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Preliminary research on amino acid composition and nutritional value of clover proteins

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Abstract

The amino acid composition and nutritional value of 5 clover varieties including 3 Polish ones ('Gloria', 'Hruszowska', 'Skrzeszowicka') and 2 of foreign origin ('Rotra' and 'Violetta') were investigated. No significant differences in the total protein content (19.2—20.0% of dry matter) as well as in qualitative amino acid composition were found among the clover varieties under examination. EAA index (Essential amino acid index) calculated according to Oser for 'Gloria' and 'Hruszowska' showed the highest nutritional value was — 40. The lowest value of EAA index was found for 'Violetta' cvar. — 32, intermediate values however for Rotra and Skrzeszowicka was 37 and 36.

INTRODUCTION

The production of valuable plant protein is of great importance for feeding people and animals. Attempts to increase the nutritional value of cultivated plants are the subject of interest in many laboratories. The content of essential amino acids, namely: leucine, isoleucine, phenylalanine, lysine, methionine, threonine and tryptophan is a factor determining the nutritional value of protein. According to Oser (B l a i m 1964) the nutritional value of protein expressed as the so called EAA index (Essential amino acid index) represents a geometric mean value of the content of all essential amino acids as per cent of the standard protein that is of hen egg protein. This paper deals with the examination of the amino acid composition in 5 clover varieties including 3 Polish varieties ('Gloria', 'Hruszowska', 'Skrzeszowicka') and 2 of foreign origin ('Violetta' and 'Rotra') and also with establishing their nutritional value.

MATERIAL AND METHODS

Air dried aerial parts of clover plants were analysed. These plants were cultivated under standard conditions in the Plant Breeding Station at Nieznanice.

1. Protein determination in plant tissue

Total protein was extracted according to the Flechter et al. (1965) method. 100 mg of air dried clover tissue were treated with 5 ml of 5% TCA. After centrifuging at 3000 rpm for 10 minutes the precipitate was washed with 80% ethanol until the supernatant became colourless. Then 10 ml of 1.2 N NaOH were added and this was heated at 100° for 10 minutes. In order to obtain complete protein extraction this procedure was repeated 4 times. Protein contents were determined in pooled extracts by the Lowry method (1951).

2. Analysis of amino acid composition of clover acid hydrolysate

a) Hydrolysis with acid (Fromageot et al. 1948)

A ground sample of air dried plant tissue (15—20 mg) was placed in a hard glass test tube and 2 ml of 5.5 N HCl (with a stable boiling point 110°C) were added. After sealing the tube the hydrolysis was carried out at 110°C for 24 hours. The hydrolysate was transfered quantitatively (using 3 times distilled water) to a glass basin and placed in a vacuum dessicator over P_2O_5 and KOH. After evaporation to dryness, the hydrolysate was dissolved in 2 ml of water. In order to reach a suitable pH this procedure was repeated 3 times. The hydrolysate was then dissolvent in 1 ml of H_2O and used for determination of the amino acid composition by the method of semi-quantitative electrochromatography.

b) Separation and determination of amino acids

The separation of amino acids was carried out by mean voltage electrophoretic technique. The electrophoresis was carried out in a buffer [glacial $CH_3COOH+HCOOH+H_2O=(200:60:740,\ v/v)$] at pH 1.5, potential drop 16 V/cm and linear current density 0.4 m A/cm (Wroński 1965). The separation of amino acids: leucine + isoleucine, valine, phenylalanine, methionine, tyrosine, cystine, alanine, glycine, serine, aspartic acid, lysine and proline was achieved by means of ascending chromatography. The chromatograms were developed for 7 hours. Glutamic acid, threonine, histidine, arginine, which cannot be determined under the above mentioned conditions of electrochromatographic separation, were separated in the way of electrophoresis on Whatman paper No. 3 in pyridine buffer

[pyridine + glacial CH_3COOH + H_2O = (3 : 10 : 487, v/v)] at pH 3.9 (Opieńska-Blauth et al. 1965/66) for 1 hour at potential drop 18 v/cm and linear current density 0.7—0.8 m A/cm. After drying the chromatogram, the separation of glutamic acid and threonine was obtained after developing twice for 12 hours in the butanol system [n-butanol — glacial CH_3COOH — H_2O = (4 : 1 : 1, v/v)]. The separation of basic amino acids was obtained using the solvent system: n-butanol + acetone + pyridine + H_2O + diethylamine = 15 : 15 : 9 : 16 : 10, v/v for 12 hours (Sanecka and Stryjecka, personal communications).

