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Improving the canolol amount and the yield of expressed canola oil applying combined pre-treatments

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Abstract

Canolol is a potent natural antioxidant. It exhibits anti-mutagenic and anti-carcinogenic properties, showing an even more potent anti-mutagenic activity than that of α-tocopherol and flavonoids. This compound is formed due to high temperatures during processing of some seeds of Brassica family, like canola. The nutritional value of canola oil can be increased by increasing the content of canolol, subjecting the seeds to different pre-treatments (steam, microwaves). The aim of the present work was to study the treatments applied to canola seeds in order to improve the quality of the expressed oil, increasing the amount of canolol, and also to obtain higher yields of oil by pressing. Canola seeds were subjected to different treatments: hydrothermal (HT), by exposing the grains to steam in an autoclave; microwave (MW), irradiation in a microwave oven; combination of both treatments (HT-MW), exposing the seeds to water vapour and then microwaved until grains reached 7% moisture (dry basis). Oil was extracted from pre-treated and untreated samples by pressing using a helical screw press. Oil yield was determined by evaluating the residual oil remaining in the press cake. Canolol content was determined in all samples by HPLC. HT-MW significantly increased the amount of canolol in oil (495 ppm, in contrast with 5 ppm in untreated samples) and significantly improved its oxidative stability. Moreover, HT-MW generated the highest oil yield (86%). Pressing untreated canola yielded 68%, while HT yielded 76%, and MW only yielded 8%. SEM micrographs showed a more open structure in HT-MW samples which could have improved the availability of oil, favouring the oil expression process.

Introduction

Industrially, conventional oil expression from oilseeds is carried out by pressing and/or solvent extraction. Hexane, being the food-grade solvent, is the most commonly used. Nowadays however, there is a wide market willing to pay a higher value for oils obtained by natural processes, such as the mechanical expression (Carletti, 1999). Principal reasons for this increasing interest are the excellent nutritional properties and natural flavour of the end product.

Canola oil's' beneficial health properties are related to its low content of saturated fats and significant amounts of essential fatty acids. Furthermore, it has been shown to contain greater quantity of phenolic compounds as compared to other oilseeds (Spielmeyer *et al*., 2009). In addition to its food use, it is also suitable for the production of alternative fuels due to its physicochemical properties. Besides, its cultivation has also shown great potential both for its grain yield per hectare as well as the oil yield of the seed, with a simple management scheme.

During oil extraction, seeds are subjected to a series of unit operations such as drying, storage, cleaning, grinding or rolling, heat treatment and mechanical pressing, extrusion and/or solvent extraction. Some treatments, such as hydrothermal pre-treatments, are applied to oilseeds prior to extraction with the aim of modifying or breaking the internal structure in order to facilitate the oil release, thus improving the oil yield (Fernández, 2015). In the last decades there has been a growing demand for new techniques of pre-treatment and/or extraction which shorten extraction times and diminish or eliminate the consumption of organic solvents, thereby reducing pollution. Among these pre-treatments, microwave radiation is included. The use of this technology offers reduced processing times and energy savings,

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since the energy is delivered to the material directly by molecular interaction with the electromagnetic field, so that the heat is generated through the volume of the material achieving a rapid and uniform heating of materials of a relatively higher thickness. A better oil yield can be achieved as a result of the rupture of the cellular membrane due to microwave, generating permanent pores, allowing the oil to be transported through permeable membranes (Uquiche *et al*., 2008). These pre-treatments could also affect the release of minor compounds of great importance such as tocopherols and canolol (Fernández, 2015). The positive effect of high temperatures on the production of canolol from sinapic acids of canola seeds has been widely investigated (Koski *et al*., 2003; Wakamatsu *et al*., 2005; Spielmeyer *et al*., 2009). The antioxidant activity of these compounds has been reported by several authors (Koski *et al*., 2003; Vuorela *et al*., 2004; Vuorela *et al*., 2005; Fazel *et al*., 2008) with canolol shown to be even more potent than other well-known antioxidants including α-tocopherol (Wakamatsu *et al*., 2005). Unlike crude canola oil, no canolol can be found in the refined one since this compound is completely removed during the refining process. (Zacchi and Eggers, 2008). It was found that crude canola oil with higher amounts of canolol showed remarkably higher oxidative stability when compared with completely refined canola oil (Matthäus, 2012).

