

Original article

Natural antioxidants from herbs and spices improve the oxidative stability and frying performance of vegetable oilsLucía Redondo-Cuevas,^{1,2,3*} Gloria Castellano² & Vassilios Raikos¹¹ Rowett Institute, University of Aberdeen, Foresterhill, AB25 2ZD Aberdeen, UK² Departamento de Ciencias Experimentales y Matemáticas, Facultad de Veterinaria y Ciencias Experimentales, Universidad Católica de Valencia San Vicente Mártir, Valencia 46001, Spain³ Escuela de Doctorado, Universidad Católica de Valencia San Vicente Mártir, Valencia 46008, Spain

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Summary Lipid oxidation has been identified as the major deterioration process of vegetable oils. Undesirable effects are even more profound when food processing involves high temperatures in the presence of oxygen. Natural ground herbs (black pepper, ginger, turmeric, rosemary and oregano) were assessed for their antioxidant capacity, phenolic content and ability to improve the oxidative stability of vegetable oils. The most potent herb was incorporated in vegetable oils formulations which were subjected to consecutive frying cycles. The oxidative stability of the vegetable oils, the formation of conjugated dienes/trienes and the decimation of tocopherol levels after the frying process were assessed. Rosemary powder was the most effective antioxidant among the ones tested. The oxidative stability of tocopherol-stripped corn oil with rosemary powder (1.53 ± 0.06 h) was significantly higher than the control (0.84 ± 0.02 h) and the oil with synthetic antioxidants (1.20 ± 0.03 h). Rosemary powder effectively improved the oxidative stability of sunflower (128.91%), olive (55.61%) and rapeseed (73.20%) oil during deep-frying and prevented CD and CT formation in rapeseed oil.

Keywords Antioxidants, frying, lipid oxidation, natural products, oils.

Introduction

Lipid oxidation has been identified as the major deterioration process of vegetable oils affecting both the sensory and the nutritional quality of foods. Frying is one of the most common cooking techniques used in domestic and industrial food preparation. Flavour, shelf-life and nutrient composition of fried food and vegetable oils are altered by this process, and also some of the compounds formed may have undesirable consequences on consumers' health (Hosseini *et al.*, 2016).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) or tertbutylhydroquinone (TBHQ) are often used to retard lipid oxidation in food systems. Due to their low thermal stability, the concern about their long-term effects on human health and the increasing demand by consumers for natural products, plant extracts emerged as good alternatives to synthetic

antioxidants (Taghvaei & Jafari, 2015; Sahin *et al.*, 2017). However, commercially available plant extracts are suitable mainly for food manufacturing at industrial scale, whereas the use of the whole ground plants could be a good option both for the food industry and domestic use. Moreover, there is higher antioxidant activity in whole plants than in their corresponding extracts prepared from an equivalent amount. This suggests that a plethora of antioxidant compounds are removed in the process of extraction, which may have an accumulative or synergistic effect on the oxidative stability of the end-product (Yanishlieva *et al.*, 2006).

In this study, we aimed to identify natural antioxidants from herbs and spices (black pepper, ginger, turmeric, rosemary and oregano) suitable for increasing the oxidative stability of three commercially available vegetable oils (olive, rapeseed and sunflower oil) commonly used for food applications. The effectiveness of the most potent herb as an antioxidant was further evaluated by assessing the frying performance of the aforementioned vegetable oils.

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Materials and methods

Samples

Blended olive oil, refined sunflower oil, refined rapeseed oil and fresh potatoes were purchased in Tesco supermarket (Aberdeen, Scotland). Tocopherol-stripped corn oil as control was purchased from Sigma-Aldrich, Co Ltd (Dorset, UK). Ground organic black pepper (*Piper nigrum*), ground organic ginger (*Zingiber officinale*), ground organic turmeric (*Curcuma longa*) and ground oregano (*Origanum vulgare*) were purchased from JustIngredients Limited (Monmouthshire, UK). Ground rosemary (*Rosmarinus officinalis*) was purchased in G Baldwin & Co (London, UK). All the ingredients were stored in a dark cool place to protect them from oxidation. Methanol, cyclohexane, FeCl_3 , TPTZ reagent, HCl, FeSO_4 , Folin–Ciocalteu reagent, sodium carbonate, gallic acid and butylated hydroxytoluene (BHT), were purchased from Sigma-Aldrich, Co Ltd (Dorset, UK). All reagents used were of analytical grade.

