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# Amino acid composition of coagulable protein from tubers of 34 potato varieties and its relationship with protein content

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*Zusammenfassung, Résumé p. 433.*

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## Summary

Samples of coagulable protein, from tubers of 34 varieties of *Solanum tuberosum*, were analysed for amino acids. The coagulable protein was rich in most of the essential amino acids. The essential amino acid index ranged from 86 to 93. Methionine was the first limiting essential amino acid. Its content in coagulable protein was considerably higher than values reported in literature. Lysine content was very high, which makes the protein a valuable component for mixed feed. Arginine was the second limiting amino acid. The varietal differences in amino acid composition were small. There was no correlation between protein content and content of most of the essential amino acids. Breeding for increased protein content in potato may be expected to have little effect on the nutritional value of the protein. Selection can be directed to content of coagulable protein.

## Introduction

The nitrogen compounds (crude protein) of potato tubers consist of two main fractions: true protein and non-protein nitrogen. As a whole, they are often referred to as total N, and the respective fractions as protein N and non-protein N. True protein is considered as nitrogen compounds precipitable with trichloroacetic acid. This protein fraction corresponds to the amount of protein precipitated, from acidified NaCl solutions at 100 °C (Neuberger & Sanger, 1942; Reissig, 1958), called coagulable protein. This protein is a valuable feedstuff because of its well balanced amino acid composition (Reissig, 1958; Kapoor et al., 1975).

In the Netherlands, the area under potatoes grown for the potato starch industry is 70 000 ha. Yearly, 2.5 to 3 million tonnes of potatoes are produced for the manufacture of potato starch. From the waste effluent of the potato starch industry, 25 000 to 30 000 tonnes of coagulable protein are available for recovery. At present, 8 000 tonnes of coagulable protein are produced yearly. This protein is used in animal feed. Increase in the content of coagulable protein in industrial potato varieties, will increase their value for the starch industry.

A high content of coagulable protein can be combined with high yield of dry matter (Miedema et al., 1976). But breeding of crops for increased protein content is often associated with a decrease in protein quality. For instance in barley, protein content is negatively associated with lysine content of the protein (Hagberg et al., 1970). The protein content of wheat is negatively correlated with contents of lysine and methionine in the protein (Dumanovic et al., 1970).

The purpose of this study was to estimate the amino acid composition of coagulable protein from potato tubers and to assess the relationship between amino acid composition and content of coagulable protein. Therefore analyses were done on 34 varieties with large differences in coagulable protein content.

## Materials and methods

*Sample preparation*

Freeze-dried coagulable protein was prepared from 34 potato varieties during earlier experiments. The protein contents of these varieties have already been reported (Miedema et al., 1976).

The following method was used. Extracts from potato tubers containing the total soluble N fraction were prepared as described before (Miedema et al., 1976). The coagulable protein fraction was prepared by continuously stirring 500 ml of extract, while adding concentrated acetic acid drop by drop to a pH of 4.7. The solution was kept in a boiling water bath for 30 min. The precipitated protein was filtered off, washed 4 times with 100 ml of hot water and 4 times with 75 ml of acetone. The protein was freeze-dried and ground in a mortar to a fine homogeneous powder.

*Amino acid analysis*

Duplicate samples of 10 mg protein were hydrolysed under nitrogen with 10 ml of redistilled HCl 6 mol/l for 24 h at 110 °C. Hydrolysed samples were dried at 40 °C at reduced pressure (Rotavapor) and taken up in 10 ml sodium citrate buffer pH 2.2. Amino acids were estimated with a Beckman Multichrom Amino Acid Analyzer.

Methionine content estimated from acid hydrolysates of the proteins of the 34 varieties ranged from 0.86 to 2,75 g per 100 g amino acids recovered. Methionine recovery from samples spiked before hydrolysis was on average 82 % and hardly reproducible. The poor recovery might be due to destruction of methionine during this hydrolytic procedure. As methionine is the first limiting essential amino acid in whole potato (Rios Irairte et al., 1972), an accurate assessment of the sulphur-containing amino acids in the coagulable protein was required. Therefore methionine and cystine were estimated separately by a method of Moore (1963). By this method, methionine and cystine are oxidized before hydrolysis and were measured as methionine sulphone and cysteic acid, respectively. From a preliminary experiment, it appeared that when this method was used, recovery of methionine was well reproducible and was on average 101.6 % (Table 1). The methionine and cystine values reported below were obtained by this method.

