

Changes of phenolic content in rapeseed during preliminary drying

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Abstract

Drying of rapeseed represents one of the most important elements in the complexity of actions defined as the “post-cropping” process. This work investigates how drying of the rapeseed at low and high temperature influences the bioactive compounds (phenolic acids). Samples of rapeseed (Kronos and Lisek variety) were dried at 60, 80, 100, and 120°C and low temperature (layer 2 and 12). In methanolic extracts total phenolic content was determined using Folin-Ciocalteu reagent and composition of free phenolic acids was determined by HPLC. Obtained results showed that the most unfavourable influence on the native hydrophilic antioxidant content was observed after drying at 60 and 80°C. Seed dried at these temperatures are characterized by highest decrease of total phenolic compounds and free phenolic acid content. Probably it is caused by the activity of enzymes (phenoloxidase) that display optimal functional activity at this temperature.

Key words: *Brassica napus*, phenolic compounds, drying, HPLC

Introduction

The rapeseed oil is extracted from the seed for production of edible oil, margarine, and salad dressings in the food industry and the by-product, which is rich in proteins, is used as a high-protein meal for feed in livestock production. Because of its oil content, rapeseed is extremely susceptible to spoilage, if not properly dried before or during storage. Oilseed rape is regarded as mature when the seed turned black and the moisture content of the seed is less than 15%. Harvesting too early, before seed matured sufficiently, may increase chlorophyll levels in the oil thereby reducing the quality. For storage of the harvested crop, a moisture content of 9% is required and artificial drying is required if seed has moisture above this level (Tys and Rybacki, 2001; Booth and Gunstone 2004, Janowicz, 2005). Minor bioactive constituents in rapeseed oil include polar lipids, tocopherols, sterols, carotenoids and phenolic compounds. Rapeseed is characterised by high content of phenolic compounds; their content is tenfold higher compared to other oilseeds (Zadernowski and

Kozłowska, 1983). In the future, market profitability of rapeseed oil may depend to a larger extent on content of minor bioactive constituents. Hence, there is an increasing interest in these compounds (Mollers, 2004). The most common phenolic compound is sinapic acid and its derivatives, especially sinapin, which constitutes 80% phenolic compounds in rapeseed (Kozłowska *et al.*, 1990, Lacki and Duvnjak, 1998, Zúkalova and Vaak, 1999; Thiyam *et al.*, 2004). Sinapic acid exists also as glucopyranosyl sinapate (Amarowicz and Shahidi 1994). Only a small part (<16%) of sinapic acid is present as free sinapic acid (Kozłowska *et al.*, 1990). Typically, the amount of sinapic acid derivatives in rapeseed meal varies between 6390 and 18370 µg/g, depending on the variety of the oilseed and the oil processing method (Kozłowska *et al.*, 1990). Most of the phenolic compounds are left in rapeseed meal during oil extraction. Of the phenolic compounds present in the crude rapeseed oil, the most abundant is a newly identified compound, vinylsyringol, which is a decarboxylation product of sinapic acid formed from sinapic acid during oil processing at the

elevated temperature and pressure (Koski *et al.*, 2003; Vuorela *et al.*, 2003). Its antioxidant activity is comparable to γ -tocopherol (Koski *et al.*, 2003). Drying rapeseed represents one of the most important elements in the complexity of actions defined as the “post-cropping” process. The efficiency of this process will have an impact not only on the costs of production but also on the quality of oil and post-extraction meal produced from the seed (Tys *et al.*, 2002, Tys *et al.*, 2003). Lately, attention has been paid to activity of phenolic compounds as biologically active substances which demonstrate antiviral, anticarcinogenic and anti-inflammatory activity (Vuorela *et al.*, 2005). In low concentrations, phenolic compounds act as antioxidants protecting food products against auto-oxidation. Thus, this work is aimed to investigate the content of phenolic compounds in rapeseeds which were dried at different temperatures.

Materials and Methods

Two varieties of doubly improved rapeseed (*Brassica napus* L.), Lisek and Kronos (00), were investigated. Both were dried in high as well as low temperatures till ca 6% moisture was obtained. Samples were stored at $18 \pm 1^\circ\text{C}$ in complete darkness.

