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Isolation and Identification of a Potent Radical Scavenger (Canolol) from Roasted High Erucic Mustard Seed Oil from Nepal and Its Formation during Roasting

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Supporting Information

ABSTRACT: Roasting of high erucic mustard (HEM) seed has been reported to give a typical flavor and increase the oxidative stability of the extracted oil. A potent radical scavenging compound was successfully isolated from roasted HEM seed oil in a single-step chromatographic separation using an amino solid-phase extraction column. Nuclear magnetic resonance and mass spectrometry spectra revealed the compound as 2,6-dimethoxy-4-vinylphenol (generally known as canolol), and its identity was fully confirmed by chemical synthesis. The formation of canolol during roasting was compared among HEM varieties (*Brassica juncea*, *B. juncea* var. *oriental*, *Brassica nigra*, and *Sinapis alba*) together with a low erucic rapeseed variety. HEM varieties were shown to produce less than one-third of canolol compared to rapeseed at similar roasting conditions. This observation was linked to a lower free sinapic acid content together with a lower loss of sinapic acid derivatives in the HEM varieties compared to rapeseed. Around 50% of the canolol formed in the roasted seed was shown to be extracted in the oil. Roasting of HEM seed before oil extraction was found to be a beneficial step to obtain canolol-enriched oil, which could improve the oxidative stability.

KEYWORDS: *2,6-Dimethoxy-4-vinylphenol (canolol), Brassica juncea, Brassica juncea var. oriental, Brassica nigra, Sinapis alba, mustard seed, rapeseed, antioxidant, roasting, sinapic acid*

INTRODUCTION

High erucic mustard (HEM) seed oil is one of the most common liquid cooking oils in Nepal and India. These traditional varieties contain 22−60% of erucic acid and are rich in glucosinolates.[1] HEM seed is generally roasted before oil extraction for its typical flavor.[2] In most parts of the world, high erucic traditional varieties have been replaced by new varieties, developed by different breeding practices, containing low erucic acid (<2%) and low amounts of glucosinolates (<30 μmol/g), such as Canadian rapeseed variety (canola) and European low erucic rapeseed variety.[1,3] Therefore, the consumption of HEM seed oil is very limited, only in some specific areas of the world, particularly in Nepal and India.[1]

Scientific studies on roasted HEM seed oil are very rare. A recent study has shown that roasting of HEM seed before oil extraction could significantly improve the oxidative stability of oil together with the stability of tocopherol and lutein during storage.[2,4] The increased stability was attributed to the formation of antioxidative compounds during roasting via the Maillard reaction. These authors also reported a small increase in the tocopherol content (around 40 μg/g of oil) during roasting. Because the reported increase in the tocopherol content was very low and the suggested Maillard reaction effect was not clearly demonstrated, it remains unclear which factors or compounds are responsible for the increase in oxidative stability of roasted HEM seed oil.[2] A similar roasting condition has been reported to have no effect on the tocopherol content during rapeseed roasting.[5] Roasting has multiple consequences, and the increased oil stability can be due to their overall effect. Additionally, several studies have shown a positive effect of seed roasting on oil stability by the formation of different radical scavengers. Roasting of sesame seed results in the production of sesamols, which leads to the increased oil stability.[6] Canolol (2,6-dimethoxy-4-vinylphenol) is formed by the decarboxylation of sinapic acid during rapeseed roasting.[5,7,8] Canolol formation has been shown to improve the oxidative stability of roasted rapeseed.[8] The increased oxidative stability of oil extracted from the roasted seed of different *Brassica napus* and *Brassica juncea* breeds (both low erucic varieties) has also been linked to canolol formation; however, quantitative data were not presented.[9]

The possible formation of such radical scavengers in roasted HEM seed oil remains to be investigated. Therefore, the objective of the current study was to isolate and identify a major radical scavenging compound from roasted HEM seed oil obtained from the Nepalese market and to compare different varieties on its formation during roasting.

MATERIALS AND METHODS

Materials. Roasted HEM seed oil samples were collected from the Nepalese market. The samples were stored under refrigerated conditions (below −18 °C) until analysis. HEM varieties (*B. juncea*, *B. juncea* var. *oriental*, *Brassica nigra*, and *Sinapis alba*) and low erucic rapeseed were collected from the local market in Belgium. Thin-layer chromatography (TLC) plates (Silicagel 60) were obtained from Merck (Darmstadt, Germany). The tocopherol standard was obtained

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from DSM (Parsippany, NJ). The aminopropyl solid-phase extraction (SPE) column (Extract Clean, 500 mg/4 mL) was purchased from Grace (Lokeren, Belgium). Syringaldehyde and sinapic acid were purchased from Sigma Aldrich (Steinheim, Germany). Other reagents and solvents were of analytical grade and obtained from reliable commercial sources.