Usually, amino acid contents are expressed as g amino acid per 16 g N or per 100 g protein. In this study, the amino acid composition is in g amino acid per 100 g of total amino acids recovered. So the data are not affected by losses due to humin formation

Table 1. Recovery of methionine from spiked protein samples of five potato varieties.

| Sample No[1] | Methionine in sample (μmol)[2] | | | Methionine recovered[6] | |
|---|---|---|---|---|---|
| | in protein[3] | added[4] | total amount[5] | absolute[7] (μmol) | relative[8] (%) |
| 1 | 0.577 | 0.608 | 1.185 | 1.181 | 100 |
| 2 | 0.950 | 0.130 | 1.080 | 1.128 | 104 |
| 3 | 0.542 | 0.594 | 1.136 | 1.151 | 101 |
| 4 | 1.122 | 0.130 | 1.252 | 1.287 | 103 |
| 5 | 0.980 | 0.182 | 1.162 | 1.165 | 100 |

[1] *Muster Nr. - Numéro d'échantillon;* [2] *Methionin im Muster (μmol) - Méthionine dans l'échantillon (μmol);* [3] *Im Protein - En protéine;* [4] *Zugefügt - Ajouté;* [5] *Totalmenge - Total;* [6] *Wiedergewonnenes Methionin - Méthionine récupérée;* [7] *Absolut - Absolu;* [8] *Relativ - Relatif*

Tabelle 1. Wiedergewinnung von Methionin aus Proteinproben von fünf Kartoffelsorten mit Zugabe einer bekannten Menge Methionin.
Tableau 1. Quantité de méthionine récupérée dans les échantillons de cinq variétés de pomme de terre.

during hydrolysis, or by slight variations in nitrogen content of the proteins due to differences in amino acid composition.

*Assessment of protein quality*

The relative deficit of an amino acid in a protein was given by the egg-protein ratio (EPR) (Oser, 1959), which is defined as the content of an amino acid in the protein as a percentage of its content in whole egg-protein. In our calculations, FAO data from column chromatographic amino acid analysis of whole-egg protein (FAO, 1970) were converted from mg amino acid per g N to g amino acid per 100 g amino acids recovered.

The quality of the protein was expressed as essential amino acid index (EAAI). The EAAI is defined as the geometric mean of the EPR for the essential amino acids (including histidine and arginine), in which EPR values exceeding 100 % are put at 100 % (Oser, 1959).

## Results and discussion

*Amino acid composition of coagulable protein*

Table 2 shows the amino acid composition of coagulable protein from tubers of 34 potato varieties. The varieties covered a wide range of contents of coagulable protein in fresh tuber (0.37 to 1.24 g per 100 g). There was little variation in amino acid composition between the 34 varieties (Table 2). The coefficient of variation was highest for methionine, alanine and cystine, 13.0 %, 13.1 % and 9.9 %, respectively. The variation in the contents of the amino acids is probably determined genetically, because all varieties were grown under identical ecological conditions and the tubers were fully mature.

Comparison with whole-egg protein showed that the coagulable potato protein

Table 2. Range, coefficient of variation and average of content of protein in fresh weight (g/ 100 g), of N in protein specimen (g/ 100 g dry), of amino acid N recovered of N in protein (g/ 100 g), of amino acids in total amino acid recovered of coagulable protein (g/ 100 g) and of essential amino acid index (EAAI) for tubers of 34 potato varieties. For comparison, FAO data for whole-egg protein are given.***

