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Influence of thermal treatment of rapeseed on the canolol content

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ABSTRACT

4-Vinylsyringol, also referred to as canolol, is a highly active antioxidant and potent lipidperoxyl radical scavenger found in rapeseed. The canolol content of rapeseed can be increased through the decarboxylation of sinapic acid via roasting treatments. Different roasting conditions were tested and compared and an optimum for the canolol formation was found at 160 °C. The canolol content of the rapeseed samples with optimal roasting increased by a factor of 120 in relation to the unroasted sample. The rapeseed was ground, extracted and analysed by normal-phase HPLC/UV. The structure of canolol was confirmed by NMR and MS techniques. Several rapeseed oils were purchased in German food stores and analysed. No differences in canolol content were observed in both cold-pressed and rape kernel oil samples tested. Dehulled rapeseed samples demonstrated no significant difference in canolol content when compared to unpeeled rapeseed samples.

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1. Introduction

Free radicals play a vital role in a number of biological processes, some of which are necessary for life. However, because of their high reactivity, the same species can participate in unwanted side reactions resulting in cell damage and the development of disease. In this context, three of the most important oxygen–containing free radicals are superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide ($^{\cdot}OOH$), and hydroxyl radicals ($^{\cdot}OH$) (Cadenas, 1989). They are collectively referred to as the reactive oxygen species (ROS). In human physiology, ROS formation is influenced by several endogenous and exogenous factors. ROS are generated endogenously as a natural byproduct of the metabolism of oxygen and have been determined to play an important role in cell signalling (Valko et al., 2007). In contrast, exogenous sources such as tobacco smoke or radiation result in a dramatic increase of ROS concentrations (Cadenas, 1989; Machlin & Bendich, 1987; Valko et al., 2007). The abnormal accumulation of ROS in an organism is referred to as oxidative stress and has been demonstrated to be a leading factor in the development of several diseases and accelerates aging. Generally, the cellular damage induced by oxidative stress includes lipid peroxidation of unsaturated components of the membrane lipids and the reactions of ROS with DNA that may lead to carcinogenic mutations (Marnett, 2002; Valko et al., 2007).

At the intracellular level, enzymes like superoxide dismutase can counter oxidative stress by the deactivation of the radicals (Nishikimi, Yamada, & Yagi, 1980). Extracellular, antioxidants like tocopherols, ascorbate or β-carotene can suppress lipid peroxidation by transferring the lipid peroxylradical (LOO**.**) into a saturated compound (Cadenas, 1989). Beside tocopherols, hydrophilic phenols can act as antioxidants (Cotelle, 2001; Rice-Evans, Miller, & Paganga, 1996).

Rapeseed contains more phenolic compounds than most of the other oilseeds (Naczk, Amarowicz, Sullivan, & Shahidi, 1998). In two independent studies, Koski, Pekkarinen, Hopia, Wähälä, and Heinonen (2003) and Wakamatsu et al. (2005) found a new phenolic compound in rapeseed and rapeseed oil, 4-vinylsyringol. Wakamatsu et al. (2005) designated it canolol, as the substance was isolated from crude canola oil. Canolol is a highly potent antioxidant and showed antimutagenic properties (Koski et al., 2003; Kuwahara et al., 2004; Vuorela, Meyer, & Heinonen, 2004; Vuorela et al., 2005; Wakamatsu et al., 2005). According to Kuwahara et al. (2004) the antimutagenic potency is higher than that of α-tocopherol and flavonoids and is comparable to that of ebselen.

Derivates of sinapic acid are the predominant phenolics in rapeseed. Canolol can be produced by decarboxylation of sinapic acid during the press process or roasting of the seeds (Koski et al., 2003; Wakamatsu et al., 2005). Thus the food value of the rapeseed and rapeseed oil may be enhanced by elevating the canolol content through press processing or the roasting of rapeseed before pressing.

Abbreviations: HPLC/UV, high performance liquid chromatography with UV-detector; LC/MS/MS, liquid chromatography with tandem MS/MS; MS, mass spectrometry; NMR, nuclear magnetic resonance; PMC, 2,2,5,7,8-pentamethyl-6-chromanol; ROS, reactive oxygen species.

