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Potential use of essential oil extraction residue from myrtle leaves for the stabilization of corn oil

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Abstract
This study was designed to investigate the effect of essential oil (EO) extraction residue from myrtle leaves on the oxidative stability of corn oil. To this end, the oxidative stability of corn oil was evaluated under accelerated oxidation conditions at 60 °C over time (21 days) by monitoring acidity, the peroxide index and the L*, a* and b* color parameter values. The findings demonstrated that the incorporation of myrtle leaves control and residue extracts led to a notable reduction in the values of the parameters assessed during the storage period ($p < 0.05$). Moreover, the results showed that the extraction of EO by steam distillation significantly enhanced the effect of the ethanolic extract of myrtle leaves on the stability of corn oil ($p < 0.05$). Indeed, oil sample added with myrtle leaves residue extract exhibited the lowest acidity value (0.16 ± 0.02%), the lowest peroxide index (10.67 ± 0.58 meq O2/kg), and the most stable L*, a*, and b* color parameter values (95.42 ± 0.05, -7.88 ± 0.10, 29.52 ± 0.18, respectively) compared to the oil sample added with the addition of the myrtle control extract at the end of the storage period. These results suggest the potential use of myrtle leaves EO extraction residues to improve the oxidative stability of vegetable oils in the food industry, thus contributing to environmentally and economically sustainable practices through the valorization of aromatic and medicinal plant residues from EO extraction industries.

Keywords: Myrtle, leaves, residue, essential oil extraction, vegetable oil, antioxidant

Introduction
Nowadays, vegetable oils are among the most expanding products in the functional food market [1]. These oils are derived from seeds, nuts or fruits of various raw materials such as palm, soybean, rapeseed, sunflower, coconut, olive, linseed, castor, sesame, peanut, cotton and corn [2]. Their consumption has increased worldwide due to their perceived potential health effects [3].
Nevertheless, all vegetable oils are susceptible to oxidation, a chemical reaction that can occur at any stage of the supply chain, from production to distribution, if not adequately protected [4].
In addition, the oxidation of vegetable oils may occur during a wide variety of food preparation processes, including frying, which is used in the production of a diverse range of foods [5]. The frying process, usually conducted in the presence of moisture and air, involves the application of temperatures reaching up to approximately 180 °C to foods that are fully immersed in heated oil, which results in the thermal oxidative degradation of the oil [6]. Thus, the fatty acid composition of the cooking oil may be affected, leading to physical alterations such as viscosity and color changes [6]. Furthermore, the synthesis of a range of monomeric compounds and polymers may occur [7]. These include hydrocarbons, alcohols, aldehydes, esters, lactones and ketones [5]. Such alterations can have a detrimental impact on the organoleptic and nutritional attributes of fried foods [8], particularly when the oil is subjected to repeat frying [6].
The oxidation phenomenon can be attributed to a number of factors, including the fatty acid composition of the oil and the quantities of mono- and diacylglycerol, heating, exposure to light, oxygen levels, the presence of pro-oxidants, such as metals and colorants [4, 9], and it is responsible for the rancidity and deterioration in the organoleptic and nutritional quality of oils, rendering them less acceptable to consumers [2, 4]. Furthermore, this process results in the formation of toxic compounds and oxidized polymers, which have the potential to be detrimental to human health [10]. Consequently, the oxidative stability of vegetable oil serves as an essential indicator of its quality, safety and shelf life [10].
Actually, numerous approaches have been used to enhance oil stability, including the addition of antioxidants [11], which inhibit the oxidation process by interacting primarily with the lipid free radical and forming a more stable radical that is more resistant to the oxidizing action [12]. Synthetic antioxidants are commonly used in the vegetable oil industry to prevent oxidation reactions, although their safety is a cause for concern because of their potential toxicity to consumers [13].
Therefore, natural antioxidants have been suggested as a substitute for synthetic antioxidants in protecting oils from oxidative degradation [1]. Typically, these antioxidants are derived from different plant sources [9], including medicinal and aromatic plants, which have been well demonstrated to contain various bioactive compounds, particularly phenols [4], that have been proven to have great antioxidant power and have shown promising efficiency in inhibiting free radical-induced effects [14].
In the last few years, there has been an increasing interest in the valorization of residues released into the ecosystem by agro-industries. The aim was to address the environmental pollution and the economic difficulties associated with the considerable generated quantities of these materials [15]. Indeed, it has been demonstrated that this waste can be used as a sustainable source of natural antioxidants, at minimal cost [16].
Essential oil extraction from medicinal and aromatic plants is one of the most relevant processes in the agri-food field, generating significant amounts of residues with high levels of phenolic antioxidative substances, where several plant matrices are used [17].

