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ORIGINAL PAPER

Effect of Hydrothermal Treatment of Rapeseed on Antioxidant Capacity of the Pressed Rapeseed Oil

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Abstract Response surface methodology (RSM) was used to evaluate the quantitative effects of two independent variables, rapeseed moisture content and conditioning temperature, on the antioxidant capacity (AC) and total phenolic (TPC), tocopherol (TTC), and phosphorus contents (PC) in the pressed rapeseed oils. The mean AC results for the crude rapeseed oils ranged from 199.8 to 947.2 μmolTE/100 g. TPC and PC in the crude rapeseed oils correlated significantly ($P < 0.01$) and positively with AC of oils ($R^2 = 0.9498$ and 0.4396, respectively). The experimental results of AC, TPC, and PC were close to the predicted values calculated from the polynomial response surface model equations ($R^2 = 0.9801$, 0.9747 and 0.9165, respectively). The effect of oil processing temperature on AC and TPC was about 1.5 times greater than the effect of moisture level in rapeseed.

Keywords Rapeseed · Crude rapeseed oils · Antioxidant capacity · Response surface methodology

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Introduction

Cold-pressed and refined rapeseed oils are frequently used in Eastern and Northern Europe for consumption, cooking, and as an ingredient in food products. Traditional production of rapeseed oil is based on seed crushing before oil separation. The overall process involves seed cleaning, preheating, flaking, cooking, mechanically heated screw-pressing, and solvent extraction with hexane. The oil obtained by pressing, with or without solvent extraction (crude oil), has a dark color and characteristic fragrance and taste. Oil from this stage is refined because consumers prefer oil with bland aroma and light yellow color. However, antioxidant capacity (AC) and total phenolics (TPC) in the unrefined rapeseed oils are higher than in refined oils [1, 2]. Moreover, cold-pressed oil with its high dietary functions can be consumed unrefined [3]. Recently, cold-pressed rapeseed oil has been introduced into the market; therefore improvements in oilseed processing are necessary to produce oil with high AC.

Rapeseed oil contains similar amounts of monounsaturated fatty acids as olive oil. However, rapeseed oil is a richer source of omega-3 fatty acids than olive oil. Moreover, the correct ratio of omega-6 to omega-3 fatty acids (2.2) for human health occurs natively in rapeseed oil [4]. Additionally, polyphenols, tocopherols, and other similar compounds present in rapeseeds and rapeseed oil exhibit antioxidant capacity (AC). Hence, these phenolic compounds are important in the prevention and treatment of some worldwide health-related problems such as heart diseases, neurodegenerative diseases, and rheumatoid arthritis [5, 6]. Moreover, rapeseed phenolics possess effective properties such as free-radical scavengers and antimicrobial activities. These properties are mostly attributed to such components as sinapic acid, sinapine,

vinylsyringol, choline ester, and the decarboxylation products of sinapic acid [7].

Recently, different analytical methods for determination of phenolics in rapeseeds, rapeseed oils, and their AC were applied. Among them, spectrophotometric methods including DPPH (2,2′-diphenyl-1-picrylhydrazyl) [3, 5, 6, 8, 9], FRAP (ferric-reducing antioxidant power) [1, 10], and ORAC (oxygen radical absorbance capacity) assays [1, 11] were proposed for the evaluation of the antioxidant activities of rapeseed oils. However, for separation, identification, and quantification of the individual phenolic compounds, chromatographic techniques are required [2, 3, 5, 6, 9]. Reported AC values of rapeseed oils range from 40 to 1,106 μmol/100 g, depending on the technology and analytical methods used [1, 5, 10, 11]. Also, the spectrophotometric Folin-Ciocalteu's procedure has been used for determination of TPC in rapeseed and its oils [1, 3, 8].

It has been shown that consumption of antioxidant-rich foods is associated with a lower risk of heart diseases, ischemic stroke, cancer, and other chronic diseases. Although phenolics are relatively thermally stable compounds, the technological processes applied in the production of rapeseed oil affect the level of AC and TPC of the final product [1, 2, 8]. The increases in health-promoting factors are the main reasons for the optimization of processing parameters in order to produce high-quality rapeseed oil. The impact of optional tempering and hulling treatments of the seed [12], drying temperature [13], and processing conditions [14, 15] on rapeseed oil quality have been studied. It was determined that the oxidative stability increased and the triglyceride content decreased in pressed rapeseed oil with increased heat treatment of the seed before pressing. Also, it was reported that the phosphorus and chlorophyll contents in cold-pressed rapeseed oil of 13 and 7 ppm, respectively, were much lower than those obtained from hot-pressed oil at 64 and 68 ppm, respectively. Furthermore, phosphorus content was correlated with oxidative stability ($R^2 > 0.99$) [14, 15].

