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ORIGINAL ARTICLE

Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers

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Abstract The aims of this study were: to evaluate the total equivalent antioxidant capacities (TEAC) and phenolic contents of 13 plants extracts; to select the most promising extracts regarding reducing activity using cluster analysis multivariate statistical technique; and to analyse evaluate sensory acceptance of lamb burgers produced with the most promising natural antioxidants replacing sodium erythorbate. Plant extracts were evaluated regarding TEAC by DPPH$^{\bullet}$ and FRAP methods, and total phenolics contents by Folin-Ciocalteau assay. The TEAC values ranged from 0.50 to 9.06 g trolox/100 g dry weight (dw) and from 43.6 to 472.32 μmol trolox/g dw for DPPH$^{\bullet}$ and FRAP methods, respectively, and the total phenolic contents from 5.98 to 74.01 mg GAE/g dw. Extracts from *Origanum vulgare*, *Melissa officinalis*, *Origanum majorana L.* and *Rosmarinus officinalis* were grouped as the ones with higher antioxidant capacities by cluster analysis. All burgers produced with each one of these four plant extracts or with sodium erythorbate showed no differences ($P>0.05$) regarding consumers' sensory acceptance. In conclusion, it is possible to replace sodium erythorbate in lamb burgers by any of the four natural extracts selected without compromising sensory acceptance of this meat product.

Keywords Equivalent concentrations · Extraction · Ovine hamburgers · Radical-scavenging activity · Sensory acceptance

Research highlights • Antioxidant potential of 13 herbs through of Folin-Ciocalteau, FRAP and DPPH
• Oregano, marjoram, lemon balm and rosemary had higher antioxidant activity
• Cluster analysis grouped the herbs, according to results of capacity antioxidant
• Concentration of extract determined through equivalence to sodium erythorbate
• Use of natural antioxidants without compromising sensory acceptance of lamb burgers

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Introduction

The demand for healthier products represents a major trend worldwide and industries have been seeking new ways to reduce the use of chemical additives, replacing them with natural alternatives. This approach could increase the shelf life of food besides reducing the incidence of several diseases, also leading to flavor enhancement (Castellini et al. 2002; Brewer 2011; Hayes et al. 2011).

Nowadays there is also a great demand for convenience products like hamburgers, which, if associated with the use of natural ingredients, could represent a manner for meat industry to offer an alternative to fulfill this trend of health and natural food consumption.

Plant extracts, foodstuffs and some beverages are now regarded as important sources of dietary antioxidant, exerting positive effects on human health and in the aging process (Dorman et al. 2004). Consumption of herbs and spices has been implicated in the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis, etc. (Srinivasan 2005). Such properties have been attributed to the presence of several compounds such as vitamins, terpenoids, polyphenols, including flavonoids (Suhaj 2006).

With the development of functional foods having specific health effects, interest in plant antioxidant is increasing among scientist, food manufacturers and consumers. From ancient times that herbs and spices have been used due to their culinary qualities and medicinal properties, including antioxidant activity (Pateiro et al. 2014; Lorenzo et al. 2014). So the use of herbs and spices in food is increasing, especially since consumers have questioned the use of the synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroxyquinone (TBHQ) and sodium erythorbate in food products (Madsen and Bertelsen 1995; Capitani et al. 2009).

Despite the approval of the use of these synthetic additives to prevent undesirable reactions and lengthen the product's shelf life, carcinogenic and/or mutagenic potential caused by the intake of high doses is known, and due to this, in several countries their use have been limited or even prohibited, stimulating the search for natural antioxidant (Capitani et al. 2015).

Several in vitro methodologies have been used to measure the antioxidant capacity of these synthetic and natural sources before addition to a food matrix. Folin-Ciocalteau, DPPH$^{\bullet}$ and FRAP (Ferric Reducing Antioxidant Power) assays are examples of methods that determine stability of free radicals or a transition metal by a transfer of electrons or of hydrogen atoms. Different methods determine different results for a given sample. Results obtained from several samples by different methods could be analyzed using descriptive procedures of multivariate statistical techniques, for example, cluster analysis, that to classify these samples into groups according to similar or different characteristics, thus minimizing the differences among them (Capitani et al. 2009). This approach could be applied in order to select extracts with better antioxidant capacity.

However, besides presenting good antioxidant capacity, herbs and spices extracts should also not impair the sensory acceptance of foods in which they are added. Thus, the purposes of the present study were: a) to evaluate a variety of plants with respect to antioxidant activity by three different methods, b) to apply cluster analysis multivariate statistical technique to select the extracts with better antioxidant capacity, taking into account the results of the three different methods performed and c) to evaluate the sensory acceptance of lamb burgers produced with the plant extracts with better antioxidant capacity replacing the synthetic sodium erythorbate, aiming to find new potential sources of natural antioxidants to meat industry.