The aim of the present work was therefore to improve the oil extraction yield and canolol content in expressed canola oil applying hydrothermal and microwave combined pre-treatments to canola seeds, and also to assess their effects on tocopherol content, fatty acid composition and oxidative stability index.

Materials and methods

Raw material characterisation

Canola seeds were stored below 8°C until further use. They were characterised according to the standard methods for determining moisture (ASAE S352.2 DEC 97), oil (IUPAC 1.122, 1992), protein content (AOCS Ai 4-91, 1998) using a BÜCHI distiller (Model 435, Switzerland), neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, lignin and cellulose (Goering and Van Soest, 1970).

Conditioning essays

Drying by hot air

A total of 100 g of entire seeds were dried at 35°C in forced air oven (DHG-9123, China) up to a moisture content of 7% dry basis (d.b.) which is a safe storage moisture (Cassells *et al*., 2003). It was also reported that the moisture content for an adequate pressing operation is in the range of 5.0-6.5% (5.3-7.0% d.b.; Unger, 1990). Drying temperature of 35°C was selected in order not to affect the oil quality characteristics.

Hydrothermal pre-treatment (HT)

A total of 200 g seeds were subjected to a hydrothermal pre-treatment with water steam in an autoclave (VZ, Argentina). The seed samples were placed in trays with a metallic mesh base (149 μm opening) to facilitate the generation of steam from the bottom of the container. The hydrothermal treatment was carried out using the entire seeds at 130°C for 5 min. The samples were then dried up to a moisture content of 7% d.b. at 35°C in a forced circulation tunnel dryer (Armfield, England).

Microwave pre-treatment (MW)

Calibration of the microwave oven power

For the application of the pre-treatment by microwave radiation, a BGH Quick Chef microwave oven (model 36960, Argentina) was used. The microwave was calibrated to verify the powers by heating a fixed amount of water in the apparatus for 60 s, and then measuring the difference in temperature. The procedure was performed in triplicate. The power absorbed by the water was calculated by Equation 1:

$$W = m_w C_{pw} \frac{\Delta T}{\Delta t} \qquad \text{(Equation 1)}$$

where W was the power absorbed by the water (Watts), m_w was the mass of water (kg), C_{pw} was the specific heat (J/°C kg), ΔT was the difference in temperature (°C), and Δt was the exposure time (seconds).

The mass of water used (≈ 500 g) was distributed into a Pyrex tray (30 cm diameter), in a similar manner as canola samples were arranged in the subsequent experiments. The absorbed powers by the water obtained was 491 W for 100% microwave nominal power, respectively (herein after referred to as 100% microwave power).

Pre-treatment with microwaves

Samples of 100 g was distributed into a Pyrex tray, and placed inside the microwave (BGH Quick Chef, model 36960, Argentina). The samples were treated for 5 min at a frequency of 2,450 MHz and at 100% (491 W of absorbed power, referred to the calibration mentioned in detail in section Calibration of the microwave oven power). The temperature of the samples was measured with an infrared thermometer (CEM DT-812, China).

Combined pre-treatment (HT-MW)

Samples of 200 g of entire canola seeds were subjected to hydrothermal treatment at 130°C for 5 min in the apparatus described in the section Hydrothermal Pre-treatment (HT). Next, samples were distributed into a Pyrex tray, placed inside the microwave (BGH Quick Chef, model 36960, Argentina) and irradiated at 100% of nominal power until its moisture reached 7% d.b.

