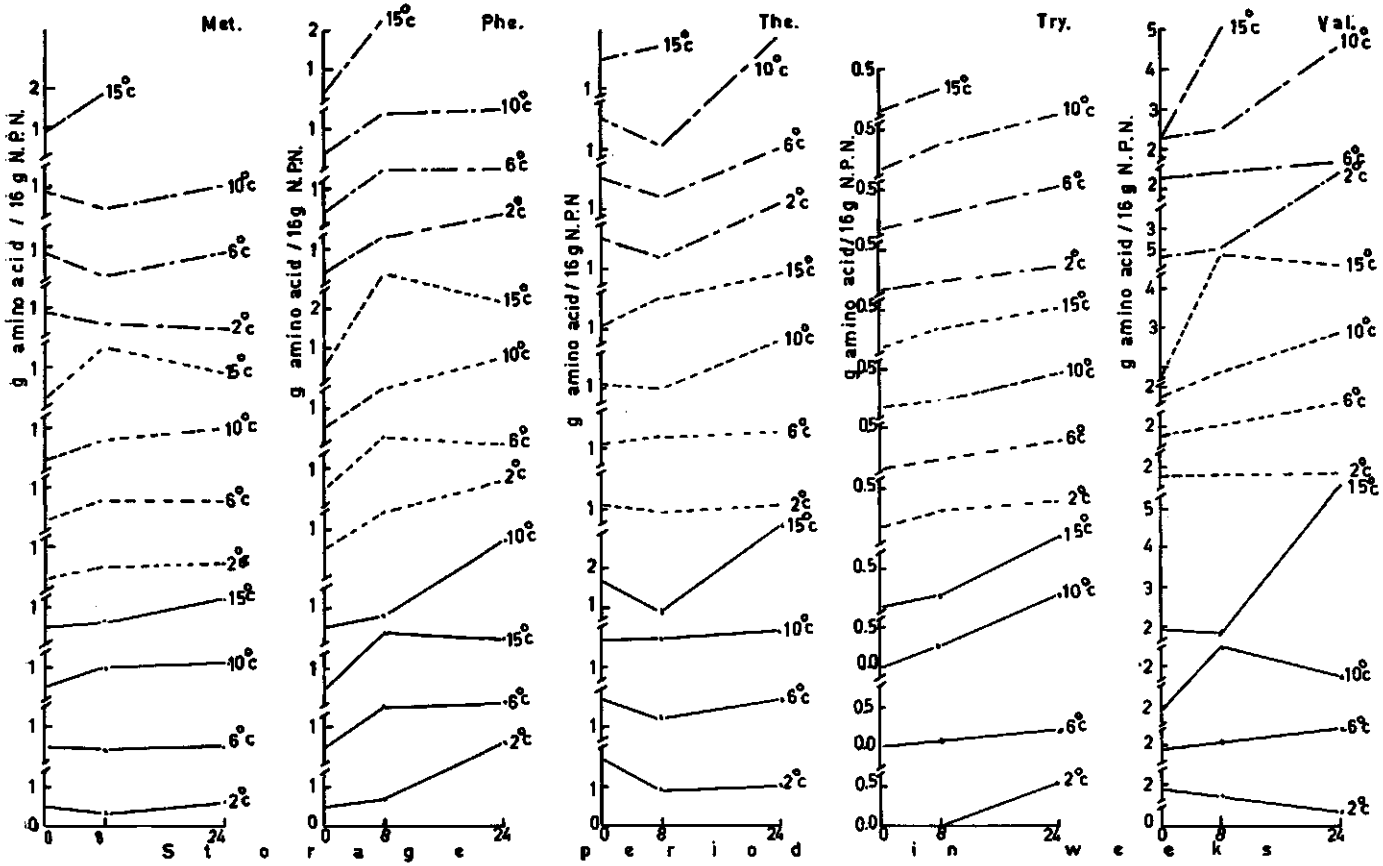


FIGURE 23. The effect of storage period on the non-protein amino acids: methionine, phenylalanine, threonine, tryptophan and valine in the varieties Alfa ———, Eigenheimer ------- and Kwinta —·—·—·—, stored at 2°, 6°, 10° and 15° C.

If protein synthesis occurs, the amides will be the major source. The decrease in amide content will indirectly make the amino acid content of the N.P.N. fraction rise. Moreover, protein breakdown will supply the N.P.N. with more free amino acids. For the transport of nitrogen to the sprouts the amides also play an important part and in this way the pool of amides is depleted too. Together with protein synthesis, however, catabolic processes take place. Part of the amino acids which result from protein breakdown, is desintegrated into ammonia, that is stored in the amides, and carbohydrate residues that are believed to be respired. Thus the incorporation of ammonia into amides counteracts the depletion of the amide pool. If the above mentioned reactions are the only operating processes with regard to the amides during storage, it can be concluded from the rise of the free amino acid content found, that those reactions which results in a depletion of amides predominate.

V. Conclusions And Summary

From the nutritional point of view the amino acids are of basic importance in human and animal nutrition. This chapter dealt with the distribution of the nitrogenous substances in potato tuber, viz. total nitrogen (T.N.), protein nitrogen (P.N.), and non-protein nitrogen (N.P.N.), and the amino acid pattern of the two nitrogenous fractions P.N. and N.P.N. The influence of nitrogen fertilization and the effect of the storage period and temperature on the nitrogen distribution and on the amino acids' patterns, were also studied. All evidence indicates that during storage the nitrogenous substances seem to play an important part in the biochemical changes occurring in the potato tuber. A short discussion of such physiological processes was given. However, a detailed description of the mechanism of these biochemical reactions, although of great interest is beyond the scope of this work.

As regards the total nitrogen, an increase was recorded with the increase of nitrogen fertilization. Total nitrogen expressed as a percentage of dry matter, increased after an 8 weeks' storage period at the high nitrogen supply levels. This was attributed to the high respiration rate in potatoes excessively dressed with nitrogen, as a result of which marked losses of carbohydrates took place. After 24 weeks T.N. decreased as compared with that at 0 time. This was thought to be due to the loss of nitrogen in favour of the new sprouts. In this respect Pol (1960) found that the highest increase in T.N. during storage occurred in those potatoes which were most heavily dressed with nitrogen. According to his experiments the loss in total carbohydrates ranged from 0.5 % at 0 kg N/ha supply to 4.2 % at a nitrogen supply of 100 kg N/ha. This is in agreement with the results of the present study which indicate an increase in T.N. after 8 weeks' storage when no appreciable sprouting occurred. In the same direction Shallenberger (1955) found a higher T.N. content in potatoes stored at room temperature, than in those stored at 32—40° F. On the other hand, Street, Kenyon and Watson (1946) observed a great loss of nitrogen after storage. Their samples were probably in an advanced stage of germination. Their results never-

theless agree with the findings in the present study after a storage period of 24 weeks.

The effect of nitrogen supply on the ratio of P.N. : T.N. in potato tubers was also studied and discussed. The highest percentage of protein was found at the 40 kg N/ha sample, the lowest at the sample representing the level of 200 kg N/ha. This is in agreement with the results of MULDER and BAKEMA (1956) and of MULDER (1949). The changes due to storage were, however, more pronounced than those due to nitrogen fertilization. It was shown that after 8 weeks' storage the variations in the P.N. : T.N. ratio were most pronounced in samples with a high nitrogen supply.

After 8 weeks the highest protein content was recorded at the 200 kg N/ha level. This was in contrast with what was found at 0 time storage, when the 200 kg N/ha level showed the lowest P.N. : T.N. ratio. In this respect it has been suggested that protein synthesis is more active at higher nitrogen levels. Moreover, it should be mentioned here that the N.P.N. fraction on a high nitrogen supply is for a great part composed of amides (MULDER and BAKEMA, 1956). Consequently, it may be expected that protein synthesis takes place during storage. The amides act as a store of ammonia easily available for protein synthesis. Furthermore, it has been assumed by many workers that respiration is closely connected with protein synthesis. In this connection POL (1960) found that respiration is higher in potatoes with higher levels of nitrogen. Altogether it may, therefore, be concluded that after 8 weeks more protein synthesis occurred in samples with higher levels of nitrogen supply. On the other hand, P.N. as a percentage of T.N. showed a declining ratio after 24 weeks' storage period. This may be attributed to the breakdown of proteins after a long storage. POL (1960) showed that at higher levels of nitrogen supply the decrease in P.N. as a percentage of the T.N. was more pronounced than at lower levels of nitrogen supply with the advance of storage period. Several factors are believed to control the behaviour of P.N. ratio. These factors, variety, storage period, storage temperature and germination, were discussed. Apart from other unknown factors, these four are believed to interact to produce the complicated picture of the protein obtained in the present investigation.

The influence of the nitrogen fertilization on the amino acid pattern of potato protein is rather small. During storage, however, some changes took place. As the potato protein is composed of at least six components, it is not strange that a breakdown or synthesis of one of these six may change the amino acid pattern of the protein mixture. When the end of the storage period approached, the content of nearly all the amino acids in the N.P.N. fraction increased. This was attributed to both protein breakdown and the withdrawal of the amides by the sprouts during storage and germination. The changes of the N.P.N. due to nitrogen fertilization or to storage were apparent. In general the alteration in the concentration of the amino acids in the N.P.N. fraction may be attributed to the amides. The change of the amides concentration in the N.P.N. due to protein synthesis or to nitrogen withdrawal from the tuber by the sprouts, may therefore be of importance for the free amino acid concentration in the potato tuber.

CHAPTER FOUR

THE NUTRITIONAL VALUE OF POTATO PROTEINS

I. INTRODUCTION

A. PROTEINS IN NUTRITION

Since proteins are essential components of the animal cell and since they have the widest biochemical functions in the living body, it is not strange that many investigators were attracted by research work on proteins so that about 50 % of the nutritional, biochemical and physiological publications covers this field. It is a fact that, if reproduction and growth are considered, the proteins undoubtedly occupy a dominant position.

The number of protein functions in the living cell is enormous. The body needs nitrogen to replace the depleted protein tissues in the steady state of synthesis and breakdown. This need of nitrogen in the form of protein is called the requirement of the body for maintenance. The nitrogen needs for maintenance can be measured from the nitrogen excreted in the urine and the faeces when a protein-free diet is consumed. The adult human and animal generally need protein for maintenance mainly, while the growing one needs it not only for maintaining the nitrogen balance in equilibrium but also for building up new tissues.

In case of infection, the γ-globulins fulfil a specific function in defending the body against the infective agent and its toxins. They produce antibodies which conjugate with the attacking bodies and so neutralize their harmful effect.

Many biochemical changes occurring in the cell are catalyzed by enzymes. For each specific enzymatic reaction a proper enzyme is required. All these enzymes are proteins or possess a substrate specific protein part. These enzymes are distributed all over the body. The breakdown of food materials to their building units and their resynthesis, as well as the oxidation of carbohydrates, proteins and fats are largely enzymatical processes.

Hormones with their vital, regulating functions are often proteins in nature too. The transport of oxygen in the blood also depends on a protein-like carrier.

