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Antioxidant activity of herbs and extracted phenolics from oregano in canola oil

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Abstract

Canola oil is a rich source of PUFA for human diets, which makes it highly susceptible to oxidative degradation (rancidity). Herbs from the Lamiaceae family named basil *(Ocimum basilicum)*, oregano *(Origanum vulgare)*, rosemary *(Rosmarinus officinalis)* and sage *(Salvia officinalis)* were evaluated by total phenolic content and DPPH assay. Oregano presented better results in the antioxidant tests, and was also studied in a bulk oil system for monitoring antioxidant properties in a fatty matrix. Different levels of methanolic or ethanolic oregano extract were added to formulate canola aromatic oil (AromOil) in order to evaluate the efficacy in avoiding lipid oxidation under accelerated storage (60°C) up to 10 days. A 23 full factorial design with central point was accomplished by studying the factors solvent type, storage time at 60°C, and extract concentration to evaluate the influence of these factors, and their interaction, in the effectiveness of the antioxidant in protecting lipid oxidation in AromOil. The parameters TBARS and acidity were used as response, and also peroxides and fatty acids were evaluated. The storage time at 60°C was significant for TBARS formation, while all the factors had an effect on acidity formation. Methanolic oregano extract seemed to provide powerful antioxidant substances willing to protecting the PUFA in the AromOil storage study more efficiently than the ethanolic oregano extract formulation. These results agree with the polar paradox theory, describing that polar compounds are more effective in nonpolar media. Finally, samples prepared with ethanolic oregano extract at 1.00% (w/v) were well evaluated by sensorial analysis, showing a potential market for oils enriched with natural phenolic compounds.

Introduction

Herbs from the Lamiaceae family such as basil, oregano, sage, rosemary, marjoram, peppermint, thyme, etc., are used fresh or as dried leaves in small quantities in food preparation to provide changes in organoleptic properties of foods, such as, color, aroma and flavor (Kozlowska *et al*., 2015). They can also present other qualities as antibacterial properties (Khaled *et al.* 2016), and their remarkable antioxidant properties.

Canola oil is considered an important source of oleic acid (18:1n-9) and also polyunsaturated fatty acids (PUFA), such as alpha-linolenic acid (LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6) (Flakelar *et al.*, 2015). Lipid autoxidation that causes a free radical-mediated chain reaction has been a major cause of food quality deterioration, especially in food matrices rich in PUFA (Shahidi and Zhong, 2011). Antioxidants provide a way of inhibiting or avoiding those reactions by breaking one of the stages of initiation or propagation by hydrogen donating or electron transference. The food industry has also looked forward to using herbs as substitutes for synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in preventing lipid oxidation in foods, warranting quality and safety and keeping nutritional value (Akbarirad *et al.*, 2016).

Antioxidant activity of herbs has been studied by a large portion of methods that may be helpful in elucidating the mechanisms of action and

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effectiveness. Phenolic compounds in herbs are the major components responsible for scavenging radical species by donating hydrogen atom or an electron in order to form stable compounds. Antioxidant assays such as 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), and ferric reducing ability of plasma (FRAP) evaluate the antioxidant power in in vitro experimental conditions. Most of the cited antioxidant assays are carried out in highly polar environments, such as in water or methanol media. Other tests evaluate the antioxidant properties in a real environment, using food samples such as oils, meat, sauces, etc., which usually brings additional information concerning potential uses of antioxidants. Besides this, antioxidants of varying structure and concentration behave differently in polar solutions, bulk oil and oil-in-water emulsion systems (Shahidi and Zhong, 2011). Lipophilic antioxidants are more active in emulsions while polar antioxidants are more active in bulk fats and oils (Frankel, 2005).

Forced oxidation of oils in an oven at 60°C for 10 to 20 days has commonly been investigated to study the effectiveness of antioxidants in lipid protection (Chong *et al.*, 2015). An advantage of using this test is the speed with which results are obtained and the smart reagent consumption. Other methods also used are the active oxygen method (Swift), differential scanning calorimetry and the Rancimat method (air flow 20 L h^{-1} at 98°C).