Myrtus communis L. (*M. communis*) in Latin, myrtle in English is one of the most abundant medicinal and aromatic plants in the Mediterranean region that is being utilized for essential oil extraction [18]. Many scientists have investigated the composition and biological potential of the essential oil and extracts of different parts of the myrtle plant [19, 20, 21]. It was found that the leaves extracts are rich in phenolic compounds from different groups with high antioxidant potency [22, 23]. In addition, certain researchers have studied the effect of enriching vegetable oils with myrtle phenolic leaves extracts on their antioxidant activity and oxidative stability [22, 24]. Nevertheless, no studies have been carried out to establish the potential use of myrtle essential oil extraction residues to delay the oxidation of vegetable oils.
This is a pioneering study that was conducted to investigate the valorization of essential oil extraction residues from plants as an oxidative stabilization agent for vegetable oils. For this purpose, the effect of incorporating myrtle leaves ethanolic extract, obtained from the distillation residue, on the oxidative stability of corn oil was determined by monitoring the evolution of acidity, peroxide index, and color parameters of the oxidized oil under an oven test. A comparative study was performed with raw myrtle leaves.

Materials and methods

Chemicals

The chemical reagents and solvents employed in the study include sodium hydroxide, phenolphthalein, potassium iodide, sodium thiosulfate, trichloroacetic acid, which were obtained from Sigma-Aldrich Chemie (Steinheim, Germany), and methanol, ethanol, diethyl ether, chloroform and acetic acid, which were obtained from Merck (Darmstadt, Germany).

Preparation of plant material

Myrtle leaves utilized in this study were collected in April 2022 in north-western Tunisia (Ain Drahem, Jendouba). The leaves were identified according to the Tunisian flora and a reference sample was deposited in the Tunisian Higher School of Food Industries (ESIAT) herbarium [25]. Subsequently, the plant leaves were subjected to microwave drying until a constant weight was attained, in accordance with the methodology outlined by Snoussi *et al.* [22]. After drying process, two batches of samples were prepared. The first was kept as a reference for comparison purposes, while the second underwent steam distillation until no significant increase in essential EO levels was observed. The residual leaves from the EO extraction were then dried under the same conditions as the reference and stored for future use.

Preparation of myrtle leaves ethanolic extracts

Myrtle leaves extracts were prepared by stirred maceration at room temperature. During 72 h, 10 g of plant material was macerated in 80% ethanolic aqueous solution, with solvent changes every 24 h (3 x 100 mL). The resulting extracts were mixed, filtered, and concentrated using a rotavapor [22].

Evaluation of the effect of myrtle leaves extracts on the oxidation stability of corn oil

The effect of myrtle leaves extracts on the oxidative stability of corn oil was studied using the Schaal oven essay, by monitoring the evolution of acidity, peroxide index and color parameters during 21 days of storage at 60 °C, after the addition of the ethanolic extract at 100 ppm to oil samples. A control assay was conducted under the same conditions without any antioxidant addition [22].

Acidity measurement

Acid-base titration was utilized to conduct the acidity measurement [26]. A mixture of ethyl alcohol and diethyl ether was prepared in a ratio of 1/2 (*v/v*), and 3-5 g of fat was added to 50 mL of the mixture, which was previously neutralized. Subsequently, 2-3 drops of phenolphthalein were added followed by titration with the potassium hydroxide solution (0.1 N). Acidity values (A) were obtained as follows:

$$A\,\% = 0.5 \times V$$

Where, V: Volume of basic solution (mL).