In order to unify amino acid spots electrochromatograms were detected with 0.5% acetone solution of ninhydrin (proline with isatin). Wet paper sheets were saturated with pyridine vapour (pyridine + glacial CH_3COOH + H_2O = 25 : :15 : 960, v/v) and dried for 3 hours at 45°C. The elution of respective amino acids was carried out with a 0.005% solution of $CuSO_4 \cdot 5H_2O$ in 75% ethanol. A measurement of absorption of the amino acids detected with ninhydrin was made by means of the photocolorimeter „Specol". Detected spots of proline were treated with absolute ethanol and extracted with 90% phenol. The absorption of eluates was measured at 510 nm.

3. Colorimetric determination of tryptophan in alkaline hydrolysate

Before alkaline hydrolysis dried clover plants were decolorized by several extractions with a mixture of chloroform with acetone (1 : 5) at temperature 0°C, and then at the boiling temperature with a mixture of ethanol and ether (3 : 1). 15—20 mg samples of material prepared in this way were used for alkaline hydrolysis in 0.8N $Ba(OH)_2$. Tryptophan was determined by the Eckert method (1943) modified by Zalta (personal communication) based on the condensation of product resulting from the action of nitrous acid on tryptophan with N-1-naphtylethylenediamine.

RESULTS AND DISCUSSION

We did not find any significant difference in protein contents in air dried clover plants of 5 varieties (Tab. 1).

Protein makes up 19.2—20% of dry matter. Tryptophan content calculated for 100 g of clover protein is practically the same in all varieties under examination (2.2—2.7%) (Tab. 3).

No qualitative differences in the amino acid composition were observed among the clover varieties under examination (Fig. 1 and 2). In all electrochromatograms of respective clover varieties protein we iden-

Table 1

Contents of protein in varieties of air dried clover

Variety	Dry matter %	Water and lipid free material %	Total protein in dry matter %
Gloria	91.8	72.7	19.2
Hruszowska	93.2	73.0	20.0
Skrzeszowicka	93.7	68.5	19.3
Violetta	93.6	74.1	19.3
Rotra	92.7	72.1	19.8

tified a spot corresponding to γ-aminobutyric acid (according to the standard amino acid separation). The presence of this compound (in trace quantities) was also proved in the course of electrophoresis at pH 3.9 (Fig. 3).

Table 2 shows the amino acid contents expressed as a percentage of dry matter, and table 3 — amino acid contents calculated for the total protein content of 5 clover varieties under examination. (We could suppose that in the course of 24 hour hydrolysis with 5.5 N HCl free amino acids and low molecular peptides were destroyed). Low contents of aro-

Table 2

Amino acid contents in 5 varieties of air dried clover (weight %)

Amino acid	Gloria	Hruszowska	Skrzeszowicka	Violetta	Rotra
Leucine	2.0	1.9	1.4	1.5	1.8
Valine	1.0	0.9	0.9	0.7	0.8
Phenylalanine	0.3	0.5	0.3	0.2	0.3
Methionine	—	—	—	—	—
Proline	1.6	1.3	1.5	1.5	1.4
Alanine	1.1	0.9	0.8	0.7	0.8
Glycine	0.7	0.5	0.5	0.5	1.0
Aspartic acid	1.9	1.6	1.8	1.6	1.6
Glutamic acid	1.3	1.4	1.1	1.1	1.3
Tyrosine	0.3	0.3	0.2	0.2	0.2
Serine	1.0	0.9	0.9	0.8	0.9
Threonine	1.2	1.1	1.1	0.9	1.2
Cystine			traces		
Histidine	0.7	0.6	0.9	0.4	0.7
Arginine	2.0	1.7	1.9	1.9	1.7
Lysine	1.3	1.1	1.1	1.1	1.1
Tryptophan	0.5	0.5	0.5	0.5	0.4

