Effect of α-tocopherol, α-tocotrienol and Rosa mosqueta shell extract on the performance of antioxidant-stripped canola oil (Brassica sp.) at high temperature

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Abstract

The antioxidant effects of tocols (α-tocopherol and α-tocotrienol) and Rosa mosqueta shell extract added to antioxidant-stripped canola oil (TCO) were evaluated and compared with the non-stripped oil (CO) under the same conditions. Seven oil systems were subjected to thermal treatment at 180 °C for 18 h. Polar compounds formation, degradation of tocols and carotenoid pigments were studied. The addition of Rosa mosqueta shell extract gave a great stability to TCO, similar to CO, with a low polar compound formation and a high retention of α-tocopherol compared with other TCO samples, which suggested the protective action of the minor components present in the extract. Alpha-tocopherol showed a higher effectiveness than α-tocotrienol at high temperature. However, an increase in the level of α-tocopherol did not improve its action.

Keywords: Antioxidant; α-Tocopherol; α-Tocotrienol; Canola oil; Carotenoids; Rosa mosqueta; Thermal oxidation

1. Introduction

Synthetic antioxidants are being questioned while natural antioxidants such as tocopherol, polyphenols and carotenoid pigments are having a greater relevance in the protection against lipid oxidation. Alpha and gamma tocopherols are the most abundant natural antioxidants in seed oils. Tocopherols act as antioxidant by donating a hydrogen atom to peroxyl radicals of unsaturated lipid molecules, forming a hydroperoxide and a tocopheroxyl radical, which reacts with other peroxyl or tocopheroxyl radicals forming more stable adducts (Lampi, Kataja, Kamal-Eldin, & Piironen, 1999).

In processes carried out at high temperatures, such as the process of frying, it is of primary interest to know about the behavior of the natural antioxidants in the oils and to define their significance in connection with oil deterioration (Barrera-Arellano, Ruiz-Méndez, Márquez Ruiz, & Dobarganes, 1999). Some studies have investigated the behavior of tocopherol in different unsaturated oils during frying (Barrera-Arellano, Ruiz-Méndez, Velasco, Márquez-Ruiz, & Dobarganes, 2002; Lampi et al., 1999; Lampi & Kamal-Eldin, 1998; Normand, Eskin, & Przybylski, 2001; Romero et al., 2004).

The use of natural compounds to prevent degradation during frying has been reported earlier, e.g. an ethanol extract from the spice Satureja hortensis L. (summer savory) improved the oxidative stability of sunflower oil at frying temperature (Yanishlieva, Marinova, Marekov,
& Gordon, 1997); a rosemary extract showed a protective action against dimer formation and tocopherol degradation in rapeseed oil used in potato frying (Gordon & Kourisma, 1995) and in another study it improved the resistance to oxidative rancidity in a vegetable oil used in deep frying (Lalas & Dourtoglou, 2003).

Carotenoids are present in a wide variety of foods and they are responsible for the red to yellow coloration in fruits and vegetables. The role of pro-vitamin A in some carotenoids has been known for a long time, but carotenoids also have an important antioxidant role in the cellular protection against lipid peroxidation (Basu, Del Vecchio, Flider, & Orthefer, 2001), thus preventing the risk of degenerative illnesses such as cancer, cardiovascular disease and macular degeneration. It also reduces the risk of cataracts and strengthens the immune system (Bermudez, Ribaya-Mercado, Talegawkar, & Tucker, 2005).

Rosa mosqueta (Rosa rubiginosa) is widely spread throughout the world. Its fruit has highly interesting carotenoid pigments such as β-carotene, lycopene and rubixanthin; and it can be used as a natural source of carotenoids in food technology (Hornero-Méndez & Minguez-Mosquera, 2000; Robert, Carlsson, Romero, & Masson, 2003). Robert et al. reported lycopene, β-carotene and rubixanthin as the main carotenoid pigments in commercial shell and fruits (Robert et al., 2003).