Oil expression

Oil from pre-treated and untreated samples was extracted using a helical screw press IMEGEN (Córdoba, Argentina, Figure 1), with a capacity of 3 kg/h. During pressing, the temperature was kept below 40°C. Following pressing, oils were separated by centrifugation in a refrigerated centrifuge (Presvac, Argentina) at 12°C and 5,438 g, and collected in order to proceed with subsequent analysis. Expressed oil yield was calculated from the determination of the expeller residual oil content: extraction of the oil from 5 g of ground expeller was carried out with hexane for 3 h using a Butt apparatus (AOCS Ba 3-38, 1998).

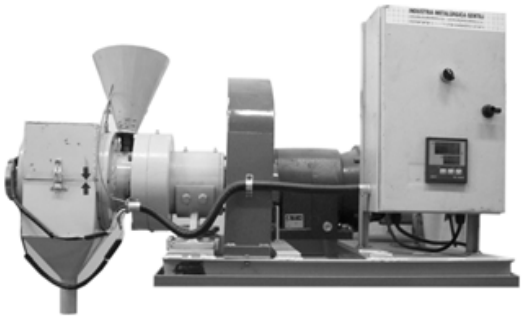

Figure 1. Helical screw press IMEGEN (3 kg/h)

Canolol and tocopherol concentration by HPLC

The synthesis of canolol standard from syringaldehyde (3,5-dimethoxy-4-ydroxybenzaldehyde) was carried out following the steps described in previous work (Sánchez *et al*., 2017).

Canolol and tocopherol concentration in the oil was determined by normal-phase ultra high-performance liquid chromatography (UHPLC) following a modified procedure from Wakamatsu *et al*. (2005). A Dionex Ultimate 3000 chromatograph (Thermo Scientific, Germany) with fluorescence detector (Agilent, 1100 Series Fluorescence Detector G1321A, Palo Alto, CA, USA) with excitation/emission wavelengths of 290/330 nm, equipped with a Luna NH2 100 column (250 × 4.6 mm i.d., particle size 5 µm; Phenomenex) was used. Hexane:isopropanol (96:4 v/v) was used as the mobile phase, with a column flow of 2mL/min. Canolol was quantified on the basis of the calibration curve using the synthesised standard (Sánchez *et al*., 2017) while levels of α-tocopherol were quantified according to AOCS standard method Ce 8-89 Note 3 (1998). All determinations were performed in duplicate.

Oxidative stability index (OSI)

The oxidative stability index, reported as induction time in hours, was measured according to AOCS official methods Cd 12b-92 (AOCS, 1998) using a Metrohm 679 Rancimat (Metrohm, Switzerland) at standards conditions (110°C and 20 L/h airflow).

Fatty acid composition

The fatty acid composition was measured by gas chromatography according to the method described by Izquierdo *et al*. (2002).

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to determine the effect of the pre-treatment on the structure of canola seeds. SEM studies of the untreated and treated seeds were carried out using a scanning electron microscope (LEO, model EVO40 VP, England).

Statistical analysis

The experimental data were analysed by Analysis of Variance (ANOVA) followed by Tukey's comparison test. Differences were considered significant at $p < 0.05$. For this purpose, Infostat software package was used (INFOSTAT, 2004).

Results and discussion

Characterisation of raw material

Table 1 shows the moisture content, percentage of oil, protein content, neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, lignin and cellulose contents. All values were expressed in dry basis (d.b.). The oil content fell within the range reported by Ramos *et al*. (2017). The protein content corresponded to a value of 29.6% in dry defatted meal, lower than that reported in previous work (Thakor *et al*., 1995). The NDF content of 30.9% was similar to that found in the literature (25.7% d.b.; Slominski *et al*., 1994).

Effect of pre-treatments on oil yield

The oil yield (% d.b.) of canola seed obtained by pressing can be observed in Figure 2 for samples dried in an oven (untreated, UT), samples irradiated with microwave (MW), pre-treated hydrothermally

and subsequently dried in air forced dryer (HT) and subjected to combined pre-treatment (HT-MW).

