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HALINA LEMAN
HENRYK KOSTYRA
BOGUSŁAWA SIELAWA
HALINA KOZŁOWSKA

LOW MOLECULAR WEIGHT NITROGEN COMPOUNDS IN RAPESEED

Department of Food Technology Foundations,
Center for Agrotechnology and Veterinary Medicine,
Polish Academy of Sciences, Olsztyn

Key words: rapeseed, non-protein nitrogen compounds

Non-protein nitrates, nitrate nitrogen, and amide nitrogen compounds in thermally processed and untreated rapeseeds were investigated. No significant differences were found in the distribution of these compounds in the various anatomic parts of the seeds. The studied compounds remained stable during thermal treatment. Seed coats contained the largest amounts of non-protein nitrogen and nitrate nitrogen, and their elimination could be one way of improving the nutritional value of rapeseed.

INTRODUCTION

The non-protein fraction (NPF) in rapeseed is a mixture of compounds, of which carbohydrates, nitrogen and phenolic compounds, glycosides and other specific compounds have not been researched sufficiently [5, 13]. The physico-chemical properties of these compounds significantly affect the functional properties of rapeseed protein products. Some of the NPF glycosides, phenols and oligo- and glycopeptides are antinutrional substances [13]. The existing publications concern mainly phenols and glycosides [4, 8, 16, 18], with only a handful devoted to non-protein nitrogen compounds (NPN) [2, 3]. In plant physiology NPN serve diverse important functions in protein biosynthesis and cellular enzymes activation and inhibition [5, 6]. These compounds also play a part in the formation of melanoid compounds lending rapeseed products a dark-brown colour, thereby reducing their quality [14]. The physiological and technological functions of these compounds fully justifies their investigation, and in this research we strove to analyse the contents of non-protein nitrogen, amide nitrogen and nitrate nitrogen compounds in

rapeseed. We also studied the effect of hydrothermal processing on the amounts of these compounds in rapeseed, and performed qualitative analysis of non-protein nitrogen compounds soluble in ethyl alcohol.

MATERIAL

Double improved Jantar *Brassica napus rapeseed* was studied. Seeds were steamed in an experimental steamer for 10 min in a steam stream at 100°C, and then separated into cotyledons, germs and seed coats with a Rumex Redical device (PZZ Track), with each fraction being subsequently additionally cleaned by hand.

The seeds and their individual anatomic parts were ground in a laboratory grinder, defatted with petroleum ether in a Soxhelet unit for 8 h and dried at room temperature (ca. 20°C).

METHODS

Non-protein nitrogen compounds were extracted from the material with 80% ethanol solution (1:40 w/v) by shaking in a reciprocating shaker for 60 min [3]. Insoluble material was removed by filtration through glass wool. Total nitrogen compounds content was determined in the filtrate by the Kjeldahl method.

Nitrate nitrogen was determined potentiometrically (N 5170A apparatus manufactured by Elwro Wrocław, equipped with RDJ 21 electrode) in an extract obtained by shaking the material with 2% acetic acid solution (1:250 w/v) for 30 min.

Amide nitrogen was determined in a hydrolysate obtained by refluxing 50-mg portions of material with 2 N hydrochloric acid (3 ml/mg protein) for 90 min [12]. The hydrolysate was filtered through glass wool, its pH adjusted to 9-10 with 2 N sodium hydroxide solution, and the liberated ammonia was distilled into a boric acid solution in a Parnas-Wagner apparatus.

The contents of nitrogen compounds were expressed in per cent of total nitrogen (N_t) in the material.

