Processing of oilseed rape using the enzyme based technique and a simple step to removal of minor parts of the dietary fibres result in appreciable increased digestibility of rapeseed protein and energy

Charlotte BJERGEGAARD, Lisbeth INGVARDSEN and Hilmer SØRENSEN

Chemistry Department, Royal Veterinary and Agricultural University,
40-Thorvaldsensvej, DK-1871 Frederiksberg C (Denmark)

INTRODUCTION

The high quality of rapeseed protein for animal feed is due to the well-balanced amino acid composition and high BV (Bille *et al.*, 1983a) compared with other plant protein, *e.g.* soybean and pea (Mortensen and Sørensen, 1985; Arentoft *et al.*, 1990). However, various rapeseed constituents present in too high concentration reduce the product quality (Eggum *et al.*, 1985a). Especially the glucosinolates and their degradation products are antinutritive and toxic compounds of interest. The effects have been documented for eighteen individual glucosinolates in trials with rats fed different concentrations of the glucosinolates +/- myrosinase added to a standard diet without other rape constituents. Great variations in antinutritional effects were seen for the individual glucosinolates, and generally the most pronounced effects were caused by the degradation products as revealed from effects on biological value (BV), palatability, feed consumption, and effects on internal organs (Bille *et al.*, 1983b; Bjerg *et al.*, 1989; Jensen, 1990; Jensen *et al.*, 1991; Loft *et al.*, 1992; Michaelsen *et al.*, 1994).

Traditional rapeseed meal obtained from oil extraction with hexane is the prevalent rapeseed protein product used for feeding. However, this product has often too high concentration of products from transformation of glucosinolates during processing. This has been revealed from several trials performed with different animals for testing the acceptable amount of rapeseed meal which could be included in the diets. Trials with mink (Henriksen *et al.*, 1987), pigs (Eggum *et al.*, 1985b), sows (Danielsen *et al.*, 1987), young bulls (Andersen and Sørensen, 1985), and dairy cows (Emanuelson *et al.*, 1993) showed that the acceptable level of rapeseed meal strongly depend on glucosinolates present in the diet, and especially the degradation products of glucosinolates influenced the utilisation of nutrients and thereby the growth rate. The lowest acceptable level of glucosinolates was found for mink and sows (Bjerg *et al.*, 1987), and levels below 1 μmol/g diet are recommended for pigs (Sørensen, 1988).

In addition to the nutritive problems caused by the glucosinolates, the digestibility of both protein (TD) and energy (DE) in rapeseed meal are for other reasons relatively low compared with TD and DE for other plant protein sources. A part of this problem can be reduced by dehulling, as the hulls have a high content of dietary fibres (DF; Bell and Shires, 1982; Bille *et al.*, 1983a; Bell, 1984; 1993). However, Bjergegaard *et al.* (1991) studied the effect of insoluble (IDF) and soluble (SDF) DF isolated from whole seed, dehulled seed, and hulls, and they found a significant

effect of DF from all seed components, especially with respect to decrease in TD for both IDF and SDF, whereas only IDF significantly influenced DE. The negative effect caused by IDF from dehulled rapeseed material demonstrated the importance of DF located in other parts of the seed than the hulls. This may be of interest as far as yellow seeded rapeseed is concerned, because the reduced hull DF (Bell and Shires, 1982) is partially offset by an increased content of embryo DF (Bell, 1993; Slominski *et al.*, 1994). Thus, protein products obtained from dehulled yellow-seeded rape may be of lower TD, DE, and biological value (BV) than the corresponding values obtained for products (embryo) of brown-seeded rape.

