Research Article

A polyphenol extract of tara pods (*Caesalpinia spinosa*) as a potential antioxidant in oils

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Tara is a native species of Peru and is widely distributed in Latin America; its fruits (pods) have a high potential for medical, industrial and food uses. A supercritical tara polyphenol extract (STPE) was obtained from tara pods by supercritical fluid extraction (SFE) with CO_2. The antioxidant activity of the STPE was studied in two oils, regular and high-oleic sunflower oil (SO, HOSO), with and without their natural antioxidants. Under accelerated conditions of oxidation, a linear relationship was observed in antioxidant-stripped oils between the polyphenol content of the STPE and the induction period.

The antioxidant effect of STPE on the HOSO was studied at 60°C. The highest polyphenol concentration of STPE showed the greatest α-tocopherol degradation and the lowest hydroperoxide formation, which implies that α-tocopherol causes the regeneration of the polyphenols that protect the TAGs in HOSO.

Tara pods combined with SFE method could be used as a source of antioxidants in oils.

Keywords: Antioxidant / Polyphenols / Sunflower oil / Supercritical extraction / Tara pods

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1 Introduction

The tara tree, which is in the Caesalpinaceae family, is a native species of Peru that is widely distributed in Latin America between 4° and 32°S, covering several arid regions from Venezuela, Colombia, Ecuador, Peru and Bolivia to northern Chile [1]. Tara is a small tree that is 2 to 3 m in height. Its flowers are reddish-yellow and are arranged in clusters of 8 to 15 cm in length. Its fruits are flat, oblong indehiscent orange pods that are 8 to 10 cm long and approximately 2 cm in width, containing 4 to 7 rounded seeds.

Tara pods have a high potential for use in medical, industrial and food applications because they can be used as a raw material to produce gums (or hydrocolloids), gallic acid and tannins, among other products [1]. Tannic acid obtained from tara pods is widely used in the pharmaceutical, chemical and paint industries, among others, and it is especially useful in the tannery industry [2]. A gum is obtained from the seeds by a thermal-mechanical process, and it can be used as an alternative to traditional gums for use in food, paint and varnish products [1]. Extraction with solvents of different polarities is the most commonly used method for obtaining antioxidant compounds from plants; however, this method has the disadvantage of requiring large volumes of solvents and is both expensive and inconvenient [3, 4].

In recent years, there has been an increasing interest in the use of supercritical fluid extraction (SFE), which uses the properties of gases above their vapour–liquid critical points to extract specific soluble components from a raw material [4]. SFE can be interpreted as a series of partitions or extractions of a solute between the mobile and stationary phases [5]. The basic principle of SFE is that the solubility of a given compound (solute) in various solvents can be adjusted by small changes in the pressure and temperature of extraction [6]. Many studies have used SFE to obtain volatile compounds, flavonoids, triterpenoids and other substances [6]. The solubility of polar substances in a non-polar SFE solvent, such as CO_2, is very low; however, if a small amount of polar

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Abbreviations: STPE, supercritical tara polyphenol extract; **SFE,** supercritical fluid extraction; **SO,** sunflower oil; **HOSO,** high-oleic sunflower oil; **TSO,** antioxidant-stripped sunflower oil; **THOSO,** antioxidant-striped high-oleic sunflower oil; **GAE,** gallic acid equivalents; **IP,** induction period; W_{InH}, mean rate of inhibitor consumption

cosolvent is added, the solubility of polar solutes can be significantly increased [7].

The cosolvent should be selected to interact strongly with the solutes of interest to facilitate the extraction of these solutes. Methanol and ethanol are capable of hydrogen bonding and forming dipole–dipole interactions with phenols, and they have proven to be effective carbon dioxide cosolvents in the extraction of phenolic compounds from grape seeds [7].

Currently, there is a strong global interest in exploring new sources of natural antioxidants that are safe, cheap [8] and do not cause the adverse effects produced by synthetic antioxidants [9]. Therefore, research on the development of antioxidants from natural plants is highly desirable. Phenolic compounds in vegetable oils can generally exert beneficial effects on the oxidative stability of the oils. For example, sesame oil, which contains sesamine, sesaminal and sesamole, is known to be highly stable. Similarly, olive oil is a rich source of tyrosol and hydroxytyrosol, which are known to possess high antioxidant activities [10]. Therefore, tara pods, which contain tannins, in combination with the SFE technique may provide a good alternative for obtaining extracts with antioxidant properties for use in foods. The goal of this work was to obtain a polyphenol extract from tara pods by SFE and to determine its antioxidant potential in two oil matrices.