Ferric reducing ability of plasma (FRAP) assay in ground plants

Extraction and testing sample preparation was adapted from Wojdyło *et al.* (2007) with some modifications. In brief, 1 g of each ground aromatic plant was weighed into a test tube. A total of 10 mL of 80% aqueous methanol were added, and the suspension was gently mixed. Tubes were sonicated twice for 15 min and then centrifuged for 10 min at 1500 *g* using a CompactStar CS4 centrifuge (VWR International Ltd, West Sussex, UK). Supernatants were collected and stored at 4 °C prior to use within 24 h.

The total antioxidant potential of the five ground plants was determined using the FRAP assay adapted from Benzie & Strain (1996) as a measure of antioxidant power. The FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of a solution of 10 mM TPTZ in 40 mM HCl, and 2.5 mL of 20 mM FeCl_3 and once prepared was kept in a water bath at 37 °C. The following were added directly to each cuvette in a Pye Unicam UV-4 UV-VIS scanning spectrophotometer (Spectronic Cam-spec Ltd., Leeds, UK) thermostated at 37 °C: 90 μL H_2O , 900 μL FRAP reagent and 30 μL of sample (diluted 25 times), standard or H_2O as a blank. Absorbance readings were taken at 593 nm after 4 min at 37 °C. A solution of 1 mM FeSO_4 was used as standard. All determinations were performed in triplicate. The results were corrected for dilution and expressed in $\mu\text{M Fe}^{+2}$ per g.

Total phenolic content (TPC) of ground plants

Extraction and testing sample preparation was performed as follows: 1 g of each ground plant was weighed into a test tube and 20 mL of methanol was added. The test tube was vortexed, homogenised for 2 min at 8000 rpm, left at a tube roller mixer for 1 h and then centrifuged at 1100 *g* for 15 min. The supernatant was collected, and the procedure was repeated one more time with another 20 mL of methanol. The two methanol extracts were combined, and the final volume was brought up to 40 mL with methanol.

The total phenolic contents of the samples were determined using the Folin–Ciocalteu reagent as described by Parry *et al.* (2005). In brief, the reaction mixture contained 50 μL of testing sample solutions, 250 μL of the Folin–Ciocalteu reagent, 0.75 mL of 20% sodium carbonate and 3 mL of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured by a Pye Unicam UV-4 UV-vis scanning spectrophotometer (Spectronic Cam-spec Ltd., Leeds, UK) and was used to calculate the phenolic contents of oils using gallic acid as standard. Measurements were taken in triplicate. The results were expressed as mg gallic acid equivalents (GAE) per g of powder.

Rancimat analysis – Induction period (IP)

Herbs and spices were used at a concentration of 0.5% (w/v) and BHT at maximum permitted levels of 100 mg kg^{-1} (Official Journal of the European Union, 2011). Each of these materials were added to tocopherol-stripped corn oil and then mixed using a tube roller for 1 h at room temperature before initiating the IP determination. The most potent plant in terms of increasing the oxidative stability was mixed with olive, sunflower and rapeseed oil following the same procedure, and they were analysed before and after the frying process.

Oxidative stability of oils was determined by the oxidation induction period (IP) in a 743 Rancimat apparatus (Metrohm Ltd., Herisau, Switzerland) according to the AOCS Official Method Cd 12b-92 (AOCS, 1998). Oil samples (3 g) were heated at 120 °C with a constant airflow of 20 L h^{-1} . The times required for a sharp increase in water conductivity is calculated automatically by the software and corresponds to the IP in hr. Measurements were taken in quadruplicate.

Frying protocol

The frying process was carried out on commercially available oils namely olive, sunflower and rapeseed oil. Rosemary powder (0.5% w/v) was allowed to mix for

1 h using a tube roller. Fresh potatoes were peeled, washed and sliced into discs of 3 mm thickness using a Swan SFS102 food slicer (Swan Products Ltd, Staffordshire, UK). After equilibrating 2.5 L of oil for 10 min at 180 °C in an electric deep-fryer (Tesco 3L Pro Fryer), four batches of potato slices (150 g per batch) were consecutively introduced into the oil for 5 min each; this protocol was used based on Akil *et al.* (2015). The oil was then allowed to cool down for 2 h at room temperature. Following the frying process, samples were taken immediately for Rancimat analysis and remaining sample was frozen at -20 °C for further analyses. Fresh oil (control) was also stored at -20 °C for subsequent analyses.