Ethanol-soluble compounds were analysed by spectrophotometry, paper electrophoresis, and paper and thin-layer chromatography as follows:

— UV spectra were studied with a Specord UV VIS spectrophotometer manufactured by Carl Zeiss, using a O.5 cm cuvette;

— high-voltage electrophoresis (1400 V, 60-80 mA) was carried out for 90 min on Whatman 3 paper using pyridineacetic acid-water (100:10:890) buffer (pH 6.5) and 0.2% ninhydrin in alcohol for staining;

— ascending paper chromatography was carried out using n-butanol-acetic acid-water solution (4:1:1) for development and 0.2% ninhydrin in alcohol for staining ;

— thin-layer chromatography was carried out on 20 × 16 cm plates coated with 1 mm of Kieselgel G silica gel. Plates were developed with n-butanol-acetic acid-water (4:1:1) solution), stained with 0.2% ninhydrin in alcohol and viewed in UV light (Emita VP-60 apparatus). The ninhydrin-stained fractions were marked on the unstained part of plates and extracted with 80% ethanol. Their UV spectra were analysed with a Specord UV VIS spectrophotometer (Carl Zeiss) using a 0.5 cm cuvette.

RESULTS AND DISCUSSION

LOW MOLECULAR WEIGHT NITROGEN COMPOUNDS

The analysis of low molecular weight nitrogen compounds comprised determinations of nitrate nitrogen and nitrogen in amide and non-protein compounds. The relevant figures for steamed and unsteamed rapeseeds and their anatomic parts are given in Table. Low molecular nitrogen compounds accounted for 3-14% of total nitrogen, this being in agreement with figures

Table. Nitrogen content in selected low molecular weight compounds in rapeseeds and their anatomic parts

Material	Non-protein nitrogen	Nitrate nitrogen	Amide nitrogen
		% N_t	
Before steaming:			
whole seeds	3.50	1.13	13.79
cotyledons	2.39	0.78	13.27
germs	2.69	0.99	16.98
seed coats	5.35	1.97	19.33
After steaming:			
whole seeds	3.52	1.02	13.84
cotyledons	2.46	0.61	15.00
germs	3.20	0.93	16.98
seed coats	5.56	1.90	21.44

given by other authors [2, 3]. The highest figure (13.8% N_t) was for nitrogen in amide compounds originating not only from low molecular wieght amide compounds but also from low molecular weight proteins present in rapeseeds. The lowest figures (1.0—1.1% N_t) were for nitrate nitrogen. Nitrogen of

non-protein compounds comprised 3.5% of total nitrogen substances. The quantities of these compounds depend on the method of extraction and the solvent, among other things [2, 3]. In most cases non-protein compounds are extracted with concentrated ethanol or trichloroacetic acid solutions, with the latter giving higher yield [2]. However, highly concentrated solutions of this acid (10-20%) are required to minimized proteins extraction. Another drawback of this solvent is that it has to be removed from the extract by repeated washing with ethyl ether, and this is conducive to losses of non-protein nitric compounds. Compared with trichloroacetic acid, ethanol extracts smaller quantities of basic amino acids. According to Bhatty and Finlayson [3] the nitric compounds in the ethanol extract correspond to actual non-protein compounds since the rapeseed reserve proteins are insoluble in alcohol. Because of this we used 80% ethanol to extract NPN.

The various anatomic parts of seeds differed as to the content of low molecular weight nitric compounds. The nitric substances in seed coats, both of steamed and unsteamed seeds, contained about two times more NPN and nitrate nitrogen than those in cotyledons and germs. The content of amide nitrogen in nitric substances in seed coats was also higher-by about 6% than in cotyledons, and about 2.5% than in germs.

The different contents of the studied nitric compounds in the various parts of rapeseeds are due to the different physiological functions of these parts [5]. As to the distribution of these compounds in the seeds, no qualitative differences were found. Steaming had no effect on the content or distribution of low molecular weight nitric forms in rapeseeds.

UV SPECTRA AND ELECTROPHORETIC AND CHROMATOGRAPHIC CHARACTERISTICS OF ETHANOL-SOLUBLE COMPOUNDS

UV spectra of non-protein compounds in seeds and their anatomic parts are shown in Fig. 1. The spectra of steamed seeds and their anatomic parts were identical; examplary spectra of seeds are given in the figure.