This work aimed at evaluating the antioxidant properties of oregano and the other herbs basil, rosemary and sage. Oregano was also evaluated against an oil model using canola oil (AromOil) through an experimental design analyzing the effects of solvent, extract concentration and oxidation time, as well as their interaction, in preventing lipid oxidation. Finally, sensorial properties of AromOil were evaluated.

Materials and Methods

Sampling and chemicals

Dried herbs basil *(Ocimum basilicum)*, oregano *(Origanum vulgare),* rosemary *(Rosmarinus officinalis)* and sage *(Salvia officinalis)* were obtained from a local market in Maringá in three different lots (200 g each one). The samples were powdered, sieved at 80 mesh, and kept at 4°C protected from light exposition. Canola oil samples free of antioxidants (12 L, same lot) were donated by COCAMAR (Cooperativa de Cafeicultores e Agropecuarias de Maringá), located in Maringá (23º25'S 51º57'W), Paraná, Brazil.

2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and fatty acid methyl ester mixture standard 189-19 were purchased from Sigma-Aldrich (São Paulo, Brazil). Folin–Ciocalteu's phenol reagent was purchased from Merck (São Paulo, Brazil). All other solvents and chemicals were of analytical grade.

Phenolic extract and antioxidant activity

Methanolic extracts were prepared by adding methanol to dried herbs (1 : 10, w/v) under magnetic stirring (Fisatom model 732) at 200 rpm for 1 h (Boroski *et al.*, 2011). After filtering, the extracts were concentrated under reduced pressure at 40°C and used to determine antioxidant activity by the DPPH method and Folin–Ciocalteu's assay.

Methanolic extract was solubilized with distilled water followed by fractionation steps using solvents with increasing polarity such as hexane, ethyl acetate and butanol. The first and most highly apolar, hexane, was added in small aliquots of 15 mL in a total of 200 mL into the separating funnel. In each extraction, the upper fraction (hexane fraction) was collected and submitted to rotary evaporation in order to eliminate the hexane solvent and to obtain the extract. A similar procedure was performed with the solvents ethyl acetate and butanol, to obtain acetate and butanol fractions, respectively. The last fraction corresponded to the aqueous fraction. Each fraction was subjected to analysis of total phenolic content and antioxidant activity by the DPPH method.

Ethanolic extract of oregano was prepared as described for the methanolic extract, fractionated and evaluated by the DPPH method.

Total phenolic content was determined according to the Shahidi and Naczk (1995) method with slight modifications described by Boroski *et al*. (2011). The results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE g^{-1}).

The antioxidant capacity of the herb extracts was studied through the evaluation of their free radical scavenging effect on the DPPH radical based on the methods proposed by El-Massry *et al.* (2002) and Boroski *et al.* (2011). The results were expressed as percentage inhibition of the DPPH radical (% inhibition DPPH). The analyses were carried out in triplicate, and the extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting the concentrations of the extract solutions versus percentage inhibition (Equation 1).

$$\%Inhibition\ DPPH^{\bullet} = \frac{\left(Abs_{DPPH}{}^{\bullet} - Abs_{sample}\right)}{Abs_{DPPH}{}^{\bullet}} \times 100$$

The antioxidant activity index (AAI) was investigated by Scherer and Godoy (2009). AAI is

calculated considering the DPPH radical mass and the mass of the extract under study (Equation 2). It allows comparison of results despite the variety of methodologies proposed to determine the antioxidant potential in relation to DPPH, and regardless of DPPH concentration and reaction time (Boroski *et al.,* 2011).

$$AAI = \frac{DPPH^{\bullet}_{concentration}}{IC_{50}} \quad (2)$$

Aromatic canola oil

Aromatic canola oil (AromOil) was prepared using the most powerful antioxidant herb. Methanolic and ethanolic oregano extracts were prepared as described above. Different types of AromOil were produced in three repetitions, using both extracts at 0.00%, 0.50%, 0.75% and 1.00% (w/v). Stability experiments of inducing lipid oxidation by the Schaal Oven Test method were applied by heating 30.0 mL of each sample at 60 ± 5°C up to 10 days exposition. Samples were collected at 2, 6 and 10 days for evaluation of TBARS, acidity, peroxides and fatty acid composition.