Determination of conjugated dienes (CD) and trienes (CT)

Ultraviolet absorption at 232 and 270 nm of fresh and fried oils was determined according to the Regulation (EU) No 299/2013 method (Official Journal of the European Union, 2013) for measuring the presence of conjugated diene and trienes systems, respectively. Oils were dissolved in the required solvent (cyclohexane), and the absorbance of the solution was measured at the specified wavelength (232 or 270 nm) with reference to pure solvent. The results were expressed as Ks values (specific extinction coefficients), which are calculated for a concentration of 1% w/v sample dissolved in cyclohexane in a 10 mm cell. Measurements were taken in triplicate by a Pye Unicam UV-4 UV-vis scanning spectrophotometer.

Determination of tocopherols

The tocopherol content of vegetable oils was determined in both fresh and fried oils. A reverse phase HPLC method was used to quantify alpha-tocopherol, gamma-tocopherol and delta-tocopherol in oils according to Hess *et al.* (1991).

Tocopherols were extracted from the oil phase as follows: 20 mg of oil was mixed with 280 µL H₂O and 400 µL ethanol. Each tube was vortexed for 10 s, and 700 µL of hexane (containing BHT) and 100 µL of echinone were added and the samples were shaken for 10 min in the vortex genie before centrifugation for 5 min. The supernatant hexane layer (600 µL) was removed and dried down on the speed vacuum for 10 min. Each sample was then dissolved in 200 µL of DEA (20% (v/v) 1,4 dioxan, 20% (v/v) ethanol, 60% (v/v) acetonitrile) and was shaken for 5–10 min before injected for HPLC analysis. HPLC analysis was performed using a Waters 717 plus Autosampler Module (Waters Corporation, Milford, USA) equipped with a Waters 2475 scanning fluorescence detector, a 2487 UV/vis absorbance detector and a C-18 silica

(Beckman Ultrasphere ODS) analytical column (250 × 4.6 mm ID 5 µm particle size). Elution flow rate was 1.1 mL min⁻¹, sample run was 30 min and injection volume was 150 µL. Measurements were determined with mixed standards containing tocopherols at appropriate concentrations, and results were expressed in µg g⁻¹ of oil. Echinone was used as an internal standard. The measurements were taken in triplicate.

Statistical analysis

All sampling and chemical analyses were performed in triplicate or quadruplicate. Results of this study were expressed as a mean with standard deviations. To identify statistical differences between herbs and spices, factor analysis was performed and a new variable was obtained (antioxidant power index) from the z-score of the three variables described. Significant differences between mean values were evaluated by variance analysis (ANOVA), Student's *t*-test (for independent variables) and two-tailed *t*-tests (for dependent variables). All analyses were performed using SPSS statistical software, and the level of statistical significance was set at *P* < 0.05.

Results and discussion

Characterisation of herbs and spices as natural antioxidants

Antioxidant activity (FRAP assay) and total phenolic compounds of black pepper, ginger, turmeric, rosemary and oregano, and also IPs of corn oil with added spices and herbs at 0.5% (w/v) are presented in Table 1. Tocopherol-stripped corn oil was used as a model system at this stage to identify the most potent antioxidant powder. Rosemary powder was initially incorporated at different concentrations in corn oil in order to determine the optimum formulation for assessing IP: 0.25%, 0.5%, 0.75% and 1% generated an IP of 1.05 ± 0.04, 1.53 ± 0.06, 2.06 ± 0.05 and 2.07 ± 0.09 h, respectively (data not shown). However, at 0.75% and 1% concentration, the powder was not adequately mixed, and therefore, 0.5% was chosen as optimum for further studies. Moreover, the IP of corn oil mixed with 0.5% w/v rosemary was significantly higher (*P* < 0.05) compared to the control and the oil containing BHT at the legal limit concentration of 100 mg kg⁻¹ (Table 1).

The mean of the score of the 'antioxidant power' was significantly higher in rosemary than the other plants, whereas black pepper had a significant lower score compared with the rest of the samples (Table 1). There was no significant difference between ginger, turmeric and oregano.

