

Detoxification of Rapeseed Meals by Steam Explosion

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Abstract Steam explosion was evaluated as a detoxification method for rapeseed meals. A series of two factor experiments were performed with different steam pressures (1.0, 1.2, 1.4, 1.6, 1.8, 2.4, 3.2 and 4.0 MPa) and different treatment times (30, 60, 90, 120, 150 and 180 s). When the steam pressure was increased to 1.6 MPa or higher, 99% glucosinolates could be removed within 180 s. Their toxic breakdown products, including isothiocyanates, oxazolidinethiones and nitriles, were also reduced by 97, 93 and 59%, respectively. A model experiment confirmed that they were taken away by the steam. The effect of steam explosion on amino acids was also investigated. Lysine, arginine, aspartate, cysteine, methionine and tryptophan contents were reduced by 21, 24, 14, 13, 17 and 8%, respectively. Steam explosion is a fast and simple detoxification method, but it negatively affects amino acids.

Keywords Steam explosion · Rapeseed meals · Glucosinolates · Detoxification

Introduction

Rapeseed meal is a potential protein resource due to its high protein content and balanced amino acids, but the presence of anti-nutritional factors limits its inclusion in animal feed [1]. Among them, glucosinolates, whose metabolites are toxic, were the primary limiting factor

before the double low varieties, especially canola, were successfully developed. Although glucosinolate content was considerably decreased in contemporary commercial varieties, low levels still influence palatability, growth performance, reproduction, thyroid function, liver and kidney function of non-ruminant animals and fish [2–8]. Therefore, it is necessary to further reduce the glucosinolate content to improve the feeding value of rapeseed meals.

Many glucosinolate detoxification procedures have been reported, including autoclaving, toasting, chemical treatments, solid-state fermentation, water extraction, solvent extraction and extrusion. However, all these techniques have major drawbacks, such as being difficult to scale up, loss of protein content and high operating costs. A new strategy is to produce transgenic *Brassica napus* plants with seeds that lack myrosin cells using a genetic ablation technology [9]. But intact glucosinolates can also be hydrolyzed by myrosinase of bacterial origin within the gastrointestinal tract [1]. In summary, none of these methods has produced satisfactory results and better solutions are needed.

Steam explosion is typically initiated at a temperature of 160–260 °C (corresponding pressure, 0.69–4.83 MPa) for several seconds to a few minutes before the material is quickly exposed to atmospheric pressure [10]. Steam explosion is usually used for pretreating lignocellulosic biomass [10], but seldom used for treating rapeseed meals and no detailed detoxification results have been reported for steam explosion. Steam explosion can provide high temperatures, which may be effective in inactivating glucosinolates since they are heat-labile [11]. In order to develop a more satisfactory detoxification method, the effects of steam explosion on glucosinolates and their toxic breakdown products were investigated.

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Materials and Methods

Materials

Rapeseed meals were collected from an oil mill (Xishan, Xinjian, Jiangxi Province, China). They were sun-dried to 6.5% moisture before steam explosion.

All chemicals were analytical grade unless otherwise specified. Internal standards, including sinigrin and butyl isothiocyanate, and sulfatase, *Helix pomatia* type H1 (EC 3.1.6.1, aryl-sulfate sulfohydrolase), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Glucosinolate Analysis

The glucosinolate content was determined by HPLC according to 'ISO 9167-1-1992'. Qualitative analyses were performed with a Thermo LTQ XL linear ion trap. A Hypersil GOLD column (100 mm × 2.1 mm, 5 μm; Thermo) was used to separate the desulfoglucosinolates. Negative ion mass spectrometry was conducted to detect them. The mass spectrometry conditions were as follows: ESI ion source; ion source temperature, 300 °C; and ion spray potential, −4000 V. Product ion scanning was performed to confirm the identities of desulfoglucosinolates. Quantitative analyses were carried out with a Waters 1525 Binary HPLC Pump equipped with a Waters 2998 Photodiode Array Detector (Milford, MA, USA). Separation of desulfoglucosinolates was performed on a Waters SunFire C18 column (250 mm × 4.6 mm, 5 μm) (Milford, MA, USA).

