Research Article

Rosemary powder filtrate improves the oxidative stability and antioxidant properties of rapeseed oil: potential applications for domestic cooking.

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Keywords: Rosemary; rapeseed oil; antioxidant; oxidation stability.

Abbreviations: CD, conjugated diene, **FRP**, ferric reducing power**, IP**, induction period**, MDA**, malondialdehyde**, TBARS**, thiobarbituric acid reactive substances.

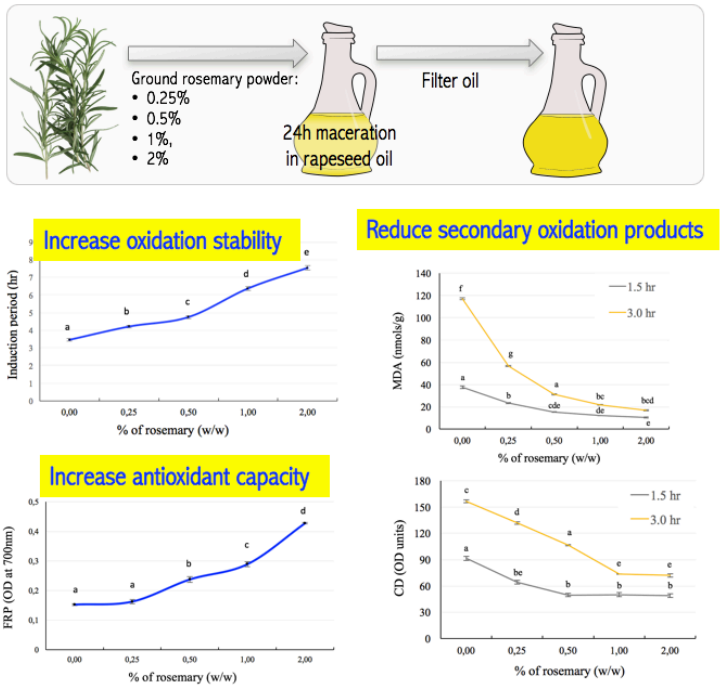
ABSTRACT

Lipid oxidation has been identified as the major deterioration process of vegetable oils. Rapeseed oil is an important source of edible oil for the human diet but is also highly susceptible to oxidative deterioration. Previous research has demonstrated that rosemary powder and its extracts can increase the oxidative stability of rapeseed oil. In this study, the protective effect of ground rosemary powder (0.25% - 2.0%) against the oxidative degradation of filtered rapeseed oil was assessed using temperature-controlled tests. Rapeseed oil filtrate showed enhanced antioxidant capacity and higher induction periods (IP) based on Rancimat analysis. The addition of rosemary powder was beneficial for the oxidative stability of rapeseed oil and effectiveness was more profound at concentrations above 0.5%. Lower conductivity changes and total colour differences, reduced malondialdehyde and conjugated dienes content, and higher levels of tocopherols and carotenoids were observed for the oils formulated with rosemary

powder after 1.5 hr and 3 hr of exposure to accelerated oxidation conditions using the Rancimat apparatus (120ºC with a constant airflow of 20 L/h).

Practical applications: The use of rosemary powder filtrate could be an effective way to protect rapeseed oil from oxidation during storage or cooking with domestic and commercial applications.

GRAPHICAL ABSTRACT



The addition of rosemary powder was beneficial for the oxidative stability and antioxidant capacity of rapeseed oil. Lower conductivity changes and total colour differences, reduced malondialdehyde and conjugated dienes content, and higher levels of tocopherols and carotenoids were observed for the oils formulated with rosemary powder after 1.5 hr and 3 hr of exposure to accelerated oxidation conditions using the Rancimat apparatus.

1. Introduction

Lipid oxidation is one of the major causes of vegetable oils degradation during processing and storage and is affecting a range of parameters related to food quality and consumers' acceptance including nutritional value, safety, color and development of off-flavors. Many of the oxidation products are highly reactive and may be responsible or contribute to undesirable *in vivo* effects in human health such as cancer, atherosclerosis, heart disease and allergic responses [1,2].

Rapeseed oil is a very important source of edible oil in many regions including Europe, China, United States and India. Together with soybean and palm oil, rapeseed is among the most widely consumed vegetable oils around the world. Rapeseed oil is rich in

omega-3 (linolenic acid) and omega-6 (linoleic acid) fatty acids which are considered beneficial for human health upon consumption and is therefore widely used as a cooking oil. On the other hand, due to its unsaturated nature, rapeseed oil is particularly prone to chemical changes, especially oxidation, during processing and storage [3].

Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) or tertbutylhydroquinone (TBHQ), are often used in many foods to retard lipid oxidation. Due to their low thermal stability and increasing consumer concerns about their long-term effects on human health, the food industry is in pursue of alternative options from natural sources. Recently, the use of extracts from a plethora of herbs and spices is gaining popularity as good alternatives to synthetic antioxidants for inhibiting the development of oxidative reactions in food systems and thus extending shelf-life [4,5,6]. However, the replacement of synthetic antioxidants with natural products is challenging for the food industry for numerous reasons including rising cost, consistency of production, antioxidant effectiveness and undesirable effects on organoleptic properties.

Rosemary is one of the most popular commercially available antioxidants on the market among herbs and spices due to its strong antioxidant capacity and cost effectiveness [7,8]. The major compounds in rosemary associated with the strong antioxidant properties include carnosic acid, carnosol and rosmarinic acid [9]. Rosemary extract has been adopted formally into the European regulations as new food additive suitable for use in foodstuffs and assigned E 392 as its E number [10,11].

