

# Canola Seed Meal as a Potential Source of Natural Antioxidant

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## ABSTRACT

In the present study seeds of *Brassica napus* L. (canola) and its defatted meal were utilized separately in order to obtain methanol extract which used after lyophilization for total phenols content (TPC), total flavonoids (TF) and  $\alpha$ -Tocopherol contents, antioxidant activity determination and phenolic compounds profiling with HPLC. The results showed a high total phenols, total flavonoids and  $\alpha$ -Tocopherol contents with superior antioxidant activity for canola meal extract (32% increase than seeds extract). The major phenolic compounds were Vanillic acid and Syringic acid with levels of 783.66 and 52.24 ( $\mu\text{g/g}$  extract); respectively, while, the major flavonoid compound was Rutin (631.13  $\mu\text{g/g}$  extract). Scientific researches should be focused on the utilization of Canola seed meal (CM) as a potential natural source of such phytochemicals.

**Key words:** canola- Antioxidant- phenols- flavonoids

## INTRODUCTION

*Brassica napus* L. (canola; also named oilseed rape, rapa and rapeseed) is one of the most important worldwide oilseed crops and its oil processing is an important section of the canola industry in Canada (Unger 2011). Canola is mainly used in vegetable oil production due to its high oil content (Shahidi 1990). The high anti-oxidant capacity of canola oil seeds is attributed to its high content of vitamin E (tocopherols) and phenolic compounds (Frag et al. 2013). Production of oil from canola produces canola meal (CM) as a by-product which represent about 60% of the whole canola seed (Unger 2011). The complex mixture of phytochemical constituents present in canola vegetables is responsible for their health promoting action (Vallejo et al. 2003). Recently, there is growing interest to finding naturally occurring antioxidants to be used in food industry as a substituent to deleterious synthetic antioxidants (Riaz et al. 2012; Rozan et al. 2018).

Phenolic compounds could be classified according to their structure into simple phenols, phenolic acids, flavonoids and hydroxycinnamic acid derivatives (Cartea et al. 2010). Reviewing the available literature showed that the seeds of *Brassica* species are rich in phenolic compounds with levels ranging from 82 to 122 mg/g dry matter (Jun et al. 2014; Chandrasekara et al. 2016).

The objective of the study was to investigate the major phytochemicals of rapeseed seeds and meal as a natural source of antioxidants and their antioxidative capacity.

## MATERIALS & METHODS

### Material:

The Egyptian origin serw 4 cultivar of *Brassica napus* L. was collected from the Agricultural Research Station in Etay El-Barod, El-Behaira Governorate, Egypt. Seeds were collected when fully ripe.

### Methods:

#### Preparation of phenolic extract from canola seed:

Canola seeds were finely grinded using a grinder (Moulinex AR1044, France). Then passes through 40 mesh sieve; from which 200 g were extracted with n-hexane. The solvent was evaporated at temperature not exceeding 40°C using rotary-evaporator (Hei-VAP Value, Germany) and then stored away from light at 4 °C. The canola seed flour along with another sample defatted one were extracted according to procedure described by (Frag et al. 2013), where 100g of the two samples flour was added to 0.5 L of 70% aqueous methanol and was stirred using ultra sonication with an ultrasonic bath (Elma, Germany) at a fixed power (200 W) for yield enhancing (Teh and Birch 2014). The extract was centrifuged at 5,000×g for 15 min, then, a filter paper (Whatman No. 1; Whatman, Little Chalfont, UK) was used in filtering supernatant. The filtrates were then pooled, concentrated by evaporation at 40 °C and lyophilized. The yields of these lyophilized two extracts were 11.54% & 17.3% for seeds and defatted meal, respectively.

#### Total phenolic content estimation:

Total phenolic content was determined using (Dewanto et al. 2002) method. Folin-Ciocalteu's reagent (0.2 mL) and 5% sodium carbonate (3 mL) were added to each sample (0.1mL) in glass tubes. The tubes were shaken and hold in the dark at room temperature for 1 hour. Then, the absorbance was measured at 725 nm using spectrophotometer (UV1650PC; Shimadzu Co.). Total phenolic contents were expressed as  $\mu\text{g}$  trans sinapic acid equivalents /mg plant dry wt.

