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CARUM COPTICUM ESSENTIAL OILS AS NATURAL ANTIOXIDANT IN DRESSING

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Abstract

In the present study, *Carum copticum* essential oils (EOs) were extracted by Ohmic assisted hydro distillation (OAHD) and Ohmic ultrasonic extractor (OUE), and extraction parameters and EOs were compared.

OUE method extracts EOs faster (17 min. vs. 20 min.) with approximately same yield, physical properties and chemical composition. Subsequently, dressing with different formulation was produced, stored at 37 ^{0}C and oxidative stability and sensory effects of produced sauce with various concentrations of EO (0.015%, 0.03% and 0.045%) which extracted by OUE and OAHD were compared to that produces by different concentrations of Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT). Peroxide value (PV) and anisidine value (AnV) measurement in sauce indicated that all concentrations of EO had antioxidant effect in comparison to synthetic antioxidants. Samples added with EOs at 0.045% were the most stable during storage.

Therefore, the results of this study showed that two extraction method of EOs have the approximately same antioxidant activity in the studied food system and also EOs can be used as an alternative for synthetic antioxidants in dressing formulation.

***Key words**: Food formulation, dressing, Carum copticum essential oil, Ohmic ultrasonic extractor, Ohmic assisted hydrodistillation, GC-MS.*

1. Introduction

Progress in the development of fatty foods like dressing with desirable nutritional and physical attributes depends on the availability of improved methods of controlling their oxidative stability, which in turn relies on a thorough understanding of the mechanisms of lipid oxidation [12]. Emulsified lipids are often oxidized more quickly than bulk oil because of the larger exposure area with air; the mechanisms of flavor deterioration in emulsions are also more complex. Studies of lipid oxidation in oil-in-water emulsions and aqueous colloidal systems suggest that the interaction between lipid hydroperoxides located at the droplet surface and transition metals originating in the aqueous phase are the most common cause of oxidative instability [3].

According to the codex standard for dressing, utilizing of some chemical antioxidants at defined concentration is permitted. In addition, among the synthetic antioxidants, BHA and BHT are widely used in food industry [13]. Despite universal application of these antioxidants, some studies reported synthetic antioxidants like BHA and BHT may have side effects and being carcinogenic [11]. In addition, nowadays there is a growing tendency from consumers to the natural ingredients. The substitution of synthetic antioxidants by natural antioxidants in food formulation may have some benefits, due to health effects and consumer perception. Plant materials contain many compounds with antioxidant activity. Various herbs and spices have been studied as sources of possibly safe natural antioxidants for the food applications [9, 17].

Hydrodistillation (HD), steam distillation and organic solvent extraction are the traditional isolation methods of Essential oils from plant materials. However, losses and degradation of some volatile compounds due to long extraction times and elevated temperatures, toxic solvents residue, consuming large amounts of solvents are the main disadvantages of these methods. Also these methods are known to be time and energy intensive. The recent advances in the extraction of EOs resulted in development of novel techniques like OAHD and OUE to overcome some of these drawbacks [4, 5, and 7].

Carum copticum is a widely distributed annual herbaceous plant which grows in the east of India, Iran and Egypt. The fruits of *C. copticum*, commonly known in Iran as 'Zenyan', have been used extensively in Iranian folk and traditional medicine to treat several disorders like gastrointestinal, rheumatic and inflammatory disorders. *C. copticum* were also used for its therapeutic effects such as diuretic, anti-vomiting, and carminative effects [6].

To the best of our knowledge, there is no report available on the application of EOs as a natural antioxidant in dressings. In addition, *C. copticum* is abundant herb in Iran and some other country (like India). So because of healthy aspects and beneficial possibility of commercial application, the aims of this study are determination of antioxidant properties of EOs, substitution of common synthetic antioxidants in sauce (BHA and BHT) with EOs and evaluating oxidation stability of the product with new formulation and also to compare the antioxidant activity of the obtained EOs by two extraction methods (OUE and OAHD) in a real food system.

2. Material and Methods

2.1 Materials

C. copticum were collected from the suburb of Kazerun city, Fars province, Iran. The species was identified and authenticated by A.R. Khosravi, a plant taxonomist, at Shiraz University, Herbarium, Shiraz, Iran. Voucher specimen (No. 24985) has been deposited in the herbarium. Certified specimens were dried in a dark room (approximately temperature was 30 ^{0}C) for 5 days, packed in high density poly ethylene (HDPE)/cardboard box and kept in a dark and cool place for further experiments. The moisture content of the herbs was measured in triplicate using a laboratory oven by drying until constant weight and was 9.8 ± 0.5%. Chemicals such as methanol, acetic acid, chloroform, sodium iodide, sodium thiosulfate, iso-octane and *p*-anisidine were obtained from Merck (Darmstadt, Germany). DPPH, BHT and BHA were purchased from Sigma Chemical Company (Sigma-Aldrich GmbH, Sternheim, Germany). Refined, bleached, and deodorized sunflower oil with no additives was purchased from a local Narges Oil Company, Shiraz, Iran. Other raw material for dressing preparation obtained from a local market.