TLC Analysis. The oil sample was dissolved in hexane and applied on a TLC plate. A mixed tocopherol standard solution (containing α-, β-, γ-, and δ-tocopherols) was also spotted. The plate was developed with hexane/diethyl ether/acetic acid (80:30:1, v/v/v). Afterward, it was sprayed uniformly with 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in hexane to give a slightly pink background and held for 5 min. The radical scavengers appeared as white spots on the pink background.

Isolation and Purification of the Compound of Interest. Roasted HEM seed oil (1 g) was dissolved in 2 mL of hexane and extracted 3 times with 2 mL of methanol. On the basis of TLC analysis, the compound of interest (R_f = 0.15) was found to be in the methanol fraction. Therefore, the methanol fraction was dried under reduced pressure, and the residue was dissolved in 5 mL of 0.9% isopropanol in hexane (v/v). This solution (0.5 mL) was brought on an aminopropyl SPE column (preconditioned with the same solvent mixture). The SPE column was eluted with the same solvent mixture, and the first 6 mL was discarded. Afterward, 12 fractions of each of 4 mL were collected. Finally, the column was eluted with 5% isopropanol in hexane (v/v), and three more fractions (each of 4 mL) were also collected (fractions 13−15). The compound of interest was observed in the fractions 1−5, via TLC analysis. These fractions were combined and dried under nitrogen. The procedure was repeated to obtain around 5 mg of white dry compound. The purity of this compound was confirmed by TLC and high-performance liquid chromatography (HPLC) analysis.

Identification of the Isolated Compound by Spectroscopic Measurements. The identification of the isolated compound was carried out on the basis of nuclear magnetic resonance (NMR), mass spectrometry (MS), and ultraviolet−visible (UV−vis) spectroscopic measurements.

NMR Spectroscopy. The purified compound was dissolved in deuterated chloroform. The proton (^{1}H) and carbon (^{13}C) NMR spectra were taken using a Jeol EX300 Eclipse NMR (300 MHz) spectrophotometer (Japan).

Liquid Chromatography−Mass Spectrometry (LC−MS) Spectroscopy. The LC−MS analysis was carried out using UltiMate 3000 ultrahigh-pressure liquid chromatography (UHPLC, Dionex) equipped with a degasser, four solvent delivery modules, an autosampler, a column oven, and an UV detector coupled with a MicroTOF MS instrument (Bruker). The purified compound was dissolved in an isopropanol/water/acetic acid (90:10:0.1, v/v/v) mixture (10 μg/mL) and then injected on a C8 Zorbax 300 SB column (Agilent, Santa Clara, CA). Mobile phase A was a water/acetonitrile/acetic acid mixture (90:10:0.1, v/v/v), and mobile phase B was an acetonitrile/water/acetic acid mixture (90:10:0.1, v/v/v). The method was run with 10% mobile phase B for 1 min, then a gradient was applied to reach 100% of mobile phase B in 11 min, which was held for 5 min. Afterward, the initial conditions were reached in 0.5 min, and the column was allowed to equilibrate for 4.5 min before a subsequent analytical run. The solvent flow rate was 0.2 mL/min. Electrospray ionization (ESI) in positive-ion mode was used, and m/z values were scanned from 50 to 1000. The capillary voltage was set at 4500 V, and the end plate offset was at −500 V. The nebulizer pressure was 0.5 bar and was heated to 190 °C with dry nitrogen at a flow rate of 4 mL/min.

UV−Vis Spectroscopy. The UV and visible absorption spectra were taken in hexane using a Cary 50 UV−vis spectrophotometer (Varian) in a quartz cuvette.

Synthesis of 2,6-Dimethoxy-4-vinylphenol (Canolol). The method described for the synthesis of 4-vinylphenols from 4-hydroxy-substituted benzaldehydes under microwave irradiation was followed.[10] Syringaldehyde was chosen as an appropriate benzaldehyde for the synthesis of 2,6-dimethoxy-4-vinylphenol. The synthesized compound was purified on a silica gel column using the procedure described in the same method.[10] Crystallization was applied as an additional step to increase the purity. A saturated solution of the synthesized compound in hexane was prepared at ambient temperature, and crystallization was induced by storing it inside the freezer (−28 °C). Crystals (white) were separated from the mother liquor and dried under nitrogen. The purity of the compound was confirmed by NMR spectroscopy.