A 23 full factorial design was developed to evaluate the influence of the solvent applied to prepare the oregano extracts (A), storage time at 60°C (B), and oregano extract concentration (C), and the interaction between those factors, in the effectiveness of the antioxidant components in protecting the lipid oxidation in AromOil. Each factor was assessed in two levels with a center point. The solvent employed as extractor was methanol as lower level (-) and ethanol as higher level (+); storage time at 60°C was evaluated during 2 days (-) and 10 days (+); and oregano extract concentration at 0.50% (-) and 1.00% w/v (+). The center point (0) was set as 6 days in factor (A) and as 0.75% (w/v) in factor (C); both was evaluated in ethanol or methanol solvent. TBARS and acidity response values were evaluated in a partial least squares (PLS) regression model performed by Matlab R2007b with PLS-Toolbox 5.2. The control sample (free of antioxidant) was considered in this experimental design.

Evaluation of lipid oxidation

The presence of thiobarbituric acid reactive substances (TBARS) was determined spectrophotometrically after solubilization and extraction of the sample with trichloroacetic acid (10%). The complex observed, consisting of a rosy-red color obtained by the reaction of TBARS and thiobarbituric acid, was determined at 532 nm. The molar extinction coefficient or molar absorptivity (1.56×10^5 L mol^{-1} cm^{-1}) was used for quantification, and the values expressed as mol kg^{-1} fat (Sinnhuber *et al.*, 1958).

Free fatty acids in AromOil were determined according to AOCS (2000) by the titrimetric method using NaOH and phenolphthalein indicator, and the results expressed as the percentage of acidity in oleic acid (w/w).

Peroxide indices from samples before and after adding herb extracts submitted to storage at 60°C were performed according to AOCS (1998) by reaction with potassium iodate, and titrimetric analysis with sodium thiosulfate in the presence of starch. The results were expressed as milliequivalent kg^{-1} fat.

Lipids and fatty acid analysis

Moisture content was determined in accordance with AOAC (1998). Total lipids were extracted by the Bligh and Dyer (1959) method using methanol, chloroform and water at 2 : 2 : 1.8 (v/v/v), respectively, and measured by the gravimetric method.

Fatty acid methyl esters (FAME) in canola oil, AromOil and herbs were prepared by methylation of total lipids as described by Hartman and Lago (1973). Methyl esters were separated by gas chromatography (GC/FID) in a Varian model 3380 equipped with flame ionization and a cyanopropyl capillary column (100 m, 0.25 i.d., 0.25 µm film thickness, CP-7420 Varian, EUA). The gas flow rates used were 1.2 mL min^{-1} carrier gas (H_2), 30 mL min^{-1} make-up gas (N_2) and 300 mL min−1 flame gases (H_2 and synthetic air, respectively). The sample splitting rate was 1 : 100 and the samples (2 µL) were injected in triplicate. The operation parameters were as follows: detector temperature 245°C and injection port temperature 225°C. In order to separate FAME in lipids from herbs, the column temperature was kept at 165°C for 12 min, then programmed to increase at 40°C min^{-1} to 180°C and kept at this temperature for 15 min; next, it was increased to 240°C at 15°C min^{-1} and kept at this temperature for 18.62 min, in a total of 50 min.

The peak areas were determined by the Workstation 5.0 (Varian) acquisition program. For the fatty acid identification, retention times were compared with those of standard methyl esters. For the purpose of quantification, the relative concentration of fatty acids was expressed by relative area percentage (%) considering the relative area of each peak per the sum of all the peak areas.

Sensory analysis

A 50-member untrained panel of healthy adults and potential consumers evaluated the AromOil attributes of color, texture, aroma and flavor based on

a hedonic scale of food acceptability of nine points (far left, 1 = dislike extremely; far right, 9 = like extremely; middle point, 5 = neither like nor dislike) (Stone and Sidel, 2004). The control formulation was not sensorially evaluated since the aim of this analysis was to evaluate the acceptability of the different formulations with the range 0.50%, 1.00% and 5.00% (w/w) of ethanolic oregano extract, and not to compare these with the control formulation. The panelists' ages ranged from 16 to 50, with an average age of 25 years. The range of the panelists' level of education was mostly from an incomplete undergraduate degree to a postgraduate degree.

Statistical analysis

The results were submitted to variance (ANOVA) analysis and Tukey's test (5% probability) using Statistica 5.0 software (StatSoft, 1996). A 23 full factorial design with central point was performed and the responses were evaluated in a PLS regression model performed by Matlab R2007b with PLS-Toolbox 5.2.