### Quantitative estimation of total flavonoid content of *Brassica napus* L. seeds & meal:

Total flavonoids content was determined following the procedures described by (Marinova et al. 2005) using  $AlCl_3$  colorimetric assay. Ten grams of seeds or meal were extracted with 70% methanol till exhaustion; the total volume was adjusted to 300 mL. 1 mL of the methanolic extract or standard quercetin flavonoid solution (0.01 - 1 mg/mL) was added to 4 mL double distilled (dd)  $H_2O$  in 10 mL volumetric flask, followed by 0.3 mL 5 %  $NaNO_2$  solution, 0.3 mL 10 %  $AlCl_3$ , and 2 mL 1 M  $NaOH$ , respectively. The flask was then completed with double distilled  $H_2O$  to adjust the total volume to 10 mL. Then the mixture was vortexed, and measured spectrophotometrically at 390 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE)/g sample. Each measurement was carried out in triplicate.

### Quantitative estimation of $\alpha$ -Tocopherol content in *B. napus* L. seeds & meal:

Tocopherol was extracted from seeds & meal using the modified procedure of (Weber 1987). A 600 mg of finely powdered plant material (seeds & meal) were extracted separately, in 10 mL of ethanol using a 70 °C water bath for 15 min. A 180  $\mu$ L of 80% potassium hydroxide was added to each tube upon removal from the water bath. Tubes were vortexed and then saponified in a water bath at 70 °C for 30 min. Samples were put into an ice bath where 2.5 mL of double-distilled, deionized water and 2.5 mL of hexane were added. Tubes were vortexed for 20 sec, and then centrifuged at (2000 rpm) for 5 min. After, the hexane fraction was separated in a test tube; this process was repeated two more times. The obtained hexane fractions were dried under vacuum, reconstituted in 400  $\mu$ L absolute ethanol prior to injection (20  $\mu$ L) onto the liquid chromatography. Tocopherol concentration was determined using reverse phase, high-performance liquid chromatography (Agilent technologies 1200 series HPLC). The system consists of a model G1311A quaternary pump and a model G1314B variable wavelength detector (VMD). A C18 reverse phase 250  $\times$  4.6 mm 5  $\mu$  (Agilent ZORBAX C8) was used. The mobile phase used was a combination of acetonitrile/methanol 50:50 (v/v). Flow rate was set to 1 mL/min, and absorbance was measured at 300 nm. Vitamin E (Sigma Chemical Co., St. Louis, MO, USA), standard was prepared in absolute ethanol. Quantification was done by comparison with standard curve of different concentrations of standard vitamin (ranges from 0.01 to 5 mg/mL) against peak area. Results are expressed as  $\mu$ g/g plant dry wt. Each measurement was performed in duplicate.

### Antioxidant Activity:

#### Antioxidant Activity using (DPPH) Assay:

Antioxidant activity using (DPPH) was measured following the procedures described by (Shimada, Fujikawa et al. 1992). 5 mL DPPH solution were added to 50  $\mu$ L of each test solution at a concentration range of 1–100 mg lyophilised extract/mL 70% methanol. The concentration of the DPPH solution was 0.004 % in methanol. The mixture absorbance was measured against a blank at 514 nm spectrophotometrically after 30 min incubation at room temperature. The used positive control was vitamin E (Sigma-Aldrich) at a concentration of 1–100 mg/mL. The antioxidant activity was expressed as  $IC_{50}$  value which means the concentration of sample inhibiting DPPH free radical formation by 50% relative to the blank. The DPPH free radical inhibition percent (I %) was estimated using the following formula  $I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$ , where  $A_{blank}$  is the control absorbance, and  $A_{sample}$  is the absorbance of the extract or vitamin E absorbance. Results are expressed as  $IC_{50}$  ( $\mu$ g/mL). Each measurement was carried out in triplicate. Samples with high antioxidant activity showed low  $IC_{50}$  value.

#### Antioxidant Activity by $\beta$ -Carotene-Linoleic Acid Method:

Antioxidant activity by  $\beta$ -Carotene-Linoleic Acid was measured according to the procedures described by (Taga et al. 1984). 10 mg of  $\beta$ -carotene was dissolved in 10 mL of chloroform. The carotene-chloroform solution, (0.2 mL) was pipette into a boiling flask containing 20 mg linoleic acid (Sigma-Aldrich) and 200 mg Tween 80. Chloroform was removed using a rotary evaporator at 40 °C for 5 min. 50 mL of distilled water were added to the residue with vigorous agitation, to form an emulsion. Five milliliters of the emulsion were added to a tube containing 0.2 mL of extract prepared at a concentration of 2 mg/mL in 70 % methanol, and the absorbance was immediately measured at 490 nm against a blank, consisting of an emulsion without  $\beta$ -carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 490 nm over a 120 min period. Control samples containing 0.2 mL 70 % methanol instead of extract. The same procedure was repeated with the positive control vitamin E. Antioxidative capacities of the extracts were compared with that of vitamin E. The antioxidant activity was expressed as inhibition percentage with reference to the control after 120 min incubation using the following equation:  $AA = 100(DRC - DRS)/DRC$ , where AA is the antioxidant activity %; DRC is the degradation rate of the control =  $[\ln(a/b)/60]$ ; DRS is the degradation rate in the presence of the sample =  $[\ln$

(a/b)/60]; a is the absorbance at time 0; b is the absorbance at 120 min. Each measurement was performed in triplicate (n=3).

