

## Effect of Dehulling Treatment on the Oxidative Stability of Cold-Pressed Low Erucic Acid Rapeseed Oil

Mei Yang · Changsheng Liu · Fenghong Huang ·  
Chang Zheng · Qi Zhou

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**Abstract** This study compared the oxidative stability of cold-pressed rapeseed oil (Copro) and dehulled cold-pressed rapeseed oil (DCopro) in the dark at 60 °C and monitored the evolution of minor constituents (tocopherols, phytosterols, phenolics). The results showed that dehulling significantly influenced the oxidative stability of the oils, the DCopro was more easily oxidized. During the autoxidation, the peroxide value (PV) and anisidine value (p-AV) of the DCopro ranged from 2.38 to 95.97 mequiv O<sub>2</sub> kg<sup>-1</sup> and from 1.20 to 30.75, whereas those of the Copro ranged from 3.80 to 46.17 mequiv O<sub>2</sub> kg<sup>-1</sup> and from 2.69 to 14.87, respectively. Dehulling affected the contents and the rates of decrease of tocopherols and phytosterols of the cold-pressed oils, and the rates of decrease of tocopherols and phytosterols of the Copro were lower than those of the DCopro (10% less, on average). The rancimat induction periods (IPs) were positively correlated with the concentrations of the total tocopherols (For DCopro,  $R^2 = 0.9622$ , For Copro,  $R^2 = 0.8334$ ). The total phenolics contents as determined by spectrophotometry first increased and then decreased. Tocopherols and phytosterols had a greater effect on oxidative stability of the rapeseed oils during the first 30 days, and phenolics had a greater effect in the 30–40 day period.

**Keywords** Oxidative stability · Food and feed science · Nutrition and health

### Introduction

About 20 million tons of rapeseed oil is produced every year in the world, and in China large amounts of the oil have been consumed for many years. However, the oil from traditional rapeseed has a high content of erucic acid as well as a strong pungent flavour, so its value as an edible oil is low. During the past 20 years, the quality of rapeseed varieties have been continuously improved, and now low-erucic acid rapeseed oil has entered the Chinese market. Low-erucic acid rapeseed oil is rich in oleic acid, and contains 15–30% of linoleic acid and 5–14% of linolenic acid. Low-erucic acid rapeseed oil is exceptionally rich in n-3 fatty acid, and is considered quite nutritious [1, 2].

The oxidative stability of oils is an important quality parameter that influences their utilizations in foods. The oxidative stability of canola oil can be improved by decreasing readily oxidizable fatty acids such as linolenic and linoleic, and increasing the oxidation-resistant fatty acids such as oleic and saturated acids. There may be value in increasing oleic and decreasing linoleic on the sn-2 carbon of glycerol [3]. However, the oxidative stability of oils can also be influenced by non-triglyceride components such as tocopherols and other phenolic antioxidants [4]. Rapeseed contains more phenolic substances than other commercial oilseeds [5, 6] and these phenolics often exert an antioxidant activity [7, 8]. The polar phenolic content of the rapeseed oil is correlated with its oxidative stability. The most active antioxidant component of the polar fraction was identified as vinylsyringol, a decarboxylation product of sinapic acid [9]. However, refining removes

M. Yang · C. Liu · F. Huang (✉) · C. Zheng · Q. Zhou  
Department of Product Processing and Nutriology,  
Institute of Oil Crops Research, Chinese Academy  
of Agricultural Sciences, Wuhan 430062, China  
e-mail: oiljgzx@gmail.com

M. Yang  
e-mail: yangmei612@yahoo.com.cn

C. Liu  
e-mail: jasonberg@163.com

great amounts of phenolic compounds. Phospholipids also have been extensively studied as antioxidants, and the oxidative stability of canola press oil was found to be significantly correlated with its phosphorus content. Phospholipids act as potent synergists with tocopherols at concentrations ranging from 0.025 to 0.22%, but above this level, increasing the phospholipid content did not significantly improve the oxidative stability [10].

