International Food Research Journal 20(6): 3189-3195 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Ohmically extracted Zenyan essential oils as natural antioxidant in mayonnaise

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Article history

Received: 6 June 2013
Received in revised form: 9 July 2013
Accepted: 11 July 2013

Keywords

Food formulation
Mayonnaise
Zenyan essential oil
Hydrodistillation
Ohmic assisted hydrodistillation
GC-MS

Abstract

In this study, essential oils (EOs) were extracted from Zenyan by Ohmic assisted hydro distillation (OAHD) and conventional hydro distillation (HD) methods and extraction parameters and extracted oil were compared. Mayonnaise with different formulation stored at 38°C and oxidative stability and sensory effects of produced sauce with different concentrations of EO (0.015%, 0.03% and 0.045%) which obtained by OAHD and HD were compared BHA and BHT. Quality parameters in mayonnaise showed that all concentrations of EO had antioxidant effect in comparison to BHA and BHT. Added with EOs at 0.045% were the most stable during storage. EOs also was able to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with a 50% inhibition concentration (IC_{50}) (IC_{50} = 25 µg/mL, approximately). extraction of EOs by OAHD have the approximately same antioxidant activity in the studied food system and also Zenyan EOs can be used as an alternative for synthetic antioxidants in mayonnaise formulation.

Introduction

Nowadays, Mayonnaise is one of the most widely used sauces in the world. It has been in existence for centuries and was first produced commercially in the early 1900s, becoming popular in America from 1917 to 1927 (Harrison and Cunningham, 1985), in Japan where sales increased by 21% in the years from 1987 to 1990 (Brabant *et al*., 1992) and it is generally believed that mayonnaise is one of the most popular dressing in many countries. Mayonnaise, similar to many dressing, is a food emulsion (Caballero *et al*., 2003).

Progress in the development of fatty foods like mayonnaise 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 (Mcclements and Decker, 2000). 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 (Coupland McClements, 1996).

According to the codex standard for mayonnaise, 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 (Sanhueza *et al*., 2000). Despite universal application of these antioxidants, some studies reported synthetic antioxidants like BHA and BHT may have side effects and being carcinogenic (Labrador *et al*., 2006). 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 (Yanishlieva and Marenova, 2001).

Hydrodistillation (HD), steam distillation and organic solvent extraction are the traditional isolation methods of Essential oils (EOs) 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

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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 Ohmic assisted hydrodistillation (OAHD) to overcome some of these drawbacks (Gavahian *et al*., 2011; Gavahian *et al*., 2012).

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. Zenyan were also used for its therapeutic effects such as diuretic, anti-vomiting, and carminative effects (Goudarzi *et al*., 2011).

To the best of our knowledge, there is no report available on the application of Zenyan EOs as a natural antioxidant in mayonnaise or any other dressings. In addition, Zenyan 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 Zenyan EOs, substitution of common synthetic antioxidants in mayonnaise (BHA and BHT) with Zenyan 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 (HD and OAHD) in a real food system.

Material and Methods

Zenyan (35.6% initial moisture content) 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°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, thiobarbituric acid, 1-butanol, 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 Mayonnaise preparation obtained from a local market.

Essential oil preparation

For HD method, fifteen grams of the areal parts of the Zenyan and 0.5 L water were hydrodistillated for 4.5 h using a Clevenger-type apparatus. To remove water, the extracted EOs were then dried over anhydrous sodium sulphate and stored in amber vials at 4°C for further experiments and use. OAHD was performed according to the method described by Gavahian *et al.* (2011) 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°C (similar to initial temperature of material in HD method). The extraction process continued until no more essential oils were obtained. In order to remove water, the extracted essential oils were then dried over anhydrous sodium sulfate and stored in amber vials at 4°C for further experiments.

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°C/min and finally held for 10 min; transfer line temperature was 250°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 ionising voltage of 70 eV and an ionisation 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°C at a rate of 4°C/min and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280°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 (Adams, 2007) and their mass spectrum was compared with the Wiley Library (Wiley 7.0).