#### Phenolic compounds profiling using High Performance Liquid Chromatography (HPLC):

Determination of polyphenols in both canola seeds & meal extracts was carried out according to the protocol of Agilent Application Note, publication number 5991-3801EN described by (Sohaimy, Mohamed et al. 2018), A 0.1 g sample was soaked overnight in 25 ml 80% methanol followed by centrifugation at 4000 rpm for 20 min. The supernatant was evaporated till dryness then the residue was reconstituted in 5 ml HPLC grade methanol and filtered with 0.45  $\mu\text{m}$  PTFE syringe filter. The HPLC system was equipped with a quaternary pump, a Zorbax Eclipse Plus C<sub>18</sub> column (100×4.6 mm id) operated at 25°C. The chromatographic separation was achieved using a ternary linear elution gradient with (a) HPLC grade water with 0.2% v/v H<sub>3</sub>PO<sub>4</sub>, (b) Methanol and (c) Acetonitrile. The injected volume was 20  $\mu\text{l}$ , and VWD detector was set at 284 nm. Identification of isolated compounds were achieved by comparison with available standard of phenols (Gallic acid, Catechol, p-Hydroxy benzoic acid, caffeine, Vanillic acid, Caffeic acid, Syringic acid, Vanillin, p-Coumaric acid, Ferulic acid, Ellagic acid, Benzoic acid, o-Coumaric acid, Salicylic acid and Cinnamic acid) and quantification was done according to standard calibration curves which were prepared in the same conditions.

#### Flavonoids profiling using HPLC:

Identification of flavonoids in both canola seeds & meal extracts was carried out according to the protocol of Agilent Application Note, Publication number 5990-9547EN, 2011, described by Mothibedi et al. 2011. HPLC, Smart line (Knauer, Germany), equipped with binary pump, a Zorbax Eclipse plus C<sub>18</sub> column (150 mm× 4.6 mm i.d.), (Agilent technologies, USA), operated at 35 °C. Eluent: methanol: H<sub>2</sub>O with 0.5% H<sub>3</sub>PO<sub>4</sub> 50:50 with flow rate 0.7 ml/min, the injected volume was 20  $\mu\text{L}$ . Detection was achieved using UV detector set at 273 nm and data integration by claritychorm@ software. Identification of isolated flavonoids was achieved by comparison with available standard flavonoids (rutin, myricetin, quercetin,

naringenin, kaempferol and apigenin) and quantification was done according to standard calibration curves which were prepared in the same conditions.

## RESULTS & DISCUSSION

The TPC that amounts 33.49  $\mu\text{g}/\text{mg}$  in whole seed approximately increased markedly by 34% in the canola seeds meal due to expected reason: the concentration of phenolic compounds embedded in protein matrix and the pressing method of defatting has no effect on phenolic compounds. The same trend is also observed for total flavonoids since the TF contents were 12.17 and 16.38  $\mu\text{g}/\text{mg}$  of canola seeds and its defatted meal, respectively, as shown in Table 1. The percentage of increase amounts 34% close to the increase in TPC. This finding confirms that canola seeds meal is a good source of phenolics and flavonoids, which coincide with the previously published results for canola seeds (Jun et al. 2014).  $\alpha$ -tocopherols content which represent 355.12  $\mu\text{g}/\text{g}$  canola seeds increased markedly to 448.65  $\mu\text{g}/\text{g}$  canola seeds defatted meal. This finding reflects the advantage of canola seeds defatted meal as a good source of  $\alpha$ -tocopherols (Flakelar et al. 2015). the percentage of increase in meal is 31% than seeds.

Phenolics, flavonoids and  $\alpha$ -tocopherols play an important role in the antioxidant activity of canola seeds (Jun et al. 2014; Flakelar et al. 2015). It has been observed that due to the apparent increase in TPC, TF and  $\alpha$ -tocopherols in rape seed defatted meal compared to the seeds itself; the antioxidant activity (measured by DPPH) was markedly higher in defatted meal. The percent of increase amount 32%. This trend is compatible to the changes of TPC, TF and  $\alpha$ -tocopherols contents during defatting the determination of antioxidant activity by  $\beta$ -Carotene-Linoleic Acid method confirms the aforementioned data.