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Generally, rapeseed oil is considered to be one of the most important vegetable oils in the world (Koski et al., 2002). It is rich in unsaturated fatty acids which makes it susceptible to oxidation. The increase of rapeseed canolol content would theoretically produce oil with enhanced food value and longer shelf life. Yet the refinements of rapeseed oil aims at the reduction of substances that affect flavour or taste of the oil (Nogala-Kalucka, Korczak, Wagner, & Elmadfa, 2004). However, the content of non-triacylglycerol compounds like tocopherols or hydrophilic phenols decrease with each refining step (Gogolewski, Nogala-Kalucka, & Szeliga, 2000) and these compounds are not detectable in purified oil (Koski et al., 2003; Vuorela, Meyer, & Heinonen, 2003).

This study was performed to determine the optimal roasting conditions to produce the highest concentration of canolol in rapeseed and to determine the concentration of this valuable antioxidant in the commercially available German rapeseed oils.

2. Materials and methods

2.1. Materials

Rapeseed samples were procured from the Thuringian State Institute for Agriculture (Jena, Germany) and the Teutoburger Ölmühle GmbH (Ibbenbüren, Germany). Rapeseed oils (11 samples) were purchased in German food stores. All solvents and chemicals utilised in the study were of analytical grade and purchased from various commercial suppliers.

4-Vinylsyringol utilised as a calibration standard was synthesised according to Rein, Ollilainen, Vahermo, Yli-Kauhaluoma, and Heinonen (2005).

2.2. Methods

For each sample, 200 g of whole rapeseed were roasted in a domestic microwave oven (Sharp R-24 W, 2450 MHz, 800 W) at 70% power for a roasting period between 1.5 and 9 min (interval: 1.5 min). The seeds were stirred for 5 s after each interval. After the last interval the temperature of the seed was determined. The ground seeds were treated with 100 ml petroleum ether for 1 min to remove the fat residue. Polar compounds were extracted with 70% methanol at 75 °C for 20 min (Cai & Arntfield, 2001; Vuorela et al., 2003). In addition, an extraction procedure that employed ultrasound for 10 min was tested. For both procedures a solvent-to-seed ratio of 14:1 was used (Naczk, Shahidi, & Sullivan, 1992). The solvent contained 1.4% ascorbic acid (m/v) and PMC (Aldrich, Germany) as internal standard. The solvent was removed down to 3 ml via a rotary evaporator and canolol was extracted three times with hexane/isopropanol (4:1 v/v). The canolol content of each rapeseed sample was determined in triplicate. Sample extracts were stored at −80 °C until analysis.

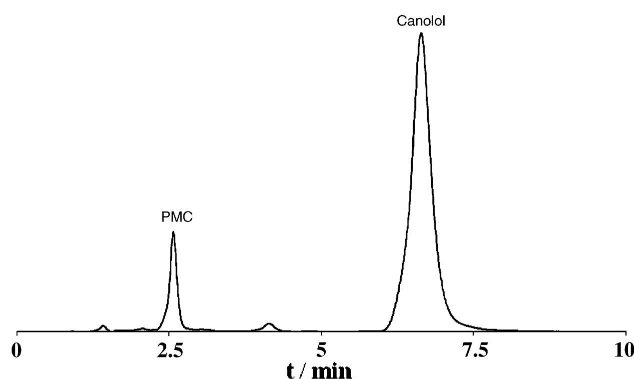


Fig. 1. HPLC-chromatogram of a rapeseed extract, extraction procedure at 75 °C utilised.

For the analysis of the rapeseed oils an aliquot (2 g) of oil was dissolved in 10 ml hexane. Triplicate samples were prepared for all oils.