Seed drying conditions

Low temperature drying of rapeseeds in a thick, motionless layer was carried out on a specially designed and constructed stand (Gawrysiak-Witulska and Ryniecki, 2001). The seed being dried on a stand built of segments, each 0.1 m high, all together forming a 1.2 m thick layer. Apparent velocity of the air flowing through the rapeseed layer was 0.14 m/s in all experiments. Relative humidity and temperature of air being sucked in by the ventilator were changing at random, like during the typical low temperature air drying. In order to avoid moistening of seed, a very simple, electronic humidistat was applied to monitor air heating and keep the relative humidity of the air blown into the seed mass at the level not exceeding 40%. Every 8h, the segments were weighed to determine

changes in seed humidity in layers 2 and 12. Drying continued until 6% humidity was reached in layer 12. Time of experiments ranged from 48 to 56 h.

High temperature drying proceeded in a laboratory drier. The seed were dried at 60, 80, 100 and 120°C , in a thin layer of ca 0.5 cm, on a fine sieve. Laboratory drier was controlled by a computer program which calculated a current seed humidity taking as a basis the mass loss and the indicated initial humidity. Drying continued until 6% moisture of the seed was obtained. Drying time was 8-10 min for 120°C , 12-14 min for 100°C , 17-20 min for 80°C and 35-40 min for 60°C .

Methanol extract preparation

All samples were defatted by shaking six times at room temperature with hexane followed by Soxhlet extraction. To obtain rapeseed phenols, each sample was extracted three times with 80% methanol. The samples were mixed with the solvent (1:3), shaken for 30 min, filtered through anhydrous sodium sulfate, and vacuum-evaporated. The residue was dissolved in 80% methanol.

Total content of phenolic compounds

The content of total phenolic compounds in methanolic extract was determined by the Folin-Ciocalteu method. An aliquot (0.2 cm^3) of the methanolic extract was placed in a volumetric flask (10 cm^3). Diluted Folin-Ciocalteu reagent (0.5 cm^3) was added. After 3 min, saturated sodium carbonate (1 cm^3) was added. The flask was filled with water up to 10 cm^3 . After 1 h, absorbance at $\lambda_{\text{max}} 725\text{ nm}$ against a reagent blank was measured using a UV-Vis spectrophotometer SP 8001 (Metertech Inc., Taipei, Taiwan). Total phenolic compounds were determined after preparation of a standard curve and on that basis total phenolic compounds were measured as sinapic acid equivalents.

Composition of phenolic acid

The analysis of phenolic acid present in methanolic extracts was performed by RP-HPLC. Crude seed extracts were purified and phenolic acids were isolated on a quaternary amine Bakerbond SPE

column (GBowniak *et al.* 1996). Separation and identification of phenolic acids were carried out by high performance liquid chromatography (HPLC – Waters Milford, MA, USA). For separation, a NovaPak[®]C₁₈ (3.9 x 150 mm; 5µm) column was used. Solvent (A) was methanol; solvent (B) – 2.5% acetic acid in water. The flow rate was 1 cm³/min. The gradient profile was: 10% (A) (0 – 10 min); 10 – 20% (A) (10 – 22 min); 20 – 70% (A) (22 – 45 min). The chromatograms were recorded at 250 and 320nm (UV-VIS Waters). The identification was carried out by retention time and the amount of phenolic acid was determined using external and internal standards of the individual phenolic acids.

Statistical analysis

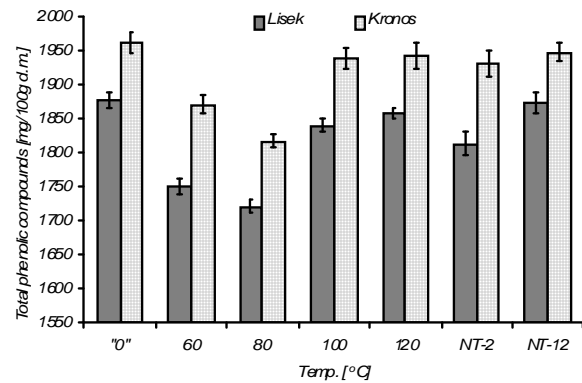
Results are presented as means ± standard deviation from three replicates of each experiment. A *P*-value < 0.05 was used to denote significant differences among mean values determined by the analysis of variance (ANOVA) with the assistance of statistical package Statistica 7.1 (StatSoft, Inc., Tulsa, OK) software.