So far, there are only a few studies about the antioxidant effect of carotenoid-containing plant extracts at high temperature (Henry, Catignani, & Schwartz, 1998a). Rosa mosqueta shell is an under utilized by-product, that remains after the extraction of the seed form the fruit, for the oil preparation. This by-product rich in carotenoid pigments presents an opportunity for the obtaining of extracts with antioxidant and pigmenting properties and therefore to increase the economic value of the Rose mosqueta and its utilization for any related industries.

The objective of this work was to study the antioxidant effect of α-tocopherol, α-tocotrienol and a Rosa mosqueta shell extract on the stability of antioxidant-stripped canola oil at high temperature.

2. Materials and methods

2.1. Materials

Rapeseed oil (canola variety) or canola oil (Brassica sp.) (CO) was supplied by Agromaule S.A. (Santiago, Chile). Commercial dried shells of Rosa mosqueta (Rosa rubiginosa) were purchased from Noveltec S.A. (Santiago, Chile). DL-α, β, γ, δ-tocopherols and tocotrienols were obtained by Calbiochem (Merck, Darmstadt, Germany). Solvents used were HPLC-grade or analytical grade.

2.2. Samples and treatments

Oil samples. Seven canola oil experimental systems were studied:

(a) Canola oil (CO).
(b) Antioxidant-stripped canola oil (canola oil treated with alumina to remove tocopherols) (TCO).
(c) TCO supplemented with 155 mg/kg of α-tocopherol (TCO + AT).
(d) TCO supplemented with 138 mg/kg of α-tocotrienol (TCO + AT3).
(e) TCO supplemented with a mixture of 66 mg/kg of α-tocopherol and 72 mg/kg of α-tocotrienol (TCO + M).
(f) TCO supplemented with Rosa mosqueta shell extract (TCO + RME) containing carotenoid pigments (465 mg/kg) and α-tocopherol (432 mg/kg).
(g) TCO supplemented with 432 mg/kg of α-tocopherol (TCO + 432AT).

The levels used of α-tocopherol and α-tocotrienol in the samples (c), (d) and (e) were chose according to previous reports about of their antioxidant effect (Fuster, Lampi, Hopia, & Kamal-Eldin, 1998), considering to study the antioxidant behaviour of each one in independent form and together.

As the RME supplied 432 mg/kg of α-tocopherol to TCO + RME was necessary to supplement a sample of TCO with 432 mg/kg of AT isolating thus the carotenoid pigments effect.

2.3. Preparation of antioxidant-stripped canola oil

Canola oil was removed of its antioxidants via adsorption chromatography using a glass column packed with activated alumina (Merck, Darmstadt, Germany), according to Yoshida, Kondo, and Kajimoto (1992). The absence of tocopherols in the antioxidant-stripped canola oil was confirmed by HPLC, according to AOCS standard method (1993).

2.4. Preparation of Rosa mosqueta shell extract

Ground Rosa mosqueta (Rosa rubiginosa) shell (3.2 kg) was extracted with hexane (3.2 L) and shaked in multi-wrist shaker at room temperature by 2 h. The hexane containing the carotenoid pigments was filtered by bûchner funnel using vacuum. Three consecutive extractions were made as the cycle described above until the pulp was light red. Extracts were combined; the solvent was removed in a Büchi rotatory evaporator at 40 °C and finally diluted to 500 ml volumetric flask with hexane.

2.5. Preparation of TCO supplemented with Rosa mosqueta shell extract

380 g of TCO was mixed with 200 ml of Rosa mosqueta shell extract. The solvent was then evaporated; first in a Büchi rotatory evaporator at 40 °C and then under a stream of nitrogen.
2.6. Thermal oxidation assays

Standard Rancimat tubes containing 10 g of oil were heated at 180 ± 1 °C in a Rancimat apparatus (Metrohm Ltd, Herisau, Switzerland). Air was not bubbled during heating and the tubes were left open. Independent tubes containing TCO + RME and TCO + 432AT were heated for 0.5, 1, 1.5, 2, 8 and 18 h, while the rest of the samples were heated for 2, 4, 6, 8, 10, 14 and 18 h. Thermal oxidation was carried out in triplicate.

