CHANGES IN ESSENTIAL OIL OF *ORIGANUM VULGARE* L. AFFECTED BY DIFFERENT EXTRACTION METHODS

Kátia Yuri Fausta Kawase [1,*], Cheila Gonçalves Mothé[2], Filipe Arantes Furtado[3] & Gerson Luiz Vieira Coelho[4]

[1,2] Federal University of Rio de Janeiro (UFRJ), 21949-900 Rio de Janeiro, Brazil
[3,4]Federal Rural University of Rio de Janeiro (UFRRJ), 23890-000 Seropédica, Brazil

ABSTRACT

Phytochemicals from oregano essential oil (*Origanum vulgare L.*) obtained from supercritical fluid extraction (SFE), hydrodistillation, Soxhlet (ethanol and hexane) were determined by GC-MS. Antioxidant activity and thermal analysis were the methods used to evaluate the extracts obtained. Synthetic antioxidant ascorbic acid and butyl hydroxyl toluene (BHT) were used as the standard phenol. Higher concentration of carvacrol and thymol was observed in the oil obtained by hydrodistillation, on the other hand, the better result in antioxidant activity was observed in Soxhlet with ethanol for 6 h (IC_{50} = 130.69 ± 1.21 µg/ml), this results indicates that the antioxidant effectiveness of the oregano essential oil was not related to a higher concentration of these phenolic compounds. Finally, this study compares the thermal analysis of oregano essential oil with BHT and ascorbic acid. The results indicate that oregano essential oil (oil resin) obtained by Soxhlet could be used to substitute synthetic antioxidants in foods products, therefore other compounds that are extracted together should be studied to clarify the higher antioxidant activity of this extract.

Keywords: *Phenolic compounds, GC-MS, Supercritical extraction, Thermal analysis.*

1. INTRODUCTION

Herbs have been widely used to extend the shelf life of food products. Oregano (*Origanumvulgare*) is an important herbaceous plant, rich in phenolic compounds with powerful anti-bacterial and anti-fungal properties [1-2]. Oregano essential oil presents numerous types of antioxidants with diverse activities, such as the ones identified as carvacrol and thymol [3]. And, the use of oregano essential oil as antioxidants in food are being describe, like in functional dairy beverage formulations and in meat [4-5].

According to Vijaya and Reddy [6], recent studies indicate that the current human lifestyle cause an increased production of free radicals and oxidative compounds. These same studies suggest that the increase of natural products consumption with antioxidant compounds decrease the risk of some degenerative diseases. Before this, consumers generally perceive natural antioxidants as better than synthetic additives.Due to this, natural antioxidants are being explored as alternatives to synthetic food antioxidants.

However, the applicability of natural products such essential oils in the development of new pharmaceuticals, food additives and industrial products require an appropriate extraction method that doesn't alter the properties. Due to this, supercritical fluid extraction (SFE), that is an alternative to conventional extraction methods, was used in this work; supercritical fluid extraction avoids thermal degeneration of thermolabile compounds and avoids solute contamination with solvent residues when compared to steam distillation or Soxhlet extraction [7-8]. The characteristics of supercritical fluids allow for more effective penetration in the plant matrix with relatively high density which improves the extraction [9-10].

Phenols are one of themost important groups of natural antioxidants and occur only in material of plant origin, and it is because the phenolic content and activity antioxidant is normally correlated in various study [11]

The techniques of Thermal Analysis like the thermogravimetry (TG), derivative thermogravimetry (DTG) and differential thermal analysis (DTA) can show more about the thermal stability of substances or materials, moisture and volatiles content of materials, oxidative stability of substances, and composition of systems. These characteristics could be important to available the essential oils obtained by different processes [12-13].

Therefore, the aim of the present study was to evaluate the changes in essential oil of *origanum vulgare L.* as caused by different extraction methods (hydrodistillation, Soxhlet and supercritical fluid extraction) and compare these extracts with synthetic antioxidant ascorbic acid and BHT (2,6-bis (1,1-dimethylethyl)-4-methylphenol), according their phenolic content, antioxidant activity and thermal decomposition behavior.

2. MATERIAL AND METHODS

2.1. Reagents and Material

Samples *of Origanumvulgare Linnaeus* were collected at Rio de Janeiro - Brazil (Rei do Mato Farm) at post-flowering stage. The sample was collected in June–August 2010, during autumn and winter**.** The reagents used were purchased from Sigma-Aldrich Chemical, except carbon dioxide, which was obtained from Linde Gases Ltda and ascorbic acid (VETEC).

The plant material was separated into leaves and branches, sifted to remove dirt, dried in an oven for a period of 24 hours and stored in airtight plastic bags. The Sauter mean diameter of the dried leaves was found to be 1.69 mm from the screen analysis using the following equation:

$$\overline{D} = \frac{1}{\sum_{i=1}^{n} \frac{x_i}{\overline{d}_i}}$$

Where n is the number of increments, x_i is the mass fraction in a given increment and d_i is the average particle diameter taken as the arithmetic mean of the openings of two consecutive screens.

