Exploring the effect of combined hydrothermal-microwave pretreatment operating conditions on canola oil expression.

Cortese CM[1], Portela G[1,2], Sanchez RJ[1], Fernandez MB[1,2*]

[1]Nucleo TECSE, Facultad de Ingenieria, Universidad Nacional del Centro de la Provincia de Buenos Aires, Av. Del Valle 5737, Olavarria, Argentina

[2]CIFICEN, Universidad Nacional del Centro de la Provincia de Buenos Aires-CONICET-CICPBA, Pinto 399, Tandil, Argentina

Abstract

The effect of the combined hydrothermal-microwave (HT-MW) pretreatment operating conditions on the oil extraction yield by cold pressing was evaluated, as well as its canolol and tocopherols content.
Entire and broken canola seeds were subjected to hydrothermal pretreatment at different temperatures (110ºC and 130ºC), then they were irradiated in a microwave oven until reaching a moisture content of 6.5 % (dry basis), that it is a safe storage moisture and it is adequate for the pressing process. Entire seeds exposed at a HT temperature of 130ºC and microwaved for 2.98 mins exhibited the best canola oil expression yield (34.9 %, d.b) and the highest amount of canolol (533 µg/g oil), maintaining the total tocopherol content invariant with respect to oil from samples subjected to the other pretreatment operating conditions and from untreated seeds. However, individual tocopherol isomers presented different contents when varying pretreatment conditions, showing a differential extraction due to the factors analysed (seeds granulometry/breakage and HT temperature)
At the optimum conditions quality as means of acidity, peroxide index and p-Anisidine value were determined. Acidity index slightly increased due to the pretreatment and peroxide index significantly diminished, being both value below the limits preestablished by the Alimentarius Codex. Meanwhile, p-Anisidine value notably increased, slightly affecting TOTOX value.

Keywords: Canola oil, Canolol, Tocopherols, Expression, Hydrothermal pretreatment, Microwave.

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Introduction

Canola oil is considered one of the most important vegetable oil in the world after palm oil and soybean oil [1]. It ranks third in world production, being the main producers the European Union, China, India and Canada. It is characterized by an oleic acid high content and a high relative content of α-linolenic acid. This, together with a 2:1 ratio between ω-6 (C18: 2 α-linoleic) and ω-3 (C18: 3 linolenic) fatty acids, make canola oil nutritionally attractive.

Also, it is relatively rich in phenolic compounds, tocopherols and phytosterols [2], which are important for health and oxidative stability of vegetable oils. The high percentage of polyunsaturated fatty acids would make canola oil prone to oxidative deterioration. For this reason, the presence of natural antioxidant substances such as tocopherols, phytosterols and canolol are essential for better in-vitro conservation.

The extraction of vegetable oil is carried out by pressing and/or solvent extraction. Although solvent extraction is more efficient, it presents safety problems, emissions of volatile organic compounds, high operating costs and high temperature product exposures that decrease the final quality of the product [3]. Press extraction is a simpler process which has lower operating costs, less aggressive to the environment and generates chemical-free oil [4]. Due to the lower efficiency of that process, it has been advanced in the application of pretreatments, which modify or break the cellular structure facilitating the oil release and improving the extraction yield [3-5]. Conventional pretreatment includes shelling, size reduction, breakage, shredding, thermal treatments and enzymatic hydrolysis [3,4]. Nowadays emerging technologies are being considered for this purpose, like microwave radiation [5].

Ghazani, García-Llatas, and Marangoni [6] found that the type of process (cold-pressing, hot-pressing or solvent extraction) affected the concentration of unsaturated fatty acids. Cold-pressed canola oils had higher linoleic and linolenic acid contents, and much lower oleic acid contents to those extracted by solvent. This implies a better nutritional quality (greater amount of ω-3 fatty acid) but lower oxidative stability.

Some years ago, a synapic acid derivative, commercially known as canolol, has been identified as having high antioxidant activity, antimutagenic and anticarcinogenic properties [7,8]. Koski, Pekkarinen, Hopia, Wähälä, and Heinonen [9], showed that canolol contributes to the better stability of crude canola oils and has lipid antioxidant activity comparable to that of γ-tocopherol. The increase of canolol in canola oil would produce oil with higher nutritional value and shelf life [10]. Although it is not naturally present in rapeseed, canola and other varieties of Brassica, it can be generated by the exposure to high temperatures and consecutive

decarboxylation of synapic acid. The temperatures generated during the pressing process [9], grain roasting [11] and pretreatments with heat (steam) and microwave [12], improve the concentration of canolol in the oil. In addition, Wakamatsu [11] and Spielmeyer [10] both found no significant differences in the tocopherol content of canola oil from unroasted and roasted raw material. This may be because the canolol acts as an antioxidant protecting the tocopherols from degradation during the heating process.

