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Studies on enzymatic fractionation, chemical composition and biological effects of dietary fibre in rape seed (*Brassica napus* L.)

1. Chemical composition of seeds and characteristics of soluble and insoluble dietary fibre of spring and winter type varieties of double improved oilseed rape

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ABSTRACT

Dark-seeded double improved Polish rape seed varieties (*Brassica napus* L.) (38 samples) were included in this study, with the main purpose of investigating their dietary fibre (DF) components. The chemical composition of unprocessed defatted seeds was determined, including analyses of neutral (NDF) and acid (ADF) detergent fibre, as well as insoluble (IDF), and soluble (SDF) dietary fibre. In eight samples (four spring type and four winter type rape seed) the prevalent DF fraction was IDF occurring in amounts from 27.6 to 34.0% of dry defatted matter (DDM), while SDF accounted for 3.8 to 6.1% of DDM. Arabinose and galactose were the prevalent monosaccharides in both fractions of DF. The DF fractions were associated with protein which was not digested by pepsin and pancreatin used in the isolation procedure. Protein associated with SDF ranged from 7.6 to 18.1% while protein associated with IDF ranged from 28.4 to 41.5% of the total protein content in rape seed. The amino acid composition of protein associated with IDF differed distinctly from that associated with SDF. Protein bound to IDF had an amino acid composition similar to that of whole rape seed, while protein associated with SDF had an extremely high content of lysine, cysteine, and serine, but a low content of methionine and phenylalanine.

KEY WORDS: double low rape oilseed, detergent fibre, dietary fibre, monosaccharides, protein

INTRODUCTION

The oilmeal from double improved rape seed is a valuable source of plant protein for supplementation of feed mixtures (Bille et al., 1983). The amount of the meal that can be added is limited, not only by the concentration of glucosinolates and products thereof, but also by the relatively high content of DF (Bjergegaard et al., 1991; Danielsen et al., 1994; Michaelsen et al., 1994). There are several proposed methods for lowering the level of DF by technological processing, such as dehulling, air stream fractionation, or the mycoenzyme degradation of DF components. Even the most advanced techniques have, however, not yet solved the problems of low digestibility of rape seed products (Jensen et al., 1990; Michaelsen et al., 1991). The most promising method at present seems to be the genetically based selection of rape seed with a low DF content, "the triple low varieties". However, the breeding achievement in developing yellow-seeded varieties of the spring type Canola with thinner hulls in which the crude fibre was reduced in the meal from about 12 to 8% did not satisfactorily improve meal utilization by poultry (Slominski and Campbell, 1990) and pigs (Bell, 1984), because the DF content increased in cotyledons.

It should be recognized that DF consists of a group of components of different structures which influence its physiological effects in animal feeding (Bjergegaard et al., 1991; Potkins et al., 1991; Eastwood and Morris, 1992; Asp et al., 1993; Hopewell et al., 1993). The analytical methods for dietary fibre (DF) estimation include ones which give different results (Bjergegaard, 1993), e.g. crude fibre determination using the classic Weende method, the detergent methods (NDF and ADF) (Van Soest, 1963; Van Soest and Wine, 1967), and the enzymatic gravimetric DF analysis, TDF = total dietary fibres (Asp et al., 1983). The variations in the obtained results occur because the different methods determine different DF components (Bjergegaard, 1993).

The purpose of these studies was fractionation of the DF components for further chemical analysis and biological evaluation of their effects in model animals with the aim to identify substances which should be selected against in plant breeding programs. The first part of this study examined the composition of different strains of dark-seeded rape seed, the characteristics and isolation of the soluble and insoluble fractions of DF for further biological investigations.

MATERIAL AND METHODS

Four double low ("00"-low erucic acid and low glucosinolate content) winter type , dark seeded rapeseed varieties (*Brassica napus* L.): Mar, Ceres, Librawo, Panter, and four spring type varieties: Bronowski, J0023-1-2-5, J0024-1-3-3,

J0024-1-5-2, as well as 30 new strains of *B. napus* L. winter type, selected by Polish breeders, were used in this study. All cultivars were grown in Poland in 1991 and 1992. Seed samples of winter type varieties were harvested from plants grown in two different places. Bronowski, three spring type and 30 winter type strains selected by the breeders were grown in experimental fields of the Plant Breeding Station Małyszyn. One kilogram samples from each site were mixed, and subsamples of 200 g were used for analysis.