Material and methods

Reagents and solvents

Radical DPPH$^{\bullet}$ (1,1-Diphenyl-2-picrylhydrazyl), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and Folin-Ciocalteau were purchased from Sigma-Aldrich (Germany). Sodium acetate trihydrate ($C_2H_3NaO_2$), sodium hydroxide (NaOH), methanol, acetone, acetic acid glacial, chloridric acid, sodium carbonate were purchased from Synth (Brazil) and gallic acid was from Vetec (Brazil).

Plant material

Thirteen plant material [basil leaves (*Ocinum basilicum*), chamomile flower (*Matricaria camomila*), fennel seeds (*Pimpinella anisum*), ginger (*Zingiber officinalis*), laurel leaves (*Laurus nobilis*), lemon balm leaves (*Melissa officinalis*), marjoram leaves (*Origanum majorana L.*), mint leaves and stalk (*Mentha piperita*), mustard seeds (*Brassica hirta*), oregano leaves (*Origanum vulgare*), rosemary leaves (*Rosmarinus officinalis*), sage leaves (*Salvia officinalis*) and turmeric (*Curcuma longa L.*)], were acquired from the local market (Piracicaba, São Paulo, Brazil), packed a vacuum and stored in a dry, dark place at room temperature until the preparation of plant extracts (Table 1).

Preparation of plant extracts for antioxidant property analysis and total polyphenol content

To obtain the extracts the methodology previously described by Michiels et al. (2012), with some modifications, was followed. One gram of dry plant material was homogenized with 20 mL of solvent solution [acetone/ultrapure water/glacial acetic acid, 70:28:2 % (*v*/*v*)] using an Ultra-Turrax TE-102 (Tecnal, Brazil) for 2 min. The mixture was shaken in an incubator shaker TE-424 (Tecnal, Brazil) during 1 h at

Table 1 Total phenolic content in 13 selected plants

Plant	Family	Total phenolic content (mg GAE/g dw)
Oregano (*Origanum vulgare*)	Lamiaceae	74.01±7.33[a]
Marjoram (*Origanum majorana L.*)	Lamiaceae	48.66±3.03[b]
Rosemary (*Rosmarinus officinalis*)	Lamiaceae	46.18±3.02[b]
Lemon balm (*Melissa officinalis*)	Lamiaceae	42.86±3.04[bc]
Laurel (*Laurus nobilis*)	Lauraceae	37.26±2.12[c]
Sage (*Salvia officinalis*)	Lamiaceae	26.89±0.49[d]
Basil (*Ocinum basilicum*)	Lamiaceae	20.42±0.79[de]
Mustard (*Brassica hirta*)	Brassicaceae	20.40±0.37[de]
Turmeric (*Curcuma longa L.*)	Zingiberaceae	17.53±0.25[ef]
Chamomile (*Matricaria camomila*)	Asteraceae	16.77±0.62[ef]
Mint (*Mentha piperita*)	Lamiaceae	10.69±0.33[fg]
Ginger (*Zingiber officinalis*)	Zingiberaceae	8.93±0.62[g]
Fennel (*Pimpinella anisum*)	Apiaceae	5.98±0.08[g]
Significance	–	***

[a–g] Means in the same column not followed by a common number are significantly different ($P<0.05$; Tukey test)
Significance: *** ($P<0.001$), ** ($P<0.01$), * ($P<0.05$), n.s. = $P>0.05$
n=09 samples by extract

4 °C and centrifuged using a refrigerated centrifuge 5810 R (Eppendorf, Germany) at 4.500 rpm for 15 min. The supernatant was filtered with a Whatman n°1 filter paper and final volume was adjusted to 50 mL with solvent solution. The extraction was carried out in triplicate for each sample. The analysis was conducted within 3 days.

Estimation of total polyphenol content

Total polyphenol content was measured using Folin-Ciocalteau colorimetric method according to Singleton and Rossi (1965). Plant extracts (100 μL) were mixed with 500 μL of 10 % Folin-Ciocalteau reagent, and incubated at room temperature for 2 min. Following the addition of 400 μL of 7.5 % sodium carbonate to the mixture, total polyphenols were determined after incubation in a water bath at 50 °C for 15 min. After cooling, the absorbance of the resulting blue colour was measured at 760 nm with a SP-22 UV–VIS (Biospectro, Brazil) spectrophotometer. Quantification was done with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), milligrammes per gram of dry weight (dw). Analyses were performed in triplicate on each extract.

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma FRAP assay by Benzie and Strain (1999) as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (*v*/*v*/*v*). The reagent (3.400 μL) and sample solutions (100 μL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 30 min. Standard curve was prepared using different concentrations of trolox. All solutions were used on the day of preparation. The results were expressed as μmol trolox equivalent/g dw. Analyses were performed in triplicate on each extract.