HPLC Analysis of Tocopherols and Canolol in Oil. Tocopherol and canolol contents of the oil were analyzed on Agilent 1100 series HPLC equipped with a degasser, four solvent delivery modules, an autosampler, a column oven, and a fluorescence detector. A mobile phase containing 0.9% isopropanol in hexane (v/v) was used in isocratic conditions at a flow rate of 1 mL/min. Separation was carried out on a LiChroCART 250-4,6 Purospher STAR Si (5 μm) column (Merck, Darmstadt, Germany) with a precolumn containing the same phase. The temperature of the column was maintained at 35 °C, and the chromatogram was obtained with a fluorescence detector (excitation at 285 nm and emission at 325 nm). The fluorescence emission spectra (310−400 nm) were also obtained at a 285 nm excitation wavelength. Analysis of each sample was carried out in triplicate. Because the reference canolol compound was not commercially available, the synthesized canolol was used as the standard for quantification.

Roasting of Seed and Powder Samples of HEM and Rapeseed and Oil Extraction. A seed sample (80 g) was added to a heated beaker (250 mL) placed on the oil bath maintained at 180 °C. Both the heating oil and seed sample were continuously mixed with electric mechanical stirrers. The temperatures of the heating oil and seed sample were continuously monitored using Testo thermostat probes. The oil temperature was maintained at 180 °C, and roasting was carried out for 10 min. The seed temperature reached 162 ± 3.5 °C in 10 min. The seed temperature profile with time followed the equation $T = 32.796 \ln(t) + 89.422$ ($R^2 = 0.99$), where T is the seed temperature (°C) and t is the time (min).

The same setup could not be used for homogeneous mixing of seed powder. Therefore, 4 g of seed powder was taken in a test tube and roasted in an oil bath maintained at 180 °C. The thermostat probe was kept in the center of the test tube containing the seed powder, and the temperature was monitored. After heating for 6 min (holding the oil bath temperature constant at 180 °C), the seed powder temperature reached 160 °C with the temperature profile represented by equation $T = 36.578 \ln(t) + 96.097$ ($R^2 = 0.99$), where T is the seed powder temperature in the center (°C) and t is the time (min). During 6−10 min of heating time, the oil bath temperature was gradually lowered to 172 °C and the seed powder temperature profile followed the equation $T = 15.569 \ln(t) + 134$ ($R^2 = 0.97$). Roasting of both seed and powder were carried out in three batches for each variety, and further analyses were performed independently on each batch.

The oil was extracted 3 times from 30 g of ground sample using 80 mL of petroleum ether. The sample−solvent mixture was kept in a shaker for 15 min and then centrifuged at 9000g for 10 min. The supernatant was filtered through a filter paper and evaporated under reduced pressure at 35 °C. The oil sample was dried overnight under nitrogen and stored in the freezer (−28 °C) until further analysis.

Analysis of the Free Sinapic Acid (FSA) Content. Seed samples were finely ground using a coffee grinder. The seed powder (4 g) was mixed with 25 mL of methanol/water/acetic acid (70:30:0.2, v/v/v) using an ultra turrax at 10 000 rpm for 2 min. The ultra turrax probe was washed with 20 mL of the same solvent, which was added to the mixture, and the volume was topped up to 50 mL. After centrifugation at 2800g for 10 min, the supernatant was filtered through a Millex-LCR filter [0.45 μm polytetrafluoroethylene (PTFE) membrane, Millipore, Ireland]. The filtrate was diluted 4 times with 0.2% acetic acid in water (v/v) before injecting on an UHPLC system.

Analysis of the Total Sinapic Acid Content after Basic Hydrolysis (TSAH). Alkaline hydrolysis of the esterified phenolic compounds were carried out using a previously described method, with slight modifications.[11] The supernatant phenolic extract (3 mL) was mixed with 3 mL of distilled water and 1.5 mL of 10 M NaOH.

The sample was flushed with nitrogen, covered with aluminum foil, and kept on a shaker for 4 h at room temperature. Afterward, the pH of the solution was adjusted to 2 using 8 M HCl, and the phenolic compounds were extracted 3 times each with 3 mL of ethyl acetate. The extracts were combined and dried under nitrogen. The residue was redissolved in 5 mL of methanol, and the volume was topped up to 15 mL with 0.2% acetic acid in water. The solution was diluted 5 times with methanol/water/acetic acid (20:80:0.2, v/v/v) solvent mixture before injecting on an UHPLC system.

UHPLC−Diode Array Detector (DAD) Analysis for the Quantification of Sinapic Acid and Canolol. The analysis was carried out using UltiMate 3000 UHPLC (Dionex), equipped with a degasser, four solvent delivery modules, an autosampler, a column oven, and a DAD. The separation was carried out using a 2.1 × 150 mm, 1.8 μm, Zorbax Eclipse Plus C18 column (Agilent, Santa Clara, CA) maintained at 30 °C. The injection volume was 10 μL, and the compounds were eluted with 0.2% acetic acid in water (v/v) (mobile phase A) and 100% methanol (mobile phase B) at a flow rate of 0.3 mL/min. The gradient program was as follows: 20−40% B (5 min), 40% B (1 min), 40−50% B (1 min), 50% B (1 min), 50−60% B (5 min), 60−70% B (2 min), 70% B (1 min), 70−20% B (1 min), and 20% B (5 min). The chromatograms were recorded at 330 and 280 nm absorbances. The sinapic acid and canolol contents were quantified on the basis of the calibration curves using a chromatogram at 330 and 280 nm, respectively. All of the analyses were carried out in triplicates.