To the best of our knowledge, only Guderjan et al. [8], by using pulsed electric fields (PEF), examined the influence of heat on rapeseed oil yield and total content of antioxidants, including tocopherols, polyphenols, and phytosterols. However, the impact of heating conditions on the AC of pressed rapeseed oil has not been reported.

Therefore, this work was focused on determining the effect of hydrothermal treatment on the crude oil quality of rapeseed before pressing in order to produce edible rapeseed oil with a maximum content of AC and total phenolic compounds (TPC). Residual oil (RO) content in the cake was also analyzed and correlations among TPC, TTC, PC, and AC of the pressed rapeseed oils were studied and discussed.

Experimental Procedures

Reagents

All reagents were of analytical or HPLC grade. 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ, 99%); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox (TE), 97%]; Folin-Ciocalteu reagent (FC reagent, 2N); caffeic acid (CA, 98%); α-, β-, γ-, and δ-tocopherols; iron(III) chloride hexahydrate; sodium acetate; sodium carbonate; ammonium molybdate; ammonium metavanadate; magnesium oxide; acetic acid; hydrochloric acid; nitric acid; methanol (99.8%); hexane (99%); and tetrahydrofuran (THF, 99.8%) were purchased from Sigma-Aldrich. Redistilled water was used for preparation of solutions.

Materials

Seeds of *Brassica napus* var. *oleifera* from the 2007 harvest were provided by a local vegetable-oil factory. Seeds were stored in the dark at ambient temperature until treatment and further analysis.

Oil Pressing

Whole rapeseeds with 37.8% oil and 6.4% moisture were equilibrated at room temperature in closed containers for 24 h to 7, 9, and 11% moisture contents. Then, the seeds were ground using a Retsch grinder (1.5 mm) and the flour transferred to a jacketed glass reactor connected to an oil bath. The rapeseed flour was stirred and heated to the experimental temperatures of 90, 105, and 120°C. After 30 min, the oil was pressed from the flour on a small capacity hydraulic press. The obtained crude rapeseed oil was stored in closed glass bottles below 10°C in the dark.

Methods

Determination of Antioxidant Capacity

The extracts of the crude pressed oils were obtained in methanol as described previously [1]. The spectrophotometric FRAP method proposed by Szydłowska et al. [1] was used for AC determination of the crude-pressed rapeseed oils. Briefly, the FRAP reagent (2.5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.1 mol/L acetate buffer, pH 3.6) was heated at 37°C for 15 min. Then, 0.05, 0.15, or 0.3 mL of methanolic extracts of oil samples and 2 mL of FRAP reagent were transferred to a 10-mL volumetric flask and brought up to the volume with redistilled water. The blue solutions obtained were kept at room temperature for

20 min, and absorbance was measured at 593 nm and compared to a reagent blank using a Cary 50 Scan UV–Vis spectrophotometer in a 1-cm quartz cuvette.

Determination of Total Phenolics

Total phenolic content was determined spectrophotometrically at 725 nm using the Folin-Ciocalteu reagent, according to the procedure described by Haiyan et al. [16].

Determination of Total Phosphorus

Total phosphorus content in the crude rapeseed oils was determined according to the official (vanadomolybdate) method PN-88/A-86930 [17]. Five milliliters of HNO_3 (6 mol/L), 10 mL ammonium molybdate (4.05×10^{-2} mol/L), and 10 mL of ammonium metavanadate were added to ashed samples, and the absorbance was measured at 460 nm against a reagent blank after 20 min.

Determination of Tocopherols

Tocopherol content was determined according to the Bunge Europe Research and Development Center in-house method [1]. Oil samples were dissolved in hexane (0.5000 g in 5 mL) and injected (5–20 μL) into a LiChrospher 100 Diol (125 × 4 mm, 5-μm particle size, Agilent Technologies, Palo Alto, CA, USA) column and analyzed by an Agilent 1100 HPLC system with an autosampler and fluorescence detector (FLD). The mobile phase was hexane with tetrahydrofuran (96:4 v/v) and a flow rate of 0.8 mL/min. Excitation and emission wavelengths at 280 and 340 nm, respectively, were used. The concentrations were calculated from the calibration curves prepared for α-, β-, γ-, and δ-tocopherol isomers.

Determination of Oil Content in Pressed Cake

Cake residual oil was determined by solvent extraction according to AOCS method Ba 3–38 [18].

Experimental Design and Statistical Analysis

The AC of the studied crude rapeseed oils were determined (five portions of each oil extracted with methanol, analyzed within 1 day) by the FRAP method. The results of AC, TPC, TTC, and PC obtained in the study are presented as mean (c) ± confidence limits (μ). The Pearson correlation test was used to determine the correlations between FRAP and TPC, TTC, and PC results in the pressed rapeseed oils. Mean differences were considered significant at the $P < 0.05$ level.