Steam Explosion

The steam explosion device was provided by a heavy machine factory (Zhengdao, Hebi, Henan Province, China). It consists of four main parts: (1) a boiler to supply steam; (2) a 5-L processing chamber in which samples are treated by steam; (3) a 100-L recovery tank which is open to the air; (4) a valve to connect the processing chamber with the recovery tank. At first, high-pressure experiments were carried out at high steam pressures (2.4, 3.2 or 4.0 MPa) for different short retention times (30, 90 or 150 s). Then low-pressure tests were conducted with lower pressures (1.0, 1.2, 1.4, 1.6 or 1.8 MPa) for longer times (60, 120 or 180 s).

Glucosinolate Breakdown Products Analysis

The analysis of isothiocyanates (ITC) was carried out according to the standard GB 13087-91 'Method for determination of isothiocyanates in feeds' authorized by AQSIQ (General Administration of Quality Supervision,

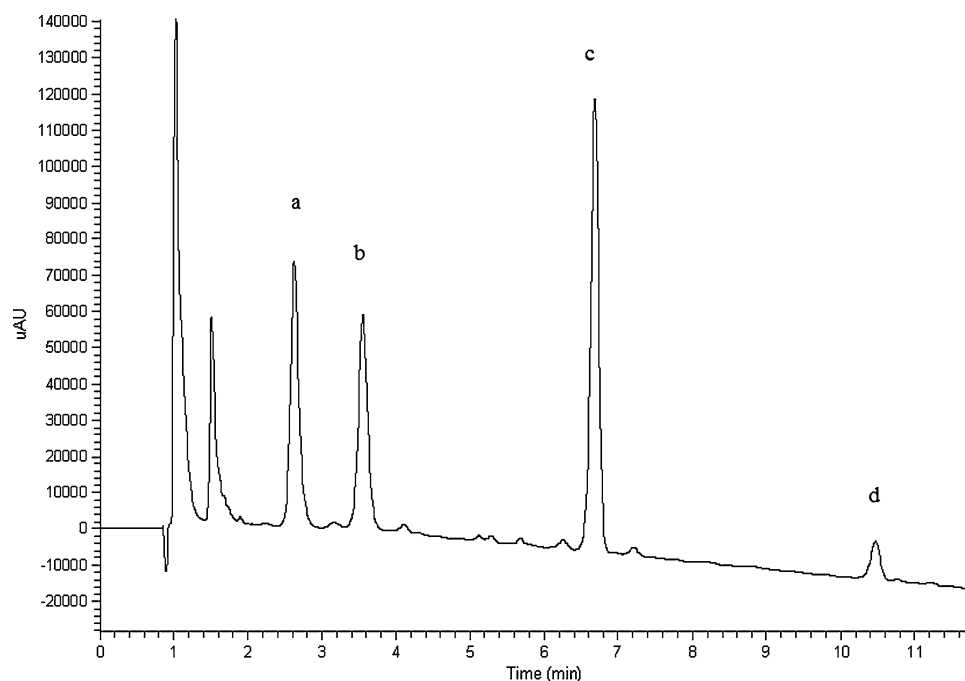
Inspection and Quarantine of the People's Republic of China). Rapeseed meal was first ground in a coffee grinder to a fine powder. A 2.2 g sample of powder was weighed into a 25 mL conical flask followed by 5 mL of a citrate–Na₂HPO₄ buffer (pH 7 buffer), 1 mL of myrosinase (Sigma, St. Louis, MO, USA) and 10 mL of an internal standard solution (0.100 mg mL^{−1} butyl isothiocyanate in methylene chloride). The flask was sealed and shaken in an orbit shaker for 2 h at 200 rpm. Following extraction, the mixture was centrifuged to collect the methylene chloride phase. Then the organic phase was dried by 0.4 g anhydrous sodium sulfate for at least 1 h and filtered through 0.45 μm filters (FP-450 Gelman Sciences, Ann Arbor, MI, USA). The filtrate was analyzed by HP 5890A gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector, using a DB-5 capillary column (30 m × 0.32 mm, 0.25 μm film, J&W Scientific, Folsom, CA, USA) and a temperature program from 35 to 200 °C. The carrier gas was helium. Temperatures of injection port and detector were 230 and 250 °C, respectively. The content of ITC was calculated based on response factors provided in the standard.

Total contents of oxazolidinethiones (OZT) and nitriles in the meals were determined by methods of Wetter LR [12] and Whitehurst DH et al. [13], respectively.