UV spectra of non-protein compounds of cotyledons and germs did not differ in a way suggesting the presence of glycosidic, peptidic and phenolic compounds responsible for absorption at 200-240 and upwards of 300 mn [19]. The UV spectrum of non-protein compounds of seed coats featuring strong absorption at about 250 nm and weak bands at 220-240 and 260-280 nm wouls suggest the presence inter alia of flavonic glycosides absorbing at 205-210 and 230-260 nm [19] and phenolic glycosides producing absorption bands at 270-280nm. Such an interpretation of UV spectra is supported by reports of other authors [4, 9, 15] who found that alcohol removes phenols and glucosinolates from rapeseed flour.

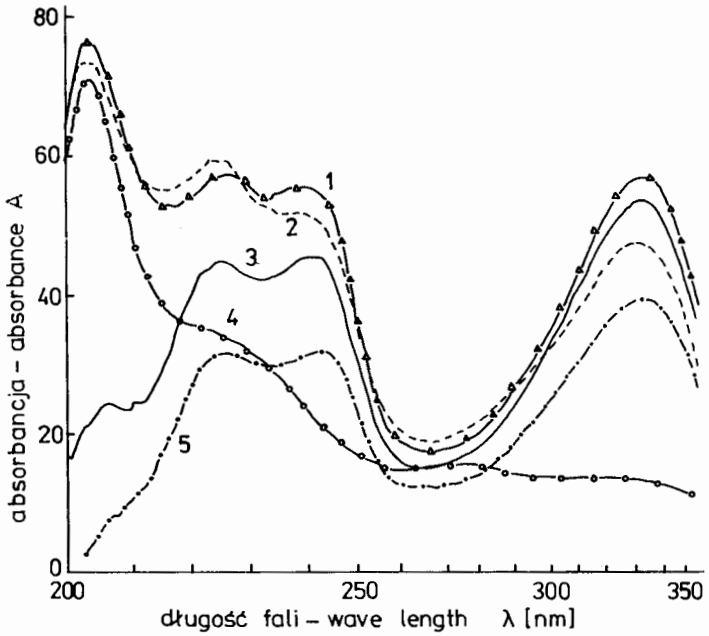

Fig 1

The electrophoretogram of non-protein compounds of steamed and unsteamed seeds and their anatomic parts featured three highly mobile amine fractions shifting towards the anode, and one amine fraction moving towards the cathode (Fig. 2.) The material from the start line shifted slightly towards the cathode. Electrophoretic separations of non-protein compounds showed no differences in distribution of amine compounds. All anatomic parts of the seeds were found to contain one basic and three acidic fractions whose electrophoretic mobility corresponds to that of acidic and basic amino acids, among others [11]. There was no visible effect of steaming on the number or electrophoretic mobility of fractions, and so further analyses were limited to unsteamed rapeseeds. The electrophoretograms, after being dried, were chromatographed with n-butanol-acetic acid-water (4:1:1) normally to electrophoretic separation in order to achieve the separation of neutral amine fractions. This chromatography revealed four amine fractions in seeds, cotyledons and germs and three in seed coats (Fig. 3), corresponding to peptidic compounds. This supposition i encouraged by the UV spectrum and by previous studies [7] which demonstrated the presence of five neutral peptides and the absence of acidic and basic peptides in rapeseed.

Thin-layer chromatography of rapeseed non-protein compounds produced six ninhydrin-positive spots and four spots fluorescing in UV light located on