Results and Discussion

Extraction in aqueous methanol is the most common extraction method. Cai and Arntfield (2001) found refluxing with 100% methanol to be as effective as extraction using 70% methanol at 75°C. According to Naczka *et al.* (1992), 70% aqueous methanol is twice as efficient in extracting rapeseed phenolics as is pure methanol. However, the solvent-to-meal ratio was lower, which, according to Cai and Arntfield (2001), may be one reason for this difference. Wang *et al.* (1998) investigated the effect of number of extractions and the extraction time on the recovery of extracted phenolics varying the number of extractions between 1 and 5 and using extraction time of 10, 30, 60, and 90 min. They found no statistical differences in the amount of extracted phenolics with the number of extractions, or the extraction time.

On the basis of the obtained results, it was found that Kronos variety had higher total phenolic content (1962.1 mg/100g d.m.) than Lisek (1876.3 mg/100g d.m.), which is shown in fig. 1. Drying seed until assessing humidity of about 6% caused the highest decrease of total phenolic content in seed

dried at 60 and 80°C (by 6.7 and 8.2% also 4.7 and 7.4% for Lisek and Kronos, respectively (fig. 1).



“0” – not dried; NT-2 – low temperature drying layer 2; NT-12 – low temperature drying layer 12

Figure 1: Total phenolic compounds in rapeseed variety – Lisek

The lowest decrease of total phenolic content (1%) was observed in seed dried at low temperature (layer 12). Shahidi and Naczka (1992) reported phenolic compounds content in rapeseed meal at the level of 1080.2 – 1807 mg/100g. Cai and Arntfield (2001) obtained 17.2 to 22.9 mg/g total phenolic compounds in rapeseed meal depending on the temperature and time of extraction using 70% and 100% methanol. Amarowicz *et al.* (2000) examined antioxidant properties of tannin extract in rapeseed hulls, obtained from 128 to 296 mg/g of total phenolic compounds as sinapic acid equivalent.

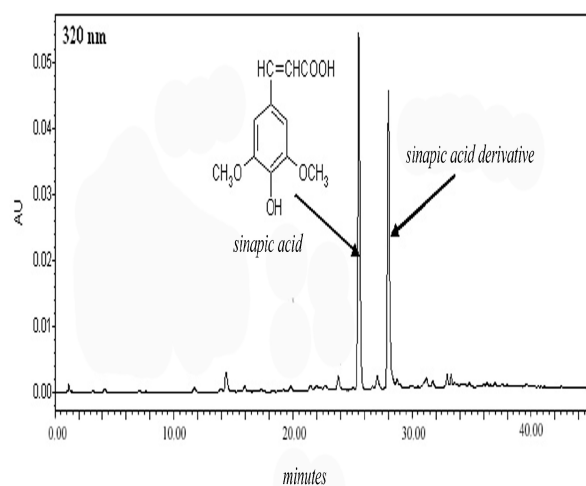


Figure 2: HPLC chromatogram of phenolic acids

Free phenolic acid content of not-stored seed of sampled varieties are given in table 1, 2. Example of the chromatograph is presented in fig 2.

The predominant phenolic acid was sinapic acid and its low molecule derivative. Drying caused decrease of free phenolic acid content. The highest decrease