2.3. HPLC analysis

Separation was performed using a LC-10AD (Shimadzu, Japan) with a SPD-10A UV–Vis and a SPD-M10AVP diode array detector equipped with a Nucleosil 100 NH_2 column (150 × 4 mm, 5 μm, Knauer, Germany). Elution was isocratic with a hexane/isopropanol mixture (96:4 v/v) with the flow rate 1.0 ml/min. The chromatograms were recorded at 275 nm (Thiyam, Stöckmann, & Schwarz, 2006) (Fig. 1). Calibration employing PMC as internal standard and a synthetic canolol standard was utilised for canolol quantification. The results are expressed as μg canolol per gram rapeseed or rapeseed oil.

2.4. Isolation of canolol for structure determination

Hexane/isopropanol sample extracts with high canolol content were extracted three times with 0.1 M hydrochloric acid solution. The canolol was isolated from the aqueous solution by solid phase extraction (Chromabond® HR-P, 3 ml/200 mg, Macherey–Nagel, Germany). The elution of canolol was carried out with methanol and the eluate was extracted with hexane. The hexane extracts were concentrated and purified using thin layer chromatography (Kieselgel 60, Merck, Germany) with hexane/ethyl acetate mixture (7:3 v/v). The canolol bond observed via UV (Rf 0.21) was extracted with chloroform.

The purified sample was reduced to dryness in argon atmosphere and subjected to 200 MHz NMR spectroscopy and LC/MS/MS. For ^{1}H-NMR spectroscopy an aliquot was dissolved in $CDCl_3$ and analysed with a Bruker AC 200 spectrometer. For mass spectral analysis an aliquot of the purified canolol was dissolved in methanol and the sample was applied to reversed-phase HPLC (Luna C18(2), 250 × 3 mm, 5 μm, Phenomenex, USA). The LC/MS/MS method required the isocratic elution of methanol/water (80:20 v/v) solvent system with the flow rate 0.4 mL/min. Perkin Elmer API 365 (Perkin Elmer, USA) was used for mass analysis with electron spray ionisation (ESI, positive mode) and product ion scan (*m/z* 181).

2.5. Statistics

The results were expressed as means with standard deviation (SD). All statistical analyses were performed using the SPSS® software package, version 14.0 (SPSS Inc., Chicago). Scheffé- and Dunnett-T3-tests were employed to compare canolol contents of different roasting conditions. Differences were considered tendentious at $P < 0.1$ and significant at $P < 0.05$.

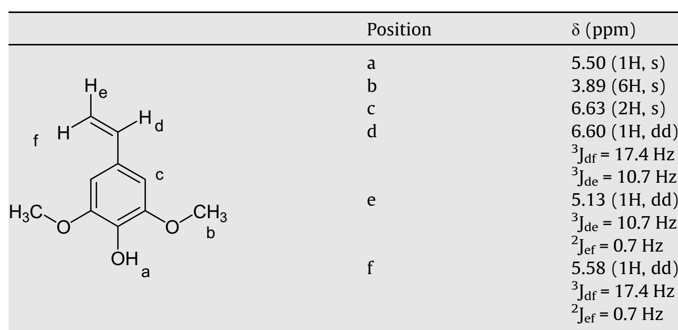
3. Results and discussion

3.1. Structure of canolol

The structural elucidation and positive identification of the isolated and purified canolol sample fraction was determined using NMR spectroscopy (Table 1) and mass spectrometry [MS (ESI$^+$) *m/z* (rel. intensity): 181 (64), 166 (16), 149 (100), 121 (84), 103 (54), 93 (66), 91 (34), and 77 (15)].

UV-spectrum was recorded for the synthesised canolol (Specord S600, Analytik Jena, Germany) and the absorption maxima were determined (220 nm and 270 nm). The extinction coefficient in

Table 1
Structure and ^{1}H-NMR data of canolol



Position	δ (ppm)
a	5.50 (1H, s)
b	3.89 (6H, s)
c	6.63 (2H, s)
d	6.60 (1H, dd) $^3J_{df}$ = 17.4 Hz $^3J_{de}$ = 10.7 Hz
e	5.13 (1H, dd) $^3J_{de}$ = 10.7 Hz $^2J_{ef}$ = 0.7 Hz
f	5.58 (1H, dd) $^3J_{df}$ = 17.4 Hz $^2J_{ef}$ = 0.7 Hz

s, single, dd, doublet of doublet.

water was 26,400 at 220 nm and 11,600 at 270 nm (ε in mmol/cm^2; d = 1 cm). These results are comparable with those reported by Wakamatsu et al. (2005).