The use of the whole plant has attracted less attention, both from an academic as well as industrial research perspective. In previous studies, we have demonstrated that rosemary powder (used directly as ground plant) showed the best potential to protect rapeseed oil from oxidation compared with black pepper, ginger, turmeric and oregano [12]. Moreover, rosemary was the most effective herb to increase the oxidative stability of rapeseed oil during frying and conferred better stability against the Rancimat test compared to the formulations containing BHT at maximum permitted levels [12].

As an extension of these studies, this research aimed to assess the potential of rosemary powder filtrate as an antioxidant of rapeseed oil. In particular, rapeseed oil was incubated with varying amounts of rosemary powder and was subsequently filtered to remove any solid residues. The oxidative stability of the filtered oil samples was determined using accelerated oxidations tests. Furthermore, the concentration effect of rosemary powder addition during the incubation stage was assessed by the quantitative determination of oxidation products as well as of natural antioxidants at different stages of the oxidation process. This study provides useful insights for the development of a reformulated food product of improved oxidative stability with possible industrial and domestic applications.

2. Materials and methods

2.1. Samples

Refined rapeseed oil was purchased from Tesco supermarket (Aberdeen, Scotland). Ground rosemary *(Rosmarinus officinalis)* was purchased from G Baldwin & Co (London, UK). The ingredients were stored in a dark cool place to protect them from oxidation. Rapeseed oil was mixed with the following concentrations of rosemary powder: 0.25%, 0.5%, 1.0% and 2.0% (w/w), and plain rapeseed oil was used as control. The samples were mixed for 24 hr on a Stuart SRT6 tube roller (Cole-Palmer, Staffordshire, UK) at room temperature and then filtered with Whatman filter paper no. 1 (Sigma-Aldrich, St Louis, MO, USA) to remove solids. Samples were immediately used for Rancimat analysis and the remaining amounts were stored at -20°C for further analysis.

2.2. *Fatty acid composition analysis*

The fatty acid composition of rapeseed oil was determined by analyzing their methyl ester derivatives with gas-liquid chromatography [13]. Analysis of the fatty acid methyl esters (FAMEs) was carried out using a gas chromatograph (HP6890, Hewlett Packard, Avondale, PA) using 50 m × 20 mm Chrompac CP7488 CP Sil-88 capillary column (film thickness 0.20 μm). Helium was used as carrier gas at a rate of 0.5 ml/min, and the split/splitless injector was used at a split ratio of 20:1. The injector and detector temperatures were 250°C. The column oven temperature was maintained at 80°C for 1 min after sample injection and was programmed to increase then at 25°C/min to 160°C where it was maintained for 3 min. Temperature was then increased to 190°C at 1°C/min and then to 230°C at 10°C/min. The temperature was maintained at 230°C for 30 min. Separation was recorded with HP GC Chemstation software (Hewlett Packard, Avondale, PA). The FAMEs were identified by comparison to previously essayed standards. Measurements were taken in duplicate. Results are expressed as % of total fatty acids.

2.3. Rancimat

A 743 Rancimat apparatus (Metrohm Ltd., Herisau, Switzerland) was used both to assess the induction period and to create temperature-controlled accelerated oxidation conditions for predefined periods of time. Oil samples (3 g) were heated at 120°C with a constant airflow of 20 L/h. Induction periods (hr) of samples were recorded as the times required for a sharp increase in water conductivity (calculated automatically by the software). For the accelerated oxidation test, the samples were placed into the Rancimat vessels and were heated at 120°C with a constant airflow of 20 L/h for 1.5 and 3 hr. In this case, the water conductivity was manually reported and the samples were removed from the vessels at the specified time intervals for further analysis. Measurements were taken in quadruplicate.

2.4. Ferric reducing power (FRP) assay

The reducing power of samples was measured as described by Siddhuraju and Becker [14] with slight modifications. 0.1 mL of sample was dissolved in 0.25 ml phosphate buffer (0.2 M, pH 6.6), mixed with 0.25 mL potassium ferricyanide (1% w/v) and

incubated at 50ºC for 20 min. Next, 0.25 mL of trichloroacetic acid (10% w/v) was added and mixed thoroughly followed by centrifugation at 650xg for 10 min. 100µl of distilled water and 25µl of ferric chloride (0.1% w/v) were added to 100µl of supernatant on a 96 well plate and mixed for 10s. The absorbance was read spectrophotometrically at 700 nm after 10 min, using a SpectraMax® 190 Absorbance Plate Reader (Molecular Devices, CA, USA). Measurements were taken in quadruplicate. The results are expressed in as optical density (OD) at 700 nm. A higher absorbance of the reaction mixture indicated greater reducing power.

2.5. Tocopherol and carotenoid content

A reverse phase HPLC method was used to quantify *beta*-carotene, *alpha*-tocopherol, *gamma*-tocopherol and *delta*-tocopherol in oils using fluorescence and visible detection according to Hess et al. [15]. Carotenoids and tocopherols were extracted from the oil phase as follows: 20 mg of oil was mixed with 280 µL H_2O and 400 µL ethanol. Each tube was vortexed for 10 seconds 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. The 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). The eluent used was acetonitrile 67.4%, tetrahydrofuran 22%, methanol containing BHT 6.8%, 1% (w/v) and ammonium acetate 3.8%. Elution flow rate was 1.1 mL/min, sample run was 30 min and injection volume was 150 µL. Measurements were determined with mixed standards containing carotenoids and tocopherols at appropriate concentrations and results were expressed in µg/mL of oil. Echinone was used as an internal standard. The measurements were taken in duplicate.