The main objective of this work was to study the effect of combined hydrothermal-microwave pretreatments operating conditions on oil yield, canolol and tocopherol contents. Besides, in optimum conditions quality of expressed canola oil was evaluated as a function of acidity, peroxide p-anisidine values.

Materials and Methods

Raw material characterization

Winter canola (Brassica napus L.) seeds supplied by Al High Tech Argentina. They were stored at temperatures below 8°C until further use.

Conditioning essays

Entire and broken canola seeds were used, some untreated and others undergoing pretreatment.

Untreated entire samples

The untreated entire seeds were dried at 35 °C in forced air tunnel (Armfield, England) up to a moisture content of 6.5%, dry basis (d.b.), being safe storage moisture [13] and adequate moisture content for pressing operation [14]. Drying temperature of 35 °C was selected in order not to affect the oil quality characteristics. Then, they were stored in hermetically containers with nitrogen in their head space to prevent oxidative deterioration by the presence of oxygen at a temperature of 5 °C until further use.

Broken samples

A horizontal blade laboratory grinder (Moulinex, Argentina) was used to break the seeds to a particle size ranging from 1.00 to 2.00 mm. Then, untreated samples (PB) were subjected to drying in a tunnel drier at 35 °C to a humidity of 6.5-7% d.b. And they were stored in the same way in container with the addition of nitrogen in the headspace at 5°C.

Moisture content

The moisture of the entire seeds was determined in duplicate according to ASAE S352.2 DEC 97. The moisture of broken seeds was determined in duplicate according to AOCS Ba 2a-38 [15].

Hydrothermal treatment (HT)

Both entire and broken seeds were subjected to a hydrothermal pretreatment with water steam in an autoclave (VZ, Argentina). 200 gram samples per lot were placed in trays with a metallic mesh base (149 μm opening). The hydrothermal treatment was carried out at 110 and 130ºC during 5 min.

Sample temperature measurements were performed with an infrared thermometer CEM DT-812 (China) at the end of the pretreatment in five regions of the sieve. The selected points were the center of the sieve, and four points located at the border of the same.

Microwave treatment (MW)

Samples, previously hydrothermally treated, were submitted to irradiation in a domestic microwave (BGH Quick Chef, model 36960, 2450 MHz, Argentina). The seeds were placed in monolayers of 100 grams per lot in circular pan trays (Pirex, Brazil) and subjected to the maximum operating power (900 W powers) up to reaching a humidity of 6.5% d.b.

The temperature at the exit of microwave treatment was measured using the same methodology and equipment than hydrothermal output temperature.

The samples subjected to the combined hydrothermal-microwave pretreatment were stored in hermetically containers with the addition of nitrogen in their headspace at 5 °C.

Oil extraction by cold pressing

All samples were pressed with an IMEGEN helical screw press (Córdoba, Argentina) with a capacity of 3 kg/h. The discs inside the press were optimally arranged for canola pressing based on the methodology used by Cortese et al. [16]. The collected press oil was stored in caramel glass containers with nitrogen gas in the headspace to prevent deterioration of the sample by the presence of oxygen. The harvested press meal was stored in containers with nitrogen gas in the headspace, as they will be subjected to solvent extraction in Soxhlet to determine residual oil. The outlet temperature of the pressing cake was maintained in a range between 50 and 60 °C.

Clarification of the pressing oil

The oil obtained from the press was subjected to clarification in a centrifuge cooled at 10 °C, at a speed of 4500 rpm during 40 minutes. The resulting clarified oil was stored in caramel colored glass containers with nitrogen gas in the headspace, in a refrigerator at 5 ° C, protected from light.

Quality indices: Acidity, peroxide and p-Anisidine values

The acidity, peroxide and p-Anisidine values were determined following the methods IUPAC 2.201 [17], IUPAC 2.501 [17] and AOCS Cd 18-90, respectively. All determinations were carried out in duplicates.

Determination of tocopherol content

The tocopherol content was determined in duplicated by AOCS Ce 8-89 [15] using a Dionex Ultimate 3000 Ultra HPLC chromatograph, with a Lichrosorb Si 60 column, 25 to 0.4 cm and 5 mm particle size. To prepare the calibration curve, a standard of α-tocopherol (Sigma T#3251, 95% purity) was used; the other isomers are calculated according to AOCS [15].