Response surface methodology (RSM) was used to study the simultaneous effects of the rapeseed moisture content (MC) and the temperature (T) of its conditioning on AC, TPC, TTC, and PC in the pressed rapeseed oils. RSM allowed the development of a predictive mathematical model based on the experimental data, which can be employed for interpolation.

The experimental design employed for the analysis was a central composite design with two factors and three levels. In this experimental design, there were three coded factor levels: −1, 0, +1 where −1 corresponds to the low level of each factor, +1 to the high level, and 0 to the mid-level. The factors and respective coded and uncoded levels are given in Table 1. The experiments consisted of 14 runs with two factors and five replicates of the central point for the estimation of pure error (Table 1). The effect of the two independent variables (X_1-moisture content and X_2-temperature) on the responses (Y_n, AC-Y_1, TPC-Y_2, TTC-Y_3, and PC-Y_4) was modeled using a polynomial response surface. The second-order response function for the experiments was predicted by the following equation:

$$Y_n = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11}(X_1)^2 + \beta_{22}(X_2)^2 + \beta_{12} X_1 X_2 \quad (1)$$

where

Y_n	is one of the four responses.
X_1 and X_2	represent the independent variables.
β_0	is the constant.
β_1, β_2	are the linear-term coefficients.
β_{11}, β_{22}	are the quadratic-term coefficients.
β_{12}	is the cross-term coefficient.

The fitness of the model was evaluated by the determination coefficient R^2, the fraction of the variation explained by the model, and by analysis of variance (ANOVA). The F test was applied to confirm whether the variance explained by the regression model was significantly larger than the variance of the residual and to evaluate the model lack of fit (model error).

The effects of two factors (X_1-MC and X_2-T) and their interactions on AC, TPC, TTC, and PC in the pressed rapeseed oils were evaluated using Pareto charts, which present important factors on the responses in the form of graphs. Moreover, the effects of the variables were displayed in surface and contour plots.

Regression and analysis of variance (ANOVA) were carried out using the Statistica Windows software package.

Table 1 Central composite design and experimental results for the responses: antioxidant capacity (FRAP) and total phenolic (TPC), tocopherol (TTC), and phosphorus (PC) contents in the crude rapeseed oils

Exp.	Coded level		Independent variables (actual values)		Dependent variables (experimental data)							
	X_1	X_2	Moisture content (%)	Temperature (°C)	FRAP[a] ± μ (μmolTE/100 g)	RSD (%)	TPC[a] ± μ (mg CA/kg)	RSD (%)	TTC[a] ± μ (mg/kg)	RSD (%)	PC[a] ± μ (mg/kg)	RSD (%)
1	−1	−1	7	90	199.8 ± 4.8	1.92	114.3 ± 4.3	2.97	730 ± 6	0.68	91 ± 3	2.58
2	−1	0	7	105	235.1 ± 5.3	1.82	137.5 ± 4.4	2.55	740 ± 10	1.07	171 ± 4	2.07
3	−1	+1	7	120	499.2 ± 11.5	1.86	359.4 ± 4.5	1.00	730 ± 11	1.22	208 ± 6	2.15
4	0	−1	9	90	246.6 ± 3.9	1.26	181.6 ± 1.3	5.62	720 ± 10	1.09	117 ± 5	3.20
5	0	0	9	105	478.5 ± 14.8	2.49	274.5 ± 16.9	4.95	730 ± 10	1.08	326 ± 6	1.58
6	0	+1	9	120	821.5 ± 13.6	1.34	508.6 ± 26.1	4.13	710 ± 7	0.80	313 ± 9	2.40
7	+1	−1	11	90	302.9 ± 4.3	1.15	197.1 ± 6.4	2.64	715 ± 7	0.77	388 ± 10	2.08
8	+1	0	11	105	625.9 ± 18.4	2.36	429.7 ± 26.7	5.00	710 ± 8	0.95	431 ± 7	1.32
9	+1	+1	11	120	947.2 ± 17.5	1.49	647.9 ± 47.7	5.93	720 ± 5	0.54	444 ± 7	1.30
10	0	0	9	105	411.6 ± 9.7	1.89	235.0 ± 6.7	2.29	730 ± 5	0.59	331 ± 11	2.60
11	0	0	9	105	439.2 ± 5.4	0.98	268.8 ± 3.6	1.08	725 ± 6	0.69	325 ± 8	2.10
12	0	0	9	105	445.4 ± 7.6	1.38	259.4 ± 8.8	2.72	730 ± 6	0.63	311 ± 7	1.72
13	0	0	9	105	475.2 ± 4.4	0.74	241.9 ± 10.2	3.39	730 ± 8	0.94	359 ± 8	1.87
14	0	0	9	105	466.1 ± 8.6	1.48	230.4 ± 13.9	4.87	730 ± 11	1.18	357 ± 5	1.18