2.6. Colour analysis

The color parameters L* (lightness/darkness), a* (redness/greenness), b* (yellowness/blueness) values were measured by using a Konica Minolta CR1 10 colorimeter (Konica Minolta Solutions Ltd, Basildon, UK). Three replications were conducted. The colour changes during the accelerated oxidation test are expressed as ΔE with the colour of the fresh rapeseed oil (before the oxidation test) used as a reference sample. ΔE is the total colour change calculated from:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \textbf{(1)}$$

2.7. Thiobarbituric acid reactive substances (TBARS)

The lipid peroxidation inhibition capacity was determined by TBARS. Sample (150µl, accurately weighed) was mixed with 4 ml distilled water before addition of 1 ml of thiobarbituric acid (Sigma) (0.34% w/v in 50% v/v acetic acid). Samples were boiled for 30 min in a water bath (VWR International Ltd, Leicestershire, UK), allowed to cool and centrifuged at 2500xg for 15 min. Supernatant (200µl) was transferred to a 96 well plate and the absorbance measured at 532nm (SpectraMax190, Molecular Devices Ltd, Wokingham, UK). Concentrations of TBARs were calculated from a standard curve prepared with malonaldehyde bis (dimethyl acetal) standard (Sigma) in the range of 0.1 to 10 µM.

2.8. Determination of conjugated dienes (CD)

The oxidative stability of the emulsions was determined by monitoring the formation of conjugated dienes [16]. Oil samples were diluted 1:100 ml in ethanol and their oxidative state was determined by monitoring absorbance at 233 nm with a UV–vis Spectrometer (Spectronic Camspec Ltd., Leeds, UK). This solution was further diluted as necessary to achieve spectrophotometric readings in the target absorbance range of 0.2-0.8 at 233 nm The amount of conjugated dienes per 100 g of sample was calculated using the relative molecular mass (280 g mol^{-1}) and the molar absorptivity of linoleic acid (ε=26,000). Ethanol was used as the blank. CD levels are expressed in units of raw absorbance.

2.9. Determination of rosmarinic acid

2.10. Statistical analysis

All sampling and chemical analyses were performed in duplicate, triplicate or quatriplicate. Results of this study were expressed as a mean with standard deviations. Significant differences between mean values were evaluated by variance analysis (ANOVA). All analyses were performed using SPSS statistical software and the level of statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Characterization of rapeseed oil

The fatty acid profile, tocopherol and carotenoid content and induction period (IP) of fresh rapeseed oil are summarized in Table 1. The fatty acid and tocopherol composition is in accordance with the *Codex Alimentarius* Standards [17] and is indicative of the health benefits associated with its consumption. Rapeseed oil is characterized by low levels of saturated fatty acids (SFA) and high levels of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. The findings of the present study are in close

agreement with previously published results, which indicated oleic acid (55.2 g/100g oil) as the major fatty acid in rapeseed oil, followed by alpha linoleic acid (19.4 g/100g oil) and linoleic acid (7.8 g/100g oil) [18]. Gamma-tocopherol and xanthophyll were the predominant forms of tocopherol and carotenoid respectively in this type of oil.

Previous studies have reported similar induction periods for rapeseed oil [5]. Rapeseed oil has lower oxidative stability compared with other vegetable oils such as olive oil, red palm oil or coconut oil, which is attributed to the high content of unsaturated (mono- and poly-) fatty acids of the former [3]. Thus, there is scope to improve the oxidative stability of rapeseed oil, particularly for cooking or processing applications which are likely to deteriorate its quality and involve exposure to high temperature treatments.

3.2. Effect of rosemary addition on oxidative stability and antioxidant capacity of rapeseed oil filtrate

The Rancimat method aims to measure the IP of vegetable oils by the quantitative determination of volatile compounds formed as a result of lipid oxidation. The results showing the effect of rosemary on the oxidative stability of filtered rapeseed oil are presented in Figure 1. The IP values of oils with added rosemary were significantly higher ($P<0.05$) with increasing rosemary concentration. In agreement with these findings, previous analysis in our lab indicated that rosemary powder added to a tocopherol-stripped corn oil (unfiltered) at increasing concentrations (0%, 0.25%, 0.5%, 0.75% and 1%), also improved the IP as follows: 0.84±0.02, 1.05±0.04, 1.53±0.06, 2.06±0.05 and 2.07±0.09 hr respectively [12]. Other studies reported that 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 [19].

Changes in antioxidant capacity of the samples were measured by the Ferric Reducing Power assay (FRP) (Figure 2). The FRP value of rapeseed oil with 0.25% (w/w) of rosemary was not significantly different when compared with plain rapeseed oil (control). However, at concentrations 0.5% and above the FRP showed a significant ($P <0.05$) increase with increasing rosemary concentration.

To explore the relationship between the IP values and the antioxidant power of filtered rapeseed oil, a linear regression analysis was performed (Figure 3). A significant correlation was observed ($r = +0.969$, $p = 0.006$), confirming that a linear correlation is observed between the antioxidant properties of rosemary powder and the oxidative stability of rapeseed oil (as determined by Rancimat analysis). Linear associations of the oxidative stability of vegetable oils with antioxidant capacity have been previously reported [20,21,22]. It is known that the antioxidant capacity plays a prominent role in the protection of oil against thermal and oxidative deterioration [23].

3.3. Effect of rosemary during accelerated oxidation conditions

3.3.1. Conductivity

The Rancimat apparatus was used to create accelerated oxidation conditions (120ºC with a constant airflow of 20 L/h) and samples were removed for analysis at 1.5 hr and 3.0 hr intervals. Under the experimental conditions, fatty acids can be degraded and secondary oxidation products are formed, including low-molecular weight, volatile organic acids such as acetic and formic acid. These are transported by the airstream to a vessel containing distilled water, where conductivity is continuously measured.