From this and many other functions and shapes of proteins that are left unmentioned here, it follows that providing the body with materials which can be used as a source for protein synthesis, is of primary importance. As neither the human nor the animal body has the capacity of drawing the nitrogen from the air or from mineral sources to build up its proteins, such provisions must be taken in from nitrogenous materials originating from plant or animal foods,

i.e., from proteins. If man or animal does not receive adequate quantities of protein to fulfil their requirements for maintenance, they will lose weight due to the depletion of the body proteins. Should such depletion continue, the final consequence will be death.

The proteins are not absorbed as such but are first digested to their building units, the amino acids, from which the body proceeds to manufacture its specific proteins. Not all the proteins can supply the body with its requirements, so there are adequate proteins and poor ones. The best proteins will be those which provide the body with amino acids of a composition or a pattern similar to that of the tissue proteins to be synthesized.

Studies with rats, chicks and human subjects revealed that not all naturally occurring amino acids are needed. There is only a fixed number which cannot be synthesized partly or wholly in adequate quantities from other amino acids. In view of this character ROSE (1938) gave them the name of "Essential Amino Acids". These amino acids are not essential for all species. For instance arginine is not essential for the human subject, while for chicks it is of absolute requirement. Its defficiency in the chicks' diet will even cause muscle paralysis and other irregularities of muscle reactions. Tyrosine is not essential for human beings, whereas in human subjetcs with phenylpyruvic oligophrenia where the administration of phenylalanine aggrevates the excessive excretion of phenylpyruvate and elevates the phenylalanine blood levels, tyrosine becomes essential because of the insufficiency of the conversion of phenylalanine to tyrosine (UNDERFRIEND and BESSMAN, 1953). As has been stated before, the body requires adequate quantities of the essential amino acids to be provided in a special pattern. The other amino acids can be synthesized in the animal body from others. However, HEPBURN, CALHOUN and BRADLEY (1960) found that the omission of glutamic acid, which is not essential, from the diet of rats resulted in a great depression of growth rates, while the omission of the other nonessential amino acids had no such effect. With a glutamic acid-free diet, a gain of 14.9 g/week was recorded. A maximum gain rate of 34.9 g/week was obtained when glutamic acid represented 5.1 % of the diet.

ROSE, JOHNSON and HAINES (1950) stated that the ten amino acids which are essential for the rat, also seem to be indispensable for the maintenance of nitrogen equilibrium in human adults. From a series of studies on human subjects, ROSE, JOHNSON and HAINES (1950), ROSE, HAINES and WARNER (1951, a, b) and ROSE, WARNER and HAINES (1951) found that histidine was not necessary for the maintenance of the nitrogen balance in adult man, while isoleucine, leucine, threonine, valine, methionine and phenylalanine were essential.

In (1954) ROSE, HAINES and WARNER concluded that the amino acids essential for the maintenance of the nitrogen equilibrium in normal adult man are: valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, and tryptophan. The requirements of those amino acids for man were determined by ROSE's school and are shown in table 23.

The quality of a protein is not only determined by its amino acid pattern, but also by the availability of the essential amino acids. Studies related to these

subjects revealed many conclusions. To explain the poor quality of some proteins, the investigators introduced expressions such as amino acid imbalance, amino acid toxicity and amino acid antagonism. A sharp division between these three definitions cannot be easily made.

TABLE 23. Essential amino acid requirements for the maintenance of the adult human, quoted from ROSE *et al.* (1955).

Amino acid	Range of requirements observed, g/day	minimum requirements g/day	safe intake g/day
L. tryptophan	0.15 — 0.25	0.25	0.50
L. phenylalanine	0.80 — 1.10	1.10	2.20
L. lysine	0.40 — 0.80	0.80	1.60
L. threonine	0.30 — 0.50	0.50	1.00
L. methionine	0.80 — 1.10	1.10	2.20
L. leucine	0.50 — 1.10	1.10	2.20
L. isoleucine	0.65 — 0.70	0.70	1.40
L. valine	0.40 — 0.80	0.80	1.60

Antagonism is believed to be a process in which the antagonist inhibits the metabolism or resorption of its analog or the related compound. Many workers showed that antagonism between amino acids occurs in bacteria; some examples are shown in table 24. As to animals, however, BENTON, HARPER and ELVEHJEM (1955) came to the conclusion that isoleucine is only partly available in zein, and questioned if antagonism between leucine and isoleucine would play any role. In another investigation with rats HARPER, BENTON and ELVEHJEM (1955) showed that the excess of dietary leucine apparently inhibits the utilization of isoleucine and increases the requirements of rat for this amino acid. They concluded: "since L. leucine was the effective form, since the reversal of isoleucine was quite specific, since a relatively small amount of isoleucine overcame the effect of a much greater quantity of leucine and since the two amino acids are so similar structurally, it is suggested that an excess of L. leucine retarded growth by acting as an antimetabolite of isoleucine".

TABLE 24. The antagonism between amino acids in bacteria.

Amino acid	Analog	References
Arginine	Lysine	HUNTER and DOWNS (1945)
Isoleucine	Isoleucine	DOUDOROFF (1943)
Leucine	Leucine	DOUDOROFF (1943)
Isoleucine	Valine	DOUDOROFF (1943)
Leucine	Valine	BRICKSON, HANDERSON, SOLHJELL and ELVEHJEM (1948)
Methionine	Threonine	DOUDNEY and WAGNER (1952—1953)
Phenylalanine	Tyrosine	BEERSTECHER and SHIVE (1947)

Few workers investigated amino acid toxicity. JONES (1961) observed that 1.5 to 2 % of L. arginine added to an adequate protein diet fed to one day old chicks showed symptoms of toxicity after 14 to 21 days. Excess methionine in the diet causes hypertrophy of the kidney and relatively high liver protein stores in animals losing nitrogen from some other tissues (BROWN and ALLISON, 1948). In practice, however, such high quantities of a single amino acid are not likely to occur.

The protein quality in nutrition is not only dependant on its essential amino acid pattern. The protein is said to be an adequate one if it can supply the required essential amino acid pattern in an available form to the body. In recent years the availability of the amino acids received a renewed attention of the investigators. For instance, lysine in many foods is only partially available. CARPENTER (1957) showed that if ground casein was kept with a small amount of glucose and water to be just moist at blood temperature for five days, only half the weight gain would be obtained when fed to rats, as compared with untreated casein. This was attributed to the liability of the free amino group of lysine to react or bind with other components in the food. The resulting complex compounds may partly be destroyed by acid hydrolysis, but are stable to enzymatic digestion.

The processing of foods does not always yield products inferior in proteins to the mother product. Corn treated with lime has a better protein value than corn itself. Many workers on this subject found the same results but drew contradictory conclusions. Some changes are, however, likely to occur resulting in a release of some amino acids which are not available in the untreated corn or in blockade of some excessive amino acids which may lead to a correction of the amino acid pattern in the corn. In this respect BENTON, HARPER and ELVEHJEM (1955) found that isoleucine is a limiting amino acid for growing rats fed corn diets and it has already been cited that HARPER, BENTON and ELVEHJEM (1955) suggested that antagonism between leucine and isoleucine is apparent in untreated corn. BRESSANI and SCRIMSHAW (1958) carried out experiments in vitro and showed that 1.5 to 2 times more isoleucine was released by enzymes from the lime-treated corn than from the untreated corn. They also observed a loss of more than 20 % of the leucine when corn was treated with lime. Such changes would increase the availability of isoleucine. Soybean meal received much attention. Fresh soybean did not support growth, but soybean cooked for three hours did (OSBORN and MENDEL, 1917). TAGNON and SOULIER (1946) reported that the factor which inhibits the utilization of soybean proteins is a trypsin inhibitor.

Amino acid imbalance was defined by HARPER (1959) as any change in the properties of the amino acids in the diet resulting in an adverse effect which can be prevented by supplementing the food with relatively small amounts of the most limiting amino acid or acids. The imbalance of corn protein was treated by BRESSANI *et al.* (1958). They obtained a negative balance when two boys 22 and 18 months old received a diet providing 2.0 g of protein/kg body weight, where corn was the only protein source. They could show that when the diet

was supplemented with tryptophan and lysine, the nitrogen balance and the nitrogen retention in the body improved. If isoleucine was also added, an even better retention was observed. FISHER *et al.* (1960), when studying the lysine deficiency in chicks, could show that the imbalance was due to an altered ratio of the limiting amino acid to the total available protein or the essential amino acid mixture. They added that imbalance was most pronounced in cases where the level of the limiting amino acid was such as to permit growth above maintenance but below the optimum growth rate. They stated that the imbalance manifested itself in a reduced feed consumption and consequently in a reduced consumption of the most limiting amino acid. HARPER (1959) stated "the magnitude of the growth depression caused by the addition of gelatine to a diet of 6 % casein increased as the level of this poor protein was increased, which showed a real imbalance of the gelatine". In this connection, it seems that an amino acid imbalance will cause a severe growth retardation when large amounts of proteins with an imbalanced amino acid pattern are consumed. SIDRANSKY's (1960) experiments demonstrated that within three days rats forced-fed with diets of corn, rice and cassava showed a development of periportal fatty livers, excess hepatic glucogen and atrophy of the spleen, pancreas and submaxillary gland, which resembled the symptoms of Kwashiorkor. He reported less marked changes with wheat and milo diets. On the other hand, he observed that animals fed the same diets *ad libitum,* consumed less food and showed less pathological changes. In their study on the imbalance induced by adding phenylalanine and methionine to a diet containing 6 % fibrin, KUMTA and HARPER (1960a) reported that imbalance reduces the efficiency of the utilization of the limiting amino acid or acids. They found that the depression caused by the addition of phenylalanine and methionine only occurs when the diet contains 6 % fibrin, which involves a delicate balance. The degree of the imbalance of a protein depends on the limiting amino acid. In a poorly balanced protein it is easy to calculate the sequence of the most limiting amino acid from the amino acid composition of the protein. With a protein such as fibrin, however, there is no sharp distinction in the order in which the amino acids are limiting. KUMTA and HARPER (1960b) found that a calculation of the sequence of limitation from the amino acid composition of fibrin does not give the same results as an experimental approach of the problem. Whilst calculation revealed that methionine and phenylalanine are the most limiting amino acids, growth experiments indicated leucine, isoleucine, valine and histidine as the most limiting ones. A special case of imbalance was noticed by MORRISON and HARPER (1960). Addition of threonine to a diet deficient in tryptophan and nicotinic acid caused an imbalance which could be corrected by the addition of either tryptophan or nicotinic acid.