The new oil extraction method based on aqueous enzymatic degradation of cell walls (Olsen, 1988; Jensen *et al.*, 1990) results in a hull-free proteinrich meal (PRM) with a very low content of glucosinolates. With test of this product in balance and/or production trials with rats, broilers, mink, and piglets (Hillemann *et al.*, 1988; Jensen, 1990; Jensen *et al.*, 1991; Danielsen *et al.*, 1994), it has been revealed that an increased acceptable content of rapeseed protein can be used in the diets. When PRM was included in the diets to mink, corresponding to a level of respectively 54% and 41% of the total protein and fat, no harmful effects from glucosinolates were observed in these otherwise rather glucosinolate-sensitive animals (Hillemann, 1985; Bjerg *et al.*, 1987; Henriksen *et al.*, 1987), The positive effects on mink fur quality obtained from feeding rapeseed oil (Hillemann, 1987) and rapeseed protein rich in sulphur containing amino acids (Glem-Hansen, 1980; Glem-Hansen and Hansen, 1981) could thus be attained without additional negative side effects. Although rapeseed protein quality has been improved by the glucosinolate reduction, relative low values of TD and DE are still problems of concern (Jensen *et al.*, 1990; Danielsen *et al.*, 1994). The main reason for this is related to the presence of DF in the product, which strongly associate with the proteins and in this way reduce TD (Bjergegaard *et al.*, 1991).

The highly complex nature of the cell wall structure may explain the observed advantage of using a mixture of cell wall degrading enzymes instead of a purified enzyme for aqueous extraction of rapeseed oil. Besides the improved oil release, the partly enzymatic degradation of DF is nutritional desirable (Düsterhöft *et al.*, 1993a, Düsterhöft *et al.*, 1993b) and it might probably influence the digestibility of other nutrients in the diet owing to, for instance, reduced fibre associated protein and fat, and increased ratio of mono- and oligosaccharides to polysaccharides. However, treating rapeseed with cell wall degrading enzymes for four hours did not significantly improve TD of the protein meal obtained (Jensen *et al.*, 1991; Danielsen *et al.*, 1994). This demonstrated the necessity of using more radical process conditions for either alteration of DF composition toward compounds of increased digestibility or reduction of DF content in rapeseed products, or both.

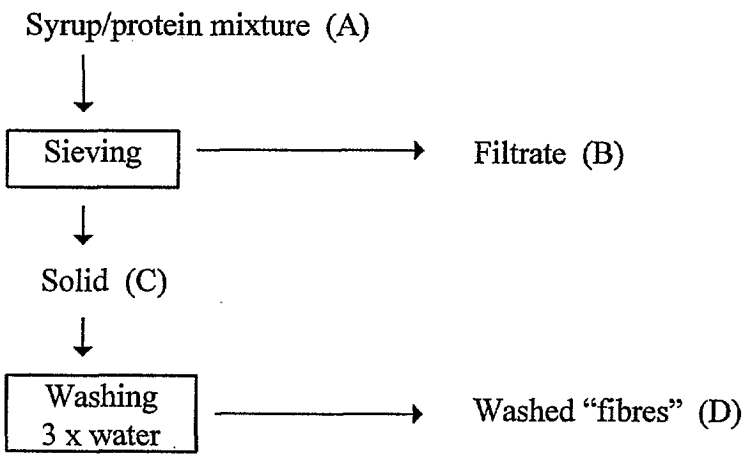
The present work comprises extended process reaction time (from four to twelve hours) in attempt to further degrade DF, and use of sieving the enzyme treated, defatted and dehulled rapeseed suspension have resulted in removed "fibre" substances from the products obtained by use of the developed pilot plant scale process. The products obtained have been tested in rat trials and by various analytical methods including determination of DF as IDF and SDF, respectively. Moreover, characterisation of total DF (TDF) in the products related to the sieving process, *i.e.* original proteinrich meal (PRM), "fibre-reduced" PRM (FR-PRM), and the washed "fibre" material

obtained by the sieving step (Fibre-PosD) has been performed by determination of the relative composition of pectic material (PEC), hemicellulose (HEM), cellulose (CEL), and lignins (LIG).

MATERIALS AND METHODS

Rapeseed used in the present work was a Danish grown double low spring rape variety Maribo line no. 1-9018 obtained from DANISCO (Maribo Seed, Holeby, Denmark). Rapeseed processing to proteinrich meal (PRM), syrup, and hulls was performed as described elsewhere (Olsen, 1988; Jensen *et al.*, 1990).