Fat, protein and moisture content of whole seeds were determined on a Near Infrared Analyzer (Infratec, Tecator Sweden) by the method used by breeders. All samples were ground in a coffee-mill and defatted with hexane in a Soxhlet apparatus. DF fractions were determined as SDF and IDF according to the method of Asp et al. (1983) in four selected winter type and four selected spring type rape seed varieties in order to allow analysis of the composition of saccharides and associated compounds, and as ADF and NDF by the methods described by Van Soest (1963) and Van Soest and Wine (1967) in all samples.

Tannin determinations were performed according to the AOAC (1965) spectrophotometric method, based on formation of coloured products between the Folin-Denis (or Folin Ciocalteu's) reagent and phenolic components, with the following modification: the waiting time between mixing and measuring absorbance was extended from 30 min to 2 h to improve colour development.

The monosaccharide composition of non-cellulosic polysaccharides in defatted samples and in isolated DF fractions was determined on a Varian 3700 Gas Chromatograph on DB-Wax 30m, ID 0.53 mm column. Hydrolysis was performed according to Albersheim et al. (1967) by 2 M trifluoroacetic acid (TFA) 1 h at 121^{0}C, followed by evaporation to dryness, and derivatization to aldononitrile acetates as described by McGinnis (1982). Nitrogen content was determined by the Kjeldahl method. Analysis of the amino acid composition of protein hydrolysates was performed on a Beckman 119CL amino acid analyzer, after acid hydrolysis in 6 M HCl as described by Mason et al. (1980). The S-containing amino acids were oxidised prior to hydrolysis by use of formic acid and hydrogen peroxide mixture.

Glucosinolates were determined as individual trimethylsilyl (TMS) derivatives (Olsen and Sørensen, 1980) by GLC on a Hewlett Packard gas chromatograph 5980A model.

RESULTS AND DISCUSSION

In all samples the moisture content ranged from 5.4 to 7.4%, the oil content varied between 41.3 and 44.9%, and the protein content varied from 20.2 to 23.5% (Table 1). With regard to the glucosinolates (Table 2), the new strains

TABLE 1

Moisture, fat, and protein content in whole seeds of double low rapeseed, %

Sample	Moisture	Fat	Protein
Bronowski	6.2	44.9	22.2
J0023-1-2-5	6.3	42.4	22.1
J0024-1-3-3	6.2	42.4	22.3
J0024-1-5-2	6.8	41.3	22.6
Mar	5.7	43.9	20.2
Ceres	5.9	43.6	20.2
Librawo	5.7	44.2	20.5
Panter	5.4	42.1	20.6
Mean of 30 winter-type strains	6.6±0.3	43.2±0.7	22.7±0.3
(Min – Max)	5.9 – 7.4	41.7 – 45.4	22.0 – 23.5

selected by the breeders showed a lower content (3.4 to 11.3 μmol/g) of the sum of progoitrin, gluconapin and glucobrassicanapin than the commercial varieties (7.4 to 15.3 μmol/g). The content of the sum of above mentioned three alkenyl glucosinolates up to 15 μmol/g of defatted seed is a limit for double improved rape seed. According to Polish standards, all the samples were considered double improved. The total content including the indole glucosinolate varied from 8.0 to 17.0 μmol/g and in the commercial varieties 13.3 to 20.1 μmol/g. The relationship between individual glucosinolates also varied, with progoitrin dominating in the commercial varieties, whereas 4-hydroxyglucobrassicin was present in the highest concentration in the new strains. The change in level and profile of glucosinolates can affect the physiological effect of the rape seed obtained (Bjerg et al., 1989; Jensen et al., 1991).

Some commonly used methods for fibre determination include the crude fibre method (not used in these studies), the detergent methods (NDF and ADF), and the enzymatic gravimetric methods resulting in IDF and SDF. The NDF and ADF methods are primarily applied for analyses of feedstuffs of vegetative parts of plants (straw, hay, leaves, etc.) and comprise only insoluble residues of plant tissues. The enzymatic gravimetric DF methods with determination of IDF and SDF are of special interest in relation to evaluation of feed and food used for monogastric animals and man. It should be applied to grains and seeds in which the DF components are located in hulls (protective tissues) and endosperm (grains) or cotyledons cell walls. The enzymatic gravimetric DF methods are, however, time consuming and expensive (pure enzymes, ethanol), but can provide a clue regarding the physiological activity caused by IDF and SDF.