The cold-pressed oil has a natural flavor, and can be used as an edible oil without further refining, and the value of rapeseed meal is significantly increased [1, 11–13]. Cold-pressing oil involves only washing and filtering without solvents or further processing, so minor components with antioxidative capability such as tocopherols, phenols, and phospholipids are not removed, and these minor constituents can effect oxidative stability of the cold-pressed oil [14, 15].

Rapeseed contains 14–18% hulls, which may contain 14–19% of the oil. Dehulling can improve the color, and initial quality of rapeseed oil and the nutritional value of the rapeseed meal. However, the effect of dehulling on the oxidative stability of the cold-pressed oil is not yet clear, and it is a problem for efficient utilization of the rapeseed hulls. In this study rapeseed with low erucic acid was used and cold-pressed rapeseed oil was prepared both before and after dehulling. The samples were stored at 60 °C in the dark, and their oxidative stability, and contents of tocopherols, phytosterols, and phenols were determined.

## Materials and Methods

### Samples

Locally grown *Brassica napus* cultivar Zhongshuang No.10 was obtained from Wuhan Zhongyou of Sunshine Seeds Co., Ltd (Wu-han, Hu-bei, China).

### Preparation of Cold-Pressed Rapeseed Oil

The rapeseeds were cleaned, and dehulled in 10 kg lots using a rapeseed dehuller (HEF2000, HEF Group, Andrezieux-Boutheon, Loiret, France), which produced 83.5% clean dehulled seed. Both the dehulled and whole rapeseeds were pressed at room temperature with a cold-pressing machine (CA59G, Komet Co., Stuttgart, Baden-Wuerttemberg, Germany), and the cold-pressed oils were centrifuged.

### Chemicals

$\alpha$ ,  $\beta$ ,  $\gamma$ -Tocopherol were purchased from Sigma-Aldrich (Saint Louis, Mississippi, USA). Standard samples of

phytosterols were obtained from Xi-an Blue-sky Biological Engineering CO., Ltd (Xi-an, Shan-xi, China). Chromatographic grade methanol was purchased from Merck (Darmstadt, Hesse, Germany). Folin-Phenol was purchased from Sigma-Aldrich (Saint Louis, Mississippi, USA). Other chemicals and reagents were of analytical grade and were used without further purification.

### Fatty Acid Composition

Fatty acid methyl esters (FAME) were prepared from oils according to Animal and vegetable fats and oils—Preparation of methyl esters of fatty acids (GB/T 17376, ISO5509:2000, IDT) and analyzed by standard methods [16, 17]. Using an Agilent GC-6890N with a Flame Ionization Detector (FID) and an Agilent 7638 series autosampler (Agilent, Santa Clara, California, USA). The column was an HP-INNOWAX (Agilent, Santa Clara, California, USA), 30 m × 0.32 mm i.d. with a 0.25  $\mu$ m film thickness. Nitrogen was the carrier gas at 1.5 mL min $^{-1}$ . Injection volume was 1  $\mu$ L at a split ratio of 80:1. Oven temperature was programmed from 210 °C for 9 min to 230 °C at 20 °C min $^{-1}$  and held for 10 min. Identifications of the individual fatty acids (FA) were accomplished by comparison with commercial standards.

### Analysis of Tocopherols by Ultra-High-Pressure Liquid Chromatography (UPLC)

#### Extraction of Tocopherols

Oil samples (5 g) were weighed into a flask, 30 mL anhydrous ethanol and 20 mL 1 g L $^{-1}$  potassium hydroxide solution were added and the mixture was heated in a boiling water bath for 1 h. The saponified fatty acids were transferred to a separatory funnel and partitioned between deionized water and diethyl ether. After dehydration of the ether with anhydrous sodium sulfate, the ether fraction was collected in a 100 mL volumetric flask and diluted to 100 mL with ether, and in a 10 mL aliquot of the ether extract were taken out, the solvent was evaporated to dryness using nitrogen at 40 °C, and the residue was redissolved in ethanol (1 mL) for the tocopherols analysis.