2.2. Essential Oil Extraction

2.2.1. Hydrodistillation

Conventional hydrodistillation was carried out with a Clevenger-type apparatus and using samples of at least 10 g of dried leaves. The experiments were performed in triplicate for 2 h. The essential oil obtained was collected in vials, dehydrated with anhydrous sodium sulfate and kept under refrigeration until being analyzed.

2.2.3. Soxhlet extraction procedure

Dried oregano samples were placed in a cellulose thimble and inserted into a Soxhlet assembly fitted with 250 ml flasks. Different procedures were performed in triplicate for 4-6 h using ethanol and hexane as solvents. A 150 ml portion of solvent was added and the whole assembly was heatedand maintained in continuous reflux.Subsequently, the extracts were concentrated using a rotary evaporator connected with a vacuum pump to remove the solvent, stored in amber bottles and kept under refrigeration until being analyzed.

2.2.4. Supercritical CO_2 extraction

The oregano oil was extracted by supercritical CO_2. An 18 ml extractor vessel was packed with 3 g of dried sample. The experiment was conducted in a continuous process with the passage of carbon dioxide in the solid matrix.The pressure and temperature in the extraction vessel was adjusted to 120 bar and 50 ºC with a pump and a thermostatic bath, respectively The process was performed for approximately 2 h.The extracted essential oil was collected through a stainless steel capillary tube in a cold trap containing isopropanol. The extraction was performed in triplicates and the samples were stored under refrigeration before analysis.

2.3. Essential Oil Analyses

2.3.1. Gas chromatography

GC analyses were carried out on Shimadzu GCMS-QP 2010, with a DB-5 MS capillary column (30 m x 0.25 mm x 0.25 µm of cross-linked Phenyl-Methyl Siloxane) and equipped with a flame ionization detector (FID). The column temperature was programmed from 60 to 250 °C at a rate of 5 °C/min. The injector and detector temperatures were programmed at 260 and 280 °C, respectively. Helium was used as carrier gas, flow rate 1.1 ml/min.

2.3.2. Gas chromatography-mass spectrometry

GC-MS of the essential oils was carried out using a Shimadzu GCMS-QP 2010 operating in EI mode (equipped with a DB-5 MS capillary column 30 m x 0.25 mm x 0.25 µm). Helium (1.1 ml/min) was used as carrier gas in both cases. Injector and transfer line temperatures were 260 °C and 230 °C; detector temperature was set at 280 °C. The initial temperature of the column was 60 °C at 5 C/min for 5 min; then it was raised to 250 °C for 17 min. The ionization energy was 70 eV. Signals were recorded in full scan mode (m/z 35-450).

2.3.3. Identification of components

All components were identified by comparison of theirs mass spectra with those obtained from authentic samples and/or the NIST05 mass spectral database. They have been also confirmed by comparison of their retention indices (RI) and retention times, with published data; and MS data with those obtained from authentic references standards (thymol and carvacrol, Sigma-Aldrich) [14-15].

2.3.4. Determination of the total phenolic content

The amount of total phenolic content was determined by the Folin–Ciocalteau method, according to Woisky and Salatino (1998), with some modifications [16]. Ethanolic extracts (1.5 ml volume with two replicates) at 0.02-0.5 mg/ml were introduced into the test tubes containing 1.5 ml of Folin-Ciocalteau reagent (Sigma-Aldrich¸Germany) diluted in water (1:10). After 8 min, 1.2 ml of 4% sodium carbonate was added. The tubes were mixed and allowed to stand for 2h. The absorbance was measured at 740 nm in a Bell 1105 spectrophotometer. A blank measure, for which the sample was replaced by water, was subtracted from the absorbance at 740 nm. Quantification was obtained from a calibration curve using butylatedhydroxytoluene (BHT) as the standard phenol (R^2 = 0.998). Results were expressed in mg of BHT/100 g of dry sample.

2.3.5. Radical scavenging activity and antioxidant content

The scavenging activity of samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Meda *et al.*(2005), with some modifications [17]. Ethanolic extracts (3 ml) at different concentrations (0.04 to 1.00 g/ml) were added to 0.1 ml of DPPH (Sigma-Aldrich, Germany) ethanolic solution (0.1 mM), with ethanol serving as the blank sample. The mixtures were maintained in the dark for 30 min at room temperature. The antioxidant content was determined using standard curves for BHT and ascorbic acid. The radical scavenging activity was calculated as follows as:

% Inhibition = [(blank absorbance - sample absorbance)/blank absorbance] x 100.

The mean of three IC_{50} (concentration causing 50% inhibition) values from each sample was determined. Concentrations were expressed in µg/ml.

2.3.6. Thermal analysis

Thermoanalytical techniques, in particular Thermogravimetry Analysis (TG), Derivative Thermogravimetry (DTG), were used in this study. Non-isothermal TG, DTG and DTA were performed using a (TA Instruments) SDT 2960 DTA-TGA analyzer with temperatures programmed at 10 ºC/min heating rate, from a room temperature at 25 ºC to 800 ºC in a N_2 atmosphere. Samples around 10 mg were used.

