The proteins in rapeseed, composition and characterization

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Zusammenfassung

Aus der Rapssorte Jet Neuf wurden die Samenproteine aufgrund ihrer verschiedenen Löslichkeit kontinuierlich extrahiert und als fünf definierte Fraktionen gesammelt (wasser-, salz-, detergens-, puffer- und alkalilösliche, Fig. 3). Die Zusammensetzung und die Ausbeute der Extraktion zeigen Fig. 2 und Fig. 4. Für die schnelle und empfindliche Proteinbestimmung wurde der Farbtest nach BRADFORD (1976), geeicht über Kjeldahl-N, benutzt (Fig. 1). Die ersten Reinigungs- und Konzentrationsschritte erfolgten durch Gelfiltration (Sephadex G25) und Ultrafiltration (Ausschlußgrenze 10000, Fig. 5). Diese Proteinkonzentrate wurden weiter fraktioniert (Fig. 6). Als erstes Enzym wurde in der wasserlöslichen Fraktion die Myrosinase nachgewiesen und ihre spezifische Aktivität gemessen (Fig. 7). Versuche zur weiteren Auftrennung der Proteine und deren enzymatischen Aktivität sind im Gange.

Introduction

It is well known that the protein from rapeseed has a high nutritive value for animal feeding. Therefore rapeseed is of economical importance (RÖBBELEN and RAKOW, 1979). As a seed protein it has manyfold physiological functions, as storage protein, which will be mobilized for the seedling and as enzymes of the basic metabolism of the dormant embryo. From this there could result some restrictions for the breeder, while he is effording to improve the quality of rapeseed. For this reason a comprehensive knowledge about the protein content, its composition, physico-chemical and biochemical properties is necessary.

Materials and Methods

Mature seeds of a homogeneous line from the cv. "Jet Neuf" were obtained from the Norddeutsche Pflanzenzucht, Hohenlieth. Milling and defatting was carried out according to GUPTA and RÖBBELEN (1986). Meal was stored

in a vaccum desiccator (p = mbar, 4° C). Nitrogen was determined as total N by elementary analysis (volume detection, Rapid-N, Heraeus Hanau). Protein-N was measured after Kjeldahl-microdigestion, titration of the ammonium borate-complex with n/200 H_2SO_4, endpoint pH=4,85 (BREIDERT and SCHÖN 1979a). The protein assay was a dye binding according to BRADFORD (1976) calibrated by protein-N. Protein extraction was carried out continuously on a column packed with the meal sample and SiO_2 in ratio 1:100 modified after BREIDERT and SCHÖN (1979b). Enrichment and purification: 1. Concentration by UF, a stirring-cell with cellulose acetate filter (SCHLEICHER and SCHÜLL, Dassel), exclusion limit 10^3 D, 4,5 bar, 3° C (± 0,5); 2. GF on Sephadex G25 fine; 3. GF on Sephadex G100, detection T=284 nm.

Myrosinase activity:
Incubation: Sinigrine (31µ Mole/ml, Soerensen buffer, pH=6,8). Source of enzyme (H_2O soluble protein, after UF and CF, G25); 1 ml, and 0,5 ml. Reaction vol. 5 ml, temp. 30° C (± 0,05).
Inactivation: After the corresponding reaction time 0,5 ml reaction mixture to 0,5 ml perchloric acid (5 %), temp. 2° C.
Detection: 0,4 ml inactivated reaction mixture to glu-hexokinase and glu-6-ph-dehydrogenase-system, E 340 nm.

Results

Adjustment of protein determination:

The protein assay by dye binding according to BRADFORD (1976) is much simpler to handle and quicker in comparison with other techniques. Because of the wide variation of specifity and sensitivity the linear range of optical density (E) has to be improved by corresponding dilution. The dilution factor and light pass (d) is given as E_{cor} (Fig. 1). For measuring the protein content the samples are diluted as shown in the calibration curves (Fig. 1).

Relation of total N and protein content

As demonstrated in Fig. 2, the protein N (precipitated by TCA) represents 94 % of total N.

Composition of the proteins:

The proteins consist of at least 5 fractions which can be simply characterized by their solubility (Fig. 3). The distribution of these fractions and the recovery of extraction is demonstrated in Figure 4.