TABLE 2

Individual and total glucosinolate content (μmol/g seeds) in double low rapeseed determined by GLC of trimethylsilylated glucosinolates. GLN = gluconapin, GBN = glucobrassicanapin, Prog = progoitrin, Nplf = napoleiferin, Ind = glucobrassicin, 4-OH–ind = 4–hydroxyglucobrassicin

Variety	GLN	GBN	Prog	Nplf	Ind	4-OH-ind	Total
Bronowski	3.9	1.0	9.4	0.5	0.5	3.8	19.1
J0023-1-2-5	2.1	1.5	9.0	0.3	0.9	3.7	17.5
J0024-1-3-3	1.4	0.5	6.2	0.2	1.2	6.1	15.5
J0024-1-5-2	1.3	0.4	5.7	0.1	0.5	2.6	10.7
Mar	2.5	0.7	5.6	0.4	0.4	3.7	13.3
Ceres	3.0	0.9	9.3	0.6	0.4	2.5	16.8
Librawo	4.3	1.2	10.3	0.5	0.5	3.3	20.1
Panter	3.3	1.1	8.1	0.5	0.5	2.6	16.1
3508B/92	1.8	0.7	2.4	0.1	0.5	4.2	9.7
3513B/92	1.5	0.2	1.7	0.1	0.3	5.4	9.2
3515B/92	2.1	0.4	2.5	0.0	0.4	6.6	12.0
3523B/92	2.5	0.3	3.5	0.1	0.4	4.0	10.8
3524B/92	2.9	0.4	3.9	0.1	0.5	5.8	13.6
3532B/92	3.2	0.4	4.1	0.1	0.4	5.3	13.5
3533B/92	2.8	0.4	4.4	0.1	0.3	4.5	11.5
3534B/92	2.5	0.4	3.7	0.1	0.3	4.5	11.5
3540B/92	3.1	0.5	3.4	0.1	0.8	5.0	12.9
3544B/92	1.3	0.2	1.6	0.0	0.4	4.6	8.1
3547B/92	2.2	0.4	2.5	0.0	0.4	5.5	11.0
3553B/92	2.1	0.4	2.1	0.1	0.4	4.7	9.8
3559B/92	3.7	0.7	4.2	0.1	0.3	6.6	15.6
3560B/92	2.7	0.5	3.0	0.1	0.2	4.5	11.0
3562B/92	2.6	0.4	3.3	0.1	0.2	5.1	11.7
3563B/92	1.9	0.3	2.1	0.0	0.3	5.1	9.7
3565B/92	3.7	0.8	4.1	0.1	0.3	5.5	14.5
3569B/92	3.1	0.5	3.4	0.1	0.3	4.5	11.9
3570B/92	2.0	0.3	2.5	0.1	0.2	3.8	8.9
3572B/92	2.6	0.4	2.9	0.0	0.4	5.6	11.9
3600B/92	2.5	0.5	3.1	0.1	0.4	6.2	12.8
3603B/92	3.9	0.5	3.9	0.1	0.3	4.8	13.5
3613B/92	1.6	0.3	1.8	0.0	0.2	4.4	8.3
3625B/92	4.3	0.6	6.4	0.2	0.4	5.1	17.0
3640B/92	2.0	0.5	2.0	0.0	0.3	5.4	10.2
3648B/92	2.7	0.4	3.0	0.1	0.2	3.8	10.2
3656B/92	1.6	0.3	1.9	0.0	0.3	3.9	8.0
3669B/92	2.5	0.4	3.6	0.1	0.4	4.9	11.9
3670B/92	2.4	0.6	2.9	0.1	0.3	5.3	11.6
3696B/92	2.1	0.3	2.4	0.07	0.2	4.6	9.7

TABLE 3

Content of DF in defatted rape seed, determined as SDF, IDF, NDF, ADF, and hemicellulose (HEM). All data are expressed on air-dry matter basis (8% moisture). SDF and IDF were corrected for ash and protein, NDF and ADF were corrected for ash. HEM = NDF–ADF