Table 1 Antioxidant capacity and total phenolic content of herbs and spices, and induction period (IP) of a tocopherol-stripped corn oil (plain oil) formulated with ground herbs and spices (0.5% w/v) and BHT (100 mg kg⁻¹)

	Antioxidant capacity $\mu\text{M Fe}^{2+}$ per g	Total phenolic compounds mg GAE per g	Induction period of plain oil mixed with antioxidants h	Antioxidant power index*
Black pepper	82.78 $\pm$ 1.42	50.16 $\pm$ 13.89	0.79 $\pm$ 0.03 ^a	-1.00 $\pm$ 0.00 ^a
Ginger	252.04 $\pm$ 11.55	121.53 $\pm$ 3.88	0.84 $\pm$ 0.08 ^a	-0.36 $\pm$ 0.02 ^b
Turmeric	166.01 $\pm$ 0.69	239.96 $\pm$ 32.17	0.86 $\pm$ 0.09 ^a	-0.27 $\pm$ 0.04 ^b
Rosemary	573.35 $\pm$ 72.74	410.16 $\pm$ 22.39	1.53 $\pm$ 0.06 ^b	1.84 $\pm$ 0.07 ^c
Oregano	290.20 $\pm$ 2.73	155.25 $\pm$ 17.79	0.86 $\pm$ 0.06 ^a	-0.21 $\pm$ 0.06 ^b
Plain			0.84 $\pm$ 0.02 ^a	
BHT			1.20 $\pm$ 0.03 ^c	

Results are expressed as mean $\pm$ SD (standard deviation).

*A new variable obtained from a factorial analysis from the z-score of the three variables described. Values from this new variable with different lower case letter are significantly different ($P < 0.05$). GAE, gallic acid equivalents; BHT, butylated hydroxytoluene.

Rosemary powder showed the highest antioxidant capacity and total phenolic content and the best potential to increase the oxidative stability of corn oil of the studied herbs and spices. When rosemary powder was mixed with oil, 0.5% w/v concentration was the best option in terms of increasing oxidative stability and also mixing efficiency. In agreement with these findings, when rosemary extract is included in bran, soya bean and cottonseed oil, the IP values are significantly increased compared to control oils or oils containing synthetic antioxidants (Yang *et al.*, 2016). Rosemary extract is popular as a natural antioxidant in vegetable oils due to its strong antioxidant capacity, which is attributed to the presence of phenolic diterpenes such as carnosic acid and carnosol (Yanishlieva *et al.*, 2006; Terpin *et al.*, 2009). In fact, it has been adopted formally into the European regulations as new food additive for use in foodstuffs and assigned E 392 as its E number (Commission Directive 2010/67/EU and 2010/69/EU).

Effect of deep-frying on vegetable oils

Oxidative stability (IP)

Results of the IP of fresh and fried sunflower, olive and rapeseed oils with and without rosemary are shown in Fig. 1. IP values of fresh plain oils (without rosemary) were in the following order: olive oil (9.02 $\pm$ 0.07 h), rapeseed oil (4.95 $\pm$ 0.24 h) and sunflower oil (2.41 $\pm$ 0.06 h), with significant differences ($P < 0.05$) detected between the samples. Frying significantly reduced the oxidative stability of rapeseed and sunflower oil (3.47 $\pm$ 0.12 and 1.28 $\pm$ 0.65 h, respectively), whereas the IP of olive oil was not significantly affected by the process of deep-frying (9.35 $\pm$ 0.33 h). According to the nutritional information provided by the manufacturers, olive oil contains 14.3% saturated, 73% monounsaturated and 8.2% polyunsaturated

fatty acids; sunflower oil contains 12% saturated, 23% monounsaturated and 65% polyunsaturated fatty acids; and rapeseed oil contains 7% saturated, 57% monounsaturated and 28% polyunsaturated fatty acids. Similar findings indicating that olive oil is more resistant to degradation under domestic frying conditions compared with vegetable oils with high polyunsaturated fatty acid content have been reported (Casal *et al.*, 2010; Marinova *et al.*, 2012).

Adding rosemary powder into the oils significantly ($P < 0.05$) increased the IP of all samples (Fig. 1). The IP of olive, rapeseed and sunflower oil increased to 15.71 $\pm$ 0.37, 7.61 $\pm$ 0.29 and 3.87 $\pm$ 0.07 h, respectively, after rosemary addition. During frying, IP of these oils was reduced to 14.55 $\pm$ 0.89, 6.01 $\pm$ 0.37