Peroxide value index measurement

The peroxide value was estimated using an iodometric method that was validated by the organization of international standards in 2017 [27]. A saturated potassium iodide solution of 1 mL volume was introduced to 1-3 g of the oil previously dissolved in 20 mL of a 2/3 (*v/v*) solution of chloroform and acetic acid. The oil-containing mixture was incubated away from light for 5 min, followed by titration with 0.01 N sodium thiosulfate solution until a pale-yellow color appeared. After adding 0.1 ml of starch solution, the sodium thiosulphate titration continued until complete discoloration. A parallel oil-free blank test was conducted under identical conditions. The peroxide value (PV) given in milli-equivalents of oxygen per kilogram of fat is calculated from the following formula:

$$PV\ (\text{meq}\frac{O_2}{\text{kg}}) = ((V - V1)/M) \times 10$$

Where, V: volume of thiosulfate used for the sample (mL); V_1: volume of thiosulfate used for blank test (mL); M: mass of the test portion (g).

Color parameters measurement

The color parameters a*, b* and L were measured using a Lovibond PFX195 tintometer (Salisbury, UK). According to the International Commission on Lighting, the value of the parameter L* that measures the lightness of the sample varies between 0 for pure black and 100 for pure white, while the values of the parameters a* and b* are between -60 and 60, where the positive values represent the reddish and yellowish colors, respectively, and the negative values refer to the greenish and bluish colors, respectively [28].

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics software, version 22 (IBM Corp, Armonk, NY, USA) with an ANOVA test at 95% confidence level ($p<0.05$) to indicate significant differences between samples. The data presented in this study are expressed as means in triplicate ± standard deviation.

Results

The oxidative stability of corn oil was evaluated under accelerated oxidation conditions at 60 °C for 21 days. The findings revealed that the addition of control and residual myrtle leaves extracts at 100 ppm had a significant influence on total acidity, peroxide value, L*, a* and b* color parameters ($p < 0.05$).

Effect of myrtle leaves extracts on corn oil Acidity

The acidity values of the corn oil samples are presented in Fig. 1. The results demonstrated that the acidity values of the corn oil control sample were significantly lower in comparison with the corn oil sample added with myrtle leaves extracts ($p < 0.05$). Indeed, after 21 days of storage at 60 °C, a significant increase in acidity values was observed for corn oil control (O. Control), from 0.05 ± 0.02% to 0.74 ± 0.02% ($p < 0.05$). However, the increase was less significant for corn oil added with myrtle leaves control extract at 100 ppm (O. EE_MC), reaching 0.22 ± 0.03% ($p < 0.05$), and for corn oil added with myrtle leaves residue extract at 100 ppm (O. EE_MR), which presents more stable values, reaching 0.16 ± 0.02% ($p < 0.05$).

