Effect of Rosa Mosqueta (*Rosa rubiginosa*) Extract on the Performance of Chilean Hazelnut Oil (*Gevuina avellana* Mol.) at High Temperature

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ABSTRACT: The effect of the addition of rosa mosqueta husk extract (RME) on thermal oxidation of nontreated (HZO) and treated (THZO) Chilean hazelnut seed oil was evaluated at 180°C for 18 h. THZO to which was added 339 mg/kg of α-tocopherol was used as a comparison model because RME supplied 314 mg/kg of α-tocopherol. Formation of polar compounds and degradation of tocols and carotenoid pigments were studied in these model systems. Degradation of *trans*-rubixanthin, *trans*-lycopene, and *trans*-β-carotene followed a pseudo first-order kinetics model. These pigments showed the same degradation rate in both HZO and THZO. The addition of RME to HZO and THZO decreased significantly ($P < 0.05$) the formation of polar compounds, lead to less degradation of tocols, and improved their oxidative stability with respect to oils without RME. This behavior can be attributed to carotenoid–tocopherol interaction, suggesting that these pigments can protect tocols against degradation at high temperature.

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KEY WORDS: Carotenoid pigments, Chilean hazelnut oil, polar compounds, *Rosa rubiginosa*, thermal oxidation.

Vitamin E compounds (tocopherols and tocotrienols) are well known for their effective inhibition of lipid oxidation in food and biological systems (1). The antioxidant activity of these tocols is due to their ability to donate phenolic hydrogens to peroxyl radicals. Tocopherols play an important role in the autoxidation of lipids, and they are reported to have effects on hydroperoxide formation, on the relative hydroperoxide isomer distribution, and on the decomposition rate and reaction routes of hydroperoxides (1,2). Some studies have also considered the effects of tocopherols on the formation of polar compounds (PC) (3,4).

Carotenoids may behave as antioxidants in lipid systems by quenching singlet oxygen or by trapping free radicals (5). It has been proposed that the mechanism of the reaction between carotenoids and radical species (peroxyl) can involve the formation of an adduct and/or the abstraction of a hydrogen atom (6). Antioxidant or pro-oxidant action of carotenoid pigments on autoxidation in different lipid models has been reported in methyl linoleate or FA in organic solvent and also in TAG of crude, refined, or purified oils (7–9). However, the antioxidant and pro-oxidant effects of carotenoids are controversial. Discrepancies in some studies could be due to differences in the lipid systems, the presence of other antioxidants in natural food lipids, the concentration of carotenoids, experimental conditions, and the different methods used to monitor the oxidation process, which include PV, O_2 concentration, and content of volatile compounds (6–9).

The antioxidant effect of carotenoids produces pigment degradation as well as color losses. Degradation of carotenoids in methyl linoleate and oils generally follows a pseudo first-order kinetic model, where the degradation rate depends on the structure of the carotenoid. The degradation rates reported are as follows: lycopene > *all-trans*-β-carotene > α-carotene in methyl linoleate (7) and lycopene > *all-trans*-β-carotene = *cis*-β-carotene > lutein in safflower seed oil (10). However, fewer studies have been carried out on the effect of carotenoids in oils at high temperatures (10).

In vegetable foods, carotenoids and tocopherols generally are present together. Studies on carotenoid–tocopherol interactions in food lipids have been reported (9,11). Lutein, canthaxanthin, β-carotene, septrapen, lycopene, 7,7-dihydro-β-carotene, and zeaxanthin are able to regenerate tocopherol from its radical tocopheroxyl in solvent models (12).

Rosa mosqueta (*Rosa rubiginosa*), a member of the Rose family, grows in Chile as a wild plant. The oil extracted from the seeds is included in many cosmetic preparations. The dried husk of rosa mosqueta fruit is an interesting source of natural pigments such as lycopene, β-carotene, and rubixanthin (13,14).

The objective of this work was to study the effect of the addition of rosa mosqueta husk extract (RME) to crude and tocol-stripped Chilean hazelnut oils (*Gevuina avellana* Mol.) on the thermal oxidative stability of the oil at 180°C.