[a] $n = 5$; μ confidence limit; $P = 0.05$

Results and Discussion

Composition Analysis of the Crude Rapeseed Oils

The results of AC, TPC, TTC, and PC in the crude rapeseed oils, obtained from flour after hydrothermal treatment are listed in Table 1. The rapeseed oil pressed from 120°C-heated flour with 11% humidity revealed the highest FRAP (947.2 μmol TE/100 g), TPC (647.9 mg CA/kg), and PC (444 mg/kg) values. AC (199.8 μmol TE/100 g), TPC (114.3 mg CA/kg), and PC (91 mg/kg) were the lowest for the oil obtained at $T = 90$°C and MC = 7%. These results suggest that AC, TPC, and PC in the resulting oils increased with an increase in temperature of flour conditioning and moisture before pressing (Table 1). For comparison, AC of cold-pressed rapeseed oils reported by others [1, 5, 11] were in a somewhat lower range (80–297 μmol TE/100 g). Moreover, AC and TPC in oil extracted from hulled and nonhulled rapeseeds after PEF treatment increased significantly from 45 to 67% and from 50 to 185 mg/L, respectively [8]. However, TPC determined in the pressed rapeseed oils (Table 1) were lower than in the post-expelled crude rapeseed oil (1,066 mg CA/kg) [2] but similar to results (439 mg sinapic acid/kg) reported by Vuorela et al. [7].

The PC levels discussed here were similar to or somewhat higher than those obtained by other authors (120–291 mg/kg) [19, 20], although Prior et al. [14] obtained a significantly lower content of phosphorus in rapeseed oils (12.6–64.2 mg/kg). The PC in rapeseed oil was reduced by decreasing the moisture content in seeds before oil pressing [14]. Phospholipids possess antioxidative properties and enhance the activity of antioxidants (tocopherols, phenolic compounds) present in oil. Antioxidants show synergism with phospholipids [21]. The most important antioxidants in rapeseeds are tocopherols [8], and their contents in rapeseed oils pressed from hydrothermally treated flour ranged from 710 to 740 mg/kg (Table 1). The three isomers, α-tocopherol (280–320 mg/kg), γ-tocopherol (410–420 mg/kg), and δ-tocopherol (10 mg/kg) were found in all pressed oils, whereas β-tocopherol was not detected. Similar concentrations of individual α-, γ-, and δ-tocopherols (295–300, 400–404, and 15 mg/kg, respectively) have been reported [2, 8]. However, TTC in the oils in this study were somewhat higher than Tuberoso et al. [5] (625 mg/kg), Goffman and Möllers [22] (650–707 mg/kg), and Prior et al. [14] (472–642 mg/kg).

Rapeseed oil pressed from hydrothermally treated flour (MC = 7% and $T = 90$°C) had fivefold lower FRAP, TPC, and PC values than the crude rapeseed oil extruded from flour with 11% moisture and heated to 120°C (Table 1). The values of RSD ranged between 0.54 and 5.93% (Table 1), indicating reasonable repeatability of the AC, TPC, TTC, and PC determinations in all studied oils.

Correlation of Antioxidant Capacity with Antioxidant Content

Regression analysis was performed for correlation among TPC, TTC, PC, and AC of the pressed-crude rapeseed oils.

The results of TPC in all oils correlated significantly positively with their AC determined by FRAP method ($R^2 = 0.9498$, $P < 0.00001$) (Fig. 1). Also, significant positive correlation ($R^2 = 0.4396$, $P < 0.01$) between PC values and FRAP of the studied crude rapeseed oils was observed (Fig. 1). This correlation confirms moderate antioxidant activity of phospholipids, especially in the presence of phenolic antioxidants and/or acidic synergists [21]. Furthermore, the obtained results indicate a nonsignificant negative correlation ($R^2 = 0.2577$, $P = 0.064$) between TTC in all pressed oils and their FRAP values (Fig. 1). Also, Hay et al. [11] did not find a linear correlation between TTC and antioxidant capacity determined by the ORAC method ($R^2 = 0.375$). Tuberoso et al. [5] demonstrated significant correlations between free-radical scavenging activity of different vegetable oils analyzed by the DPPH method and TTC ($R^2 = 0.4900$ and 0.5625 for methanolic and ethyl acetate extracts).