Steam Condensate and Residue Analysis

Steam condensate was produced by a model system. A 500-mL flask containing distilled water was heated to provide steam. A glass tube (10 cm × 4 cm ID) was connected vertically on the top of the flask. A 10-g sample of meal was placed into the tube and supported by gauze. After passing through the meal, the steam was introduced into a condenser and the condensate was collected. Two hundred milliliter of condensate was extracted three times by 100 mL of methylene chloride. Extracts were dried by anhydrous sodium sulfate and filtered by Whatman No. 3 (Whatman Scientific Ltd, Maidstone, UK). The filtrate was concentrated by rotary evaporation (45 °C) to about 50 mL and analyzed by GC–MS. GC–MS was performed with a HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and a capillary fused silica DB-5MS column (0.25 μm film thickness, 30 m × 0.25 mm ID; J & W Scientific, Folsom, CA, USA). Helium was used as the carrier gas. Temperatures of the injector and the detector were set at 210 and 230 °C, respectively. The oven temperature was increased from 50 to 200 °C at 5 °C min^{−1}. A HP 5989A quadrupole mass detector (Hewlett-Packard, Avondale, PA, USA) was used for MS analyses under the following conditions: interface temperature, 280 °C; repeller, 7 V, emission,

Fig. 1 HPLC Chromatograms of glucosinolates in rapeseed meals. **a** progoitrin, **b** sinigrin (internal standard), **c** gluconapin, **d** glucobrassicinapin



300 V, electron energy, 70 eV; source temperature, 200 °C. Individual peaks were identified by comparing mass spectra with literature data [14, 15]. The residue was combined with methylene chloride in a conical flask. The flask was sealed and shaken in an orbit shaker for 2 h at 200 rpm. Following extraction, the mixture was centrifuged to collect the methylene chloride phase. Then it was treated and analyzed as above.

Amino Acid Analysis

Amino acid analyses were performed after acid hydrolysis of proteins in rapeseed meals. The samples, acidified with 6 mol L⁻¹ HCl, were sealed in tubes under nitrogen and incubated in an oven at 110 °C for 24 h. After cooling, the hydrolysate was washed in a distilled water filter and dried in a rotary evaporator at 60 °C. The dried samples were then dissolved in 0.01 mol L⁻¹ HCl. The amino acids in hydrolysate were separated and quantified by injecting 5 µL into a Hitachi 835-50 amino acid analyzer (Minato-ku, Tokyo, Japan) equipped with a 2.6 × 150 mm ion-exchange column. The column temperature was 53 °C. Sodium citrate buffer was used as the eluent at 0.225 mL min⁻¹ flow rate. The light absorbance of amino acids was detected at 570 nm and the amino acids were quantified by comparing them with amino acid standards.

Sulfur-containing amino acids were determined after performic acid oxidation. Tryptophan was measured after alkaline hydrolysis according to the standard GB/T 18246-2000 'Determination of amino acids in feeds'.

Statistical Analysis

Each steam explosion treatment was performed three times. Every sample was analyzed in triplicate. The reduction ratios of glucosinolates after steam explosion were compared by two-way analysis of variance with steam pressure and time as main effects using the GLM procedure of the SPSS version 13.0 (SPSS Inc, Chicago, IL, USA). The contents of glucosinolate breakdown products and amino acids after steam explosion were compared to those of the control by one-way analysis of variance to determine the treatment effects. Differences were considered significant when $P < 0.05$.

Results and Discussion

Glucosinolate Analysis Before Steam Explosion

There were three kinds of glucosinolates including progoitrin, gluconapin and glucobrassicinapin in rapeseed meals used in this work (Figs. 1, 2). Their total content was 18.61 µmol g⁻¹ (Table 1).

Effects of Steam Explosion on Glucosinolates

High steam pressures were tested first. High pressures had remarkable effects on the glucosinolate content (Fig. 3). The reduction of glucosinolates was always >90% within 30 s at 2.4, 3.2 or 4.0 MPa. When processing time was