2 Materials and methods

2.1 Materials

Tara pods were obtained from Fundación Chile (Santiago, Chile). The pods were stored frozen at -20°C.

The sunflower oil (SO) and high-oleic sunflower oil (HOSO) used in this study were supplied by Camilo Ferrón S.A. (Santiago, Chile).

2.2 Samples and treatments

Oil samples were treated with supercritical tara pod polyphenol extract (STPE) at different concentrations of polyphenols, expressed as gallic acid equivalents (GAE/kg oil) as follows:

A) SO supplemented with STPE at levels of 0, 12, 58, 117 and 233 mg GAE/kg oil.
B) Antioxidant-stripped sunflower oil (TSO) supplemented with STPE at the same concentrations of SO.
C) High-oleic sunflower oil (HOSO) supplemented with STPE at concentrations of 0, 12, 58, 85, 117, 200 and 400 mg GAE/kg oil.
D) Antioxidant-stripped high-oleic sunflower oil (THOSO) supplemented with STPE at concentrations of 0, 12, 58 and 117 mg GAE/kg oil.

2.3 The production of STPE

Tara pods were dried (1 kg) at RT in dark conditions until they reached a moisture content of 8%. The dry pods were ground in a hammer mill (Biber Wien VII, Austria) and these were sieved through a battery of sieves and mean particle size was calculated (173 μm). The powder was stored at -20°C. Several 5 g portions of ground tara pods were extracted with a Spe-ed SFE system, model 7071, (Applied Separations, Inc., Allentown, PA, USA) equipped with a 50 mL extraction cell. Ethanol (Merck, Darmstadt, Germany) was used as a modifier, and it was spiked (5 mL) directly into the sample 16 h prior to extraction. The experiments were carried out at 40°C, between 350 and 550 bars of CO_2 (99.5%; AGA, Santiago, Chile), at an output flow rate of 2.5 L/min and a metering valve temperature of 120°C. The samples were subjected to static SFE for 30 min followed by dynamic extraction for 30 min, time considered appropriate according to preliminary assays. Each extract was collected in 20 mL glass tubes (Applied Separations, Inc.), immersed in an ice water bath and then transferred into 10 mL volumetric flasks. Ethanol was added to each flask to reach a total volume of 10 mL, and the extracts were stored at -4°C until measurement. The extractions were carried out in triplicate.

A pool of tara pod polyphenol extracts obtained under supercritical conditions (STPE) was collected from several extractions carried out at 500 bars and 40°C following the same methods as above.

2.4 The preparation of the antioxidant-stripped oils

Oils were stripped of their tocopherols by adsorption chromatography using a glass column packed with activated alumina (Merck) according to the method of Yoshida et al. [11]. The absence of tocopherols in the antioxidant-stripped oils was confirmed by high-performance liquid chromatography (HPLC) according to the Ce 8-86 AOCS standard method [12].

2.5 The preparation of oils supplemented with STPE

For oxidative stability study, natural (HOSO or SO) or antioxidant-stripped (THOSO or TSO) oil (15 g) was mixed with an aliquot of STPE (5.2 mg GAE/mL), to obtain each supplemented oil described in Section 2.2. The solvent was then removed under a stream of nitrogen.

For the storage stability study, HOSO (200 g) was mixed with different amounts of STPE to obtain levels of 85, 200 and 400 mg GAE/kg oil, and the solvent was removed under a stream of nitrogen.

2.6 Oxidative stability

The antioxidant activity of different concentrations of STPE was tested on the oils by subjecting them to forced dynamic

oxidation. A Rancimat Oxidative Stability Instrument (Metrohm Ltd., Herisau, Switzerland) was used at 100°C with an air flow of 20 L/h. Five grams (±0.1 g) of oil was weighed in each glass tube. Induction periods (IP) were determined in triplicate according to the Cd 12b-92 AOCS standard method [12].

