Influence of roasting on the thermal degradation pathway in the glucosinolates of fragrant rapeseed oil: Implications to flavour profiles

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ABSTRACT

Purified desulphated glucosinolates (GSLs) were subjected to thermal treatment in model systems without interference to investigate the formation of volatile components derived from GSLs only. Desulphated progoitrin (PRO), desulphated gluconapin (GNA), and desulphated glucobrassicanapin (GBN) were isolated from rapeseed (Brassica napus L.). Their structures were identified via spectroscopic data. According to the final thermal degradation compounds of the desulphated GSLs, the thermal degradation pathways of the GSLs identified herein in rapeseed during roasting were speculated. PRO degradation formed 2,4-pentadieninetrile by eliminating the hydroxy group of the R group to generate the double bond at C-2. GNA degradation produced 4-isothiocyanato-1-butene. Two degradation pathways were possibly involved in the degradation of GNA and GBN. The main precursors that produce the pungent flavour of rapeseed oil were obtained by exploring the relationship between the degradation of the GSLs and the volatile flavour of this vegetable oil during roasting.

1. Introduction

Rapeseed (Brassica napus L.) is a major oilseed crop in the world, and it is predominant in North America, Eastern Europe, and Asia. The consumption of rapeseed oil has reached almost 8.5 million tons, and fragrant rapeseed oil (FRO) accounts for almost 30% of the total consumption of domestic rapeseed oil (Liu et al., 2018). FRO is a type of hot-pressed oil that has a strong characteristic flavour, a smooth taste and an intensive colour (Zhang et al., 2020b). It is produced via roasting, pressing and filtering to preserve its high amounts of unsaturated fatty acids, tocopherols and sterols (Kasprzak et al., 2020). During roasting, numerous complex reactions occur, including glucosinolate (GSL) degradation, lipid oxidation, and Maillard reaction, which improve the flavour of FRO (Zhang, Wang, Yuan, Yang, & Liu, 2016; Zhou et al., 2018). The products of GSL degradation are the major volatile flavour substances of FRO that endow it with a pungent aroma, and they are also the main features that distinguish it from other flavour oils (Zhang et al., 2020a).

GSLs are nitrogen- and sulphur-containing secondary metabolites in which the sole structural variant is the nature of the aglycone. GSLs can be subdivided into different classes, namely, aliphatic (alkyl and alkenyl), benzenic and indole GSLs (Mikkelsen, Petersen, Olsen, & Halkier, 2002). The basic structure of GSLs is composed of a β-d-glucopyranose moiety linked via a sulphur atom to a (Z)-N-hydroximinosulphate ester and a variable R group (Blazevic et al., 2020). Rapeseed contains 13 kinds of common GSLs, among which the contents of progoitrin (PRO), gluconapin (GNA) and glucobrassicanapin (GBN) account for over 75% of the total content (Mao, Zhao, Huyan, Liu, & Yu, 2019). In intact rapeseed cells, GSLs and myrosinase coexist but are physically separated by tissues. As shown in Fig. 1, when rapeseed cells are damaged, GSLs come into contact with myrosinase and are rapidly hydrolysed by myrosinase to form various hydrolytic products, such as thiocyanates, nitriles and isothiocyanates (Ortner & Granvogl, 2018; Wu et al., 2021). Aside from enzymatic degradation, GSLs can also be subjected to chemical and thermal degradation to generate volatile compounds during the production of rapeseed oils (Nugrahedi, Dekker, & Verkerk, 2017). Given that myrosinase loses its activity at 100 °C within 5 min (Oliviero, Verkerk, & Dekker, 2012), thermal degradation is the main degradation pathway of GSLs and desulphated GSLs (ds-GSLs) are important intermediates (Hanschen et al., 2012). Researchers have found that the thermal degradation of GSLs provides the building blocks...
for the generation of simple nitriles under different conditions, although they did not explicitly focus on volatile flavours (Williams, Critchley, Pun, Chaliha, & O’Hare, 2009). In addition, Hanschen, Rohn, Mewis, Schreiner, and Kroh (2012) heated broccoli sprouts containing five sulphur-containing aliphatic GSLs and four indole GSLs at 130 °C to induce the thermal degradation of GSLs. They reported that GSLs are stable in neutral and slightly acidic environments but undergoes rapid degradation in a basic environment. In our previous study, we investigated the influence of GSL degradation on the flavour of FROs during heat treatment by selecting five rapeseed varieties grown in the important rapeseed production areas in China. We speculated that GSL degradation may promote the generation of nitriles and isothiocyanates during heating, which are vital to the production of sulphur and the pungent flavour of FROs (Mao, Zhao, Huyan, Liu, & Yu, 2019). Previous studies largely focused on the influence of GSL degradation on the flavour of FROs during heating. However, the specific pathways involved in the thermal degradation of individual GSLs are poorly known. Therefore, the evolution of individual GSLs during heat treatment must be elucidated because this information will provide further insights into the formation of volatile flavour compounds in FROs.