Physical constants

Specific gravity and the refractive index of the EOs from the Zenyan samples were measured according to Food Chemical Codex (FCC) at temperature of 25 and 20°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 and Papadakis (2004). In addition, the color of the oils was determined visually as directed in FCC.

DPPH assay

The radical scavenging capacity of EOs from *C. copticum* for DPPH was monitored according to the method described by Burits and Bucar (2000). Fifty micro liters of different concentrations of the EOs samples in methanol (15, 25, 35, 45 and 55 µg/mL) were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature under dark condition, the absorbance of the samples was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = (A_{blank} - A_{sample}) / A_{blank} \times 100,$$

Where A_{blank} is the absorbance of the control reaction (containing all reagent except the test compound), and A sample is the absorbance of the test compound. EOs concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against essential oil concentration. BHT was used as a control and all tests were carried out in triplicates.

Preparation of sauce

Production of sauce was performed in a local factory (Reyhan mikhak Food Industry, Shiraz, Iran). Mayonnaise recipe was very little modified from that recommended by Worrasinchai *et al.* (2006). 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, hydro 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 mayonnaise formulation is presented in Table 1. Then produced mayonnaise was aseptically transferred in sterile high density poly ethylene recipients (100 gram in each container) and stored for more experiments.

Table 1. Variations in produced mayonnaise 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 HD	0.015%
Z-0.03%	Zenyan essential oil obtained by HD	0.03%
ZO-0.045%	Zenyan essential oil obtained by HD	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%

Storage

Many times, mayonnaise is stored at room temperature which for most purposes can be considered to be approximately 37°C (especially in south of Iran). In addition, for transportation purpose the temperature of mayonnaise may reach to higher temperatures due to special environmental conditions. The temperature chosen for the present study was 38°C which seems to be the common temperature in mayonnaise transportation and exhibition in stores. Storage time was 11 weeks and sampling performed each seven days.

Color measurement of mayonnaise

L, a and b parameters of mayonnaise samples determined according to Yam and Papadakis (2004) two days after production.

Separation of mayonnaise oil

Required amounts of oil for experiments were isolated from mayonnaise by breaking the emulsion followed by ultracentrifugation according to Jacobsen *et al.* (1998). The mayonnaise was frozen at -40°C for 24 h to separate the emulsion. Afterward, frozen mayonnaise was thawed for 4 h at 5°C and then centrifuged for 10 min at 25,400 x g. Due to these operations; oil was separated from aqueous phase and then collected by pipetting for further experiments.

Chemical analyses of mayonnaise samples separated oil

AnV and TBA value analyses were performed according to the American Oil Chemist's Society (1998). PV was measured using a spectrophotometric method according to Shantha and Decker (1994). 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 cuvette. Measurement of TBA value was done by heating a 5 mL aliquot of a solution of sample (50–200 mg) in 25 ml 1-butanol with 5 ml TBA reagent at 95°C for 120 min and reading the absorbance at 530 nm using distilled water in the reference cuvette. Determinations were carried out in triplicates.

Determination of totox index

The totox value was determined from the equation (Allen and Hamilton, 1989):

$$\text{Totox} = 2(\text{PV}) + \text{AV}.$$

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.

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).

Results and Discussion

The extraction kinetics of EOs from Zenyan using OAHD was compared with that of HD (Figure 1). Extraction with OAHD started much earlier than that with HD (about 4 min vs. 20 min, respectively). This is due to the more efficient heating in the ohmic system. Unlike the classical conductive heating methods, ohmic heating can heat the entire sample almost simultaneously and at a higher rate, therefore it is able to generate heat inside products rapidly. As the data shows, by the time the extraction of EOs with HD started (i.e. 20 min), almost all of EOs (8.4% v/w) had been extracted with OAHD. After extraction time of 20 min, OAHD resulted in a similar essence recovery to that obtained by HD after about 2 h.

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

Table 2. Physical properties of extracted EOs from Zenyan by OAHD and HD

Specific	$0.912^{a*} \pm 0.013$	$0.912^{a*} \pm 0.001$
Refractive index	$1.49^{a} \pm 0.01$	$1.49^{a} \pm 0.01$
Appearance	Pale yellow	Pale yellow
L#	$54.7^{a} \pm 1.5$	$53.0^{b} \pm 5.0$
a	$-24.0^{a} \pm 1.0$	$-25.0^{a} \pm 1.0$
b	$9.3^{a} \pm 2.1$	$11.7^{a} \pm 1.5$

* 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.