The Food and Agriculture Organization of the United Nations (FAO) (1957) introduced an amino acid pattern which was proposed as a provisional reference of a balanced protein. This pattern may be used to correct the protein amino acid composition of the food. Generally speaking, it has been found that animal proteins show a better balanced amino acid proportion than plant proteins. As,

As has been shown before, proteins differ in their adequacy of supporting growth or maintenance. To evaluate these differences a number of methods have been worked out to measure and express the adequacy of a protein in numerical terms. It is obvious that the determination of the nutritive value of a protein depends on many factors, i.e. not only the digestibility of the protein and the degree of amino acid balance after proteolyses, but also on the accompanying

the reasons why plant proteins are inferior are wellknown, it is possible to correct their imbalance by a proportional addition of the limiting amino acids according to the amino acid reference proposed by the FAO. SQUIBB *et al.* (1959) composed a mixture of vegetable products containing 50 % lime-treated corn, 35 % sesame flour, 9 % cotton seed flour, 3 % kikynleaf meal and 3 % torula yeast. They reported that this mixture was palatable and resulted in good growth and high efficiency of food utilization in rat trials. They found that neither the addition of 0.45 % lysine nor the subsititution of part of the corn by skimmed milk improved the growth in rats; that is why they recommended such mixtures for

nutrients and the total amount of calories consumed. Therefore, the variables in such determinations are many.

The adequacy of a protein to support growth can be more or less predicted from its amino acid composition. Such predictions are of restricted value because they are based on equal digestibility of all proteins, which is not the case. Methods for determining the nutritional value have recently been reviewed by EL-SAMMAN (1961), FROST (1959) and ALLISON (1955). RIPPON (1959) carried out a comparative study as to the different methods for evaluating the biological value of proteins.

When discussing the methods of determining the protein value in nutrition, some terms should be defined first.

Nitrogen balance (N.B.):

By definition, N.B. is the difference between nitrogen intake (N) and nitrogen excreted. If the nitrogen intake equals the excreted nitrogen (urine + faecal nitrogen), the animal will neither gain nor lose nitrogen. This equilibrium status is known as the nitrogen balance equilibrium.

Faecal nitrogen (F):

The faecal nitrogen is not only derived from non-resorbed food residues but also from some metabolic products excreted via the intestines. This part of faecal nitrogen, which can be determined in faeces when nitrogen-free diets are consumed, is called the metabolic faecal nitrogen (Fm).

Urine nitrogen (U):

FOLINE (1905) introduced the terms endogenous nitrogen (E) and exogenous nitrogen (Ex). The endogenous nitrogen is the nitrogen excreted during a period when a protein-free diet is consumed. This nitrogen fraction originates from body cellular metabolism. The exogenous nitrogen comes from the food. From the nitrogenous substances in the urine, creatinine excretion is rather constant and the output of urea is quite variable, the urea being both exogenous and endogenous.

1. Direct Methods.

a. The nitrogen balance method:

THOMAS (1909) defined the term biological value of proteins (BV) as the percentage of absorbed nitrogen which a human can retain in the body. He carried out the experiments on himself and assuming that the Fm = 1.0 as in the case of vegetables, came to equation (1)

$$BV = \frac{E + [N - (F + U)] + 1.0}{N - F + 1.0} \times 100 \qquad (1)$$

or

$$BV = \frac{E + [N - (F + U)] + Fm}{N - F + Fm} \times 100 \qquad (1)^1$$

MITCHELL (1924) who carried out the biological value determinations of the proteins on rats, changed the equation of THOMAS (1) on the base of his own formula (2) to result in equation (3)

$$BV = \frac{N - (F + U)}{N - F} \times 100 \qquad (2)$$

$$BV = \frac{N - (F - Fm) - (U - E)}{N - (F - Fm)} \times 100 \qquad (3)$$

Recently BROUWER (1959) suggested another arrangement of the Thomas-Mitchell's formula to result in equation (4)

$$BV = 100 - 100 \frac{U - E}{N - (F - Fm)} \qquad (4)$$

in which 100 — exogenous nitrogen as percentage of the (N — F + Fm) = the absorbed nitrogen (AN), will indicate the amount of nitrogen used by the body, which is known as "nitrogen retained" in the literature.

The measurement of E and Fm is carried out by feeding the animal a protein-free diet. These two nitrogen fractions are independent of the nitrogen intake. This fact led ALLISON and ANDERSON (1945) to suggest that the relationship between NB and absorbed N (AN) must be linear, as could also be concluded from the THOMAS-MITCHELL equation (3). In experiments with normal dogs they, indeed, found this relation to be linear in the region of negative and low positive nitrogen balance. This relation was indicated by:

$$NB = K\ (AN) - BN_0 \qquad (5)$$

where K is a constant.

BN_0 is the sum of urinary nitrogen and faecal nitrogen when the absorbed nitrogen was zero (E + Fm).

BRICKER, MITCHELL and KINSMAN (1945) and BRICKER and MITCHELL (1947) had also found a linear relationship between AN and NB in man and in rat respectively.

ALLISON and ANDERSON's (1945) equation (5), showed that BN_0 equaled (E + Fm), which is the constant amount of nitrogen excreted from the body at all nitrogen intakes; K will then represent the BV.

ALLISON, ANDERSON and SEELEY (1946) defined K as the rate of change of nitrogen balance with respect to absorbed nitrogen and as a function of the retention of both dietary and body nitrogen. They, therefore, concluded that the value is a valuable measure of the B.V. of proteins under any experimental design and called it the nitrogen balance index of absorbed nitrogen.

b. Protein Efficiency Ratio (PER):

OSBORNE, MENDEL and FERRY (1919), feeding their rats albumin and casein diets, found that absolute gain of the body weight might differ, even by 75 %, from one rat to the other, while the differences between the gains in body weight per gram of protein consumed are much less. This method involving the growth promoting efficiency of protein was developed into a much simpler screening method. It is true that the ex-

periment lasts longer than the determination of BV, but it is far less laborious. It is, however, doubtful if a gain in weight would merely implicate a retention of protein. The PER is highly dependent on the protein level in the diet. At higher levels not all protein absorbed is used for growth (tissue building), but part of it is also used as a source of energy, thus lowering the efficiency of the protein. In fact each protein gives its maximum efficiency at a specific nitrogen level of the diet. This optimal level is higher for poor proteins. When comparative studies are carried out on the base of 10 % protein in the diet, this level might be less optimal for one protein than for another which makes the comparison difficult.

RIPPON (1959) reported that he could successfully use the method of OSBORNE *et al.* (1919), if the technique of depletion-repletion described by FROST and SANDY (1949) was employed.

c. Net Protein Utilization (NPU):

In (1953) BENDER and MILLER introduced a simple and adequate method depending on the gain of carcass nitrogen of rats. With this method one group of rats is fed a non-protein diet and the other group an isocaloric diet with the test protein. They developed the following equation:

$$NPU = \frac{B_N - (B_K - N_K)}{N} \qquad (6)$$

where B_N = Body nitrogen of the group fed the test protein.
B_K = Body nitrogen of the group fed the free-protein diet.
B = Body nitrogen at the start of the experiment.
N_K = Nitrogen intake of the free-protein-fed group.

It can be shown that NPU is obtained when BV and digestibility are multiplied.

BENDER (1954) reported:

$$NPU = \frac{N - (F - Fm) - (U - E)}{N - (F - Fm)} \times \frac{N - (F - Fm)}{N}$$

$$= \frac{N - (F - Fm) - (U - E)}{N} \qquad (7)$$

$$= \frac{\text{Nitrogen Retained}}{\text{nitrogen intake}}$$

When $\frac{N_K}{N}$ is substracted from both sides of the equation, it can be arranged as follows:

$$NPU - \frac{N_K}{N} = \frac{(N - F - U) - (N_K - Fm - E)}{N} \qquad (8)$$

it can be seen that in equation (8)

$N - F - U =$ the gain in nitrogen by the animal fed on a protein diet
$= $ final body nitrogen — initial body nitrogen
$= B_N - B$

and $N_K - Fm - E =$ the change in body nitrogen induced by feeding the non-protein diet
$= B_K - B$

Then from equation 8 the following can be extracted

$$NPU - \frac{N_K}{N} = \frac{(B_N - B) - (B_K - B)}{N} \qquad (9)$$

$$NPU = \frac{(B_N - B) - (B_K - B) + N_K}{N}$$

$$= \frac{B_N - B_K + N_K}{N} \qquad (6)$$

MILLER and BENDER (1955) could correlate the nitrogen content of the body with its moisture conent, which simplified the method considerably.

d. Net Protein Ratio (NPR):

BENDER and DOELL (1957) gave another modification of the PER method. In this method paired litter mate groups, one of which is a control group receiving a protein-free-diet, whereas the other group or groups received the test proteins at 10 % of the diet. The difference between the body weight of the test group and that of the control group, divided by the total protein consumed by the test group, is called the net protein ratio. Protein retention efficiency, as a percentage, can be obtained when nitrogen is expressed as protein, so when NPR is multiplied by 16. This value correlates well with the values of the carcass analysis methods, which will support the idea of OSBORNE *et al.* (1919) that a gain in weight is a rather reliable reference for the biological value of the proteins.

e. Minimal Protein Requirements (MPR):

MELNICK, COWGILL and BURACK (1936) as well as MELNICK and COWGILL (1937) determined the protein minimum amounts to maintain the nitrogen equilibrium. Although their method did not receive much attention, it seems to be in close connection with the views of ALLISON and ANDERSON (1945). ALLISON (1957) stated that the amount of dietary nitrogen necessary to meet with the requirements for maintenance and growth varies with the BV of the protein in the diet. It can be easily seen that the determination of the requirements minimally needed to keep NB in equilibrium, will give figures, which can be correlated with the nitrogen balance index, i.e. the slope of the tangent representing the nitrogen balance as a function of the nitrogen absorbed.

2. Indirect Methods.

a. Liver Xanthine Oxidase:

Xanthine Oxidase is a flavoprotein enzyme which contains flavine adenine dinucleotide as the prosthetic group. It catalyzes the oxidation of hypoxanthine to xanthine and the latter to uric acid. DJU, BAUR and FILLER (1957) found that liver xanthine oxidase activity reflects the BV of the dietary proteins. A decrease in total liver nitrogen content is accompanied by a decrease in liver xanthine oxidase activity. This method was used to evaluate processed egg and milk proteins.

b. Liver Cytoplasm:

KOSTERLITZ and CAMPBELL (1946) demonstrated that the amount of cytoplasm in liver is dependent on the quality and the quantity of the protein in the diet. They employed two methods. With the first method the rats were transferred from the stock diets to the test diet for one week. The sum of the liver proteins, phospholipids and nucleic acids was determined. In the second method the rats were given a protein-free-diet for four days in which period the livers were completely deprived of their labile cytoplasm; next, they were transferred to the test diet for another four days. The non-glycogen-non lipid liver solids were estimated. The results of the two methods showed no significant differences.

c. The Percentage of Creatinine in Urine:

MURLIN, HAYS and JOHNSON (1953) found that with a very poor protein in the diet the amount of nitrogen appearing in the urine is large, so that the creatinine nitrogen as a percentage of total urine nitrogen is small, and vice versa. The percentage of creatinine in urine was found to correlate well with the biological value of the proteins fed to the animal. Especially because such a method can give results within 3 days, which as has been stated, are close approximations of BV, it was frequently suggested to be used in evaluating proteins.