Results and Discussion

Phenolic extract and antioxidant activity

The antioxidants in herbs are bioactives produced by plants, and exhibit a protective role. Table 1 shows phenolic compounds and antioxidant activity of herbs. Oregano extract presented high phenolic content and also showed IC_{50} (concentration necessary to inhibit 50% of DPPH radical absorbance) values smaller than the other herbs, considering all the fractions except for the hexane fraction.

According to Scherer and Godoy (2009), plant extracts presenting AAI values lower than 0.5, which means IC_{50} values higher than 94 g mL^{-1}, can be considered weak antioxidants. Values between 0.5 and 1.0 (IC_{50} values between 94 and 47 g mL^{-1}) are considered moderate, from 1.0 to 2.0 (IC_{50} values from 47 to 23 g mL^{-1}) are considered strong antioxidants, and over 2.0 very strong (IC_{50} values smaller than 23 g mL^{-1}). Regarding these parameters, the acetate fractions of basil, oregano and rosemary had the highest AAI, thus being considered foods with strong antioxidant activity. They also had the largest amount of phenolic compounds.

Fractionation is a useful tool when you are interested in estimating a polarity of antioxidants and evaluating which solvent can be applied in order to have the extract with the greatest antioxidant capacity. This process starts by chosen the solvent prone to solubilize the antioxidants present into the matrix. Fractionation of the methanolic extract targeted semi-purification for knowledge of the distribution of antioxidant compounds in solvents of increasing polarity. The acetate fraction showed higher potential against DPPH radicals for all the herbs except sage. As intermediate ethyl acetate and butanolic fractions are considered to be higher antioxidant potential (Aguiar *et al.*, 2011).

The nature of the solvent employed in producing the extracts influences the extracted antioxidant compounds and therefore their antioxidant activity. Use of the acetate fraction of herbs is limited due to its low yield. Thus, it was decided to continue the work using the methanolic oregano extract, and this was the first step of this study, a strategy for a triage analysis.

The ethanolic fraction of oregano was also evaluated to be applied in food, because the methanolic oregano extract is restricted in food because of methanol toxicity, even at residual levels, thus the study by using methanol solvent was conducted as a triage analysis in order to verify which herb present suitable results. Moreover, methanolic extract and antioxidant activity is extensively present on scientific literature (Habtemarian and Varghese, 2017; Nahid *et al.,* 2017; Luís *et al.,* 2016; Ghlissi *et al*., 2016; Vaithiyanathan and Mirunalini, 2016; Eid *et al*., 2015; Kunyanga *et al.*, 2012). However, methanol and ethanol solvents are similar in their polarities and solubility properties (ethanol Snyder polarity parameter for partition 4.3 and adsorption 0.88, methanol Snyder polarity parameter for partition 5.1 and adsorption 0.95; ethanol and methanol Hildebrand solubility parameter 12.7 and 12.9, respectively) (Collins *et al*., 2006). Ethanolic oregano extract was able to inhibit 50% of DPPH absorbance at 78.7 μg mL^{-1} (ethanolic fraction), 208.5 μg mL^{-1} (hexane fraction), 19.0 μg mL^{-1} (acetate fraction), 22.4 μg mL^{-1} (butanolic fraction) and 16.8 μg mL^{-1} (aqueous fraction).

Methanol and ethanol are polar solvents acting as proton donors that solubilize antioxidants due to the solvation promoted by interactions (hydrogen bonds), between the polar sites of the antioxidant molecules and the solvent. Methanolic oregano extract showed better results as antioxidants compared to ethanol oregano extract. The presence of the ethyl radical that is longer than the methyl radical present in methanol results in a lower solvation of antioxidant molecules (Boeing *et al.*, 2014).

Kozlowska *et al.* (2015) found smaller phenolic values for oregano methanolic aqueous extract (70%), corresponding to 137.60 mg GAE g^{-1} extract, while for the oregano aqueous ethanolic extract (70%), the phenolic values found was 204.60 mg GAE g^{-1}.