#### Analysis of Tocopherols

An autosampler fitted with a 20  $\mu$ L loop was used to inject 2  $\mu$ L of the treated samples (Waters, Milford, Massachusetts, USA). The column was a Waters Acquity BEH C<sub>18</sub> column (100 mm × 2.1 mm diameter and 1.7  $\mu$ m particles) (Waters, Milford, Massachusetts, USA). Tocopherols were detected with a photodiode array (PDA) detector (Waters, Milford, Massachusetts, USA) at a wavelength of

300 nm. The isocratic mobile phase was a mixture of methanol and high-purity water (95:5, v:v) at a flow rate of 0.30 mL min<sup>-1</sup>. Each tocopherol was quantified using an external standard method with reference samples of tocopherols. Waters Empower 2 software (Waters, Milford, Massachusetts, USA) was used to calculate the peak areas.

#### Analysis of Phytosterols by Gas Chromatography

##### *Extraction of Phytosterols*

The saponification and extraction of oils for the preparation of phytosterols was similar to that for tocopherols. The ether extracts were evaporated to dryness in a rotavapor at 40 °C and the residues were dissolved in 5 mL of ethanol.

##### *Analysis of Phytosterols*

Gas chromatography (GC) on a DB-5 column (Agilent, Santa Clara, California, USA), 15 m × 0.32 mm i.d. with a 0.1-μm film thickness, was used with nitrogen as the carrier gas at a flow rate of 1.5 mL min<sup>-1</sup>. The injection volume was 1 μL and the split ratio was 10:1. The oven temperature was programmed from 180 °C to 243 °C at 3 °C min<sup>-1</sup> and held for 0.5 min. Then the temperature was increased 50 °C min<sup>-1</sup> to 340 °C and held for 0.5 min. Identification of the individual phytosterols were accomplished by GC chromatograms of standards [18].

#### Analysis of Phenolic Compounds

Oil samples (2.5 g) dissolved in 3 mL of hexane were extracted with 3 mL of aqueous methanol by vortexing for 2 min. After centrifugation at 3,500 rpm for 10 min, the aqueous phase was separated and transferred to a 50-mL colorimetric tube, and dissolved in 25 mL of water. Next 2.5 mL of Folin-phenol reagent and 10 mL of saturated sodium carbonate solution were added, and diluted to 50 mL with water. After 30 min, the absorbance was measured at 765 nm with a UNICO 2802PCS UV/VIS spectrometer (Shanghai, China). Tannic acid was used for calibration, and the results of duplicate analyses are reported as ppm of tannic acid.

#### Peroxide Value (PV), Acid Value (AV) and P-Anisidine Value (p-AV)

The method used to measure the PV and AV was that of the National Standard of the PRC (GB/T 5538, ISO 3960:2001, IDT; GB/T 5530, ISO 660:1996, IDT) [19, 20]. The p-AV was determined following method ISO 6885 [21].

#### Oil Stability

The oxidative stabilities of the two oils samples were determined with a Rancimat 743 (Metrohm KEBO Lab AB, Herisau, Switzerland). Vegetable oils samples (3 g) were weighed into the reaction vessel, and heated to 110 °C, while blowing clean air at 10 L h<sup>-1</sup> through the samples. Volatile products released during the oxidation process were collected in a flask containing distilled water. The oxidation process was recorded automatically by measuring the change in conductivity of the distilled water caused by the formation of volatile compounds. The induction periods (IPs) are defined as the point of rapid change in the rate of oxidation, and the results were expressed in hours (h).

#### Oil Autoxidation

One thousand grams of the CPRO and DCPRO, respectively, were placed in four closed 500-mL grinding mouth glass vials and stored at 60 °C in the dark. Sample aliquots were periodically withdrawn for analyses. At the beginning, the samples filled the glass bottles. About 30 g of oil was taken out for analysis every time, so the head-space of the glass vials became greater and greater. The peroxide value (PV) was determined according to GB/T 5538. When the PV of any one of the oil samples reached 100, the autoxidation test was finished. Changes in the amounts of tocopherols, phytosterols, phenolic compounds and IPs were also monitored.