The fibre content of PRM was reduced by simply sieving the defatted rapeseed slurry and named FR-PRM. The removed material was subsequently washed with water as outlined below. Details on process conditions are described in The Whole Crop Biorefinery Project - Final Research Report (Bioraf Denmark Foundation, 1994).



Analysis of glucosinolates (Sørensen, 1988; 1990), aromatic choline esters (Bjergegaard *et al.*, 1993), and phenolic carboxylic acids (Bjergegaard *et al.*, 1992) was performed for PRM, syrup and hulls, whereas Soxleth fat (extracted with diethyl ether for four hours), SDF and IDF (Asp *et al.*, 1983; Bjergegaard, 1993), protein (AOAC, 1975), and amino acid composition (Eggum and Sørensen, 1989) were determined for all samples used in the trials. IDF and SDF residues were corrected for their content of ash and protein (N x 6.25) prior to calculation of the final level as discussed in Bjergegaard (1993). SDF were recovered by dialysis in preference to precipitation by alcohol, as this reduced the ash content in the residue considerably. Unless otherwise stated, all results are average values of double determinations, and dry matter content corresponds to the dry material obtained after freeze drying to constant weight. Fractionation of total dietary fibres (TDF) into four fractions named pectic material (PEC), hemicellulose (HEM), cellulose (CEL), and lignins (LIG) followed the procedure described by Bjergegaard (1993).

Balance trials with rats with determination of true protein digestibility (TD), biological value (BV), net protein utilisation (NPU), and digestible energy (DE) were performed at the Department of Animal Physiology at Research Centre Foulum. The trials were performed according to Eggum (1973) and Bille *et al.* (1983a) using groups of five Wistar male rats (average weight 70 g) housed in individual metabolic cages. The trials lasted nine days, which included a preliminary period of four days followed by a balance period lasting five days. During the latter, urine and faeces were collected separately, and feed residues were weighed. The rats were anaesthetised in carbon dioxide after the end of the balance period. Throughout the trial each animal received 150 mg N in 10 g dry matter (DM) daily. The diets were composed of different rapeseed products, both as singly and in mixture, and the N-content was adjusted by use of a nitrogen-free diet of autoclaved maize starch (80.6%), sucrose (9.0%), cellulose powder (5.2%), soybean oil (5.2%), mineral mixture (4.0%), and vitamin mixture (1.6%). A control diet of casein fortified with 1% DL-methionine was used.

RESULTS

Chemical composition of the protein sources or products evaluated are shown in Tables 1 - 5. The protein content determined as N x 6.25 was high in syrup (27%, Table 1). Fat and IDF were low in the syrup preparation, whereas the syrup had a relatively high content of SDF (11%).

PRM had a high content of protein (46-48%) and fat (27%). IDF and SDF constituted respectively 10% and 4% of the PRM.

"Fibre-reduced" PRM (FR-PRM) had an increased protein and a relatively high fat content. IDF and SDF were reduced to approximately one half and two third, respectively.

Rapeseed hulls were rich in DF (55%) of which IDF constituted more than 92%, but also a relative high content of protein (23%) was found. Fat following the hull fraction amounted 9%.

Table 1. Content (% DM) of protein, fat, IDF, and SDF in the products.

Product	Protein[a]	Fat	IDF[b]	SDF[b]
Syrup	27.7	0.94 (0.23)	0.0 (0.0)	11.2 (0.1)
PRM	46.9	27.44 (0.11)	10.0 (0.1)	4.3 (0.2)
FR-PRM	57.1	29.59 (0.16)	5.0 (2.2)	2.9 (0.2)
Hulls	23.5	9.17 (0.14)	51.3 (0.4)	4.1 (0.3)

a) calculated as N x 6.25; b) corrected for protein and ash

Table 2. Distribution (g per16 g protein N) of the limiting amino acids in the products.