Fitting the Models

Response surface analysis was applied and the second-order polynomial response surface models were fitted to each response variable: FRAP, TPC, TTC, and PC (Table 1). Regression analysis and analysis of variance (ANOVA) of the experimental data (Table 1) were performed for the mathematical-model fitting, determination of regression coefficients, and statistical significance examination of the model terms. Multiple regression coefficients were determined by the least-squares technique to predict quadratic polynomial models for the studied response variables. The following regression equations were obtained:

$$\mathrm{FRAP} = 3430.5 - 71.43 \cdot X_1 - 74.14 \cdot X_2 - 8.437 \cdot X_1^2 + 0.3102 \cdot X_2^2 + 2.874 \cdot X_1 \cdot X_2 \quad (2)$$

$$\mathrm{TPC} = 3867.7 - 168.1 \cdot X_1 - 70.47 \cdot X_2 + 2.412 \cdot X_1^2 + 0.3162 \cdot X_2^2 + 1.714 \cdot X_1 \cdot X_2 \quad (3)$$

$$\mathrm{TTC} = 456.50 - 17.56 \cdot X_1 + 7.118 \cdot X_2 + 0.4779 \cdot X_1^2 - 0.0359 \cdot X_2^2 + 0.0417 \cdot X_1 \cdot X_2 \quad (4)$$

$$\mathrm{PC} = -4429.3 + 56.59 \cdot X_1 + 75.90 \cdot X_2 + 3.493 \cdot X_1^2 - 0.3201 \cdot X_2^2 - 0.5083 \cdot X_1 \cdot X_2 \quad (5)$$

The ANOVA results describe the effect and the regression coefficients of the individual linear, quadratic, and interaction terms that were individually determined. The quadratic polynomial models represent responses of AC, TPC, and PC in the pressed oils with the coefficients of determination, $R^2 = 0.9801$, 0.9747, and 0.9165, respectively (Table 2).

The R^2 values for these response variables were above 0.90, thus ensuring a satisfactory fit of the regression models to the experimental data. However, the R^2 value for the response of TTC in the crude rapeseed oils was 0.7072, suggesting that a high proportion of variability (29%) cannot be explained by the model (Table 2) because R^2 should be at least 0.80 for a good fit [23]. Moreover, R^2 and the adjusted R^2 values for studied response variables (except TTC) were higher than 0.80 (Table 2), hence there is a close agreement between the experimental results and theoretical values predicted by the proposed models.

The model adequacy was tested using the lack-of-fit F test, which was not significant for $P > 0.05$. The ANOVA results of responses for AC and TPC revealed insignificant lack of fit ($F = 4.27$ and 5.41, $P > 0.05$, respectively) (Table 2). Therefore, these models were adequate for prediction within the range of variables employed. However, significant lack of fit of the models for PC and TTC indicates that other factors are affecting the total phosphorus and tocopherol contents in the studied oils. The model error of PC, however, is not significant in relation to the pure error at the 0.01 level, as is evident from the calculated F value (10.27; $F_{5,3,0.01} = 12.06$).

Moreover, the linear and quadratic effects of the independent variables (X_1-MC and X_2-T) and their interactions on the response variables (FRAP, TPC, TTC, PC) were analyzed by ANOVA and are shown in a Pareto chart (Fig. 2).

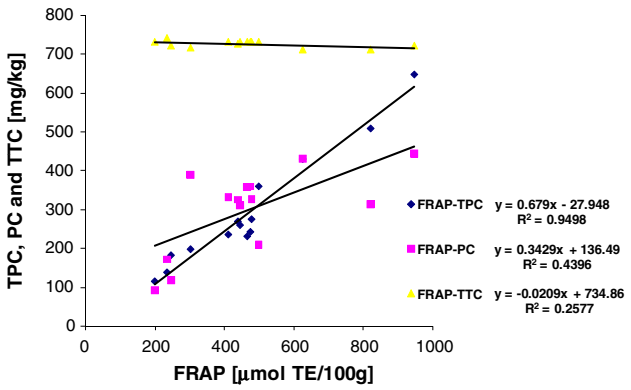

Fig. 1 Correlations between total phenolic (*TPC*), phosphorus (*PC*), and tocopherol (*TTC*) contents in the crude rapeseed oils and their antioxidant capacities (*FRAP*)

Table 2 ANOVA results for the responses: FRAP, TPC, TTC, and PC in the pressed rapeseed oils