3. Chemical Methods.

As has been shown before, the amino acids pattern of a protein greatly determines its balance or imbalance, or in other words its nutritive value. On this base some investigators developed a method for calculating the nutritive value of proteins from their amino acid composition.

a. The Chemical Score Method (C.S.):

MITCHELL and BLOCK (1946) stated: "The relationship of the amino acid constituents of a protein or of the protein components of a food product, to its nutritive value (NV) for the growing rat can be best revealed in the absence of accurate values for the amino acid requirements, by computing for each protein or protein moiety, the percentage deviations of the contents

of each essential amino acid, expressed per 16 gram of nitrogen, from the corresponding contents of a protein mixture, such as that of the whole egg, that is almost completely digestible by the rat and utilizable in adolescent metabolism." In such a computation the essential amino acid most limiting to the nutritive efficiency of the protein, will be revealed as the one the percentage deficit of which, as compared with that of the standard protein (here the whole egg protein was taken as a standard), is the greatest. These percentages (for 28 different proteins and protein mixtures) were found to be highly correlated ($r = -0.86$) with the corresponding biological value determined by the nitrogen balance. This relation between CS and BV is expressed in the empirical formula:

$$Y = 102 - 0.634\ X \qquad (10)$$

where Y = N.V. of a protein

and X = maximum deficit in an essential amino acid

b. Essential Amino Acid Index (EAAI):

MITCHELL and BLOCK's (1946) method only takes the most limiting amino acid into account.

OSER (1951), however, introduced a method by which the rating of a protein quality was based on the contribution of all the essential amino acids. The EAAI of OSER (1951) is derived from the ratios of the essential amino acids in a protein relative to their respective amounts in the whole egg protein according to equation (11):

$$EAAI = \sqrt[n]{\frac{Lys_p}{Lys_e} \times \frac{Try_p}{Try_e} \times \ldots\ldots\ldots \times \frac{His_p}{His_e}} \qquad (11)$$

where n = the number of amino acids
p = refers to the food protein
e = refers to the standard protein (in this case the whole egg)

For the relation between EAAI and the BV of proteins, MITCHELL (1954) reported a correlation coefficient of $r = 0.948$; the regression line is represented by:

$$BV = 1.0747\ (EAAI) - 13.74 \qquad (12)$$

OSER's results (1951) yielded the following relation:

$$BV = 1.1403\ (EAAI) - 8.415 \qquad (13)$$

OSER (1959) combined the two equations (12) and (13) to give:

$$BV = 1.09\ (EAAI) - 11.73 \qquad (14)$$

c. FAO Protein Score:

The committee of protein requirements of the Food and Agriculture Organization of the United Nations (1957) suggested a provisional pattern based on the knowledge of the requirements of healthy human beings for essential amino acids. MITCHELL and BLOCK (1946) and OSER (1951, 1959) used the whole egg protein pattern as a referance protein to predict the nutritive value of the protein. The FAO committee suggested to use the provisional pattern in computing the nutritive value of a protein or protein mixtures in foods from their amino acids composition. In this method the extent to which a food or food combinations supply the limiting amino acids as compared to the provisional pattern is calculated. The values obtained are in good agreement with the biological values determined on man. It has been stated that a protein or a mixture of proteins having a BV of 70 or more, is satisfactory, whereas foods with BV 60 or less tend to be unsatisfactory.

d. Other Methods:

BUNYAN and PRICE (1960) found that the content of available lysine is of particular significance, as in rations based on maize, wheat and cereal products it is the amino acid that is most commonly deficient. MCLAUGHLAN *et al.* (1959) showed that most common foods are deficient in either lysine or methionine, or in methionine and cystine. Based on this assumption a "Simplified Chemical Score" (SCS) was adopted. The correlation coefficient for the relation between SCS and PER of proteins deficient in lysine was found to be $r = 0.96$. In the case of methionine (+ cystine) deficiency, a coefficient of $r = 0.83$ was obtained. They suggested that the simplified method can predict the nutritive value of a protein in a short time.

4. Microbiological Methods.

This "direct" method of evaluating the quality of proteins will be considered in Part III of this Chapter.

II. MATERIALS

The materials used in the present study were the same as described in Chapter Three, (see page 30).

III. METHODS

The nutritive value of the potato nitrogenous substances was determined on the soluble nitrogen, including the non-protein nitrogen and the soluble protein nitrogen.

A. MICROBIOLOGICAL METHODS USED FOR DETERMINING THE NUTRITIVE VALUE (NV) OF PROTEINS

Laboratory animals are generally accepted organisms for measuring the NV of proteins. The limitations to which animal experiments are bound, however, are in many respects enormous. Speaking of limitations of laboratory animal experiments, many workers in this field think them to be laborious, expensive and too much time consuming for routine work. These difficulties can any how be overcome, but if the protein samples to be examined are available in too small quantities, the objections become serious and the application of micro methods is inevitable. For the last reason and also because a considerable number of samples had to be examined, a microbiological technique was employed in the present investigation.

With the advance in the microbiological technique of the determination of amino acids, it has been found that many bacteria and protozoa need the amino acids, essential for rat and man, in their media. This fact was a stimulus for some workers to investigate the usefulness of these microorganisms for the estimation of the NV of proteins. HORN *et al* (1952) carried out an experiment to evaluate the quality of processed cotton seed meal proteins. They used both rat feeding methods and microbiological methods. In the microbiological experiments they submitted the protein to an enzymatic digestion and evaluated the NV in the resulted hydrolyzate. The results they obtained from the microbiological evaluation correlated well with those of the rat feeding method. The work in this respect progressed and resulted in the introduction of a suitable method to measure the NV of proteins with the aid of microorganisms. HORN, BLUM, and WOMACK (1954) described a rapid method for the determination of the NV of the proteins of processed cotton seed meals, involving the use of *L. mesenteroides* P. 60, as an experimental bacteria. HALEVY and GROSSOWIEZ (1953) suggested the use of *S. faecalis* for the determination of the NV of proteins. They also used an enzymatic hydrolyzate of the protein to be tested and the medium was devoided of the 10 essential amino acids. Their results were in agreement with those obtained with rats. TEERI, VIRCHOW, and LOUGHLING (1956) found that *S. faecalis* (ATCC 9790) gave much better results than *L. mesenteroides* P. 60. In their method they used a mixture of enzymes under conditions comparable with those of the human digestive tract. They demonstrated that the method proved itself reliable and a classification of the proteins in good, bad and intermediate could be made. NEHRING and WUNSCHE (1959) determined the NV of fish meals with *L. mesenteroides* P. 60 (ATCC 8042) in pepsine and trypsine hydrolyzates. The results they obtained were in good agreement with the BV estimated according to the method of THOMAS-MITCHELL. Recently FORD (1960) developed a new microbiological method for protein evaluation using *S. zymogenes* (NCDO-592). This microorganism can use directly the non-hydrolysed protein. He evaluated the NV of the proteins by the growth response of the microorganism, which was measured in three ways, namely, turbidimetric, titrimetric and oxidimetric by measuring the reduction of 2, 3, 5-triphenyltetrazolium chloride. He claimed that the values obtained with a variety of food proteins had been found to correlate closely with those obtained in the biological tests with rats.

KIDDER and DEWEY (1945) when studying the biochemistry of *Tetrahymena*, showed that arginine, histidine, methionine, tryptophan, lysine, isleucine, leucine, threonine, valine, phenylalanine and glycine had to be regarded as essential for growth of these protozoa. DUNN and ROCKLAND (1947) developed a titration method for the determination of the response of *T. pyriformis* H. to intact proteins in their media. They found that the estimated NV was dependent on the length of the incubation periods. They stated: 'The BV found at some levels of the samples are in approximate agreement with those obtained with other procedures'. In another publication ROCKLAND and DUNN (1949) wrote that with relatively long incubation periods the NV determined with the aid of *Tetrahymena pyriformis* H. was in close agreement with values obtained with the nitrogen balance methods. ANDERSON and WILLIAMS (1951) introduced a method for measuring the growth of *T. pyriformis* W. in protein suspensions. The outline of their method is the reduction of the colourless 2, 3, 5-triphenyltetrazolium chloride to the red triphenylformazan, a reaction effected by the respiratory enzymes of the organism. They also reported that the method successfully reflected the response to graded levels of the four different proteins tested. PLICHER and WILLIAMS (1954) reported similar results. FERNELL and ROSEN (1956) and ROSEN and FERNELL (1956) studied the medium required for *T. pyriformis* W. in microbiological evaluation of proteins. A recent review of the microbiological assay of protein quality was given by ROSEN (1960). He stated that, according to the present knowledge, the use of *T. pyriformis* appeared to be preferable to the other microorganisms.

B. THE METHOD EMPLOYED IN THE PRESENT STUDY.

A great number of samples had to be examined and the quantities of samples available were small, so it was decided to apply a microbiological method. The method described by FERNELL and ROSEN (1956), and ROSEN and FERNELL (1956) was applied after some modifications, and is described below:

1. The microorganism:

The organism used for the evaluation of the nitrogenous substances in potato tubers was *Tetrahymena pyriformis* W., obtained from the 'Culture Collection Of Algae And Protozoa, The Botany School, Cambridge, England'. The stock cultures were grown on a nutrient broth composed of:

proteose peptone (Difco)	2.0 g
Yeast extract	0.1 g
glucose	0.5 g
sodium chloride	0.1 g

suspended in water, boiled for three minutes, adjusted to pH 7.1 and completed to 100 ml. Stock cultures were transferred at 15 days' intervals. For inoculation in the experiment, 5 day old cultures were used. The cultures were kept at 25°C in 1 oz screw-capped bottles containing 10 ml of the nutrient broth.

2. The medium:

The medium used was devoid of protein, and its composition is shown in table 25. This medium is double strength, and of a pH 7.1.

TABLE 25. The composition of the medium used for *T. pyriformis* W.

Ingredients		Ingredients	
Pantothenic acid	625.0 µg	Glucose	750.0 mg
Nicotinic acid	625.0 µg	$MgSO_4 . 7H_2O$	140.00 mg
Pyridoxine	6250.0 µg	$Fe(NH_4)_2 . 6H_2O$	62.50 mg
Pyridoxal	625.0 µg	$Mn\ Cl_2 . 4H_2O$	1.25 mg
Pyridoxamine	625.0 µg	$Zn\ Cl_2$	0.125 mg
Riboflavine	625.0 µg	$Ca\ Cl_2 . 2H_2O$	30.00 mg
Folic acid	62.5 µg	$Cu\ Cl_2 . 2H_2O$	3.00 mg
Thiamine	6250.0 µg	$Fe\ Cl_3 . 6H_2O$	0.75 mg
Inesitol	625.0 µg	$K_2H_4PO_4$	875.00 mg
Choline	6250.0 µg	KH_2PO_4	875.00 mg
p-amino benzoic acid	625.0 µg	Guanylic acid	150.00 mg
Biotin	62.5 µg	Adenylic acid	100.00 mg
Lipoic acid	2.0 µg	Cytidylic acid	125.00 mg
		Uracil	50.00 mg

3. The reference protein:

Casein (B.D.H.) was taken as a reference protein. A solution containing 0.4 mg N/ml with a pH of 7.1 was prepared.

4. Preparation of the sample:

The nutritive value was determined in the soluble nitrogen fraction of the potato tuber (see Chapter Three, page 31). As this solution contains between 0.250 and 0.370 mg N/ml, it was used at the original strength, after the pH had been adjusted to 7.1.