3.2. Influence of roasting temperature

The extraction procedure that employed ultrasound yielded lower canolol contents with larger standard deviations for identical samples tested (Table 2). The ultrasound extraction was conducted at room temperature. This is probably the reason why this technique was less effective than the extraction procedure at 75 °C. According to Cai and Arntfield (2001), the temperature has an influence on the extraction efficiency. The extraction procedure at 75 °C was utilised for further experiments.

The unroasted samples possessed 5.8 μg canolol per gram of rapeseed (Table 2). The roasting treatments significantly increased the concentration of canolol in rapeseed. After exposure to a microwave roasting treatment of 4.5 min duration with a final temperature of 135 °C, the canolol content increased from 5.8 μg/g to 340.1 μg/g (Table 2). After 7.5 min at 160 °C the maximum canolol content of 720 μg/g was observed. The optimal roasting conditions produced increases in the rapeseed canolol content of 120 times to that found in the unroasted samples. Further roasting leads to a considerable loss of canolol. The rapeseed sample subjected to a 9.0 min duration roasting treatment (171 °C) possessed 320 μg/g. This decrease of canolol content must result from potential side reactions of canolol with other rapeseed components like lipidperoxyl radicals that accumulate during the heat treatment. Furthermore, pyrolysis or thermal decomposition of canolol can occur within the rapeseed. Prolonged roasting resulted in changes in the colour of the rapeseed and sample extracts from a light yellow to a brown colour. Larrauri, Rupérez, and Saura-Calixto (1997) found for red grape pomace peels that drying temperatures of 100 or 140 °C lead to a considerable loss of antioxidative activity and a reduction of the polyphenol content. The authors suggested that the main mechanism for this loss is thermal degradation.

Samples 6 and 8 were roasted for 7.5 and 6.0 min with intervals of 1.5 and 1.0 min, respectively. At the end of the roasting, both samples were found to have the same temperature and canolol content (Table 3). Thus, for the roasting treatments tested in this study an optimum temperature regarding the formation of canolol was at approximately 160 °C. These results are consistent with the results of Wakamatsu et al. (2005) who roasted rapeseed at 165 °C for 5 min and achieved increases of up to 10–250 times.

In addition to the roasting in a microwave oven, another technique of heat input was tested. Rapeseeds were put into a test-tube (7 g per tube) and heated in a heating block at 40 and 80 °C, respectively (sample 9–14, Table 4). Only a slight increase of the canolol content was observed for all samples. The results were not significantly different from the result of sample 2 at 73 °C, although the heat was supplied via different techniques. Therefore, the temperature seems to be more critical to increasing rapeseed canolol content than the manner in which the heat energy is supplied to the rapeseed.

At present the mechanism for the formation of canolol in plants has not been determined. It is not known whether canolol is synthesised directly or is a byproduct of decarboxylations. To retrace the decarboxylation of sinapic acid and formation of canolol 15 mg sinapic acid (Merck, Germany) were added to 1 ml water and heated in a microwave oven for 5 min. Afterwards the smell of canolol was noticeable (comparable to smoked meat), but only 7.0 μg/ml were detected by HPLC/UV in this solution. Feasible reasons might be the low solubility of sinapic acid in water and the

Table 2
Canolol content of rapeseed (canolol μg/g of rapeseed) depending on roasting time and extraction conditions (n = 3)

Sample number	Total roasting time (interval units of 1.5 min)	Extraction conditions	
		Water bath (75 °C) Mean ± SD	Ultrasound (25 °C) Mean ± SD
1	0	5.8 ± 0.2[a]	5.0 ± 0.7[f]
2	1.5	11.1 ± 0.9[b]	12.0 ± 2.0[f]
3	3.0	54.4 ± 0.3[c]	34.5 ± 8.7[f]
4	4.5	340.1 ± 30.1[d]	317.4 ± 72.7[g]
5	6.0	592.6 ± 57.9[e]	475.6 ± 111.6[g]
6	7.5	720.0 ± 127.6[d,e]	572.6 ± 103.2[g]
7	9.0	320.2 ± 30.6[d]	341.9 ± 45.0[g]

[a–g] Values in a column without a common superscript are tendentiously different (P < 0.1).