2.7 Storage stability test

Open glass tubes with 10 g of HOSO mixed with STPE (prepared as described in Section 2.5) were stored in an oven in the dark at 60°C for 39 days. Tubes were removed every 7 days, and HOSO was used as a control. The stability experiments were carried out in triplicate, and the peroxide value (PV) and α-tocopherol concentrations were evaluated over time.

2.8 Analytical Assays

2.8.1 Fatty acid composition

Fatty acids were analysed as methyl ester derivatives [13] by GC (GLC) using an 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. A temperature increase was programmed between 160 and 230°C at 2°C/min, and the samples were run with hydrogen as the carrier gas. Standard FAME (Merck) were used for identification.

2.8.2 The analysis of the tocopherols

Tocopherols were evaluated using HPLC with fluorescence detection according to the Ce 8-86 AOCS standard method [12] for non-esterified tocopherols. The HPLC system consisted of a Merck-Hitachi L-6200 pump (Merck) equipped with a Rheodyne 7725i injector with a 20 μL sample loop and a Merck-Hitachi F-1050 fluorescence detector. The signal was recorded and processed using Clarity software. Each sample was analysed in duplicate. The 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) 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. Tocopherols were identified and quantified using Calbiochem tocopherols (Merck) as external standards.

2.8.3 The characterisation of the STPE extract pool

The total phenolic content was determined according to the Folin–Ciocalteau colorimetric method, as modified by Chun et al. [14], and the results were expressed as milligrams of GAE according to a calibration curve (133.8–428.0 μg/mL; $R^2 = 0.9901$).

The antioxidant activity was evaluated according to the radical scavenging DPPH method [4]. All of the analyses were carried out in triplicate, and the data are reported as averages.

2.8.4 Peroxide value

The PV was determined according to the Cd-8-53 AOCS standard method [12].

2.9 Statistical analysis

A linear regression (95% confidence limit) was used to determine the reaction order and degradation rate constant of α-tocopherol. The values of IP, W_{InH} and PV for a set time were analysed by a one-way ANOVA. Statistical analyses were carried out with Statgraphics Plus software, version 7.0 (Manugistics Inc., Statistical Graphics Corporation, Rockville, MD).

3 Results and discussion

Table 1 shows the composition of major fatty acids of the oils studied. HOSO and its respective antioxidant-stripped oil (THOSO) primarily showed a monounsaturated profile,

Table 1. Fatty acid composition and α-tocopherol of SO, TSO, HOSO and THOSO

	SO	TSO	HOSO	THOSO
Fatty acid (%)				
Palmitic (14:0)	6.5 ± 0.01	6.4 + 0.02	3.9 ± 0.02	3.9 ± 0.01
Stearic (18:0)	3.9 ± 0.01	3.9 ± 0.01	3.5 ± 0.02	3.3 ± 0.01
Oleic (18:1w9)	26.2 ± 0.03	25.4 ± 0.04	81.1 ± 0.12	79.6 ± 0.07
Linoleic (18:2w6)	57.1 ± 0.11	57.3 ± 0.08	7.3 ± 0.02	7.2 ± 0.02
Linolenic (18:3w3)	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.3 ± 0.01
Tocopherols [mg/kg]				
Alpha tocopherol	681 ± 13	nd	619 ± 15	nd

nd, not detected; SO, sunflower oil; HOSO, high-oleic sunflower oil; TSO, antioxidant-stripped SO; THOSO, antioxidant-stripped HOSO. Values are reported as means ± SD ($n = 3$).

whereas SO and its respective antioxidant-stripped oil (TSO) showed a more polyunsaturated profile. Similar fatty acid compositions were reported by Smith et al. [15] and Marinova et al. [16]. Both regular and high-oleic sunflower oils had high contents of α-tocopherol, and a similar result has been reported by Angelo and Jorge [17] in sunflower oil.