Model parameters	Degrees of freedom	Sum of squares	Mean square	*F* value
Antioxidant capacity (FRAP)				
Regression	5	575,839.1	115,167.8	176.7**
Residual	8	11,670.9	1,458.9	
Lack-of-fit	3	8,394.7	2,798.2	4.27
Pure error	5	3,276.2	655.2	
Total	13	587,510.0		
R^2		0.9801		
Adjusted R^2		0.9677		
Total phenolic content (TPC)				
Regression	5	277,972.7	55,594.5	160.6**
Residual	8	7,214.0	901.8	
Lack-of-fit	3	5,513.8	1,837.9	5.41
Pure error	5	1,700.2	340.0	
Total	13	285,186.7		
R^2		0.9747		
Adjusted R^2		0.9589		
Total tocopherol content (TTC)				
Regression	5	707.3	141.5	34.10*
Residual	8	292.7	36.59	
Lack-of-fit	3	271.9	90.65	21.75*
Pure error	5	20.8	4.17	
Total	13	1,000.0		
R^2		0.7072		
Adjusted R^2		0.5243		
Total phosphorus content (PC)				
Regression	5	144,014.2	28,802.8	78.39**
Residual	8	13,127.8	1,641.0	
Lack-of-fit	3	11,295.0	3,765.0	10.27*
Pure error	5	1,832.8	366.6	
Total	13	157,142.0		
R^2		0.9165		
Adjusted R^2		0.8643		

*$P < 0.05$ level, **$P < 0.001$ level

The standardized Pareto charts for each of the four responses illustrate the importance and the statistical significance of the studied parameters in the models. The length of each bar (Fig. 2) is proportional to the absolute value of the associated regression coefficient (Table 2) and to the estimated main effect. The vertical line on the chart corresponds to the 95% limit, indicating statistical significance. A positive or negative sign demonstrates the enhanced or reduced response, respectively, when passing from the lowest to the highest level of a specific variable. The model is highly significant when the computed *F* value is greater than the tabulated and the probability value is low ($P < 0.0001$). Both linear (X_1, X_2), quadratic (X_2^2) and interaction ($X_1 \times X_2$) parameters of the models were highly significant (*F* values ranged between 586.6 and 21.07, $P < 0.001$ and $P < 0.05$) for AC and TPC in the studied pressed oils (Fig. 2a, b). However, the interaction between X_1 and X_2 did not produce a significant effect on TTC ($F = 1.50$, $P > 0.05$) and PC ($F = 2.54$, $P > 0.05$) in the crude rapeseed oils (Fig. 2c, d). The variable with the largest positive effect on AC ($F = 586.6$, $P < 0.001$) and TPC ($F = 512.9$, $P < 0.001$) was the linear term of temperature (X_2), while moisture content (X_1) in rapeseeds was most effective on PC ($F = 285.9$, $P < 0.001$). Only the linear term of moisture content ($F = 121.0$, $P < 0.001$) and the quadratic term of temperature ($F = 44.49$, $P < 0.05$) had significant negative effects on TTC in oils (Fig. 2c). The results indicate that linear (X_1 and X_2) and quadratic (X_2^2) parameters of independent variables are the primary determinants causing significant impacts on the