Table 3
Canolol content of rapeseed (means: canolol μg/g of rapeseed) depending on roasting conditions (n = 3)

Sample number	Number of interval units (×1.5 min)	Total roasting time (min)	Temperature (°C)	Canolol content
1	0	0	20	5.8
2	1	1.5	73	11.1
3	2	3.0	114	54.4
4	3	4.5	135	340.1
5	4	6.0	146	592.6
6	5	7.5	160	720.0
7	6	9.0	171	320.2
8	6(×1.0 min)*	6.0	161	703.2

* Sample 8 was roasted with a 1.0 min base roasting interval instead of the 1.5 min base roasting interval utilised in all the other trial runs.

Table 4
Canolol content of rapeseed (means: canolol μg/g of rapeseed) depending on heating conditions (n = 3)

Sample number	Heating conditions			Canolol content
	Microwave oven*		Heating block	
	Temperature (°C)	Time (×1.5 min)	Time (min)	Means ± SD
1	20	0	–	5.7 ± 0.2[a]
2	73	1.5		11.1 ± 0.9[b]
9	40		2.5	8.8 ± 1.2[ab]
10	40		5.0	8.3 ± 0.2[b]
11	40		7.5	9.1 ± 0.8[ab]
12	80		2.5	8.7 ± 1.3[ab]
13	80		5.0	10.3 ± 0.8[b]
14	80	–	7.5	8.9 ± 0.7[ab]

[a,b] Values without a common superscript are significantly different (P < 0.05).
* The microwave oven possessed a 560 W rating.

volatility of canolol. When the experiment was repeated with sinapic acid in refined rapeseed oil, no canolol was detected. These results underline the importance of the rapeseed matrix for the canolol formation during roasting. In addition, the main mechanism for the formation of canolol in rapeseed could result through the conversion of sinapic acid derivatives.

The tocopherol content of the roasted samples was not significantly different from the unroasted sample. The ratio of γ- to α-tocopherol was constant (data not shown). These results are in accordance with the results presented by Wakamatsu et al. (2005). Therefore, roasting of rapeseed can lead to higher canolol content without a negative effect on both tocopherol content and profile.

Rapeseed contains high concentrations of unsaturated fatty acids which can undergo lipid peroxidation during exposure to heat. Thus, further research to investigate the influence of the roasting treatments on the fatty acids profile of rapeseed is required. Comparable roasting experiments were conducted on sesame and sunflower seeds which studied, amongst other aspects, peroxide and carbonyl values (Yoshida, Hirakawa, Abe, & Mizushina, 2002; Yoshida, Hirakawa, Tomiyama, & Mizushina, 2003). Those studies reported the tendency for increases in both values with prolonged roasting treatments at a temperature of 135 °C after 30 min. In this study the optimal roasting temperature exceeded 135 °C. Thus, roasting may result in similar increases in the peroxide and carbonyl values in roasted rapeseed samples.

3.3. Rapeseed oils

As expected no canolol was detected in refined rapeseed oils. For both the commercially prepared kernel and cold-pressed rapeseed oil products tested no distinct differences in the canolol content was observed. The canolol content ranged from 6.7 to 81.4 μg canolol per gram of rapeseed oil (Table 5). Sinapic acid and its derivatives are believed to be the basis for the formation of canolol. Thus, the variant levels of the canolol content observed in these commercial oil products are most likely the result of the natural variant levels of sinapic acid and its derivatives in the rapeseed material used. Therefore, rapeseed with a low content of these sinapic acid related compounds produce oil products with low canolol content.