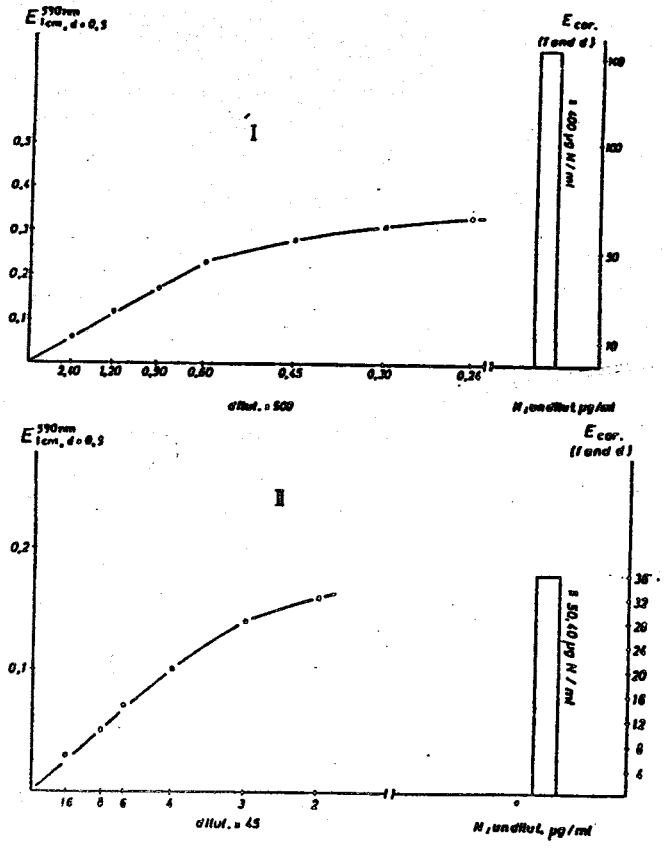

Fig.: 1
N-CONTENT AND PROTEINDETERMINATION IN
H_2O- (I) AND TRITON X- (II) DISS. FRAKT. (BRADFORD 1976)

Enrichment and purification:

Water soluble fraction: the concentration was increased fivefold by UF. But a complete seperation of the proteins from other compounds as AA, KH, Sinapine and other substances with a low molecular weight can only be achieved by GF (Fig. 5a).

Sodium chloride soluble fraction is treated as discribed above, Fig. 5b. The low molecular weight substances are assumed to be AA, which are probably released by proteolytic activity of this fraction (autolysis?). This assumption will be proved by the authors. All other fractions contain only proteins (Fig. 5c). We have experimental evidences that only the water soluble fractions contains myrosinase activity.

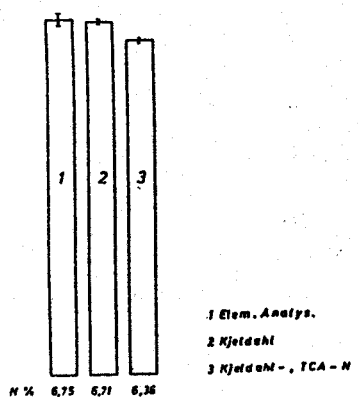

Fig.: 2
TOTAL- AND PROTEIN- N (TCA-7,5%)

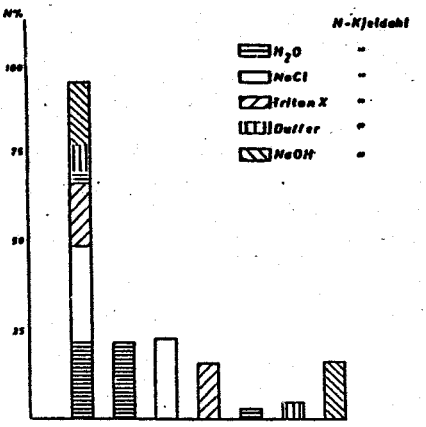

Fig.: 4
RECOVERY AND DISTRIBUTION OF TOTAL PROTEIN (%)

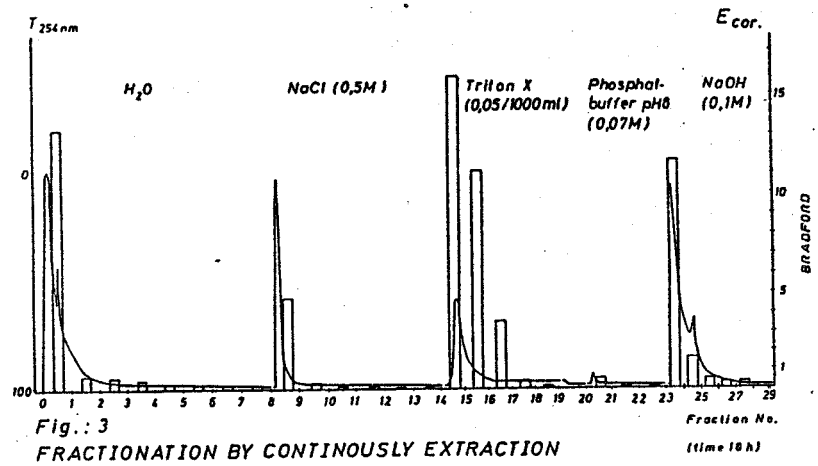

Fig.: 3
FRACTIONATION BY CONTINOUSLY EXTRACTION
(SAMPLE 100 mg/10 g SiO_2)

By GF (Sephadex G100) the water soluble fraction contains two or three components. In sodium chloride soluble fraction two distinct peaks were seperated (Fig. 6). By co-chromatography with Et.-DH, RSA and Cyt. C the mol. wt. can be estimated in the water soluble component 150 000 D and approximately 12 000 D, in sodium chloride soluble components 66 000 D and 12 000 D. More detailed results are expected from further experiments.