Sample	Dietary fibres, %		Detergent fibres, %		
	SDF	IDF	NDF	ADF	HEM
Bronowski	3.1	33.0	26.4	22.8	3.6
J0023-1-2-5	4.9	27.5	21.7	18.2	3.5
J0024-1-3-3	5.0	29.2	22.8	18.3	4.5
J0024-1-5-2	5.2	28.7	23.3	20.1	3.2
Mar	5.2	33.3	27.9	23.7	4.2
Ceres	3.0	32.0	23.6	18.6	5.0
Librawo	4.2	30.9	21.6	17.8	3.8
Liporta	5.1	31.3	22.5	17.0	5.5
Mean of 30 winter-type strains	nd	nd	21.5±1.6	16.6±1.3	4.9±1.3
(Min – Max)			18.6–25.4	13.7–19.4	1.6–7.5

The results obtained with the different methods of DF analyses are shown in Table 3. DF determined enzymatic-gravimetrically is found in considerably higher amounts than revealed from the detergent methods, emphasizing the appreciable difference in DF values obtained by these two types of analyses. The shortcoming of the detergent methods is the loss of the more soluble DF components, whereas the analytical approach of the enzymatic gravimetric method comprise recovering of both insoluble and soluble DF components (IDF and SDF). These methods have, on the other hand, been criticized for overestimation of the DF content, by including components in the residue different from the traditional DF components (non-starch polysaccharides and lignins), e.g. cell wall proteins, Maillard reaction products, tannins, low molecular weight phenolic acid derivatives, ash, steroids etc. The data in Table 3 were corrected for protein and ash content, and a great part of the discrepancy in level found for the two DF methods lies probably in the difference in determination of soluble DF components. Other factors may, however, also be involved, as the level of IDF is higher than that of NDF in all samples.

Table 4 shows the level of ash, protein, IDF and SDF in isolated fractions. As revealed, the SDF fraction contained much more ash, compared to ash in IDF. The high level of ash in the SDF fraction is a result of the analytical procedure, with ethanol precipitation of the soluble part of DF resulting in co-precipitation of buffer salts (Prosky et al., 1984; Mañas and Saura-Calixto, 1993). To avoid this, dialysis may be used as an alternative procedure for recovering SDF.

TABLE 4

Content of protein (N x 6.25), ash and dietary fibre in rapeseed meals, expressed as % of air-dry defatted matter

Variety	IDF fraction, %			SDF fraction, %		
	Protein	Ash	IDF	Protein	Ash	SDF
Bronowski	13.8	2.6	33.0	6.2	7.4	3.1
Mar	12.8	2.9	33.3	5.2	5.7	5.1
Ceres	14.0	2.9	32.0	3.5	7.6	3.0
Librawo	13.3	2.4	30.9	2.4	5.6	4.2
J0023-1-2-5	13.0	2.1	27.5	6.2	6.7	4.9
J0024-1-3-3	11.8	2.9	29.3	4.8	7.2	5.0
J0024-1-5-2	12.3	2.3	28.7	6.7	6.3	5.2

* – ash content determined gravimetrically (550°C, 20h)

** – protein content (N x 6.25) determined by the Kjeldahl method

TABLE 5

The content of amino acids in protein associated with soluble (SDF) and insoluble fibre (IDF) [g/16 g N] compared to rapeseed meal. Mean value of four varieties

Amino acid	SDF	IDF	Meal
Cys	2.97	2.03	2.10
Asp	13.39	7.53	7.63
Met	0.83	1.67	2.33
Thr	3.85	4.78	4.42
Ser	6.26	5.20	4.83
Glu	15.89	11.72	19.40
Pro	3.00	6.01	7.89
Gly	6.01	5.03	5.25
Ala	3.37	4.43	4.56
Val	2.77	5.82	5.15
Ile	2.20	5.15	4.18
Leu	2.73	8.92	7.89
Tyr	1.31	2.74	3.23
Phe	1.44	4.40	4.53
His	4.28	2.74	2.90
Lys	11.25	5.78	5.74
Arg	6.82	5.58	6.73
NH_3	2.21	2.02	1.68
A-acid recovery, %	90.5	91.2	100.4

The level of protein was higher in IDF than in SDF. Recalculation of these data showed that 40 to 50% of the protein in rape seed meal could be associated with the DF fractions. This may partially explain the low rape seed protein digestibility. This was also the conclusion from previous studies with isolated IDF and SDF fed to rats in balance trials (Bjergegaard et al., 1991).