5. Measuring the growth:

The method used in this investigation was the one described by ANDERSON and WILLIAMS (1951), in which the reduction of 2,3,5-triphenyltetrazolium chloride by respiratory enzymes of the organisms was employed as indicator for growth. The red colour was extracted with acetone, and the extraction was measured with the aid of a Bekmann Spectrophotometer at a wave length of 485 mµ.

6. The set up of the assay:

The assay was carried out on 4 ml final volume. In preliminary experiments it appeared that the diameter of the containers influenced the results. To overcome this interference tubes of the same diameter (± 18 ml) were selected. As the growth of the organism was found to be stimulated by a large surface of the medium, the

racks of the tubes had been hung in the incubator with special hangers. In this way the same sloping position was obtained in all tubes, which caused the surfaces of the media to be of the same enlarged magnitude. A solution of 1% of 2, 3, 5-triphenyltetrazolium chloride in 0.2 M potassium phosphate buffer of pH 7.6, was prepared.

For each sample four tubes were used, each containing 2 ml of the sample and 2 ml of the medium. In preparing a standard curve four nitrogen levels each in four-fold, were taken. The four levels were composed of 0.5, 1.0, 1.5 and 2.0 ml of the standard protein solution and made up to a volume of 2.0 ml with water. After 2 ml. of the basal medium had been added, the tubes were plugged with cotton wool, autoclaved at 110° C for 10 min., allowed to cool, and then inoculated with one loopful of a five-day-old culture. Next, the tubes were incubated at 25° C for 72 hrs. At the end of the incubation period one of the four tubes of each sample or standard curve level was sterilized to inactivate the microorganisms, and, consequently, the respiratory enzymes. Subsequently, 1 ml of the 2, 3, 5-triphenyltetrazolium chloride solution was added to each tube and all the tubes were incubated for another hour at 37° C. After this treatment the growth of the organisms was stopped by adding 0.4 ml of 0.1 M acidic $HgCl_2$ (prepared by using 20 ml of concentrated HCl per liter). The contents of each tube were transferred to a centrifuge tube and centrifuged. The supernatants were discarded and the tubes were allowed to drain. The triphenylformazan thus formed and precipitated, was dissolved in 10 ml acetone and the tubes were centrifuged again in order to obtain clear solutions. The red clear solutions were transferred to cuvettes and the transmission was determined in a Beckmann spectrophotometer.

The standard curve was prepared by plotting the estimated transmission of the standard tubes against the quantity of casein nitrogen in each level. From this curve and the transmission values obtained for the test protein, the quantity of casein nitrogen that produced the same growth as that of the sample could be calculated. Assuming the NV of casein to be 100, the relative values of the proteins examined can be estimated.

IV. Results And Discussion

The NV of potato nitrogenous substances was investigated by several workers. It has been frequently claimed that potato proteins are of a better quality than most other plant proteins. Kon and Klein (1928) reported that two adults, a man and a woman, lived over a period of 167 days in nitrogen balance and in good health on a diet in which the nitrogen was almost exclusively derived from potatoes. The daily nitrogen intake was 5.7 g for the man and 3.8 g for the woman. Kon (1928) extracted the proteins of potato and examined their biological value according to the method of Mitchell. He came to the conclusion that the potato protein appeared to be a good, well-balanced protein. Chick and Cutting (1943) reported that the growth promoting value of the nitrogenous substances of potatoes in young rats exceeded that of whole wheat, but was inferior to that of milk. They assumed

that part of the non-protein nitrogen (N.P.N.) fraction of the potato tuber complemented the amino acids of the potato protein to produce a mixture of a biological value no less than that of the protein itself. Moreover CHICK and SLACK (1949) demonstrated that the potato proteins themselves were not superior to the mixture of the protein and the N.P.N. substances as far as their nutritional value is concerned. SCHUPHAN and POSTEL (1957) showed that the analysis of 134 samples of potato tubers gave an Essential Amino Acid Index (EAAI) of 72. SCHUPHAN (1959, a) found that the EAAI of 258 German potatoes averaged with 72. On the other hand HARTWELL (1927) found that potato proteins could not support the growth of rats but suggested that the quantity rather than the quality of potato proteins was responsible for the slow growth observed.

The aim of the present investigation was to trace the biological value of the potato nitrogenous substances as a whole. Four different varities were studied with the view to the effect of nitrogen fertilization and storage on the nutritive value of the nitrogenous fraction of the tuber.

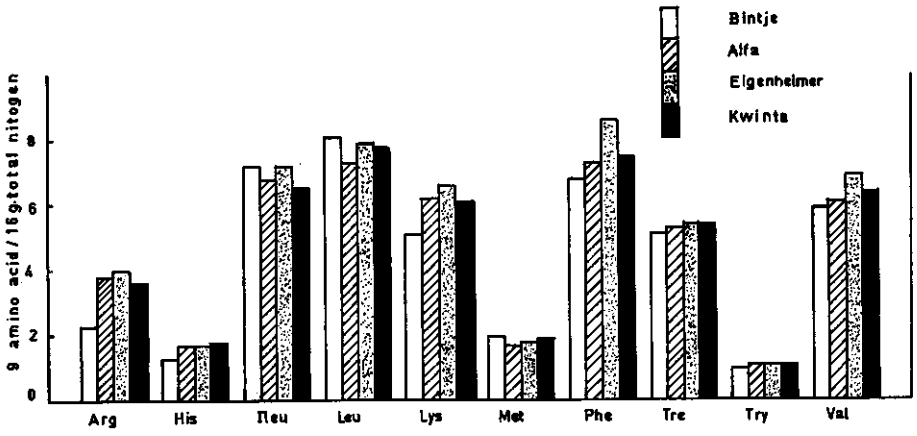


FIGURE 24. The average values of the amino acids in the whole potato, in varieties: Alfa, Eigenheimer, Kwinta and Bintje, expressed as g amino acid per 16 g total nitrogen.

A: GENERAL AMINO ACID CONTENT.

In Chapter Three the amino acid content was discussed at length. When the NV of a combination of proteins and of free amino acids is considered, the amino acid composition of the whole is of importance. The average values of the amino acids determined, in respect to the whole potato, is illustrated in figure 24. The variations between the average values of the four varieties were not great. If the essential amino acid pattern of the whole egg is compared with the pattern of the four potato varieties, it will especially be noticed that methionine and tryptophan are the limiting amino acids. Moreover a comparison of the FAO provisional pattern showed that tryptophan was a limiting factor. It should be stated, however, that the tryptophan content of the FAO pattern can be lowered somewhat without reducing the BV,

(SWENDSEID *et al.*, 1961). Although from the sulphur containing amino acids only the methionine was determined in the present study, it could be shown that the methionine is a limiting one. Table 26 shows the amino acid pattern of FAO, whole egg, and of the four potato varities.

TABLE 26. The amino acid pattern of the whole egg (OSER 1951), the provisional pattern of FAO (1957) and the essential amino acid composition of the four potato varities studied, expressed as gram amino acid per 100 gram protein.

Animo acid	Whole egg	FAO	Potato varieties			
			Bintje	Alfa	Eigenheimer	Kwinta
Isoleucine	7.7	4.2	7.2	6.3	7.2	6.5
Leucine	9.2	4.8	8.1	7.3	7.9	7.8
Lysine	7.0	4.2	5.1	6.2	6.6	6.1
Methionine	4.0	2.2	2.0	1.7	1.8	1.9
Phenylalanine	6.3	4.2	6.8	7.3	8.6	7.5
Threonine	4.3	2.8	5.1	5.3	5.4	5.4
Tryptophan	1.5	1.4	1.0	1.1	1.1	1.1
Valine	7.2	4.2	5.9	6.1	6.9	6.4

B. EFFECT OF NITROGEN FERTILIZATION AND STORAGE ON THE NV OF THE NITROGENOUS SUBSTANCES IN THE POTATO VARIETY BINTJE.

In his study on the BV of potato proteins, SCHUPHAN (1959) concluded that low BV of potato nitrogenous substances may sometimes be caused by nitrogen deficiency or nitrogen surplus in plant-nutrition. He reported that in relation to other nutrients, unbalanced nitrogen supplies may produce a BV of only 50.

In the discussion of the amino acids and the nitrogen distribution in potato tubers it was shown that these two were influenced both by nitrogen fertilization and by storage. These factors will, therefore, be discussed together. The influence of storage on potatoes dressed with different quantities of nitrogen was found to be different in respect of nitrogen distribution. It is not surprising that the same was found with regard to the NV of the nitrogenous substances.

At 0 time of the experiment, the NV decreased at the level of 40 Kg N/ha, a rise and rather stable level could be noticed at 80, 120, 160, and 200 Kg N/ha supply. After 8 weeks' storage, a different picture was observed. The maximum NV was recorded with the sample of 80 Kg N/ha supply. After 24 weeks, the NV first dropped reaching a minimum at 80 kg N/ha, but afterwords increased and showed a rather stable level. This is illustrated in figure 25.

In figure 26, in which each nitrogen supply level was drawn separately, it can be noticed that after 8 weeks' storage the nitrogen fertilization levels of 0, 40, and 80 Kg N/ha showed an increased NV, whereas a decrease in the NV was seen with the levels 120, 160 and 200 Kg N/ha. After a storage period of 24 weeks an increase in the NV was observed in all the levels of nitrogen supply except that of 80 kg N/ha, which indicated a decrease.

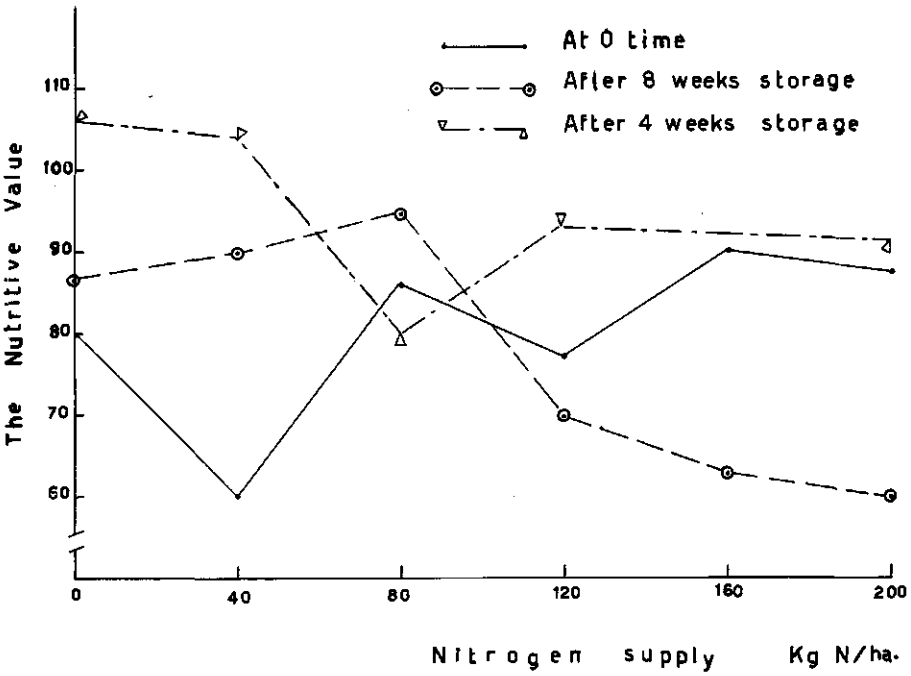


FIGURE 25. The effect of nitrogen fertilization on the NV of potato nitrogenous substances as determined microbiologically, in three storage periods, viz, 0 time, 8 and 24 weeks, in the variety Bintje.