3.1 A preliminary assay of polyphenol extraction from tara pods by SFE

A preliminary assay was performed to extract polyphenols from tara pods by SFE. Table 2 shows the effect of pressure on the polyphenol extraction yield. A proportional effect was observed between pressure and yield up to 500 bars, at which point the extraction yield decreased significantly. This effect could be explained by the presence of carbohydrates, fibre and other compounds contained in tara pods, which at high pressures can obstruct the frits in the lid of the extraction cell, hindering the polyphenol extraction. When this occurs, substances other than polyphenols are also extracted. This behaviour was observed in a pressurised liquid extraction process in carob pods in which compounds such as sugars, coloured substances, lipids and resins were coextracted with polyphenols [18]. In the present study, a great effect of pressure on the extraction yield was shown between 400 and 500 bars, and no significant effect was observed between 350 and 400 bars.

3.2 The characterisation of the STPE

The STPE showed a total polyphenol content of 5.2 mg GAE/mL of extract (44 mg GAE/g tara). Haslam et al. [19] reported that the principal components of tara polyphenols are tannins, which are based on a galloylated quinic acid structure. The antioxidant activity (DPPH) of STPE expressed as an EC_{50} value was 0.83 μg GAE/mL extract, which is higher than the reported activity of both supercritical oregano extract (53.8 μg GAE/mL) [4] and supercritical olive leaf extract (0.244 mg CAE/mL) [20].

Table 2. Influence of the pressure on the polyphenols extraction from tara pods by CO_2 SFE

Pressure (bar)	Polyphenols content (μg GAE/g$^{\#}$)
350	291 ± 44^{a}
400	309 ± 98^{a}
500	4107 ± 80^{b}
550	3408 ± 64^{c}

GAE, Gallic acid equivalents; g$^{\#}$, ground tara pods with 8% of moisture.
Values are reported as means ± SD ($n = 3$).
Different letters mean significant differences between polyphenols content ($p < 0.05$).

The differences in antioxidant activity could be attributed to the individual polyphenol profiles of each extract. The structure–activity relationship of flavonoids has been clearly established [21].

3.3 The influence of STPE on the oxidative stability of oil under accelerated conditions

Table 3 shows the oxidative stability of SO and HOSO (regular and antioxidant-stripped) supplemented with different amounts of STPE. The IP values in the TSO and THOSO (without STPE) were 1.4 and 4.0 h, respectively. The addition of STPE at concentration of 12 mg GAE/kg oil and higher THOSO and at 58 mg GAE/kg oil and higher for TSO significantly improved their oxidative stabilities. The IP values of the TSO and THOSO showed significant differences ($p < 0.05$) at almost all levels of STPE supplementation.

In THOSO and TSO, the difference between the IP values was small; however, upon the addition of antioxidants, the differences between the IP values increased considerably, the THOSO IP values were several times greater than those of the TSO. This behaviour may be explained by the different unsaturation ratios of the matrices. Such an effect has been observed in olive oil matrices with different degrees of unsaturation spiked with antioxidants [22].

As expected, the HOSO without added antioxidants showed a higher IP value (48.7 h) than the SO (11.3 h), which correlated with its high level of MUFA. Lower IP values have been observed in HOSO of 19.1 h [23] and in SO of 8.7 h [17] under similar conditions. The addition of the STPE (12 mg GAE/kg oil) significantly improved ($p < 0.05$) the IP value of the SO; however, non-statistically significant differences were observed when greater amounts of extract were added. A similar behaviour has been described by Farag et al. [10] in SO supplemented with both olive fruit extract and olive leaf extract at similar levels to those used in this study. Angelo and Jorge [17] assayed SO supplemented with coriander extract at a concentration much greater than the amount of STPE used in the present study (1,600 mg GAE/kg oil); under the same conditions, these authors reported a similar IP value to those of this study (9.81 h), which indicates that STPE has a higher antioxidant activity than coriander extract.

A different behaviour was observed for HOSO, in which the IP values increased slightly with increasing concentrations of the extract until reaching a statistically significant difference at an STPE concentration of 117 mg GAE/kg oil ($p < 0.05$).

The addition of STPE to the antioxidant-stripped oils at the levels used in this study did not produce the level of protection provided by their natural antioxidants, but a higher concentration of extract may be able to provide an equivalent level of protection.