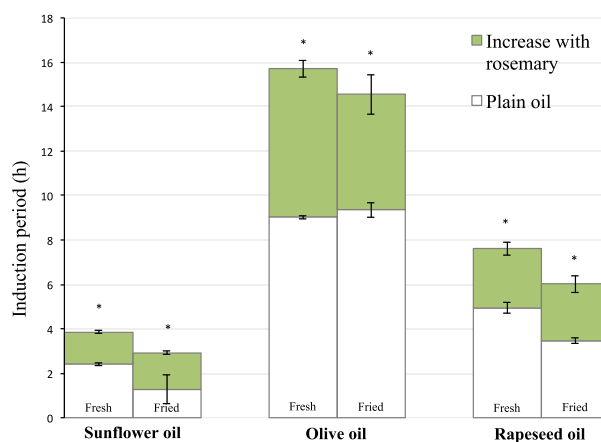


Figure 1 Effect of rosemary powder (0.5% w/v) addition on vegetable oil stability (IP) during a deep-frying process. Results are presented as mean $\pm$ SD. * denotes significant differences at $P < 0.05$ for samples before/after rosemary addition. [Colour figure can be viewed at wileyonlinelibrary.com]

and 2.93 ± 0.08 h, respectively. It is worth mentioning that the IP of all fried oils with rosemary powder was higher than the one shown by the plain oils which have not been subjected to the deep-frying process. This indicates the protective effect of rosemary against oxidation. Addition of rosemary into sunflower, olive and rapeseed oil during the frying process led to an increase of IP of 128.91%, 55.61% and 73.20%, respectively. Our results suggest that rosemary powder is more effective in vegetable oils with a high polyunsaturated fatty acid content. Tohma & Turan (2015) investigated the effects of rosemary powder (and its alcoholic extracts) on the oxidative stability of hazelnut oil during deep frying. The rosemary concentration used for the frying procedure was 10 or 50 g kg⁻¹ and 1000 ppm for the plant powder and its alcoholic extracts, respectively. This study indicated that rosemary additives were effective against lipid oxidation during frying of hazelnut oil. Rosemary additives inhibited the formation of oxidation products, increased the IP and improved the frying performance of hazelnut oil. For rosemary powder, this effect was more profound when used at high concentrations (50 g kg⁻¹).

The use of rosemary extracts for increasing the oxidative stability of vegetable oils has been extensively documented. The addition of rosemary extracts to frying oils reduced both primary and secondary oxidation products during deep frying of potato slices (Alizadeh *et al.*, 2016). Upadhyay *et al.* (2017) reported that oleoresin rosemary was able to significantly increase the frying stability of sunflower oil blends with ascorbyl palmitate. Moreover, the use of a rosemary extract in soya bean oil heated for 10 h at 180 °C led to a significant increase of IP of 75.24%, as reported by Casarotti & Jorge (2012).

Measurement of IP can be indicative of quality deterioration of vegetable oils during a frying process and a linear correlation with some parameters of oil

oxidation and IP has been established (Farhoosh & Moosavi, 2007; Farhoosh & Tavassoli-Kafrani, 2011; Upadhyay *et al.*, 2017). Farhoosh & Moosavi (2007) proposed that assuming 24% of total polar compounds are the maximum permitted levels in frying oils (i.e. discard point level), the IP of used oil should be ≥ 2.32 h. In the present study, fresh sunflower oil had an IP approximating the discard limit and fried sunflower oil was below the minimum recommended value. Adding rosemary powder to sunflower oil increased the IP above the limits, both for fresh and fried oil (Fig. 1). However, additional parameters should be considered when monitoring the oxidative stability of oils during frying, particularly for assessing their safety for human consumption (Nayak *et al.*, 2016).

Conjugated dienes (CD) and trienes (CT)

Conjugated dienes (CD) and trienes (CT) are formed as secondary products from the oxidation of polyunsaturated fatty acids, and their levels increase with frying time. Results from changes in CD and CT values in vegetable oils during the deep-frying process and the effect of rosemary powder addition are shown in Table 2. Rapeseed oil showed a significant increase ($P < 0.05$) for both CD and CT levels during frying. Olive and rapeseed oil showed an increase of CT (only significant for olive oil), whereas CD remained stable in these oils during frying. Rapeseed oil is more susceptible to the formation of secondary products of oxidation during the frying process due to its high polyunsaturated fatty acids content, according to the supplier's information. Olive oil had the lowest CD and CT values compared with sunflower and rapeseed oil before frying. Moreover, the CT value was maintained below the maximum permitted levels (≤ 0.90) for olive oil (Official Journal of the European Union, 2013) during frying.