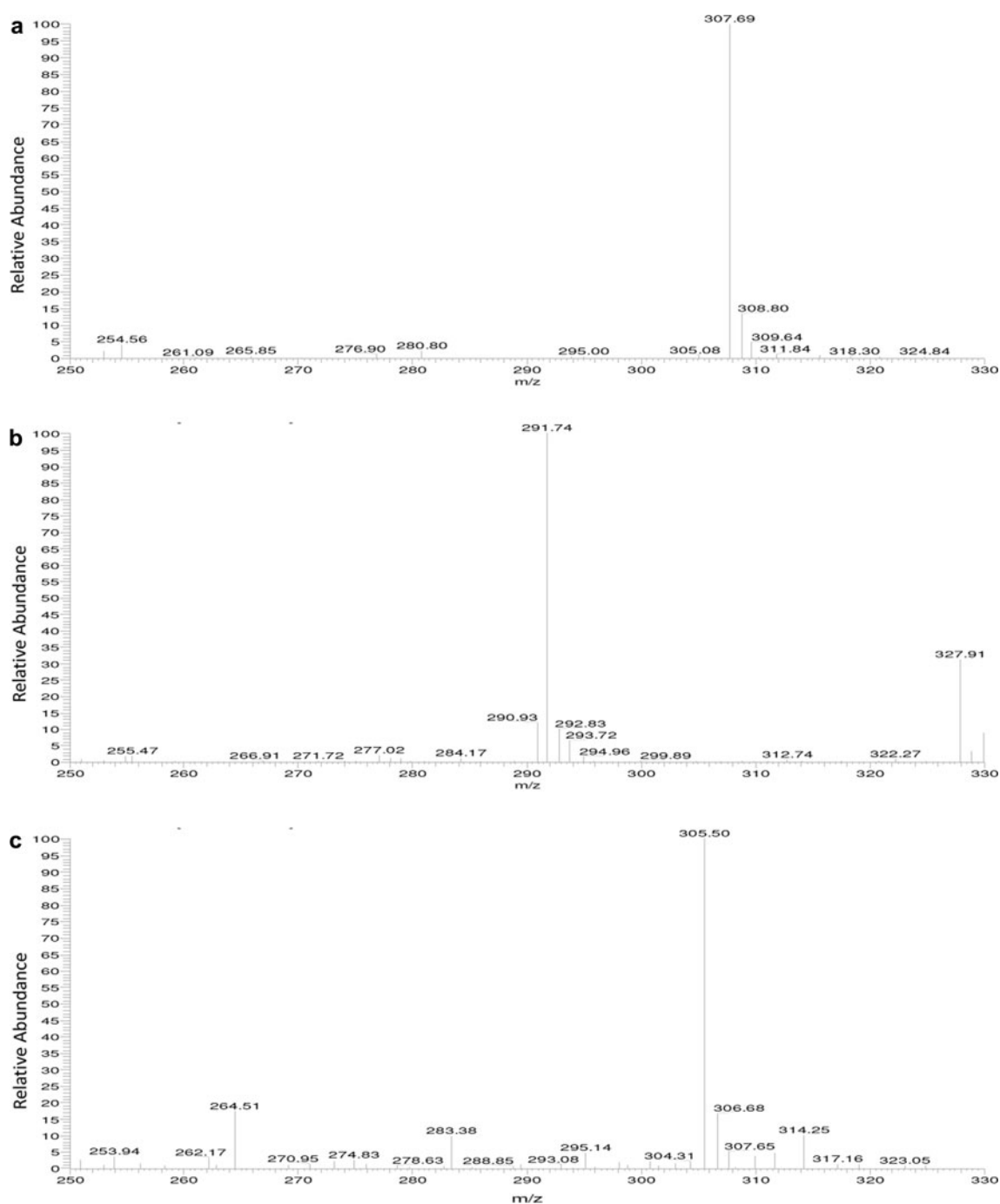


Fig. 2 MS Chromatograms of glucosinolates in rapeseed meals. **a** Progoitrin, **b** gluconapin, **c** glucobrassicinapin

extended to 150 s, 99% reduction was achieved. These reductions were attributed to the thermolysis nature of glucosinolates. Steam explosion was a fast and effective method for detoxifying rapeseed meal, but high pressures may not necessarily be needed. Overheating leads to undesirable Maillard or browning reactions and reduced amino acids, especially lysine [1]. Therefore, lower steam pressures were attempted in the following work to avoid negative effects as much as possible.

Low pressures were less effective than higher pressures. Glucosinolate reduction was only 62% after treating at 1.0 MPa for 60 s, but when the steam pressure was increased to 1.6 MPa or higher, the reduction in glucosinolate content reached 99% within 180 s (Fig. 4). The effects of steam pressure, time and their interaction on glucosinolates are significant (Fig. 5). Compared with high pressures, low-pressure treatments avoided excessive heat and were suitable for detoxification.