EXPERIMENTAL PROCEDURES

Materials. Crude hazelnut oil from the seed (*G. avellana* Mol.) (cold pressed without preservatives) and commercial dried husks of rosa mosqueta (*R. rubiginosa*) were purchased from Noveltec S.A. (Santiago, Chile). DL-α-, β-, γ-, δ-Tocopherols and DL-α-, β-, γ-, δ-tocotrienols were obtained from Calbiochem (Darmstadt, Germany). Solvents used in the mobile

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phase for liquid chromatography were HPLC-grade, and other solvents were analytical grade.

Samples and treatment. Three hazelnut oil (HZO) systems were studied: (i) crude HZO with the addition of RME (HZO ± RME); (ii) treated HZO with the addition of 339 mg/kg of α-tocopherol (THZO ± T); and (iii) treated HZO with the addition of RME (THZO ± RME).

The FA composition of crude HZO was 2.3% (C16:0); 0.6% (C16:1n-7); 21.1% (C16:1n-5); 1.7% (C18:0); 8.7% (18:1); 34.6% (C18:1n-9); 6.4% (C18:2n-6); 0.2% (C18:3n-3); 2.0% (C20:0); 9.7% (C20:1); 2.8% (C22:0); 9.0%(C22:1); 0.6% (C24:0); and 0.2% (C24:1).

Oil treatment. HZO was purified *via* adsorption chromatography using a glass column packed with activated alumina (Merck, Darmstadt, Germany), as described by Yoshida *et al.* (15). The treated oil was analyzed by HPLC to confirm the absence of tocols and PC.

Preparation and addition of RME. Ground rosa mosqueta husks were extracted with hexane (1:1 wt/vol) at room temperature for 2 h. Three consecutive extractions were made until the pulp was light red. Extracts were combined; the solvent was removed in a Büchi rotary evaporator and finally diluted to a known volume with hexane.

The addition of RME to HZO was performed as follows: 200 mL of RME in hexane was mixed with 380 g of HZO or THZO. The solvent was evaporated first in a Büchi rotary evaporator and then under a stream of nitrogen.

Thermal oxidation assays. Open glass tubes (14.5 × 2.5 cm) with 10 g of each oil system were heated at 180 ± 1°C for 18 h in a heating block. Tubes were removed every half hour up to 3 h, and then at 8 and 18 h, to determine the loss of tocols and carotenoid pigments and the formation of PC. Thermal oxidation was carried out in triplicate.

Analytical methods. Tocopherols and tocotrienols were determined according to AOCS (16) by HPLC using a LiChroCART Superspher Si-60 column (5 µm particle size, 4 mm i.d. × 25 cm; Merck); the mobile phase was propan-2-ol/hexane (0.5:99.5 vol/vol) at a flow rate of 1 mL/min. The HPLC system consisted of a Merck-Hitachi L-6200A pump with a 20 µL injection loop, and a Merck-Hitachi F-1050 fluorescence detector. Peaks were detected at 290 and 330 nm (excitation and emission wavelengths, respectively). Identification and quantification were accomplished by using tocopherols and tocotrienols as external standards.

PC were determined by adsorption column chromatography and the distribution of PC by high-performance size exclusion chromatography, according to Dobarganes *et al*. (17). The HPLC system consisted of a Merck-Hitachi L-6200A pump with 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 in series 500 and 100 Å columns (PLGEL, 5 µm particle size, 0.8 cm i.d. × 30 cm; Hewlett-Packard, Amherst, MA). The mobile phase was THF at a flow rate of 1 mL/min. The quantification of each compound was based on peak areas, assuming equal detector response.

Oxidative stability. Induction periods were determined by a Rancimat Oxidative Stability Instrument (Metrohm Ltd., Herisau, Switzerland), at 100°C and an air flow of 20 L/h, according to AOCS (16).