2.2 Essential oil preparation

For OUE method, 15 grams of the areal parts of the *C. copticum* and 0.5 L water were hydrodistillated for 17 min using an Ohmic ultrasonic apparatus [7]. OAHD also was performed according to the method described by Gavahian *et al.* [4] by using an ohmic distillator device. The device operated at 220 v, 50 Hz. In OAHD procedure, 15 g of dried herb and 0.5 L salted water (1% NaCl, w/v) were heated in the apparatus flask for up to 1.5 h from initial temperature of 28 ± 1 ^{0}C (similar to initial temperature of material in OUE method). Two extraction processes continued until no more essential oils were obtained. In order to remove water, two extracted essential oils were then dried over anhydrous sodium sulfate and stored in amber vials at 4 °C for further experiments.

2.3 Identification of EO components

The EOs were analyzed by GC-MS. The analysis was carried out on a Thermoquest - Finnigan Trace GC-MS instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed to increase from 60 to 250 °C at a rate of 4 ^{0}C/min and finally held for 10 min; transfer line temperature was 250 ^{0}C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35 - 465 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA. GC-FID analyses of the oil were conducted using a Thermoquest - Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 µm). Nitrogen was used as the carrier gas at the continuous flow of 1.1 mL/min; the split ratio was the same as for GC-MS. The oven temperature was raised from 60 to 250 ^{0}C at a rate of 4 ^{0}C/min and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 ^{0}C, respectively. Semi quantitative data were obtained from FID area percentages without the use of correction factors. Retention indices (RI) were calculated by using retention times of n-alkanes (C6 - C24) that were injected after the oil at the same temperature and conditions. Compounds were identified by comparison of their RI with those reported in the literature [1] and their mass spectrum was compared with the Wiley Library (Wiley 7.0).

2.4 Physical constants

Specific gravity and the refractive index of the EOs from the *C. copticum* samples were measured according to Food Chemical Codex (FCC) at 25 and 20 ^{0}C, respectively. The color parameters of the EOs (L: lightness, a: redness-greenness and b: blueness-yellowness) were determined according to the method described by Yam & Papadakis [16]. In addition, the color of the oils was determined visually as directed in FCC.

2.5 Preparation of sauce

Production of sauce was performed in a local factory (Reyhan mikhak Food Industry, Shiraz, Iran). Sauce rec-

ipe was very little modified from that recommended by Worrasinchai *et al.* [15]. The recipe contained the following ingredients in percentage (w/w): pure egg yolk 17, vinegar (5% (w/v) acetic acid) 13, sun flower oil 68%, salt 0.8, mustard 0.5, sugar 0.4, and ground pepper 0.3. The preparation process had the following steps: First of all, pure egg yolk and vinegar were mixed together then other ingredients (except oil, essential oil, BHT and BHA), then proposed antioxidant of each formulations (BHT, BHA, ultra and ohmically distillated EOs) was added to the sunflower oil to ensure even distribution in the final mix and finally mixture of oil and essential oil that was very slowly added and mixed for 5 minutes. The formulations of all products were same except the type and concentration of antioxidants. The concentration of each antioxidant in the sauce formulation is presented in Table 1. Then produced dressing was aseptically transferred in sterile high density poly ethylene recipients (100 gram in each container) and stored at 37 ^{0}C. Storage time was 11 weeks and sampling performed each seven days**.**

2.6 Color measurement of dressing

L, a and b parameters of dressing samples determined according to Yam & Papadakis [16] two days after production.

2.7 Separation of dressing oil

Required amounts of oil for experiments were isolated from dressing by breaking the emulsion followed by ultracentrifugation according to Jacobsen *et al.* [10]. The dressing was frozen at -40 ^{0}C for 24 h to separate the emulsion. Afterward, frozen dressing was thawed for 4 h at 5 ^{0}C and then centrifuged for 10 minutes at 25,400 x g. Due to these operations; oil was separated from aqueous phase and then collected by pipetting for further experiments.