This study intends to (1) explore the influence of roasting on the thermal degradation pathway in the GSLs of rapeseed; (2) three ds-GSLs, namely, desulphated PRO (ds-PRO), desulphated GNA (ds-GNA) and desulphated GBN (ds-GBN) were isolated from rapeseed; (3) investigate the nitriles and isothiocyanates derived from the thermal degradation of the three ds-GSLs; (4) determine variations in the nitriles and isothiocyanates in FROs determined by volatile component and sensory evaluation analysis. A better understanding of the flavour characteristics of FROs from the roasted rapeseeds will be helpful in monitoring their flavour profiles when they are processed in order to develop new products, which would be more accepted by consumers.

2. Materials and methods

2.1. Materials and chemicals

Certified seeds of the rapeseed variety Zhongyou 821, which is rich in GSLs (99.01 µmol/g), were harvested from Dali County, Shaanxi Province, China. It is one of the major rapeseed producing districts in the country (Zhang et al., 2022a). Chromatographic-grade acetonitrile was obtained from Tianjin Chemical Company, Ltd. N-Alkanes (C7–C30) for calculating retention index (RI) and standard 2-octanol were supplied by Sigma (St. Louis, MO, USA). All other chemicals utilized were of an analytical reagent grade and obtained from Shanpu Chemical Co., Ltd.

2.2. Purification of ds-GSLs and determination of GSLs

The method for ds-GSL extraction and purification was modified from that of Kim et al. (2010). Defatted seed meal (50 g) was extracted with 70% (v/v) boiling methanol. An aliquot of each methanol extract was applied onto the DEAE-Sephadex A-25 column, and eluted with 3 mL of distilled water. Subsequently, sulphatase was loaded onto each column, and desulphation reaction was carried out overnight (16 h) at 35 °C. The purified ds-GSLS were eluted with 5 mL of distilled water. The obtained materials were then freeze-dried to recover the purified ds-GSLs.

The purified ds-GSLs were separated via semipreparative high-performance liquid chromatography (HPLC) by using an Agilent 1100 series system with a SinoChrom ODS-BP column (10 mm × 250 mm, 5 µm). The HPLC mobile phases were composed of acetonitrile and water for solvents A and B, respectively. The gradient elution conditions were as follows: 0–20 min, linear gradient 5–100% A; 100% A in 20–50 min. The flow rate was 2 mL/min at the ultraviolet wavelength of 229 nm and controlled temperature of 30 °C. An aliquot of the ds-GSLs (200 µL) was directly injected into the semipreparative HPLC sample loop by using the gradient elution described above. According to the elution chromatogram, peak fractions were collected manually. Meanwhile, the method for the GSL determination was modified according to Mao, Zhao, Huyan, Liu, and Yu (2019). The GSL content of the samples was quantified using allyl GSLs as internal standard and relevant relative response factors. GSL contents are expressed in micromoles per gram of dry matter weight (µmol/g of dw).