Free radical-scavenging ability by the use of a stable DPPH$^{\bullet}$ radical

The DPPH$^{\bullet}$ radical-scavenging activity was determined using the method proposed by Brand-Williams et al. (1995). Aliquots (25–170 μL) of the tested sample was placed in a cuvette, and 3.15 mL of 72 μM methanolic solution of DPPH$^{\bullet}$ radical was added. Absorbance measurements commenced immediately. The decrease in absorbance at 515 nm was determined after 3 h for all samples, period determined from kinetics and variation of absorbance studies in relation to time to prove the stability of 13 herbs. Methanol was used to zero spectrophotometer. The absorbance of the DPPH$^{\bullet}$ radical without antioxidant (control) was measured daily. Methanolic solutions of trolox were tested. Analyses were performed in triplicate on each extract. The results were corrected for dilution and expressed as g trolox equivalent/100 g dw.

Preparation of plant extracts to manufacture of lamb burgers

The extracts were obtained according described above, using at a ratio of the 1:50 in weight of plants (g) per volume of solvent (mL), being these samples subjected to grinding, agitation, centrifugation and filtration, according described above, and concentration in a rotary evaporator MA 120 (Marconi, Brazil), lyofilization process and ressuspension in ultrapure water.

Processing of the burgers

Burgers formulation consisted of lamb neck meat (84 %) and lamb fat trimmings (14 %), acquired from the local fridge, 2 % salt and the antioxidant. The meat and fat were thawed at 4 °C for 12 h and minced separately using disc of the 4 mm. Five treatments were processed, being four extracts with natural antioxidants and one with synthetic antioxidant sodium erythorbate (Kerry, Brazil). The quantities of natural extracts to be used were determined by converting the average values obtained for the three methods of antioxidant capacity, compared to the values obtained for the sodium erythorbate concentration of 500 ppm.

The burgers were formed using a manual molder HP 112 (Picelli, Brazil) of 112 mm diameter×2 cm height and individually separated with polyethylene films, weighing 95–100 g. The samples were frozen in ultra freezer UCE-10 (EcoClima, Brazil) for 40 min and packed in air-permeable polypropylene bags, being immediately stored at −18 °C in a vertical freezer flex frost free BVR 28/127 (Brastemp, Brazil) for 5 days. A total of 50 burgers were analyzed (5 treatments× 10 samples of each treatment).

Sensory analysis

Sixty consumers were recruited among the University's students, staff and faculty where the selection criterion was just to like lamb meat. The recruited consumers were given a free and informed consent form to be read and signed prior to performing the tests.

The burgers were evaluated after 5 days of elaboration by affective an acceptance test using a 9-point hedonic scale (1 - "extremely dislike" to 9 - "extremely like"), for the consumption of cooked samples as described by Meilgaard et al. (2006). The samples were cooked at 180 °C for 8 min using an electric griddle (Croydon, Brazil), turned over every 2 min interval until the internal temperature reached 72 °C. After, were stored at 60 °C for a maximum of 30 min, being the samples cut in six pieces at diagonal and served individually to the panelists, inside disposable plastic cups that were coded by three-digit numbers. The test was conducted in individual booths that was illuminated by white light and a randomized complete block design was used to assess the attribute overall acceptability.

Statistical analysis

To analyze the antioxidant capacity parameters of the extracts, the assays of the three replications were performed in triplicate, and the results were expressed by means ± SD (Standard Deviation). For the sensory analysis, expressed by means ± SE (Standard Error) were evaluated 10 samples of each of the treatments after storage of 5 days by 60 consumers.

Experimental data were subjected to Analysis of Variance (ANOVA), using the statistical package SAS (Statistic Analysis System) version 9.1.3 and the differences among means were evaluated by Tukey's test at a level of 5 % significance level. Linear regression analyzes were performed to correlate the total phenolic content with the antioxidant capacity (DPPH$^{\bullet}$ and FRAP), being calculated the correlation coefficients (r) for the 13 extracts evaluated.

Additionally, to select the most promising extracts in relation to antioxidants properties, the results obtained to in the three methods of antioxidant capacity for all the extracts were grouped according to the similarity in each method using Multivariate Technique from groupings (Cluster Analysis), that grouped values based on the similar measurements, recognizing the existence of homogeneous and heterogeneous groups among the 13 extracts evaluated.