The recorded conductivity values for plain rapeseed oil after 1.5 hr and 3.0 hr of incubation in accelerated oxidation conditions were 5.08 and 17 μS/cm respectively. Rosemary addition to rapeseed oil prevented the formation of oxidation products, as determined by the changes in conductivity at the specified time intervals (Figure 4). The more pronounced protective effect of rosemary was shown after 3 hr of the accelerated oxidation process: the addition of 1% and 2% w/w of rosemary was able to reduce conductivity by 80% and 75% respectively.

The reduction in conductivity measured with Rancimat when rosemary powder was incorporated can be related to the activity of antioxidant compounds. The inclusion of filtered rosemary resulted in the release of active compounds with known antioxidant properties, such as carnosic acid and carnosol, into rapeseed oil. [9]. These compounds can inhibit or retard the oxidative decomposition of rapeseed oil.

3.3.2. Total colour difference (ΔE)

The oil appearance (colour) is one of the traditional methods used for assessing its quality. The Hunter scale, which evolved during the 1960s, is based on the opponent-colour theory. This theory assumes that the human eye receptors perceive colour as the following pairs of opposite: the L value is an estimation of the luminosity and indicates dark (0-50) or white (51-100); a positive number indicates red and a negative number indicates green for the a scale, whereas the b value is positive for yellowish colors and negative for the bluish ones.

ΔE is a single value that takes into account the differences between the L, a, and b of the sample and standard. ΔE is a combination of the parameters L, a and b values and is extensively used to characterize the variation of colour in oils during processing. It was calculated from Eq. 1 (described in Materials and Methods) where L_0, a_0 and b_0 correspond to the measurements of the fresh sample and L, a and b to the measurements after 1.5 hr and 3.0 hr of exposure to accelerated oxidation conditions.

The ΔE of the oil after accelerated oxidation conditions are shown in Table 2. A remarkable reduction in ΔE was observed with rosemary addition, indicating a protective effect against the the oxidative degradation of the oil. It has been reported that rosemary extract can effectively protect oil colour changes during a deep-frying process in hazelnut [24] and sunflower oil [25].

3.3.3. Malondialdehyde (MDA) and conjugated diene (CD) content

Both, MDA and CD are formed as secondary oxidation products as a result of the oxidation of polyunsaturated fatty acids. Results from changes in MDA and CD values in rapeseed oil during the accelerated oxidation process and the effect of rosemary addition are shown in Figure 5.

All the rosemary concentrations incorporated in rapeseed oil, exhibited a significant ($P< 0.05$) decrease in MDA formation during the accelerated oxidation conditions. The more pronounced effect was observed for samples incubated with 1% and 2% (w/w) of rosemary powder. These samples showed a decrease of 81% and 86% in the MDA content respectively at the end of the incubation period (3 hr). The effect of rosemary addition on the MDA levels of lipids has been documented previously. Gallego et al. [26] stated that rosemary extract can prevent MDA formation in oil-in-water emulsions. Furthermore, Zhang et al. [27] investigated the effect of carnosinic acid on the stability of sunflower oil incubated at 60°C for a total period of 21 days; carnosinic acid was capable to inhibit the formation of MDA at all concentrations.

Following exposure to accelerated oxidation conditions for 1.5 hr, rapeseed oil samples incubated with 0.5% and 1% (w/w) rosemary showed a decrease of 45% in CD content, meanwhile higher rosemary concentrations (2%) led to a further decrease of 46% (compared to the control). After 3 hr of exposure, the addition of 1% and 2% (w/w) of rosemary powder, decreased the CD content of the rapeseed samples by 53% and 54% respectively compared with the plain oil. The protective effect of rosemary addition against CD formation in vegetable oils has been previously reported. The addition of unfiltered rosemary powder (0.5% w/w) to rapeseed oil prevented the formation of CD during deep frying [12]. CD levels increased by 29% when the plain rapeseed oil was used for frying; meanwhile, when rosemary powder was added, the corresponding increase was only 8%. Rosemary extract addition in sunflower oil decreased CD formation by 49% after 20 deep-frying cycles [25]. Furthermore, the addition of rosemary extract in soybean oil decreased CD formation by 43% following a heating process at 180ºC for 2 hr [28].

Antioxidant compounds from rosemary such as rosmarinic acid, carnosic acid and carnosol [9], could be responsible for the observed reduction in CD and MDA formation (secondary oxidation products) in rapeseed oil during accelerated oxidation conditions. Carnosic acid and carnosol contain a single aromatic ring with two −OH groups that can serve as H* donors and can also chelate pro-oxidative metals, thus preventing oxidation via two mechanisms [29]. Furthermore, carnosic acid is a reactive oxygen species quencher [30]. However, a synergistic effect from the antioxidant compounds present in rosemary is more likely since rosemary extracts demonstrate higher antioxidant activity than do the individual phenolic compounds separately [29].

3.3.4. Rosmarinic acid

3.3.5. *Tocopherol and carotenoid content analysis*

Tocopherols and carotenoids are naturally present in most vegetable oils and they may influence the oxidative stability of oils, even if present at minor quantities.

Tocopherols are the most important antioxidants present in rapeseed oil. These compounds exhibit their antioxidant activity by donating a phenolic hydrogen atom to lipid free radicals, thereby retarding the autocatalytic lipid peroxidation processes [31]. Heating treatment resulted to a significant decrease ($P<0.05$) of alpha tocopherol levels after 1.5 hr and 3 hr of exposure and the same effect was observed for gamma tocopherol after 3 hr (Figure 6). There were no significant changes in gamma tocopherol levels after 1.5 hr of heat treatment and delta tocopherol levels remained unaffected at all treatments. Alpha-tocopherol was the form that degraded faster (66% in the first 1.5 hr and non-detected after 3 hr), meanwhile delta-tocopherol seems to be the most stable of all tocopherols, under the specified oxidation conditions. In agreement with this study, previous reports denote that tocopherol content decreases during heating or frying [32,33,34,35] and alpha-tocopherol was shown to be the least stable form [35,36,37].