2.3. Elucidation of ds-GSL structures

For qualitative analysis, the ds-GSLs were separated through a C18 column (50 mm × 2.1 mm, 1.7 µm) by using a UPLC system (Waters Technology Co., Ltd., Shanghai, China). The mobile phase consisted of water (A) and 20% acetonitrile (B) with gradient elution and the flow rate was 0.3 mL/min. The conditions were the following: 0–2 min, 15–85% B; 2–4 min, 85–15% B; 4–5 min, 15% B. The UV-visible detector was set at 229 nm. Mass spectrometric analysis was done on a quadrupole mass spectrometer (LC-MS2010A; Shimadzu) and operated with the positive electrospray ionisation mode. Ionization source parameters were set to: capillary temperature, 250 °C; spray voltage, 4.5 kV. The scan range ranged from 100 to 700 m/z (Chevolleau, Debrauwer, Boyer, & Tulliez, 2002).

1H and 13C nuclear magnetic resonance (NMR) spectra were analyzed in deuterium oxide (D2O) by using an Agilent DD2400–MR NMR spectrometer (Santa Clara, CA, USA) at 600 and 154 MHz (Lin et al., 2021).

2.4. Thermal treatment of purified ds-GSLs

Rapeseed can produce a multitude of volatile compounds under thermal conditions (Zhang et al., 2022b). To exclude the interference of other factors, the purified ds-GSLs (100 µg) were separately placed in a 20 mL vial sealed with an aluminum cap provided with a silicon septum, and placed into an electric blast drying oven at 150 °C for 60 min. A headspace glass vial without any added ds-GSL served as the control group. After the desired reaction time, the samples were immediately cooled with an ice-water bath for 20 s, and subsequently their volatiles were analyzed. All of the tests were conducted with no less than three parallel samples.

2.5. Thermochemical analysis

Thermal analysis was performed by using a thermal gravimetric analysis (TGA) and a differential scanning calorimetry (DSC) system (SETARAM, France). The experiments were performed in a parallel system with a SinoChrom ODS-BP column (10 mm × 250 mm, 5 µm). The HPLC mobile phases were composed of acetonitrile and water for solvents A and B, respectively. The gradient elution conditions were as follows: 0–20 min, linear gradient 5–100% A; 100% A in 20–50 min. The flow rate was 2 mL/min at the ultraviolet wavelength of 229 nm and controlled temperature of 30 °C. An aliquot of the ds-GSLs (200 µL) was directly injected into the semipreparative HPLC sample loop by using the gradient elution described above. According to the elution chromatogram, peak fractions were collected manually. Meanwhile, the method for the GSL determination was modified according to Mao, Zhao, Huyan, Liu, and Yu (2019). The GSL content of the samples was quantified using allyl GSLs as internal standard and relevant relative response factors. GSL contents are expressed in micromoles per gram of dry matter weight (µmol/g of dw).
2.5. Seed roasting and oil extraction

The seeds were roasted according to the procedure described in our previous study (Zhang et al., 2022a). Seeds (1.5 kg) were roasted in a continuous and circular monolayer roasting machine (Jiangsu Maisi Machinery Equipment Co., Ltd., Jiangsu, China). Temperature (150 °C) and times (10, 20, 30, 40, 50 and 60 min) were selected in accordance with commercial processes and designed to produce samples with an acceptable flavour and colour. Having cooled to room temperature, part of the seeds was ground into a fine meal for further experiments. The crude FROs were extracted from roasted seeds with a small expeller (Westinghouse, Inc., U.S.A.). Finally, the oil samples were obtained by centrifugation (2191g, 15 min) and stored at −20 °C for subsequent assays. Each experiment was carried out with three replicates, and the experiments were repeated twice.

