Glucosinolates and by-products in rapeseed meal related to hydrothermal processing

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Objectives

The study aims to propose and test methods for identifying and quantifying breakdown products from GSL, and myrosinase activity. The relationship between hydro-thermal treatment of RSM and the routes of the GSL degradation should also be helpful to predict the anti-nutritional potential of the meal in the diet.

Background

Rapeseed meal (RSM) is usually desolventized with strong hydrothermal treatments, leading to various levels of residual glucosinolates (GSL), and protein solubility. The RSM nutritional quality may be then lowered for monogastrics, due to GSL breakdown products whose reliable and "easy to use" indicators lack.

Material and methods

Isolation of glucosinolates

Isolated GSL were used to fortify RSM and to evaluate the ability of the analytical method to recover them or their degradation products. GSL were extracted from seeds of Brassica napus L., B. campestris L. and Crambe abyssinica, then purified by ion exchange and reverse phase liquid chromatography (RPLC) (Heaney et al. 1984). Several batches with different GSL profiles were obtained.

Analysis of GSL and breakdown products

***GSL** : were analyzed according to ISO 9167-1, RPLC-UV of desulfo-derivatives (DSGSL). An alternative method by Ion Pair LC-DAD-UV of the intact form according to Helboe et al.(1980) was also used. **Isothiocyanates (ITC) and nitriles** were extracted by CH_2Cl_2 and analyzed by GC-FID-MS according to Wathelet et al. (2004).*

*•**5-vinyloxazolidine-2-thione** (VOT) was analyzed by RPLC–UV according to Mabon et al. (1999) .*

Determination of Myrosinase activity in RSM

Myrosinase was extracted in neutral phosphate buffer at 4°C and kinetics of sinigrin degradation was determined by glucose-hexokinase assay Kit, (Sigma) according to Wilkinson et al. 1984. One activity unit was defined by 1 µmol sinigrin hydrolyzed per min at 25 °C and pH=7.0

Recovery of GSL breakdown products

Recovery was determined :

1) to check the ability of the methods to identify and quantify these products in complex matrix such as RSM. The experiments were :

a) degradation of pure GSL in different concentration in water by myrosinase addition, followed by extraction and analysis.

b) analyzing RSM samples fortified with pure GSL and treated by dry or wet toasting at 110°C.

2) to determine the degradation routes of GSL breakdown in RSM according to the hydrothermal treatment conditions and the yield of the reactions.

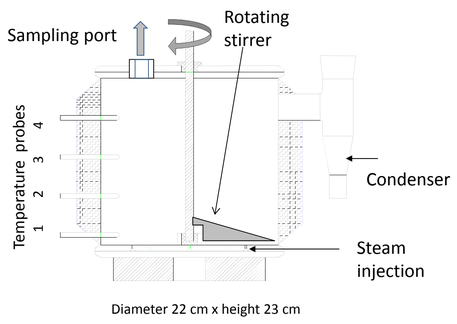
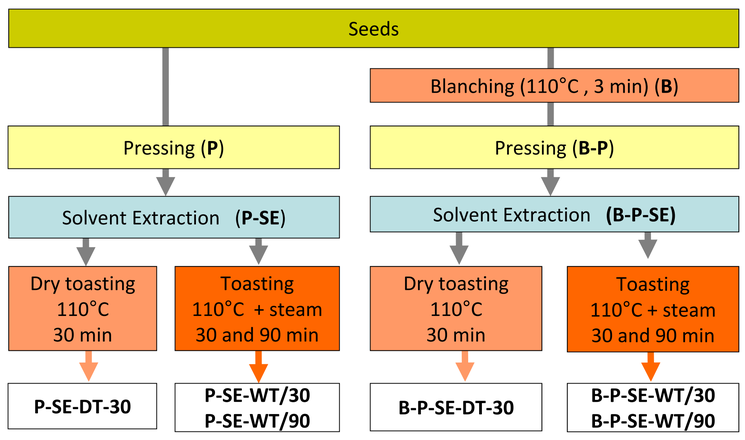


***Figure 1** : Bench-cooker for hydro-thermal treatment*

Hydrothermal treatment of rapeseed meal

Two batches of 1.5 kg rapeseed seeds (one of them was previously blanched with steam (3 min) to inactivate the myrosinase) were pressed and deoiled with hexane then desolventized at low temperature. The two RSM batches were treated by either dry or wet toasting at 110°C by the means of a bench-cooker (Fig. 1), to promote different routes for GSL breakdown.