Table 2 Changes in conjugated diene and conjugated triene value of oils during deep-frying and effect of rosemary powder addition to oils (0.5% w/v)

Oils	Conjugated diene value (K ₂₃₂)		Conjugated triene value (K ₂₇₀)	
	Fresh oil	Fried oil	Fresh oil	Fried oil
Sunflower oil	$4.62 \pm 0.19^{a,A}$	$4.65 \pm 0.02^{a,A}$	$2.64 \pm 0.08^{a,A}$	$3.23 \pm 0.23^{a,A}$
Sunflower oil + ROS	$4.70 \pm 0.15^{a,A}$	$4.66 \pm 0.23^{a,A}$	$2.79 \pm 0.14^{a,A}$	$3.44 \pm 0.23^{b,A}$
Olive oil	$2.72 \pm 0.18^{a,A}$	$2.65 \pm 0.20^{a,A}$	$0.39 \pm 0.01^{a,A}$	$0.46 \pm 0.01^{b,A}$
Olive oil + ROS	$2.34 \pm 0.19^{a,A}$	$2.26 \pm 0.09^{a,B}$	$0.45 \pm 0.03^{a,B}$	$0.64 \pm 0.07^{a,B}$
Rapeseed oil	$3.59 \pm 0.08^{a,A}$	$4.64 \pm 0.40^{b,A}$	$1.02 \pm 0.29^{a,A}$	$3.18 \pm 0.54^{b,A}$
Rapeseed oil + ROS	$3.89 \pm 0.3^{a,A}$	$4.19 \pm 0.10^{a,A}$	$0.71 \pm 0.08^{a,A}$	$1.14 \pm 0.01^{b,B}$

Results are expressed as mean $\pm$ SD (standard deviation). Means followed by the same lower case letter are not significantly ($P < 0.05$) different when comparing fresh and fried oil. Means followed by the same upper case letter are not significantly ($P < 0.05$) different when comparing with and without rosemary. ROS, rosemary.

The addition of rosemary to rapeseed oil prevented the formation of CD and CT during deep frying. CD levels increase by 29.25% for plain rapeseed oil after the frying process; for rapeseed oil containing rosemary, the corresponding increase is only 7.71%. A similar effect is observed for CT where values increase by 211.76% and 60.56% postprocessing for plain and rosemary-added rapeseed oil, respectively; meanwhile, when rosemary powder is added to the oil, the increase of CT is 60.56%. Furthermore, although the frying process did not affect the CD values of olive oil, fried samples containing rosemary had significantly lower levels compared to plain ones. Rosemary did not prevent CT formation during frying of olive or sunflower oil. In fact, the addition of rosemary unexpectedly induced CT formation in fresh and fried olive oil; however, the values obtained were always below the official limits mentioned. The protective effect of rosemary extract addition to sunflower oil has been documented for longer deep-frying process cycles (up to 20 batches of potatoes), and results indicate that it can perform better than BHA, TBHQ or mixed tocopherols (Urbančič *et al.*, 2014).

Tocopherol content analysis

Changes in alfa, gamma, delta and total tocopherol content in vegetable oils during the deep-frying process are shown in Table 3. The frying process and the addition of rosemary powder seem to have a minor effect on tocopherol levels for the three vegetable oils. Tocopherol content did not significantly decrease after the frying process; surprisingly, tocopherol levels slightly increased in olive oil after frying (alpha and total), but the observed increase was not statistically significant. Previous reports denote that tocopherol content decreases during frying or heating (Barrera-Arellano

et al., 2002; Casal *et al.*, 2010; Nayak *et al.*, 2016), and the inclusion of rosemary extract has a protective effect against this degradation (Casarotti & Jorge, 2012; Saudi *et al.*, 2016). However, all these studies are conducted for longer periods of frying or heating, so it is likely that in the present study exposure to high temperatures was not suffice to induce the chemical breakdown of the antioxidants naturally occurring in vegetable oils.

Conclusions

Rosemary powder showed the best potential to protect vegetable oils from oxidation compared with black pepper, ginger, turmeric and oregano. Rosemary demonstrated the highest antioxidant capacity (FRAP assay) and had the highest levels of phenolic compounds. Moreover, adding rosemary (0.5% w/v) was the most effective herb to increase the oxidative stability of tocopherol-stripped corn oil, and stability was superior compared to the formulations containing BHT at permitted levels.

