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CHROMATOGRAPHIC SEPARATION OF SOLUBLE PROTEINS OBTAINED IN ALFALFA JUICE FRACTIONATION

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The chloroplastic fraction of green alfalfa juice was separated by centrifugation, heating the juice to 333°K, or application of the polyelectrolytes Magnafloc LT-27 and M-22S. The cytoplasmatic fraction was precipitated by heat (358°K) or acid coagulation (pH = 3.5). The supernatants after chloro- and cytoplasmatic fraction separation (chloroplast free juice and whey) were chromatographed on a Sephadex G-100 column.

INTRODUCTION

The proteins contained in juice pressed from green plants feature two prominent fractions, namely the chloroplasmatic consisting of proteins combined with chlorophyl, and the cytoplasmatic which is a mixture of cytoplasmatic proteins and proteins liberated from chloroplasts during comminution of plants and juice pressing [12]. The juice protein may be separated in a single-stage process, or it may be fractionated. In the latter case one obtains two protein concentrates: the chloroplasmatic, green in colour, and the white cytoplasmatic [4, 12].

The fractionation of proteins from green plant juice involves precipitation and separation of the green chloroplasmatic fraction, and the subsequent separation of cytoplasmatic proteins from the obtained filtrate. Chloroplasmatic proteins may be coagulated — either thermally [4], using acids [11] or organic solvents [3, 13], or by agglomeration with polyelectrolytes [1, 2, 6, 8] — or separated in high-speed centrifuges [5]. The cytoplasmatic fraction, in its turn, may be separated from the juice deprived of chloroplasts by thermal coagulation at 358°K [4] or with organic solvents [3, 13], mineral acids [11], or it may be condensed and separated by ultrafiltration [9].

The aim of this research was to confirm the presence of soluble proteins in juice deprived of the chloroplasmatic fraction using flocculating agents, and to demonstrate that the effects of alfalfa juice fractionation are comparable to those obtained with classical method of producing protein concentrates from green plant parts.

MATERIAL AND METHODS

OBTAINING AND FRACTIONATION OF PLANT JUICE

The juice from green alfalfa (Kleszczewska variety) was obtained according to previously described procedures [1, 2].

The chloroplasmatic fraction was separated from alfalfa juice by:

(i) juice centrifugation (12 000 g for 1800 s);

(ii) heating the juice to 333°K;

(iii) adding to the juice solutions (300×10^{-6} kg/dm^3) of the following flocculants: (a) Magnafloc LT-27 (pH of juice = 7.5), or (b) Magnafloc M-22S (pH of juice = 4.5).

The obtained green concentrates sediments were separated from the parent solution by filtration. The cytoplasmatic proteins were separated from the chloroplasts-free juice by acidifying this juice to pH = 3.5 with 2N HCl, or by heating it to 358°K [1, 2].

CHROMATOGRAPHIC SEPARATION OF PROTEINS

The separation of proteins in filtrates obtained after separation of both the chloro- and the cytoplasmatic fractions was done by molecular filtration. A Pharmacia column, 1 m long and 25×10^{-3} m in diameter, filled with Sephadex G-100 gel, was used. The solution doses applied to the column contained 15×10^{-6} kg of protein. Elution was carried out with a phosphate buffer solution (0.1 M, pH = 5.8). Separation was performed on an LKB apparatus, using a 280 nm detector for recording. Protein content in the various fractions obtained in the separation was determined by Lowry's method [10].

RESULTS AND DISCUSSION

The obtained separation of soluble proteins is illustrated in Figs 1 and 2; protein content in the various fractions is given in the Table.

Nearly identical chromatogram were obtained for the three independent methods of chloroplasmatic fraction separation (centrifugation, heating, flocculant application). Proteins remaining in the juice after the removal of the chloroplasmatic fraction form three fractions in the column, differing as to protein content. In fraction I the protein content amounted to 13.2-14.4% of its total amount. Similar figures were obtained by Free and Satterlee [7] who separated proteins of the chloroplasmatic fraction by centrifugation at 12 000 g. Different chromatograms were obtained after separation of proteins contained in filtrates from which the cytoplasmatic proteins were removed (Fig. 2). As can be seen, these filtrates contain fractions II and II exhibiting 76% and 6% of initial protein content, respectively. This means that during separation of cytoplasmatic proteins, both by thermal coagulation and acid precipitation at pH = 3.5 (i.e. in