Results and discussion

1) Checking of the analytical methods

***Glucosinolates** can be easily analyzed in dry samples if the endogenous myrosinase is inactivated before the extraction. Many studies have shown the performances of HPLC of desulfoderivates (ISO 9167-1) to identify and quantify the GSL in rapeseed and other Brassicaceae even at a low level. ISO 9167-1 was then used to characterize the starting materials and to calibrate an alternative method (ion pair RPLC of intact GSL) which was used to check the different fractions during the isolation of the four purified extracts. These ones exhibited different GSL patterns and purities, the higher GSL content in the starting material, the higher purity in the extract. (table 1).*

Table 1: mass and purity of « GSL rich » extracts isolated from Brassicaceae

Extract	Seeds	GSL content (µmol/g defatted seeds)					Mass (mg)	Purity (%)	Pure GSL mass (mg)
		PRO	EPRO	SIN	GNA	Total			
A	B. napus	147.3	3.9	0	44.0	210	170	80.6	137.0
B	C. abyssinica	3.2	106	0	0.8	110	139	68.8	95.6
C	B. campestris	0.8	0.9	12.4	58.0	72	123	44.7	54.9
D	B. napus	22.7	0.6	0	10.9	40	74.4	21.8	16.2

PRO : progoitrin, EPRO : epi-progoitrin, SIN: sinigrin, GNA : gluconapin

Recovery of VOT

5-vinyloxazolidine-2-thione (VOT) is well known as one of the most harmful degradation product from GSL, so, our analytical experiment has first focused on the behavior of its precursor (progoitrin) in RSM.

The C. abyssinica extract (epi-progoitrin rich) was used to simulate the behavior of progoitrin when added to water and to 2 RSM (A : medium and B, strongly cooked) and to determine the recovery of the breakdown product (VOT) during the analysis.

It was observed that VOT added to RSM was easily recovered as the slope of the curve (orange line, Fig. 2) was very close to the standard one (blue line). In opposite, VOT was partially recovered from the epi-progoitrin addition in water or in RSM, indicating that VOT is less reactive with the matrix than its precursor. It was observed that recovery from RSM A (medium cooked) was lower than from the RSM B (strongly cooked), confirming the well known reactivity of the intermediary metabolite (hydroxyalkenylisothiocyanate) with the protein.

It was concluded from this experiment that analysis of VOT is possible in RSM but according to the heat treatment, the extraction yield could be lowered by strong interactions with the matrix.

