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The Antioxidant Effect of *Mentha Pulegium* Extracts on the Stability of Canola Oil During Storage Conditions

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ABSTRACT

Medicinal plants, as natural resources with antioxidant functionality, play an essential role in reducing free radicals activities and oxidative damages to human body. Nowadays, consumers are becoming highly interested in products containing active plant-based ingredients with high nutritional value and no synthetic chemicals, as being beneficial to human health. This study aimed at analyzing the antioxidative effects of Mentha pulegium essential oil extracted by methanol to canola oil ratio of5:1, at two concentrations levels of 400 and 800 ppm, stored at room temperature for 60 days. Total tocopherols and total phenolic contents of canola oil, which are compounds of canola oil and the mentha extract were initially assessed. Subsequently, color index, conjugated Dienes value, acid value, peroxide value,carbonyl value andtotal polar compound were also analyzed. The results indicated the better effect of oxidative stability and lower acid valueat the 800 ppm concentration in comparison to the Tertiary Butyl Hydro Quinone sample. Moreover, the high concentrations of the extract performed better on the stability and, therefore, the inhibiting of double bond conjugation formation in both concentrations during the process. It can be applied to edible oil as an antioxidant with similar performance to the synthetic antioxidant.

KEYWORDS:*Mentha pulegium*; Canola oil; Phenolic compound; Oxidative stability; Heat stability

1. INTRODUCTION

Due to the great effects of edible vegetable oils on body functions like their effects on blood pressure andcholesterol level and being a vital supplier of fatty acids, this type of oil is important for our diet [1] . Canola is one of the most outstanding resources of edible oils in the world since it has the lowest saturated fatty acids amongst vegetable oils, and it is a rich resource of mono- and poly-unsaturated fatty acids. This is because it is a rich resource of vitamin E, and it has a 1:2 linoleic and linoleic acid ratio. Due to its high unsaturated fatty acid content, the widely-used long heating periods [2,3] lead to changes in the free fatty acids (FFAs) level, formation of polymers, reduction in the unsaturation degree, changes in the peroxide value, chemical changes with off-flavor and rancid smell, changes in color, and reduction in nutritional value [4,5]. In this regard, decomposition of hydrpreoxides into smaller molecules such as aldehydes, ketones and low-concentration alcohol, extremely affects the oil flavor [6]. In the presence of hydrolysis, oxidation and polymerization reactions, a combination of dimers,polymersand cyclic monomers might be built during thermal processes, leaving harmful effects on the body [7]. Therefore, inexpensive synthetic antioxidants are usually used to minimize oxidation. Today, however, these synthetic antioxidants are known to have toxic and carcinogenic effects on humans and may cause non-alcoholic steatohepatitis or influence liver enzyme activities [8]. Butylated Hydroxyl Toluene (BHA) and Butylated Hydroxyl Anisole (BHT) have been reported to be carcinogenic, and Tert-Butyl Hydroquinone (TBHQ) has been banned in Japan and European countries. Body cellular components such as protein, lipid and nucleic acid are sensitive to free radicals from oxidation reactions. These free radicals have an important role in causing heart diseases, cancer and aging [9] and even atmosphere pollution [3]. Moreover, synthetic antioxidants affect body adversely. Against this background, there is large body of research on using herbal extracts as an alternative for synthetic antioxidants.Literature shows that herbal resources are more effective than synthetic antioxidants [10,11,12,13]. The Labiate family is also known as an important herbal resource with antioxidant activity. It is widely applied to various purposes, like producing liquid essential oils for the confectionary industry, a flavoring agent, and perfume production and also for medicinal purposes [14]. Several studies have been done on antimicrobial and antioxidant activities of some species of Mentha Longifoli [14] and MenthaPulegium [15]. However, the effects of temperature and storage conditions on the antioxidant activity of extracts from different species of mentha in food products have been

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paid less attention. The purpose of this study was to analyze the antioxidant effect of the Mentha pulegium extract obtained using methanol on the stability of canola oil (in two concentrations of 400 ppm and 800 ppm, during 60 days of storage).