Carotenoid extraction and chromatographic procedure. Pigment extraction from oils was carried out according to Henry *et al*. (10). Carotenoid analysis was carried out by HPLC using a Waters symmetry column (C18, 5 µm particle size, 4.6 mm i.d. × 25 cm; Waters, Milford, MA). The isocratic mobile phase was methanol/acetonitrile/ethyl acetate (20:65:15 by vol) at a flow rate of 1 mL/min. The HPLC system consisted of a Merck-Hitachi L-6200 pump and a Waters 996 Photodiode Array detector coupled to a computer with Millennium 32 software. Carotenoids were detected at 450 nm and identified by comparing the peak retention times with standards.

Standard solution. All-*trans*-β-carotene, all-*trans*-lycopene and all-*trans*-rubixanthin were obtained from carrots, tomatoes, and rosa mosqueta husks, respectively. The pigments were purified by open column chromatography, as described by Rodriguez-Amaya (18). Concentrations of standards in hexane were determined by spectrophotometry. Calibration curves were obtained for each carotenoid.

Kinetic analysis. The carotenoid data were best fit by a first-order kinetic model, $\ln C = \ln C_o - k(t)$. Degradation rate constants (k) were obtained from the slope of a plot of the natural log of the percentage retention of carotenoids vs. time.

Statistical analysis. Thermal oxidation experiments were performed in triplicate. The linear regression (95% confidence limit) was used to determine the reaction order and rate constants. To determine the statistical differences in the formation of PC, a multivariate ANOVA was performed by using Statgraphics, version 7.0 (Manugistics Inc., Statistical Graphics Corporation, Rockville, MD).

RESULTS AND DISCUSSION

Table 1 shows the concentrations of major carotenoid pigments in RME and in non-treated HZO ± RME and treated THZO ± RME HZO to which the extract was added. Lycopene, β-carotene, and rubixanthin were reported as the main carotenoid pigments in the fruit (13) and the commercially available husks (14) of rosa mosqueta.

Table 2 shows the initial content of tocols present in the different HZO systems studied and their initial oxidative stability measured by induction period. For comparison, previously reported results on the performance of HZO and THZO at high temperature are also considered (19). Crude HZO contains α-tocotrienol (T3) as a natural antioxidant. The alumina stripping process showed a high efficiency in removing tocotrienol from crude HZO. It was necessary to add α-tocopherol (T) (339 mg/kg) to treated oil (THZO ± T) because the RME supplied had 314 mg/kg of T, thus allowing us to isolate its effect.

HZO has a high oxidative stability (30.8 h), probably due to the presence of T3 or other minor compounds in the crude oil and its high level of monounsaturated FA. Addition of RME to samples of both nontreated and treated HZO improved their

TABLE 1
Composition[a] of Major Carotenoid Pigments in Rosa Mosqueta (*Rosa rubiginosa*) Husk Extract and in Nontreated and Treated Hazelnut Oil When the Extract Was Added

Carotenoid	RME (μg/mL)	HZO ± RME[b] (μg/g)	THZO ± RME[b] (μg/g)
trans-Rubixanthin	207	100 ± 2	122 ± 8
isom-Rubixanthin[c]	146	67 ± 1	85 ± 5
trans-Lycopene	81	24 ± 2	23 ± 2
cis-Lycopene	55	39 ± 1	31 ± 7
trans-β-Carotene	251	144 ± 2	122 ± 8
cis-β-Carotene	85	39 ± 1	30 ± 3

[a]Values are expressed as mean ± SD (n=3).
[b]200 mL of the RME was added to 380 g of HZO or THZO.
[c]Corresponding to gazanianxanthin, according to Reference 13. HZO, crude hazelnut oil; THZO, alumina-treated hazelnut oil; RME, rosa mosqueta (*Rosa rubiginosa*) husk extract.

TABLE 2
Initial Tocols Content and Stability (induction period) of Hazelnut Oil Systems[a]

Oil	T3 (mg/kg)	T (mg/kg)	IP (h)
HZO[b]	152 ± 5	ND	30.8 ± 0.2
HZO ± RME	152 ± 5	314 ± 1.1	39.0 ± 0.6
THZO[b]	ND	ND	3.5 ± 0.1
THZO ± T	ND	339 ± 0	27.9 ± 1.0
THZO+ RME	ND	314 ± 1.1	36.0 ± 0.3

[a]Values are expressed as mean ± SD (n = 3).
[b]From Reference 19. T3, α-tocotrienol; T, α-tocopherol; IP, induction period (100ºC); ND, not detected. For other abbreviations see Table 1.

oxidative stability compared with the oil without RME; HZO ± RME showed the highest value (39.0 h). THZO ± RME and THZO ± T had similar T contents; nevertheless, the model with added RME showed better oxidative stability than the model with T. The greater stability could be attributed to antioxidant compounds present in the extract, among which carotenoid pigments and other minor compounds should be considered.