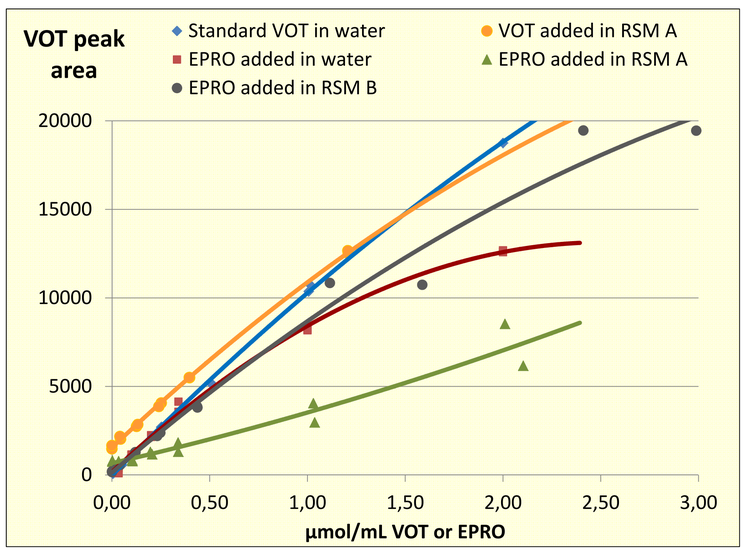


Figure 2 : recovery of VOT from VOT added to RSM A or from epi-progoitrin added to water, RSM A (medium cooking) or RSM B (strong cooking).

Identification and recovery of isothiocyanates (ITC) and nitriles

Degradation of purified GSL (extract D) added to RSM mainly led to ITC and nitriles which were easily identified and determined by GC-FID (Fig 2).

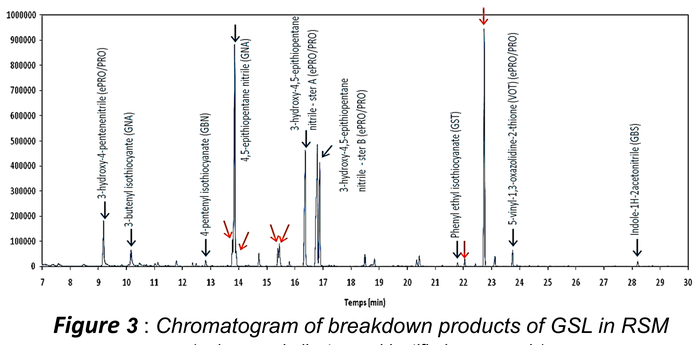


***Figure 3** : Chromatogram of breakdown products of GSL in RSM (red arrows indicate non identified compounds)*

Addition of different GSL mixture (Extracts A and D) to RSM in water with myrosinase led to different recovery yields of the GSL degradation products (GSL-DP) according to the level of added GSL (tab. 2). Recoveries for low levels of GSL-DP (extract D) were lower showing a typical matrix effect, the active sites being not saturated by a low content of GSL-DP.

Detection and recovery of the GSL Degradation Products

Only degradation products released from alkenyl-GSL (VOT, ITC and nitriles) were monitored because of their well-known harmful effects on animal physiology. Higher VOT contents were detected in non-blanched RSM (0.22 to 1.22 µmol/g) than in the blanched ones (0.05 to 0.27 µmol/g) indicating the role of the myrosinase in the degradation route.

However, a small increase of the VOT content in the blanched RSM was observed during the toasting (from 0.08 to 0.27 µmol/g). That could mean that VOT could be released despite the myrosinase inactivation. ITC were only detected in the non-blanched RSM containing a high myrosinase activity. The content was yet low (0.07 µmol/g) confirming that ITC would strongly interact with the matrix to become non-extractable. Nitriles were detected in every case in very low amounts.

Table 2: Recovery of ITC, VOT and Nitriles (content in µmol/g) from extract of purified GSL (A and D) added to RSM with myrosinase

Extract	Precursor	GSL broken down	ITC or VOT	Nitriles	Epithio-nitriles	Total GSL DP	% recovery
A	PRO	118	74.1	0.85	Nd	75	64
A	GNA	45.8	40.2	0.64	0.61	41	90
A	GBN	8.2	7.6	0.03	Nd	7.6	93
A	**Total**	**172**	**122**	**1.52**	**0.61**	**124**	**72**
D	PRO	18.6	0.97	0.62	Nd	1.6	8.5
D	GNA	6.9	0.26	0.41	3.5	4.2	61
D	GBN	3	0.32	0.16	Nd	0.48	16
D	**Total**	**28.5**	**1.55**	**1.19**	**3.5**	**6.3**	**20**

GSL DP : degradation products from GSL % recovery = Total GSL DP x 100 / GSL broken down

Myrosinase activity measurement

The method was tested on different types of seeds and meals. The relative standard deviation of repeatability was 6% and the limit of quantification was 0.05 U/g. Myrosinase activity in Brassica seeds and meals was found around 1 – 2 and 0.05 – 1.2 U/g ranges, respectively. Purchased purified myrosinase exhibited a very high activity (176 U/g). The method was found sensitive enough to be applied to heated meals.