#### Statistical Analysis

Statistical analysis was carried out with SPSS 15.0 for Windows software. Differences were considered significant at  $P < 0.05$ .

### Results and Analysis

#### Compositional Analysis of the Oils

The fatty acid composition of CPRO and DCPRO are given in Table 1, while their tocopherol and phytosterol contents along with initial data readings of stability tests are given in Table 2. The rapeseed oil used in these experiments was chosen because it was low in saturated fatty acids and high in oleic acid, a composition regarded as having health benefits. The cold-press procedure used to produce the oils required no solvents or further processing besides filtering.

The fatty acid composition of the CPRO and DCPRO showed slight differences. The polyunsaturated fatty acids/

**Table 1** Fatty acid composition (%) of the rapeseed oil samples

	C PRO	D C PRO
C16:0	4.43 ± 0.20	3.93 ± 0.20
C18:0	1.87 ± 0.04	1.84 ± 0.05
C18:1	60.37 ± 0.50	62.17 ± 0.49
C18:2	19.35 ± 0.10	19.18 ± 0.10
C18:3	7.82 ± 0.32	7.71 ± 0.35
C20:0	0.58 ± 0.01	—
C20:1	2.23 ± 0.02	2.12 ± 0.02
C22:0	0.28 ± 0.01	—
C22:1	3.15 ± 0.02	3.06 ± 0.02
P/S	3.8 ± 0.23	4.7 ± 0.25

C PRO cold-pressed rapeseed oil, D C PRO dehulled cold-pressed rapeseed oil, P/S polyunsaturated fatty acid/saturated fatty acid ratio

**Table 2** Contents of tocopherols, phytosterols, and chlorophyll, initial peroxide value (PV), acid value (AV), anisidine value, rancimat induction period, of the rapeseed oil samples

	C PRO	D C PRO
α-Tocopherol (mg/100 g)	18.2 ± 1.12	16.8 ± 1.05
γ-Tocopherol (mg/100 g)	32.4 ± 1.98	30.8 ± 2.01
Total tocopherols (mg/100 g)	50.6 ± 1.55	47.6 ± 1.53
Brassicasterol (mg/100 g)	290 ± 13.0	276 ± 17.0
Campesterol (mg/100 g)	751 ± 20.4	628 ± 19.4
Sitosterol (mg/100 g)	1348 ± 98.6	1137 ± 91.5
Total phytosterols (mg/100 g)	2389 ± 59.5	2041 ± 55.1
Total phenols (mg/kg tannic acid)	8.0 ± 0.03	9.3 ± 0.04
Chlorophyll (mg/kg)	11.8 ± 0.10	18.9 ± 0.19
Initial peroxide value (mequiv/kg)	3.96 ± 0.03	2.98 ± 0.01
Initial acid value (mg KOH/g)	2.60 ± 0.01	1.25 ± 0.01
Initial anisidine value	2.68 ± 0.05	1.20 ± 0.03
Initial induction time (110 °C, h)	8.58 ± 0.43	9.76 ± 0.42

saturated fatty acids ratio of D C PRO was 4.7 and higher than that of C PRO.

The individual and total tocopherol contents are different in the fresh C PRO and D C PRO. The α-tocopherol and γ-tocopherol of C PRO were 18.2 mg/100 g oil and 32.4 mg/100 g oil, respectively, and higher than those of D C PRO. Brassicasterol, campesterol, sitosterol were identified in both of the oil samples. Sitosterol was the dominant phytosterol in the rapeseed oil, accounting for 56% of the total phytosterols content. Dehulling had a significant effect on the phytosterols content. The phytosterols content of D C PRO was 348 mg/100 g oil less than that of C PRO. The total phenol and chlorophyll contents of the C PRO and D C PRO differed. The C PRO had 1.3 mg tannic acid per kg of the sample and 7.1 mg chlorophyll per kg of the sample less than D C PRO. The initial peroxide

and anisidine values of the D C PRO were lower than those of C PRO, and the induction period of D C PRO was higher. Thus, dehulling improved the initial quality of the cold-pressed oils.