The addition of rosemary powder to sunflower, olive and rapeseed oil during a deep-frying process led to a significant increase in their oxidative stability. Rosemary had an additional beneficial effect when incorporated in rapeseed oil, as it prevented CD and CT formation. The frying process and the addition of rosemary powder seem to have a minor effect on tocopherol levels for the three vegetable oils, possibly due to the short frying time applied which was not adequate to induce the chemical breakdown of the antioxidants naturally occurring in vegetable oils.

Results of this study have important food processing applications for domestic settings as direct rosemary addition to a vegetable oil can inhibit lipid oxidation during frying. However, further elaborate studies on

Table 3 Changes in tocopherol levels during deep-frying process and effect of rosemary powder addition to oils (0.5% w/v)

Oils	Tocopherols ($\mu\text{g mL}^{-1}$)							
	Alpha		Gamma		Delta		Total	
	Fresh oil	Fried oil	Fresh oil	Fried oil	Fresh oil	Fried oil	Fresh oil	Fried oil
Sunflower oil	565.16 $\pm$ 55.71	489.37 $\pm$ 58.30	21.92 $\pm$ 2.12	19.33 $\pm$ 1.64	6.26 $\pm$ 0.45	5.28 $\pm$ 0.16	593.73 $\pm$ 58.36	513.98 $\pm$ 60.03
Sunflower oil + ROS	536.80 $\pm$ 62.36	488.01 $\pm$ 28.93	21.34 $\pm$ 2.65	19.19 $\pm$ 0.87	5.46 $\pm$ 0.33	5.30 $\pm$ 0.48	563.60 $\pm$ 65.32	512.50 $\pm$ 30.27
Olive oil	143.83 $\pm$ 16.76	169.88 $\pm$ 10.31	11.42 $\pm$ 0.97	11.42 $\pm$ 0.62	0.56 $\pm$ 0.10	0.59 $\pm$ 0.02	155.81 $\pm$ 17.80	181.89 $\pm$ 10.94
Olive oil + ROS	155.97 $\pm$ 13.97	167.55 $\pm$ 26.50	12.17 $\pm$ 1.13	11.04 $\pm$ 1.86	0.71 $\pm$ 0.02	0.63 $\pm$ 0.12	168.85 $\pm$ 15.12	179.21 $\pm$ 28.49
Rapeseed oil	230.27 $\pm$ 21.55	213.80 $\pm$ 4.98	331.13 $\pm$ 30.94	313.97 $\pm$ 9.00	9.09 $\pm$ 0.90	8.64 $\pm$ 0.26	570.49 $\pm$ 53.37	536.40 $\pm$ 14.24
Rapeseed oil + ROS	199.58 $\pm$ 4.38	197.06 $\pm$ 13.08	286.88 $\pm$ 7.78	283.53 $\pm$ 15.79*	7.90 $\pm$ 0.28	7.85 $\pm$ 0.49	494.35 $\pm$ 12.15	488.44 $\pm$ 29.28

Results are expressed as mean $\pm$ SD (standard deviation).

*Means significant differences ($P < 0.05$) when comparing with and without rosemary oils. There were no significant differences in any data at $P < 0.05$ when comparing fresh and fried oil. ROS, rosemary.

lipid oxidation are needed to establish the effect of rosemary powder during frying in vegetable oils.

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Conflict of interest

The authors declare no conflict of interest.