Results and discussion

Total phenolic content

The amount of total phenolics in the evaluated plants, measured by Folin-Ciocalteau method, varied significantly ($P<0.001$) from 5.98 to 74.01 mg GAE/g dw (Table 1). The highest level of phenolics was found in *Origanum vulgare*, while the lowest was in *Pimpinella anisum*, *Origanum majorana L.* (48.66 mg GAE/g dw), *Rosmarinus officinalis* (46.18 mg GAE/g dw), and *Melissa officinalis* (42.86 mg GAE/g dw) also had very high levels of phenolics. Other herbs with high levels of phenolics were *Laurus nobilis* (37.26 mg GAE/g dw), and *Salvia officinalis* (26.89 mg GAE/g dw). *Ocinum basilicum* (20.42 mg GAE/g dw), *Brassica hirta* (20.40 mg GAE/g dw), *Curcuma longa L.* (17.53 mg GAE/g dw) and *Matricaria camomila* (16.77 mg GAE/g dw) had relatively low levels of phenolics, whereas in *Mentha piperita* (10.69 mg GAE/g dw) and *Zingiber officinalis* (8.93 mg GAE/g dw) phenolics were quite low. Among 6 families tested in this study, *Lamiaceae* (seven tested spices), *Zingiberaceae* (two tested spices), *Lauraceae* (one tested spice), *Brassicaceae* (one tested spice), Asteracae (one tested spice) and *Apiaceae* (one tested spice), only *Lamiaceae*

exhibited high levels of polyphenols (until 74.01 mg GAE/g dw). There have been extensive studies on antioxidant activity of many spices in the *Lamiaceae* family. The findings obtained in the present study showed that the spices were relatively high but not very high in polyphenols. Total phenolic contents of the seven spices decreased in the following order: oregano > marjoram > rosemary > lemon balm > sage > basil > mint. Statistical analysis showed significantly ($P<0.001$) differences among these spices and could be due to genotypic and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested, time of taking samples and determination methods (Kim and Lee 2004; Shan et al. 2005). These outcomes are in agreement with those reported by Gawlic-Dziki (2012) who noticed higher phenolic content from oregano extract compared with rosemary extract (177 vs. 94 mg GAE/g dw, respectively). Contrary, Dorman et al. (2003) observed higher phenolic amount in extracts from rosemary (185 mg GAE/g dw) compared with extracts from oregano and sage (149 and 166 mg GAE/g dw, respectively).

On the other hand, *Zingiberaceae* and *Apiacea* families displayed the lowest phenolic amount (8.93 and 5.98 mg GAE/g dw, respectively). Similar results were observed by Wongsa et al. (2012) who noticed values of 12.81 mg GAE/g dw in ginger. These results are in agreement with those observed by Chan et al. (2008) who find lower phenolic content in Zingiberaceae family (2.91, 2.42 and 1.62 mg GAE/g fresh weight (fw), for *Zingiber officinale*, *Z. spectabile* and *Z. ottensii*, respectively). So, the differences in the amounts of bioactive compounds may be affected by the origins of the samples.

Antioxidant capacity

Two methods have been used to measure the antioxidant activity of plants: FRAP and DPPH$^{\bullet}$ radical scavenging assays (Table 2). Total antioxidant activity, measured by the DPPH$^{\bullet}$ method, varied significantly ($P<0.001$) from 0.50 to 9.06 g trolox/100 g dw with an overall mean of 2.87 g trolox/100 g dw, after study of the samples in relation to reactional stability.

The absorbance decay over time for the reaction between DPPH$^{\bullet}$ stock solution and each one of the 13 herbs extracts were evaluated to establish the reaction time (25 to 170 μL), as presented in Fig. 1 for oregano extract, and to all the samples in Fig. 2 with volumes of the 170 μL. All the herbs showed non-linear behavior over time with similar decay tendency for the dilutions tested (data not shown). These tests allowed assessing the variation of absorbance versus time at different sample volumes (Fig. 3), setting 180 min as a period that absorption was stabilized regardless the amount extract used, being this time used to evaluate all samples.

Among six families tested in this study, *Lamiaceae* (seven tested spices) and *Lauraceae* (one tested spice) showed high mean antioxidant capacity (general means of 4.29 and 3.23 g trolox/100 g dw, respectively). However, total antioxidant capacity mean values of the other four families were significantly ($P<0.001$) lower (Table 2). *Origanum vulgare* presented the highest antioxidant amounts, considering both methods of analysis, and also it showed the highest total phenolic content measured by Folin-Ciocalteau method (74.01 mg GAE/g dw). Our comparative results of the seven spices in the *Lamiaceae* family indicated that their total antioxidant capacity decreased in the following order: oregano > lemon balm > rosemary > marjoram > salvia > basil > mint. Oregano exhibited the most powerful antioxidant capacity among the four *Lamiaceae* species, approximately seven times greater than mint. These findings are in agreement with those reported by Shan et al. (2005) who noticed higher antioxidant activity from oregano extracts (1.01 mmol trolox/g dw) compared with sage (0.52 mmol trolox/g dw), rosemary (0.38 mmol trolox/g dw) and sweet basil (0.30 mmol trolox/g dw) extracts. In addition, Tusevski et al. (2014), also observed higher antioxidant activity in extract of *Origanum vulgare* (714.15 μmol trolox/g dw) than in lemon balm (406.03 μmol trolox/g dw). In opposite, Wojdylo et al. (2007) showed higher antioxidant activity in *Rosmarinus officinalis* (5.13 μM trolox/g dw) than in *Origanum vulgare* (0.80 μM trolox/g dw) and *Melisa officinalis* (0.36 μM trolox/g dw). Finally, the other plants studied had antioxidant values between 1.25 and 5.57 g trolox/100 g dw, while only in 5 plants were the values lower than 1 g trolox/100 g dw, measured by DPPH$^{\bullet}$ method.

