4-HYDROXYGLUCOBRASSICIN AND DEGRADATION PRODUCTS OF GLUCOSINOLATES IN RELATION TO UNSOLVED PROBLEMS WITH THE QUALITY OF DOUBLE LOW OILSEED RAPE

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INTRODUCTION

Double low rape varieties have the potential for increased importance as oil and protein sources for food and feed (Larsen and Sørensen, 1985), if still unsolved problems with the quality of rapeseed products can be solved (Eggum et al., 1985a). The well balanced amino acid composition and thereby high biological value (BV) of rapeseed proteins (Bille et al., 1983a) have caused special attention toward rapeseed meal as animal feed (Schultz and Petersen, 1981; Thomke et al., 1983; Bell, 1984; Bourdon et al., 1985; Vermorel et al., 1987). The low digestibility of energy (DE) and protein (TD) in rapeseed meal is, however, a serious problem partly connected to the hull fraction rich in dietary fibres (DF) (Bille et al., 1983a; Bell and Keith, 1987), but the DF level in meal of dehulled rapeseed is also high and cause strong negative effects on DE and TD (Bjergegaard et al., 1991). Another main problem, limiting optimal use of double low rapeseed for animal feed and eventual use of rapeseed protein as food, is the glucosinolates (Sørensen, 1988).

Products formed as a result of glucosinolate degradation e.g. during processing of rapeseed, seems to be important in relation to some unsolved quality problems (Jensen et al., 1990; Michaelsen et al., 1991a). Traditional processing of rapeseed in the oil mills results, thus often in appreciable degradation of 4-hydroxyglucobrassicin and other unstable glucosinolates (Olsen and Sørensen, 1980; Bille et al., 1983a; Sang and Truscott, 1984; Slominski and Campbell, 1989). As 4-hydroxyglucobrassicin is quantitatively dominating in double low rapeseed (Eggum et al., 1985b; Bjerg et al., 1987a), it is important with investigations of this compound, even if it gives special requirement to analytical methods (Bjerg and Sørensen, 1987b; Buchner, 1987; Sørensen, 1990). The thiocyanate ion is easily formed from indolylglucosinolates together with various indolyl derivatives (vide-infra; Gmelin and Virtanen, 1961). The thiocyanate ion is assumed to have goitrogenic properties and indolylderivatives seems to be involved in effects on liver enzymes (Eggum et al., 1985b; McDanell et al., 1988; Fenwick et al., 1989). In the work now presented, several grams of 4-hydroxyglucobrassicin has been isolated and used in studies of the chemical- biochemical- and physiological properties of this glucosinolate and degradation products thereof.

MATERIALS AND METHODS

Seeds of double low rapeseed (Brassica napus L.; lines 1-9004 and 1-9011) were obtained from Maribo Seeds A/S, Holeby, Denmark.

Methods and equipment used for the glucosinolate analyses and isolation have been described elsewhere; paper chromatography (PC), high voltage electrophoresis (HVE), UV- and NMR-spectroscopy (Olsen and Sørensen, 1981), high performance liquid chromatography (HPLC) for determination of intact and desulfoglucosinolates (Sørensen, 1990), high performance capillary electrophoresis (HPCE) (Michaelsen et al., 1991c).

Myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1) preparation and characterization have been performed and described elsewhere (Michaelsen et al., 1991b).

The technique used for isolation of 4-hydroxyglucobrassicin was a modification of the original ion-exchange procedure (Olsen and Sørensen, 1979; Bjerg and Sørensen, 1987a) comprising flash chromatography on an Amberlite IR 120(H^+), 3 cm x 12.5 cm i.d., column and a DE Cuno Zetaprep® assembly 100 modular DEAE(AcO^-) column. Portions of 1 kg rapeseed meal were extracted three times with boiling ethanol-water (7:3; 2 l), 5 min. using Ultra Turrax homogenization, cooling, centrifugation (4000 x g; 60 min.) and filtration. The combined filtrates were evaporated to approx. 200 ml, which were extracted with chloroform (3 x 200 ml), stored at 4°C overnight, re-centrifugated and transferred to the columns which were washed with 500 ml water. Effluent from the Amberlite column was transferred to the DEAE column. Flow > 20 ml/min. The DEAE column was finally washed with 2M HOAc-EtOH (1:1; 0.5 l), water-EtOH (1:1; 0.2 l), 2M HCOOH-EtOH (1:1; 0.6 l), water-EtOH (1:1; 0.2 l) and eluted with 2M ammonia-EtOH (1:1; 0.2 l fractions). The eluates were immediately (< 15 min.) evaporated to dryness under vacuum at 40°C. The glucosinolates were transformed into potassium salt (Bjerg and Sørensen, 1987a; with column sizes as above) and nearly pure 4-hydroxyglucobrassicin (contained less than 5% of other glucosinolates) could be obtained by SPE-flash chromatography (Bjerg and Sørensen, 1987a).