| | Range[1] | Coeff. of variation[2] | Average[3] | Whole egg[4] |
|---|---|---|---|---|
| Coagulable protein[5] | 0.37- 1.24 | 27.1 | 0.76 | - |
| N in protein[6] | 14.3-14.9 | 1.1 | 14.6 | - |
| N recovery[7] | 85.8-92.6 | 2.3 | 89.3 | - |
| *Amino acid*[8] | | | | |
| Threonine* | 4.64- 5.94 | 5.17 | 5.42 | 5.04 |
| Valine* | 5.77- 6.88 | 4.21 | 6.42 | 6.74 |
| Methionine* | 1.60- 2.64 | 12.99 | 2.15 | 3.31 |
| Isoleucine* | 4.81- 6.77 | 7.17 | 5.29 | 6.19 |
| Leucine* | 9.44-12.14 | 5.18 | 10.28 | 8.67 |
| Phenylalanine* | 5.53- 7.61 | 5.42 | 6.53 | 5.64 |
| Lysine* | 6.85- 8.57 | 5.19 | 7.64 | 6.86 |
| Histidine** | 1.90- 2.65 | 7.32 | 2.06 | 2.39 |
| Arginine** | 4.32- 5.80 | 6.04 | 4.95 | 6.00 |
| Aspartic acid | 11.56-13.72 | 4.23 | 12.64 | 9.46 |
| Serine | 5.03- 5.81 | 4.10 | 5.40 | 7.52 |
| Glumatic acid | 9.42-11.06 | 3.94 | 10.23 | 12.53 |
| Proline | 4.21- 5.36 | 4.75 | 4.83 | 4.09 |
| Glycine | 4.85- 5.47 | 4.41 | 5.03 | 3.26 |
| Alanine | 3.77- 6.05 | 13.14 | 4.73 | 5.82 |
| ½ Cystine | 0.64- 0.92 | 9.92 | 0.77 | 2.39 |
| Tyrosine | 4.46- 6.48 | 5.93 | 5.62 | 4.09 |
| EAAI[9] | 86- 93 | 2.08 | 89 | 100 |

*Essential amino acids - *Essentielle Aminosäuren - Acides aminés indispensables.*

**Growth promoting amino acids - *Wachstumsfördernde Aminosäuren - Acides aminés favorables.*

***An extended table presenting (a) each variety by name, (b) coagulable protein for each variety as % of the wet and dry matter of the tuber, (c) the amino acid composition for each variety, is lodged at the Foundation for Agricultural Plant Breeding (SVP). This table can be obtained on request - *Eine ausführliche Tabelle bestehende aus (a) die Namen der Sorten, (b) koagulierbares Protein für jede Sorte als % der Frisch- und Trockensubstanz der Knolle, und (c) die Zusammensetzung der Aminosäuren für jede Sorte, können Sie beim Foundation for Agricultural Plant Breeding (SVP) erhalten - Un tableau étendu presentant (a) les noms des variétés, (b) la protéine coagulable pour chaque variété comme % de la matière mouillé et sèche du tubercule et (c) la composition de l'acide aminé pour chaque variété, peut être obtenu à Foundation for Agricultural Plant Breeding (SVP).*

[1] *Bereich - Ecarts;* [2] *Variationskoeffizient - Coefficient de variation;* [3] *Mittelwert - Moyenne;* [4] *Ganzes Ei - Oeuf entier;* [5] *Koagulierbares Protein - Protéines coagulables;* [6] *Stickstoff im Protein - Azote protéique;* [7] *Wiedergefunden - Retrouvé;* [8] *Aminosäuren - Acides aminés;* [9] *Index der essentiellen Aminosäuren - l'index des acides aminés indispensables*

Tabelle 2. Bereich, Variationskoeffizient und Mittelwert des Gehaltes an Protein im Frischgewicht (g/ 100 g), an N in getrockneten Protein (g/ 100 g), an wiedergefundenen Aminosäure-N des N im Protein (g/ 100 g), an Aminosäuren im Aminosäuretotal des koagulierbaren Proteins (g/ 100 g) und des Indexes der essentiellen Aminosäure (EAAI) in Knollen von 34 Kartoffelsorten. Als Vergleich werden die FAO-Daten des Volleiproteins angegeben.***

Tableau 2. Ecarts, coefficient de variation et moyenne des concentrations en protéines par rapport au poids frais (g/ 100 g), en acides aminés par rapport aux acides aminés totaux récupérés dans les protéines coagulables (g/ 100 g) et de l'index des acides aminés indispensables (EAAI) pour 34 variétés de pommes de terre. Par comparaison, les normes FAO pour les protéines de l'oeuf entier sont données.***

contained substantial levels of most of the essential amino acids. However methionine content was relatively low (2,15 g per 100 g).

The amino acid composition corresponded closely to values from pure protein reported for 7 potato varieties by Wünsch (1975), except that contents of methionine and cystine were even lower in Wünsch's study, probably because of losses during the non-protected acid hydrolysis that he used. Our values also largely agree with data of Desborough & Weiser (1974), except for valine, methionine and isoleucine, which were lower in their study. The discrepancy might be due to genotypic differences between the *Solanum tuberosum* varieties used in our study and the Phureja-Tuberosum hybrids they used. However, in a preliminary experiment, we found quite similar amino acid compositions for protein samples from tubers of *S. tuberosum* and *S. phureja*.