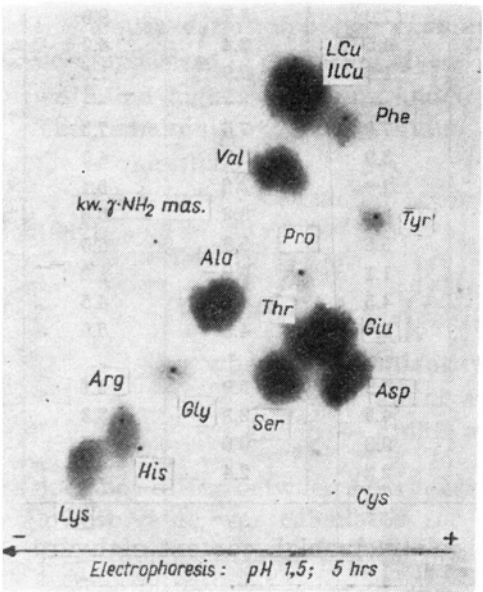

Fig. 1

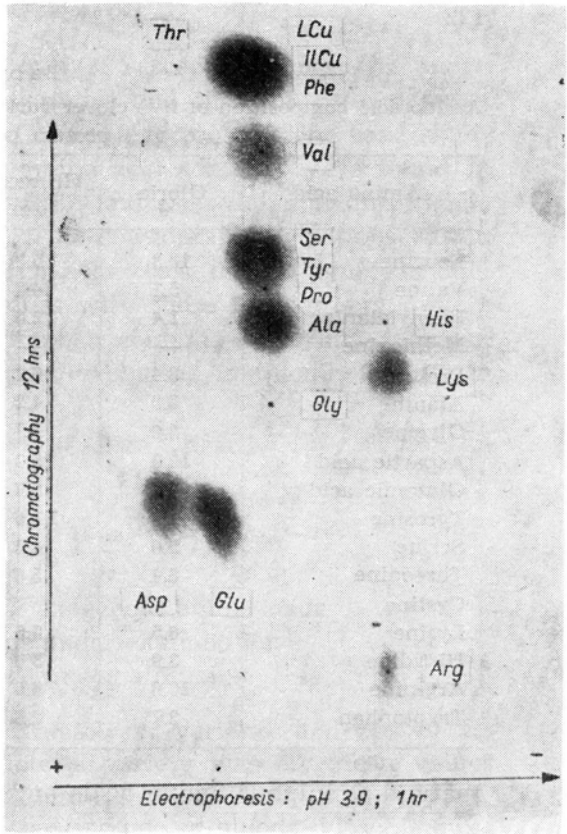

Fig. 2

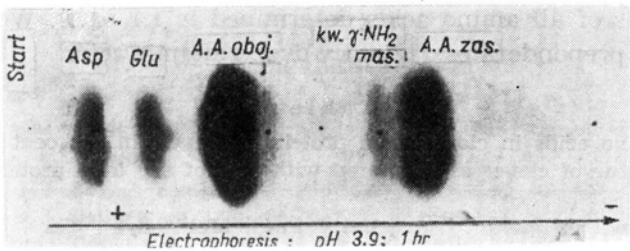

Fig. 3

1. Electrochromatographic separation of amino acids in acid hydrolysate of clover (cv. Hruszowska). ophoresis: pH 1.5, potential drop 16 V/cm, linear current density 0.4 mA/cm, 5 hours. Chromatography: n-butanol-glacial acetic acid — water (4 : 1 : 1.5) 7 hours.

2. Electrochromatographic separation of amino acids in acid hydrolysate of clover (cv. Hruszowska). rophoresis: pH 3.9, potential drop 18 V/cm, linear current density 0.7—0.8 mA/cm, 1 hour. Chromatography: n-butanol — acetone — pyridine — H_2O — diethylamine (15 : 15 : 9 : 16 : 10, v/v), 12 hours.

Fig. 3. Electropherogram of amino acids in the acid hydrolysate of clover (cv. Hruszowska) Electrophoresis: pH 3.9, 18 V/cm, 0.7—0.8 mA/cm, 1 hour.

Abbreviations: *LCu* — leucine; *IlCu* — isoleucine; *Val* — valine; *Phe* — phenylalanine; *Tyr* — tyrosine; *Ser* — serine; *Pro* — proline; *Ala* — alanine; *His* — histidine; *Lys* — lysine; *Gly* — glycine; *Asp* — aspartic acid; *Glu* — glutamic acid; *Arg* — arginine; *Cys* — cystine; *Thr* — threonine; *kw. γ-NH₂mas.* — γ-NH_2-butric A.; *A, A. oboj.* — Neutr. A. A.; *A, A. zas.* — Basic A. A.