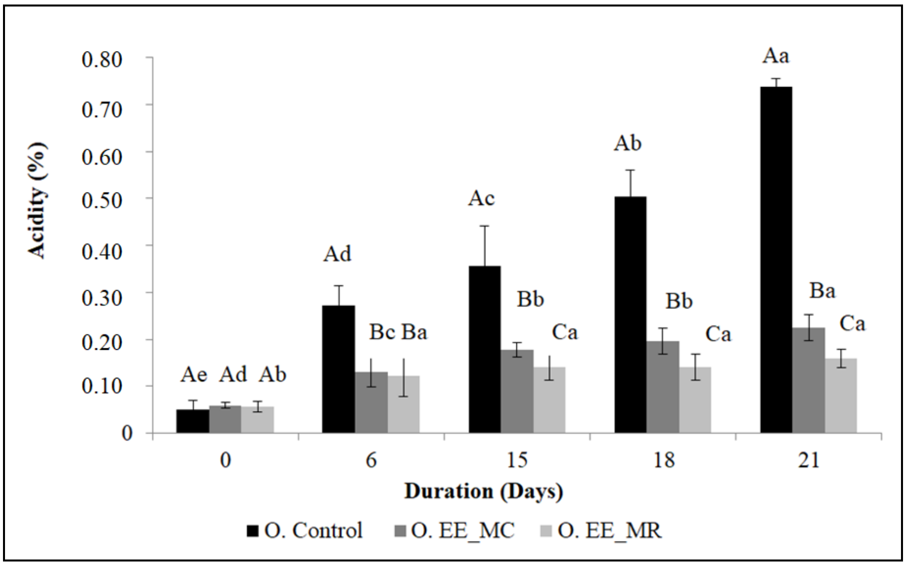

Fig 1: Acidity evolution (%) for corn oil samples added with myrtle leaves extracts at 100 ppm (O. Control: oil corn without extract; O. EE_MC: oil corn with ethanolic extract of myrtle control leaves; O. EE_MR: oil corn with ethanolic extract of myrtle leaves residue; mean values referring to the same day of analysis and followed by different superscript capital letters were found to be significantly different. ($p<0.05$); mean values referring to the same oil simple and followed by different lower-case superscript letters are significantly different ($p<0.05$).

Effect of myrtle leaves extracts on corn oil peroxide value

The resulting peroxide values are presented in Fig. 2. Following a three-week storage period at 60 °C, a significant increase in peroxide values was observed, reaching a maximum value of 20.56±1.92 meq O_2/kg for the O. Control ($p<0.05$). The increase in peroxide value for O. EE_MC was less pronounced, reaching 12.78±1.39 meq O_2/kg ($p<0.05$), while for O. EE_MR, it did not exceed 10.67±0.58 meq O_2/kg ($p<0.05$) at the end of the storage period, indicating more effective protection against the oxidation process.

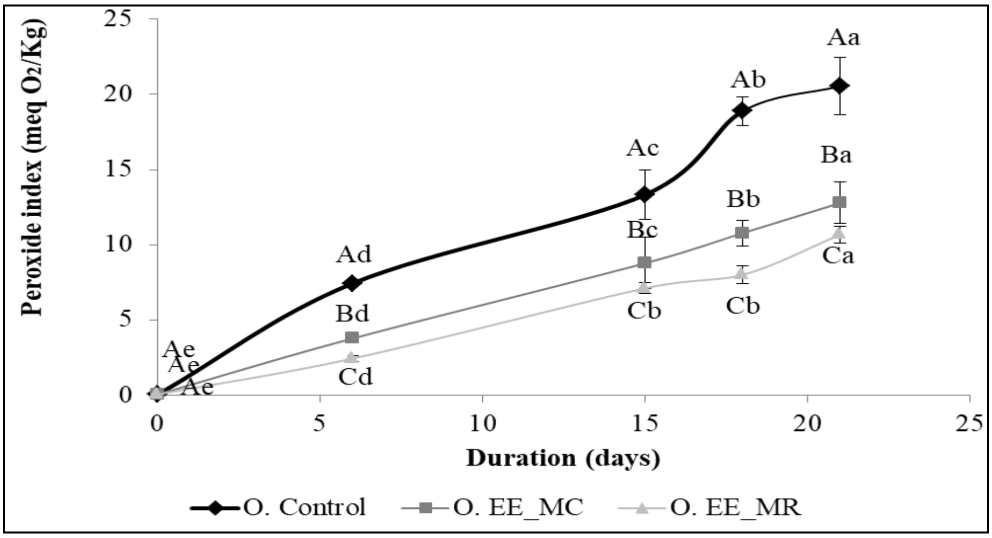

Fig 2: Peroxide values (meq O_2/kg) for corn oil samples added with myrtle leaves extracts at 100 ppm (O. Control: oil corn without extract; O. EE_MC: oil corn with ethanolic extract of myrtle control leaves; O. EE_MR: oil corn with ethanolic extract of myrtle leaves residue; mean values referring to the same day of analysis and followed by different upper-case superscript letters, are found to be significantly different. ($p<0.05$); mean values referring to the same oil simple and followed by different lower-case superscript letters are significantly different ($p<0.05$).