2.6. Analysis of volatile components

According to the method of Zhang et al. (2022b), the volatiles of the samples were analyzed via headspace solid–phase microextraction/gas chromatography–mass spectrometry (HS–SPME/GC–MS) device (Shimadzu-QP2010, Kyoto, Japan). The separation was done on DB-17MS column (60 m × 0.25 mm id × 0.25 μm). Each sample was placed in a 20 mL vial and incubated at 50 °C for 30 min. Immediately following the extraction, the SPME fibre was inserted into a GC–MS sample inlet after being desorbed for 3 min at 250 °C. The carrier gas was helium (flow rate, 1.0 mL/min). The temperature program was as follows: initial temperature set at 40 °C was on hold for 3 min, after which it was increased to 120 °C at 4 °C/min; next, the speed was set as 6 °C/min, and finally, the column temperature was increased to 240 °C and held for 9 min. By comparing the RIs of volatile compounds with real reference compounds and using the NIST14 library (NIST, Gaithersburg, MD, USA) of volatile compounds, only the volatile compounds that had >85% similarity with NIST14 library were used. The quantitation of volatile compounds in FROs was determined by GC–MS with 2-octanol (4 μL of a 0.49 mg/mL solution in methanol) as an internal standard.

2.7. Sensory evaluation

Descriptive sensory analysis was conducted by ten highly-trained panelists (five males, five females; aged 22–31 years old). Each sample (15 g) was placed in a 50 mL cup at room temperature. Prior to the analysis, six sensory qualities, namely, nutty-like, burnt-like, pickled-like, pungent-like, green-like, and fatty-like, were evaluated via a descriptive test. The intensity of the sensory attributes was scored using a scale from 0 to 10 (Zhang et al., 2022b).

2.8. Statistical analysis

Results are presented as means ± standard deviations. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Duncan’s test with SPSS version 11.5 (SPSS, USA). Differences were considered significant when p < 0.05.

3. Results and discussion

3.1. Purification of ds-GSLs from rapeseed extracts

The GSL compounds obtained via column chromatography were isolated by reversed-phase HPLC. Four peaks were found in the eluate absorbance elution profile of the rapeseed extracts (Fig. S1). All ds-GSLs were identified via UPLC following the method of Mao, Zhao, Huyan, Liu, and Yu (2019). Three ds-GSLs, namely, ds-PRO, ds-GNA and ds-GBN, corresponded to peaks 1, 2 and 4, respectively (Fig. S1). According to the relative peak areas, the purity of the individual ds-GSLs was >90% (Fig. S2). The advantage of the extraction and purification adopted herein was that the ds-GSLs had been purified via reversed-phase HPLC without using ion-pair reagents (West, Tsui, & Haas, 2002), thereby obtaining high-purity ds-GSLs while avoiding the high cost associated with the use of such reagents.

3.2. Verification of ds-GSL structures

The 1H NMR and 13C NMR spectral data obtained for each ds-GSL are presented in Table S1. D2O served as the solvent, and the active H of OH in glucopyranoside was replaced with D. The NMR data of the three ds-GSLs, i.e., ds-PRO, ds-GNA and ds-GBN, were in good agreement with previous results reported in references (Frechard et al., 2001; Ibrahim et al., 2018; Kiddle et al., 2001; Wang, Liang, & Yuan, 2014). The structural parameters of these ds-GSLs are provided in Figs S3–S5.

The GSLs were detected in the positive ion mode, which illustrates the molecular ion [M + H]+ in the first stage of MS. MS/MS experiments of the GSLs led to the production of some structurally diagnostic fragments according to previously described MS fragmentation patterns (Fabre et al., 2007; Guo, Sun, Tang, Zhang, & Cheng, 2020). The MS data of the ds-GSLs are provided in Table S2. On the basis of differences in the R-side chain, these characteristic fragments allowed the preliminary distinction of the GSLs from the other types of components. The base peaks in the spectrum of ds-PRO, ds-GNA and ds-GBN were at 310, 294 and 308 m/z, respectively, which had also been reported as the major fragment spectrum of the same compound. This result was in agreement with that of previous works (Guo, Sun, Tang, Zhang, & Cheng, 2020; Zimmermann, Gerendas, & Krumblein, 2007). These results further confirmed the accuracy of the proposed compounds.