Canolol content

A pure canolol standard previously isolated by Sanchez et al. [18] was used for the calibration curve. An aliquot of 0.04 grams of canolol standard was weighed into a 25 mL volumetric flask and routed with HPLC grade hexane. It was homogenized in an ultrasonic bath for 1 minute; maintaining at all times the flask wrapped in aluminum foil protected from sunlight. The calibration curve was assembled by dissolving the above solution in decreasing concentrations of canolol. For the determination of canolol, the same samples as those used for the determination of tocopherols were used. Except for whole grains submitted to combine hydrothermal (130 ºC) - microondas (E130) pretreatment, which had a canolol content wich exceeds the scale of the calibration curve. For the analysis of the E130 sample, 0.075 grams of oil was weighed into a 25 ml volumetric flask and basified with HPLC grade hexane. It was homogenized in ultrasonic bath for 1 minute, keeping the flask wrapped in aluminum foil. All measurements were performed in triplicate.

Residual oil in pressing cake

The determination of the residual oil content in the pressing flour was carried out in a similar way to the determination of total oil in grains. 10 grams of previously crushed flour was weighed and brought to Soxhlet for 8 hours at 80 °C and atmospheric pressure. The solvent was removed by rotating the miscella at a temperature of 50 °C under vacuum for 30 minutes. The remaining solvent was removed in a hot air oven for 2 hours at a temperature of 105 °C. And then the balls were cooled in a desiccator until constant weight. The determination was performed in duplicate.

Statistical analysis

The results were analyzed by analysis of variance (ANOVA) in conjunction with Tukey´s Test ($p<0.05$), using Software Infostat.

Results and Discussion

Influence of combined hydrothermal-microwave pretreatments on operating variables

Table 1 shows the moisture and temperature after hydrothermal pretreatment, the microwave irradiation time and the temperature after microwave treatment (MoHT, ToHT, tMW and ToMW, respectively).

Table 1: Moisture and temperature after hydrothermal pretreatment, microwave irradiation time and temperature after microwave treatment.

	Hydrothermal Treatment HT		Microwave MW	
Sample	Exit temperature ToHT (ºC)	Final moisture MoHT (% d.b.)	Irradiation time tMW (min)	Output temperature ToMW (ºC)
E110	68.4 a 1.67	13.9 a 0.01	2.95 b 0.22	82.6 b 6.31
E130	69.6 a 3.13	14.3 a 1.55	2.98 b 0.29	85.8 b 8.11
P110	65.6 a 3.65	14.6 a 1.24	2.21 a 0.14	62.8 a 4.76
P130	64.4 b 2.97	16.6 b 1.88	2.32 a 0.22	61.8 a 3.77

E110: entire canola seeds pretreated, HT temperature: 110ºC; E130: entire canola seeds pretreated, HT temperature: 130ºC; P110: broken canola seeds pretreated, HT temperature: 110ºC; P130: broken canola seeds pretreated, HT temperature: 130ºC

Different letters in the same column indicate significant (ANOVA, Tukey´s Test, $p<0.05$)

Respect to moisture contents MoHT, significant differences were observed only in P130 sample (broken seeds hydrothermally treated at 130 ºC), being the sample with the higher MoHT. At the HT-temperature of 110ºC, the effect of granulometry on MoHT was not notable, meanwhile, it presented a considerable influence at 130ºC, indicating an interaction between these two factors. This fact could be due to the protective coating of the entire seed not allowing the temperature increment to modify the water capture by the seed. However, when the shell was broken the effect of temperature was noticeable, increasing the seed moisture with the increment of this variable. It is worth mentioning that as temperature increases, pressure and, consequently the amount of vapor to which seed was exposed were higher too.

Autoclave output temperatures (ToHT) showed similar values to each other, according to Tukey´s test ($p> 0.05$), so that the grain size and moisture content did not influence the temperature of the sample at the outlet of the autoclave.

Irradiation times (tMW) were affected by the granulometry of the samples, but not by the HT (hydrothermal treatment) temperature. Entire seeds required more time to reach the target humidity than broken ones, and consequently the final temperatures achieved ToMW were higher. The broken samples removed water faster than entire seeds, probably due to their size, morphology and the broken shell. The intact structure of the entire seeds implies a greater resistance for the diffusion of the water vapor towards the surface of the seeds.

Although moisture content was different (MoHT=14.6 and 16.6% d.b.) when studying broken seeds (P110 vs P130), tMW were similar.