The test in animal experiments were performed as standard balance trials with young growing rats as described previously (Bjerg et al., 1989).

RESULTS

4-Hydroxyglucobrassicin was isolated as potassium salt in an amount of 11 g from 12 kg of rapeseed meal. The purity of the compound was tested by HPLC, HPCE, UV- and NMR-spectroscopy (Sørensen, 1990). The preparation containing less than 3% progoitrin and 2% of other glucosinolates, was added to the standard diet giving test diets with the 4-hydroxyglucobrassicin levels shown in Table 1.

Table 1. The effect of dietary 4-hydroxyglucobrassicin (26) (± myrosinase: Enz.) on feed utilization and protein quality (true protein digestibility (TD); biological value (BV); net protein utilization (NPU)) in rats.
(Figures in parenthesis stand for standard error of means).

Diet	1	2	2[1)]	3	3[1)]	4	4[1)]	5
(26) (μmol/g DM)	0	0.5	0.5	2.5	2.5	12.5	12.5	2.5
Enz. (U/g DM)	-	-		-		-		0.15
Consumed diet	49.9	48.8	52.7	48.2	52.7	49.6	51.9	49.9
(g DM in 5 days)	(0.29)	(2.71)		(3.94)		(0.42)		(0.10)
Weight gain	352	291	331	310	312	346	304	371
(mg/g DM eaten)	(87)	(86)		(62)		(70)		(73)
TD (%)	95.3	95.4	94.4	95.3	94.3	94.9	90.9	95.0
	(1.53)	(1.31)	(1.33)	(1.90)	(0.93)	(1.18)	(0.78)	(1.00)
BV (%)	99.2	99.1	94.4	98.0	92.7	97.9	92.3	97.3
	(0.72)	(1.01)	(1.27)	(1.41)	(0.75)	(1.25)	(1.02)	(1.34)
NPU (%)	94.5	94.5	89.1	93.4	87.4	92.9	84.0	92.4
	(1.44)	(1.01)	(2.12)	(2.52)	(1.52)	(1.91)	(1.26)	(1.76)

[1)] 4-Hydroxyglucobrassicin thermally degraded in rapeseed meal

4-Hydroxyglucobrassicin used in 2[1], 3[1], and 4[1] (Table 1) was thermally degraded in rapeseed meal (3.56 g portions) at 140°C as described elsewhere (Michaelsen et al., 1991a). This degradation of 4-hydroxyglucobrassicin resulted in dark coloured products corresponding to oxidation and formation of "melanine-type" products (vide infra). The amount of degraded glucosinolate used for each portion and animal trial are shown in Table 1 and correspond to the levels used of intact 4-hydroxyglucobrassicin.

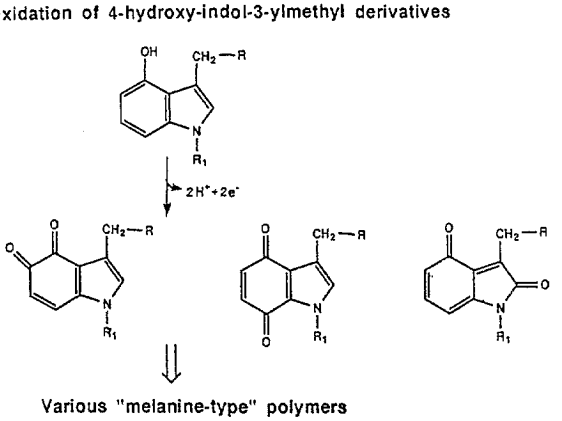

Fig. 1. Oxidation of 4-hydroxyglucobrassicin results in dark coloured products with the quinoid-type compounds as expected precursors for "melanine-type" polymers.