Statistical Analysis. All data are presented as mean values ±95% confidence interval of the mean based on three independent experiments. Data were transformed into a logarithmic scale before statistical analyses to have homoscedasticity. Analysis of variance (ANOVA) and post-hoc Tukey test were performed using TIBCO Spotfire S+ 8.1 software. The significance level is $p < 0.05$, unless otherwise indicated.

RESULTS AND DISCUSSION

Isolation and Identification of a Potent Radical Scavenger from Roasted HEM Seed Oil Obtained from the Nepalese Market. The qualitative identification of the different tocopherols present in roasted HEM seed oil was carried out using normal-phase HPLC analysis. The α-, γ-, and δ-tocopherols were identified comparing the retention time and fluorescence spectra to the tocopherol standard. One small peak eluting before β-tocopherol and another peak eluting after δ-tocopherol were additionally observed in the chromatogram (Figure 1). Both peaks showed similar fluorescence spectra as tocopherol and did not show any tocotrienol using known standards. Both of these peaks were observed in a number of HEM seed oil samples analyzed on HPLC. After linseed oil was injected, which contains plastochromanol-8,[1] the small peak before the β-tocopherol position was identified to be plastochromanol-8. The major peak was more polar than all of the tocopherols and also showed a slight tailing (Figure 1).

The oil sample was subjected to a separation on silica using TLC. Radical scavenging compounds were detected simply by spraying a DPPH solution on the TLC plate after development. Tocopherols eluted at the R_f value of 0.3−0.5. Another radical scavenging spot was observed at a R_f value of 0.15, which was isolated from the TLC plate and injected on HPLC. It was proven that the isolated compound had the same elution behavior as that of the unknown compound eluting at 14 min, as shown in Figure 1.

Using an aminopropyl SPE column, the compound of interest could easily be purified by washing the column with 6 mL of 0.9% isopropanol in hexane (v/v) and then eluting the compound with 20 mL of the same solvent mixture. The UV absorption spectra of the isolated compound showed peaks at 222 and 272 nm in hexane.

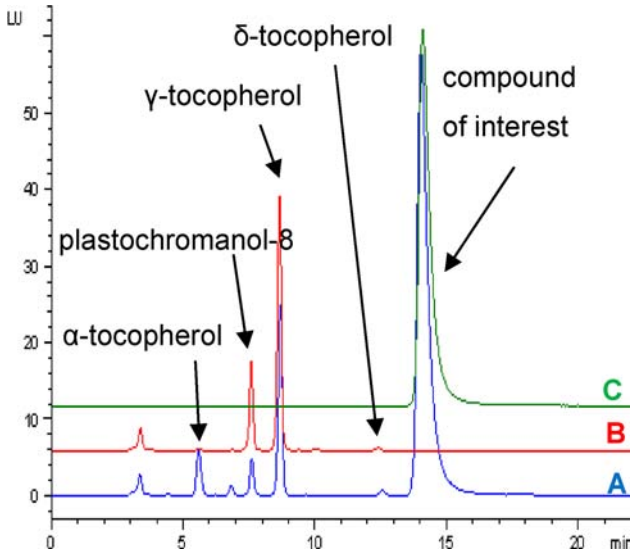


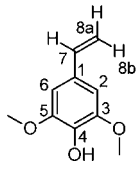
Figure 1. Normal-phase HPLC chromatogram of (A) roasted HEM seed oil, (B) flax seed oil, and (C) isolated compound of interest, using a fluorescence detector (excitation at 285 nm and emission at 325 nm). The time axis is in minutes.

The mass spectrum of the compound of interest showed the ions (with relative intensity) with m/z values of 181.08 (100), 149.06 (32), 203.06 (15), 121.06 (11), 166.06 (3), and 103.05 (2). The NMR chemical shifts of the compound of interest are given in Table 1. The compound of interest was identified as 2,6-dimethoxy-4-vinylphenol, and the fragmentation pattern completely supported the proposed structure (Figure 2).