Total antioxidant activity, measured by the FRAP method, varied significantly ($P<0.001$) from 43.61 to 472.32 μmol trolox/g dw with an overall mean of 214.28 μmol trolox/g dw. According to their reducing ability/antioxidant power, the 13 species can be divided in five groups: (a) very low FRAP (<10 μmol/g), $n=0$; (b) low FRAP (10–50 μmol/g), $n=3$; (c) good FRAP (50–100 μmol/g), $n=2$; (d) high FRAP (100–400 μmol/g), $n=5$; very high FRAP (>400 μmol/g), $n=3$. The strongest antioxidant properties, measured by FRAP assay, were in three species of *Lamiaceae* (*Origanum vulgaris*, *O. majorana* L. and *M. officinalis*) herbs. All plants from these families exhibited higher capacity in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) than to scavenging free radicals. Wojdylo et al. (2007) noticed that *Syzygium aromaticum*, out of 32 selected herbs, exhibited the highest antioxidant activity measured by the FRAP method, followed by *R. officinalis* and *Tanacetum vulgare*. In the present study, most of the plants reduced ferric ion (Fe^{3+}) to 100–400 μmol/g dw. In contrast, the weakest abilities to reduce ferric ion were exhibited by *Pimpinella anisum*, *Mentha piperita*, *Zingiber officinalis*, *Curcuma longa* L. and *Brassica hirta*, as in previous DPPH$^{\bullet}$ method. It was interesting that, among 13 plants analyzed, no herbs were able to reduce ferric ion below 10 μmol/g dw.

Table 2 Antioxidant capacity in 13 selected plants

Plant	Family	TEAC	
		DPPH• g trolox/100 g dw	FRAP µmol trolox/g dw
Oregano (*Origanum vulgare*)	Lamiaceae	9.06±0.10[a]	472.32±15.96[a]
Marjoram (*Origanum majorana L.*)	Lamiaceae	4.62±0.35[c]	463.96±28.49[a]
Rosemary (*Rosmarinus officinalis*)	Lamiaceae	5.07±0.35[bc]	361.57±33.72[b]
Lemon balm (*Melissa officinalis*)	Lamiaceae	5.57±0.46[b]	464.83±25.96[a]
Laurel (*Laurus nobilis*)	Lauraceae	3.23±0.17[d]	157.15±8.98[d]
Sage (*Salvia officinalis*)	Lamiaceae	2.59±0.17[e]	231.60±9.50[c]
Basil (*Ocinum basilicum*)	Lamiaceae	2.26±0.15[e]	213.28±11.47[c]
Mustard (*Brassica hirta*)	Brassicaceae	0.57±0.01[g]	45.90±0.97[e]
Turmeric (*Curcuma longa L.*)	Zingiberaceae	0.96±0.02[fg]	45.25±0.29[e]
Chamomile (*Matricaria camomila*)	Asteraceae	1.25±0.09[f]	153.94±5.80[d]
Mint (*Mentha piperita*)	Lamiaceae	0.84±0.02[fg]	67.94±0.75[e]
Ginger (*Zingiber officinalis*)	Zingiberaceae	0.74±0.03[fg]	64.36±2.79[e]
Fennel (*Pimpinella anisum*)	Apiaceae	0.50±0.01[g]	43.61±0.89[e]
Significance	–	***	***

[a–g] Means in the same column not followed by a common number are significantly different ($P<0.05$; Tukey test)
Significance: *** ($P<0.001$), ** ($P<0.01$), * ($P<0.05$), n.s. = $P>0.05$
$n=09$ samples by extract

Relationship between total antioxidant capacity and total phenolic content

To correlate the phenolic compounds concentrations with the antioxidant capacities, the linear correlation coefficients (r) were calculated for the 13 plants analyzed (Fig. 4). The r between the antioxidant capacities obtained from FRAP assay and phenolic contents was 0.8866 (Fig. 4a) and the r between the antioxidant capacities obtained from DPPH• assay and phenolic contents was 0.9706 (Fig. 4b). Such high r value suggested that the DPPH• radical scavenging activity could be credibly predicted on the basis of the Folin-Ciocalteau assay for total phenolic content and directly confirmed that the phenolic compounds in the 13 plants were responsible for their antioxidant capacity. These results are in agreement with those reported by Wojdylo et al. (2007) who observed good correlations between the antioxidant capacity obtained from DPPH• assay and phenolic contents ($r=0.8352$) and between FRAP assay and phenolic contents ($r=0.9100$) for species of the *Lamiaceae* family.