2.4. Statistical Analysis

All data were expressed as means ± standard deviation (n = 3). Data were analyzed by means of the one-way ANOVA test (using XLSTAT for Windows2010). Significant differences between the means of parameters were determined using Duncan's test (p< 0.05).

3. RESULTS AND DISCUSSION

3.1. Phytochemistry

In this study, 14 compounds in the highest percentage of oregano essential oil (*Origanum vulgare L.*) are shown in table 1. The major component of the essential oil obtained by Soxhlet and SFE methods of extraction was cis-sabinene hydrate, and this compound was obtained in high amount by hydrodistilation (37.5%). Sabinene is used in the preparation of various artificial essential oils [18].

Table 1. Composition (% w/w) of Origanum vulgare L. oils obtained by hidrodistilation, SFE and Soxhlet.

Name of Compound	RI [a]	RI[b]	Extraction methods Concentration (%w/w)					
			Hydrodistillation (2h)	CO_2 SFE (120bar/50°C/2h)	Soxhlet (ethanol)		Soxhlet (hexane)	
					4 h	6 h	4 h	6 h
Monoterpene hydrocarbons								
α-Tujene	930	920*	0.03	1.80	-	-	0.25	-
Sabinene	977	972*	0.10	7.54	-	0.21	1.00	0.98
β-Myrcene	993	991*	0.03	3.54	-	0.19	0.63	0.74
p-Cymene	1029	1024*	0.11	8.50	0.34	0.47	0.90	0.72
Trans-β-ocymene	1041	1044**	0.05	7.34	0.71	0.50	2.86	2.31
γ – terpinen	1063	1064*	0.10	9.80	-	-	-	-
Trans-Sabinen hydrate	1072	1074**	0.57	-	0.32	0.13	-	0.68
Cis-sabinene hydrate	1105	1010**	37.53	17.94	24.24	32.61	27.16	27.74
Oxygenated monoterpene								
4-Terpineol	1185	1183**	8.03	0.44	0.40	0.45	0.34	0.31
α-Terpineol	1197	1190*	4.51	0.46	1.07	1.88	1.21	1.25
Geraniol	1260	1256*	0.60	1.70	5.84	4.35	5.70	7.35
Thymol	1298	1292*	39.15	0.93	17.84	21.27	23.23	20.96
Carvacrol	1307	1300*	0.84	-	0.27	0.45	0.31	0.33
Sesquiterpene								
Spathulenol	1596	1587**	0.37	-	0.28	0.92	0.18	0.35

[a] *Kovats retention indices on DB-5MS column.*
[b] *Database of Kovats retention indices on DB-5MS column .* NIST. ** Novak et al. (2002) [14-15].*

The hydrodistillation of dried samples for 2 h produced a low yield in essential oil (0.45% ± 0.02, w/w) (table 2). The GC and GC/MS analysis of this essential oil led to the identification and quantification of 14 components (figure 1a) which accounted for 92.2% of the total oil. Thymol (39.15%) was the most abundant component in the essential oil and similar to carvacrol (0.84%), it is a phytochemical that presents antioxidant activityand effective antimicrobial activity against food pathogens i.e. *Salmonella typhimurium*, *Bacillus cereus* and *Staphilococcus aureus* [19-21]. This method of extraction produced the higher yield (%) of these main biologically active compounds compared with the other methods used in this study. The GC/MS analysis of authentic samples of thymol and carvacrol are shown in figure 1b.

Table 2. Extraction yield and phenolic content of essential oil extracted from Origanumvulgare L essential oil obtained by different methods.

Extraction methods	Extraction yield (%w/w ± SD)	Phenolic content (mg BHT/100g ± SD)
Hydrodistillation	0.45 ± 0.02 a	7.74 ± 0.12 a
Soxhlet (ethanol/ 4h)	19.25 ± 1.39 b	1.80 ± 0.09 b
Soxhlet (ethanol/ 6h)	26.49 ± 1.71 c	3.92 ± 0.12 c
Soxhlet (hexane/ 4h)	8.67 ± 1.36 d	-
Soxhlet (hexane/ 6h)	7.17 ± 1.04 d	-