Table 1. NMR Chemical Shifts of the Isolated Compound of Interest

Position	^{1}H δ (ppm)	^{13}C δ (ppm)
1	-	129.28
2,6	6.65 (2H, s)	103.08
3,5	-	136.92
4	5.54 (1H, s)	134.87
7	6.62 (1H, dd)	147.15
	$J_{7, 8b}$ = 17.1 Hz	
	$J_{7, 8a}$ = 11.0 Hz	
8a	5.15 (1H, dd)	
	$J_{7, 8a}$ = 11.0 Hz	
	$J_{8b, 8a}$ = 1.1 Hz	
8b	5.61 (1H, dd)	111.95
	$J_{7, 8b}$ = 17.1 Hz	
	$J_{8b, 8a}$ = 1.1 Hz	
OCH_3	3.91 (6H, s)	56.35

Molecular Structure



The final confirmation of the identity of the isolated compound of interest was performed by synthesizing 2,6-dimethoxy-4-vinylphenol from syringaldehyde (Figure 3). The isolated and synthesized compound had similar MS, NMR, UV, and fluorescence spectra, along with the same retention time in HPLC, and was also in line with the existing literature

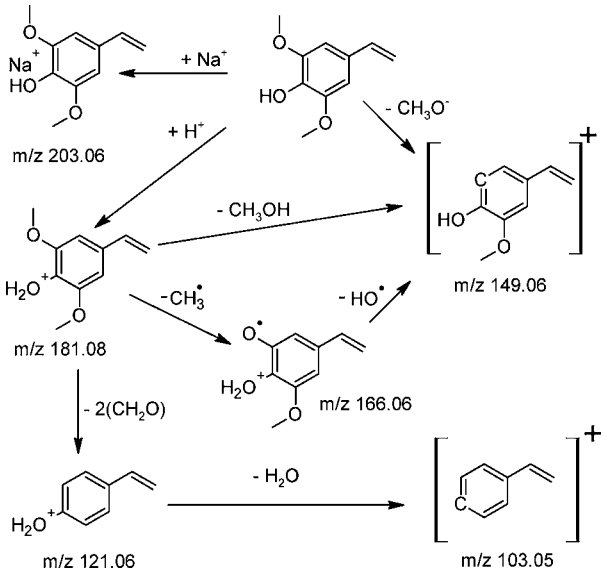


Figure 2. Possible fragmentation pattern and formation of positive ions from canolol as detected in the MS spectra.

data.[5,7,8,12] These observations fully confirmed the identification of the isolated compound.

Canolol was previously isolated from roasted rapeseed oil using different techniques. Most of these methods were long and tedious. The first method consisted of the fractionation of roasted rapeseed oil on a silica SPE column by eluting with heptane/diethyl ether (90:10, v/v), followed by separation on a C18 SPE column by eluting with methanol.[8] The second isolation method consisted of the extraction with methanol by liquid−liquid partition, followed by fractionation with silica gel chromatography by sequential elution with hexane/diethyl ether (9:1, v/v), chloroform, acetone, and methanol. The chloroform fraction was then separated by preparative TLC, and purification was carried out by HPLC.[5] Another method comprised the extraction of roasted rapeseed with methanol and separation on an aminopropyl SPE column by sequential elution with hexane/isopropanol (98.8:1.2, v/v), followed by a mixture in a 95:5 (v/v) ratio. Afterward, the canolol-rich fraction was collected with isopropanol.[9] This fraction contains other polar compounds together with canolol. In a more recent isolation method, roasted ground rapeseed was extracted with 70% methanol after washing with petroleum ether to remove the oil. The extract was concentrated and extracted with hexane/isopropanol (4:1, v/v). Afterward, it was further extracted with a 0.1 M hydrochloric acid solution, and separation was carried out using SPE (Chromabond HR-P) by eluting with methanol. The methanol fraction was concentrated and further extracted with hexane. Finally, canolol was purified using TLC by developing with a hexane/ethyl acetate (7:3, v/v) mixture.[7] In comparison to all of these previous isolation procedures, the method developed in our laboratory could be considered as one of the simplest methods currently available. The developed method was close to the one previously described,[9] with the additional benefit of obtaining canolol without eluting other polar compounds together in the same fraction. Furthermore, this method was also applicable for the isolation of canolol from roasted rapeseed oil.

Comparison of Canolol Formation among Different Varieties of HEM and Rapeseed during Roasting. Roasting is generally practiced on either the seed form or the flaked form. To study the effect of the physical integrity of the seed, canolol formation was studied during both seed roasting and seed powder roasting. The formation of canolol after roasting could be observed in Figure 4. The amount of canolol formed during roasting of different varieties of HEM seed and seed powder is shown in Table 2. The data on the rapeseed are also presented together for comparison. Canolol formation was observed in all varieties, during both seed and seed powder roasting; however, canolol formation was far lower in all of the HEM varieties compared to that in rapeseed. The highest canolol formation during seed roasting among HEM varieties was observed in *B. juncea*, followed by *B. nigra*, *B. juncea* var. *oriental*, and *S. alba*.