*Quality of coagulable protein*

Assessment of protein quality by calculating the EPR and the EAAI from the chemical composition of a protein is only an approach to the real nutritional value of the protein. Nevertheless, it is a useful tool when biological methods are not feasible.

The egg-protein ratios (Table 3) show that in coagulable protein of the potato, methionine is the first limiting amino acid, although an EPR of 65 % is a fairly high score compared to other plant proteins. Arginine is an amino acid essential to monogastric animals during growth. In coagulable potato protein it proved to be the second limiting amino acid compared to whole-egg protein; its EPR was 82 %. The

Table 3. Range and average of the egg-protein ratio (EPR) for essential amino acids of coagulable protein from tubers of 34 potato varieties.

| Amino acid [1] | EPR (%) | |
|---|---|---|
| | range [2] | average [3] |
| Threonine | 92-118 | 108 |
| Valine | 86-102 | 95 |
| Methionine | 48- 80 | 65 |
| Isoleucine | 78-109 | 86 |
| Leucine | 109-140 | 119 |
| Phenylalanine | 98-135 | 116 |
| Lysine | 100-125 | 111 |
| Histidine* | 80-111 | 86 |
| Arginine* | 72- 97 | 82 |

*Growth promoting amino acids - *Wuchsfördernde Aminosäuren - Acides aminés stimulant la croissance.*

[1] *Aminosäure - Acide aminé;* [2] *Bereich - Ecarts;* [3] *Mittel - Moyenne*

Tabelle 3. Bereich und Mittelwert des Ei-Proteinverhältnisses (EPR) für essentielle Aminosäuren von koagulierbarem Protein aus Knollen von 34 Kartoffelsorten.
Tableau 3. Ecarts et moyenne pour les acides aminés indispensables extrait des protéines coagulables de 34 variétés de pommes de terre par rapport à ceux de l'oeuf (EPR).

EPR for lysine was very high, 111 %. The high lysine content makes potato protein a valuable component of animal feed, in which cereals with low lysine content are often used.

The slight variation in amino acid composition of coagulable protein hardly affects the EAAI (Table 2). The average EAAI was 89, which is high for plant proteins. These data agree with those reported by Reissig (1958) for six potato varieties.

*Relation between coagulable protein content and amino acid composition*

Table 4 shows correlation coefficients between content of coagulable protein for the 34 varieties and of the essential amino acids in the protein. There was no correlation between protein content and the first limiting amino acid, methionine. Thus methionine seems to be independent of content of coagulable protein. Also the content of lysine, which makes potato protein useful in mixed feed, seems to be independent of the coagulable protein content. A significant positive correlation was found for threonine ($r = +0.455^{**}$). Significant negative correlations were found for histidine ($r = -0.411^{*}$) and for the second limiting essential amino acid, arginine ($r = -0.589^{**}$). For most feeding purposes of potato protein these amino acids are of minor importance, except to poultry which has a very high arginine requirement. Between coagulable protein content and the EAAI a low negative correlation ($r = -0.263$) was found, which was not significant. When histidine and arginine (growth promoting amino acids) were omitted from computation of the EAAI, no correlation was found between protein content and EAAI ($r = 0.069$).

It can be concluded that the amino acid composition of potato protein is largely the same in varieties with low and high protein content. However methionine shows

Table 4. Correlation coefficients between content of coagulable protein in fresh matter and of essential amino acids in coagulable protein of 34 potato varieties.

| Amino acid [1] | Coagulable protein [2] |
|---|---|
| Threonine | 0.445** |
| Valine | 0.138 |
| Methionine | 0.091 |
| Isoleucine | -0.240 |
| Leucine | -0.011 |
| Phenylalaline | 0.245 |
| Lysine | -0.007 |
| Histidine | 0.411* |
| Arginine | -0.589** |

*, **Significant correlations at $P \leq 0.05$ and $P \leq 0.01$ respectively - *Signifikante Korrelationen bei $P \leq 0.05$ bzw. $P \leq 0.01$ - Les corrélations significatives à $P \leq 0.05$ et $P \leq 0.01$ respectivement.*

[1] *Aminosäure - Acide aminé;* [2] *Koagulierbares Protein - Protéine coagulable*

Tabelle 4. Korrelationskoeffizienten zwischen den Gehalt an koagulierbarem Protein in der Frischsubstanz und den essentiellen Aminosäuren im koagulierbaren Protein von 34 Kartoffelsorten.