Alpha-tocopherol levels remained unaffected ($P>0.05$) from the heating process (1.5 hr) regardless the rosemary concentration (0.25%-2.0%). After 3 hr exposure, 0.25% of rosemary powder added to the oil was not suffice to prevent the total degradation; at 0.5% concentration, the alpha-tocopherol levels were significantly higher, and at concentrations above 1% the alpha-tocopherol was preserved at a level similar to the one detected in the fresh (unheated) oil (Figure 6A). With respect to gamma-tocopherol levels, the addition of rosemary at concentrations of 0.5% or above prevented the degradation to not significant levels ($P>0.05$) after 3 hr of heating (Figure 6B). It has been previously reported that the inclusion of a rosemary extract has a protective effect against tocopherol degradation [32,38,39]. Specifically, the incorporation of rosemary extract to soybean oil preserved approximately 50% of the total tocopherols when heated at 180ºC for 18 hr [32]. Beddows et al. [39] observed that rosemary, thyme, turmeric, sage, oregano and cumin extracts preserved alpha-tocopherol in sunflower oil when subjected to heating at temperatures ranging from 85ºC to 105ºC in a Rancimat apparatus.

Carotenoids are also known to protect lipids from free radical auto-oxidation by reacting with peroxyl radicals, inhibiting their propagation and promoting termination of the oxidation chain reaction [31,40]. The heating treatment significantly decreased ($P<0.05$) both xanthophyll and beta carotene content after 1.5 hr (46% and 47% respectively) and 3 hr (88% for xanthophyll and non-detected for beta carotene) of exposure (Figure 7). The present findings are in accordance with previous studies [40], which reported a thermal degradation of approximately 50% for beta carotene in rapeseed oil when heated at 110ºC for 1 hr.

On one hand, the addition of rosemary seems to have a protective but non-significant ($P>0.05$) effect against the degradation of xanthophyll after exposure to oxidation conditions for 1.5 hr. On the other hand, under more severe oxidation conditions (3.0 hr) the addition of rosemary to rapeseed oil at concentrations above 0.5% (w/w), has a significant beneficial effect on the stability of xanthophyll (Figure 7A). Similarly, rosemary powder protected beta carotene from degradation after 1.5 hr of exposure at any concentration ($P<0.05$), whereas this effect was significant at concentrations >0.5% (w/w) after 3 hr of exposure (Figure 7B).

Phenolic diterpenes (carnosol, carnosic acid) are the main antioxidant compounds found in rosemary plant. These compounds exert their antioxidant activity through different mechanisms [30]. The findings of the present study suggest that compounds present in rosemary can prevent lipid oxidation of vegetable oils by acting as secondary antioxidants and stabilizing indigenous antioxidants such as tocopherols and carotenoids. Moreover, it has been shown that certain polyphenols exhibit the ability to repair alpha-tocopherol by reducing the alpha tocopheroxyl radical [29].

4. Conclusions

The effectiveness of rosemary powder at different concentrations to protect rapeseed oil filtrate from oxidation was evaluated in this study. The addition of rosemary powder improved the induction period of the filtrate. An increased oxidative stability of rapeseed oil incubated with rosemary is indicated by lower conductivity changes, total colour differences, and reduced malondialdehyde and conjugated dienes content after exposure for 1.5 hr and 3 hr to accelerated oxidation conditions in Rancimat apparatus. Moreover, the formulated oils showed higher antioxidant capacity and a protection in the decimation of tocopherol and carotenoid content during the accelerated oxidation process, which could at least partially explain the observed oxidative stability. The beneficial effects of rosemary powder on the above parameters were more profound at concentrations above 0.5% (w/w). This study suggests that the incubation of rapeseed oil with rosemary powder offers significant benefits to the nutritional value and safety of the vegetable oil, even if the solid residue is removed. This could lead to alternative reformulation strategies for oil manufacturers, with potential domestic or industrial applications.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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TABLES

Table 1

Rapeseed oil characteristics.

Main fatty acids (% of total fatty acids)		**Tocopherols (μg/mL)**		**Carotenoids (μg/mL)**		**IP (hr)**
Palmitic acid (C16:0)	4.63±0.03	Alpha-tocopherol	132±1.41	Xanthophyll	13.0±0.27	3.46±0.17
Stearic acid (C18:0)	1.61±0.02	Gamma-tocopherol	235±6.79	Beta-carotene	1.61±0.22	
Oleic acid (C18:1n9)	61.4±0.01	Delta-tocopherol	4.71±0.32			
Alpha-linoleic acid (C18:2n6)	19.2±0.00					
Linolenic acid (C18:3n3)	11.3±0.00					

Results are expressed as mean ± SD (standard deviation).