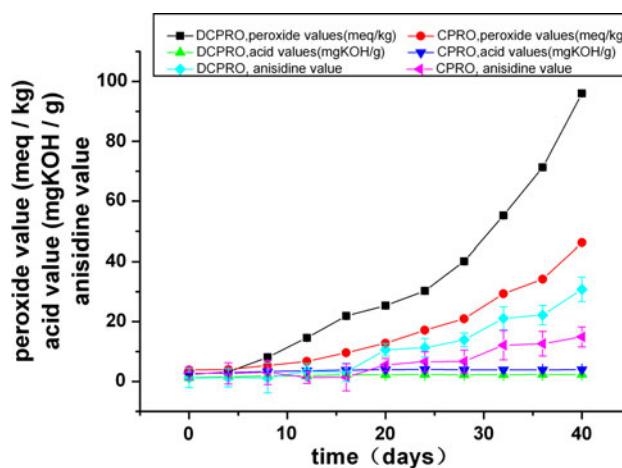
A low P/S ratio in the oil made it less susceptible to oxidation as expected [22]. However, despite the lower P/S of the C PRO, it was less stable than the D C PRO as shown by initial the induction time. This may reflect the C PRO's relatively high content of hydroperoxide, free fatty acids and chlorophyll.

#### Peroxide Value, Anisidine Value and Acid Value

The trends in peroxide value, anisidine value, and acid value in the two different oil batches during autoxidation at 60 °C in the dark are shown in Fig. 1. These parameters were significantly affected after dehulling: very low peroxide levels were detected in the fresh samples. These increased during autoxidation and the PV exceeded 20 mequiv O<sub>2</sub> kg<sup>-1</sup> after 16 d. However, the PV of the D C PRO ranged from 2.38 to 95.97, whereas those for the C PRO were lower ( $P < 0.0001$ ) and ranged from 3.80 to 46.17. These differences might arise from the higher contents of tocopherols and phytosterols in the C PRO.

During autoxidation at 60 °C in the dark, the anisidine value increased in the samples. The increases were slow before the 16th day. However, after the 20th day, the increases were obvious. The rates of increase in anisidine value were higher for D C PRO than for C PRO. The values ranged from 1.20 to 30.75 in the D C PRO, and from 2.69 to 14.87 in the C PRO.

Acid value did not distinctly increase during the 40 days of autoxidation and remained under the legal limit (<4.0 mg KOH g<sup>-1</sup>) (Fig. 1). The free fatty acids levels were still able to meet the CODEX STAN 210-1999 for



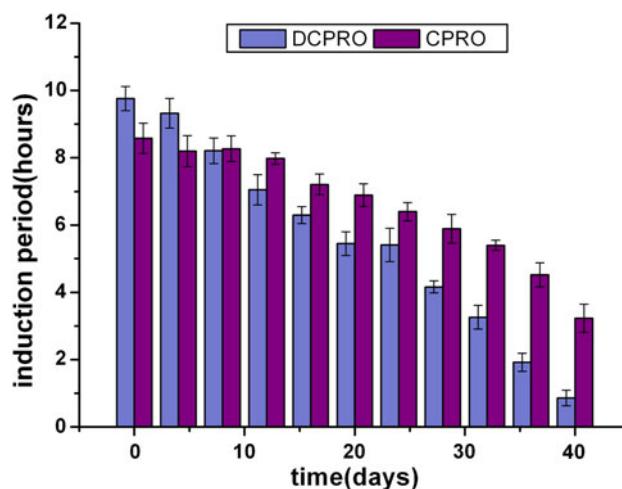
**Fig. 1** Changes in peroxide value, anisidine value and acid value during the autoxidation of rapeseed oils at 60 °C in the dark

cold-pressed oil. The DCPRO had significantly lower AV ( $P < 0.0001$ ) than the CPR, ranging from 1.34 to 1.72 mg KOH g<sup>-1</sup>.