In addition, Katalinic et al. (2006) also noticed a higher correlation between the amount of total phenols and the antioxidant capacity obtained from FRAP assay ($r=0.9825$). However, Wongsa et al. (2012) did not find a good correlation between the antioxidant capacity obtained from DPPH• assay and phenolic contents ($r=0.02$), indicating that phenolic compounds were not likely to contribute to the antioxidant activity. The antioxidant activity of plant extracts is not limited to phenolics (Javanmardi et al. 2003). The antioxidant activity may also contribute from the other antioxidant secondary metabolites such as volatile oils, carotenoids and vitamins (Wongsa et al. 2012).

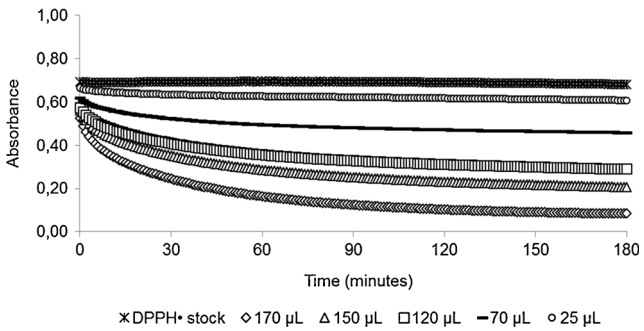

Fig 1 Curves for the DPPH• assay with different volumes of extract of oregano over time ($n=09$ samples of extract)

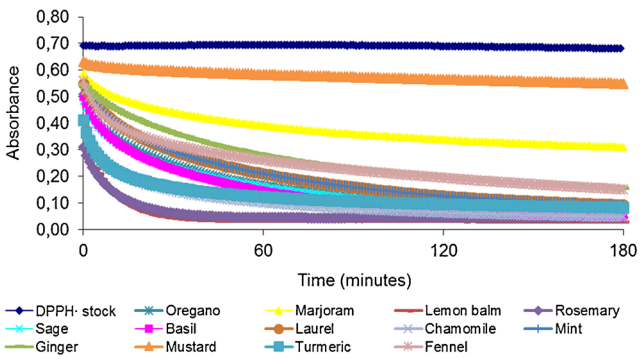

Fig 2 Curves for the DPPH• assay with different extracts of herbs and same volumes (170 µL) over time ($n=09$ samples by extract)

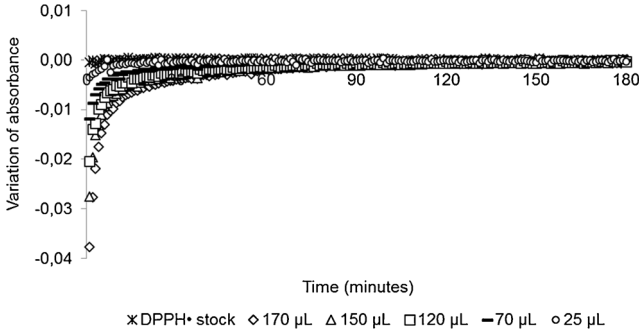

Fig 3 Variation of absorbance for volumes of oregano extract over 180 min (n=09 samples of extract)

Cluster analysis

Data on total phenolic contents and antioxidant capacity measured by the DPPH• and FRAP methods were used to carry out a cluster analysis of the 13 plants selected, and the dendrogram that was generated by cluster analysis showed three well defined groups (Fig. 5), in descending order. The first group clearly discernible is composed of oregano, marjoram, lemon balm and rosemary. These species are associated with high total phenolic content and strong antioxidant activity as measured by DPPH• and FRAP methods, and therefore this group was considered the most promising. A second group consists of laurel, chamomile, basil and sage, which is characterized by moderate phenolic amount and antioxidant capacity, while mint, ginger, fennel, turmeric and mustard belong to the third group with low phenolic content and weak antioxidant capacity.