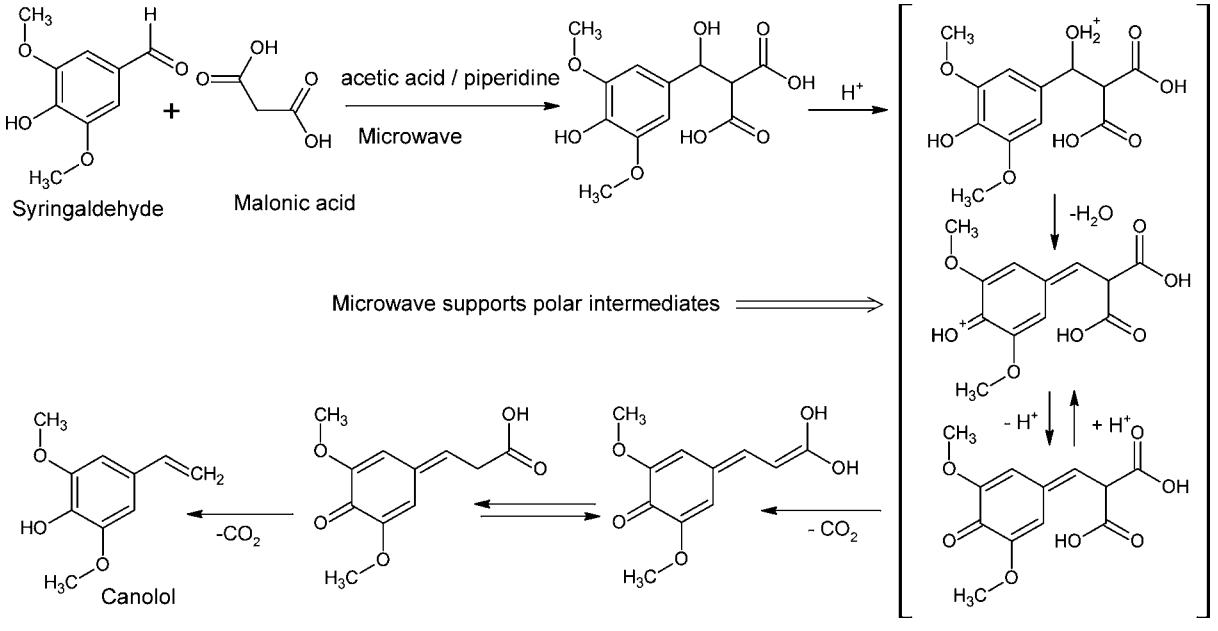


Figure 3. Synthesis of canolol from syringaldehyde (adapted from ref 10).

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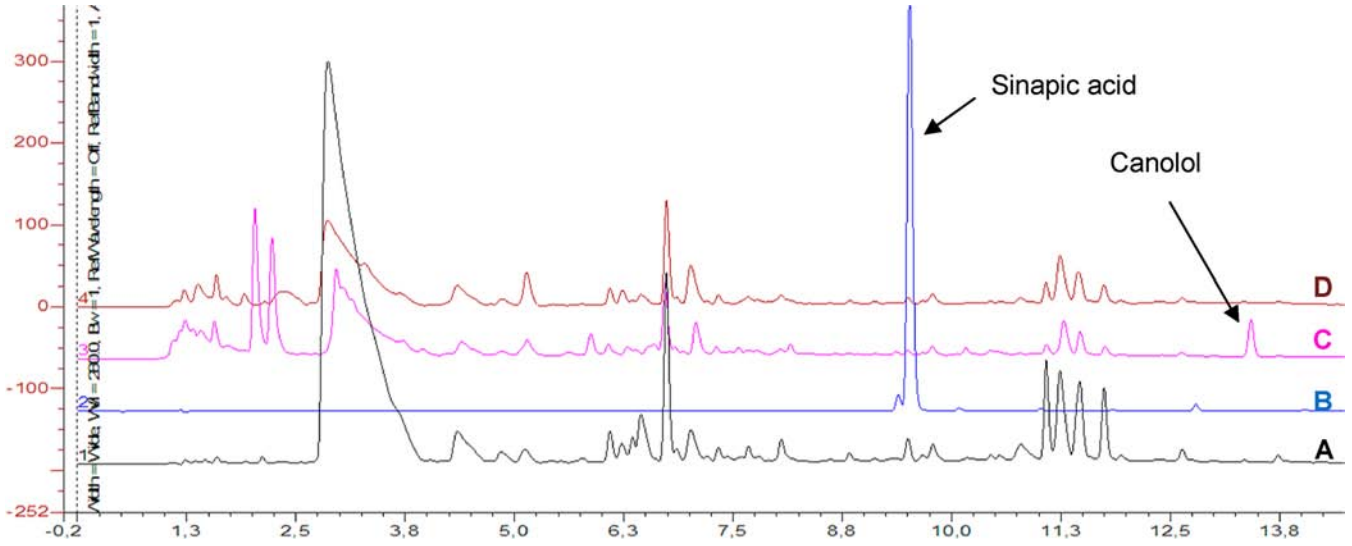

Figure 4. Reverse-phase UHPLC chromatogram of the 70% methanol extract of the *B. juncea* var. *oriental* variety: (A) unroasted seed measured at 330 nm, (B) after basic hydrolysis measured at 330 nm, (C) 10 min roasted seed measured at 280 nm, and (D) unroasted seed measured at 280 nm. The time axis is in minutes.

