

Nutritional value of cruciferous oilseed crops in relation to profile of accumulated biomolecules with especial regard to glucosinolate transformation products

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Abstract

Cruciferous oilseed crops accumulate relatively high concentrations of nutritional high quality oil and proteins in their seeds. In addition to these major seed components, their co-occurrence with high concentrations of dietary fibre (DF) and various bioactive components as glucosinolates/glucosinolate products is decisive for the nutritional value of the seed meal or products obtained from it. Depending on structural types and concentration of glucosinolates and glucosinolate derived products, these compounds can be either health beneficial or act as antinutrients. The effects of these components depend, however, strongly on the type of animal and development of the animals fed with the diets based on these compounds. Results from studies based on differently treated and processed seeds and from use of individual isolated seed components included in standard diets are evaluated and treated in relation to literature data as a basis for recommendations of acceptable concentrations of glucosinolates/glucosinolate products in animal diets. The relation between these recommendations of acceptable concentrations in feed to different animals and those reported as necessary for plant pathogen control (biofumigation) and health beneficial effects (chemoprotection) is discussed.

Introduction

The nutritional value of cruciferous oilseed crops has been a hot subject for a long time, with research being intensified some decades ago as a result of the success plant breeders had in connection with the production of double low oilseed rape (Bunting, 1981; Sørensen, 1985). Advantages of double low oilseed rape cultivars are related to the opportunities for an optimal utilization of relatively high yielding oil- and protein rich agricultural crops with ca. 45 % oil and 20 % protein in seed dry matter (DM), and less than 0.5% erucic acid in the oil and less than 20 µmole of glucosinolates per g. of seed DM (GCIRC, conf., 2003). Both of these major seed components have a high nutritional value and quality if problems caused by dietary fibres (DF) and especially glucosinolates can be solved (Bille et al. 1983a; 1983b; Bjergegaard et al. 1991). This gives the need for consideration of effects caused by processing and biorefining (Bagger et al. 2007) as well as the need for taking into account the variation in effects caused by structurally different glucosinolates and glucosinolate derived products (Bille et al. 1983; Bjerg et al. 1987a; Loft et al. 1992; Bonnesen et al. 1999; Bjergegaard et al. 2000; Vang et al. 2001). The great variation in structurally different glucosinolate derived products reflects primarily the glucosinolate structures (Bellostas et al. 2007) and the reaction conditions during non-enzymatic and enzyme/myrosinase (EC 3.2.1.147) catalysed reactions (Bjergegaard et al. 1994; Agerbirk et al. 1998; Bjergegaard et al. 1999; Buskov et al. 2000a; 2000b; 2000c; Barba et al. 2005; Bellostas et al. 2006). Thereby, it is also expected and found that effects on:

- feed and food quality,
- animal or human health and diseases and
- the value of final products,

are a function of structure and concentrations for both the glucosinolates and products thereof (Bille et al., 1983b; Bjerg et al., 1989; Jensen et al., 1991; Michaelsen et al., 1994; Bonnesen et al., 1999; Bjergegaard et al., 2001; Vang et al., 2001; Bellostas et al., 2007).

Materials and Methods

Details and comprehensive descriptions of the applied analytical methods are presented elsewhere (Sørensen et al., 1999; Sørensen 2001; Bellostas et al., 2003; Bellostas et al., 2006) as are applied procedures for evaluations of nutritional values and effects of allelochemicals/xenobiotics, N-balance trials with rats (Bille et al., 1983b; Bjerg et al., 1989), mink trials (Danielsen et al., 1987), young bull trials (Andersen and Sørensen, 1985) and evaluations of health and anticarcinogenic effects (Loft et al. 1992; Bonnesen et al., 1999; Vang et al., 2001).