Table 1. Characterisation of canola seeds

Components (% d.b.)	Canola
Moisture	8.0 ± 0.01
Oil	51.5 ± 0.28
Protein	14.4 ± 0.08
NDF	15.0 ± 0.06
ADF	11.9 ± 0.06
Lignin	4.7 ± 0.08
Cellulose	7.3 ± 0.15
Hemicellulose	3.1 ± 0.15

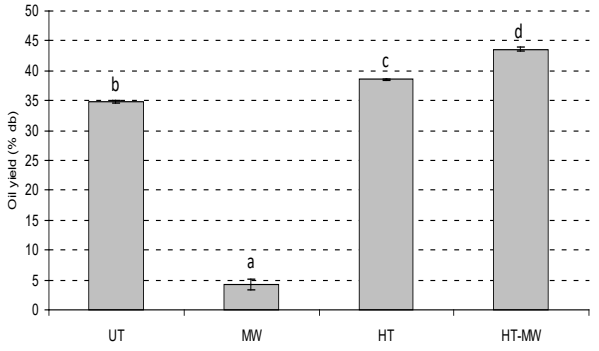

Figure 2. Oil yield by pressing from untreated (UT), microwaved (MW), hydrothermally treated (HT) and combination of hydrothermal pre-treatment and microwave (HT-MW). *Different letters indicate significant differences (Tukey's Test, $p < 0.05$*

Expression of UT samples yielded 68% of the oil contained in grains (51.5%, d.b., Table 1), while HT and HT-MW samples yielded 76% and 86% of the total oil from the seed, respectively. However, MW samples only yielded 8% of the total oil. It is worth mentioning that these samples, after pre-treatment, achieved a moisture content of 1.3% (d.b.). The higher value obtained by HT-MW could be explained by a modification of internal structure of the seeds, acquiring a larger porosity or by a higher plasticity achieved by the canola seeds, allowing a more efficient expression (Fornal *et al*., 1996). Furthermore, HT samples also presented an increase in oil yield when compared to UT samples; but this effect was not as pronounced as in HT-MW. Regarding the low level of extraction obtained from MW samples, it could be explained by the notable diminution of moisture due to the treatment, this value being out of the suggested range of moisture for pressing (Unger, 1990). It is worth mentioning that when extracting by solvent, positive effect of MW was observed in which it achieved a faster oil extraction (Ramos *et al*., 2017).

Effect of pre-treatments on minor compounds tocopherols and canolol

Table 2 shows the expressed oil tocopherol and canolol contents from untreated (UT), hydrothermal treated (HT), microwaved (MW) and conditioned by combined pre-treatments with vapour and microwave (HT-MW) seeds.

The content of each one of the tocopherols in oil obtained did not vary significantly among treated samples (HT, MW y HT-MW). Likewise, γ-tocopherol, δ-tocopherol and total tocopherols of treated samples has not been modified significantly respect to untreated seeds (UT). Only the quantity of α and β-tocopherols were slightly affected when compared to UT in all cases. However, this variation is not perceived in the amount of total tocopherol response.

Regarding to the canolol content, a marked variation is observed due to the pre-treatments applied, significant differences were also found among treatments. Combined pre-treatment (HT-MW) generated the largest amount of canolol, followed by pre-treatment with microwave (MW) and the hydrothermal pre-treatment (HT).

On the other hand, oil extracted by pressing of untreated samples (UT) presented minimum canolol content (5µg/g oil). The increase of this compound in oil from heat-treated seeds has been widely reported attributing the formation of the canolol to the decarboxylation of the sinapic acid present in the grain, due to the exposition of the canola seeds to high temperature during conditioning. After microwave treatment temperature reached up to 190ºC, meanwhile samples submitted to HT processing achieved a temperature of only 130ºC.

Table 2. Tocopherols and canolol content (µg/g oil) of the oil extracted by pressing from untreated and treated samples.