#### Effect of Tocopherols on Oxidative Stability

The  $\alpha$ - and  $\gamma$ -tocopherols are the most abundant antioxidants in plant lipids and significantly retard their oxidation. The tocopherol contents were also monitored during the autoxidation test (Fig. 2). On the whole, the rates of consumption for  $\alpha$ -tocopherol were higher than those of  $\gamma$ -tocopherol. In the two cold-pressed rapeseed oils, all of the  $\alpha$ -tocopherol was consumed within 36–40 days while 5–30% of the initial  $\gamma$ -tocopherol was still present after 5 weeks of oxidation at 60 °C in the dark. Lampi et al. [4] showed that  $\gamma$ -tocopherol was a more effective antioxidant than  $\alpha$ -tocopherol at levels above 100 µg g<sup>-1</sup>. The contents of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol were 182 µg g<sup>-1</sup>, and 324 µg g<sup>-1</sup> in the CPR, these were 168 µg g<sup>-1</sup>, and 308 µg g<sup>-1</sup> in the DCPRO, respectively (Table 2). Therefore, our results were in accord with the research conclusions of Lampi et al.

The oxidative stabilities of the oils were measured as the induction time in response to forced oxidation, and the results are shown in Fig. 3. A decrease in the oxidative stability during storage at 60 °C in the dark occurred for the two samples. Interestingly, in the first 8 days of autoxidation, the induction periods of the DCPRO were higher than those of the CPR, the possible reason was the initial contents of hydroperoxide, free fatty acids and chlorophyll of the CPR were higher than those of the DCPRO. However, the rate of decreases in the induction period was obviously ( $P < 0.001$ ) greater for the DCPRO than for the CPR. On the 40th day, the induction period

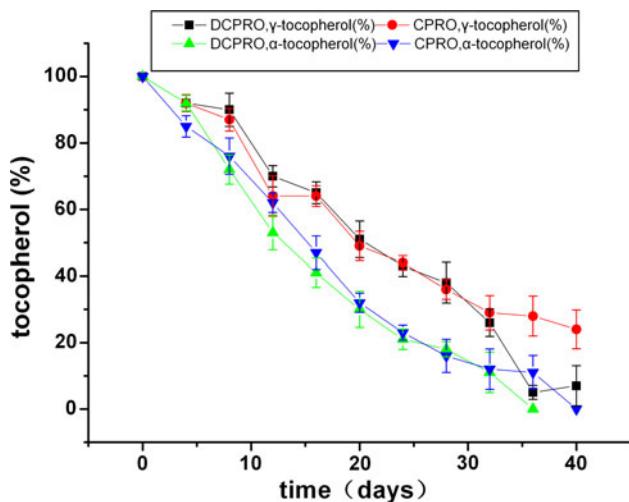


**Fig. 3** The induction times of oil samples during autoxidation at 60 °C in the dark

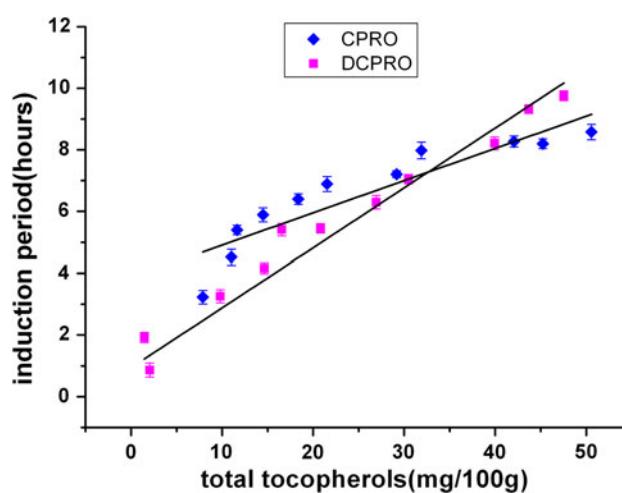
of the DCPRO was less than 1 h, but that of the CPR still exceeded 3 h. The CPR (with high total tocopherol contents) showed greater oxidative stability than the DCPRO (with low total tocopherol contents).