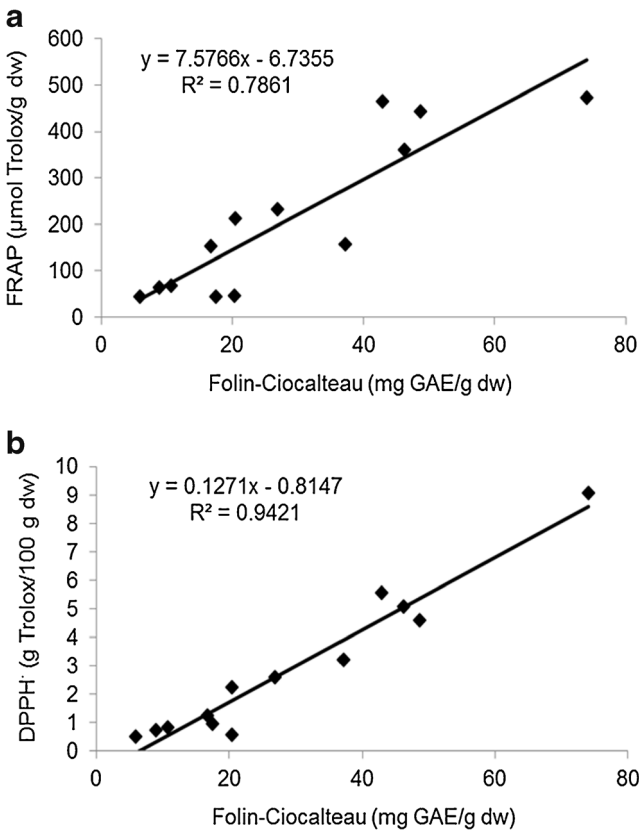

Fig 4 Linear correlation between the amount of total phenols and antioxidant capacity measured by the FRAP (**a**) and DPPH• methods (**b**) (n=09 samples by extract)

Calculate volumes of natural extracts selected to manufacture of lamb burgers

According to results of cluster analysis, the first group composed for oregano, marjoram, lemon balm and rosemary was selected to manufacture the lamb burgers (Fig. 5). Using the same colorimetric methods of antioxidant capacity, results obtained for sodium erythorbate were 608.83±46.65 mg of GAE/g dw, 100.24±30.8 g trolox equivalent/100 g dw and 7044.53±266.34 µmol trolox equivalent/g dw for Folin-Ciocalteau, inhibition of DPPH• radical and FRAP, respectively.

Table 3 shows the quantities (ppm) of natural extracts to be added to lamb burgers calculated in order to present similar antioxidant capacities to 500 ppm of sodium erythorbate. These quantities were determined by converting the average values obtained by the three methods of antioxidant capacity (Folin-Ciocalteau, DPPH• e FRAP) compared to the values obtained for antioxidant capacity of 500 ppm of sodium erythorbate (concentration usually applied to meat products). In this case, was calculated the ratio between the values obtained to the sodium erythorbate and the natural extracts, to each colorimetric method. These results were converted separately to ppm for each extract, using the conversion factor 500, to define the equivalent concentrations to be used, and consequently to be valid for comparison to synthetic antioxidant. Thereafter, the volumes of each extract were calculated in mL/kg to manufacture of the burgers from the mean values obtained from the three different colorimetric methods. For this, it was considered in the calculations the initial ratio of plant mass (g) per amount of solvent (ml) [1:50], the relationship between amounts of extract obtained initially and after concentration [25:7 (v/v)], and lyofilization process and ressuspension in ultrapure water (ml) [1:4 (v/v)].

Other authors have evaluated the application of natural antioxidants in meat products. However, these works usually do not bring information about how the quantities added were defined or calculated. In most studies the concentrations applied followed percentages based on satisfactory results obtained by other studies or indications of commercial extracts (Akarpat et al. 2008; Trindade et al. 2009; Ozogul and Uçar 2012).

Sensory analysis

For sensory evaluation (Fig. 5), among the selected participants, 71.67 % were female and 61.67 % were between 20 and