Table 2

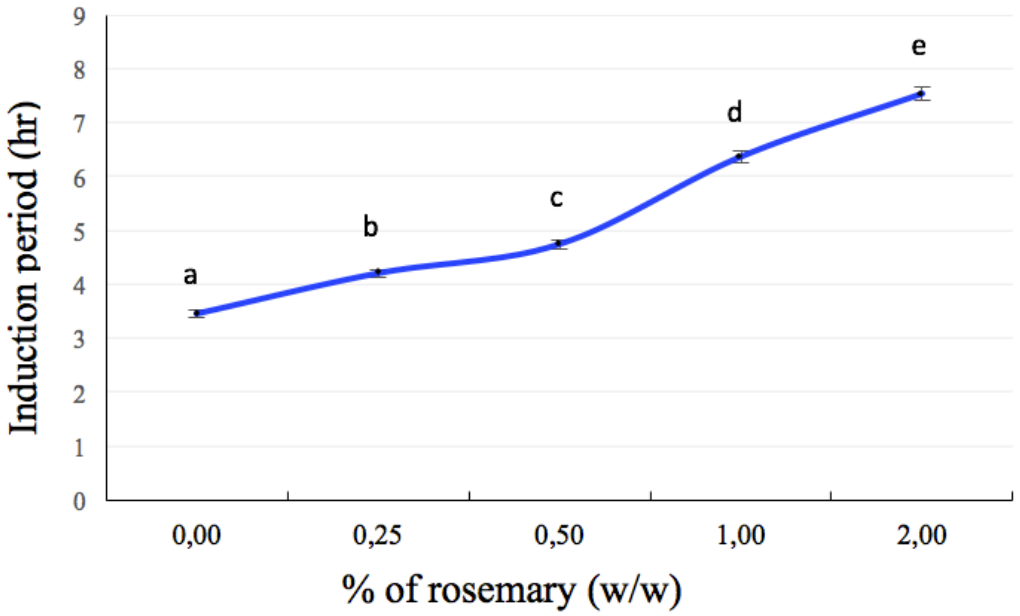
Total colour differences (ΔE) of the oils after accelerated oxidation conditions.

	ΔE	
% of rosemary (w/w)	**1.5 hr**	**3 hr**
0	5.99±0.08[a]	14.7±0.12[a]
0.25	2.78±0.08[b]	8.69±0.26[b]
0.5	2.42±0.12[c]	7.66±0.28[c]
1.0	0.93±0.11[d]	1.60±0.09[d]
2.0	0.84±0.05[d]	2.79±0.12[e]

Results are expressed as mean ± SD (standard deviation). Means followed by the same lower case letters are not significantly (P>0.05) different between treatments (columns).

FIGURES

Figure 1.



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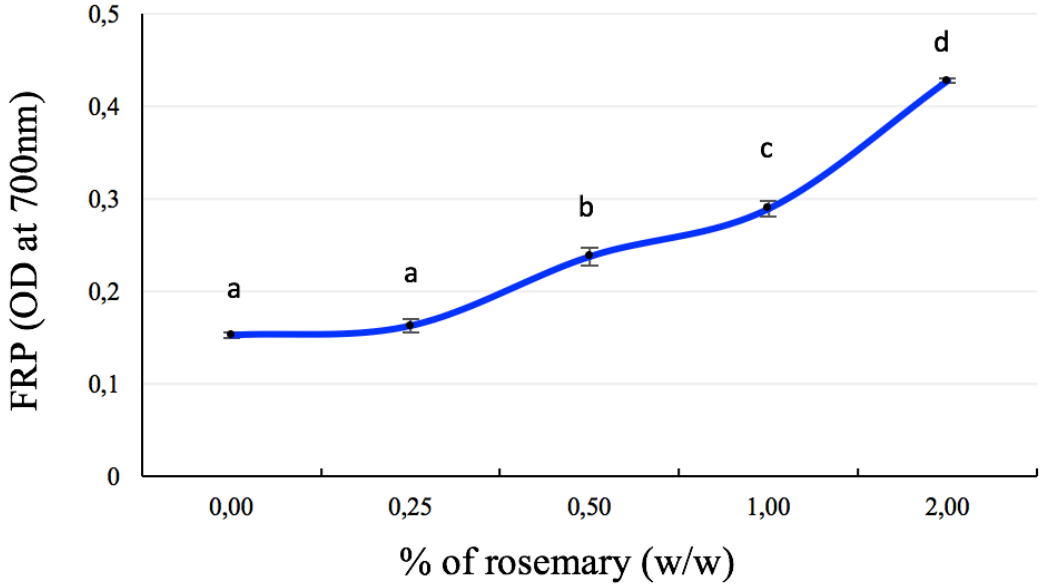

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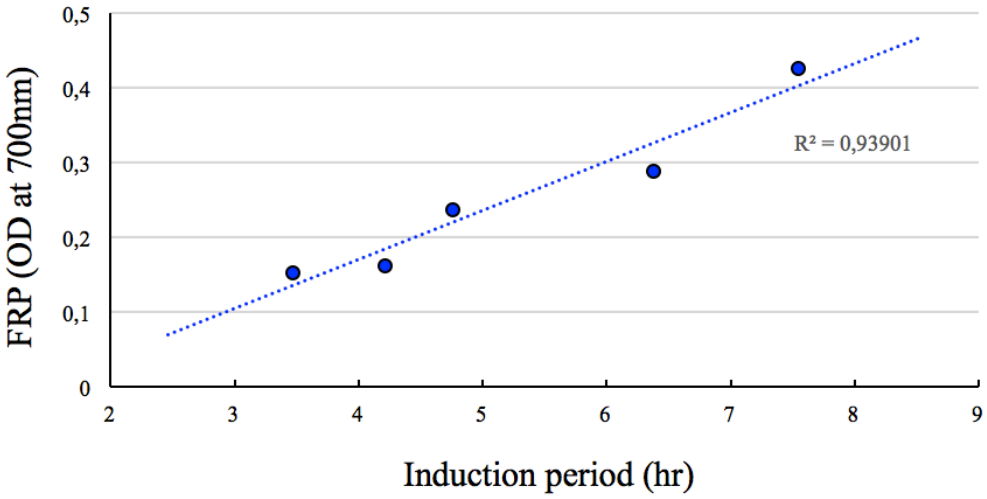