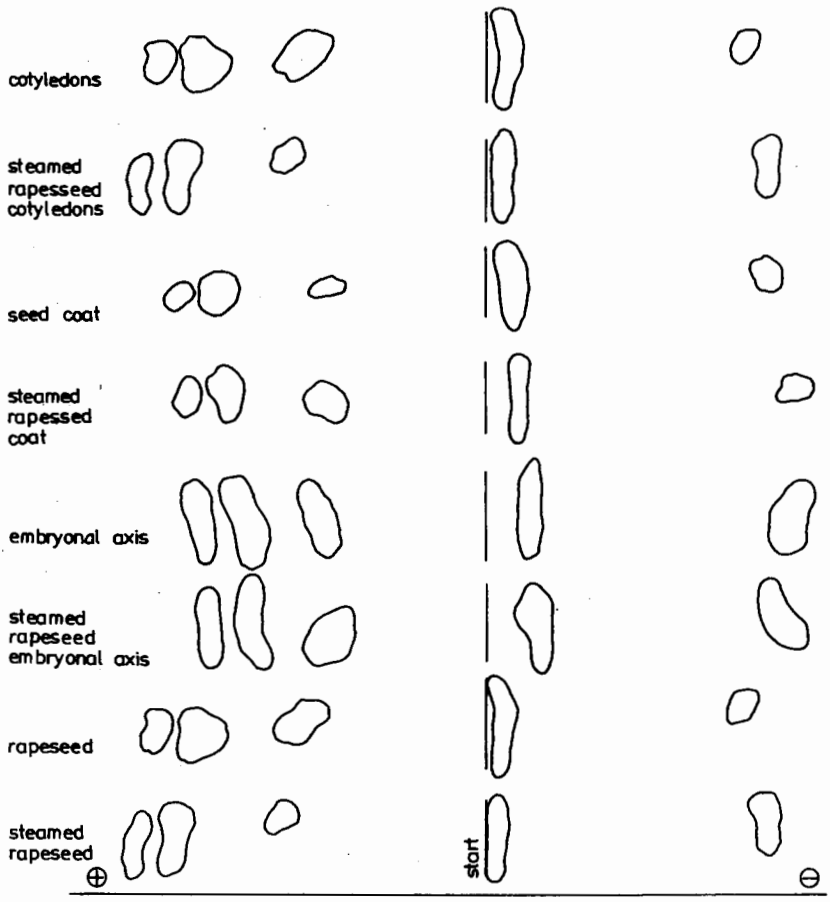

Fig. 2

the start line, between the fifth and the sixth spot, as well as on and above the sixth spot (Fig. 4). These fluorescing spots are probably indicative of the presence of phenolic compounds [17] and their complexes with amine compounds. Fig. 5 shows UV spectra of amine fractions and the fluorescing fraction from the start line. The absorption curves of amine fractions were all similar, featuring an absorption band at 200-240 nm. Compounds from the start line demonstrated strong absorption at 205, 230 and 340 nm, wavelengths characteristic for peptidic, glycosidic and phenolic compounds, among others.

Analysis of ethanol-soluble non-protein compounds in rapeseeds and their anatomic parts appears to show the presence of amino acids, peptides, phenols and glycosides, among other things. One should thus also reckon with their interactions. No significant differences were found in the distribution of these compounds in the distribution of these compounds in the various anatomic parts of the seeds. Also, steaming did not lead to qualitative changes in the