Table 3. Yield and chemical compositions of EOs obtained by OAHD and HD from Zenyan using GC-MS

Formulation Parameters	Control	Z-0.045%	ZO-0.045%
L#	$65.7^{a} \pm 2.1$	$66.3^{a} \pm 2.5$	$65.5^{a} \pm 2.3$
a	$-15.3^{a} \pm 0.6$	$-15.0^{a} \pm 1.0$	$-15.4^{a} \pm 1.2$
b	$-9.0^{a} \pm 1.0$	$-8.7^{a} \pm 1.2$	$-8.8^{a} \pm 1.5$

* 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.

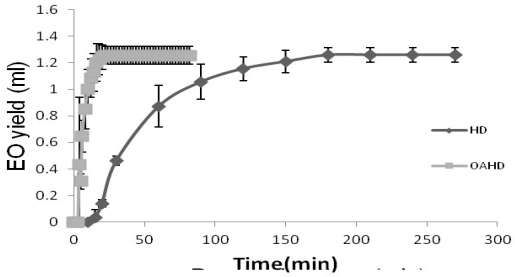

Figure 1. Extraction yield (ml essential oils/15 g dried herb) as a function of time for HD and OAHD of essential oils from Zenyan

to those obtained by HD. From the physical tests of the extracted EOs it can be concluded that OAHD, as a novel extraction technique, did not introduce any considerable changes to the studied physical properties of the extracted Zenyan EOs.

The identified components in the extracted essential oils of Zenyan by HD 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 HD and OAHD were almost similar and as a result, components extracted by HD 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 HD. There is no significant difference between two studied methods and EOs yield was about 8.4 (%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 (Hashemi *et al*., 2011ab).

The antioxidant activity of the EOs obtained by two extraction methods, against DPPH free radicals showed that both extracted EOs had similar activity) OAHD: $IC_{50} = 25.1 \pm 1.2$ µg/mL and HD: $IC_{50} = 25.2$