Results and Discussion

Cruciferous oilseed crops are important sources for both vegetable oil (40-45% of seed DM) and protein (20-25 % of seed DM). Oil from these crops has been used as energy sources from ancient times, and in the recent years, additional focus has been directed at this oil for its use as fuels of biodiesel (Knothe, 2006; GCIRC conf., 2003). With introduction of double

low oilseed rape, an increased interest in the oil has been focused on the nutritional applications, as it has been found to be high quality oil (Bunting, 1981; Sørensen, 1985; GCIRC conf. 2003). The result of a large and increasing production of oils from cruciferous seeds is thus a great amount of deoiled meal for applications as feed, food and non-food, where the main limitations in its uses are caused by dietary fibres (DF, Bjergegaard et al. 1991) and especially glucosinolates and glucosinolate derived products. The lipophilic and volatile glucosinolate derived products may also create environmental (smell, taste, toxicity) and oil-quality problems, depending on structures of the glucosinolate products (Sørensen, 2001), which again is linked to the plant source and glucosinolate type. Cruciferous plant sources most often used belong to genera of the family Brassicaceae, and these sources are quantitatively dominated by Brassica cultivars; *B. napus* and *B. rapa* (Table 1).

Table 1. Quantitatively dominating seed glucosinolates in different Brassicaceae species.

Species	Glucosinolate dominating
<i>Brassica napus</i> L.	
<i>B. rapa</i> L.	
<i>B. oleracea</i> L.	
<i>B. nigra</i> (L.) Koch	
<i>B. carinata</i> Braun	
<i>B. juncea</i> (L.) Czern & Coss	
<i>Crambe abyssinica</i> Hochst. ex R.E. Fries	(2S)-2-Hydroxybut-3-enylglucosinolate
<i>Barbarea vulgaris</i> R. Br.*	(2S) and (2R)-2-hydroxy-2-phenylethylglucosinolate
<i>B. verna</i> (Miller) Ascherson*	Phenethylglucosinolate
<i>Lepidium campestre</i> (L.) R. Br.*	Sinalbin
<i>Camelina sativa</i> (L.) Crantz	(R)-Methylsulphinyl-(CH ₂) _n -glucosinolates#
<i>Sinapis alba</i> L.	Sinalbin

Ref. (* Andersen et al., 1999; Sørensen, 2001) # Dominating glucosinolates with n=9 and 10.

The cruciferous meal available for feed, food and non-food applications after deoiling contains thus various types and concentrations of glucosinolates defined by the applied type of seeds, and depending on storage and processing conditions a great number of glucosinolate derived products can be present in the meal. When applied at few percent in diets to monogastric animals, this will give reduced nutritional value for the cultivars with more than 20 µmol/g seed DM. A great number of oilseed rape cultivars – even double low oilseed rape – also have a level and composition of glucosinolates that give nutritional problems (Bille et al., 1983; Bjerg et al., 1987; Jensen et al., 1991).

The acceptable level of glucosinolates in diets to monogastric animals needs to be below 2nd level (Table 2) or 1-2 µmol/g DM to avoid nutritional problems. However, as described elsewhere (Bjerg et al., 1987), the acceptable level depend on the type of animal, its age/development and especially the structure of the glucosinolate/glucosinolate products.

Table 2. Glucosinolate level in seed, seed meal and diets (DM) to monogastric animal without the presence of glucosinolate transformation products

	level 1**	level 2	level 3**	level 4
Diets (µmol/g DM)	0.5	2.5	12.5	
Feed with 20 % rapeseed meal: (µmol/g rapeseed meal)	2.5	12.5	62.5	as level 2 but with active myrosinase added
Seed before deoiling: (µmol/g seed; depend on oil and water content)	1-2	6-8	30-40	

** Generally safe. ** Create nutritional problems.

The quantitatively dominating glucosinolates in double low oilseed rape are (2R)-2-hydroxybut-3-enylglucosinolate (progoitrin) and 4-hydroxyglucobrassicin, which as result of hydrolysis give (5S)-5-vinyloxazolidine-2-thione (goitrin) and thiocyanate ion, respectively. Both hydrolysis products have strong effect on the thyroid gland and thereby on the metabolism, growth and development of the animals. The indolyl group of 4-hydroxyglucobrassicin gives, as result of transformations, appreciable negative effects on rapeseed products caused by a complex group of structurally different compounds (Jensen et al. 1991; Belostas et al., 2007). Appreciable variations are also found for other types of glucosinolate transformation products as isothiocyanates (ITC's; R-N=C=S) and nitriles (RCN):

Table 3. LD₅₀ of glucosinolate hydrolysis products on rats and mice.