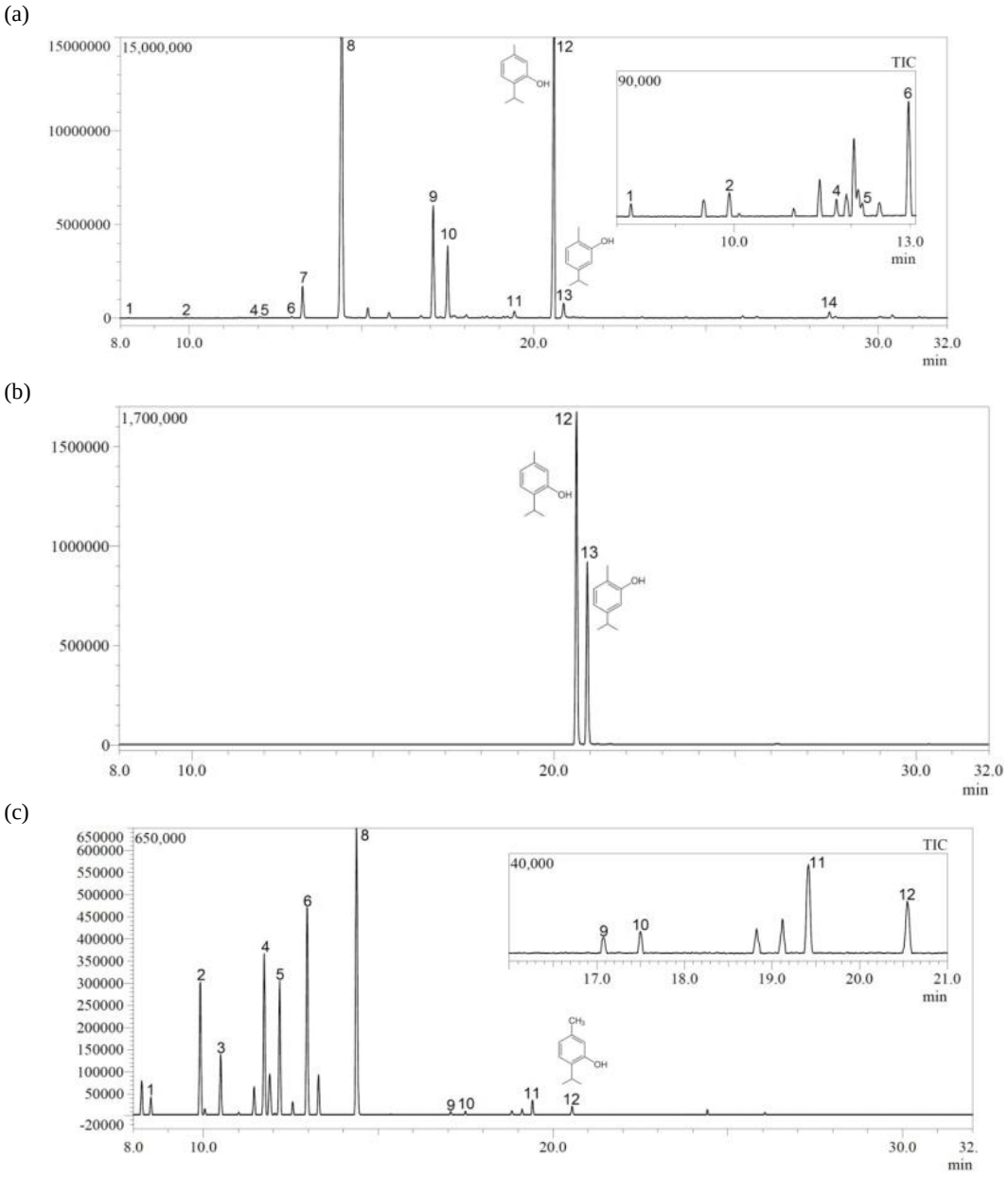

Figure 1. Total ion chromatogram (obtained by GC-MS analysis) of oregano essential oil extracted by hydrodistillation (2h) (a), authentic thymol and carvacrol (b), and SFE (50 °C/120 bar/ 2h) (c), considered similarities higher than 90%; and of the (1: α–thujene, 2: sabinene, 3: β–myrcene, 4: p–cymene, 5: β–trans–ocimene, 6: γ – terpinene, 7: trans-sabinene hydrate, 8: cis-sabinene hydrate, 9: 4–terpineol, 10: α–terpineol, 11: cis-geraniol, 12: thymol, 13: carvacrol, 14: spathulenol.

The experimental conditions for the SFE in this study (120 bar, 50 ºC, 2h) did not show good results in terms of yield of antioxidant compounds. This method given a low concentration of thymol (0.93%) and did not extract carvacrol (table 1, figure 1c). It was more selective in extracting volatile compoundsas myrcene and cis-sabinene hydrate.

The results indicate the need to improve the conditions applied in the SFE in order to obtain more antioxidants compounds. The use of a density higher than the one used in this study (around 0.58 g/cm^3), exposed to lower temperature and/or higher pressure could improve the yield of the phenolic monoterpenescarvacrol and thymol [22-24].

The Soxhlet extraction with ethanol for 6 h produced higher yield in oil/dried matter (26.49% ± 7.71) compared with the time of 4 h (19.25% ± 1.39), however with hexane the yield did not share significant differences at $p < 0.05$ between 4 and 6 h (8.67% ± 2.36 and 7.17% ± 1.04) (table 2). These conditions (Soxhlet extraction with ethanol for 6 h) also showed a better thymol (21.27%) and carvacrol (0.48%) yield in this study. The extractions with ethanol solvent yielded higher phenolic compounds concentration with the increase of the extraction time [25].

However the integrity of the analyte may not be maintained under the conditions used in the Soxhlet extraction, i.e., thermal decomposition or reaction with the solvent may occur [26]. This can explain the lower concentration of thymol and carvacrol obtained with this method when compared with the method of hydrodistillation.

Beside this, conditions different than the ones used (around 45 ºC and 24 h) to dry the oregano plant parts can be tested to avoid the loss of interesting compounds since drying the samples in a convection oven (using hot air) leads to significant volatile losses [27].