Amino acid	Syrup	PRM	Hulls	FR-PRM
Lys	6.40	5.39	7.10	5.36
Thr	4.99	4.62	4.93	4.62
Cys	3.17	2.06	3.13	1.86
Met	2.12	2.18	2.08	2.03
Trp	0.73	1.54	1.53	1.54

Apart from a relatively low content of lysine in the rapeseed products PRM and FR-PRM, a well-balanced amino acid composition was found (Table 2). Concerning the real content of protein N (determined as the sum of the individual amino acids), a variation from 73 to 97% of total N was observed with the highest content in PRM. The high content of non-protein N in syrup was explained by the presence of glucosinolates, sinapine, and amide N released from the basic amino acids during hydrolysis.

Hulls are rich in sinapine and products thereof, which may explain parts of the high content of non-protein N occurring therein.

Regarding the content of limiting amino acids, syrup was particularly rich in cysteine, but also the content of threonine and methionine was high corresponding to a relatively high content of the storage protein napin in this fraction (Ochodzki *et al.*, 1994). Tryptophan, however, was present in low concentration in syrup. PRM was rich in limiting amino acids except for lysine, and only minor reduction of cystine and methionine was observed after fibre reduction of the meal.

Analyses for LMW compounds in the rapeseed products obtained by the enzymatic oil extraction process gave results presented in Table 3. The glucosinolates (illustrated by the quantitatively dominating compounds; gluconapin (**2**), progoitrin (**4**), glucobrassicin (**23**), and 4-hydroxyglucobrassicin (**26**)) were concentrated in the syrup together with the aromatic choline esters (determined as the quantitatively dominating compound sinapine), which were high in the hull fraction as well.

Table 3. Content (μmol/g DM) of glucosinolates, aromatic choline esters, and phenolic carboxylic acids in the rapeseed products obtained from the process.

Product	Glucosinolates[a]					Aromatic choline esters[b]	Phenolic carboxylic acids[c]
	2	**4**	**23**	**26**	Total		
Syrup	2.93	5.01	0.60	2.60	11.14	91.58	0.18
PRM[d]	0.44	0.80	0.32	1.16	2.72	10.23	0.12
Hulls	0.15	0.10	0.11	0.16	0.52	82.84	0.40

a) **2** = gluconapin, **4** = progoitrin, **23** = glucobrassicin, and **26** = 4-hydroxyglucobrassicin.

b) determined as sinapine; c) determined as sinapic acid; d) freeze dried

Further investigation of the DF composition of the products related to the "fibre-reducing" process was performed by determination of the relative composition of the fibre components: pectins, hemicelluloses, cellulose, and lignins, as shown in Table 4. Additionally, the fat content in the starting materials are given.

Table 4. Content (%) of fat in rapeseed products[a] and relative composition (%) of polysaccharides and phenolic polymers in DF from these divided into pectic material (PEC), hemicelluloses (HEM), cellulose (CEL), and lignins (LIG).

Rapeseed product	Fat (% of product)	Relative composition (%)			
		PEC	HEM[b]	CEL	LIG
PRM	27.4	24.8	22.7	19.0	33.5
FR-PRM	29.6	29.7	25.5	23.1	21.3
Fibre - PosD	53.8	16.8	12.6	13.4	57.1

a) abbreviations as in Table 9.1.; b) HEM-A included for FR-PRM

The sum of PEC, HEM, CEL, and LIG accounted for 58%, 62%, and 92% of TDF in PRM, FR-PRM, and Fibre-PosD, respectively.

The results obtained from balance trials with rats are shown in Table 5. The low BV obtained by use of syrup was caused by the content of glucosinolates. TD and DE values were, on the other hand, high. Use of the hulls resulted in accordance with the amino acid composition in high BV, whereas TD, NPU, and DE were rather low as expected from the high content of DF. Concerning the PRM, and both TD, and DE were high, whereas BV was lower than seen for other PRM products (Danielsen *et al.*, 1994) in accordance with the relatively low content of lysine in the rapeseed used in these present trials. An exceptional improvement of PRM was obtained by the FR-PRM product obtained from the new step included in the process which resulted in the product with some special types of fibres removed and thereby an increased TD value from ca. 90 to 95% and besides, the DE was raised from ca. 85 to 94%.

Table 5. Nutritional value of the rapeseed products in balance trials with rats.