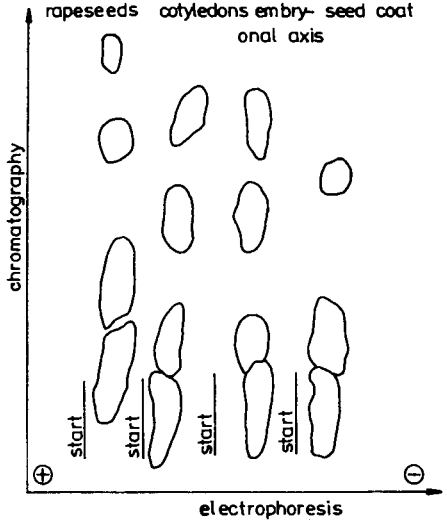

Fig 3

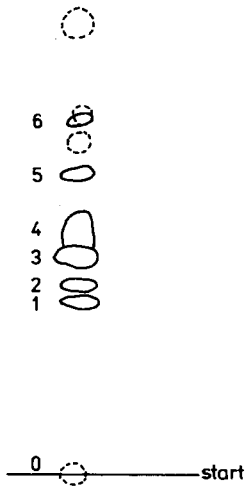

Fig. 4

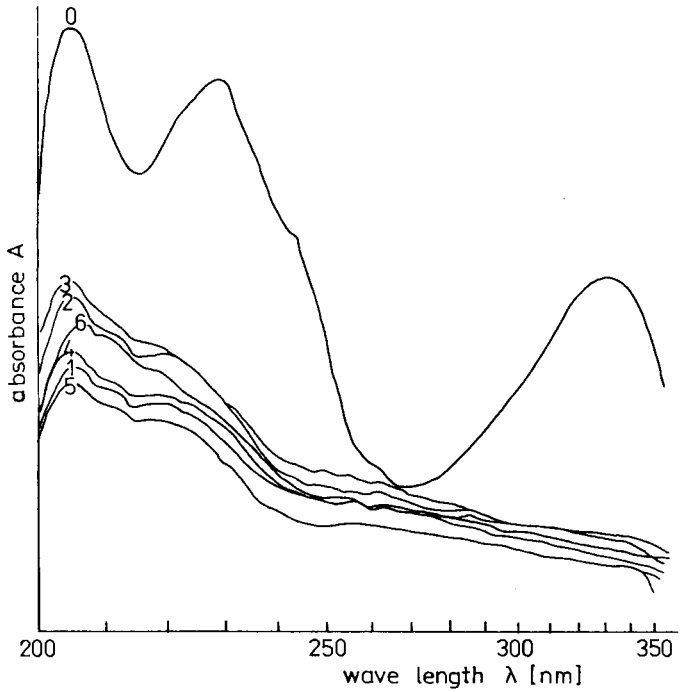

Fig 5

fraction of non-protein nitrogen compounds. Our results encourage furtherstudies, the more so since the nutritional value of double-improved rapeseed varieties is being put in doubt in view of the presence of antinutritional substances in the ethanol-soluble non-protein compounds fraction [10]. One way of improving the nutritional value of rapeseed is the elimination of seed coats [1]. In this research we demonstrated that the nitric substances in seed coats are marked by the highest content of non-protein compounds. Chromatographic analysis and UV spectra also suggest that nitric compounds in the ethanol extract, or some of them at least, occur in bounded form. For this reason it would be interesting to identify the nitric non-protein compounds and determine their effect on the metabolism of rapeseed products. Another interesting problem is the role of these compounds in seed physiology in the context of identifying rapeseed cultivars.

CONCLUSIONS

1. There were no qualitative but only quantitative differences in the distribution of nitrate, amide and non-protein nitrogen in Jantar rapeseeds.
2. Low molecular weight nitrogen compounds stable under steaming for 10 min at 100°C.
3. The ethanol extract of rapeseeds contained other constituents apart from amine compounds.

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Authors address: 10-718 Olsztyn Kortowo 43.

H. Leman, H. Kostyra, E. Sielawa, H. Kozlowska

NISKOCZĄSTECZKOWE ZWIĄZKI AZOTOWE NASION RZEPAKU

Centrum Agrotechnologii i Weterynarii PAN, Olsztyn

Streszczenie

W nasionach rzepaku odmiany podwójnie ulepszonej Jantar i ich częściach anatomicznych mierzono ilość azotu azotanowego, amidowego i niebiałkowego. Określono również wpływ operacji parowania nasion na stabilność i rozmieszczenie niskocząsteczkowych związków azotowych w poszczególnych częściach anatomicznych nasion. Związki wyekstrahowane z nasion rzepaku 80% roztworem alkoholu etylowego badano spektrofotometrycznie, elektroforetycznie i chromatograficznie. Nie stwierdzono jakościowego zróżnicowania pod względem rozmieszczenia niskocząsteczkowych związków azotowych w nasionach rzepaku. Ilość tych związków oraz ich rozmieszczenie w poszczególnych częściach anatomicznych nie zmieniały się również pod wpływem operacji parowania nasion. Związki niebiałkowe, rozpuszczalne w etanolu, charakteryzowały się absorcją światła UV w zakresach długości fal 200-210, 220-250 i 320-330 nm. We frakcji związków niebiałkowych wykryto jeden zasadowy, trzy kwaśne i cztery obojętne związki aminowe. Frakcja związków niebiałkowych zawierała również inne składniki polarne m.in. fluoryzujące w świetle UV.