The lower concentration of monoterpene hydrocarbons (i.e. cymene) obtained from the hydrodistillation and the Soxhlet methods is due to their instability when exposed to heat which can cause their degradation by thermal or hydrolytic effects [28]. However the decrease of cymene can implicate in an increase of carvacrol, because cymene is a carvacrol biological precursor [29]. These inferences do not apply to the SFE method since it uses lower temperatures.

3.2. Total Phenolic Content Determination

The evaluation of phenolic compounds was performed only for samples extracted by hydrodistillation and by Soxhlet with ethanol. The extract obtained by the SFE was not evaluated due to the low yield of the target compounds. The essential oil obtained by Soxhlet with hexane, even showed similar concentrations of these compounds with ethanol, was not assessed due to toxicity of the solvent that would prevent its application in food products. The total phenolics content was measured by the Folin-Ciocalteau method using a standard curve of BHT solutions. BHT is a synthetic antioxidant, used since 1950 in food products to stabilize the freshness and to avoid nutritive value, flavor and color losses [30-31].

The Folin-Ciocalteau method was used in this work to evaluate the phenolic compounds and the means was to compare essential oil extraction methods as Soxhlet with ethanol and the hydrodistillation.
The amount of total phenolics content in the extracted essential oil using hydrodistillation was 700.74 ± 0.12 (mg BHT/100g), the extract obtained using Soxhlet with ethanol for 4h and 6h were 100.80 ± 0.09 (mg BHT/100g) and 300.92 ± 0.12 (mg BHT/100g) (table 2).

Phenolic substances such as phenolic acids, exhibit antioxidant activity mainly due to its reducing properties and chemical structure that act by neutralizing or sequestering free radicals preventing the propagation of the oxidation process [32].

3.3. Antioxidant Activity – DPPH Free Radical-Scavenging Assay

This method was used in the essential oil obtained from the hydrodistillation and Soxhlet with ethanol extractions. The IC_{50} are presented in table 3. The antioxidant activities of these oils obtained were compared with the standard commercial synthetic antioxidants BHT and ascorbic acid, carvacrol, thymol and oregano essential oil. Thymol and carvacrol (IC_{50} = 0.42 ± 0.00 µg/ml and 0.45 ± 0.01 µg/ml, respectively) were more effective than synthetic antioxidants BHT and ascorbic acid (IC_{50} = 26.23 ± 0.20 µg/ml and 19.44 ± 0.26 µg/ml, respectively); and the essential oil obtained were less effective than these four standard samples.

Table 3. Antioxidant activity (DPPH free radical-scavenging assay) of carvacrol, thymol, ascorbic acid, BHT, commercial oregano essential oil and essential oil extracted from Origanumvulgare L essential oil obtained by different methods.

Compound	**Antioxidant activity (DPPH) - IC_{50} (µg/ml ± SD)***
Carvacrol	0.42 ± 0.00 a
Thymol	0.45 ± 0.01 a
Ascorbic acid	19.44 ± 0.26 a
BHT	26.23 ± 0.20 a
Commercial essential oil	392.93 ± 14.00 b
Essential oil (Soxhlet/ethanol/4h)	435.80 ± 4.85 c
Essential oil (Soxhlet/ethanol/6h)	130.69 ± 1.21 d
Essential oil (hydrodistillation)	509.90 ± 23.01 d

**IC_{50}, 50% inhibitory concentration; SD, standard deviation.Different letters indicate significant differences (P<0.05 ANOVA).*

Higher antioxidant activity of extracted essential oil was the oil obtained by Soxhlet with ethanol for 6 h (IC_{50} = 130.69 ± 1.21), that showed higher antioxidant activity in comparison with the oil obtained with lower time (4h) and with commercial oregano essential oil (IC_{50} = 392.933 ± 14.00 µg/ml). The hydrodistillation for 2 h extracted more antioxidants compounds (carvacrol and thymol) that are in agreement with the higher amount of total phenolics comparing with other oils extracted; however this essential oil showed a low antioxidant activity as demonstrated by the high IC_{50} value (509.90 ± 23.01 µg/ml).

The antioxidant synergism between thymol and carvacrol was undoubtedly confirmed by Milos and Makota (2012), so a higher concentration of both compounds is positive do antioxidant activity [33]. Moreover, the study of other components is necessary to evaluated possible synergistic effects on the total oil antioxidant activity potential that can explain the higher amount of phenolics and low antioxidant activity of essential oil obtained by hydrodistillation. The antioxidant activity is not only dependent on a different chemical constitution and on the concentration of phenolic compounds, but also other than phenolic compounds, which also function as oxidation inhibitors, such as carotenoids and ascorbic acid [34].

Besides this, combinations of natural antioxidants with synergistic action to increase the likelihood of acceptance in the sensory analysis are important. According Govaris*et al.* (2010), the increase from 0.6% to 0.9% essential oil of oregano in the meat has a negative impact on sensory properties [1].

3.4. Thermal analysis

The thermal analysis was performed on the synthetic antioxidants ascorbic acid and BHT and on the essential oil obtained by Soxhlet with ethanol for 6 h (sample that presented the higher antioxidant activity in this study). The TG, DTG and DTA curves of these samples are presented in figures 2-4.