3.3. Formation of volatile components during the thermal treatment of the ds-GSLs

GSLs reportedly decrease because of myrosinase hydrolysis or thermal degradation (Rungapamesty, Duncan, Fuller, & Ratcliffe, 2007). Given that myrosinase is inactivated at 150 °C, we considered the thermal degradation of the GSLs. In the model systems, the high temperature led to the production of various carbohydrate breakdown products, which can subsequently generate different odorants. These compounds had been recently investigated by Ortner and Granvogl (2018). In the present study, the purified ds-GSLs were subjected to thermal treatment to explore the formation of volatile components, namely, nitriles and isothiocyanates, derived from the GSLs only. Each purified ds-GSL was placed in a headspace vial, and placed into an oven controlled at 150 °C for 60 min. After cooling, qualitative analysis of GSL degradation was performed by GC–MS. Five nitriles and one isothiocyanate were identified in the model systems, including 2,4-pentadieninitrile, 2-pentenenitrile, 3-methyl-2-butenenitrile, 4-isothiocyanato-1-butene, 2,4-hexadieninitrile, and 5-methyl-hexeninitrile (Table 1). Therefore, the three GSLs may be the main precursors for the pungent flavour exuded by the roasted rapeseeds.

Table 1: Nitriles and isothiocyanates derived from the thermal degradation of the ds-GSLs

| Ds-GSL compounds | Volatile components | Molecular formula | RI |
|------------------|---------------------|-------------------|----|
| Ds-PRO           | 2,4-Pentadieninitrile | C6H8N             | 762 |
| Ds-GNA           | 2-Pentenenitrile     | C6H8N             | 772 |
|                  | 3-Methyl-2-buteninitrile | C6H9N          | 731 |
|                  | 4-Isothiocyanato-1-butene | C6H7NS       | 1050 |
| Ds-GBN           | 2,4-Hexadieninitrile | C7H8N             | 879 |
|                  | 5-Methyl-hexeninitrile | C7H9N           | 898 |

Abbreviations: ds-GSL, desulphated glucosinolate; ds-PRO, desulphated progoitrin; ds-GNA, desulphated gluconapin; ds-GBN, desulphated glucobrassicanapin; RI, retention index.
3.4. Changes in GSLs of rapeseeds during roasting

The contents of the GSLs in rapeseed during roasting were measured (Table 2). The total GSL amount in the raw rapeseeds was 99.01 μg/g. The content of 12 GSLs from the high to the low was as follows: PRO (56.19 μg/g), GNA (25.43 μg/g), GBN (4.87 μg/g), 4-hydroxyglucobrassicin (4.75 μg/g), epi-progoitrin (3.81 μg/g), gluconapoleiferin (2.06 μg/g), glucoraphanin (0.89 μg/g), glucobrassicin (0.89 μg/g), glucostain (0.33 μg/g), glucotropaeolin (0.25 μg/g), glucoraphasin (0.20 μg/g), 4-methoxyglucobrassicin (0.14 μg/g) and glucobrassicin (0.09 μg/g). Notably, the three initial reactants, namely, PRO, GNA and GBN, in the raw rapeseeds accounted for 87.36% of the total GSL content. Roasting the rapeseeds substantially depleted the contents of the GSLs. By the end of the heat treatment, the total amounts of GSLs decreased by 30.48%. After 60 min of roasting, the contents of PRO, GNA and GBN decreased by 28.62%, 25.68% and 23.00%, respectively, whereas those of the other GSLs only slightly decreased.