Myrosinase activity:

The activity was determined as described in Materials and Methods. To prove the relationship between enzyme concentration, substrate saturation and the rate of reaction, the turnover was measured by drawing the

tangent to the curve of glu-release. To obtain the specific activity the resulting dates are simple divided by the protein content. Figure 7 demonstrates this expected relation.

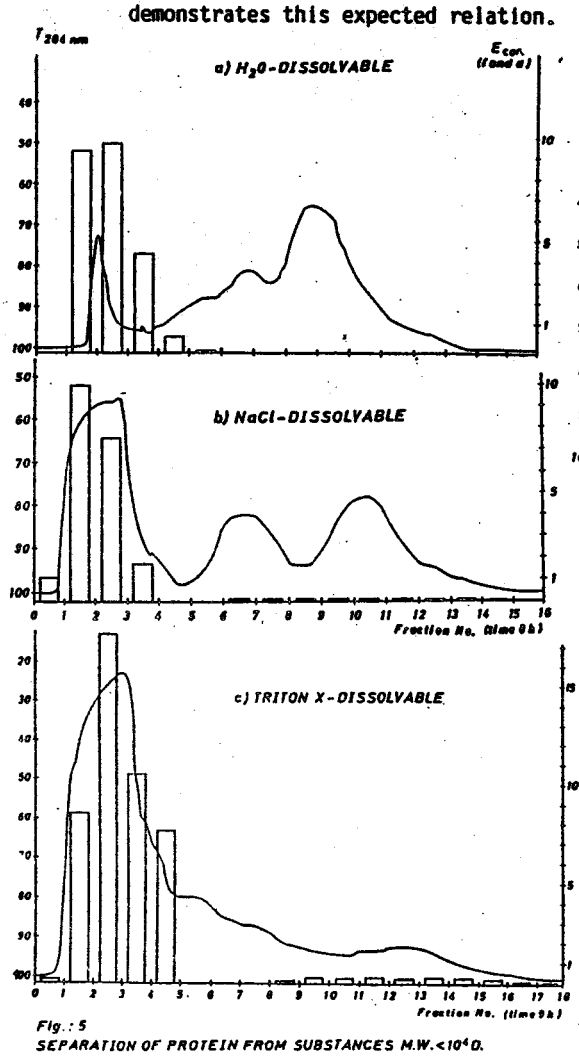

Fig.: 5
SEPARATION OF PROTEIN FROM SUBSTANCES M.W.<10^4 D.

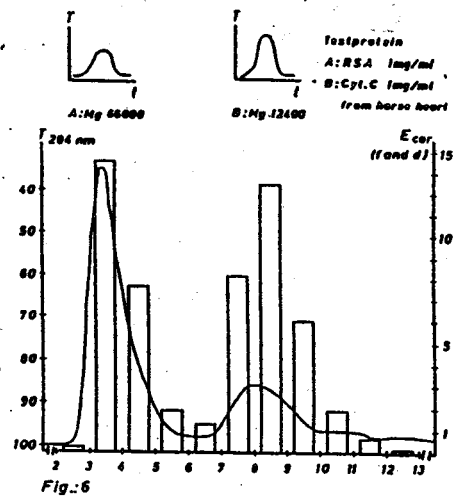

Fig.: 6
SEPARATION OF TWO PROTEIN COMPONENTS FROM NaCl-DISSOLV. FRACTION, GF, SEPH. G100

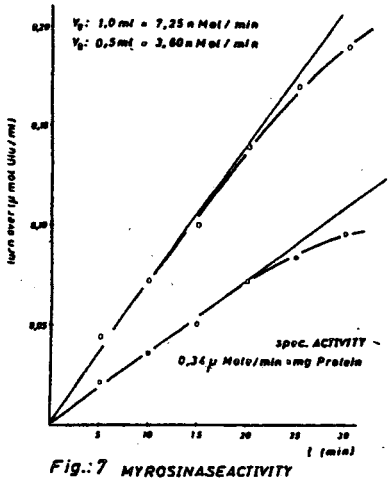

Fig.: 7 MYROSINASEACTIVITY (UF AND GF, SEPH. G 25)
Substrate: Sinigrine
Testsyst.: Hexokinase / Glu-6-ph-dehydrogenase, ΔE 340 nm

Discussion

These preliminary results demonstrate that such investigation can contribute biochemical facts to characterize the rapeseed proteins. They support the evaluations by sophisticated physico-chemical techniques (SCHWENKE et al., 1983). But for the breeder it is important to know whether these results can be applied to other cultivars of Brassica

napus. In particular, it is of considerable interest, whether the content and the distribution of the proteins is similar or significant different (SCHWENKE et al., 1973). If there exists another pattern of protein distribution, it must be proved, whether this can influence breeding programs.

Abbreviations

AA	= amino acid(s)	F	= grade of dilution
Cyt. C	= Cytochrom C from horse heart	glu	= glucose
		GF	= gelfiltration
d	= light pass (cm)	KH	= carbohydrates
E	= optical density	RSA	= Bovine serum albumin
Et-OH	= Ethanol dehydrogenase	TCA	= Trichloracetic acid
		UF	= ultrafiltration

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