2.7. Fatty acid composition

Fatty acids were analyzed as methyl esters derivatives (AENOR, 1991) by gas chromatography (GLC) using a HP 5890 chromatograph (Hewlett-Packard, Palo Alto, CA, USA). A fused silica capillary column BPX70 (50 m, 0.25 μm film; SGE, Incorporated, Austin, TX, USA) was used. Temperature increase was programmed between 160 and 230 °C at 2 °C/min, and samples were run with hydrogen as carrier gas. Standard fatty acid methyl esters (FAME) from Merck (Merck, Darmstadt, Germany) were used for identification purposes.

2.8. Analysis of tocopherols and tocotrienols

Tocols were determined by high-performance liquid chromatography (HPLC) with fluorescence detection, according to AOCS standard method (1993) for non-esterified tocopherols and tocotrienols. The HPLC system consisted of a Merck-Hitachi L-6200 A pump (Merck, Darmstadt, Germany) equipped with a Rheodyne 7725i injector with 20 μl sample loop, a Merck-Hitachi F-1050 fluorescence detector and a Merck-Hitachi D-2500 chromato-integrator. Peaks were detected at 290 nm (excitation wavelength) and 330 nm (emission wavelength). A LiChroCART Superspher Si 60 column (25 cm × 4 mm id, 5 μm particle size; Merck, Darmstadt, Germany) was used. The mobile phase was propan-2-ol in hexane (0.5:99.5 v/v) at a flow rate of 1 ml/min. Tocols were identified and quantified using external standards. Standards for all-trans-β-carotene, all-trans-lycopen and all-trans-rubixanthin were obtained from carrots, tomatoes and Rosa mosqueta shells, respectively. The pigments were purified by open column chromatography, as described by Rodriguez-Amaya (1999). Concentrations of standards were determined by spectrophotometry using their respective \( A_{\text{1%}}^{1%}\). Calibration curves were determined for each compound. Cis-isomers were identified according to elution order as reported by Henry et al. (1998b).

2.9. Analysis of polar compounds

Quantification and distribution of polar compounds (PC) were determined by a combination of adsorption column chromatography and high-performance size exclusion chromatography (HPSEC) according to Dobarganes, Perez-Camino, and Marquez-Ruiz (1988). The HPLC system consisted of a Merck-Hitachi L-6200 A pump, a 20 μl injection loop, a Merck RI-71 refractive index detector and a Merck-Hitachi D-2500 chromato-integrator. The separation was performed on two serial 500 and 100 Å columns (PLGEL, 30 cm × 0.8 cm id, 5 μm particle size; Hewlett Packard, Amherst, MA, USA). The mobile phase was tetrahydrofuran at a flow rate of 1 ml/min. Identification was performed in agreement with Dobarganes et al. (1988) and the quantification of each compound was based on peak areas assuming equal detector response.

2.10. Oxidative stability

Induction period (IP) was determined using a Rancimat Oxidative Stability Instrument (Metrohm Ltd., Herisau, Switzerland) at 100 °C and an air flow of 20 l/h, according to AOCS standard method (1993).