References

- Akil, E., Castelo-Branco, V.N., Costa, A.M.M., Vendramini, A.L.A., Calado, V. & Torres, A.G. (2015). Oxidative stability and changes in chemical composition of extra virgin olive oils after short-term deep-frying of French fries. *Journal of the American Oil Chemists' Society*, **92**, 409–421.
- Alizadeh, L., Nayebzadeh, K. & Mohammadi, A. (2016). A comparative study on the in vitro antioxidant activity of tocopherol and extracts from rosemary and *Ferulago angulata* on oil oxidation during deep frying of potato slices. *Journal of Food Science and Technology*, **53**, 611–620.
- AOCS (1998). Method Cd 12b-92. In: *Official Methods and Recommended Practices of the American Oil Chemists' Society* (edited by D. Firestone). 5th ed. Champaign, Illinois: AOCS Press.
- Barrera-Arellano, D., Ruiz-Méndez, V., Velasco, J., Márquez-Ruiz, G. & Dobarganes, C. (2002). Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content. *Journal of the Science of Food and Agriculture*, **82**, 1696–1702.
- Benzie, I.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, **239**, 70–76.
- Casal, S., Malheiro, R., Sendas, A., Oliveira, B.P. & Pereira, J.A. (2010). Olive oil stability under deep-frying conditions. *Food and Chemical Toxicology*, **48**, 2972–2979.
- Casarotti, S.N. & Jorge, N. (2012). Antioxidant activity of rosemary extract in soybean oil under thermoxidation. *Journal of Food Processing and Preservation*, **38**, 136–145.
- Commission Directive 2010/67/EU of 20 October 2010 amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners.
- Commission Directive 2010/69/EU of 22 October 2010 amending the Annexes to European Parliament and Council Directive 95/2/EC on food additives other than colours and sweeteners.
- Farhoosh, R. & Moosavi, S.M.R. (2007). Rancimat test for the assessment of used frying oils quality. *Journal of Food Lipids*, **14**, 263–271.
- Farhoosh, R. & Tavassoli-Kafrani, M.H. (2011). Simultaneous monitoring of the conventional qualitative indicators during frying of sunflower oil. *Food Chemistry*, **125**, 209–213.
- Hess, D., Keller, H.E., Oberlin, B., Bonfanti, R. & Schüep, W. (1991). Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *International Journal for Vitamin and Nutrition Research*, **61**, 232–238.
- Hosseini, H., Ghorbani, M., Meshginfar, N. & Mahoonak, A.S. (2016). A Review on frying: procedure, fat, deterioration progress and health hazards. *Journal of the American Oil Chemists' Society*, **93**, 445–466.
- Marinova, E.M., Seizova, K.A., Totseva, I.R., Panayotova, S.S., Marekov, I.N. & Momchilova, S.M. (2012). Oxidative changes in some vegetable oils during heating at frying temperature. *Bulgarian Chemical Communications*, **44**, 57–63.
- Nayak, P.K., Dash, U., Rayaguru, K. & Krishnan, K.R. (2016). Physio-chemical changes during repeated frying of cooked oil: a review. *Journal of Food Biochemistry*, **40**, 371–390.
- Official Journal of the European Union (2011). European Union Regulation No. 1129/2011. L 295, 12 November 2011.
- Official Journal of the European Union (2013). European Union Regulation No 299/2013, L 90, 28 March 2013.
- Parry, J.W., Su, L., Luther, M. et al. (2005). Fatty acid content and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. *Journal of Agricultural and Food Chemistry*, **53**, 566–573.
- Sahin, S., Bilgin, M., Sayim, E. & Güvenilir, B. (2017). Effects of natural antioxidants in the improvement of corn oil quality: olive leaf vs. lemon balm. *International Journal of Food Science & Technology*, **52**, 374–380.
- Saoudi, S., Chammem, N., Sifaoui, I. et al. (2016). Influence of Tunisian aromatic plants on the prevention of oxidation in soybean oil under heating and frying conditions. *Food Chemistry*, **212**, 503–511.
- Taghvaei, M. & Jafari, S.M. (2015). Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. *Journal of Food Science and Technology*, **52**, 1272–1282.
- Terpinc, P., Bezjak, M. & Abramović, H. (2009). A kinetic model for evaluation of the antioxidant activity of several rosemary extracts. *Food Chemistry*, **115**, 740–744.
- Tohma, S. & Turan, S. (2015). Rosemary plant (*Rosmarinus officinalis* L.), solvent extract and essential oil can be used to extend the usage life of hazelnut oil during deep frying. *European Journal of Lipid Science and Technology*, **117**, 1978–1990.
- Upadhyay, R., Schwag, S. & Niwas Mishra, H. (2017). Chemometric approach to develop frying stable sunflower oil blends stabilized with oleoresin rosemary and ascorbyl palmitate. *Food Chemistry*, **218**, 496–504.
- Urbančić, S., Kolar, M.H., Dimitrijević, D., Demšar, L. & Vidrihb, R. (2014). Stabilisation of sunflower oil and reduction of acrylamide formation of potato with rosemary extract during deep-fat frying. *LWT- Food Science and Technology*, **57**, 671–678.
- Wojdyło, A., Oszmian'ski, J. & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, **105**, 940–949.
- Yang, Y., Song, X., Sui, X. et al. (2016). Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. *Industrial Crops and Products*, **80**, 141–147.
- Yanishlieva, N.V., Marinova, E. & Pokorny, J. (2006). Natural antioxidants from herbs and spices. *European Journal of Lipid Science and Technology*, **108**, 776–793.