Table 3. Oxidative stability and rate of antioxidant consumption of, natural and antioxidants-stripped, sunflower oils (SO, TSO) and high-oleic sunflower oils (HOSO; THOSO) with addition of different concentrations of polyphenols extract from STPE at 100°C

Oil system	α-tocopherol (mg/kg)	Polyphenols added from STPE (mg (GAE)/kg oil)	Total antioxidants ($M \times 10^4$)	Molar ratio (α-T)/(STPE)	IP (h)	W_{InH} ($\times 10^8$) (M/s)
TSO	50[a)]		1.2		3.2	1.2
	125[a)]		3.0		5.5	1.61
	250[a)]		6.0		7.4	2.33
		0	0		1.4 ± 0.03[a]	
		12	0.6		1.9 ± 0.05[ab]	0.95 ± 0.02[a]
		58	3.2		2.7 ± 0.20[b]	3.29 ± 0.24[b]
		117	6.4		4.8 ± 0.10[c]	3.65 ± 0.07[b]
		233	12.7		7.1 ± 0.83[d]	5.01 ± 0.59[c]
SO	681	0	14.7		11.3 ± 0.42[a]	3.63 ± 0.14[a]
	681	12	15.3	1:0.05	12.4 ± 0.10[b]	3.44 ± 0.03[a]
	681	58	17.9	1:0.2	12.6 ± 0.05[b]	3.96 ± 0.02[b]
	681	117	21.1	1:0.5	12.3 ± 0.35[b]	4.78 ± 0.14[c]
	681	233	27.4	1:1	12.0 ± 0.05[b]	6.38 ± 0.03[d]
THOSO		0	0		4.0 ± 0.01[a]	
		12	0.6		8.1 ± 0.54[b]	0.218 ± 0.02[a]
		58	3.2		28.4 ± 2.42[c]	0.313 ± 0.03[b]
		117	6.4		44.9 ± 1.19[d]	0.394 ± 0.01[c]
HOSO	619	0	13.4		48.7 ± 2.25[a]	0.76 ± 0.04[a]
	619	12	14.0	1:0.05	48.5 ± 0.45[a]	0.80 ± 0.01[a]
	619	58	16.6	1:0.25	49.2 ± 1.60[ab]	0.94 ± 0.03[b]
	619	117	19.7	1:0.5	52.7 ± 0.35[c]	1.04 ± 0.01[c]

STPE, supercritical tara polyphenols extract; α-T, alpha tocopherol; GAE, gallic acid equivalent.
Values are reported as means ± SD ($n = 3$). Different letters for IP and W_{InH} for the same oil mean significant differences between concentrations ($p < 0.05$).
[a)] Data from study of Marinova et al., 2008.

A linear relationship was found between the polyphenol content of the STPE and the IP values for both the TSO and the THOSO ($R^2 = 0.99$). These results indicate that polyphenols, or other minor components present in the extract, could exert a protective effect against the thermoxidation of oils under accelerated conditions, which would occur in a concentration-dependent manner. The slope of the polyphenol content-IP value relationship of the THOSO was 14 times greater (0.357 vs. 0.025) than that of the TSO, indicating that at the same concentration of antioxidants, monounsaturated oil reaches a higher stability than polyunsaturated oil.

Farag et al. [10] found a linear relationship when the relative concentrations of phenolic compounds were plotted against the IPs ($r^2 = 0.90$–0.99) in SO.

These results differ from those presented by Mateos et al. [22] at similar antioxidant concentrations in olive oil matrices, in which a linear relationship was observed at a low concentration, and a trend towards constant IP values was observed at higher concentrations of hydroxytyrosol.

Table 3 shows the oxidation rates during the IP in the presence of an inhibitor (W_{InH}), which were calculated as defined by Marinova et al. [16], and represent the mean rate of inhibitor consumption according to the following equation:

$$W_{InH} = \frac{[InH]o}{IP_{inh}}$$

where [InH]o represents the initial molar concentration of the antioxidant or inhibitor, and IP_{inh} is the duration of the IP in the presence of the inhibitor (in seconds). A lower value of W_{inH} indicates a lower oxidation rate (i.e. better antioxidant behaviour). It was observed that SO which contains 681 ppm of α-tocopherol (in the absence of STPE) presented a W_{InH} of 3.63 M/s; this value is higher than those obtained by Marinova et al. [16] at lower concentrations of α-tocopherol, which supports the concentration-dependent effect of α-tocopherol on the TAGs of sunflower oil (TGSO) (indicating the participation of its molecules in a side reaction with