Table 1 Structures and contents of glucosinolates ($\mu\text{mol g}^{-1}$) in rapeseed meals

Trivial name	Basic structure	Structure of R	Content ^a
Progoitrin		$\text{H}_2\text{C}=\text{CH}-\text{CH}(\text{OH})-\text{CH}_3$	6.55 ± 0.03
Gluconapin		$\text{H}_2\text{C}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_3$	10.96 ± 0.02
Glucobrassicinapin		$\text{H}_2\text{C}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$	1.11 ± 0.03
Total			18.61 ± 0.04

^a Means \pm standard deviations ($n = 3$)

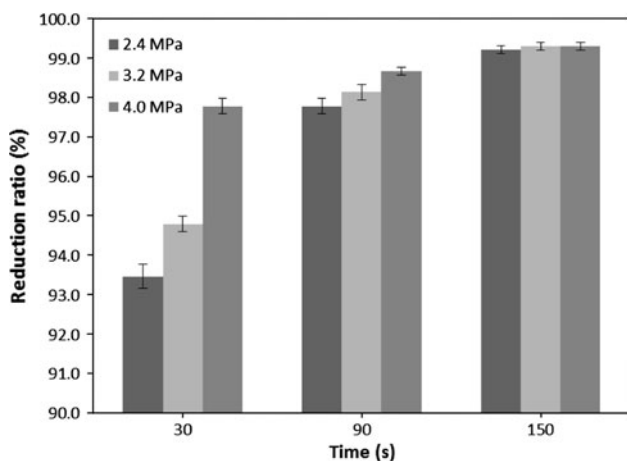


Fig. 3 Effects of high pressure steam explosion on the reduction of glucosinolates in rapeseed meals

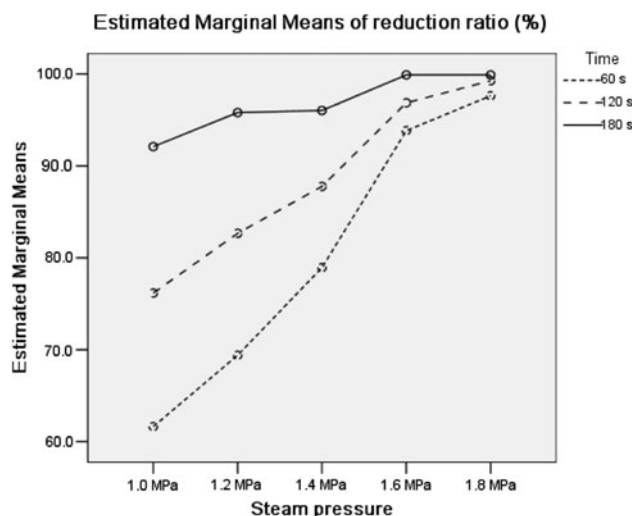


Fig. 5 Profile plot of steam pressure and time

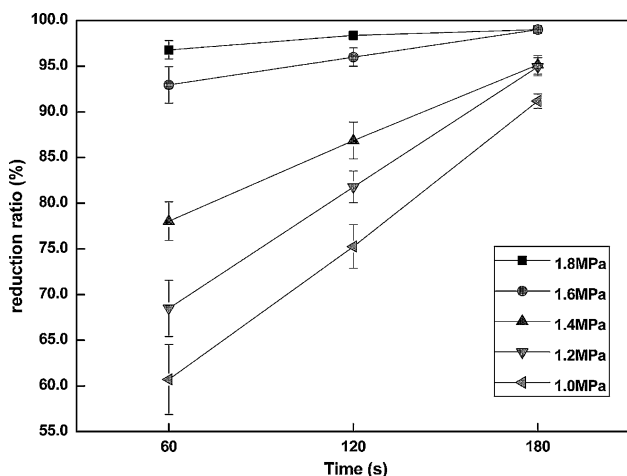


Fig. 4 Effects of low pressure steam explosion on the reduction of glucosinolates in rapeseed meals

Common thermal treatments including toasting and autoclaving have been used to reduce glucosinolates [3, 17]. These processes usually need relatively long times (1 h or longer), which make them commercially infeasible when treating large amounts of rapeseed meals. As far as