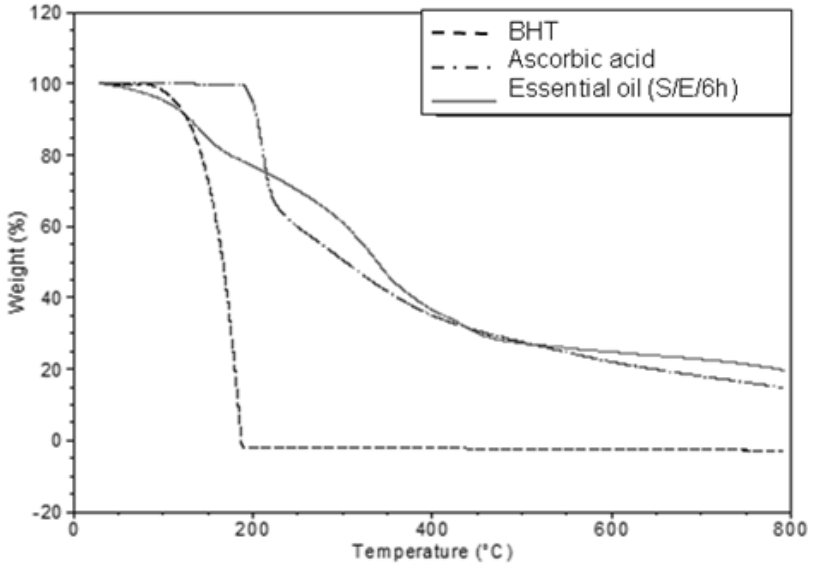

Figure 2.Thermogravimetric curves of oregano essential oil obtained by Soxhlet with ethanol for 6 h, BHT and ascorbic acid.

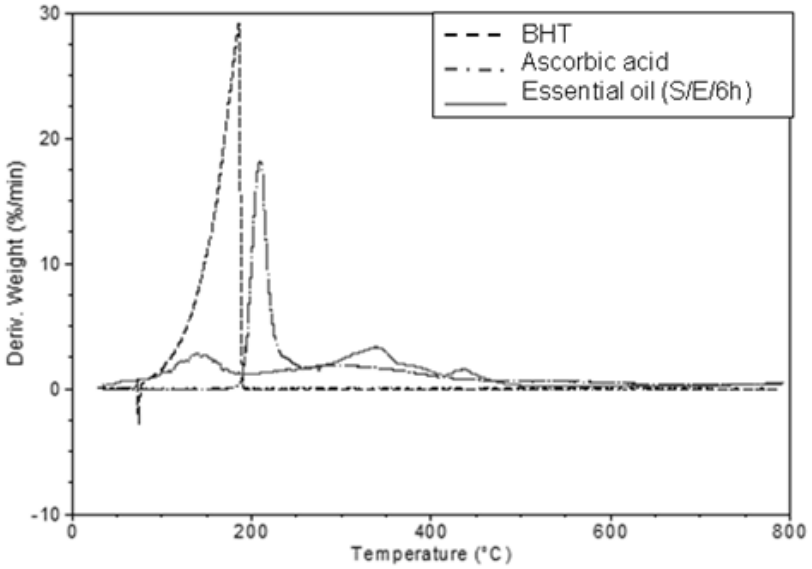

Figure 3. Derivative thermogravimetric curves of oregano essential oil obtained by Soxhlet with ethanol for 6 h, BHT and ascorbic acid.

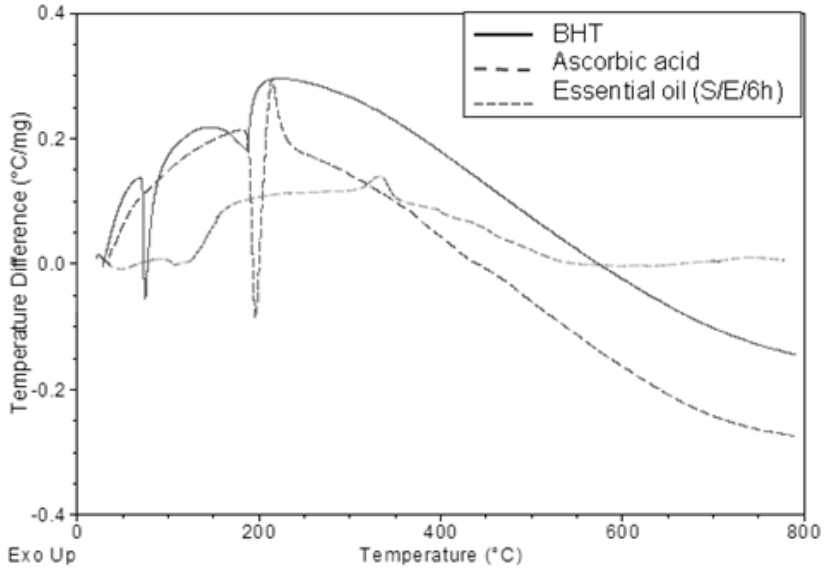

Figure 4. Differential thermal analysis curves of oregano essential oil obtained by Soxhlet with ethanol for 6 h, BHT and ascorbic acid.