Treatment	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols	Canolol
UT	253 ± 5^{a}	24 ± 3^{a}	499 ± 19^{a}	11 ± 4^{a}	787 ± 26^{a}	5 ± 0.2^{a}
HT	275 ± 3^{b}	47 ± 1^{b}	506 ± 5^{a}	16 ± 10^{a}	844 ± 18^{a}	373 ± 8.4^{b}
MW	268 ± 1^{b}	45 ± 1^{b}	495 ± 14^{a}	7 ± 3^{a}	815 ± 15^{a}	466 ± 6.5^{c}
HT-MW	271 ± 2^{b}	47 ± 1^{b}	492 ± 13^{a}	7 ± 2^{a}	816 ± 16^{a}	495 ± 5.4^{d}

UT: Untreated; HT: Hydrothermal treatment; MW: microwave; HT-MW: combination of hydrothermal pre-treatment and microwave. *Different letters in the same column indicate significant differences (Tukey's Test, $p < 0.05$)*

Effect of combined pre-treatment (HT-MW) on oxidative stability, fatty acid composition and structure

Table 3 shows the results of oxidative stability index and fatty acid composition of oil expressed from UT and HT-MW samples. It was found that HT-MW significantly improved oxidative stability of the oil when compared to UT samples. This fact was expected because of the increased canolol content. On the other hand, fatty acid composition did not vary significantly due to the combined pre-treatment applied.

Table 3. Oxidative stability index (OSI) and fatty acid composition of the expressed oil from untreated (UT) and combination of hydrothermal pre-treatment and microwave (HT-MW) samples.

Determination	UT	HT-MW
OSI (h)	4.96 ± 0.01	14.10 ± 0.01
C18:0 (%)	1.48 ± 0.02	1.34 ± 0.52
C18:1 (%)	69.24 ± 0.03	68.88 ± 1.17
C18:2 (%)	17.75 ± 0.23	17.92 ± 0.14
C18:3 (%)	6.81 ± 0.17	6.87 ± 0.39
C22:1 (%)	0.50 ± 0.02	0.68 ± 0.17

Micrographs of UT and HT-MW samples are shown in Figures 3 (a) and (b), respectively. Different structural configurations can be observed. In HT-MW samples, the structure was more open as compared to that in UT samples. The HT-MW was found to improve the availability of the oil because the solid matrix was modified due to the denaturalisation of the protein, and this also reduced the viscosity of the oil by the effect of temperature. These observations are in agreement with those reported in the literature (Uquiche *et al*., 2008; Zárate *et al*., 2015; Ramos *et al*., 2017) when employing hydrothermal pre-treatments and microwaves.

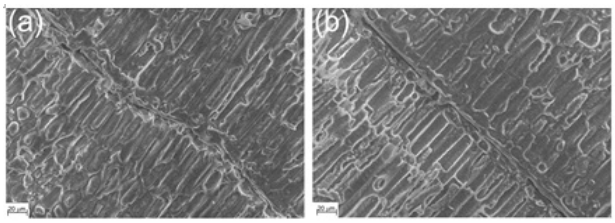

Figure 3. Micrograph of (a) the untreated canola sample, and (b) the pre-treated canola sample with Hydrothermal-Microwave combined treatment.

Conclusion

Combined hydrothermal-microwave pre-treatment applied to canola seeds significantly improved the oil extraction by pressing when compared to untreated sample. The effect of hydrothermal pre-treatment also exhibited similar trend but was less pronounced. However microwaved canola was shown to be the less effective during pressing, since the oil yield markedly diminished. This behaviour could be attributed to the notable decrease of the seed moisture. On the other hand, canolol content also increased due to the pre-treatments applied in all cases, especially in hydrothermally-microwave pre-treated samples, where the value achieved (495 µg/g oil), and was significantly higher than those obtained when the other treatments were applied. The combined treatment also improved the oxidative stability of the expressed oil. In general, the tocopherol content did not vary due to the pre-treatments the seeds were subjected to. In either case, the fatty acid composition was not affected due to the combined pre-treatment.

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