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Leaf proteins in five varieties of red clover cultivated in Poland

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Abstract

Protein fractions: albumins, globulins, gluteins and prolamins were extracted from the leaves of 5 varieties of red clover. 'Skrzeszowicka' and 'Hruszowska' showed the highest content of total protein, 'Rotra' however — the highest globulin level.

Globulins were fractionated on DEAE cellulose column into 3 fractions. Globulins from 'Rotra' and 'Hruszowska' varieties were separated into 4 fractions.

INTRODUCTION

In the presence of an overall world shortage of protein, the problem of seeking further sources of protein showing a high nutritional value is of great importance. Particulary plant proteins deserve notice. Their main supply comes from cereal grains and legume seeds, but recently attention has been also devoted to leaf proteins (P i r i e 1969a, 1969b). The green leaves of cultivated plants are an abundant potential source of proteins. Therefore the search for the most valuable feed to cattle becomes one of the main problems in the field of plant proteins.

This paper deals with proteins of five red clover varieties cultivated in Nieznanice — Plant Breeding Station. This institution is interested in the selection of the most valuable clover varieties from among red clover varieties of Polish and foreign origin. The selected clover varieties could be in the future a starting material for genetic investigation and crossing experiments. S c h w a n e (1958) suggests that the nutritional value of protein is a variety feature.

MATERIAL AND METHODS

The leaves of five varieties of red clover cultivated in Nieznanice under standard experimental conditions were analyzed. The following varieties were examined: 'Skrzeszowicka', 'Hruszowska', 'Rotra', 'Violetta', 'Gloria'.

The plants were harvested from a stubble field in autumn. Leaves were separated. Our investigation included fresh and air-dried material. We obtained from Nieznanice samples containg air-dried powdered aerial parts of clover (leaves and stems).

Proteins were fractionated by the method of Pleszkov (1968) with successive use of: 1M NaCl in 0,5M phosphate buffer at pH 7,3. After dialysis, albumins and globulins were obtained. The globulins were dissolved in 0,2M NaCl in the same phosphate buffer, glutelins — in 0,2% NaOH, prolamins — in 80% ethanol. The protein residue was extracted with 1M NaOH in a water bath at 100°C for 10 min. Total protein was extracted by the method of Fletcher and Osborne (1965). All manipulations were done in a cold room. The extracted protein fractions as well as total protein were determined by the Lowry method (1951). The globulin fraction exstracted in the above mentioned way was placed in DEAE cellulose column (Serva Laboratory). Columns were prepared after Gorman and Levine (1966). The following eluents were applied successively: 0,2M NaCl in phosphate buffer, pH 7,3, 1M NaCl in the same buffer, 0,1M NaOH and 1% NaOH. The different zones were collected into the fraction collector. Proteins were determined in the corresponding samples by the Lowry method.

RESULTS AND DISCUSSION

The results of analysis showed a total protein content of 11,89 - 15,2% in 5 clover varieties (Table 1). This content is very high when compared with cereals where it does not exceed 16% (Pleszkov 1965).

Comparing the respective varieties we see that 'Skrzeszowicka' variety shows the highest portein content, 'Gloria' however — the lowest one.

Taking into consideration the respective protein fractions, the great similarity between 'Skrzeszowicka' and 'Hruszowska' varieties should be emphasised. The only difference between them consists in the content of the proteins which cannot be extracted with the extractants used in our experiments — namely proteins tightly bound with the leaf tissues. The high content of globulins — the most biologically valuable proteins — found in the leaves of 'Rotra' variety should be also stressed. Moreover, this variety showed a relatively low glutelin content. Glutelins are considered as less valuable proteins.