Figure 2 shows a comparison of TG curves for synthetic antioxidant ascorbic acid, BHT and essential oil using Soxhlet with ethanol for 6 h. TG curve for essential oil using Soxhlet presents two decomposition stages at 70 and 250 ºC, suggesting the decomposition of ethanol and essential oil respectively and it was observed 20 % of residual at 800 ºC. Ascorbic acid showed higher thermal stability until the temperature of 190 ºC; two decomposition stages could be observed at 200 and 300 ºC, with 38 % of mass loss at the first stage referent to the organic compounds, at 800 ºC it was observed 16% of residual, suggesting the presence of inorganic compounds. BHT sample a mass loss of approximately 100% from 150 to 190 ºC was observed, and it did not present residual at 800 ºC; indicates a pure product. But, even ascorbic acid have low purity related with BHT, this acid showed a straight lower antioxidant activity compared with BHT, but with no significance at $p < 0.05$ (table 3).

The DTG curves (figure 3) confirmed one decomposition stage for BHT and it showed the temperature in which the rate of mass change is maximum at 180 ºC. For ascorbic acid it exhibited a maximum temperature at 200 ºC and three small decomposition stages at 140, 340 and 440 ºC (due the resin degradation) for essential oil using Soxhlet, suggesting a different thermal behavior.

The DTA curve (figure 4) of oregano essential oil (Soxhlet with ethanol for 6h) showed two endothermic peaks, at 50 ºC and 130 ºC; and one exothermic at 350 ºC due the triglyceride oxidation. The DTA curve of BHT showed two endothermic peaks at 75 ºC and 195 ºC, due to fusion and degradation respectively, while the DTA curve of ascorbic acid showed one endothermic peak at 195 ºC due to fusion and one exothermic peak at 220 ºC due to oxidative degradation.

These results indicate need to improve the extraction process (Soxhlet) in order to obtain a pure oregano essential oil. However, the influence of these other compounds presents in the extract should be study to explain increasing of antioxidant activity of the oregano essential oil (oil resin) obtained.

4. CONCLUSIONS

It is concluded that the conventional methods applied in this study were promising and the extracted oil presented a high percentage of thymol and carvacrol in its composition. On the other hand, the antioxidant activity was not high in the oils with the greatest concentrations of these compounds.

Dates obtained in this study indicate that oregano oil could be used as an alternative to other synthetic antioxidant. However, the study of the synergism of the phytochemical compounds and the influence of other compounds extraction with oregano essential oil by Soxhlet is necessary to explore antioxidant effectiveness.

5. ACKNOWLEDGEMENTS

We acknowledge financial support from CNPq and FAPERJ.

7. REFERENCES

[1] A. Govaris, N. Solomakos, A. Pexara, P. S. Chatzopoulou, The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella* Enteritidis in minced sheep meat during refrigerated storage, *Int. J. Food Microbiol.* **13**7, 175-180 (2010).

[2] A. Figiel, A. Szumny, A. Gutirrez-Ortz, A.A. Carbonell-Barrachina, Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method, *J. Food Eng.* **98**, 240-247(2010).

[3] A.P. Raina, K.S. Negi, Essential oil composition of *Origanum majorana* and *Origanum vulgare* pp. *Hirtum* growing in India, *Chem. Nat. Comp.* **47**, 1015-1017 (2011).

[4] M. Boroski, H.J. Giroux, H. Sabik, H.V. Petit, J.V. Visentainer, P.T. Matumoto-Pintro, M. Britten, Use of oregano extract and oregano essential oil as antioxidants in functional dairy beverage formulations, *LWT- Food Sci. Technol.* **47**, 167-174 (2012).

[5] M.K. Fasseas, K.C. Mountzouris, P.A. Tarantilis, M. Polissiou, G. Zervas, Antioxidant activity in meat treated with oregano and sage essential oils, *Food Chem.* **106**, 11881194 (2007).

[6] C. Vijaya, K. Reddy, Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Res. Int.* **3**, 385-388 (2010).

[7] A. Ocaa-Fuentes, E. Arranz-Gutirrez, F.J. Seorans, G. Reglero, Supercritical fluid extraction of oregano (*Origanumvulgare*) essential oils: Anti-inflammatory properties based on cytokine response on THP-1 macrophages, *Food Chem. Toxicol.* **48**, 1568-1575 (2010).

[8] M. Herrero, A. Cifuentes, E. Ibanez, Sub and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review, *Food Chem.* **98**,136-148 (2006).

[9] G.L.V. Coelho, Modelagem do processo de extração com fluido supercrítico, *Cinc. Tecn. Alim.***14**, 186-196 (1994).

[10] G. Brunner, "Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes". Steinkopff Darmstadt Springer, New York (1994).

[11] M. Karpińska, J. Borowski, M. Danowska-Oziewics. The use of natural antioxidants in ready-to-serve food, *Food Chem.* **72**, 5-9 (2001).