Clearly, the content of tocopherols correlated with the level of oxidation: the induction periods were positively correlated with the concentrations of the total tocopherols (Fig. 4) (For DCPRO,  $R^2 = 0.9622$ ; For CPR,  $R^2 = 0.8334$ ), and the rates of decreases of the induction periods also were positively correlated with the rates of consumption of the total tocopherols (For DCPRO,  $R^2 = 0.9852$ ; For CPR,  $R^2 = 0.8341$ ).

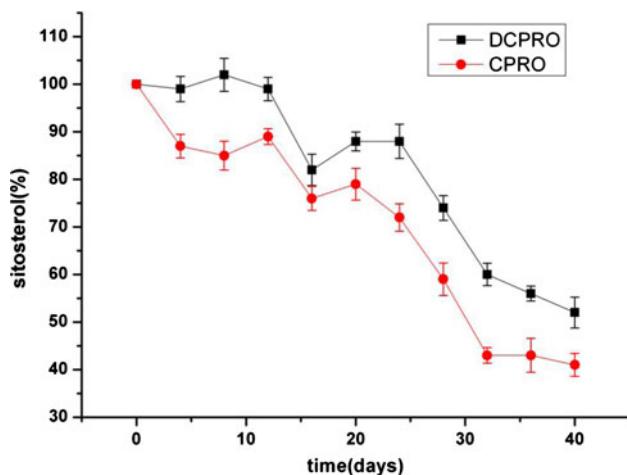
Even when the two batches of oils were prepared from the same rapeseed, dehulling only slightly influenced the fatty acid compounds of the oil samples: The CPR had lower P/S ratios than the DCPRO (Table 1). Thus, the



**Fig. 2** The tocopherols contents of the rapeseed oils during autoxidation at 60 °C in the dark



**Fig. 4** Correlations of total tocopherol with oxidative stability in the DCPRO and CPR



**Fig. 5** Changes in sitosterol contents during autoxidation of rapeseed oils at 60 °C in the dark

induction periods were not only correlated with the total tocopherols contents for the two kinds of oils, but also well correlated with the P/S ratios.

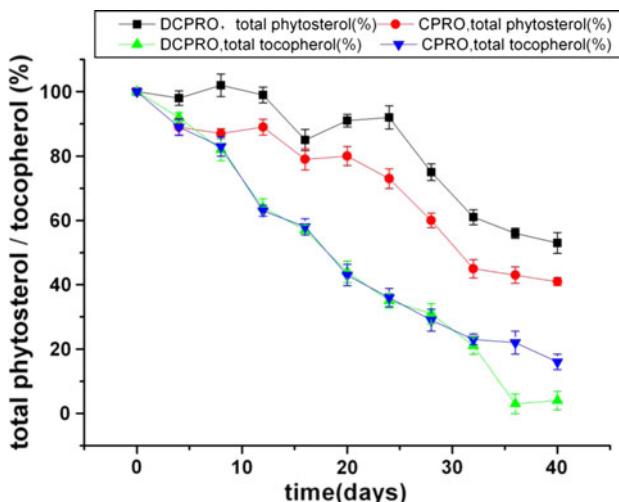
#### Evolution of Phytosterols

Phytosterols contained in vegetable oils are known to exert a hypocholesterolemic function. Like unsaturated fatty acids, phytosterols are prone to oxidation, especially when subjected to heat treatment or long-term storage [22, 23]. Since sitosterol is the major phytosterol in low-erucic acid rapeseed oil, we examined the changes in sitosterol during the accelerating oxidation process (Fig. 5). On the whole, during autoxidation at 60 °C in the dark, the rates of consumption of sitosterol were 10% higher for CPRO than for DCPRO, which were the reverse of the rates of consumption of tocopherols in the two oil samples. At the 12th day, the content of sitosterol of the DCPRO almost did not change, while 10% of the initial content was consumed in the CPRO. When the autoxidation test was finished (on the 40th day), approximately half of sitosterol was present in the DCPRO, while only 40% of the original content was present in the CPRO.