Product	TD (%)	BV (%)	NPU (%)	DE (%)
Syrup	86.6	68.3	59.1	90.4
PRM	90.8	88.0	79.9	84.9
Hulls	47.9	91.6	43.9	23.9
FR-PRM	94.8	85.7	81.3	93.8

DISCUSSION

The processing performed, based on aqueous enzymatic rapeseed extraction, followed with some modifications the procedure described earlier (Olsen, 1988; Jensen *et al.*, 1990). The changes now included in the new-developed process conditions may explain some of the changes seen in product composition and subsequent values obtained for TD, BV, NPU, and DE. However, it is also obvious that the relative low BV obtained with the rapeseed products now investigated can be explained by the relative low content of lysine in the rapeseed compared to the content of lysine in the rapeseed used in the previous studies (Jensen *et al.*, 1991; Danielsen *et al.*, 1994).

In agreement with earlier results, the glucosinolates and aromatic choline esters were concentrated in the syrup. The glucosinolate contents in syrup and PRM obtained from the production performed in Bioraf were lower compared with the corresponding contents obtained by Jensen *et al.* (1990), while the sinapine content was nearly doubled in all the fractions. These changes may be explained by the plant variety used.

Concerning the original rapeseed products obtained from the aqueous enzyme based extraction process, they were all used singly for evaluation of the quality now obtained in the Bioraf production compared to the results obtained in the production previously performed at Novo Nordisk A/S. The analyses data for hulls were unchanged, and use of this fraction as the only protein source for monogastric animals is not recommendable, because the digestibility of both protein and energy were poor. Syrup used as the only protein source resulted in a low BV reflecting the too high content of some of the glucosinolates. Part of a reasonable explanation for this result can also be problems caused by the high content of SDF (11%). As far as PRM is concerned, both TD and DE were ca. 7% higher than the values obtained by use of the products from the original Novo process, probably owing to the prolonged reaction time with the cell wall degrading enzymes (from four to twelve hours). The DF were also changed to consist of relatively more SDF and less IDF after long-time hydrolysis which may be the reason for a more favourable TD of the new PRM products. Increment of DE may also be related to changed DF compositions as these constituents bind fat, which is high in energy. The oil yield, and hence fat-residue left in the PRM, was correspondingly influenced by the changed procedure. This agrees with results found by others (Sosulski *et al.*, 1988). The high fat content and its availability in the PRM here used for investigations contribute to the high DE. Regarding digestibility of rapeseed fat, this problem has previously been studied in trials with mink and a value approximately 40% lower than the usual values for plant oils fed to mink was found (Børsting, 1991). Effects caused by the rapeseed DF on the fat digestibility were found to be a likely explanation (Michaelsen, 1992; and refs. cited herein) including lower lipase activity, changed diffusion/absorption conditions in the intestine, change in intestinal structure and function, fibre-binding to the intestinal surface and/or the abilities of DF to entrap, absorb, bind and/or destabilise enzymes, lipids and bile salts. If the degradation of fibre components is enhanced by prolonged enzyme reaction time the negative effects mentioned above or some of them may be reduced and digestibility of both fat and protein become improved. FR-PRM gave additional support to the ideas behind special effects of some rapeseed DF.

FR-PRM was found to have the highest TD and DE for all of the rapeseed products investigated up to now including dehulled protein rapeseed meal with TD and DE values of ca. 85% and 70%, respectively. By the simple fibre reduction procedure now performed in Bioraf, values of 95% for TD and 94% for DE were obtained. This makes the product of considerable interest for feeding purposes because exactly these values have been claimed decisive for substituting rapeseed

protein in diets based on other protein sources of higher TD. By the sieving technique used, a product with strong binding capacity for protein and especially fat was removed. The FR-PRM product had a changed content of both SDF and IDF, which both have a strong negative effect on TD, BV, and DE according to Bjergegaard *et al.* (1991). However, some other antinutritional compounds may possibly follow the removed "fibres", and detailed studies of the removed "fibres" are now under investigation in the laboratory. More specific details related to the fibre reduction procedure will be published separately in connection with the Final Research Report (Bioraf Denmark Foundation, 1994).

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