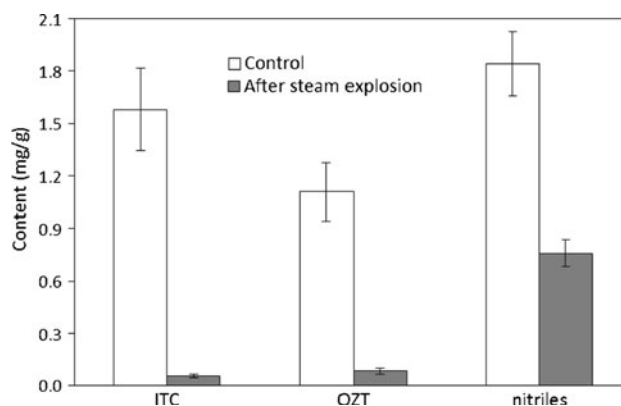
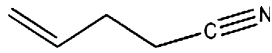
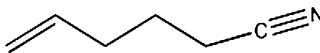
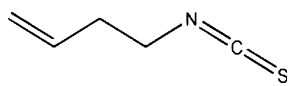
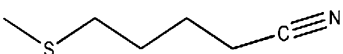
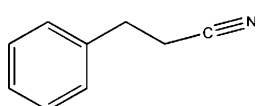
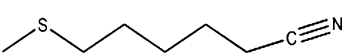
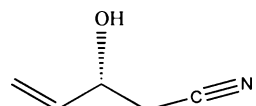
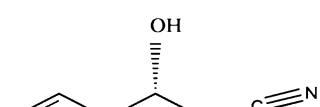
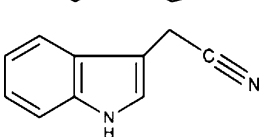


Fig. 6 Effects of steam explosion (1.6 MPa, 180 s) on the contents of toxic glucosinolate breakdown products in rapeseed meals

treatment time is concerned, steam explosion has an advantage, which results from high temperatures used compared to the low temperatures (100, 110 or 121 °C) of toasting or autoclaving.

In addition to heat treatments, many other detoxification methods have also been reported such as chemical treatment [18], solid-state fermentation [19], water extraction [20], solvent extraction [21] and extrusion [22]. All these

Table 2 Main toxic breakdown products of glucosinolates in steam condensate and the residue from the model system

Source	Chemical name	Structure	MS spectral data m/z
Condensate	4-Pentenitrile		81, 54, 41
	5-Hexenenitrile		95, 94, 80, 67, 55, 41
	3-Butenyl isothiocyanate		113, 85, 72, 55, 39
	5-(Methylthio)-pentanenitrile		129, 82, 61, 55
	Benzene-propanenitrile		131, 91, 65, 51
	6-(Methylthio)-hexanenitrile		143, 96, 69, 61, 55, 41
Residue	3-Hydroxy-4-pentanitrile		98, 69, 57, 41
	3-Hydroxy-5-hexenenitrile		112, 94, 70, 42, 41
	3-Indoleacetoneitrile		156, 130, 101, 77, 51

techniques have obvious drawbacks. Chemical treatment and solvent extraction need metal solutions or several kinds of solvents, which add cost. The process of solid-state fermentation is difficult to regulate and scale up. Water extraction washes away not only glucosinolates but also some crude proteins. Furthermore, the demand for large amounts of water limits commercial feasibility. Extrusion often needs ammonia pretreatments to get better results. Compared with these methods, the most obvious advantage of steam explosion is the short processing time. In addition, steam explosion is simpler because it is easy to control and there is no demand for pretreatments, a great deal of water or extra chemicals. In summary, the main advantage of steam explosion is speed and simplicity, which will bring more satisfactory results when treating large quantities of rapeseed meals.

Analyses of Glucosinolate Breakdown Products

After the steam explosion (1.6 MPa, 180 s), toxic breakdown products of glucosinolates, including ITC, OZT and

nitriles, were reduced by 97, 93 and 59%, respectively (Fig. 6). The main thermolysis products of aliphatic glucosinolates are nitriles [23–25]. Sometimes ITC can also be detected [26]. ITC and OZT were determined after myrosinase treatments so the reduction of them was mainly accounted for by the removal of glucosinolates. Nitriles had accumulated during meal production because of glucosinolate thermolysis. After the steam explosion, more nitriles must have been produced but the total content was decreased. Some of them may be taken away by the steam.