Tableau 4. Coefficient de corrélation entre les taux de protéines coagulables de la matière fraîche et les acides aminés indispensables des protéines coagulables de 34 variétés de pomme de terre.

considerable variation and requires attention in breeding for improved protein content in ware potatoes. In breeding of industrial varieties, methionine has limited significance because methionine from other sources can be added to the mixed feed in which potato protein is used. Breeding for high content of coagulable protein may be expected to have little effect on the nutritional value of the protein. Genetic variation in amino acid composition seems to be small. So selection can be directed to content of coagulable protein, for which rapid screening techniques are available (van Gelder & Krechting, 1973; Sárvári et al., 1977).

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**Zusammenfassung**

*Aminosäurezusammensetzung von koagulierbarem Protein aus Knollen von 34 Kartoffelsorten und ihr Verhältnis zum Proteingehalt*

Aus Knollen von 34 Sorten von *Solanum tuberosum* mit grossen Unterschieden im Proteingehalt wurden Muster von koagulierbarem Protein hergestellt. Die Proteinmuster wurden auf Aminosäuren analysiert. Methionin und Cystin wurden mittels einer Methode von Moore (1963) (Tabelle 1) getrennt bestimmt.

Das koagulierbare Protein weist hohe Gehalte bei den meisten essentiellen Aminosäuren auf (Tabelle 2). Der Index der essentiellen Aminosäuren (EAAI) reichte von 86 bis 93, was für ein Pflanzenprotein hoch ist. Methionin war die erste begrenzende essentielle Aminosäure (Tabelle 3), aber sein Gehalt war bedeutend höher als in der Literatur aufgeführt. Der Lysingehalt war sehr hoch, was das Protein zu einem wertvollen Bestandteil für Mischfutter macht.

Arginin war die zweite begrenzende Aminosäure. Zwischen Arginin- und dem Proteingehalt wurde eine signifikant negative Korrelation ($r = -0{,}589$) festgestellt. Dies ist wichtig, wenn das Protein im Futter für Geflügel, das einen hohen Argininbedarf hat, verwendet wird. Die sortenbedingten Unterschiede in der Zusammensetzung der Aminosäure waren relativ klein (Tabelle 2). Es gab keine Korrelation zwischen Proteingehalt und dem Gehalt an den meisten essentiellen Aminosäuren (Tabelle 4). Der Züchtung auf erhöhten Proteingehalt in Kartoffeln wird nur wenig Einfluss auf den Nährwert des Proteins haben. So kann die Auslese auf den Gehalt an koagulierendem Protein ausgerichtet werden.

**Résumé**

*La composition en acides aminés des protéines coagulables des tubercules de 34 variétés de pomme de terre et sa relation avec la teneur en protéines*

Dans le but d'étudier les protéines coagulables on a préparé des échantillons avec 34 variétés de *Solanum tuberosum* ayant de grandes différences dans leur teneur en protéines. A partir de ceux-ci on a effectué l'analyse des acides aminés. La méthionine et la cystine ont été évalués séparément par la méthode de Moore (1963) (tableau 1).

Les protéines coagulables avaient une teneur élevée pour la plupart des acides aminés indispensables (tableau 2). Leur index se situait entre 86 et 93, ce qui est élevé pour des protéines de plantes. La méthionine a été le premier acide aminé indispensable limitant (tableau 3) mais sa teneur était considérablement plus élevée que celle donnée dans la littérature. La teneur en lysine était très haute, faisant de ces protéines un constituant valable

pour les aliments composés. L'arginine a été le second acide aminé limitant. Une corrélation négative ($r = -0{,}589$) entre l'arginine et le taux de protéines s'est avérée significative. On doit en tenir compte lorsque ces protéines sont utilisées comme aliments destinés aux volailles, qui nécessitent un taux élevé d'arginine. Les différences variétales en acides aminés ont été relativement faibles (tableau 2). Il n'existe pas de corrélation entre la teneur des protéines et la plupart des acides aminés indispensables (tableau 4). L'hybridation dans le but d'augmenter le taux de protéines dans les pommes de terre peut être considéré comme n'ayant que peu d'effet sur la valeur nutritionnelle de ces protéines. Aussi, la sélection peut être orientée vers l'amélioration de la teneur en protéines coagulables.

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