Table 1. Variations in produced sauce formulations

Formulation	Type of antioxidant	Concentration of added antioxidant in final product
Control	No antioxidant	0
BHA - 0.006%	BHA	0.006%
BHA - 0.012%	BHA	0.012%
BHT - 0.006%	BHT	0.006%
BHT - 0.012%	BHT	0.012%
Z - 0.015%	Zenyan essential oil obtained by OUE	0.015%
Z - 0.03%	Zenyan essential oil obtained by OUE	0.03%
Z - 0.045%	Zenyan essential oil obtained by OUE	0.045%
ZO - 0.015%	Zenyan essential oil obtained by OAHD	0.015%
ZO - 0.03%	Zenyan essential oil obtained by OAHD	0.03%
ZO - 0.045%	Zenyan essential oil obtained by OAHD	0.045%

2.8 Chemical analyses of sauce samples separated oil

AnV analyses were performed according to the American Oil Chemist's Society [2]. PV was measured using a spectrophotometric method according to Shantha and Decker [14]. Determination of AnV was done by reading the absorbance of a solution of oil (0.5–4 ± 0.001 g) in 25 mL isooctane, treated with 1 mL *p*-anisidine reagent at 350 nm using solvent with *p* - anisidine reagent as blank in the reference curette. Determinations were carried out in triplicates.

2.9 Sensory evaluation

First of all, two groups of 24 members taste panel established. After two days of storage, samples were labeled using mix of numbers and letters coding and presented to the panelists. The produced sauce with EOs (Z - 0.015%, Z - 0.03% and Z - 0.045% samples) where compared with control sauce (which have no antioxidant) using Triangle test in the case of sauce color, odor (aroma indeed) and also preference.

2.10 Statistical analysis

All tests were performed in triplicates. Analysis of variance (ANOVA) was performed to determine significant differences between the means and Duncan multiple range tests was used to compare among the means using SPSS (version 19.0.0; IBM Institute Inc., USA).

3. Results and Discussion

The extraction kinetics of EOs from *C. copticum* using OAHD was compared with that of OUL. Extraction with OUL started earlier than that with OAHD (about 3 min. vs. 4 min., respectively). This is due to application of ultrasound in OUL.

The physical properties (specific gravity, refractive index and color) of EOs extracted by OAHD and OUE are shown in Table 2. There was no significant difference between OAHD and OUE for the specific gravity and refractive indices of essences. Sensory color of OAHD sample was also similar to those obtained by OUE.

Table 2. Physical properties of extracted EOs from *C. copticum* by OAHD and OUE

Physical properties	OAHD	OUE
Specific gravity	0.912$^{a^*}$ ± 0.013	0.912$^{a^*}$ ± 0.001
Refractive index	1.49^a ± 0.01	1.49^a ± 0.01
Appearance	Pale yellow	Pale yellow
L$^\#$	54.7^a ± 1.5	52.0^b ± 5.0
a	-24.0^a ± 1.0	-24.0^a ± 1.0
b	9.3^a ± 2.1	10.8^a ± 1.5

* The same letters in each row indicate that the means are not significantly different (p <0.05);

$^\#$ L: lightness,

a: redness-greenness and

b: blueness-yellowness.

The identified components in the extracted essential oils of *C. copticum* by OUE and OAHD are given in Table 3. The percentages of each component of EO were quantified by peak area using the FID detector. The 18 components presented in Table 3 comprise more than 98.32% of the total GC peak areas. The compositions of the EOs obtained by OUE and OAHD were almost similar and as a result, components extracted by OUE were also found in OAHD. As the results show, the major components of the EOs are thymol (component No. 17), γ - terpinene (component No. 10) and *p* - cymene (component No. 10). Table 3 also present the EOs yield by OAHD and OUE.

Table 3. Chemical compositions of EOs obtained by OAHD and OUE from *C. copticum* using GC-MS

No.	Component	RI	Relative peak area [%]***	
			OAHD	OUE
1	α - Thujen	931	0.33 ± 0.02	0.32 ± 0.01
2	α - Pinene	941	0.12 ± 0.01	0.12 ± 0.01
3	Sabinene	979	0.19 ± 0.03	0.19 ± 0.02
4	β - Pinene	986	0.42 ± 0.05	2.42 ± 0.07
5	Myrcene	990	0.52 ± 0.03	0.51 ± 0.03
6	α - Terpinene	1022	0.02 ± 0.01	3.16 ± 0.06
7	*p*-Cymene	1030	22.90 ± 1.03	19.20 ± 0.95
8	1,8 - Cineole	1038	0.52 ± 0.08	0.5 ± 0.06
9	Ocimene	1047	0.05 ± 0.01	0.04 ± 0.01
10	γ - Terpinene	1064	23.92 ± 0.89	22.32 ± 0.81
11	*cis*-Sabinene hydrate	1072	0.04 ± 0.00	0.04 ± 0.00
12	Linalool	1098	0.06 ± 0.00	0.00 ± 0.00
13	*trans*-Sabinene hydrate	1105	0.04 ± 0.00	0.03 ± 0.00
14	Cyclocitral	1171	0.13 ± 0.02	0.13 ± 0.03
15	Terpinen-4-ol	1185	0.13 ± 0.03	0.11 ± 0.01
16	α - Terpineol	1198	0.10 ± 0.01	0.09 ± 0.00
17	Thymol	1295	50.07 ± 1.56	49.01 ± 1.33
18	Carvacrol	1301	0.14 ± 0.01	0.13 ± 0.01