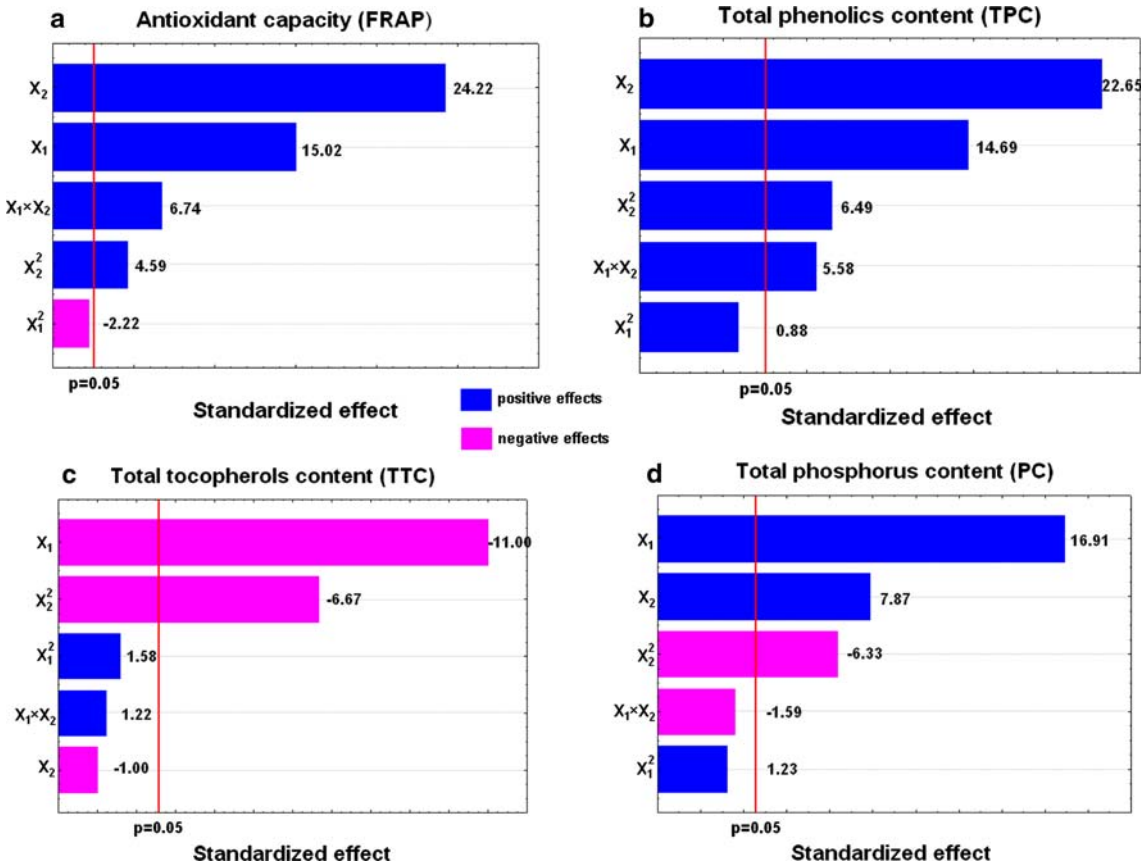

Fig. 2 Pareto charts of standardized effect of each term in the model divided by its standard error for the four response variables: **a** FRAP, **b** TPC, **c** TTC, and **d** PC. The *vertical line* indicates the confidence level of 95%, and factors with standardized effect values *to the right of this line* are statistically significant. X_1 moisture content, X_2 temperature

responses (AC, TPC, and PC), while the quadratic (X_1^2) terms were insignificant (F values ranged between 0.776 and 4.92, $P > 0.05$).

Hydrothermal treatment of flour positively affected AC and TPC in the pressed crude oils. Thus, crude rapeseed oils have higher AC and TPC when pressed from more heated flour with higher moisture contents. The effect of processing temperature (X_2) on AC and TPC in the examined oils was about 1.5 times greater than the effect of rapeseed moisture content (X_1) (Fig. 2).

Analysis of Response Surfaces

The effects of the two independent variables (MC and T) on AC, TPC, TTC, and PC on the obtained crude oils were illustrated using surface response and contour plots of the quadric polynomial models (Fig. 3).

Figure 3 shows that FRAP and TPC values in the crude oils significantly increased with the increasing conditioning temperature and moisture content (Fig. 3a, b). Therefore, these two parameters have a positive effect on AC and TPC in the pressed oils. For comparison, Koski et al. [2] and Vuorela et al. [7] reported higher contents of phenolic compounds in rapeseed oil when extraction was performed at high temperature (100°C) and pressure. Phenolic components are mainly classified as free and bound phenolic substances. Thus the increase in solubility of phenolic substances at high treatment temperatures can be related to disruption of the seed during subcritical water treatment. Subsequently, phenolic substances are released from the cell wall into the oil [24].

Figure 3d shows that a change in the moisture content of the seed (from 7 to 11%) and cooking temperature (90–120°C) could increase PC in the resulting oil. Rapeseed oil pressed from heated (80 and 100°C) flour resulted in a substantial increase in phosphorus content (22.1–64.2 mg/kg) compared to that in the cold-pressed oil (PC = 12.6 and 16.2 mg/kg) [14]. Our results agree with those of Veldsink et al. [25] who reported that phosphorus and phenolics increased significantly as the temperature increased.

The moisture content in rapeseeds has a significant linear effect on TTC in the pressed rapeseed oils, while