Effect of myrtle leaves extracts on corn oil color parameters

Color parameters values L*, a*, and b* of different oil samples during storage for 21 days at 60 °C are presented in Table 1.

Table 1: Color parameters evolution of corn oil samples during storage

Color parameter	Sample	Duration (days)				
		0	6	15	18	21
L*	O. Control	98.88±0.06^{Aa}	94.50±0.03^{Cb}	92.55±0.43^{Cc}	89.81±0.11^{Cd}	88.76±0.02^{Ce}
	O. EE_MC	98.15±0.13^{Aa}	95.63±0.12^{Bb}	95.14±0.01^{Bb}	94.92±0.01^{Bc}	94.83±0.14^{Bc}
	O. EE_MR	97.97±0.01^{Ba}	97.17±0.02^{Aa}	96.93±0.01^{Ab}	96.20±0.04^{Ab}	95.42±0.05^{Ac}
a*	O. Control	-6.09±0.01^{Aa}	-7.40±0.18^{Ab}	-8.16±0.04^{Bc}	-8.36±0.2^{Bc}	-8.67±0.15^{Bc}
	O. EE_MC	-6.08±0.01^{Aa}	-7.32±0.12^{Ab}	-7.46±0.26^{Ab}	-7.81±0.15^{Ac}	-7.88±0.10^{Ac}
	O. EE_MR	-6.06±0.11^{Aa}	-7.15±0.13^{Ab}	-7.37±0.12^{Ab}	-7.69±0,15^{Ab}	-7.88±0.10^{Ac}
b*	O. Control	22.43±0.1^{Ae}	28.74±0.03^{Ad}	29.39±0.15^{Ac}	31.25±0.08^{Ab}	39.65±0.03^{Aa}
	O. EE_MC	21.75±0.14^{Be}	25.81±0.03^{Bd}	28.84±0.44^{Bc}	30.91±0.02^{Bb}	35.55±0.01^{Ba}
	O. EE_MR	21.08±0.16^{Ce}	21.73±0.2^{Cd}	25.87±0.09^{Cc}	29.24±0.15^{Cb}	29.52±0.18^{Ca}

O. Control: oil corn without extract.
O. EE_MC: oil corn with ethanolic extract of myrtle control leaves.
O. EE_MR: oil corn with ethanolic extract of myrtle leaves residue.
Mean values in the same column followed by different upper-case superscript letters are significantly different (p<0.05).
Mean values in the same line followed by different lower-case superscript letters are significantly different (p<0.05).

The results showed a significant decrease in L* values for O. Control, from 98.88±0.06 to 88.76±0.02 (p<0.05). In contrast, the addition of myrtle leaves control extract at 100 ppm (EE_MC) to the corn oil resulted in a relatively minor decrease in L* values, from 97.97±0.01 to 95.42±0.05. Moreover, it was observed that the addition of myrtle leaves residue extract at 100 ppm (EE_MR) to corn oil resulted in a reduction in L* values, although to a lesser extent than that observed with the addition of the leaves control extract.

Furthermore, the findings showed a significant decrease in a* values from -6.09±0.01 to -8.67±0.15 for O. Control (p<0.05), while, the a* values of corn oil added with myrtle leaves extracts (100 ppm) showed a comparatively minor reduction effect, reaching -7.88±0.10 at the end of the storage period, with no significant difference observed between the values of O. EE_MC and O. EE_MR.

On the other hand, there was a significant increase in the b* value for all corn oils simples (p < 0.05). Indeed, O. Control showed the highest b* values during storage, increasing from 22.43±0.1 to 39.65±0.03. In comparison, corn oil added with myrtle leaves extracts (100 ppm) showed a less significant increase in b* values, with O. EE_MC showing a higher value (35.55±0.01) than O. EE_MR (29.52±0.18) at the end of the storage period.