Determination of the amino acid composition of the DF associated protein (Table 5) showed that the protein associated with IDF had an amino acid composition similar to that of rapeseed meal, with the exception of a slightly higher level of leucine and a slightly lower level of glutamine. In contrast, protein associated with SDF differed markedly, being extremely high in lysine (11.25 g vs. 6.73 g/16 g N), and asparagine (13.39 g vs. 7.63 g/16 g N in rapeseed meal), whereas the levels of cysteine, serine and histidine were only slightly elevated. Methionine, proline, valine, leucine, tyrosine, and phenylalanine appeared in lower amounts compared to the level in rapeseed meal protein.

The amino acid composition of protein in the SDF fraction was corrected by subtraction for amino acids deriving from the enzymes used for isolation of DF. So, the differences obtained in proteins from IDF and SDF, respectively, should not be considered an artifact. It should also be noticed that the level of protein demonstrated in DF may be of another magnitude than that found, due to the possible presence of low molecular weight N-containing compounds and the calculation of protein as N x 6.25.

TABLE 6

Composition of noncellulosic polysaccharides in SDF and IDF fraction, expressed as % of monosaccharide in air-dry defatted matter, determined by GLC as aldononitrile alditoles

Variety/Fraction		Rham	Fuc	Arab	Xyl	Mann	Gluc	Gal	Sum
Bronowski	SDF	0.02	0.08	0.60	0.07	0.08	0.25	0.45	1.6
	IDF	0.48	0.26	4.14	1.70	0.16	0.90	2.19	9.8
J023 1-2-5	SDF	0.06	0.08	1.24	0.20	0.05	0.18	0.48	2.3
	IDF	0.37	0.20	3.50	1.33	0.16	0.64	1.57	7.8
J024 1-3-3	SDF	0.42	0.07	0.88	0.15	0.04	0.18	0.40	1.8
	IDF	0.29	0.22	3.67	1.48	0.15	0.71	1.67	8.2
J024 1-5-2	SDF	0.07	0.08	1.11	0.20	0.04	0.19	0.49	2.2
	IDF	0.38	0.22	3.43	1.42	0.16	0.70	1.68	8.0
Mar	SDF	0.03	0.11	0.96	0.10	0.09	0.43	0.62	2.3
	IDF	0.48	0.33	4.08	1.70	0.17	1.26	2.58	10.6
Ceres	SDF	0.07	0.07	0.82	0.08	0.08	0.34	0.57	2.0
	IDF	0.43	0.18	3.92	1.67	0.16	0.79	2.30	9.4
Librawo	SDF	0.03	0.08	0.80	0.08	0.08	0.31	0.52	1.9
	IDF	0.47	0.29	4.01	1.77	0.93	0.94	2.48	10.2

The amount and composition of neutral non-cellulosic polysaccharides are given in Table 6. The content of noncellulosic polysaccharides in the IDF fraction was on a level between 7.8 to 10.6% DM, which constitutes about one third of IDF. The polysaccharides of the soluble fibre fraction were found in lower amounts: 1.6 to 2.3% DM. Arabinose and galactose were shown to dominate in IDF as well as in SDF, which is in agreement with previous results from the investigation of rape seed DF (Bjergegaard, 1993). The next dominating monosaccharide in SDF was glucose, whereas in IDF it was xylose. A determination of non-starch polysaccharides instead of non-cellulosic polysaccharides, as in the present study, will, however, considerably increase the proportion of cellulose in IDF.