Presently, the mechanism and location of formation and storage of canolol in rapeseed has been generally unexplored. Phenolic compounds are mainly stored in seed cotyledons (Kozlowska, Naczk, Shahidi, & Zadernowski, 1990), but canolol might also be localised in the hulls. Therefore, both samples of dried seed and rape kernels were analysed in this study. No significant difference was observed concerning the canolol content (2.8 versus 3.2 μg/g, respectively). Thus, the dehulling of the rapeseed does not significantly affect the canolol content. This is supported by the similar results obtained for rape kernel and cold-pressed oil analysis in this study.

As previously stated, canolol has been reported to be one of the most potent antioxidative and antimutagenic compounds. Therefore increase of rapeseed oil canolol content would theoretically produce oil with enhanced food value and as well as longer shelf life. Often oil products are sold in clear glass containers which permit light-induced oxidation and the formation of lipidperoxyl radicals (Hawrysh, 1990). Oils purchased in clear glass flask demonstrated the lowest canolol contents (6.7 and 10.4 μg/g). A direct light-induced oxidation of canolol is unlikely. For synthesised canolol no photosensitivity was observed, when it was stored in clear glass. No additional peaks were observed in the PDA-spectra (data not shown). Due to its antioxidative activity a reaction of canolol with lipidperoxyl radicals is more likely (Kanazawa, Sawa, Akaike, & Maeda, 2000; Kanazawa, Sawa, Akaike, & Maeda, 2002; Wakamatsu et al., 2005).

Similar reasons can be stated for older oil samples. Two samples of oils of the same brand contained 9.3 and 17.7 μg canolol per gram rapeseed oil. The oil with the lower canolol content was analysed at the end of its expiration date. Natural variations can lead to different canolol contents within one brand, but differences of 50% may result from the degradation of canolol which relate to antioxidative activity and reaction with radicals.

According to Koski et al. (2003) canolol can be formed during the press process. Teutoburger Ölmühle GmbH provided this study with samples of unfiltered rapeseed oil of the first and second press. The second press process is characterised by higher temperatures and pressure. Consequently, this oil possessed higher canolol content (16.3 μg/g) than the oil of the first pressing (9.1 μg/g). These results demonstrate the potential effects of the conditions of the press process on the canolol content of the oil.

Although canolol formation may result from sinapic acid during heating, this compound is thermally instable. When the rapeseed oil with the highest canolol content (81.4 μg/g) was exposed to the heat treatment up to a temperature of 180 °C for 20 min the canolol content decreased exponentially. After 15 and 20 min only 20.2 and 11.0 μg/g canolol remained in the oil, respectively.

Table 5
Canolol content (means: canolol μg/g of rapeseed oil) of rapeseed oils depending on processing state ($n = 3$)

Processing state (number of analysed oils)	Canolol content
Refined (2)	Not detected
Rape kernel (4)	6.7–32.6
Cold-pressed (5)	9.3–81.4

4. Conclusion

Canolol, a compound of rapeseed, can be formed by decarboxylation of sinapic acid. The endothermic reaction of pure sinapic acid in water was experimentally demonstrated. The structure of canolol was confirmed by ^{1}H-NMR and mass spectrometry.

Roasting of rapeseed led to an increase of the canolol content, whereas tocopherol content of the rapeseed was not affected by this treatment. The temperature greatly influenced the amount of canolol generated in the rapeseed and an optimum temperature for canolol formation in rapeseed was determined for the roasting conditions tested. The optimum temperature of 160 °C resulted in an increase by a factor of 120 in the canolol content. Higher temperatures led to a reduction of the canolol content. The considerable loss of canolol may be ascribed to the reaction of canolol with accumulated radicals or pyrolysis and combustion reactions.

Canolol was not detectable in refined rapeseed oils. Cold-pressed and rape kernel oils tested contained from 6.7 to 81.4 μg canolol per gram rapeseed oil without distinct differences. In addition, dehulling of the rapeseed has no influence on the canolol content as no considerable differences were observed when dried rapeseed and rape kernels were analysed.

As a result of the antioxidative and antimutagenic activity of canolol, its present in rapeseed oil is desirable. Heating of rapeseed before pressing can lead to higher canolol content in the oil. Thus, high-value oil could be produced. At this point, further research to investigate the influence of heat treatments on the fatty acids profile of rapeseed is required.

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