Tocols degradation. Table 3 shows the evolution of T3 and T during thermal oxidation of the different HZO systems studied at 180°C.

The loss of T in THZO ± T was fast, before 3 h of heating, similar to T3 behavior reported in HZO (19). The disappearance of 500 mg/kg of T before 6 h of heating has been reported under similar conditions in commercial sunflower, rapeseed, and high-oleic sunflower oils (20).

Addition of RME to crude HZO significantly modified the degradation of T3, improving its stability. Tocols in nontreated and in treated HZO containing RME persisted for more than 18 h of heating before disappearing completely. When heating was performed for 3 h, more than 80% retention of T3 and T in HZO ± RME and T in THZO ± RME was observed.

Degradation of T was similar in HZO ± RME and THZO ± RME in the first 3 h of thermal oxidation, but after this time degradation of T was lower in the HZO ± RME system. These results are in agreement with other reports where a lower degradation rate of tocopherols in nontreated oils has been observed (4).

These results indicate that the extract (carotenoid pigments or other compounds present in the extract) is probably responsible for both the increase in the retention of tocols and the high oxidative stability of the HZO.

Carotenoid degradation. When RME was added, the carotenoids rubixanthin, lycopene, and β-carotene were present too. Figure 1 shows the logarithm of the percent retention vs. time (h) for *trans*-lycopene, *trans*-rubixanthin, and *trans*-β-carotene in HZO ± RME (A) and in THZO ± RME (B). The degradation of these carotenoid pigments followed pseudo first-order kinetics for HZO ± RME and THZO ± RME. The correlation coefficient was used as a parameter to determine the reaction order. The degradation rate constants were obtained from the slopes of the plots of Figure 1.

The degradation rate constants (k) for *trans*-lycopene, *trans*-rubixanthin, and *trans*-β-carotene were 2.14 ± 0.09 (r^2 = 0.998); 1.56 ± 0.04 (r^2 = 0.998), and 1.29 ± 0.11 (r^2 = 0.990) h^{-1}, respectively, in HZO +RME and 2.07 ± 0.03 (r^2 = 0.999), 1.71 ± 0.12 (r^2 = 0.990), and 1.30 ± 0.09 (r^2 = 0.987) h^{-1}, respectively, in THZO ± RME. Similar kinetic behavior was observed in the thermal and oxidative degradation of carotenoids in safflower oil (10) and methyl linoleate (7).

The degradation of these carotenoids was rapid, occurring in less than 3 h independently of whether they were present in treated THZO or nontreated HZO, as can be seen by the degradation rate constants.

T is reportedly a protective compound in the degradation of β-carotene in food lipids (11). On the other hand, research (9) on the effect of lutein, lycopene, and γ-tocopherol on the au-

TABLE 3
Degradation of α-Tocotrienol and α-Tocopherol During Thermal Oxidation of Hazelnut Oil Systems[a] at 180ºC

Oil		Heating time (h)								
		0	0.5	1	1.5	2	2.5	3	8	18
HZO[b]	T3 (mg/kg)	152 ± 5	97 ± 5	79 ± 4	56 ± 6	40 ± 5	1 ± 2	ND	ND	ND
HZO ± RME	T3 (mg/kg)	152 ± 6	140 ± 0.9	136 ± 0.4	132 ± 1.4	128 ± 0.7	123 ± 0.2	119 ± 0.6	83 ± 1.2	24 ± 0.4
	T (mg/kg)	314 ± 1.1	294 ± 0.9	292 ± 0.5	290 ± 0.5	286 ± 0.9	281 ± 0.4	277 ± 0.7	212 ± 1.1	78 ± 0.7
THZO ± T	T (mg/kg)	339 ± 0	237 ± 1	171 ± 7	117 ± 1	3.6 ± 0.5	ND	ND	ND	ND
THZO ± RME	T (mg/kg)	314 ± 1.0	303 ± 1.0	294 ± 0.7	283 ± 1.1	277 ± 1.1	274 ± 0.8	262 ± 0.4	160 ± 0.9	17 ± 0.2

[a]Values are expressed as mean ± SD (n = 3).
[b]From Reference 19. For abbreviations see Tables 1 and 2.