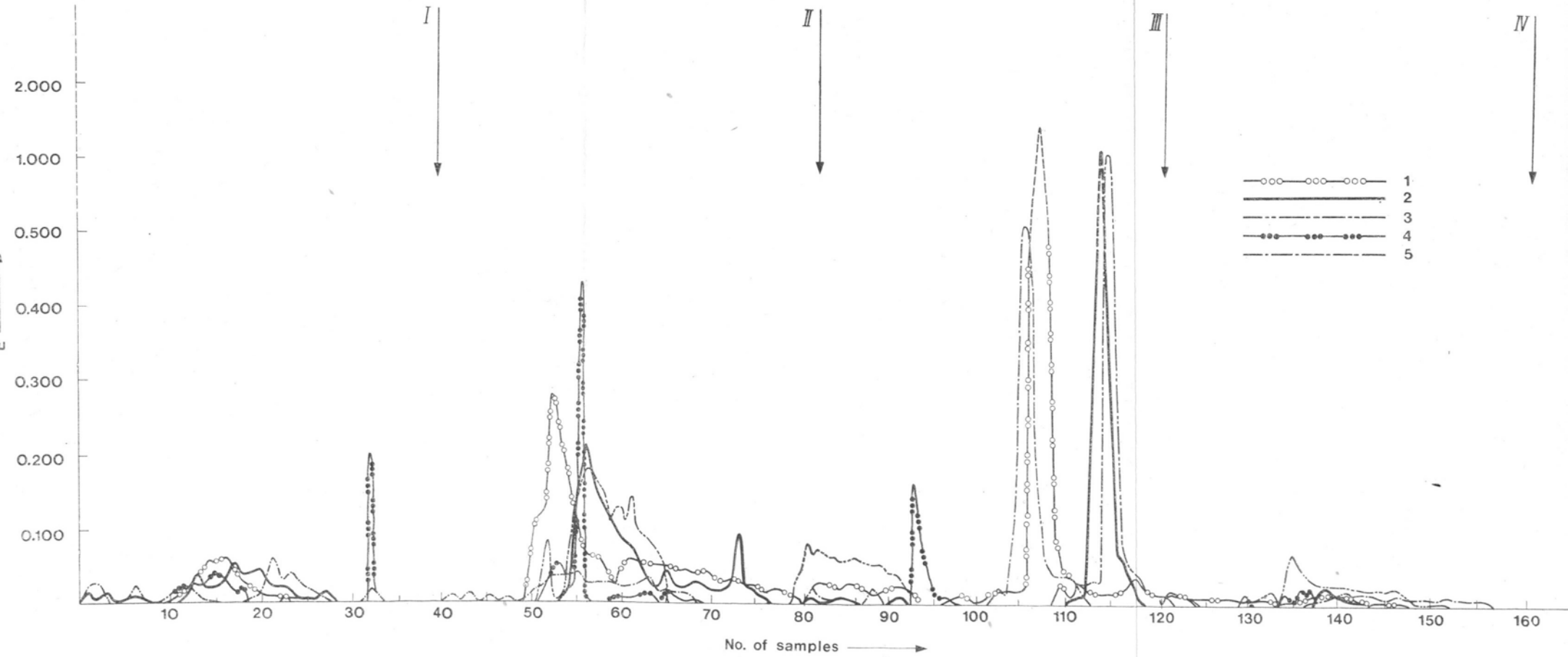

Fig. 1. Chromatographic profile of globulins in 5 red clover varieties
I — 0.2M NaCl in phosphate buffer, pH 7,3; *II* — 1M NaCl in phosphate buffer, pH 7,3; *III* — 0.1M NaOH; *IV* — 1% NaOH
1 — 'Skrzeszowicka'; *2* — 'Hruszowska'; *3* — 'Rotra'; *4* — 'Violetta'; *5* — 'Gloria'

Table 1

Protein fractions from leaves of 5 red clover varieties in mg/100 mg dry matter

Fractions	Clover varieties				
	'Skrzeszowicka'	'Hruszowska'	'Rotra'	'Violetta'	'Gloria'
Albumins	2.08	2.09	2.55	2.48	2.05
Globulins	0.13	0.14	0.40	0.15	0.07
Glutelins	4.60	4.59	2.32	2.53	4.76
Prolamins	trace amounts	trace amounts	trace amounts	trace amounts	trace amounts
Remaining proteins	8.40	6.24	7.66	7.48	5.01
Total protein	15.21	13.06	12.93	12.64	11.89

The results of the respective protein fraction separation on DEAE cellulose columns are shown in the graph representing the chromatographic profiles. We succeeded in achieving a good separation of globulins in all varieties under examination into 3 - 4 fractions. These proteins were readily elutable from cellulose columns with the following eluents: 0,2M NaCl in phosphate buffer, pH 7,3, 1M NaCl in the same buffer and 0,1M NaOH. They could not however be eluted with 1% NaOH. Moreover, the globulins of 'Rotra' and 'Hruszowska' varieties showed an additional globulin fraction elutable with 1M NaCl in phosphate buffer, pH 7,3.

Table 2

Protein fractions in air-dried aerial parts of 5 red clover varieties in mg/100 mg dry matter

Fractions	Clover varieties				
	'Skrzeszowicka'	'Hruszowska'	'Rotra'	'Violetta'	'Gloria'
Albumins	2.38	2.73	2.67	2.32	2.45
Globulins	0.09	0.08	0.10	0.07	0.08
Glutelins	5.11	4.32	5.62	5.50	4.32
Prolamins	trace amounts	trace amounts	trace amounts	trace amounts	trace amounts
Remaining proteins	7.24	7.85	7.72	8.32	8.60
Total protein	14.82	14.98	16.11	16.21	15.45

Apart from the analyses of fresh clover leaves several analyses of dried material serving as feed for cattle were made. We found that total protein content in this material was similar with that determined of dry matter 14,82 - 16,21%. As seen in Table 2, the results of analyses of dried material are similar to those obtained for fresh leaves. There are only some differences in the total protein content. Moreover, similarly as in the case of fresh material, the highest globulin content was found in the 'Rotra' variety.

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Białka liści pięciu odmian koniczyny czerwonej uprawianych w Polsce

Streszczenie

Z liści 5 odmian koniczyny czerwonej wyekstrahowano albuminy, globuliny, gluteliny i prolaminy. Najwyższy poziom globulin, a więc białek o największej wartości odżywczej występował u odmiany 'Rotra', a u odmian 'Skrzeszowicka' i 'Hruszowska' największa ilość białka całkowitego.

Wyekstrahowane globuliny poddano chromatografii kolumnowej na DEAE celulozie, w wyniku czego uzyskano 3 frakcje. Jedynie w przypadku odmian 'Rotra' i 'Hruszowska' — 4 frakcje.