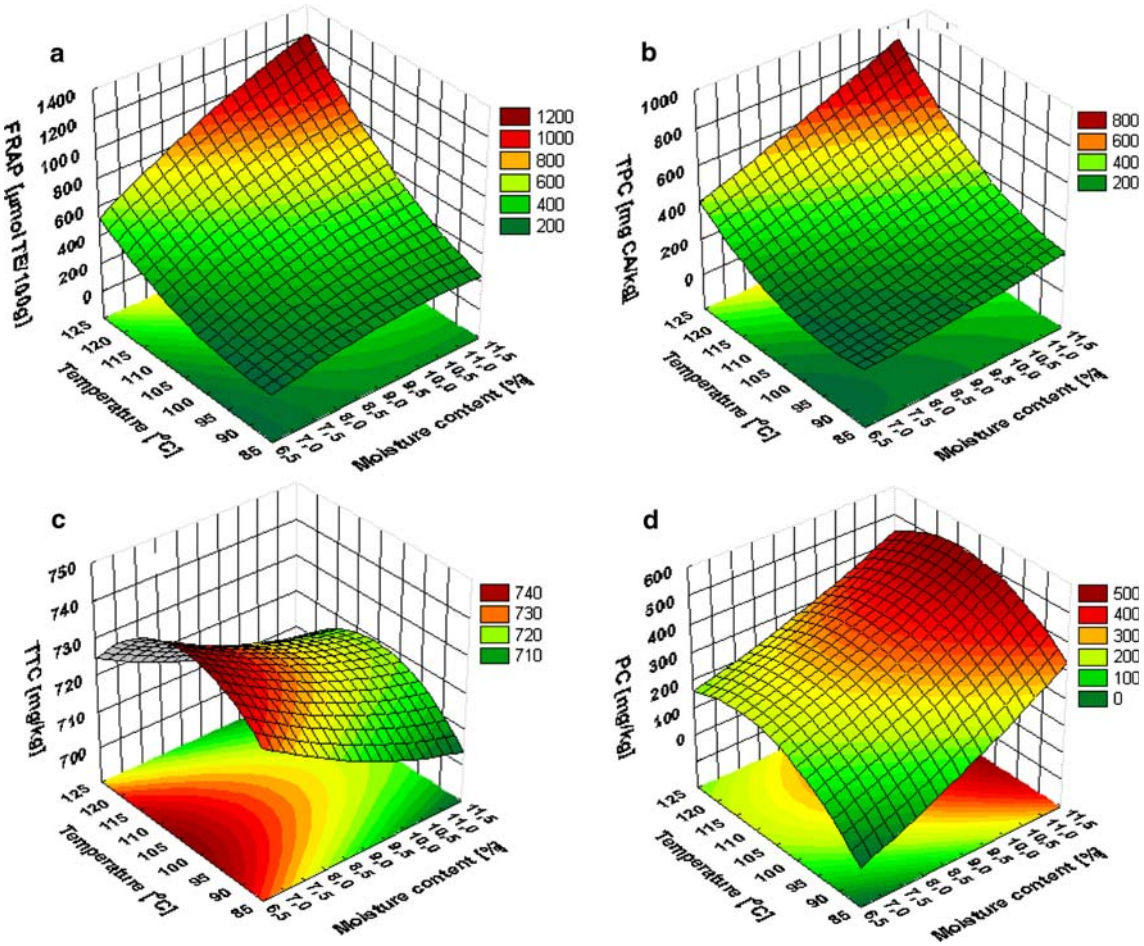

Fig. 3 Response surfaces and contour plots for **a** antioxidant capacity (*FRAP*) and **b** total phenolic (*TPC*), **c** tocopherol (*TTC*), and **d** phosphorus (*PC*) contents in the crude rapeseed oils expressed as a function of moisture content and temperature of rapeseed flour conditioning before oil pressing

the cooking temperature had a significant quadratic effect on TTC (Fig. 3c). TTC in the crude rapeseed oils was lower when moisture content in seeds and conditioning temperature were higher before pressing processes. For comparison, Goffman and Möllers [22] reported that higher temperatures combined with the availability of oxygen (air) caused decomposition of tocopherols in rapeseed.

Relationship between Residual Oil Content in Cake and Hydrothermal Treatment of Rapeseed Flour

The hydrothermal treatment of flour before oil pressing influenced the residual oil (RO) content in the pressed cake. Figure 4 shows that the increased moisture in seed from 7 to 11% reduced the RO amount.

The oil content in the cake increased with the increasing conditioning temperature of flour. Thus, the highest content of RO was determined in the cake after pressing the flour with MC = 7% at $T = 120$°C (Fig. 4).

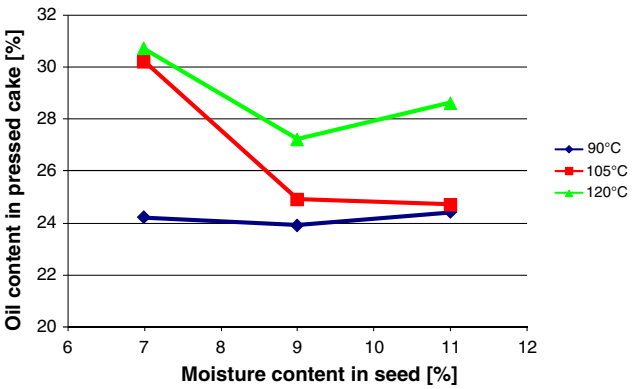

Fig. 4 Relationship between residual oil content in cake and moisture content in seeds at different pressing temperatures

Conclusion

The response surface methodology (RSM) appeared to be useful for studying the influence of the hydrothermal pretreatment of rapeseed flour on AC, TPC, TTC, and PC in

the resulting oils. The second-order polynomial model can be applied to optimize the parameters of extraction to produce an oil with high antioxidant capacity and total phenolics. Conditioning temperature has a greater effect on the AC and TPC in the crude rapeseed oils than moisture content. A linear and significant correlation between AC and TPC in the crude oils was demonstrated. The proposed FRAP method is relatively simple, precise, and convenient for the determination of antioxidant capacities of rapeseed oils, and RSM can be employed by the oil-processing industry for producing rapeseed oil with high antioxidant capacity.

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