3.5. Changes in volatile compounds of FRO during roasting

A semiquantitative method was used to determine the volatile compounds of the FROs according to internal standards. The goal of this step was to assess the change of volatile compounds of the FROs during roasting. Table S3 of the Supporting Information provides comparisons of volatile compounds from different chemical classes that were produced by the FROs during roasting. The proportions of acids, alcohols, isothiocyanates, nitriles, pyrazines, oxygen heterocycles and other sulphur-containing compounds in the FROs increased compared with those in the raw samples. Nitriles and isothiocyanates are the main characteristic flavour compounds of FROs different from other edible oils. According to our previous studies, the elevation of nitrile and isothiocyanate levels in FROs was associated with the GSL degradation during roasting (Mao, Zhao, Huyan, Liu, & Yu, 2019; Jing, Guo, Wang, & Yu, 2020). These compounds provide obvious and stimulating odours, such as pungent, cabbage-like, and onion odours (Zhou et al., 2019). After comparing their RIs and mass spectra with the data above, the nitriles and isothiocyanates in the FROs were identified from the FROs (Table 3). After roasting for 60 min, these compounds accounted for 75.87% of the total nitrile and isothiocyanate contents of the FROs according to internal standards. The goal of this step was to assess the change of volatile compounds of the FROs during roasting. Table S3 of the Supporting Information provides comparisons of volatile compounds from different chemical classes that were produced by the FROs during roasting. The proportions of acids, alcohols, isothiocyanates, nitriles, pyrazines, oxygen heterocycles and other sulphur-containing compounds in the FROs increased compared with those in the raw samples. Nitriles and isothiocyanates are the main characteristic flavour compounds of FROs different from other edible oils. According to our previous studies, the elevation of nitrile and isothiocyanate levels in FROs was associated with the GSL degradation during roasting (Mao, Zhao, Huyan, Liu, & Yu, 2019; Jing, Guo, Wang, & Yu, 2020). These compounds provide obvious and stimulating odours, such as pungent, cabbage-like, and onion odours (Zhou et al., 2019). After comparing their RIs and mass spectra with the data above, the nitriles and isothiocyanates in the FROs were identified from the FROs (Table 3).

3.6. Sensory evaluation analysis

By using descriptive sensory analysis, the sensory qualities of samples were evaluated. The main flavours contributed by the FROs were nutty-like, burnt-like, pickled-like, pungent-like, green-like, and fatty-like. The mean score of each flavour attribute was shown in Fig. 2. According to sensory analysis, the six attributes showed statistically significant differences (p < 0.05), and these differences were used to describe the flavour profiles of the FROs. The FROs exuded a strong fatty-like note because of the high aldehyde content (Wei et al., 2012; Liu et al., 2020). FROs roasted for 40–60 min had stronger nutty-like note compared to other samples, which was tightly linked to the generation of pyrazines (Wang et al., 2019). Moreover, FROs also emitted a pungent odour due to the presence of isothiocyanates and nitriles (Mao, Zhao, Huyan, Liu, & Yu, 2019). The longer the roasting time was, the higher the pungent odour was. However, ketones, acids and alcohols are not correlated with the flavour attributes of the roasted FROs due to their low relative odour activity values (Zhang et al., 2022b).
### Content of identified nitriles and isothiocyanates in FRO (mg/kg).

| Temperature | n-BuN | n-PeN | n-HxN | n-PeNN | n-OctN | n-HexN | n-PeN | n-HeCn | n-I3Cn | MMeCN | n-Pe2Cn | n-Pe2CN | n-Bu2Cn | n-Pe2CN | n-Pe2CN | n-Pe2CN | n-Pe2CN |
|-------------|-------|-------|-------|--------|--------|--------|-------|--------|--------|-------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0           | 0     | 0     | 0     | 0      | 0      | 0      | 0     | 0      | 0      | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| 10          | 0.71 ± 0.17 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 20          | 1.47 ± 0.34 | 0.03 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 30          | 5.1 ± 1.26 | 0.04 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 40          | 10.62 ± 2.01 | 0.05 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 50          | 20.7 ± 4.04 | 0.06 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 60          | 60.7 ± 12.08 | 0.07 ± 0.03 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Note: “–” not detected. Results are means ± SD of triplicate determinations. Values with different letters in the same column are significantly different (p < 0.05). Abbreviation: FRO, fragrant rapeseed oil.