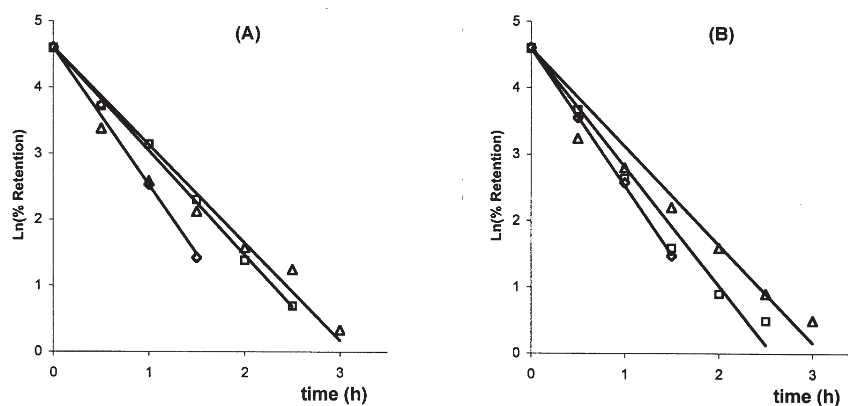

FIG. 1. First-order degradation plots for *trans*-rubixanthin (□); *trans*-β-carotene (△); and *trans*-lycopene (◇) in HZO ± RME (A) and in THZO ± RME (B) during thermal oxidation at 180°C. Each point represents an average of triplicate experiments. HZO, crude hazelnut oil; THZO, alumina-treated hazelnut oil; RME, rosa mosqueta shell extract.

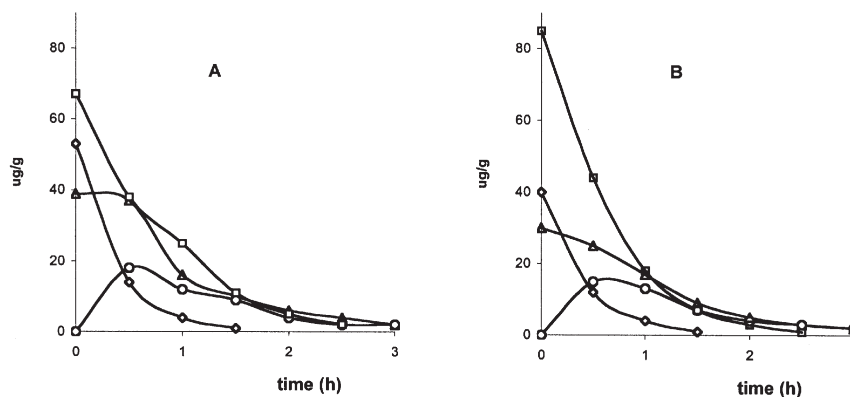

FIG. 2. Evolution of *cis*-isomers of rubixanthin (□); lycopene (◇); 13-*cis*-β-carotene (○); and 9-*cis*-β-carotene (△) in HZO ± RME (A) and in THZO ± RME (B) during thermal oxidation at 180°C. Each point represents an average of triplicate experiments. For abbreviations see Figure 1.

toxidation of purified low-erucic acid rapeseed oil showed that in the presence of γ-tocopherol, the loss of carotenoids was retarded, owing to the ability of tocopherol to inhibit carotenoid degradation. The results obtained in this study, in which the presence of carotenoid protects tocols, indicate that the temperature at which the experiment is performed may affect the antioxidant mechanism of tocopherols and carotenoids, as well as the interaction between them.