Table 3

Amino acid composition of five clover varieties in calculation for 100 g of total clover protein (weight %)

Amino acid	Gloria	Hruszowska	Skrzeszowicka	Violetta	Rotra
Leucine	10.3	9.3	7.0	7.7	9.3
Valine	5.1	4.2	4.5	3.4	4.0
Phenylalanine	1.4	2.6	1.6	0.9	1.6
Methionine	—	—	—	—	—
Proline	8.4	6.2	7.9	7.6	7.3
Alanine	5.3	4.2	3.9	3.7	4.0
Glycine	3.6	2.7	2.8	2.6	5.1
Aspartic acid	10.0	8.0	9.1	8.2	8.0
Glutamic acid	6.7	6.8	5.6	5.9	6.5
Tyrosine	1.4	1.6	1.1	0.9	1.2
Serine	5.0	4.5	4.5	4.4	4.5
Threonine	6.4	5.6	5.7	4.9	6.0
Cystine			traces		
Lysine	6.5	5.5	5.9	5.9	5.6
Histidine	3.9	3.0	4.5	2.3	3.3
Arginine	10.6	8.6	9.8	9.9	8.5
Tryptophan	2.7	2.5	2.3	2.4	2.2

matic and sulphur amino acids and a relatively high content of hydroxyaminoacids should be emphasized.

The protein of all examined clover varieties shows a slightly acid character (the ratio of acidic and basic amino acids expressed in moles per 100 moles of all amino acids determined is 1.1—1.3). We found that aspartic acid preponderates distinctly over glutamic acid.

Table 4

Exogenous amino acids in clover total protein expressed in per cent and biological value of clover as compared with that of egg total protein

Variety	Leu+Ile	Val	Phe	Met	Lys	Thr	Tyr	EAA index
Gloria	10.3	5.1	1.4	—	6.5	6.4	2.7	40
Hruszowska	9.3	4.2	2.6	—	5.5	5.6	2.5	40
Skrzeszowicka	7.0	4.5	1.6	—	5.9	5.7	2.3	36
Violetta	7.7	3.4	0.9	—	5.9	4.9	2.4	32
Rotra	9.3	4.0	1.6	—	5.6	6.0	2.2	37
Egg protein	11.7 +8.2 Ileu	7.5	5.7	4.0	7.8	4.9	1.5	97

Abbreviations:
Leu — leucine; Ile — isoleucine; Val — valine; Phe — phenylalanine; Met — methionine; Lys — lysine; Thr — threonine; Tyr — tyrosine.

Table 4 illustrates the per cent part of respective exogenous amino acids in hydrolysates of all clover varieties and their nutritional value expressed as EAA index as compared to hen egg protein. The most significant differences in the exogenous amino acid contents were found in the case of: leucine + isoleucine, valine and phenylalanine meanwhile lysine, threonine and tryptophan show no considerable deviations within the clover varieties under examination. The lack of methionine should be emphasised. Comparing these values with those for hen egg protein we found higher threonine and tryptophan contents occurring in clover. The remaining exogenous amino acids (excluding methionine) occur in lower quantities.

„EAA index" calculated according to Oser (cit. after Masłowski 1962):

$$\text{index EAA} = \sqrt[n]{\frac{100a_1}{b_1} \cdot \frac{100a_2}{b_2} \cdot \ldots \cdot \frac{100a_n}{b_n}}$$

where: n — quantity of exogenous amino acids
a — amino acids under examination
b — amino acids in egg albumin.

does not differ between Gloria and Hruszowska varieties and was 40. Its lowest value was calculated for Violetta variety was 32. Mean values were found for Skrzeszowicka was 36, and Rotra was 37.

The nutritional value of clover protein calculated on the basis of the amino acid composition seems to be a variety feature.

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Skład aminokwasowy i wartość biologiczna pięciu odmian koniczyny

Streszczenie

Badano skład aminokwasowy i wartość biologiczną 5 odmian koniczyny, w tym 3 odmian krajowych ('Gloria', 'Hruszowska', 'Skrzeszowicka') oraz 2 odmian zagranicznych ('Rotra' i 'Violetta'). Nie wykazano większych odchyleń w zawartości białka (19,2—20,0% suchej masy) ani różnic jakościowych w składzie aminokwasowym analizowanych 5 odmian. Za pomocą indeksu EAA Osera stwierdzono najwyższą wartość biologiczną 40, dla odmiany 'Gloria' i 'Hruszowskiej', pośrednią, dla 'Rotra' — 37 i dla 'Skrzeszowickiej' — 36, a najniższą — dla odmiany 'Violetta' — 32.