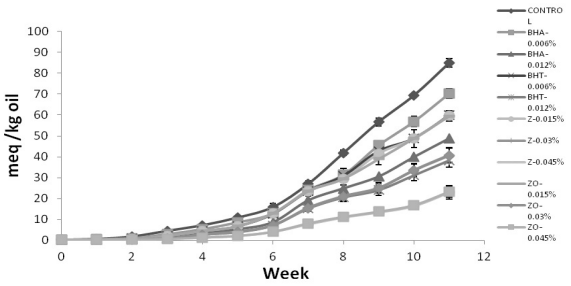

Figure 2. Variations in PVs of separated oil from mayonnaise samples at 38°C

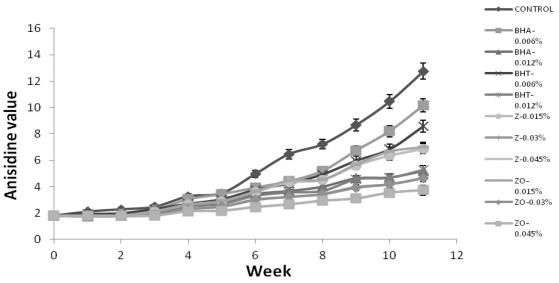

Figure 3. Variations in AnVs of separated oil from mayonnaise samples at 38°C

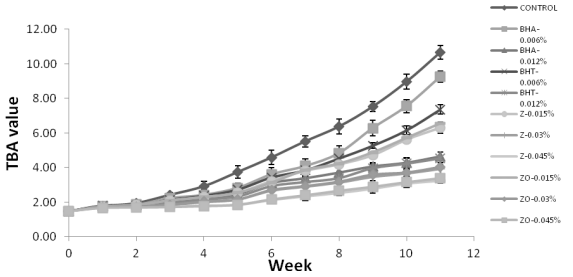

Figure 4. Results of TBA value measurements of samples

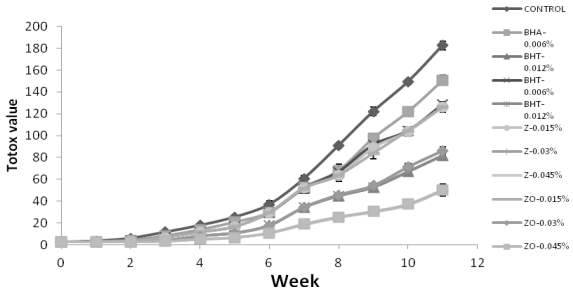

Figure 5. Results of Totox values of samples

± 1.4 µg/mL). This may be related to approximately same chemical composition, especially in the case of their main compound (Thymol), As the previous studies indicated that thymol content of EOs has pronounced antioxidant activity (Hashemi *et al*., 2011b). Therefore; OAHD could be a good alternative for the extraction of EOs from Zenyan.

Antioxidant activity of the EOs in mayonnaise was evaluated during storage and compared with other mayonnaise formulations (described in Table 1). The PVs of separated oil from mayonnaise with added antioxidants at 38°C is presented in Figure 2. As data show, all concentrations of EO extracted by two methods reduced the oxidation rate of Mayonnaise 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 were considerably higher than that of all samples ($p < 0.05$).

Anisidine value determines the level of aldehyde, principally, 2-alkenals (Hashemi *et al*., 2011a). 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 38°C. The TBA test is another empirical method widely used for the measurement of secondary product of oxidation, it relates to the level of aldehyde present in the oil by the reaction of malonaldehyde with TBA (Hashemi *et al*., 2011b). Results of TBA value measurements are very similar to results of PV measurements, as seen in Fig. 4. At 38°C, all concentrations of EO showed significant protection against secondary oxidation and samples with EO at 0.045% gave the lowest increase in TBA value. EO at 0.03% was significantly more stable than BHA and BHT at 0.006%, while samples with synthetic antioxidants at 0.012% were not significantly different with EO at 0.03% during storage at 38°C.

The combined effect of the PV and AnV of Mayonnaise samples can be studied by Totox value. The results of Totox value of Mayonnaise stored at 38°C are shown in Figure 5. As it shows, the Totox value of all samples were increased steadily during storage, but samples with EOs at 0.045% concentration have significantly better stability than other additives. No significaant differences were observed in Totox value of Mayonnaise with EO at 0.015% and BHT at 0.006%, while the Totox value of Mayonnaise with 0.006% BHA was significantly lower than 0.015% EO.

The evaluation of antioxidant effectiveness frequently corresponds to an extension of the Induction period (IP) as result of the addition of the antioxidant compound (Hashemi *et al*., 2011a, b). All concentrations of EOs increased IP of Mayonnaise during oxidation. EOs at the higher concentration was significantly more active than the lower concentration. EOs at 0.03% was not significantly different with 0.012% BHT, however EOs at this concentration more active than BHA. In addition, EOs at the maximum concentration was the most active of all.

All results of primary and secondary oxidation

measurement were shown that EOs extracted by OAHD and HD exhibit similar protection effect against lipid deterioration in mayonnaise. Results of GC-MS showed main component of both EOs are thymol. Therefore action of Zenyan EOs as antioxidant principally 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 (Hashemi *et al*., 2011ab) thereby could be retarded of lipid oxidation in mayonnaise.

The color parameters of selected mayonnaise 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 hydro distillated EOs at described concentration to mayonnaise 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.

Conclusions

In this study all concentrations of Zenyan EOs were suitable antioxidants for preserving of mayonnaise against oxidation. Synthetic antioxidants like BHA and BHT can be substituted with Zenyan EO but only if the EO is used in higher concentrations. In addition, the antioxidant properties of Zenyan EOs were almost independent from described extraction methods (HD and OAHD) and because of advantages like time and energy saving, OAHD can be proposed for extraction of EO from aromatic plants instead of traditional HD. This study about using of EO extracted from Zeynan as antioxidant so it was one of first studies which deals with natural antioxidant application of Zeynan in food industry especially Mayonnaise making.

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