Table 1. Phenolic compounds and antioxidant activity of herbs

Analysis	Basil	Oregano	Rosemary	Sage
Phenolics (mg EGA g^{-1} extract)[1]	119.5±6.1[a]	226.5±9.9[b]	141.8±2.1[c]	139.6±9.2[c]
DPPH (IC_{50}, μg mL^{-1})				
Methanolic	52.9±1.2[a]	22.3±0.1[b]	45.6 ±0.8[c]	27.1±1.1[d]
Hexane	241.4±0.9[a]	407.2±1.4[b]	34.9±0.5[c]	52.4±0.6[d]
Acetate	12.9±0.7[a]	9.3±0.3[b]	22.3±0.1[c]	31.4±1.2[d]
Butanolic	40.1±0.9[a]	13.7±0.7[b]	42.4±1.9[c]	25.1±1.7[d]
Aqueous	120.8±2.9[a]	41.3±2.1[b]	105.2±1.8[c]	56.6±1.6[d]

Mean of three repetitions ±SD. [1]Gallic acid equivalents. Equal superscripts in the same row mean no significant difference ($p< 0.05$).

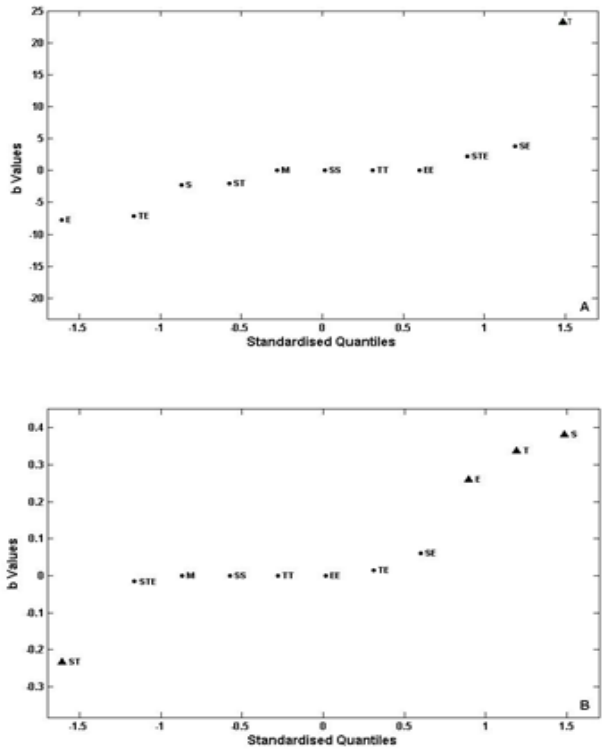

Figure 1. Standard probability distribution curve for: (A) TBARS regression coefficients and (B) acidity regression coefficients. S=Solvent, T=Storage time at 60°C, E=Extract concentration, ST=interation between Solvent and Storage time, SE=interation between Solvent and Extract concentration, TE=interation between Storage time and Extract concentration, STE=interaction between Solvent, Storage time and Extract concentration, SS=interation Solvent with yourself, TT=interation Storage time with yourself, EE=interaction Extract concentration with yourself.

These same authors also studied the methanolic aqueous extract of rosemary and methanolic aqueous extract of sage, and obtained phenolic values of 184 and 225.90 mg GAE g^{-1} extract, respectively.

Chemicals responsible for antioxidant properties of the Lamiaceae family are mainly hydroxybenzoic and hydroxycinnamic acids. Concerning oregano, the antioxidant activity observed is provided mainly for polar phenolic compounds such as caffeic acid, rosmarinic acid and luteolin-7-O-glucoside, and also 4-hydroxy-4-methyl-2-pentanone and thymol, between other compounds detected by CG/MS (Kozlowska *et al.*, 2015).

Evaluation of lipid oxidation

Herbs from Lamiacea family were evaluated and oregano was chosen to carry out the studies on the effectiveness of phenolic antioxidant in avoiding lipid oxidation in canola oil (AromOil). An experimental design was developed to study the influence of common variables, such as the solvent used to prepare the oregano extracts (A), storage time at 60°C (B), and oregano extract concentration (C), and the interaction between those factors, in the effectiveness of the antioxidant components in protecting the lipid oxidation in AromOil. TBARS formation and acidity values were assessed. A relationship between the contrast coefficients for a 23 factorial design and another matrix containing TBARS and acidity values was assessed through the PLS method (Alexandrino and Poppi, 2013).