hydroperoxides) [16, 24, 25, 26]. The polyphenols from the STPE behaved in a concentration-dependent manner as well: the W_{InH} values for and THOSO added with STPE polyphenols increased significantly with increasing concentrations ($p < 0.05$). The W_{InH} values for SO and HOSO added with STPE also behaved in a concentration-dependent manner. Contrary, a concentration-independent behaviour was observed by Marinova et al. [16] in TGSO with myricetin or with mixtures of myricetin and α-tocopherol. TSO with STPE showed higher W_{InH} values than those reported for myricetin [16], at similar molar polyphenol concentrations, indicating a higher level of permanence of the myricetin in the oil as well as a greater stability.

3.4 The effect of STPE on the oxidative stability of HOSO at 60°C

The antioxidant effect of STPE on HOSO was studied at three polyphenol concentrations. Figure 1 shows the evolution of primary oxidation compounds measured as PV during storage at 60°C. The control oil presented the highest formation of hydroperoxides during storage; in this system, the protection of its own natural antioxidants was provided mainly by α-tocopherol. The addition of STPE increased the stability of the HOSO considerably, mainly at later time points of storage, which was reflected by a lower formation of hydroperoxides ($p < 0.05$) at the three concentrations tested. When the extract was added at a concentration of 400 ppm, it protected the oil for longer than 24 days; for as long as 39 days, the PV was slightly higher than 10 $mEqO_2$/kg, whereas control oil reached this value on the 10th day of storage. After the 10th day, there was a significant ($p < 0.05$) increase in the PV. The antioxidant effect of STPE was dependent on the concentration of polyphenols, which may indicate a relationship between tocopherols and polyphenols or other minor components present in the extract.

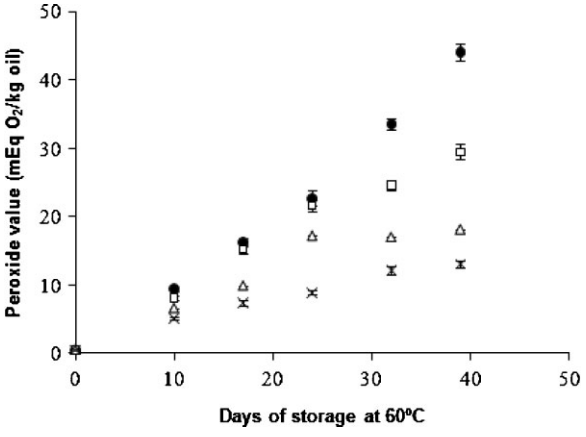

Figure 1. Effect of different concentration of polyphenols from STPE on the stability of high-oleic sunflower oil (HOSO). ● = control; □ = 85 ppm; Δ = 200 ppm; × = 400 ppm.

Table 4. Degradation rate constants (k) and coefficients of determination (r^2) from the linear regression of Ln % retention of α-tocopherol versus time in high-oleic sunflower oil added with STPE in storage condition at 60°C

STPE (mg GAE/kg)	k	r^2
0	$0.025^a \pm 7.7 \times 10^{-4}$	0.881
85	$0.015^b \pm 8.4 \times 10^{-4}$	0.958
200	$0.017^b \pm 9.5 \times 10^{-4}$	0.989
400	$0.024^a \pm 4.5 \times 10^{-4}$	0.968

For abbreviations see footnotes in Table 3.
Values are reported as means ± SD ($n = 3$).
Different letters for k mean significant differences ($p < 0.05$).

Furthermore, it was observed that the system with the highest polyphenol concentration (from STPE) presented the greatest α-tocopherol degradation (Table 4) and the lowest level of hydroperoxide formation (Fig. 1), which may indicate that α-tocopherol causes the regeneration of the polyphenols, which, in turn, protect the TAGs in HOSO. The same behaviour was reported by Marinova et al. [16] for the regeneration of myricetin by α-tocopherol.

STPE exhibits antioxidant activity against unsaturated oils under accelerated storage conditions. Therefore, tara pods combined with SFE could be a source of antioxidant compounds for use in oils.

The authors have declared no conflict of interest.

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