Glucosinolate precursor	Compound	Animal	LD ₅₀ mmol/kg	References
Sinigrin	allyl-ITC	rats	1	Langer& Geer 1968
Glucoiberin	CH ₃ SO-(CH ₂) ₃ -ITC	rats	0.5	Langer& Geer 1968
Phenethyl-glucosinolate	Phenethyl-ITC	rats	0.3-0.5	Langer& Geer 1968
Epiprotogotrin	(5R)-5-vinyloxazolidine-2-thione	mice	11-13	Van Etten et al., 1969
Epiprotogotrin	1-cyano-2-hydroxy-3,4-epithiobutane	rats	1.5-2.0	Van Etten et al., 1969
Epiprotogotrin	1-cyano-3,4-epithiobutane	rats	0.8	Van Etten et al., 1969
Epiprotogotrin	(2S)-1-cyano-2-hydroxybut-3-ene	rats	2.2-2.4	Van Etten et al., 1969
Glucobrassicin	Indol-3-ylacetonitrile	rats	1.1	Fenwick et al., 1983

Comparing the concentration levels and structures of glucosinolates and glucosinolate derived products acceptable for nutritional purposes and those needed for food, feed and plant protection (Belostas et al., this conference; 2007) gives the basis for progress in these fields.

Conclusion

Glucosinolates and transformation products of glucosinolates are key factors for the nutritional value of cruciferous oilseed crops and for the value of products produced from them. Considering the physiological effects of glucosinolates and glucosinolate products it seems to be possible to use appropriate structural types in concentrations of the compounds where they can function as food, feed and plant protection agents and/or as chemoprotection or health beneficial compounds without reaching the toxic levels of the compounds.