When the mathematical model is developed, the regression coefficients values are found. However, not all the regression coefficients are significant, and to know which were significant a standard probability distribution curve was constructed and it is shown in Figure 1. This Figure can be divided into two regions, where factors and interactions have a negative coefficients and where factors and interactions have positive coefficients. All factors and interactions that are represented by a triangle (▲) were significant and these are located outside the central line which crosses the zero value on the ordinate. The effects positioned on this line are represented by a point (●) and correspond to the estimate of the error effects, not being significant.

Considering TBARS results, the only significant factor was the storage time at 60°C (T) in Figure 1A.

Table 2. Lipids and fatty acid composition of herbs

Analysis	Basil	Oregano	Rosemary	Sage
Moisture (%)	11.64±0.09ª	10.16±0.00ᵇ	9.46±0,07ᶜ	8,87±0,05ᵈ
Total lipids (%)	3.32±0.15ª	4.21±0.02ᵇ	9.99±0.00ᶜ	8.11±0.16ᵇ
Fatty acids (wt%)				
14:0	ND	0.88± 0.04ª	ND	ND
15:0	6.32±1.14ª	0.15±0.01ᵇ	ND	ND
15:1	1.52±0.14ª	4.38±0.22ᵇ	1.80±0.04ª	3.06±0.07ᶜ
16:0	21.52±1.75ª	13.24±1.16ᵇ	16.05±2.04ᵇ	13.45±0.54ᵇ
16:1	6.41±0.65ª	0.32±0.02ᵇ	2.17±0.23ᶜ	2.05±0.29ᶜ
17:0	ND	ND	ND	0.75±0.20ª
18:0	2.39±0.24ª	2.21±0.27ª	1.59±0.21ᵇ	1.86±0.34ª,ᵇ
18:1n-9	3.47±0.38ª	1.64±0.13ᵇ	4.89±0.65ᶜ	5.37±0.32ᶜ
18:2n-6 (LA)	8.54±0.37ª	7.68±1.09ª	8.51±1.46ª	8.12±0.81ª
18:3n-3 (LNA)	18.90±1.92ª	35.51±1.63ᵇ	20.50±4.99ª	10.72±0.89ᶜ
20:0	21.43±1.62ª	5.50±0.43ᵇ	ND	ND
20:1n-9	0.90±0.05ª	0.72±0.02ª	ND	2.07±0.19ᵇ
21:0	0.46±0.04ª	0.55±0.04ª	1.11±0.02ᵇ	ND
20:3n-6	ND	0.50±0.02ª	5.51±4.27ᵇ	9.74±1.59ᵇ
22:0	3.41±0.02ª	9.65±0.29ᵇ	23.60±2.49ᶜ	14.88±0.51ᵈ
22:1n-9	0.59±0.08ª	1.52±0.14ᵇ	1.37±0.15ᵇ	3.81±0.60ᶜ
22:2n-6	0.61±0.02ª	3.31±0.11ª	ND	ND
24:0	3.59±0.35ª	12.98±0.25ᵇ	12.89±2.20ᵇ	24.16±0.62ᶜ
SFA[1]	59.13	44.51	56.64	55.10
MUFA[2]	17.96	8.55	10.08	16.36
PUFA[3]	22.98	47.10	33.26	28.58
PUFA/SFA	0.39	1.06	0.59	0.52
n-6/n-3	0.22	0.31	0.30	0.40

Mean of three repetitions ±SD. [1]SFA: Saturated fatty acids. [2]MUFA: monounsaturated fatty acids. 3PUFA: polyunsaturated fatty acids. ND: not detected. Equal superscripts in the same row mean no significant difference ($p < 0.05$).

When storage time is on the lower level the TBARS values are lower, indicating that the storage time factor in the lower level (2 days) is more effective. Increasing in storage time was found to be a significant variable in TBARS formation (Takeungwongtrakul and Benjakul, 2016). The acidity results in Figure 1B show that the factors solvent type (S), storage time at 60°C (T), extract concentration (E), and the interaction between solvent and storage time (ST) are significant in this case.