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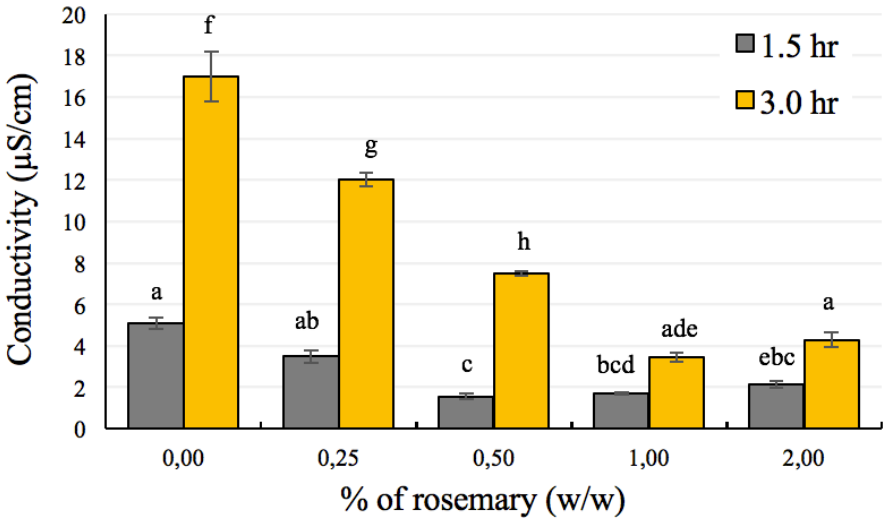

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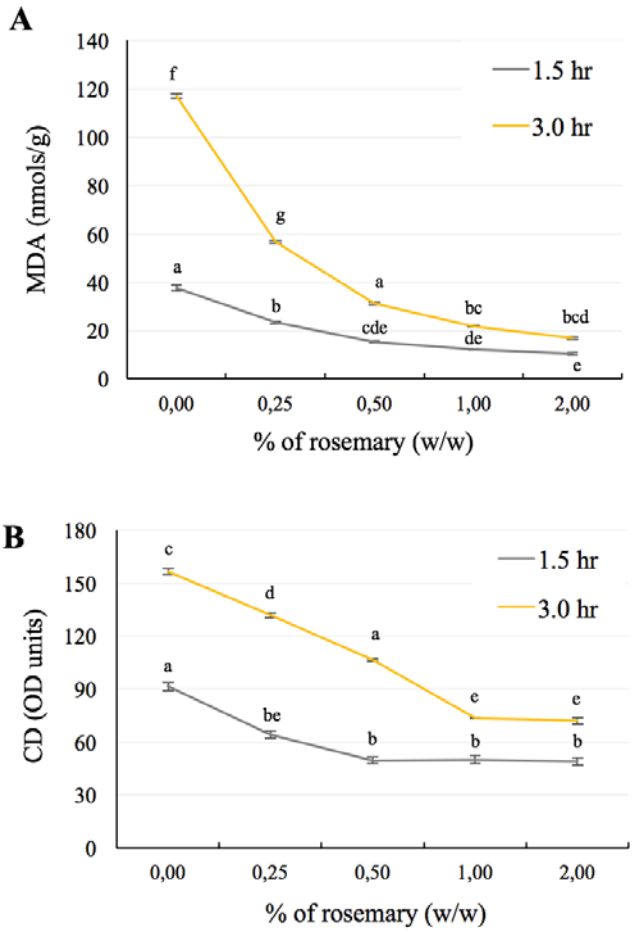
A
MDA (nmols/g)
140
120
100
80
60
40
20
0
1.5 hr
3.0 hr
f
g
a
a
b
cde
bc
de
bcd
e
0,00
0,25
0,50
1,00
2,00
% of rosemary (w/w)
B
CD (OD units)
180
150
120
90
60
30
0
1.5 hr
3.0 hr
c
d
a
a
be
b
e
b
e
b
0,00
0,25
0,50
1,00
2,00
% of rosemary (w/w)

Figure 6.