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References

- Andersen, A.A.M., Merker, A., Nilsson, P., Sørensen, H., and Åman, P.: Chemical composition of the potential new oilseed crops *Barbarea vulgaris*, *Barbarea verna* and *Lepidium Campestre*. *J. Sci. Food Agric.* (1999) 79, 179-186
- Andersen, H.R. and Sørensen, H.: Double low rapeseed meal in diets to young bulls. In: *Advances in the Production and utilization of Cruciferous Crops*. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster (1985) 11, 208-217.
- Agerbirk, N., Olsen, C.E. and Sørensen, H.: Initial and final products, nitriles and ascorbigens produced in myrosinase catalysed hydrolyses of indole glucosinolates. *J. Agric. and Food Chemistry* (1998), 46 (4), 1563-1571.
- Barba, I., Bellostas, N., Puiggrós, A., Sørensen, J.C., Sørensen, S. and Sørensen, H.: Myrosinases, glucosinolates and transformation products in *Brassica*, *Raphanus* and *Sinapis*: Physico-chemical properties and activity of myrosinase isoenzymes. *GCIRC Bulletin* (2005) 22, 6pp.
- Bellostas, N., Jørgensen, A.L.W., Lundin, N.V.F., Petersen, I.L., Sørensen, H., Sørensen, J.C., Sørensen, R. and Tidemand, K.D.: Comparison of physico-chemical properties of myrosinase isoenzymes occurring in seeds of *Brassica* species of the U triangle. In: *Proceedings of 11th International Rapeseed Congress*, 6-10 July, Copenhagen, Denmark. ISBN 87-7611-003-6 (2003) PP25, 720-723.
- Bellostas, N., Sørensen, A.D., Sørensen, J.C. and Sørensen, H.: Genetic variation and metabolism of glucosinolates in cruciferous oilseed crops. In: *Rapeseed Breeding: Advances in Botanical Research* (Ed. Dr. Surinder Gupta) Academic Press/ Elsevier, Dan Diego, Vol. 54, 2007.
- Bellostas, N., Sørensen, J.C. and Sørensen, H.: Micellar electrokinetic capillary chromatography – Synchronous monitoring of substrate and products in the myrosinase catalysed hydrolysis of glucosinolates. *J. Chromatogr. A* (2006), 1130, 246-252.
- Bellostas, N., Sørensen, J.C. and Sørensen, H.: Profiling glucosinolates in vegetative and reproductive tissues of four *Brassica* species of the U-Triangle for their biofumigation potential. *J. Sci. Food Agric.* (2007) JSFA-05-0807R1 in press.
- Bille, N., Eggum, B.O., Jacobsen, I., Olsen, O. and Sørensen, H.: The effects of processing on antinutritional rape constituents and the nutritive value of double low rapeseed meal. *Zeitschr. Tierphysiol., Tierernährung u. Futtermittelkd.* (1983a) 49, 148-163.
- Bille, N., Eggum, B.O., Jacobsen, I., Olsen, O. and Sørensen, H.: Antinutritional and toxic effects in rats of individual glucosinolates (\pm myrosinases) added to a standard diet. I. The effects on protein utilization and organ weights. *Zeitschr. Tierphysiol., Tierernährung u. Futtermittelkd.* (1983b) 49, 195-210.
- Bjerg, B., Eggum, B.O., Rasmussen, K.W. and Sørensen, H.: Acceptable concentrations of glucosinolates in double low oilseed rape and possibilities of further quality improvements by processing and plant breeding. In: *7th International Rapeseed Congress*, Poznan Poland, (1987a), VII, Vol. 7, 1619-1626.
- Bjerg, B., Eggum, B.O., Jacobsen, I., Otte, J. and Sørensen, H.: Antinutritional and toxic effects in rats of individual glucosinolates, (myrosinases) added to a standard diet (2). *Zeitschr. Tierphysiol., Tierernährung u. Futtermittel-kd.* (1989) 61, 227-244.
- Bjerg, B., Larsen, L.M. and Sørensen, H.: Reliability of analytical methods for quantitative determination of individual glucosinolates and total glucosinolate content in double low oilseed rape. In: *7th International Rapeseed Congress*, Poznan Poland, (1987b), IV, Vol. 6, 1330-1341.
- Bjergegaard, C., Buskov, S., Ib, K., Sørensen, H., Sørensen, J.C. and Sørensen, S.: Effects of various antioxidants and storage conditions on oxidative degradation of the indole glucosinolate 4-hydroxyglucobrassicin. In: *Biologically-active Phytochemicals in Food. Analysis, Metabolism, Bioavailability and Function*. The Royal Society of Chemistry, UK. ISBN 0-85404-806-5 (2001), 88-90.
- Bjergegaard, C., Buskov, S., Sørensen, H., Sørensen, J.C., Sørensen, M. and Sørensen, S.: Reactions between glucosinolate products and thiol groups in food components. *Czech. J. Food Sci.* (2000) 18, 28-30.
- Bjergegaard, C., Eggum, B.O., Jensen, S.K. and Sørensen, H.: Dietary fibres in oilseed rape: Physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diet (1). *J. Anim. Physiol. Anim. Nutr.* (1991) 66, 69-79.