[12] C.G. Mothé, A.D. Azevedo, "Análise Térmica de Materiais". Artliber, São Paulo (2009).

[13] W. Wendlandt, "Thermal analysis". Wiley Sons, New York (1986).

[14] NIST Chemistry WebBook. *National Institute of Standards and Technology*, http://webbook.nist.gov/chemistry.

[15] J. Novak, J. Langbehn, F. Panck, M. Chlodwig, C.M. Franzl, Essential oil compounds in a historical sample of marjoram (*Origanum majorana* L., Lamiaceae), *Flav. Fragr. J.* **17**, 175180 (2002).

[16] R.G. Woisky, A. Salatino, Analysis of propolis: some parameters and procedures for chemical quality control, *J. Agric. Res.* **37,** 99105 (1998).

[17] A. Meda, C.E. Lamien, M. Romito, J. Millogo, O.G. Nacoulma, Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity, *Food Chem.* **91**,571-577 (2005).
[18] M. Rossato, A.C.A. Santos, L.A. Serafini, F. Agostini, M.R. Pansera, R. Wasum, R.L. Barbieri, Avaliação do óleo essencial de Aloy siasellowii (briquet) moldenke (verbenaceae) do sul do Brasil, *Quím. Nova* **29**, 200-202 (2006).
[19] S. Delfine, F. Loreto, P. Pinelli, R. Tognttti, A. Alvino, Isoprenoids content and photosynthetic limitations in rosemary and spearmint plants under water stress, *Agric. Ecos. Environ*. **106**, 243-252 (2005).
[20] A.I. Nazer, A. Kobilinsky, J.L. Tholozan, F. Dubois-Brissonnet, Combinations of food antimicrobials at low levels to inhibit the growth of *Salmonella*sv. Typhimurium: a synergistic effect? *Food Microbiol*. **22**, 391-398 (2005).
[21] R.J.W. Lambert, P.N. Skandamis, P.J.A. Coote, Study of the minimum inhibitory concentration and mode of action of oregano essentials oil, thymol and carvacrol, *J. Appl. Microbiol*. **91**, 453-462 (2001).
[22] S. Cavero, M.R. Garca-Risco, F.R. Marn, L. Jaime, S. Santoyo, F.J. Seorns, G. Reglero, E. Ibaez, Supercritical fluid extraction of antioxidant compounds from organo – Chemical and functional characterization via LC-MS and in vitro assays, *J. Superc. Fluids* **38**, 62-69 (2006).
[23] M. Mazutti, B. Beledelli, A.J. Mossi, R.L. Cansian, C. Dariva, J.V. De Oliveira, Caracterização química de extratos de *Ocimum basilicum* L. obtidos através de extratos com CO_2 a altas pressões, *Quím. Nova* **29**, 1198-1202 (2006).
[24] E. Reverchon, G. Della Porta, Supercritical carbon dioxide extraction and fractionation of lavander essential oil and waxes, *J. Agric. Food Chem.* **43**, 1654-1658 (1995).
[25] B. Lapornik, M. Prosek, A.G. Wondra, Comparision of extracts prepared from plant by-products using different solvents and extraction time, *J. Food Eng.* 7**1,** 214- 222 (2005).
[26] S.M. Pourmortazavi, S.S. Hajimirsadeghi, Supercritical fluid extraction in plant essential and volatile oil analysis, *J. Chrom. A* **1163**, 2-24 (2007).
[27] M.C. Daz-Maroto, E. Snchez Palomo, L. Castro, M.A. Gonzlez Vias, M.S. Prez-Coelho, Changes produced in the aroma compounds and structural integrity of basil (*Ocimum basilicum L.*) during drying, *J. Sci. Food Agric.* **84,** 2070-2076 (2004).
[28] B. Bayramoglu, S. Sahin, G. Sumuru, Solvent-free microwave extraction of essential oil from oregano, *J. Food Eng.* **88**, 535-540 (2008).
[29] A. Ultee, M.H. Bennik, R. Moezelaar. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus, *Appl. Environ. Microbiol.* **68**, 1561-1568 (2002).
[30] IARC, Monographs on the evaluation of carcinogenic risk to humans. Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation, *International Agency of Research on Cancer Lyon* (1986).
[31] JECFA, Toxicological evaluation of certain food additives and contaminants in food, WHO Food Additives Series, Geneva, *Joint FAO/WHO Expert Committee on Food Additives* (1996).
[32] A. Delazar, B. Talischi, H. Nazemiyeh, H. Rezazadeh, L. Nahar, S.D. Sarker, Chrozophorin: a new acylated flavone glucoside from *Chrozophoratinctoria* (Euphorbiaceae), *Rev. Bras. Farmacogn.* **16**, 286-290 (2006).
[33] M. Milos, D. Makota, Investigation of antioxidant synergisms and antagonisms among thymol, carvacrol, thymoquinone and p-cymene in a model system using the Briggs Rauscher oscillating reaction, *Food Chem.* **131**, 296299 (2012).
[34] E. Capecka, A. Mareczek, M. Leja, Antioxidant activity of fresh and dry herbs of some Lamiaceae species, *Food Chem.* **93**, 223-226 (2005).