Discussion

In this study, the potential use of myrtle leaves essential oil extraction residue for the stabilization of corn oil was evaluated. This was accomplished by monitoring the evolution of acidity, peroxide index and color parameters in accelerated oxidation conditions at 60 °C for 21 days. Raw myrtle leaves were employed as a comparative standard. The analysis indicated that the incorporation of EE_MC and EE_MR has resulted in an improvement in the stability of corn oil under oxidative conditions.

The results of the acidity measurement showed that on day 0, all oil samples had values within the 0.06% range. This suggests that the incorporation of the myrtle leaves extracts in the oil samples did not affect their acidity values. In fact, according to ANVISA standard the acidity values for vegetable oil must remain below 0.6 mg KOH/g oil [29]. Moreover, the results showed that the acidity values of the samples increased during storage at 60 °C, which is in line with several previous studies [22, 30, 31, 32]. Furthermore, it was observed that the addition of EE_MC significantly improved the stability of the acidity levels of corn oil during the storage period.

Indeed, in a previous study on the effect of myrtle leaf extract on the oxidative stability of a vegetable oil, Snoussi *et al*. [22] found that the acidity of all soybean oil samples increased significantly during storage. However, the increase in acidity was less pronounced in the oil samples added with myrtle leaf extract. The increase was limited to 1.4-fold for the oil added with microwave-dried myrtle leaf extract compared to the oil control, which increased 4-fold.

In addition, Alves *et al* [32] also investigated the oxidative stability of corn oil enriched with hydroalcoholic extract of *Pluchea quitoc*. They found that the control oil had the highest acidity values, showing an increase in all measured values over the storage period at 62 °C, reaching the highest values of 14.26 mg KOH/g on the 28th day. However, the samples with the extract showed a less accelerated evolution of the acidity values and presented low levels compared to the oils without extracts, suggesting that the extracts contributed to stabilizing the acidification phenomenon. Besides, they showed that the highest concentration of extract added to the oils (5%) gave samples with lower acidity than corn oil with BHT standard. In fact, at the end of the analysis, the corn oil with 5% *Pluchea quitoc* extract presented an acid value of 2.72 mg KOH/g, whereas the acid value of the oil with the BHT standard was 4.75 mg KOH/g.

The results of the peroxide index measurement indicate that the incorporation of EE_MC has significantly enhanced the peroxide value stability of corn oil approximately two times and the peroxide value was limited to 12.78±1.39 meq O_2/kg for O. EE_MC compared to the peroxide value of the O. Control, which was increased to 20.56±1.92 meq O_2/kg at the end of the storage period.

The aforementioned study by Snoussi *et al* [22] showed that myrtle leaf extract had a significant effect on the stability of the peroxide value of soybean oil. In fact, the peroxide value of the control oil was the highest (200 meq O_2/kg), whereas the oil enriched with myrtle leaf extract dried in the microwave had a peroxide value 7 times lower than that of the control oil. These results of the study demonstrated the antioxidant effect of myrtle leaf extract on vegetable oil and suggested the possible use of myrtle leaf extract as a substitute for synthetic antioxidants.

Baştürk *et al*. [33] evaluated the oxidative stability of six corn oil samples and confirmed that the oxidative stability of corn oil was more important with incorporation of sumac, thyme and mint extracts. The peroxide value of control sample reached to 39.31 meq O2/kg, which was the highest peroxide value after 6 weeks of storage at 60 °C. Considering the peroxide values of the samples, the inhibitory effects of plant extracts followed the order of sumac extract >mint extract> thyme extract, in the descending order.

Moreover, Yang *et al*. [34] conducted a study to assess the effect of rosemary extract on enhancing the oxidative stability of various oils utilizing the oven test. The researchers observed that incorporating rosemary extract into oils increased both antioxidant activity and total phenolic content while decreasing the peroxide value. Thus, their findings suggest that using rosemary extract as a substitute for synthetic antioxidants is possible.