It should be emphasized that the information about the constituent monosaccharides in IDF and SDF is valuable only in conjunction with preliminary knowledge on the main types of polysaccharides present. According to Slominski and Campbell (1990), the non-cellulosic polysaccharide fraction in rape seed has a high content of uronic acids (30%), and it is likely that part of the arabinose and galactose found in SDF are constituents of acidic pectic substances. An acidic arabinogalactan was isolated from the water-soluble fraction of rape seed cotyledon meal by Siddiqui and Wood (1977). Polysaccharides of the arabinan and arabinogalactan type found in SDF are apparently also present in IDF, their solubility properties being dependent on the association with other polysaccharides in the fraction, e.g. xyloglucans. The occurrence of xylose indicate the presence of xylans, xyloglucans and amyloids, which are branched polysaccharides containing xylose, glucose, and possibly galactose. Amyloids are the third dominating polysaccharide in rape seed (Naczk and Shahidi, 1991). Fucoamyloids have also been reported (Aspinall et al., 1977; Siddiqui and Wood, 1977), their presence is related to the detection of fucose. Mannose is probably derived from galactomannans (Slominski and Campbell, 1990).

The total content of tannins in DF was similar in the four varieties tested. The tannin content in IDF was about 2.5 times higher than in SDF (Table 7). Part of

TABLE 7
Tannin content determined by the colorimetric AOAC method in SDF and IDF fractions isolated from double low rapeseed. The data are given as % of dietary fibre fraction

Variety	Tannin in SDF, %	Tannin in IDF, %
Bronowski	0.4	1.0
Mar	0.3	1.1
Ceres	0.4	0.8
Librawo	0.3	0.9

the tannin response in IDF is probably caused by lignin-type compounds, but low molecular weight phenolics, non-covalent or ester bound (Bjergegaard et al., 1993), and aromatic choline esters present in rapeseed may contribute to the level found.

CONCLUSIONS

Use of the enzymatic gravimetric method for determination of DF in rape seed samples resulted in a considerably higher level than found by the two detergent methods. The main reason for this discrepancy is probably a loss of soluble DF components with detergent methods. On the other hand, IDF and SDF, obtained by use of enzymes, contains a wide range of compounds of noncarbohydrate origin in addition to lignin and non-starch polysaccharides, which are considered traditional DF components.

One of the quantitatively dominating groups of compounds are the proteins, which was shown to occur in IDF as well as SDF fractions.The presence of this protein, not digested by pepsin and pancreatin during the isolation procedure, may explain the relatively low biological utilization of rape seed protein found in non-ruminants. Further investigations are needed in order to improve the availability of this protein.

Another problem, which also deserves attention when working with the enzymatic gravimetric method, is the high level of ash in the SDF fraction. Preferably, dialysis for isolating SDF should be used instead of the normally used precipitation technique.

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STRESZCZENIE

Badania nad enzymatycznym frakcjonowaniem, składem chemicznym i działaniem biologicznym włókna pokarmowego rzepaku podwójnie ulepszonego. 1. Skład chemiczny nasion i charakterystyka włókna rozpuszczalnego i nierozpuszczalnego jarych i ozimych odmian rzepaku podwójnie ulepszonego.

38 próbek rzepaku podwójnie ulepszonego o ciemnych nasionach poddano analizom ze szczególnym uwzględnieniem zawartości składników włókna pokarmowego. Oznaczono zawartość włókna detergentowego neutralnego (NDF) i kwaśnego (ADF) w 4 odmianach ozimych i 4 jarych, oraz w 30 wybranych rodach ozimych. Zawartość włókna pokarmowego metodą Aspa (enzymatyczną), z uwzględnieniem podziału na frakcje rozpuszczalną (SDF) i nierozpuszczalną (IDF), oznaczono w 4 odmianach ozimych i 4 jarych. We włóknie pokarmowym dominowała frakcja nierozpuszczalna (IDF) 27,6 do 34,0% suchej masy odtłuszczonej(DDM), zaś ilość SDF wahała się od 3,8 do 7,5% DDM. Arabinoza i galaktoza były cukrami dominującymi tak we włóknie SDF jak i IDF. Z obydwoma frakcjami DF związane były białka, nie poddające sie trawieniu pepsyną i pankreatyną. Ilość białka asocjowanego z frakcją włókna nierozpuszczalnego wynosila od 28,4 do 41,5%, podczas gdy z frakcją rozpuszczalną od 7,6 do 18,1% całkowitej ilości białka w rzepaku. Skład aminokwasowy białka asocjowanego z SDF i IDF różnił się wyraźnie. Białko związane z IDF miało skład podobny do składu odtłuszczonych nasion, natomiast frakcja SDF zawierała bardzo dużo lizyny, cysteiny i seryny, lecz mało metioniny i fenyloalaniny.