The consumption rates of phytosterols of CPRO and DCPRO were less than these of tocopherols (Fig. 6), which shows tocopherols can inhibit the oxidation of plant sterols. Tabee, Rudzinska et al. [22, 24] showed that addition of  $\alpha$ -tocopherol can significantly ( $P < 0.05$ ) inhibit oxidation of phytosterol in vegetable oil. These authors concluded that  $\alpha$ -tocopherol is a strong inhibitor of the formation of phytosterols oxidation products.

#### Evolution of Phenolics

Among commercial oilseeds, rapeseed contains the highest amount of phenolics: defatted rapeseed meal may contain

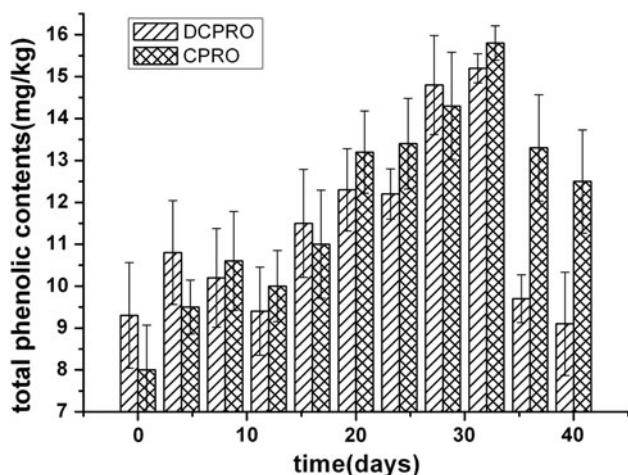


**Fig. 6** Changes in total tocopherol and total phytosterol contents during autoxidation of rapeseed oils at 60 °C in the dark

up to 2% of phenolic acids. The most abundant phenolic acid in rapeseed is sinapic acid [15]. When pressing the oil from rapeseed, most of the phenolic compounds remain in the meal, and only a small proportion is transferred to the crude oil. Many phenolic compounds have antioxidative properties. The main phenolics in rapeseed oil were vinyl-syringol, sinapine and sinapic acid [25]. Some (crude) rapeseed oils may contain as much as 100–200 mg kg<sup>-1</sup> of hydrophilic phenolic compounds as determined from methanol extracts by the colorimetric Folin-Ciocalteu method [26]. Koski et al. [15] reported that cold-pressed rapeseed oils contained only minor amounts (3–4 mg kg<sup>-1</sup>) of polar phenolics. The contents of initial total phenolic compounds in our samples were 8–9 mg kg<sup>-1</sup> (Table 2). The differences in phenolic levels might arise from factors, such as the method of analysis, the degree of maturity, cultivars and sample preparation.

Figure 7 shows the changes in the evolution of the total phenolic contents of the CPRO and DCPRO as determined through spectrophotometry. For autoxidation of the rapeseed oils at 60 °C in the dark, the total phenolics contents increased during the first 32 days. On the thirty-second day, the contents of total phenolics were highest (15.2–15.8 mg kg<sup>-1</sup>) in the oils. When the autoxidation test was finished (on the 40th day), the content of total phenolics in the DCPRO corresponded to the original content, whereas total phenolics content in the CPRO, was 4.5 mg kg<sup>-1</sup> higher than initial content.

The trends in the concentrations of the total phenols can be explained by considering the three different stages of this experimental plan: dehulling, oil extraction, and oil storage. The content of total phenols in the rapeseed is different from that of the kernel of the rapeseed. Secondly, the pressures formed under the same cold-pressing



**Fig. 7** Changes in total phenolic contents during autoxidation of rapeseed oils at 60 °C in the dark

condition are different between the rapeseed and the kernel of the rapeseed, so, the amounts of phenols transferred to the crude oils are different. Later, the lysis of the complex phenols during oil storage led to a higher content of low molecular weight phenolics, as shown by spectrophotometry [27].

Based on the changes in total tocopherols, total phytosterols, and total phenolics during autoxidation at 60 °C in the dark, tocopherols and phytosterols have a greater effect on oxidative stability of rapeseed oils during the first 30 days of oxidation, and phenolics have a greater effect later during oxidation (30–40 days).

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