The increase of the NV of the potato nitrogenous substances cannot be due to the proteins only, since the protein content as a percentage of the total nitrogen decreased at the higher levels of nitrogen supply (Chapter Three). CHICK and CUTTING (1943) and CHICK and SLACK (1949) observed that the N.P.N., even if it was poor in supporting growth by itself, improved the growth when it was added to the potato proteins.

After a 24 weeks' storage period it was shown that the content of most the free essential amino acids of the N.P.N. fraction increased. This was discussed in Chapter Three, in which it was suggested that a possible explanation of this phenomenon might be found in the fact that with a decrease in the ammonia storage substances, i.e. amides, the essential amino acids should form a higher percentage of the N.P.N. fraction.

As has been emphasized before, the nutritive value of a protein depends not only on the amino acid composition, but also on the availability of its essential amino acids; in other words, on the ease with which the amino acids become free during digestion, so that the body can use them. In this connection, it is obvious that the potato free amino acids are 100% available, this ought not to be the case with the tuber proteins. It is doubtful if (i) the six components of the potato proteins have the same digestibility, and (ii) the digestibility of one or more components in the potato proteins would amount to 100%. It is reasonable to assume that, even the six

components may have the same digestibility, the rate of releasing the amino acids would limit their availability. An increase in the N.P.N. may, therefore, have a direct and considerable influence on the NV of the total nitrogen when it is assumed that the increase did not involve the amide fraction only. During storage amides are required for protein synthesis and also to provide nitrogen to the sprouts. Consequently an increase in the percentage of the free amino acids of the tuber may be the result.

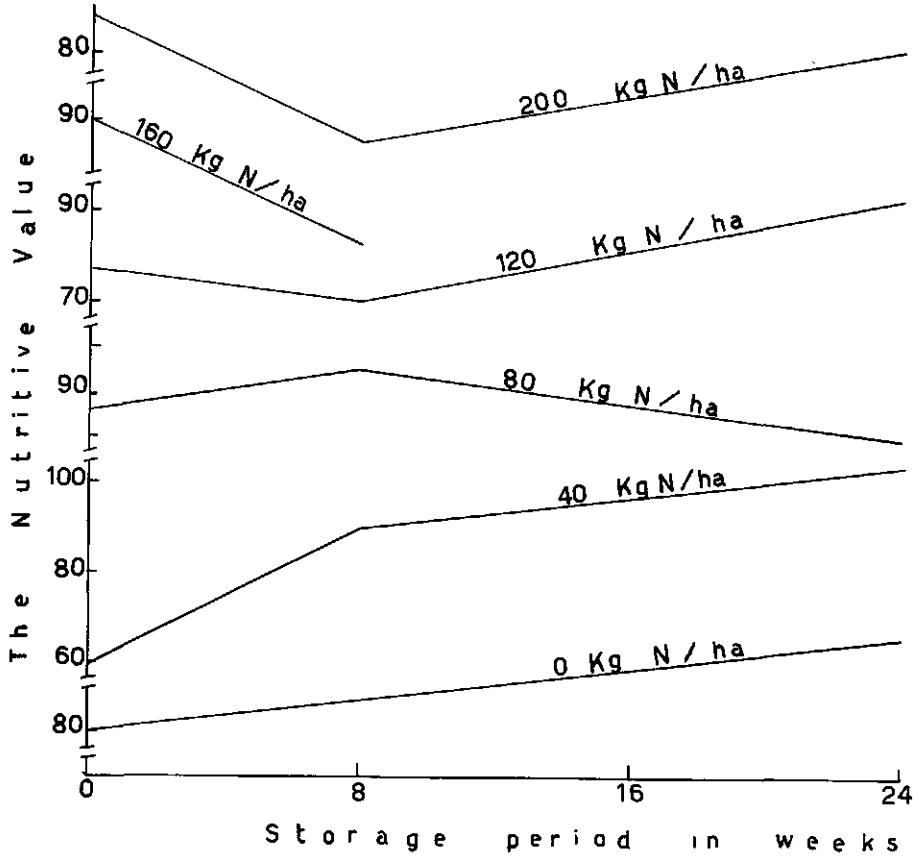


FIGURE 26. The effect of storage period on the NV of the nitrogenous substances in potatoes supplied with different quantities of nitrogen in the variety Bintje.

C. THE EFFECT OF STORAGE TIME AND TEMPERATURE ON THE NV OF THE NITROGENOUS SUBSTANCES IN THE POTATO VARIETIES ALFA, EIGENHEIMER AND KWINTA.

The NV of the nitrogenous substances of the potato varieties Alfa, Eigenheimer and Kwinta as influenced by storage period and temperature, are shown in figure 27. It can be noticed that the trends encountered in the three varieties are not quite the same.

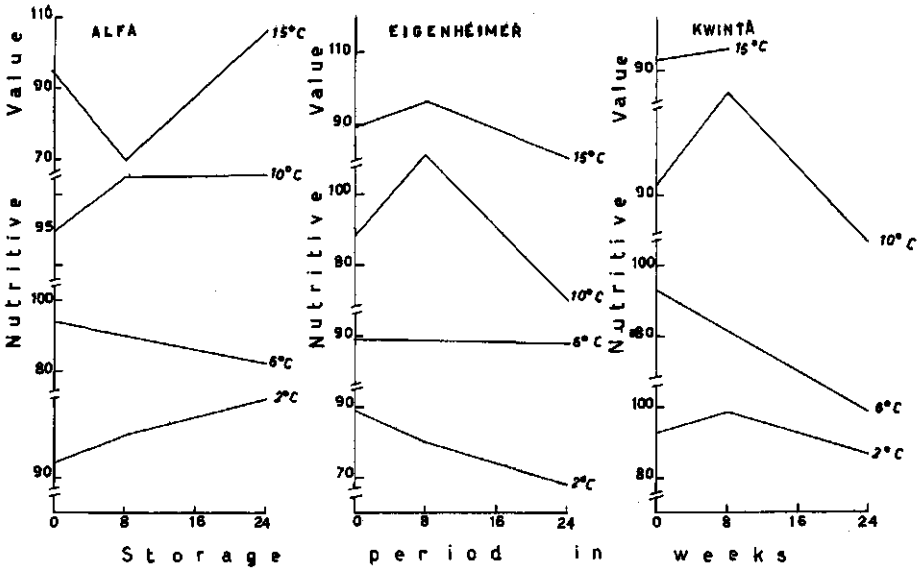


FIGURE 27. The effect of storage period on the NV of potato nitrogenous substances in the varieties: Alfa, Eigenheimer and Kwinta, stored at 2°, 6°, 10° and 15° C.

In Alfa and Kwinta an increase in the NV was observed after 8 weeks' storage when the temperature was 2° C. In Eigenheimer, however, a decrease was recorded. From figure 16 in Chapter Three, it can be seen that the protein part of the total nitrogen gave the reverse picture under the same circumstances. At 10° C all the varieties under investigation showed an increase in NV after 8 weeks' storage, whereas the protein nitrogen to the total nitrogen ratio showed a decrease under the same conditions. However, at 15° C, when both synthesis and breakdown were greatly enhanced, the NV behaved as the protein content, i.e., increased together. After 24 weeks' storage, at 2° C, Alfa showed an increase in the NV, Kwinta and Eigenheimer a decrease. At 6° C a stable level in Eigenheimer and a decreasing one in Kwinta and Alfa were noticed. After 24 weeks' storage at 10° C a decline in the NV was observed with Eigenheimer and Kwinta, a stable level being found with Alfa. At 15° C, Eigenheimer showed a decrease and Alfa an increase.

The picture described above is a very complicated one and seems to be the result of an interaction of many factors. The amino acid composition of the whole tuber, the free amino acids and the availability of the amino acids from the potato proteins are all directly involved. These direct factors are in their turn influenced by storage and germination. It has been shown before that the total nitrogenous substances of the potato tuber will give a higher NV if the free amino acid content is high. An exception was encountered in the samples after 8 weeks of storage at 15° C where protein synthesis was involved. In this case the proteins seemed to be largely responsible for the NV.

It can be seen that at higher temperatures and after longer storage periods the NV generally decreased. Alfa, however, is known to possess good storage qualities, which is reflected in its NV during storage, the reduction being less pronounced and taking place later than in Eigenheimer and Kwinta.

D. GENERAL VIEWS ON THE NV AS DETERMINED BY T. PYRIFORMIS W. METHOD AS COMPARED WITH THE EAAI.

The EAAI was estimated from the average values of the amino acids. The average values of the EAAI was estimated to be 80, whereas the microbiological experiment gave 89 as a mean value. From the EAAI, the BV can be estimated employing the equation of MITCHELL (1954):

$$BV = 1.0747\ (EAAI) - 13.74$$

thus, the BV of potato nitrogenous substances amounted to 73. The NV determined microbiologically was related to casein, that of the EAAI to the whole egg. Now it can be easily demonstrated that, if the BV of the whole egg proteins is assumed to be 100 and the casein 84 (OSER 1959), the average NV determined microbiologically will become 75 related to the egg proteins. The two values are nearly of the same order. The results of calculating the EAAI and the BV from the average amino acid composition are shown in table 27. In the same table the average values obtained with the microbiological method, corrected to the egg value are also illustrated.

TABLE 27. The EAAI and its BV values as compared to the NV determined microbiologically and corrected to the whole egg protein value.

Potato varieties	EAAI		NV
	EAAI	BV	
Bintje	83	76	71
Alfa	83	76	84
Eigenheimer	87	80	73
Kwinta	85	78	76

The discrepances between the estimated and the calculated data may be due to a neglect of the availability of amino acids when computing EAAI and to some differences in the amino acid requirements for rat and protozoa. As to the availability, the results of CARPENTER (1957) concerning the availability of lysine may serve as an example in this respect.

The short time in which the microbiological method could be carried out, the similarity of the amino acid requirements for the protozoa and rat and the fact that the digestibility is taken into account might render the microbiological assay as the method of choice for protein evaluation in spite of the weak correlation between the EAAI and the results obtained in this study. The number of objections to and the little confidence in the microbiological methods, originate from the insufficient

knowledge of the exact behaviour of the organisms. It seems possible to develop these methods in such way that more accurate results are obtained. For the time being the method used in this work can be recommended when small samples have to be examined and when an evaluation is needed for a great series of proteins, as in case of processing or storage experiments.

V. Summary And Conclusions

The nutritive value (NV) of the nitrogenous substances of the potato tuber were investigated.

The introduction included a short review of the place of proteins in nutrition and the methods used for the determination of their biological value (BV).

The method for evaluation of potato nitrogenous substances used in this investigation, was a microbiological one, in which the protozoa *T. pyriformis* W., was applied as the test organism.