Feed consumption (palatability), feed conversion, TD, BV, and NPU were not affected by any of the levels used of intact 4-hydroxyglucobrassicin, and the same were the case for liver and thyroid weights. However, with thermally degraded 4-hydroxyglucobrassicin included in the standard diets, it is seen (Table 1) that both TD and BV are reduced as a function of the amount of degradation products included. This is again reflected in a 10% reduction of NPU in the diet containing the highest amount of thermally degraded glucosinolate.

Table 2 shows results from determinations of the amounts of 4-hydroxyglucobrassicin in the digestive tract of 5 rats in one of the trials. Appreciable differences are seen among the rats. For some of the rats relatively high concentrations were found in the last part of the small intestine, whereas in caecum and in the large intestine the concentration of intact glucosinolate was close to zero.

Table 2. Concentration (μmol/g dry matter) of 4-hydroxyglucobrassicin in the content from the digestive tracts of rats fed 4-hydroxyglucobrassicin.

Rat No.	Stomach	Small intestine 1'st part	Small intestine 2'nd part	Caecum	Large intestine
1	0.45	0.38	0.03	0.00	0.00
2	0.23	1.17	1.11	0.04	0.00
3	0.08	7.15	-*	0.00	0.00
4	0.72	0.61	2.68	0.54	0.00
5	2.26	2.67	17.53	0.22	0.00

The considerable differences in the concentrations of intact glucosinolate in the digestive tract of the rats as well as the variations among the animals are in agreement with earlier findings (Eggum et al., 1985b). The disappearance of the glucosinolate in caecum and the large intestine is most likely caused by microbial activity as the case is for poultry (Slominski et al., 1987).

DISCUSSION

The present study with isolated 4-hydroxyglucobrassicin added in different levels to standard diets without other rapeseed constituents and fed to young growing rats is a continuation of our previously described work with 10 other glucosinolates (Bille et al., 1983b; Bjerg et al., 1989). These studies comprise also one level where active myrosinase is included in the diet together with the glucosinolates, for investigations of effects caused by glucosinolate degradation products. Corresponding studies based on isolated intact glucosinolates added to standard diets fed to rats have also been performed by others (Vermorel et al., 1986). The results obtained from all of these investigations show; a) different glucosinolates cause very different harmful effects, b) the intact glucosinolates cause antinutritional effects, c) glucosinolate degradation products are more harmful, antinutritional and toxic than the intact glucosinolates, d) detailed informations are needed on the various transformation products obtained as a result of glucosinolate degradation including their metabolism in animals.

The results now obtained with 4-hydroxyglucobrassicin show that this glucosinolate has only little or no detectable antinutritional effects as intact glucosinolate, whereas appreciable harmful effects of its degradation products were observed. The thermally induced degradation results obviously in oxidations with formation of dark coloured products as shown in Fig. 1. This type of oxidation occurs also easy and fast when 4-hydroxyglucobrassicin is in alkaline solutions. With myrosinase catalyzed transformation of indolylglucosinolates (McDanell et al., 1988; Sørensen, 1990; Michaelsen et al., 1991b) various products are formed. The isothiocyanates and thiocyanates lead obviously to formation of the thiocyanate ion and a indolylderivative reactive toward various nucleophilic compounds, resulting in various indolylderivatives, including ascorbigens. Not yet published results obtained with myrosinase catalyzed hydrolysis of glucobrassicin and neoglucobrassicin have shown a mixture of degradation products without a single quantitatively dominating compound. When tested toward human cancer cells, the results revealed that the degradation products were much more toxic than the intact indolylglucosinolates and the products from neoglucobrassicin were much more toxic than the products from glucobrassicin.

The results now available show clearly that it is important to include indolylglucosinolates in evaluations of the quality of cruciferous food and feed products, including rapeseed products. Different indolylglucosinolates are even more diverse in their properties than they are in their structure (Sørensen, 1990), and as 4-hydroxyglucobrassicin is quantitatively dominating in rapeseed, so other indolylglucosinolates are often quantitatively dominating in vegetative parts of cruciferous plants as rape and cabbage.

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