On the other hand, El-Malah *et al*. [35] investigated the effect of adding natural antioxidant extracts like tomato water extract, tomato ethyl lactate extract and grape ethanolic extract on the oxidative stability changes during heating of soybean oil, sunflower oil and their mixture at 60 °C for 16 days. They showed that the lower the change in peroxide values the higher the stability of oils and that the maximum of peroxide values were given after 16 days of heating duration. Peroxide values change of treated oils was found to be lower than that of control sample for all concentrations (200 ppm, 400 ppm, 600 ppm). As a result, thermal treatment of oil samples leads to important increase in peroxide values due to primary oxidation but this effect was significantly decreased when natural antioxidant extracts are incorporated to oils and improved their oxidation stability.

In general, peroxide values may be affected by the structure and reactivity of peroxides as well as reaction temperature and duration of storage. The iodometric method for determination of PV is applicable to all normal fats and oils, but it is highly empirical and any variation in procedure may affect the results [36].

The results of the color parameters measurement demonstrated a significant decrease in L* and a* values and a significant increase in b* values ($p<0.05$), indicating that the oil samples exhibited a progressive darkening with a greenish-yellow color over time. Furthermore, the addition of EE_MC has been observed to enhance the stability of the color parameters in corn oil.

The findings of Snoussi *et al*. [22] indicated that the values of the parameter L* of the various soybean oil samples decreased during storage, as a result of oxidation phenomena leading to browning of the oil samples. In addition, it was observed that L* was reduced by 8 units for the oil control, whereas the reduction was only 2 units for the oil added with the extract of myrtle leaves dried in a microwave oven. It has been suggested that the oil is maintained in a more stable state due to the presence of antioxidants that react with and stabilize free radicals.

For all the parameters measured, it was found that the addition of EE_MR was more effective for the oxidative stability of corn oil, with the O. EE_MR presenting the most stable values during the storage period (21 days) at 60 °C. This indicates that the process of extracting the essential oil has enhanced the antioxidant capacity of the myrtle leaves ethanolic extract.

In fact, it is widely accepted that the antioxidant capacity of medicinal plants is dependent on the concentration of bioactive phenolic compounds, which include phenolic acids, flavonoids, anthocyanins and tannins [37]. The findings of a multitude of research studies indicate that residues resulting from EO extraction are abundant in these compounds [38, 39, 40].

Our previous study [41], which was designed to investigate the chemical composition and biological properties of EO extraction by-products from myrtle leaves, demonstrated that the application of heat during steam distillation for EO extraction influences the levels of phenolic compounds. In particular, the by-product ethanolic extract exhibited a higher concentration of total phenolics, flavonoids and proanthocyanidins than the raw leaf ethanolic extract. The increase in phenolic compounds was attributed to the disruption of the plant material's cell structure caused by the action of drying steam at temperatures of up to 100°C during the EO extraction process, resulting in the liberation of these compounds that were previously bound to proteins or polysaccharides. Furthermore, the impact of the EO extraction process on the antioxidant activity of myrtle leaves was assessed through $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ radical scavenging assays and a FRAP test. The by-product extract demonstrated noteworthy antioxidant activity and proved more efficacious than the control in inhibiting $ABTS^{+\bullet}$ radical.

Conclusion

The plant extracts derived from agri-food industrial residues have attracted considerable interest, particularly with regard to their potential application in enhancing the quality of food products through the prevention of oxidation, with a particular focus on the inhibition of lipid oxidation. The aim of this study was to investigate the potential use of myrtle leaves residue extract, derived from steam distillation of EO, in the inhibition of lipid oxidation in corn oil. It was found that corn oil with myrtle leaves residue extract demonstrated the most stable values for acidity, peroxide value and color parameters (L*, a* and b*) when subjected to accelerated rancidity conditions at 60 °C. It can be concluded that the extraction of essential oils has enhanced the efficacy of myrtle leaves in maintaining the oxidative stability of corn oil. This suggests the potential use of essential oil extraction residues to improve the oxidative stability of vegetable oils in the food industry, thereby promoting eco-friendly and cost-effective strategies through the valorization of aromatic and medicinal plant residues from EO extraction industries.

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