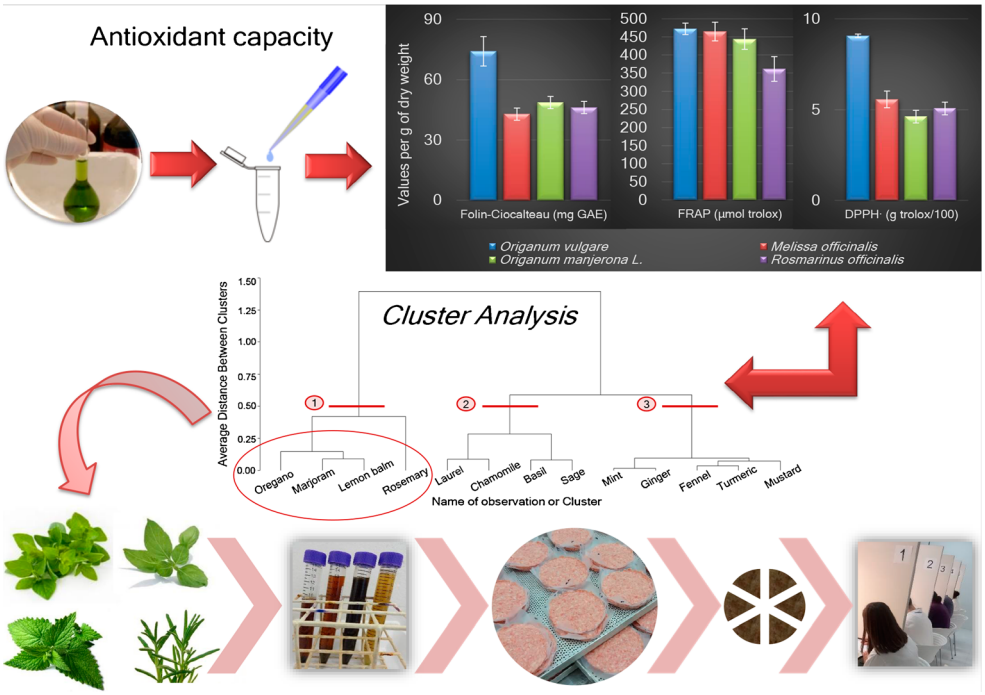

Fig 5 Steps of the study and dendrogram plot visualizing the clustering of the 13 plants studied based on their total phenolic content and antioxidant capacity measured by the FRAP and DPPH• methods

40 years old. Consumers' acceptance of different samples did not differ ($P>0.05$) for overall quality attribute (Table 4). In general, the average attributed scores were near to 7.0 ("like moderately") for all samples. Results showed that burgers with natural antioxidants were as well accepted as the one with the antioxidant usually applied by meat industry, i.e., sodium erythorbate. Also, among the four plant extracts evaluated, no one was better or worse evaluated. In other words, sensory analysis did not allow the selection of only one of the extracts, meaning that any one of them could be added to lamb burger without compromising its sensory acceptability, as well as Bozkurt (2006), who found that the addition of green tea extract, *T. spinata* oil or green tea extract combined with *T. spicata* oil in Turkish dry-fermented sausage, did not affect the overall sensory quality in day zero of storage ($P>0.05$) in comparison to control and BHT added samples.

Similarly, according to many other studies, the addition of natural extracts did not affect the overall sensory attributes in comparison of control treatment. Beal et al. (2011) evaluated effects of mate leaves extract in different concentrations, sodium erythorbate and control (without antioxidant) in fermented Italian-type sausages, stored at 18 °C for 60 days, being that the global acceptance was not affected by the addition of mate extract in relation to control and the formulation using synthetic antioxidant. According to Lara et al. (2011), differences in sensory attributes among treatments (control, BHT, extracts of rosemary and lemon balm) were also not perceived by panelists in cooked pork patties packed in Modified Atmosphere Packaging (MAP), storage for 6 days under refrigeration (4±1 °C).

Table 3 Concentrations of natural extracts calculated to be equivalent to the antioxidant capacity of 500 ppm of sodium erythorbate and the volumes of each extract necessary to reach the calculated concentrations (mean ± standard deviation)

Extract	Equivalence[1]	Volume[2]
Oregano	5710.75±159.64	18.37±1.10
Marjoram	8376.80±579.20	26.51±3.44
Lemon balm	7916.89±548.93	26.62±1.13
Rosemary	8774.63±649.02	29.69±2.23

[1] Results expressed in ppm; [2] Results expressed in mL/kg of meat product

n=09 samples by extract

Table 4 Acceptance test of burger samples with the four natural antioxidants and sodium erythorbate

Treatment	Sensory attribute Overall quality M[1]±SE[2]
Marjoram	6.95±1.44[a]
Sodium erythorbate	6.88±1.44[a]
Oregano	6.87±1.44[a]
Rosemary	6.85±1.44[a]
Lemon balm	6.42±1.44[a]

[1] Mean; [2] Standard Error

[a] Means in the same column followed by a common number not are significantly different ($P<0.05$; Tukey test)

n=60 consumers by treatment

Conclusions

In summary, this study showed that the results further support the view that some plants are promising sources of natural antioxidants. Total phenol content and total antioxidant capacity differs significantly among 13 selected plants. Then, cluster analysis was useful and allowed the selection of best plant extracts based on the results of different antioxidant capacity methods, giving that, among the 13 plant extracts evaluated, the best results were obtained for oregano, marjoram, lemon balm and rosemary.

From the results obtained in the sensory evaluation of lamb burgers made with the extracts which showed better antioxidant capacity, it can be concluded that the four aromatic herbs represent a good alternative and a quite viable solution for the replacement of synthetic antioxidant. In this case, natural antioxidants derived from spices could be able to reduce changes that cause deterioration of meat products, besides offering a good choice to the formulation of products with healthier appeal, without compromising sensory acceptance of lamb burgers.

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