There is no significant difference between two studied methods and EOs yield was about 9.1 (%v/w). It can be mentioned that the EOs content of aromatic plants may be influenced by harvest time, ecological climatic conditions and extraction method [7, 8]. Antioxidant activity of the EOs in sauce was evaluated during storage and compared with other dressing formulations (described in Table 1). The PVs of separated oil from dressing with added antioxidants at 37 ^{0}C is presented in Figure 1. As data show, all concentrations of EO extracted by two methods reduced the oxidation rate of dressing during storage in terms of formation of peroxides ($p < 0.05$). The oxidation rate of the samples with 0.03% EOs additive was not significantly different with BHA, and BHT at 0.012%. The stability of the samples with 0.045% EOs was considerably higher than that of all samples ($p < 0.05$).

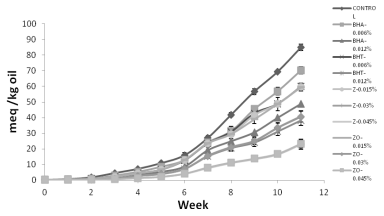

Figure 1. Variations in PVs of separated oil from sauce samples at 37 °C

AnV determines the level of aldehyde, principally, 2 - alkenals [2]. AnV of samples are shown in Figure 3. Results show that all concentrations of EO reduce this parameter ($p<0.05$). Samples with 0.03% EO were more stable than samples with 0.012%BHA and BHT ($p < 0.05$), whereas samples with added 0.045% EO had a significantly lower AnV than all samples during storage at 37 °C.

All results of primary and secondary oxidation measurement were shown that EOs extracted by OAHD and OUE exhibit similar protection effect against lipid deterioration in dressing. Results of GC-MS showed main component of both EOs are thymol. Therefore action of EOs as antioxidant mainly depends on the content of this component. Thymol is a primary antioxidant which either delays or prevents the initiation step by reacting with a lipid-free radical or prevents the propagation step by reacting with the peroxy or alkoxy radicals [8, 9] thereby could be retarded of lipid oxidation in dressing.

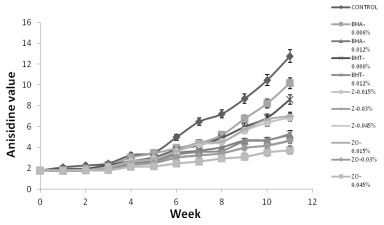

Figure 2. Variations in AnVs of separated oil from sauce samples at 38 ^{0}C

The color parameters of selected dressing samples (control, Z - 0.045 and ZO - 0.045) are shown in Table 4. As data show, there are not any significant differences between the evaluated parameters. These results indicated that addition of ohmically and ultrasound distillated EOs at described concentration to dressing formulation did not affect the color of the product.

The results of sensory evaluation indicate that in the case of color of samples, the panelist did not detect any significant differences between represented samples. It also revealed that addition of EOs in mentioned concentration did not make any considerable changes in sensory color of product.

From the stand point of sauce odor, all the Z samples (Z - 0.015%, Z - 0.03% and Z - 0.045% samples) and

ZO samples (ZO - 0.015%, ZO - 0.03% and ZO - 0.045% samples) were identified from control sample but the panelist were unable to change the difference between three Z samples and also three ZO samples.

Despite significant difference between sensory odor of new formulated sauce (Z and ZO Samples) and control sauce, there were no significant differences in the case of preference test of all represented samples. This may be due to different elegance of panelist. It seems that some people prefer sauce with new aroma and some people like classic sauce more.

Table 4. The color of selected dressing samples

Formulation / Parameters	Control	Z-0.045%	ZO-0.045%
L#	65.7^{a} ± 2.1	65.3^{a} ± 2.5	64.5^{a} ± 2.3
a	-15.7^{a} ± 0.6	-15.0^{a} ± 1.0	-15.4^{a} ± 1.2
b	-9.0^{a} ± 0.9	-8.8^{a} ± 1.2	-8.9^{a} ± 1.5

*The same letters in each row indicate that the means are not significantly different ($p<0.05$);

#L: lightness, a: redness-greenness and b: blueness-yellowness.

4. Conclusions

- In this study all concentrations of EOs were suitable antioxidants for preserving of dressing against oxidation.
- Synthetic antioxidants like BHA and BHT can be substituted with EO but only if the EO is used in higher concentrations.
- In addition, the antioxidant properties of EOs were almost independent from described extraction methods (OUEand OAHD).

5. References

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