2) GSL breakdown during RSM treatments

Steam treatment was found efficient to inactivate myrosinase as blanching decreased the activity from 1.2 to less than 0.05 u/g and wet toasting, from 0.22 to less than 0.05 U/g (tab.3). The effect of steam on the GSL content was not observed during the blanching, probably due to the short exposition time (3 min). In contrast, the alkenyl-GSL breakdown rate was higher by wet toasting after blanching (36% vs. 14%) or not (39% vs. 29%) than with dry toasting. Except for VOT in non-heated RSM (P-SE), recovery yields of GSL-DP (ratio GSL-DP/degraded GSL) were found very weak for each treatment and particularly after toasting (Tab. 3).

Table 3: Myrosinase activity, GSL and degradation products (GSL-DP) during hydrothermal treatments

Treatment/ min	MYR Activity U/g ddm	Residual alkenyl-GSL	Degraded alkenyl-GSL	GSL-DP			Recovery of GSL-DP (%)
				VOT	ITC	Nitriles	
		µmol/g ddm					
None	1.74	24.7	0,0	0	0	0	-
P-SE	1.19	16.8	2.1	1.10	0.07	0.03	57
P-SE-DT/30	0.22	13.4	5.5	0.53	-	0.04	10
P-SE-WT/30	< 0.05	11.5	7.4	0.38	-	0.03	6
P-SE-WT/90	< 0.05	5.8	13.1	0.22	-	0.06	2
B-P-SE	< 0.05	17.8	1.0	0.06	-	0.01	7
B-P-SE-DT/30	< 0.05	16.3	2.6	0.08	-	0.05	5
B-P-SE-WT/30	< 0.05	12.1	6.8	0.11	-	0.01	2
B-P-SE-WT/90	< 0.05	5.4	13.5	0.27	-	0.02	2

B: Blanching; P: pressing ; SE: solvent extraction; DT: dry toasting; WT : wet toasting
ddm : dried defatted matter

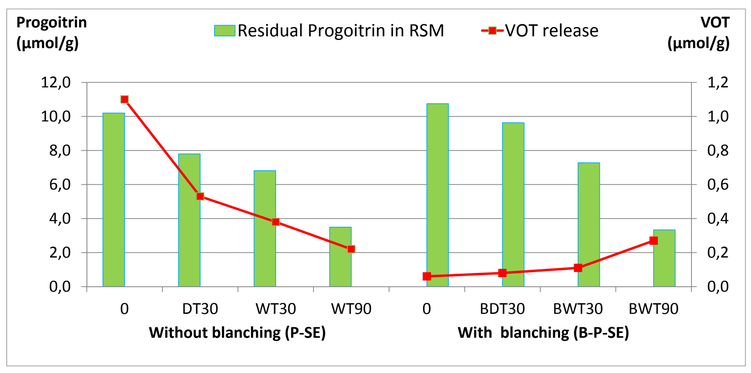


Figure 4 : degradation of progoitrin and release of VOT in RSM with or not blanching prior toasting treatments

These observations are in agreement with the literature data on the GSL degradation routes with or without myrosinase activity and the reactivity of ITC with proteins.

Conclusion

*The study showed that **myrosinase activity**, residual **GSL** and **VOT** contents can be accurately determined in RSM. In contrast, nitriles and ITC were detected at a low level, due to strong interactions with the matrix.*

Residual myrosinase activity and GSL content can give hypothesis for the GSL degradation routes and possible release of ITC. Nevertheless, as ITC can be linked to amino-acids, further studies remain necessary to evaluate their bio-availability.

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