The lower degradation of tocopherols can be explained by a competition between carotenoids and tocopherols toward peroxyl radicals, resulting from the similar concentrations used in this experiment. There is also the possibility that carotenoids regenerate tocopheroxyl radicals, as seen in organic solvent models (6,12). Nevertheless, according to our results it is not possible to distinguish the mechanism through which the protection occurs.

Figure 2 shows the evolution of *cis*-isomers of lycopene, rubixanthin, and β-carotene during thermal oxidation in HZO ± RME (A) and in THZO ± RME (B). An initial increase in 13-*cis*-β-carotene was accompanied by a decrease in all-*trans*-β carotene and no change in 9-*cis*-β-carotene followed by a degradation of all isomers. This behavior was described by Henry *et al.* (10) for β-carotene.

PC evolution. Measurement of the formation and distribution of PC represents a good way to evaluate lipid oxidation (17). Table 4 shows PC formation for all HZO systems studied. The PC slightly increased during the first hours of heating in all the tested oils, owing to the protective action of the tocols and carotenoid pigments. As can be seen, the PC formation was significantly inhibited ($P < 0.05$) when RME was added to HZO and THZO with respect to oils without RME.

The addition of T to THZO inhibited PC formation only during the first 3 h, as compared with THZO. After this time the evolution of PC in the presence of T was similar to that in the presence of THZO, corresponding to unprotected oil. Results for T loss and for evolution of PC were similar to results reported for the monounsaturated model system (triolein) (3).

Distribution of PC at 18 h of heating at 180°C is shown in Figure 3. As can be observed, added RME acts as a polymerization inhibitor during the thermal oxidation of HZO. Lampi *et al.* (20) reported that tocopherols were able to act as antipolymerization agents in purified high-oleic sunflower oil at frying temperature.

TABLE 4
Evolution of Polar Compounds[a] (PC) During Thermal Oxidation of Hazelnut Oil Systems at 180°C

Time (h)	HZO[b,c] (%)	HZO + RME[c] (%)	THZO[b,d] (%)	THZO + T[e] (%)	THZO + RME[f] (%)
0	1.8 ± 0.1	1.8 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
2	3.0 ± 0.1	2.5 ± 0.2	4.0 ± 0.3	2.1 ± 0.1	1.4 ± 0.2
3	5.9 ± 0.4	3.7 ± 0.1	6.0 ± 0.3	3.7 ± 0.2	1.6 ± 1.3
8	12.6 ± 0.4	5.0 ± 0.1	11.5 ± 1.3	13.4 ± 0.1	3.1 ± 1.3
18	22.9 ± 0.8	8.9 ± 0.2	25.3 ± 1.3	26.0 ± 0.2	7.4 ± 1.3

[a]Values are expressed as mean ± SD (n = 3).
[b]From Reference 19.
[c–f]Different letters show significant difference between treatments ($P < 0.05$). For abbreviations see Tables 1 and 2.

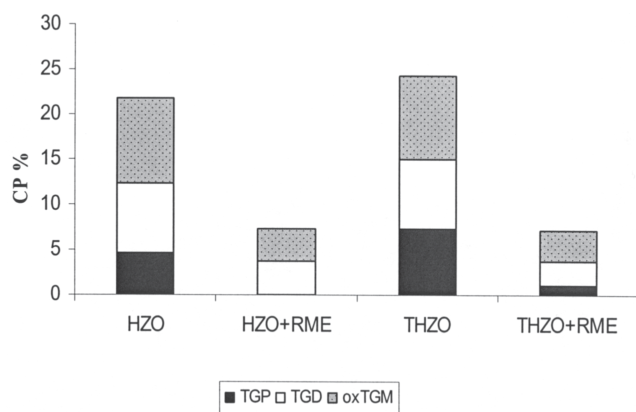

FIG. 3. Effect of RME on the alteration of polar species in nontreated (HZO) and treated (THZO) hazelnut oil heated at 180°C for 18 h. Abbreviations: TGP, triacylglycerol polymers; TGD, TAG dimers; oxTGM, oxidized TAG monomers; for other abbreviations see Figure 1.

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