### 3.7 Possible thermal degradation pathways of the purified ds-GSLs

Alliphatic GSLs have been reported to be able to be thermally decomposed into nitriles and isothiocyanates (Deng et al., 2014; Mao, Zhao, Huyan, Liu, & Yu, 2019). First, the GSLs could be degraded and converted into β-d-glucose and unstable aglycones under thermal conditions. Subsequently, the generated aglycones underwent further Lossen rearrangement to yield isothiocyanates and sulphates or were decomposed to remove sulphate ions and hydrogen sulphide, thereby forming the corresponding nitriles based on the R groups. However, the specific pathway of producing nitriles and isothiocyanates by individual GSL thermal degradation has not been reported. Thus, the relationship between purified individual ds-GSLs and their degradation volatiles was speculated on the basis of their structure, and the pathways of thermally induced degradation of individual GSLs were deduced (Fig. 3).

The possible formation pathway of PRO degradation during heating is presented in Fig. 3a. First, PRO lost glucose, sulphate ion and S and then degraded to 1-cyano-2-hydroxy-3-butene. The hydroxyl group was eliminated and a double bond formed at C-2 due to instability of 1-cyano-2-hydroxy-3-butene, resulting in the formation of 2,4-pentadienitrile. Zhang et al. (2022) studied the thermally induced degradation of PRO in different matrices (phosphate buffer at a pH value of 5.0, 7.0, or 9.0, sea sand, and rapeseed powder) at different temperatures (150–200 °C). Results showed that 2,4-pentadienitrile was the major nitrile formed, which is in line with our finding here. At the base of sulphate, glucose and hydrogen sulphide were released, and the C-4 of the R group of GNA underwent an additional reaction. Afterward, the C–C bond of C-2 was broken, thereby generating 2-pentenonitrile after dehydrogenation elimination reaction (Fig. 3b). 3-Methyl-2-butenenitrile was generated by GNA degradation, in which the double bond of R group in GNA was broken, elimination reaction occurs at position C-2, and position C-3 can be generated by C-alkylation (Mao, Zhao, Huyan, Liu, & Yu, 2019; Ros, de la Rosa, & Enfedaque, 2002). Apart from these products as above, 4-isothiocyanato-1-butenone could also be produced by Lossen rearrangement of GNA. According to research of Zhou et al. (2018), 4-isothiocyanato-1-butenone was responsible for the pungent odour in rapeseed oils from microwaved seeds. As shown in Fig. 3c, GBN reduced glucose, sulphate and sulphur, and then reduction reaction occurred to form hexanenitrile. No volatile compound of 2,4-hexenonitrile was detected in the FROs during the roasting of the rapeseeds probably because trace amounts of hexanenitrile were not detected or hexanenitrile reacted with other substances present in the rapeseeds to form unknown compounds at high temperatures. In addition, 5-methyl-hexanenitrile was produced by the degradation of GBN, in which the double bond of the R group in GBN is replaced by the methyl group. In a previous report, 5-methyl-hexanenitrile was also identified in...
commercial FROs by monolithic material sorptive extraction and GC–MS (Zhou et al., 2019).

4. Conclusions

The focus of this work was to determine the thermal degradation pathway of three individual ds-GSLs purified from rapeseed, including ds-PRO, ds-GNA, and ds-GBN. Five nitriles and one isothiocyanate were identified in the model systems. The same volatiles, including 2,4-pentadienitrile, 2-pentenonitrile, 3-methyl-2-butenenitrile, 4-isothiocyanato-1-butene, and 5-methyl-hexanenitrile, were also detected in FROs. After roasting for 60 min, these volatiles accounted for 75.87% of the total nitrile and isothiocyanate content in oil sample. Meanwhile, the longer the roasting time was, the higher the pungent odour of FRO was. This result demonstrated that the three GSLs may be the main precursors of FROs in the production of their pungent flavour. Overall, our research may form an important basis for future attempts to investigate the interactions between the GSL degradation and flavour profile of FRO, toward guiding industrial production.

CRediT authorship contribution statement

Lingyan Zhang: Writing – original draft, Investigation, Conceptualization, Methodology. Jia Chen: Investigation, Data curation, Visualization. Xingzhong Zhao: Supervision, Validation. Yimeng Wang: Methodology, Software, Supervision. Xiuzhu Yu: Funding acquisition, Writing – review & editing, Validation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100503.

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