Table 1: Free phenolic acids contents in rapeseed variety Lisek

Temp. [°C]	Free phenolic acids [mg/100g d.m.]								Sum
	Protocatechuic	p-hydroxybenzoic	Vanillic	Caffeic	p-coumaric	Ferulic	Sinapic	Sinapic acid derivative	
0	0.15 ±0.05 ^a	0.04 ±0.001 ^{b,c}	0.45 ±0.08 ^a	1.97 ±0.10 ^c	0.18 ±0.01 ^d	0.37 ±0.01 ^c	25.20 ±0.15 ^e	46.62 ±0.14 ^f	74.98 ±0.54 ^f
60°C	0.11 ±0.01 ^a	0.04 ±0.002 ^{b,c}	0.30 ±0.09 ^a	1.04 ±0.12 ^a	0.09 ±0.01 ^{b,c}	0.32 ±0.03 ^{b,c}	18.58 ±0.10 ^a	41.65 ±0.15 ^c	62.13 ±0.51 ^a
80°C	0.07 ±0.07 ^a	0.04 ±0.003 ^{b,c}	0.39 ±0.07 ^a	1.20 ±0.08 ^{ab}	0.07 ±0.02 ^{ab,c}	0.21 ±0.04 ^a	19.14 ±0.08 ^b	40.77 ±0.08 ^b	61.89 ±0.44 ^a
100°C	0.10 ±0.08 ^a	0.05 ±0.004 ^c	0.31 ±0.02 ^a	1.09 ±0.09 ^{ab}	0.04 ±0.01 ^a	0.35 ±0.02 ^{b,c}	24.59 ±0.10 ^d	45.25 ±0.16 ^e	71.78 ±0.48 ^e
120°C	0.13 ±0.01 ^a	0.05 ±0.005 ^c	0.44 ±0.04 ^a	1.28 ±0.11 ^{ab}	0.10 ±0.02 ^c	0.35 ±0.01 ^{b,c}	24.78 ±0.07 ^d	40.40 ±0.17 ^a	67.53 ±0.44 ^d
NT-2	0.14 ±0.07 ^a	0.02 ±0.007 ^a	0.41 ±0.05 ^a	1.32 ±0.07 ^b	0.06 ±0.01 ^{ab}	0.29 ±0.05 ^b	20.41 ±0.12 ^c	41.62 ±0.09 ^c	64.27 ±0.47 ^b
NT-12	0.11 ±0.02 ^a	0.03 ±0.006 ^{ab}	0.28 ±0.07 ^a	1.23 ±0.03 ^{ab}	0.06 ±0.01 ^{ab}	0.30 ±0.01 ^{b,c}	20.23 ±0.13 ^c	43.68 ±0.11 ^d	65.92 ±0.39 ^c

“0” – not dried; NT-2 – low temperature drying layer 2; NT-12 – low temperature drying layer 12; Data presents mean values from three replicates; (a,b,c,d,e,f) mean values followed by different letters are statistically significant at $p \leq 0.05$

of sinapic acid was observed in samples dried at 60°C. Decrease was equal to 5.2 and 6.6 mg/100g d.m. for Kronos and Lisek, respectively. The lowest decrement of free phenolic compounds content was observed in samples dried at 100 and 120°C (1.5 and 2.8 mg/100g d.m. for Kronos and 0.6 and 0.4

mg/100g d.m. for Lisek). Similarly, sinapic acid derivative content is characterized by the lowest decrement in the Kronos seed dried at 120°C. (decrease by 2.4 mg/100g d.m.) and Lisek dried seed at 100°C (decrease by 1.4 mg/100g d.m.). Other phenolic acids occurred in small amounts –