Tryptophan and methionine are the most limiting amino acid in the potato tuber if compared with the provisional FAO amino acid pattern and the composition of the whole egg proteins.

The effect of nitrogen fertilization and storage on the NV has been dealt with. The NV of the nitrogenous substances of potato tuber seemed to be influenced by many factors. The amount of the non-protein nitrogen as a percentage of the total nitrogen and the amino acid composition of the former fraction turned out to be a factor of considerable importance. Storage and fertilization not only influenced the protein and the non-protein nitrogen fractions markedly but also interweaved the NV. The NV mostly changed inversely with the protein content as a percentage of total nitrogen. This was related to the increased amounts of the free amino acids, which are totally available and act as a supplement for the protein animo acids. The availability of the latter, however, is not complete and depends on the digestibility, which may be different for the six components of the potato proteins.

The average EAAI of the total nitrogenous substances in potato was found to be 80, while the NV determined microbiologically averaged in 89. The BV calculated from the EAAI was 73 and the NV corrected to the whole egg proteins value was 75.

The microbiological method used in this investigation, is recommended for experiments on small samples and when great numbers of samples have to be examined on a routine base.

CHAPTER FIVE

SUMMARY

Physical and biological investigations were carried out on the nitrogenous substances in the potato tuber.

The importance of potato as food has been considered in Chapter One; The Introduction.

Chapter Two: was devoted to a study of the electropherotic properties of the potato proteins.

An accurate method for potato protein separation was developed in which the paper electrophoresis technique was used. The experiments revealed that the potato proteins consisted of at least six components, the sixth one was thought to be a mixture of at least three fractions. The results of the experiments with paper electrophoresis could be confirmed by the observations made on zone electrophoresis.

In the present study four potato varieties, viz. Alfa, Eigenheimer, Kwinta and Bintje, were examined. The six protein fractions showed the same migration rates in the electropherograms of all the varieties. The ratio of the quantities of the six fractions was, however, found to be different from one variety to another. A simple scanning technique was developed to identify the four varieties from their electropherograms.

Nitrogen fertilization had but little influence on the electrophoretic pattern, and it did not interfere with the identification.

Storage, time and temperature affected the pattern composed of the six components. A discussion was given of the possibility of protein synthesis and breakdown that may take place during storage as related to the changes into the electropherograms. The effect of storage was no reason to alter the method of identifying the four varieties proposed in this investigation.

Chapter Three: involves the distribution of the nitrogenous components in the tuber as influenced by storage and nitrogen fertilization. In the present work the nitrogenous compounds were devided into total nitrogen (T.N.), non-protein nitrogen (N.P.N.) and protein nitrogen (P.N.). The P.N. and the N.P.N. fractions were analysed for the ten essential amino acids. The amino acids were determined microbiologically. Tryptophan in proteins was determined spectrophotometrically.

A short review of the different methods for amino acids determined was included. In Chapter Three, Results And Discussion, a short account of the biochemical changes due to storage occurring in the vegetative storage organs of plants in general and in potato tubers in particular, was given.

An increase in T.N. content of the tuber as a result of increasing nitrogen ferti-

lization was recorded. At high nitrogen supply levels T.N. increased after 8 weeks' storage at 5° C. This was attributed to the high respiration rates seen in potatoes excessively dressed with nitrogen, which resulted in marked losses of carbohydrates. After 24 weeks' storage, however, the T.N. decreased, which was supposed to be due to the losses of nitrogen in favour of the new sprouts. The highest ratio of P.N. : T.N. was noticed at a nitrogen supply level of 40 kg/ha, whereas the lowest was recorded at the level of 200 kg N/ha supply. The changes, however, due to storage were found to be more pronounced than those due to nitrogen fertilization. Different results between samples with ample nitrogen supply and poorly dressed samples as to the changes into P.N. : T.N. due to storage were clearly demonstrated. The highest P.N. as a percentage of T.N. in samples dressed with 200 Kg N/ha was found after an 8 weeks' storage period. This was attributed to protein synthesis. The source of ammonia for protein synthesis is believed to be the amides. It may then be expected that the amide pool will decrease, when protein synthesis is taking place, and consequently, the total N.P.N. decreases, and the P.N. : T.N. ratio increases. With the advance of the storage period, however, the ratio P.N. : T.N. was found to decrease in potato samples excessively dressed with nitrogen. The factors that may participate in the changes of the P.N. : T.N. ratio, i.e. variety, storage period, storage temperature and germination were also discussed.

The influence of nitrogen fertilization on the amino acid composition of P.N., contrary to the influence on the N.P.N. composition, was found to be rather small, the influence of storage being more pronounced. At the end of the storage period the contents of nearly all the free essential amino acids increased. This was attributed to two factors: the breakdown of proteins dominating during that period will supply the N.P.N. pool with more amino acids, and sprouts as well as protein synthesis in the tubers will withdraw the amides preferentially, which will increase the portion of the free amino acids in the N.P.N. fraction.

The Fourth Chapter dealt with the nutritive value (NV) of potato nitrogenous substances. It was stated as a conclusion that the microbiological method in which *Tetrahymina pyriformis* W. was used as the test organism, may supply a useful means for evaluating proteins, if the laboratory animals cannot be used on routine scale and only small quantities of the protein to be examined are available.

On an average the NV of potato nitrogenous substances was estimated to be 89 when related to casein, and 75 when related to the whole egg proteins, when determined microbiologically. Comparatively, the Essential Amino Acid Index (EAAI) was calculated to be 80, and the biological value (BV) derived from the (EAAI) averaged 73. No close correlation was found to exist between the estimated and the calculated nutritive values of the nitrogenious substances in the potato varieties examined in this work. In this connection it was emphasized that in computing the (EAAI), the availability of the amino acids and the digestability of the proteins were completely neglected.

The conclusion was drawn that the NV of potato nitrogenous substances is not predominently dependent on proteins, but also to a great extent on the free amino acids.

SAMENVATTING

Een fysisch en chemisch onderzoek van de stikstofhoudende bestanddelen van de aardappel werd uitgevoerd.

Het belang van de aardappel als voedingsmiddel werd besproken in Hoofdstuk I; de inleiding.

Hoofdstuk II: behandelt de electroforetische karakteristieken van de aardappeleiwitten. Een verfijnde scheidingsmethode werd ontwikkeld, gebruik makend van de papierelectroforese techniek. Het onderzoek toonde aan, dat aardappeleiwit is samengesteld uit ten minste zes eiwitcomponenten. Het werd waarschijnlijk geacht, dat de zesde component een niet te scheiden mengsel van drie fracties was. De resultaten van deze onderzoekingen konden worden bevestigd door de waarnemingen verkregen uit een onderzoek naar de eigenschappen van aardappeleiwit in de Tiseliuscel bij vrije electroforese. Het aardappelonderzoek werd uitgevoerd op vier verschillende rassen, te weten: Alfa, Eigenheimer, Kwinta en Bintje. Bij vergelijking van de electropherogrammen bleken de zes eiwitfracties bij ieder ras dezelfde loopsnelheid te hebben. De rassen onderscheidden zich echter in de hoeveelheden die van iedere component in het totaal eiwit aanwezig waren. Een eenvoudige methodiek werd geïntroduceerd om de vier rassen aan het electropherogram te indentificeren. Stikstofbemesting had slechts weinig invloed op het karakteristieke electroforesepatroon en stoorde dientengevolge de identificatie niet. Bewaartijd en bewaartemperatuur hadden een wat grotere invloed op het patroon, samengesteld uit de zes componenten. Een bespreking over de mogelijkheid van synthese en afbraak van eiwitten gedurende de bewaring van aardappelen werd gegeven in verband met de waargenomen veranderingen in het electroforese patroon. Deze veranderingen waren echter niet van dien aard, dat de voorgestelde methode tot identificatie van de rassen Alfa, Eigenheimer, Kwinta en Bintje moest worden herzien.

Hoofdstuk III: behandelt de verdeling van de stikstofhoudende bestanddelen van de aardappel en de invloed van de bewaring daarop. In dit onderzoek werden de stikstofhoudende bestanddelen ingedeeld in verschillende fracties n.l. totaal stikstof (TN), niet-eiwitstikstof (NPN) en eiwitstikstof (PN). Langs microbiologische weg werden PN en NPN geanalyseerd op het gehalte aan de 10 essentiële aminozuren. Alleen tryptofaan in PN werd spectrofotometrisch bepaald. Een bespreking van de bestaande methodieken gaat aan de behandeling van de verkregen resultaten vooraf. Bij de bespreking van de resultaten werd aandacht geschonken aan de biochemische reacties en de daardoor veroorzaakte veranderingen, die plaatsvinden in de opslagorganen van planten in het algemeen en van de aardappel in het bijzonder. Een toename in het TN-gehalte als gevolg van toenemende bemesting met stikstof werd waargenomen. Bij een hoge stikstofgift nam het TN-gehalte toe indien het monster

8 weken bij 5° C werd bewaard. Dit werd toegeschreven aan de hoge respiratiesnelheden die men waarneemt bij overvloedig met stikstof bemeste aardappelen; deze aardappelen verademen namelijk een aanzienlijke hoeveelheid koolhydraten. Het TN-gehalte nam echter na een bewaring van 24 weken weer af. Gedacht werd, dat in dit stadium het stikstofverlies ten gunste van de nieuw gevormde spruiten groter was dan het verlies aan koolhydraten. De hoogste PN/TN-verhoudingen werden gevonden in de monsters, die een bemesting van 40 kg N/ha ontvingen; de laagste waarden werden daarentegen genoteerd bij de monsters die 200 kg N/ha ontvingen. Deze verhouding werd echter veel sterker door de bewaring dan door de bemesting beïnvloed. De veranderingen in het PN-gehalte als percentage van het TN ten gevolge van de bewaring waren in de ruim met stikstof bemeste aardappelen anders dan in aardappelen, die ontoereikend met stikstof waren bemest. In de monsters die een stikstofgift van 200 kg N/ha ontvingen werden de hoogste PN-percentages gevonden na 8 weken bewaring. Dit werd toegeschreven aan eiwitsynthese, die in deze periode plaats vond. Bij synthese zal n.l. het gehalte aan vrije amiden, die als bron van de voor de synthese vereiste ammoniak kunnen worden beschouwd, afnemen. Dientengevolge zal ook het gehalte aan totaal NPN afnemen, zodat de fractie PN uitgedrukt als percentage van het TN zal stijgen. Naarmate de bewaring echter nog langer wordt voortgezet, daalde de PN/TN-verhouding. Klaarblijkelijk zal in zo lang bewaarde aardappelen aan de aminozuurbehoefte van de gevormde spruiten worden tegemoetgekomen door snellere afbraak van eiwitten. Een en ander was duidelijker waarneembaar in overvloedig, dan in onvoldoende met stikstof bemeste aardappelen.