2.11. Analysis of carotenoids

Pigment extraction from oils was carried out according to Henry, Catignani, and Schwartz (1998b). Carotenoid analysis was determined by HPLC. The HPLC system consisted of a Merck-Hitachi L-6200 pump, a Waters 996 Photodiode Array detector coupled to a computer with Millennium 32 software and a Waters symmetry column (C18, 5 μm particle size, 4.6 mm id × 25 cm; Waters, Milford, MA). The isocratic mobile phase was methanol:acetonitrile:ethyl acetate (20:65:15 v/v) at a flow rate of 1 ml/min. Carotenoids were detected at 450 nm. Identification and quantification were carried out using external standards. Standards for all-trans-β-carotene, all-trans-lycopen and all-trans-rubixanthin were obtained from carrots, tomatoes and Rosa mosqueta shells, respectively. The pigments were purified by open column chromatography, as described by Rodriguez-Amaya (1999). Concentrations of standards were determined by spectrophotometry using their respective \( A_{\text{1%}}^{1%}\). Calibration curves were determined for each compound. Cis-isomers were identified according to elution order as reported by Henry et al. (1998b).

2.12. Statistical analysis

The linear regression (95% confidence limit) was used to determine the reaction order and degradation rate constant of tocols. To determine statistical differences in the formation of PC and their distribution a multivariate ANOVA was performed considering treatment and time as independent variables and PC as dependent variable. Induction period and PC for a same time were analyzed by one factor ANOVA. These statistics were carried out by using Statgraphics Plus, version 7.0 (Manugistics Inc., Statistical Graphics Corporation, Rockville, MD).

3. Results and discussion

Table 1 shows the concentrations of major carotenoid pigments and tocopherols in Rosa mosqueta shell extract (RME) alone, and in antioxidant-stripped canola oil with added RME extract, (TCO + RME). Lycopene, β-carotene and rubixanthin were found in RME, being \( \text{trans} - \beta\)-carotene and \( \text{trans} - \text{rubixanthin} \) the main carotenoid pigments. RME had \( \alpha\)-tocopherol too; therefore the addition of RME to
TCO supplied 432 mg/kg of \( \alpha \)-tocopherol (TCO + RME), then it was necessary to add AT (432 mg/kg) to antioxidant-stripped canola oil (TCO + 432AT) for to isolate its effect.

Table 2 shows the fatty acid composition, the tocols, the total polar compounds and the polar compound distribution in the initial CO and TCO. CO consisted of totally 61% monounsaturated fatty acids and the major fatty acid was oleic acid (total content 56%). In addition, CO had a low content of saturated fatty acids (8.1%) and a medium content of polyunsaturated fatty acids (30.7%), mainly represented by linoleic and linolenic acids (21.5% and 8.0%, respectively).

CO showed a higher concentration in total tocopherols than the content previously reported by Normand et al. (2001) and Barrera-Arellano et al. (2002). In CO, \( \alpha \) and \( \gamma \)-tocopherols were the main natural antioxidants present, with contents of 247 and 348 mg/kg, respectively.

TCO did not show important changes in its fatty acid composition compared to CO. However, it can be observed that the treatment with alumina not only removed the tocopherols, but also the polar compounds (PC) present in CO.

Oxidative stability results are shown in Table 3. A high induction period (IP) of 19.6 h at 100 °C was found for CO, which agreed with its high levels of monounsaturated fatty acids and tocopherols. Barrera-Arellano et al. (2002) found an IP value of 10.4 h for rapeseed oil under similar conditions.

The IP value in TCO was 1.4 h and reflected the importance of antioxidative compounds for oil stability. The addition of tocols and Rosa mosqueta shell extract to TCO improved the oxidative stability of the oil \( (P < 0.05) \). At similar concentrations of tocols not significant difference in stability was observed. The Rosa mosqueta shell extract gave the highest stability to treated oil compared with the other treated samples \( (P < 0.05) \) in the accelerated test, but did not reach the oxidative stability of CO.

### 3.1. Canola oil thermal oxidation assays

Table 4 shows the tocopherol degradation and the polar compound formation in all the oil systems studied. It can be observed that tocopherols remained in CO during the
whole thermal oxidation assay. After 18 h 70% of total tocopherols were remaining which was concordant with the high value for IP, as commented above. The result for tocols in CO was in agreement with the data published by Normand et al. (2001) on the frying stability of regular canola oil which presented a high level of tocopherols at 20 h of frying.