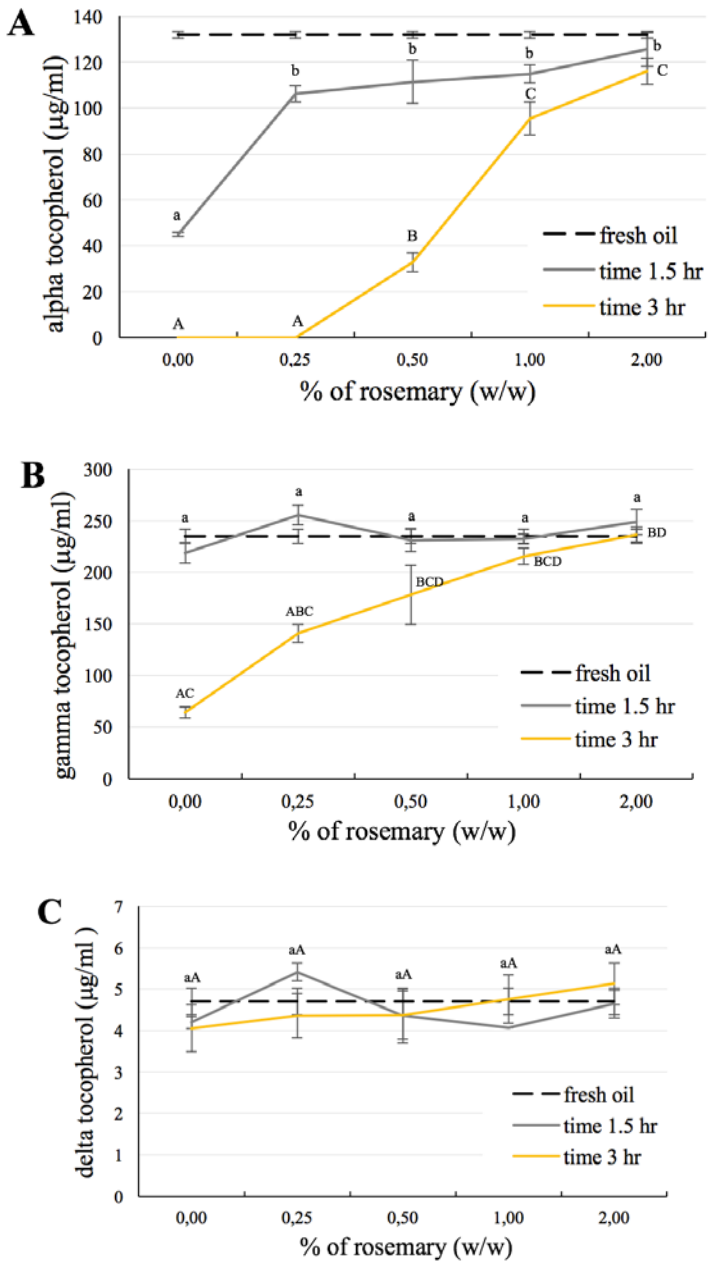
A
alpha tocopherol (µg/ml)
140
120
100
80
60
40
20
0
a
b
b
b
b
A
A
B
C
C
fresh oil
time 1.5 hr
time 3 hr
0,00
0,25
0,50
1,00
2,00
% of rosemary (w/w)
B
gamma tocopherol (µg/ml)
300
250
200
150
100
50
0
a
a
a
a
a
BD
BCD
BCD
ABC
AC
fresh oil
time 1.5 hr
time 3 hr
0,00
0,25
0,50
1,00
2,00
% of rosemary (w/w)
C
delta tocopherol (µg/ml)
7
6
5
4
3
2
1
0
aA
aA
aA
aA
aA
fresh oil
time 1.5 hr
time 3 hr
0,00
0,25
0,50
1,00
2,00
% of rosemary (w/w)

Figure 7.

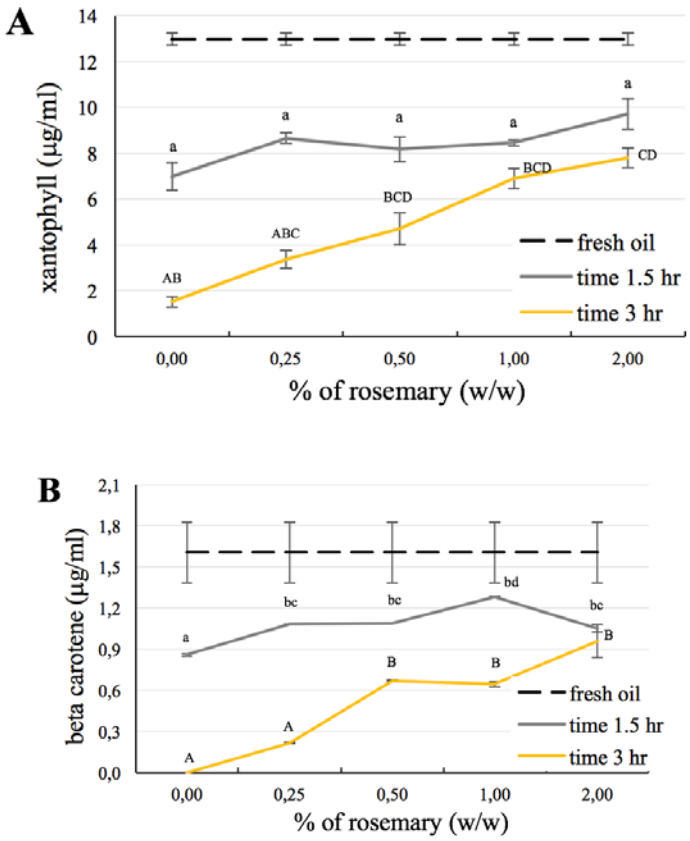

FIGURE LEGENDS

Fig. 1. Effect of rosemary concentration on the oxidative stability (IP) of filtered rapeseed oil measured by Rancimat. Results are presented as mean ± SD. Values with different lower case letter are significantly different (P <0.05).

Fig. 2. Effect of rosemary concentration on rapeseed oil antioxidant capacity measured by FRP. Results are presented as mean ± SD. Values with different lower case letter are significantly different (P <0.05). FRP; ferric reducing power, OD; optical density.

Fig 3. Linear regression analysis between FRP value and IP of rapeseed oil samples.

Fig 4. Conductivity of samples measured in Rancimat apparatus during accelerated oxidation conditions at 1.5 hr and 3 hr. Results are presented as mean ± SD. Values with different lower case letter are significantly different (P <0.05).

Fig. 5. Malondialdehyde (MDA) (**A**) and conjugated diene (CD) (**B**) content after exposure to accelerated oxidation conditions in Rancimat appararus. Results are presented as mean ± SD. Values with different lower case letter are significantly different ($P < 0.05$).

Fig. 6. Effect of rosemary concentration in rapeseed oil on alpha tocopherol (**A**), gamma tocopherol (**B**) and delta tocopherol (**C**) levels during accelerated oxidation conditions in Rancimat apparatus. Results are presented as mean ± SD. Values with different lower (for values at 1.5 hr) or upper (for values at 3 hr) case letter are significantly different ($P<0.05$).

Fig. 7. Effect of rosemary concentration in rapeseed oil on xanthophyll (**A**) and beta carotene (**B**) levels during accelerated oxidation conditions in Rancimat apparatus. Results are presented as mean ± SD. Values with different lower (for values at 1.5 hr) or upper (for values at 3 hr) case letter are significantly different ($P < 0.05$).