After a screening of the factors to determine which were significant in the process, it was possible to perform an analysis of contour maps to obtain the lowest acidity values (Figure 2). For this, the solvent was fixed on the lower level (methanolic oregano extract) and upper level (ethanolic oregano extract) to obtain the contour maps. In both cases, the lowest acidity values were obtained when storage time and extract concentration was at the lower level (2 days and 0.50% oregano extract (w/w), respectively).

The studied parameters TBARS, acidity, peroxide value and fatty acids are commonly assessed in stability tests using bulk oil models (Frankel, 2005; Chong *et al*., 2015). Lipid peroxides are the earliest oxidation products formed by radical chain breaking. The oxidative stability of the AromOil was also monitored by peroxide formation. Samples containing methanolic oregano extract at 0.50%, 0.75% and 1.00% up to 10 days at 60°C significantly reduced peroxide formation in 50%, 44% and 61%, respectively, while samples containing the same proportion of ethanolic oregano extract reduced these values in 59%, 63% and 57%, respectively. The synthetic antioxidant ascorbyl palmitate (0.050%), when added to sunflower oil, showed the ability to retard the formation of peroxides at 77% in an accelerated storage test in an oven at 60°C for 10 days (Angelo and Jorge, 2008). As the results showed, TBARS and acidity values increased with the storage time. Concerning acidity, using methanol as the solvent in oregano extraction was more effective in protecting lipid oxidation compared to ethanol. This behavior is linked with the antioxidant activity of the extract, which varies with the solvent; as shown above, methanolic extract presented an IC_{50} of 22.3 μg mL^{-1} while when ethanolic extract was tested, this value was 78.7 μg mL^{-1} (DPPH assay). Although both are considered as polar solvents, methanol worked

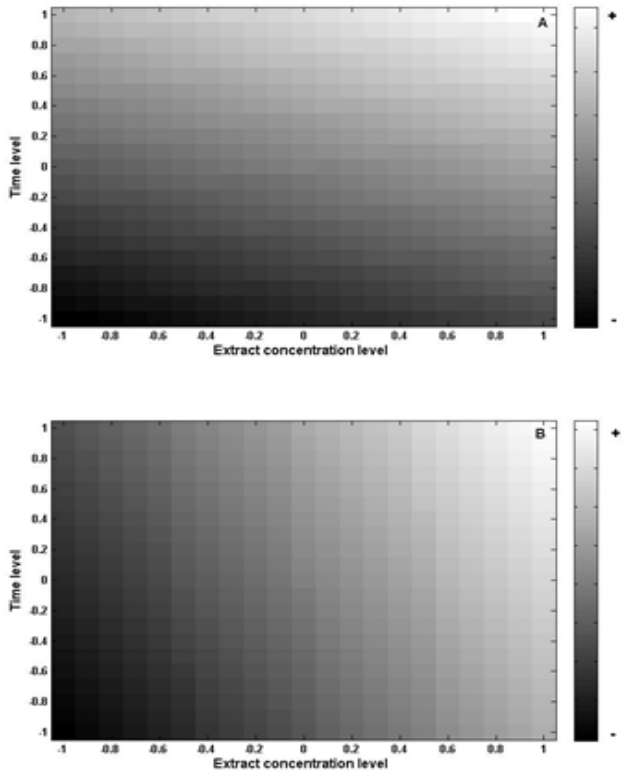

Figure 2. Contour maps for: (A) the solvent fixed on the lower level (methanol oregano extract) and (B) upper level (ethanol oregano extract).

better in the extraction of polar compounds.

According to "polar paradox theory", phenolics found in oregano are suitable for avoiding lipid oxidation of AromOil. Oxidation in vegetable oil does occur in homogeneous media, but empirical evidence has shown an important role of water and atmosphere when oxidation is initiated and propagated in vegetal oils (Li *et al*., 2015). Thus, polar antioxidants do not act dissolved in bulk oil, but are distributed at the oil–air and oil–water interfaces created by natural components, such as phospholipids, or even in lipid oxidation products named hydroperoxides (Huber *et al.*, 2009; Shahidi and Zhong, 2011; Li *et al.,* 2015). Phenolic compounds present in extracts avoid autoxidation reaction in oils by hydrogen donation to delay the initiation step of lipid autoxidation.