Table 2. Changes in FSA, TSAH, and Canolol Contents during Roasting of *B. juncea* (BJ), *B. juncea* var. *oriental* (BJO), *B. nigra* (BN), *S. alba* (SA), and Rapeseed (RS)

variety	roasting condition	FSA (μg/g of DM)[a]	TSAH (mg/g of DM)[a]	canolol (μg/g of DM)[a]
BJ	unroasted	26.55 ± 1.86 ab	8.41 ± 0.58 g	10.83 ± 0.11 a
	seed roasted	56.90 ± 1.52 f	6.30 ± 0.13 ef	212.28 ± 5.14 i
	seed powder roasted	58.52 ± 0.92 f	7.49 ± 0.11 f	159.15 ± 2.00 h
BJO	unroasted	46.92 ± 2.17 e	6.72 ± 0.09 f	nd[b]
	seed roasted	39.68 ± 1.23 cd	5.38 ± 0.06 bc	135.56 ± 2.07 g
	seed powder roasted	42.41 ± 0.45 d	5.55 ± 0.13 cd	118.97 ± 2.83 f
BN	unroasted	77.18 ± 4.11 g	5.63 ± 0.12 cd	nd[b]
	seed roasted	26.50 ± 0.72 ab	4.22 ± 0.21 a	143.00 ± 2.14 g
	seed powder roasted	35.97 ± 1.25 c	4.94 ± 0.27 b	95.50 ± 5.86 e
SA	unroasted	64.97 ± 1.74 f	7.15 ± 0.52 f	nd[b]
	seed roasted	29.58 ± 1.46 b	6.04 ± 0.08 def	75.96 ± 2.19 d
	seed powder roasted	23.10 ± 0.64 a	6.87 ± 0.29 f	56.67 ± 0.97 c
RS	unroasted	294.95 ± 4.06 j	8.48 ± 0.12 g	24.74 ± 0.45 b
	seed roasted	124.55 ± 15.71 i	5.61 ± 0.23 cd	707.69 ± 50.68 j
	seed powder roasted	106.91 ± 11.69 h	5.88 ± 0.31 cde	790.41 ± 50.78 k

[a]Values with different letters in the same column are significantly different ($p < 0.05$). [b]nd = not detected.

Canolol formation has been proposed to be formed as a result of decarboxylation of sinapic acid during roasting.[5,7−9] It is well-known that the major quantity of sinapic acid in both mustard and rapeseed is present in the form of different derivatives (sinapine, sinapoyl-hexoside, disinopoyl-dihexoside, disinopoyl-hexoside, trisinapoyl-dihexoside, etc).[8,11,13,14] Therefore, both the FSA and TSAH contents were quantified. The phenolic compound extraction was carried out with 70% methanol, because a better extractability with this solvent mixture was reported previously.[13] After basic hydrolysis, the majority of the peaks were lost, giving a single major peak of sinapic acid, indicating that most of the peaks observed before hydrolysis were sinapic acid derivatives (Figure 4).

The FSA and TSAH contents of all of the varieties during the different roasting conditions are presented in Table 2. The FSA content in different HEM varieties varied from 26.55 to 77.18 μg/g of dry matter (DM), while the rapeseed contained a higher quantity of FSA (294.95 μg/g of DM). The TSAH content varied between 5.63 and 8.41 mg/g of DM in all HEM varieties. Rapeseed contained 8.48 mg of TSAH/g of DM (13.95 mg/g of fat-free mass), which was similar to the previously reported value of 11.00−15.33 mg of total sinapic acid equiv/g of defatted meal.[13] The FSA content in the different HEM varieties and rapeseed was 0.32−1.37 and 3.48% TSAH, respectively. The FSA content was reported earlier to be 0.86−3.76% of the total phenolics in defatted canola.[13]

After roasting, the loss of the TSAH content of all of the varieties was observed. This loss was significant ($p < 0.05$) for all of the varieties, except for *S. alba*. The FSA content also decreased significantly ($p < 0.05$) after roasting, except for *B. juncea*. However, the loss of FSA was far below the amount of sinapic acid converted into canolol (the molecular weight ratio of sinapic acid/canolol is 1.24). Therefore, esterified sinapic acid derivatives must have provided FSA by hydrolysis during roasting. The significant increase in FSA in *B. juncea* during roasting also supported this fact ($p < 0.05$). The possible contribution of sinapic acid derivatives on canolol formation during roasting was also stated previously.[7,9] The presence of a

higher quantity of FSA together with a higher loss of sinapic acid derivatives in rapeseed during roasting supported the formation of a higher quantity of canolol compared to other HEM varieties.