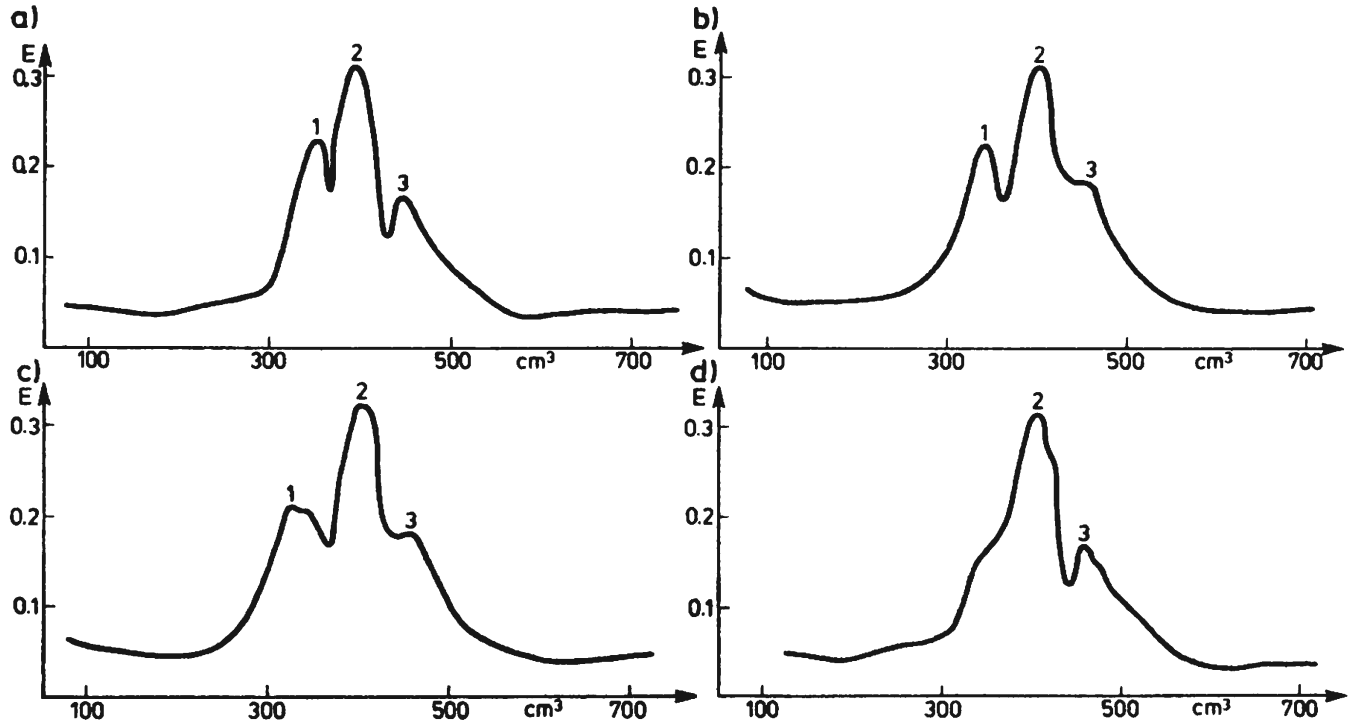

Fig. 1. Chromatographic separation on Sephadex G-100 gel of proteins in alfalfa juice following removal of the chloroplasmatic fraction by means of: a—centrifugation (12000 g, pH = 5.5), b—heating to 333°K (pH = 5.5), c—flocculant LT-27 (pH = 7.5), d-flocculant M22S (pH = 4.5)