Lipids and fatty acid analysis

Table 2 shows the values of moisture, total lipids and fatty acid composition of basil, oregano, rosemary and sage, which were significantly different between the studied herbs ($p<0.05$). Rosemary presented the highest levels of total lipids followed by sage, oregano and basil. LNA (18:3n-3) was found in all the herbs, and as the major fatty acid in oregano (35%) (Table 1). Leaf lipids usually contain large proportions of LNA, which is an important component of chloroplast membrane lipids (Simopoulos, 2002). Additionally, the PUFA and saturated fatty acid rate was superior in oregano.

Table 3. Means ± standard deviations of liking ratings of aroma and flavor of AromOil

Ethanolic extract of oregano (%)	Aroma	Flavor
0.50	6.3±1.5[a]	6.1±1.6[a]
1.00	7.4±1.5[b]	7.1±1.3[b]
5.00	7.4±1.5[b]	6.4±1.7[a]

Mean of three repetitions ±SD. Equal superscripts in the same column mean no significant difference ($p< 0.05$).

Canola oil used in the oxidative study showed fatty acid composition as C14:0 (0.04%), C16:0 (4.62%), C18:0 (2.52%), C18:1n-9 (58.65%), C18:1n-7 (10.67%), C18:2c9t12 (0.07%), C18:2n-6 (16.64%), C18:3n-6 (0.48%), C20:0 (0.42%), C18:3n-3 (5.03%), C20:1n-9 (0.76%) and C22:0 (0.09%), values very close to other studies (Flakelar *et al.*, 2015). Fatty acid composition was also evaluated in AromOil up to 10 days of storage at 60°C. As a result, fatty acid profile was not changed in canola oil free of antioxidants compared to AromOil ($p<0.05$).

Consumer acceptance

The production of AromOil using canola oil is due to the high quality of fatty acid composition, presenting a low level of saturated fatty acids (Flakelar *et al*., 2015). Canola oil has been shown to be a valuable source of n-3 fatty acids, and its n-6/n-3 ratio (2:1) allows it to be considered an important food to improve dietary health. Besides the nutritional aspects, sensory characteristics are important in determining the acceptance of a product by consumers. In this context, sensory analysis is an important tool to verify acceptance of the final product. Thus, the study of AromOil evaluates the acceptance of its differentiated characteristics to the consumer (Table 3).

The ethanol extract of oregano showed effectiveness in protecting lipid oxidation when compared to oil-free antioxidants. In addition to the prevention of oxidation reactions, the extract also modifies the organoleptic properties of the oil. The sensory acceptability of AromOil was evaluated at 0.50%, 1.00% and 5.00% ethanol extract of oregano. Table 3 shows that the aroma parameter was evaluated as better in AromOil with ethanol extract of oregano at 1.00% and 5.00%, indicating that concentrations above 0.50% positively affect the aroma of canola oil, possibly due to enhancement of the aroma characteristic of oregano essential oil.

Regarding flavor, the best consumer acceptance rate was reached at 1.00%, corresponding to 7.1 and statistically different from the values obtained with concentrations of 0.50% and 5.00%. At the 5.00% concentration it is possible that the bitterness of the oregano is related to the lower acceptance of the product. Thus, the results of sensory analysis indicate that the best formulation for AromOil is obtained with a concentration of 1.00% ethanolic extract of oregano.

Conclusion

This work pointed out a practical application of natural antioxidants to promote stabilization against lipid oxidation. Oregano showed the highest phenolic content and also better results as antioxidant evaluated by DPPH assay compared to the other herbs from Lamiaceae family. Ethyl acetate fraction of oregano had showed high potential as antioxidant, but the low yield obtained limited the applicability of this fraction. In order to be used as an ingredient in food matrix, oregano ethanolic extract was chosen to prepare AromOil. Canola oil induction time was increased in the presence of oregano extract due to lipid protection of PUFA. TBARS formation and acidity values were affected by the storage conditions, while smaller acidity values were reached when methanolic extract was used at 0.50% (w/w) (lower level). Antioxidants present in AromOil were able to prevent peroxides formation but no difference was observed regarding canola fatty acid composition up to 10 days of storage at 60°C with or without antioxidants. Oil enriched in phenolic compounds was well evaluated by sensorial analysis presenting a food preparation possible to be a component in a health-based diet.

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