Effect of combined pretreatment on oil yield, canolol and tocopherol contents

The pressing performance, the canolol and tocopherol contents in oil of untreated and treated seeds are shown in Table 2 and 3. The yields of the untreated samples EB and PB were the lowest (23.5% and 29.34%, respectively) and the higher extraction yields were obtained for pretreated entire seeds samples (E110 and E130), not showing significant differences between them. When comparing untreated samples, it is observed that seed breakage improves significantly extraction performance. It could be due to a more exposure of the oil since grinding broke the intact structure of the seed previous to pressing. However, this trend was not followed by pretreated samples, since entire seeds showed a better behavior when pressing that the broken ones.

Table 2: Oil yield and Canolol content of untreated and pretreated samples.

Sample	Oil yield (%, d.b.)	Canolol (µg/g)
EB	23.05 a 0.21	11.42 a 1.32
PB	29.34 b 0.06	22.8 b 0.33
E110	33.84 d.e 0.19	115.34 d 0.31
E130	34.91 e 0.06	532.75 f 0.54
P110	32.33 c 0.06	58.03 c 1.38
P130	32.70 c.d 0.32	341.99 e 1.75

EB: untreated entire canola seeds; PB: untreated broken canola seeds. E110: entire canola seeds pretreated, HT temperature: 110ºC; E130: entire canola seeds pretreated, HT temperature: 130ºC; P110: broken canola seeds pretreated, HT temperature: 110ºC; P130: broken canola seeds pretreated, HT temperature: 130ºC.

Different letters in the same column indicate significant (ANOVA, Tukey´s Test, $p<0.05$).

Table 3: Tocopherol contents of untreated and pretreated samples.

Tocopherol (µg/g oil)	EB	E110	E130	PB	P110	P130
α-tocopherol	226.2 b.c 6.70	212.7ª.b.c 0.23	218.1a.b.c 2.15	211.5 a.b 2.36	205.4 a 3.45	227.1c 3.84
β-tocopherol	27.8 a 0.62	34.7 c 0.84	41.6 d 0.69	31.6 b 0.03	37.0 c 0.56	39.9 d 0.90
γ-tocopherol	445.1 b 10.60	450.7 b 1.75	360.4 a 37.23	421.5 a.b 2.14	438.0 b 0.33	406.1a.b 1.85
δ-tocopherol	n/d	n/d	75.7 d 0.00	14.6 b 5.83	18.6 b 0.33	64.9 c 1.85
Tota tocopherols	704.3 a 10.50	698.1a 0.67	695.8a 38.69a	679.8 a 6.09a	699.2 a 12.94	738.1a 38.69

EB: untreated entire canola seeds; PB: untreated broken canola seeds. E110: entire canola seeds pretreated, HT temperature: 110ºC; E130: entire canola seeds pretreated, HT temperature: 130ºC; P110: broken canola seeds pretreated, HT temperature: 110ºC; P130: broken canola seeds pretreated, HT temperature: 130ºC.

Different letters in the same line indicate significant (ANOVA, Tukey´s Test, $p<0.05$).

The purpose of these pretreatments is to modify the cellular structure of the canola seed, so that it offers less resistance to the oil expression. Cortese et al. [16] determined by SEM that the pretreated seeds under superheated steam followed 5 minutes microwave irradiation showed a more open structure, improving the availability of oil, thus favoring oil extraction.

Likewise, significant differences ($p<0.05$) were observed in canolol contents of all analyzed samples. It is remarkable the difference in canolol content observed for the expressed oil from untreated seeds, with that obtained by those corresponding to the grains subjected to HT-MW treatments. The oil obtained by solid-liquid extraction has higher canolol content than the EB sample; this could be attributed to the extraction conditions (seeds temperature between 65.6 and 69.3 °C), favoring the formation of the compound. Although Mayengbam et al. [12], stated that the minimum temperature value for the decarboxylation of synapic acid is 90 °C.

For samples subjected to combined pretreatment HT-MW, substantial percentage differences are also observed; even though the difference in the temperature of the hydrothermal treatment is only 20 °C. Comparing the cases with the same granulometry and different treatment temperature, an increase in canolol formation was observed due to HT temperature (361.89% and 489.33% for entire and broken seeds, respectively). This phenomenon corroborates the assertions of previous research that the decarboxylation of the synapic acid present in canola occurs under the action of high temperatures.

Oil from entire canola seeds presented higher canolol content than their respective broken ones (98.76% difference in entire seeds and treated leaves at 110 °C, and 55.77% at 130 ° treated). It is worth mentioning that entire seeds were exposed to microwaves for a longer time, reaching to a significant increment of temperature, favoring the canolol generation. Besides, another probable cause of this decrease in canolol content in oil from broken seeds could be the exposure of the endosperm to the ambient during vapor and microwave treatments, which could destroy a fraction of the canolol being formed or its precursors by oxidation, since these compounds are thermolabile [10].