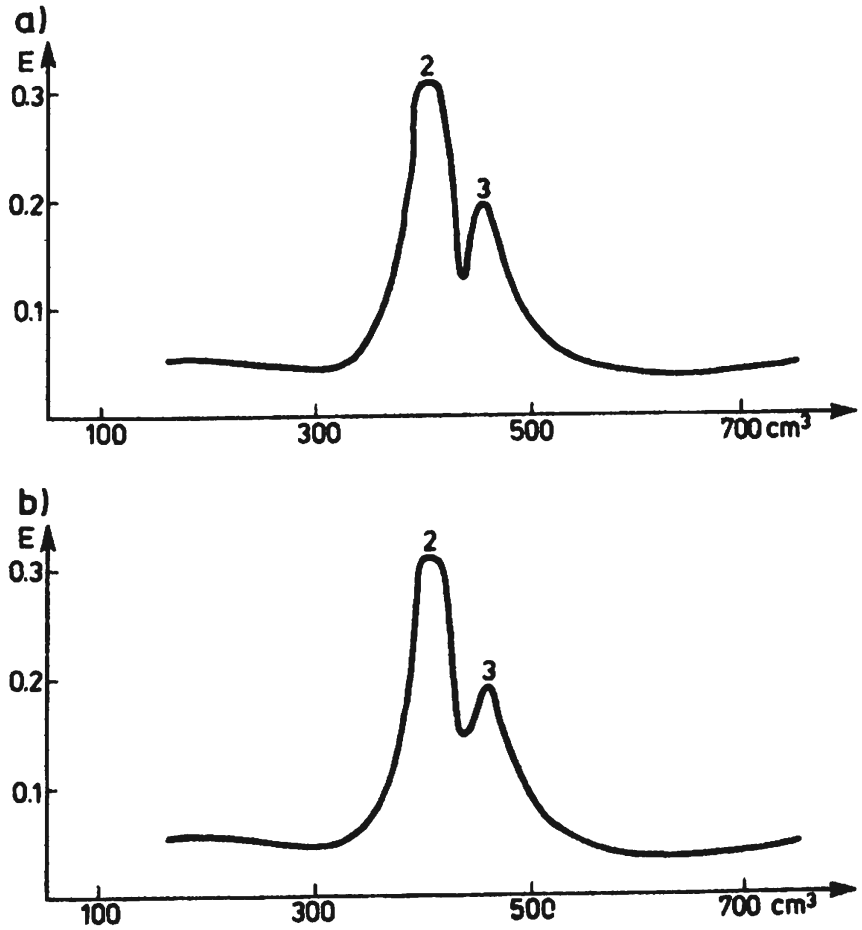

Fig. 2. Chromatographic separation of proteins in filtrate obtained after separation of the chloroplasmatic fraction with the flocculant Magnafloc LT-27 (pH = 7.5); A—cytoplasmatic fraction precipitated thermally at 358°K, B—cytoplasmatic fraction precipitated by acidification to pH = 3.5

Table. Protein content ($kg \times 10^{-6}$) in fractions obtained during protein separation on Sephadex G-100 gel

Separation solution	Fraction		
	1	2	3
Alfalfa juice with chloroplasts removed by means of:			
(a) centrifugation (12000 g)	2.1	11.7	0.8
(b) heating to 333°K	1.7	11.3	0.9
(c) flocculant LT-27 (pH = 7.5)	2.0	10.8	1.0
(d) flocculant M22S (pH = 3.5)	—	14.0	0.8
Filtrate after precipitation of cytoplasmatic proteins by			
(a) heating to 358°K	—	11.9	1.1
(b) acidification to pH = 3.5	—	11.2	0.9

the pH range close to the isoelectric point of most of these proteins), only a small proportion of these proteins is separated (14%). A similar chromatogram was obtained when separating soluble proteins in the juice treated with the flocculant Magnafloc M22S which is active in acid media (Fig. 1d). The obtained chromatograms indicate that in an acid medium (pH = 4.5) fraction I of soluble proteins precipitates together with the fraction of chloroplasmatic proteins.

The proteins contained in plant juice constitute a colloidal system, and their fractionation with flocculating agents may be carried out in neutral or weakly alkaline media. The choice of conditions accompanying the precipitation of the chloroplasmatic fraction has a crucial effect on mutual relations of chloro- and cytoplasmatic proteins obtained in the form of protein concentrates during fractionation of plant juice. The ratio of cytoplasmatic concentrate amount precipitated at pH = 3.5 to the amount of chloroplasmatic concentrate obtained from alfalfa juice by means of the Magnafloc LT-27 flocculant was 1:5 [2]

CONCLUSIONS

1. The effect of the flocculant Magnafloc LT-27 (300×10^{-6} kg/dm^3) on weakly alkaline green alfalfa juice is equivalent to the effect produced by heating the juice to 333°K, and it leads to the precipitation of the chloroplasmatic proteins fraction.

2. The application of flocculating agents in acid media leads to a joint precipitation in the alfalfa juice of cytoplasmatic and chloroplasmatic proteins.

3. Cytoplasmatic concentrates precipitated by heating the filtrate deprived of the chloroplasmatic fraction to 333°K or by acidifying it to pH = 3.5 contain only a part (ca. 14%) of the soluble proteins which are not flocculated by polyelectrolytes in weakly alkaline media.

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ROZDZIAŁ CHROMATOGRAFICZNY ROZPUSZCZALNYCH BIAŁEK OTRZYMANYCH W PROCESIE FRAKCJONOWANIA SOKU Z LUCERNY

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Streszczenie

Zielony sok z lucerny frakcjonowano uzyskując koncentraty chloro- i cytoplazmatyczne. Frakcje chloroplazmatyczne wydzielano z soku przez: odwirowanie soku (12 000 g), ogrzewanie do temp. 333°K lub stosowanie flokulantów Magnafloc LT-27 i M-22S. Frakcję cytoplazmatyczną wytrącano przez ogrzewanie soku pozbawionego chloroplastów do temp. 358°K lub jego zakwaszenie do ph = 3,5. Otrzymano przesącze (sok pozbawiony chloroplastów i odciek) rozdzielano chromatograficznie na żelu Sephadex G-100. Przeprowadzone rozdziały wykazały, że pozostające w soku pozbawionym chloroplastów białka składają się z trzech frakcji. Frakcję I stanowią wysokocząsteczkowe białka, których zawartość w stosunku do całkowitej ilości białka wynosi 13-14% i białka te mogą być wydzielone w postaci koncentratu cytoplazmatycznego.