Steam condensate was collected from a model system. The main products of glucosinolates in the condensate included five kinds of nitriles and one kind of ITC (Table 2). 4-Pentenitrile and 3-Butenyl isothiocyanate were thermolysis products of gluconapin. 5-Hexenenitrile was from glucobrassicinapin. Other nitriles may accumulate during meal production. These results confirmed toxic glucosinolate products can be taken away by the steam. The predicted production of 3-hydroxy-4-pentenitrile from progoitrin, was not detected in the condensate. It remained in the residue with other nitriles (Table 2) probably because

Table 3 Effects of steam explosion (1.6 MPa, 180 s) on amino acids (g kg⁻¹) in rapeseed meals

Amino acid	Amino acid level	
	Control	After treatment
Asp	21.9 ± 0.4 ^a	18.9 ± 0.4 ^b
Thr	12.6 ± 0.3	12.2 ± 0.3
Ser	13.6 ± 0.3	13.2 ± 0.3
Glu	56.5 ± 1.0 ^b	62.1 ± 0.9 ^a
Gly	15.0 ± 0.3	15.5 ± 0.3
Ala	12.8 ± 0.3 ^b	13.9 ± 0.3 ^a
Cys	11.5 ± 0.1 ^a	10.0 ± 0.1 ^b
Val	23.5 ± 0.5	24.0 ± 0.5
Met	8.6 ± 0.1 ^a	7.1 ± 0.1 ^b
Ile	12.4 ± 0.2 ^b	15.3 ± 0.3 ^a
Leu	23.2 ± 0.5 ^b	26.6 ± 0.5 ^a
Tyr	9.9 ± 0.2 ^b	11.1 ± 0.2 ^a
Phe	12.1 ± 0.2	12.6 ± 0.3
Lys	12.3 ± 0.2 ^a	9.7 ± 0.1 ^b
His	9.1 ± 0.2	9.1 ± 0.2
Arg	17.2 ± 0.3 ^a	13.1 ± 0.2 ^b
Pro	19.9 ± 0.4	19.7 ± 0.4
Trp	5.3 ± 0.1 ^a	4.9 ± 0.1 ^b

Values represent means ± SD, *n* = 3

Means in a row followed by different letters (a, b) are significantly different (*P* < 0.05)

the steam temperature of the model system was not high enough. Steam explosion may get better detoxification results.

Effects of Steam Explosion on Amino Acids

Table 3 shows the changes in amino acid compositions in rapeseed meals after a steam explosion (1.6 MPa, 180 s). The contents of lysine, arginine, aspartate, cysteine, methionine and tryptophan were significantly decreased by 21, 24, 14, 13, 17 and 8%. The levels of glutamate, alanine, leucine, isoleucine and tyrosine were significantly increased by 10, 8, 15, 23 and 12%, respectively. No significant differences were noted in other amino acids.

The partial loss of amino acids is inevitable because of heating effects. Lysine is usually the first-limiting amino acid in animal rations and tends to be lost during thermal processing, which has been attributed to Maillard reactions [16], so it has received more attention. The decrease in lysine observed in the present work is in agreement with previous reports [3, 17, 27], but more severe (21%), probably because the temperature used in this experiment was higher than others. The reductions of arginine, aspartate and cysteine were also reported by other researchers

[3, 17, 27] and may be attributed to Maillard or browning reactions.

The increase of some amino acids after thermal treatments also occurred in other experiments [16, 27]. However, it has never been reported that so many kinds of amino acids were simultaneously increased. It may be related to the heating effects of steam explosion. Proteins can be destroyed to become water-soluble peptides and free amino acids in the steam explosion process [28]. More amino acids may be detected in the samples treated by steam explosion than in controls. Therefore, we suggest that steam explosion just influenced the determination of amino acids rather than synthesizing new ones.

Although glucosinolates could be reduced by 99% after steam explosion, some limiting amino acids, especially lysine, were lost. In order to reduce the loss, lower steam pressures or shorter times can be adopted because it is not necessary to completely eliminate glucosinolates. For example, when the glucosinolate content was decreased to <2 μmol g⁻¹ [29] or 5 μmol g⁻¹ [30], the feeding value of rapeseed meals was marginally improved for pigs because it is replaced by the relatively low available energy level as the first-limiting factor [1]. Therefore, the steam explosion conditions should be selected according to animal tolerance to the residual content of glucosinolates.

Conclusions

Steam explosion is a fast and simple detoxification method not only for glucosinolates but also for their toxic breakdown products. It will contribute to the utilization of rapeseed meals in feed or food production. Its main drawback is the loss of some amino acids. In order to reduce this adverse effect as much as possible, steam explosion conditions should be determined according to animal tolerance to the residual content of glucosinolates.

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