Canolol formation was highly correlated with the initial FSA content (0.94), the residual FSA content after roasting (0.98), and also the loss of TSAH (0.93) during seed roasting. Similarly, canolol formation was highly correlated (0.96) with all three of these parameters during seed powder roasting. Hence, hydrolysis of sinapic acid derivatives into FSA favors canolol formation during roasting. Canolol was shown to be thermally unstable, and fast degradation during longer roasting was previously reported.[7] The degradation of canolol was proposed to be due to its involvement in possible side reactions with lipid peroxyl radicals formed during heat treatments, pyrolysis, etc.[7] Furthermore, it has recently been shown that hydroxycinnamic acids (including sinapic acid) could inhibit the Maillard reaction and color development by a radical scavenging mechanism and reacting with intermediates of the Maillard reaction.[15] The major reaction pathway with intermediates of the Maillard reaction was the formation of vinylphenol by decarboxylation (e.g., canolol from sinapic acid), which reacts with Maillard intermediates (such as 3-deoxy-2-hexosulose) to generate phenolic Maillard adducts.[15] The possibilities of these various reactions during roasting support the lower yield of canolol compared to the loss of TSAH. The maximum yield (molar basis) of canolol compared to the TSAH loss was observed for rapeseed (30.69%), while the yield was below 12.62% for the different HEM varieties during seed roasting. Seed powder roasting generally produced less canolol than seed roasting of the same variety, except for rapeseed. However, the yield (molar basis) of canolol compared to the TSAH loss was slightly higher during seed powder roasting than during seed roasting of the same variety. The highest yield was again for rapeseed (37.84%), while the yield varied from 12.71 to 25.42% in different HEM varieties during seed powder roasting. The higher conversion rate of sinapic acid to canolol during seed powder roasting could be due to the easier loss of carbon dioxide from the matrix in the powder form compared to the intact seed, favoring the decarboxylation step.

The canolol content was analyzed in oil samples extracted from roasted seeds of all of the varieties (Table 3). The highest canolol content was observed for roasted rapeseed oil, while all roasted HEM seed oils contained significantly less canolol ($p < 0.05$). Significant differences in the canolol content were also observed between the HEM varieties ($p < 0.05$). The differences in the ratio of canolol formed in the seed and that observed in the oil between different varieties were linked to the differences in the oil content of the different seeds. The fat content of rapeseed, *B. juncea* var. *oriental*, *B. juncea*, *S. alba*, and *B. nigra* was 45, 40, 37, 32, and 30% DW basis, respectively. The extracted canolol was observed to be more concentrated in the samples with a lower oil content than in the samples with a higher oil content. Moreover, only 49−56% of the canolol formed during seed roasting was observed to be extracted in oil in all of the varieties.

Table 3. Canolol Content in the Extracted Oil (μg/g of Oil) from Unroasted and 10 min Roasted Seeds of *B. juncea* (BJ), *B. juncea* var. *oriental* (BJO), *B. nigra* (BN), *S. alba* (SA), and Rapeseed (RS)

	canolol content (μg/g of oil)[a] in the extracted oil	
variety	unroasted seed	roasted seed
BJ	5.41 ± 0.01 c	297.76 ± 6.26 g
BJO	5.06 ± 0.15 c	171.64 ± 3.20 e
SA	0.48 ± 0.03 a	117.29 ± 5.68 d
BN	1.25 ± 0.01 b	266.21 ± 4.81 f
RS	5.19 ± 0.09 c	808.48 ± 24.67 h

[a]Values with different letters are significantly different ($p < 0.05$).

Canolol was confirmed to be formed in HEM varieties during seed roasting. The formation of canolol during roasting and its extraction in oil supports the increased oxidative stability of roasted HEM seed oil.[2] Canolol has been accepted to have very good antioxidative activity.[16,17] The proposed simple procedure could be useful for the isolation of canolol from both roasted HEM and rapeseed oils. The role of sinapic acid derivatives in canolol formation was further illustrated. HEM varieties were shown to produce a far lower amount of canolol compared to rapeseed, and this difference was found to be due to differences in the amount of the FSA content and a different degree of hydrolysis of sinapic acid derivatives. Roasting is a beneficial step for increasing the canolol content in the extracted HEM seed oil and, hence, could increase oxidative stability.

■ ASSOCIATED CONTENT

Supporting Information

Fatty acid composition data of the different mustard and rapeseed varieties and the MS spectra of canolol. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

DM, dry matter; FSA, free sinapic acid; HEM, high erucic mustard; TSAH, total sinapic acid after basic hydrolysis

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