Fig. 1 shows the logarithm of the percent retention vs. time (h) for α-tocopherol and α-tocotrienol in six samples.

The degradation of α-tocopherol and α-tocotrienol followed pseudo first-order kinetics in all the samples. The correlation coefficient was used as a parameter to determine the reaction order. The degradation rate constants (k) were obtained from the slopes of the plots of Fig. 1 (Robert, Romero, Ortiz, Masson, & Barrera-Arellano, 2006). The degradation rates for α-tocopherol varied greatly between the different samples containing the oil treated with alumina (P < 0.05). Alpha-tocopherol showed a lower degradation rate (P < 0.05) when it was added as the only antioxidant in TCO + AT (0.27 ± 0.004 h⁻¹, r² = 0.995) than when it was added together with α-tocotrienol in TCO + M (0.44 ± 0.01 h⁻¹, r² = 0.999). This same behaviour was observed and explained in a previous research in Chilean hazelnut oil (Romero et al., 2004). Degradation rate of AT was lower than AT₃ (P < 0.05) as can be observed in samples TCO + AT₃ (0.54 ± 0.03 h⁻¹/C₀, r² = 0.999). Different letters mean significant differences between samples for degradation rate constants (k) (P < 0.05).

![Fig. 1. Pseudo first-order degradation plots for α-tocopherol or α-tocotrienol at 180 °C in canola oil (CO), antioxidant-stripped canola oil (TCO), antioxidant-stripped canola oil with addition of α-tocopherol (TCO + AT), α-tocotrienol (TCO + AT₃), α-tocopherol and α-tocotrienol mixture (TCO + M), Rosa mosqueta shell extract (TCO + RME) and 432 mg/kg of α-tocopherol (TCO + 432AT). Different letters mean significant differences between samples for degradation rate constants (k) (P < 0.05).](image1.png)

![Fig. 2. Degradation of carotenoid pigments in antioxidant-stripped canola oil with added Rosa mosqueta shell extract (TCO + RME) during heating at 180 °C. (a) trans-isomers; (b) cis-isomers.](image2.png)
Changes in polar compound distribution (wt%) in the same samples as named in Table 4

Table 5

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<th>Samples/heating period (h)</th>
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<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>14</th>
<th>18</th>
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<td>3.2 ± 0.0</td>
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<tr>
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<td>3.0 ± 0.2a</td>
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<td>3.1 ± 0.3</td>
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<td>11.3 ± 0.3p</td>
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Abbreviations: oxTGM, oxidized triacylglycerol monomers; TGD, triacylglycerol dimers; TGP, triacylglycerol polymers. Values are reported as means ± SD (n = 3). Different letters for the same parameter mean significant differences between samples (P < 0.05).
was in according with a higher permanence of AT in its system.

Table 3 showed an IP for TCO + AT lower than TCO + 432AT (P < 0.05), suggesting differences in behaviour at high temperatures and in the conditions of the Rancimat test.

The results obtained for the distribution of polar compounds are shown in Table 5. All distribution species including oxidized triacylglycerol monomers (oxTGM), triacylglycerol dimers (TGD) and triacylglycerol polymers (TGP) increased continuously during heating in most of the oil systems. In general, low levels of polymers were found in oils with greater retention of tocols (P < 0.05). TCO + AT3 and TCO + M showed a similar behaviour to TCO (P > 0.05). Rosa mosqueta shell extract gave the highest stability to antioxidant-stripped canola oil with the lowest values of polar compounds and distribution species at 18 h (P < 0.05).

In conclusion, the addition of Rosa mosqueta shell extract gave a great stability to TCO similar to CO which suggested the protective action of the minor components present in the extract. Alpha-tocopherol showed a high effectiveness as antioxidant at high temperature, however, an increase in the level of α-tocopherol did not improve its action.

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