Tenslotte worden een aantal van de PN/TN-verhouding bepalende factoren, zoals ras, condities van de bewaring en kieming nader besproken. Wat de invloed van de bemesting op de aminozuursamenstelling van de eiwitachtige bestanddelen van de aardappel betreft, werd gevonden dat deze zich voornamelijk uitstrekte over de NPN-fractie. De invloed van de bewaring bleek echter groter te zijn. Aan het einde van de bewaarperiode van 24 weken bleek het gehalte aan vrijwel alle vrije aminozuren te zijn toegenomen. Deze bevinding werd aan twee factoren toegeschreven. Enerzijds overheerste van de anabolische en catabolische reacties in deze periode de afbraak, door welke proteolyse de NPN-fractie van meerdere aminozuren werd voorzien; anderzijds onttrekken de nieuw gevormde spruiten aan deze accumulatie van aminozuren bij voorkeur de amiden voor eiwitsynthese.

Hoofdstuk IV: behandelt de voedingswaarde van de stikstofhoudende bestanddelen van de aardappel. De conclusie luidde, dat de microbiologische methode, waarbij *Terahymena pyrifermis W.* als organisme wordt gebruikt, een bruikbaar hulpmiddel voor de waardering van eiwitten is gebleken, vooral waar het inzetten van proefdieren op zo grote schaal niet mogelijk was en bovendien slechts kleine hoeveelheden van de te onderzoeken eiwitten voorhanden waren. De bepaling van de voedingswaarde van de eiwitachtige bestanddelen leverde gemiddeld 89 op, indien de groei van de protozoa als percentage van de groei op een medium met caseïne wordt uitgedrukt. Door omrekening werd gevonden, dat indien op eiwit zou zijn betrokken een waarde van 75 gevonden zou zijn.

Ook de EAAI (eveneens betrokken op eiwit) werd berekend. Deze bleek gemiddeld 80 te zijn. Voor de hieruit afgeleide Biologische Waarde is een waarde van 73 te berekenen.

Weinig overeenkomst werd gevonden tussen de bepaalde en de berekende voedingswaarden van de onderzochte eiwitten. In dit verband werd nogmaals de aandacht gevestigd op het feit, dat bij de berekening van de EAAI slechts rekening wordt gehouden met de aanwezige — en niet met de voor het organisme beschikbare hoeveelheid aminozuren.

Geconcludeerd werd, dat de voedingswaarde van de eiwitachtige bestanddelen van de aardappel niet overwegend door de eiwitfractie wordt bepaald maar voor een belangrijk deel ook door de vrije aminozuren.

ملخص باللغة العربيـــــة

بروتينات البطاطس

قيمتها الغذائية وصفاتها الحيوية الطبيعيـــة

أولا : المقدمة

فى الجزء الاول من هذه الرسالة نوقشت أهمية البطاطس من الناحية الغذائية وأهميـــة البروتين خاصة . واشتملت هذه الدراسة على ثلاثة دراسات رئيسية .

١ ــ البحث عن طبيعة المركبات البروتينية عن درنة البطاطس .

٢ ــ دراسة الاحماض الأمينية وتوزيع الازوت فى درنة البطاطس .

٣ ــ دراسة القيمة الغذائية للمواد الازوتية فى درنة البطاطس .

ولما كانت عمليات التسميد والتخزين لمحصول البطاطس يعتبران من أهم العمليات المرتبطة مباشرة بانتاج واستهلاك البطاطس ولها بذلك تأثير مباشر وارتباط وثيق بالتغذيـــــة ــ فقد اشتملت هذه الدراسة أيضا على بحث تأثير التسميد الازوتى والتخزين على المركبـــات البروتينية والقيمة الغذائيـــــــة .

ثانيـا : صفات بروتينات البطاطس تجاه الانتقال فى الحقل الكهربائى

The electrophoretic properties of potato proteins

١ ــ باستخدام تيار كهربائى قوة ٤٠٠ فولت ومحلول منظم من حامض البربيتال وبربيتال الصوديوم قوته الايونية ٠٫٠٥ أمكن استحداث طرقة جديدة لفصل بروتينات البطاطس على الورق .

٢ ــ اثبتت الطرقة المستحدثة أن بروتينات البطاطس ليست بروتين واحد وليست مخلوط مكـــون من بروتينين كما كان معروفا بل أنها مخلوط مكون من ستة مكونات بروتينية على الاقل . ويشتبه فى أن المركب السادس خليط من ثلاثة بروتينات اخرى .

٣ ــ باستخدام طريقة الانتقال الحر فى التيار الكهربائى Free electrophoresis أثبتت صحة هذه النتائج .

٤ ــ أثبتت النتائج ان الاربعة اصناف المدروسة (ألفا ــ ايخنهيمر ــ كوينتا ــ وبينتيـــا) تحتوى بروتيناتها على الستة مركبات وان انتقال هذه المركبات تمت بمسافات ثابتة فـــى جميع الاصناف .

٥ ــ اختلفت كميات كل من الستة مركبات البروتينية باختلاف صنف البطاطس ــ وقد وجد أن نسب هذه المركبات الى بعضها فى كل صنف ثابتة ومختلفة عن الاصناف الاخرى مما أدى الى استنباط طريقة سريعة ودقيقة للتفريق والتمييز بين أصناف البطاطس الاربعة المدروسة .

٦ــ لم يؤثر التسميد الازوتي تأثيرا يذكر على عدد أو كميات البروتينات الستة الموجودة في درنة البطاطس .

٧ ــ وكان نتيجة التخزين ان اخفقت نسبة المركبات البروتينية قليلا عن مثيلاتها قبل التخزين وقد عزى ذلك الى التغيرات الفسيولوجية التي تحدث في درنات البطاطس اثنا ء التخزين مثل بنا ء وهدم البروتينات . ولم يظهر لهذا التغيير تأثير على الطريقة المستحدثة للتفريق أو التمييز بين الاصناف .

ثالثـــــا : المواد الازوتية والاحماض الامينية الاساسية في درنات البطاطس

١ ــ الازوت الكلـــــــي

ا ــ ارتفعت نسبة الازوت الكلي بالدرنات تحت تأثير زيادة التسميد الازوتي .

ب ــ بعد ثمانية أسابيع من التخزين ارتفعت نسبة الازوت الكلي في درنات البطاطس ذات التسميد الازوتي العالي وعزى ذلك الى ارتفاع نسبة تنفس الدرنات في هذه الحالة مما يتسبب عنه فقد كمية أكبر من الكربوهيدرات .

جـ ــ بعد أربعة وعشرون اسبوعا انخفضت نسبة الازوت الكلي في الدرنات وعزى ذلك الى أن النموات الجديدة استنزفت كمية من الازوت من درنة الام .

٢ ــ المواد الازوتية البروتينيـــــــــة .

ا ــ انخفضت نسبة الازوت البروتيني المئوية من الازوت الكلي نتيجة للتسميد الأزوتي .

ب ــ بعد ثمانية اسابيع من التخزين ارتفعت نسبة البروتين الازوتي في درنـــــــات البطاطس ذات التسميد المرتفع ــ وذلك نتيجة لبنا ء بروتينات جديدة في درنـــــة البطاطس ــ ولما كانت الاميدات هي مصدر الامونيا في الدرنات فقد استخلص أن التسميد الازوتي الغزير يساعد على تنشيط عملية بنا ء البروتين اثنا ء الفترة الأولـــــى من التخزين .

جـ ــ بعد اربعة وعشرون اسبوعا من التخزين انخفضت نسبة البروتين في عينات البطاطس المسمدة تسميدا غزيرا عن الفقيرة التسميد كنتيجة لزيادة النموات الحديثة في الاولـــى عن الثانيـــــــة . ومن المعروف أن النموات الحديثة تستنزف الازوت من درنـــات الام عن طريقة الازوت الغير بروتيني ــ لذلك فان هدم البروتين في هذه الفتـــــرة يسود على البنا ء حتى يوفر كميات الازوت الحر للانبات الحديث .

أما بالنسبة للأزوت الغير بروتيني فانه يأخذ طريقا عكسيا لطريقة الازوت البروتيني .

٣ ــ الاحماض الامينيـــــــــــة .

ا ــ كما هو معروف في التغذية ان قيمة البروتين الغذائية تعتمد أساسا على نوع وكميـــات الاحماض الامينية المركب منها ــ لذلك فقد اشتملت هذه الدراسة على بحث عـــــن الاحماض الامينية الاساسية الموجودة في القسم البروتيني وكذلك الاحماض الامينية الحرة الموجودة في الجز ء الازوتي الغير بروتينـــــي .

ب ـ التسميد الازوتى لا يؤثر كثيرا على كميات الاحماض الامينية الاساسية المكونـــة للبروتين ولكن بالنسبة للاحماض الامينية الحرة فان تأثير التسميد الازوتى واضح ٠

جـ ـ فى نهاية فترة التخزين ارتفعت كمية الاحماض الامينية الحرة ـ وعلل ذلك بان نسبـــة الاميدات فى هذا الجزء من الازوت تستفد اولا بأول بواسطة الانبات فى هذه الفتـــرة المتأخرة من التخزين بالاضافة الى هدم البروتين الذى يسود فى هذا الوقت ٠

د ـ اجرى تقدير الاحماض الامينية بواسطة طرق ميكروبيولوجية وانحصر الخطأ التجريبى بيـــن ٥ر١ ـ ٩ر٤ ٪ ٠

رابعـــا : القيمة الغذائية للمواد الازوتية فى البطاطس ٠

١ ـ اعطيت نبذة قصيرة عن البروتين وأهميته فى التغذية بالنسبة للانسان والحيوان ـ ونوقـش موضوع توازن وعدم توازن الاحماض الامينية وتأثير ذلك على الانسان والحيوان ـ واعطيـــت نبذة عن امكان تحسين البروتينات النباتية ذات القيمة الغذائية المنخفضة ٠

٢ ـ اعطى ملخص لأهم طرق تقدير القيمة الغذائية للبروتين وقسمت الى طرق مباشرة وغيـــر مباشرة وكيماوية وميكروبيولوجيـــة ٠

٣ ـ استخدمت فى هذه الدراسة طريقة ميكروبيولوجية لتقدير القيمة الغذائية للمواد البروتينيـــة فى البطاطس وذلك باستعمال البروتوزوا " Tetrahymena pyriformis W ".

٤ ـ لا تتوقف القيمة الغذائية للمواد الازوتية بدرنة البطاطس على البروتين ولكن ايضا على الاحماض الامينية الحرة ٠

٥ ـ أظهرت التجارب أن القيمة الغذائية للمواد الازوتية بالبطاطس تعادل ٨٨ اذا قورنـــت بالكازين ـ ٧٥ اذا قورنت ببروتينات البيض ٠

٦ ـ باستخدام طريقة (Oser) الخاصة بنسب الاحماض الامينية للبروتين الى مثيلاتهـــا فى بروتين البيض وجدت أن المواد الازوتية فى البطاطس تعادل ٧٣ اذا كانت بروتينات البيض تساوى ١٠٠ ٠

٧ ـ بمقارنة كمية الاحماض الأمينية فى البطاطس بمثيلاتها فى البيض وفى مخلوط الاحمـــاض الامينيـــــة المقترح من هيئة التغذية والزراعة لهيئة الامم المتحدة ـ اتضح أن الاحماض الامينيـــــة المحددة هى المثيونين والتربتوفان ٠

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