Table (2) represents the identification of phenolic substances in the lyophilized phenol extract. It is clear that vanillic acid 783.66  $\mu\text{g}/\text{g}$  extract followed by syringic acid (52.24  $\mu\text{g}/\text{g}$  extract) are the major phenolic substances, both acids were previously identified in seeds (Krygier et al. 1982), while the lowest one is caffeine (8.25  $\mu\text{g}/\text{g}$  extract).

**Table 1. TPC, TF,  $\alpha$ -tocopherol contents and antioxidant activity of canola seeds and its meal.**

	Whole seeds	Defatted meal
T.P.C $\mu\text{g}$ trans sinapic acid /mg plant dry wt.	33.49 $\pm$ 0.027	45.16 $\pm$ 0.03
(TF) $\mu\text{g}$ quercetin/mg plant dry wt.	12.17 $\pm$ 0.40	16.38 $\pm$ 0.24
$\alpha$ -tocopherol content $\mu\text{g}$ Vitamin E /g plant dry wt.	355.12 $\pm$ 38.25	448.65 $\pm$ 18.88
Antioxidant (DPPH) inhibition IC <sub>50</sub> (mg/mL)*	10.50 $\pm$ 0.26	7.19 $\pm$ 0.13
Antioxidant( $\beta$ -Carotene-Linoleic Acid) **	84.52 $\pm$ 0.40	89.30 $\pm$ 0.26

\* Vitamin E as standard was 0.0764  $\pm$  0.0010

\*\*Vitamin E as standard was 91.43  $\pm$  0.34

**Table 2. Phenolic compounds profiling with HPLC:**

No.	Rt.	Name	Conc. ( $\mu\text{g/g}$ extract)
1	4.354	Gallic acid	17.84
2	9.627	p- Hydroxy benzoic acid	35.34
3	10.389	Caffeine	8.25
4	10.813	Vanillic acid	783.66
5	11.755	Syringic acid	52.24
6	15.313	Ferulic acid	17.29
7	16.465	Ellagic	25.03
8	18.262	O-Coumaric acid	11.66
9	19.364	Salicylic acid	18.32

**Table 3. Flavonoids profiling by HPLC:**

No.	Rt.	Name	Conc. ( $\mu\text{g/g}$ extract)
1	3.433	Rutin	631.13
2	8.767	Naringenin	6.0

The identification of flavonoids that presented in table (3) showed that just two main components were appeared. The concentrations of the two compounds are: Rutin (quercetin- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranose) and naringenin were 631.13 and 6.0  $\mu\text{g/g}$  extract, respectively. Quercetin glycosides were previously identified in canola seeds utilizing HPLC technique (Qu, Fu et al. 2013).

### CONCLUSION

From the aforementioned data it is concluded that rapeseed meal is a good source for phytochemical like phenolic substances and flavonoids and food industries should be focused on maximize the utility of rapeseed meal in preparing a functional food.

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## الملخص العربي

### كسب الكانولا كمصدر واعد لمضادات الأكسدة الطبيعية

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جهاز كروماتوجرافيا الغاز عالي الكفاءة (HPLC) أن حمض الفانيليك هو الأكثر وجوداً بتركيز ٧٨٣,٦٦ ميكروجرام/ جم مستخلص يليه حمض السينرجيك بتركيز ٥٢,٢٤ ميكرو جم / جم مستخلص، في حين كان الروتين هو الأكثر وجوداً في تفريد المواد الفلافونويدية بتركيز ٦٣١,١٣ ميكرو جرام / جم مستخلص. لذلك توصي الدراسة بأن تركز الأبحاث العلمية علي تعظيم الاستفادة من كُسب الكانولا كمصدر واعد لمضادات الأكسدة الطبيعية.

قامت هذه الدراسة علي استخدام كل من بذور الكانولا وكسب الكانولا بعد استخلاص الزيت للحصول علي المستخلص الميثانولي من كل منهما بشكل منفصل ثم تقدير محتوى كل منهما من المواد الفينولية الكلية والمواد الفلافونويدية و الألفا توكوفيرولات ومن ثم تقدير فاعليتها كمواد مضادة للأكسدة بواسطة طريقتي (DPPH) وحمض بيتا كاروتين لينوليك حيث أظهرت النتائج المحتوى العالي من هذه الكيماويات ذات المصدر النباتي. كما أظهر المستخلص الكحولي لكسب الكانولا قدره اختزالية عالية تفوق مستخلص البذور بنسبة ٣٢%. وأظهر تفريد المركبات الفينولية بواسطة