As regards tocopherol contents, α, β and γ-tocopherol were detected in all samples analyzed; and δ-tocopherol was present in E130, PB, P110 and P130. The range of such compounds varied within the range on 679.23-877.47 µg/g.

α tocopherol content in oil showed a change due to the autoclave temperature only in broken seeds, showing that at higher temperature, better α tocopherol extraction. Granulometry did not affect the content of this isomer. Meanwhile this factor slightly affected β tocopherol content only in untreated seeds, and a little effect of HT temperature was noticed.

In the case of the other isomers, there have been found significant differences in γ and δ tocopherol oil contents among samples, indicating a differential extraction due to the treatment applied (milling, HT and MW).

Samples EB, E110, PB, P110 and P130 did not present significant differences among them in γ-tocopherol content ($p > 0.05$); as well as samples E130, PB and P130. It can be observed that an increase in the temperature of the autoclave causes a decrease in the concentration of γ-tocopherol when analyzing entire seeds. δ-tocopherol could not be detected in the EB and E110 oil samples. Of the remaining samples, it was the E130 that presented the highest contents of this isomer. The oil from sample P130 also presented significant difference; while PB and P110 were found to have similar concentrations. Temperature was the most relevant factor for the concentration of δ-tocopherol; samples of equal particle size have significantly ($p < 0.05$) higher contents as the treatment temperature increases.

On the other hand, total content of tocopherols did not vary among samples (ANOVA, Tukey´s Test, $p > 0.05$).

The sample E130 is the one which presented not only the greater content of canolol, but greater yield in the extraction by press, besides its total tocopherol content was not affected, being the sample with the best behavior for the target of this study.

Acidity, peroxide and p-Anisidine values at the best pretreatment conditions

Table 4: Quality parameters of untreated seeds and treated at the best condition (E130).

Quality parameter	Sample	
	EB	E130
Acidity index (%ácido oleico)	0.56 a 0.006	0.69 b 0.008
Peroxide index	2.45 b 0.005	1.96 a 0.003
p-Anisidine value	0.78 a 0.28	2.6 b 0.29

The values corresponding to the acid, peroxide and p-Anisidine indices (AV, PI and p-A) of the oil extracted by pressing the entire untreated seed and the combined hydrothermal (130 °C) and microwave treatment are presented in the Table 4.

EB: untreated entire canola seeds; E130: entire canola seeds pretreated HT temperature: 130ºC.

Different letters in the same line indicate significant (ANOVA, Tukey´s Test, $p<0.05$).

The three quality indices analyzed differed significantly between samples studies. Acidity and p-anisidine indices increased due to the combined pretreatment while peroxides content decreased. Both Codex Alimentaruis-preestablished parameters (AV and PI) were below the upper limit for cold pressed and virgin oils [2].

The trend showed by AV and p-A values could be due to the high humidity and temperature conditions of E130 samples resulting in the formation of secondary oxidation compounds which adversely affect the quality of the oil.

Meanwhile, calculated Totox value were 5.68 and 6.52 for EB and E130, respectively, showing a slightly change in this parameter quality due to the pretreatments applied.

Conclusion

The effect of the hydrothermal/microwave pretreatment combination on extraction yield and oil quality of entire and broken canola seeds was evaluated.

The pretreatment application positively affected the extraction performance by cold pressing in all the samples, compared to their respective untreated samples. The highest oil extraction yield by pressing was obtained when essaying entire canola seeds hydrothermally pretreated at 130 °C previous to the microwave irradiation, also presenting the highest canolol content.

The content of canolol increased markedly with the increase in the hydrothermal pretreatment temperature. The increase in temperature could influence the reaction of the synapic acid and its consequent formation of the antioxidant canolol, improving both the nutritional quality of the final product as well as its shelf life against lipid oxidation.

With respect to tocopherols the effect of pretreatment operating conditions varied from one isomer to another. The content of α and β-tocopherol did exhibited slightly variations in the analyzed samples. The γ-tocopherol content was higher for the expressed oil obtained from broken seeds. δ-tocopherol content increased in the entire seeds submitted to hydrothermal treatment at 130 °C, showing a differential extraction due to pretreatments conditions.

The quality indices were slightly affected by the thermal pretreatments. Likewise, both the acidity (0.69% oleic acid) and the peroxide value (1.96 meq O2 / kg oil) are below the limits established by Alimentarius Codex.

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***Correspondence to:**

María Belén Fernández

CIFICEN, Universidad Nacional del Centro de la Provincia de Buenos Aires-CONICET-CICPBA

Argentina

Tel: +542284451055

E-mail: mbfernan@fio.unicen.edu.ar