- Bjergegaard, C., Li, P.W., Michaelsen, S., Møller, P., Otte, J. and Sørensen, H.: Glucosinolates and their transformation products - compounds with a broad biological activity. *Bioactive Substances in Food of Plant Origin* (1994) 1, 1-15.
- Bjergegaard, C., Møller, P., Sørensen, H., Sørensen, J.C. and Sørensen, S.: Micellar electrokinetic capillary chromatography of thiocarbamoyl derivatives produced in reactions between isothiocyanates and amino acids. *J. Chrom. A* (1999) 836, 115-127.
- Bonnesen, C., Stephensen, P.U., Andersen, O., Sørensen, H. and Vang, O.: Modulation of cytochrome P-450 and glutathione S-transferase isoform expression in vivo by intact and degraded indolyl glucosinolates. *Nutr. Cancer* (1999) 33(2), 178-187.
- Bunting, E.S. *Production and Utilization of Protein in Oilseed Crops* Martinus Nijhoff Publ., The Hague/Boston/London (1981) 5.
- Buskov, S., Olsen, C.E., Sørensen, H. and Sørensen, S.: Supercritical fluid chromatography as basis for identification and quantitative determination of indol-3-ylmethyloligomers and ascorbigens. *J. Biochem Biophys Methods* (2000a) 43, 307-327.
- Buskov, S., Hasselstrøm, J., Olsen, C.E., Sørensen, H., Sørensen, J.C., and Sørensen, S.: Supercritical fluid chromatography as method of analysis for determination of 4-hydroxybenzylglucosinolate degradation products. *J. Biochem. Biophys. Methods* (2000b) 43, 281-298.
- Buskov, S., Hansen, L.B., Olsen, C.E., Sørensen, J.C., Sørensen, H. And Sørensen, S.: Determination of ascorbigens in autolysates of various *Brassica* species using supercritical fluid chromatography. *J. Agric. Food. Chem.* (2000c) 48(7), 2693-2701.
- Danielsen, V., Eggum, B.O., Rasmussen, K.W. and Sørensen, H.: Long-term studies of requirements to the quality of rapeseed meal from double low varieties used in sow diets. In: *7th International Rapeseed Congress*, Poznan Poland, (1987), V, Vol. 7, 1727-1734.
- Eggum, B.O., Just, A. and Sørensen, H.: Double low rapeseed meal in diets to growing - finishing pigs. In: *Advances in the Production and utilization of Cruciferous Crops*. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster (1985) 11, 167-176.
- GCIRC Conf., 2003: *Proceedings of 11th International Rapeseed Congress*, Copenhagen, ISBN 87-7611-003-6
- Henriksen, P., Hillemann, G., Mortensen, K. and Sørensen, H.: Requirements to the quality of oilseed rape which can be used without problems in diets to mink (*Mustela vison* Screb.). In: *7th International Rapeseed Congress*, Poznan Poland, (1987), VII, Vol. 7, 1817-1824.
- Jensen, S.K., Michaelsen, S., Kachlicki, P. and Sørensen, H.: 4-Hydroxyglucobrassicin and degradation products of glucosinolates in relation to unsolved problems with the quality of double low oilseed rape. *GCIRC - Congress*, Saskatoon, Canada. (1991), V, 1359-1364.
- Knothe, G. *Biodiesel and vegetable oil fuels: Then and now*. Inform, 2006, 17 (11), 729-731.
- Loft, S., Otte, J., Poulsen, H.E. and Sørensen, H.: Influence of intact and myrosinase-treated indolyl glucosinolates on the metabolism in vivo of metronidazole and antipyrine in the rat. *Fd. Chem. Toxic.* (1992), 30 (11), 927-935.
- Michaelsen, S., Otte, J., Simonsen, L.-O. and Sørensen, H.: Absorption and Degradation of Individual Intact Glucosinolates in the Digestive Tract of Rodents.

- Acta Agric. Scand. (1994) Sect. A, 44, 25-37.
- Petersen, I.L., Andersen, K.E., Sørensen, J.C. and Sørensen, H.: Determination of shikimate in crude plant extracts by micellar electrokinetic capillary chromatography. *J. Chromatogr. A.* (2006), 1130, 253-258.
- Sørensen, H.: Advances in the Production and utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster (1985) 11.
- Sørensen, H., Sørensen, S., Bjergegaard, C., Michaelsen, S.: Chromatography and capillary electrophoresis in food analysis. Royal Society of Chemistry, UK, (1999) 470 pp, ISBN 85404-561-9
- Sørensen, J.C. Biorefined oilseed products – high quality products with added value. Ph.D. Thesis 2001, Chemistry Department, KVL, Frederiksberg C, Denmark. 293pp.
- Vang, O., Frandsen, H., Hansen, K.T., Sørensen, J.N., Sørensen, H. and Andersen, O.: Biochemical effects of dietary intakes of different broccoli samples. I. Different modulation of cytochrome P-450 activities in rat liver, kidney and colon. *Metabolism. Clinical and Experimental* (2001) 50(10), 1123-1129