Table 2: Free phenolic acids contents in rapeseed variety Kronos

Temp. [°C]	Free phenolic acids [mg/100g d.m.]								Sum
	Protocatechuic	p-hydroxybenzoic	Vanillic	Caffeic	p-coumaric	Ferulic	Sinapic	Sinapic acid derivative	
0	0.15 ±0.01 ^a	0.05 ±0.001 ^b	1.47 ±0.04 ^e	2.05 ±0.08 ^d	0.08 ±0.01 ^c	0.36 ±0.03 ^b	29.10 ±0.14 ^e	52.31 ±0.10 ^f	85.57 ±0.40 ^e
60°C	0.15 ±0.04 ^a	0.05 ±0.002 ^b	1.14 ±0.07 ^c	1.98 ±0.10 ^d	0.03 ±0.01 ^{a,b,c}	0.34 ±0.03 ^b	23.86 ±0.10 ^a	42.98 ±0.13 ^a	70.53 ±0.48 ^a
80°C	0.14 ±0.03 ^a	0.03 ±0.007 ^a	1.34 ±0.01 ^d	1.46 ±0.11 ^b	0.01 ±0.02 ^a	0.35 ±0.01 ^b	25.13 ±0.09 ^b	44.20 ±0.08 ^b	71.66 ±0.36 ^a
100°C	0.14 ±0.06 ^a	0.05 ±0.006 ^b	0.69 ±0.04 ^a	1.68 ±0.07 ^c	0.04 ±0.01 ^{a,b,c}	0.36 ±0.01 ^b	27.56 ±0.15 ^d	44.44 ±0.09 ^b	74.96 ±0.44 ^b
120°C	0.13 ±0.01 ^a	0.05 ±0.001 ^b	0.65 ±0.03 ^a	1.79 ±0.12 ^{c,d}	0.07 ±0.02 ^{b,c}	0.36 ±0.03 ^b	26.26 ±0.07 ^c	49.88 ±0.10 ^e	79.19 ±0.37 ^d
NT-2	0.12 ±0.03 ^a	0.05 ±0.003 ^b	0.92 ±0.06 ^b	1.24 ±0.09 ^a	0.05 ±0.01 ^{a,b,c}	0.28 ±0.02 ^a	25.08 ±0.08 ^b	48.97 ±0.12 ^d	76.71 ±0.41 ^c
NT-12	0.12 ±0.03 ^a	0.02 ±0.004 ^a	0.75 ±0.01 ^a	1.21 ±0.14 ^a	0.06 ±0.03 ^{b,c}	0.31 ±0.01 ^{ab}	26.48 ±0.10 ^c	47.23 ±0.09 ^c	76.18 ±0.41 ^c

“0” – not dried; NT-2 – low temperature drying layer 2; NT-12 – low temperature drying layer 12; Data presents mean values from three replicates; (a,b,c,d,e,f) mean values followed by different letters are statistically significant at $p \leq 0.05$

less than 1 mg/100g d.m. with exception of the caffeic acid, the amount of which was about 2 mg/100g d.m. for seed of both varieties. Total phenolic acid content was greater in the Kronos variety (85.5 mg/100g d.m.), i.e., about 10 mg/100g d.m. more than in Lisek (74.9 mg/100g d.m.). The greatest decrease of total phenolic content was observed in seed dried at 60 and 80°C (table 1, 2). KozBowska *et al.* (1983b) also achieved the greatest content of sinapic acid (41.3 mg/100g) in rapeseed meal received from high erucic acid seed. Cai and Arntfield (2001) studied the content of sinapic acid in rapeseed meal which was extracted using methanol in different concentrations and at different temperatures. The level of sinapic acid varied from 0.34 mg/g (100% methanol, 75°C, 20 min.) to 0.40 mg/g (70% methanol, 75°C, 20 min.). Also KozBowska *et al.* (1983b) obtained similar levels of sinapic acid content (41.3 – 51.6 mg/100g) in the rapeseed and mustard (4.5 mg/100g). Shahidi and Naczki (1992) report sinapic acid content in rapeseed at the level of 27.6 – 67.7 mg/100g. Second dominant peak in the chromatogram (fig. 2) was a low molecular sinapic acid derivative. Most of sampled varieties were characterized by higher content of the sinapic acid derivative than of the sinapic acid itself (table 1-2). Hydroxycinnamic acid derivatives have been shown to exhibit a stronger inhibition of pancreatic lipase activity than hydroxybenzoic acid derivatives.

The inhibitory effect is influenced by the position of hydroxyl groups and the presence of methoxy groups. Phenolic acid with methoxy groups such as sinapic and syringic acids are the weakest inhibitors of lipase activity (Naczki *et al.*, 1998). Moisture content ~8-9% at temperatures below 20°C is necessary for safe storage.

The drying of rapeseed must be completed fast enough to prevent rapid product deterioration by microorganisms and enzymes. With the increasing supply of rapeseed as a source of edible oil, the quantity dried and stored and the time for which it may remain in store are also increasing. Drying process caused decrease in content of total phenolic compound in the analyzed rapeseed samples, especially in seed dried at 60 and 80°C. During storage we observed further decrease in content of

total free phenolic compounds. Probably it is caused by activity of enzymes (phenoloxidases) that show optimal functional activity in these temperatures.

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