2. MATERIALS AND METHODS

2.1 Materials

Refined, bleached and deodorized canola oil (CO) without any synthetic antioxidants was taken from Ghoncheh Co. (Sari, Iran) and was stored in a refrigerator at -18°c. Aerial parts of M. pulegium L. were collected before flowering from a farm in Amol, Iran in summer 2011, and its species was identified by Agriculture Jihad Department of Sari, Iran. Their leaves were dried in a dark place at room temperature for 72 hours.The dried leaves wereminced using an electrical device (Moulinex model 684, France) and were kept in polyethylene bags in a freezer at -18°C. All the study's chemical compositions and solvents were of analytical reagent grade and were supplied from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO).

2.2 Extract Preparation

In this stage, 30 gram dried leaf powder was combined with 150 ml methanol alcoholic solvent (HPLC grade, K20192408Merck) and were mixed in occasional shaking (Fanazmagostar, Iran) at 140 rpm for 24 hours at room temperature. The supernatant phase was removed, and was centrifuged for several times (3times) at 3000 rpm for 10 minutes (Sahand, Iran) and was then filtered through Whatman Grade 4 paper. To evaporate the solvent, the mixture was placed into a vacuum oven (Fanazmagostar, Iran) at 50°C [16]. The extract obtained through this method (1.649 g) was poured in a glass container and was then stored at -18°C.

2.3 Total phenolic contents

Tocopherol (TCP) contents of canola oil (without synthetic antioxidants) and Mentha extracts were determined using a spectrophotometer (UV-2100,China) and Folin-Ciocalteu's reagent, following the methods described in Capannesi et al., 2000 and Singhatong et al., 2010, respectively. They were then compared with the standard gallic acid curve, and the results were stated based on the gallic acid equivalent per mg of the sample.

2.4 Total Tocopherol (TT)Content

The TT contents of the oil sample (without synthetic antioxidants) and Mentha extracts were determined according to the method described in Wong et al. [17].

2.5 Stability Test

The extract was added to the canola oil without synthetic antioxidant at two different concentration (400 ppm, 800 ppm) and was then kept at room temperature. Sampling was done during Days 0, 15, 30, 45 and 60. The canola oil containing synthetic antioxidant TBHQ was purchased from Ghoncheh Co. (Sari,Iran) and was then sampled and stored based on the above-mentioned method [16].

2.6. Analytical method

The Conjugated Dienes Value (CDV) was measured spectrophotometrically at a 234nm wavelengthfollowing the method described by Saguy et al.[19] andwas read against HPLC-grade hexane as a blank. The oil sample was diluted by n-hexane using a 1: 600 ratio. The Peroxide Value (PV) was determined spectrophotometrically, according to the Shantha and Decker [20] method.Using the spectrophotometer, absorption of the oil samples at 420 nm was determined in accordance to the method reported by Saguy et al. [19], and the Color Index (CI) was read against water as a blank. The Acid Value (AV) was determined according to the AOCS Official Method Cd 3d–63 [21].

2.7 Statistical Analysis

All experiments were carried out with three replications, and the regression analysis was done using MSTAT-C and SlideWrite. Means differences were compared by Duncan's multiple range tests ($P < 0.05$).

3.RESULTS AND DISCUSSION

The primary properties of refined,bleached, deodorized CO without synthetic antioxidantare presented in Table 1. The combination of fatty acids, particularly the degree of unsaturation, is one of the most important factors for determining the oxidative stability. Here, the fatty acid composition trivially, phenolic, tochopherol, and other combinations with antioxidant function, are supposed to be analyzed [25]. Fatty acids mainly consist of oleic acid (18:1, 64%), linoleic acid (18:2, 15%) and linolenic acid (18:3, 2 %).The PV and AV for CO were 0.223 (meq O2/kgoil) and 0.312 (mg KOH/g), respectively, indicating that CO was unoxidized and that the initial quality of oil was high.

Through their reactions with free radicals,tochopherols and phenolics prevent oils from rancidity and increase the shelf life of edible oil.Besides preventing cancer, they can also reduce heart diseases and Alzheimer's disease, as major biological antioxidants. These compounds may reduce in numbers with the occurrence of oxidative and hydrolysis processes during heating.There is a biochemical relationship between the tochopherol level, the unsaturation degree of oil and vitamin E against oxidation [26,11,12].The amount of tochopherol compositions of CO and the extract were 59.321(mg/kg oil) and 635.94 (mg/kg oil)، respectively.

The amount of phenolic contents was 12.452(mg /kg oil)and 474.26(mg GAE/g extract), respectively. The results suggest that phenolic contents in CO were lowerthan those reported in the literature [13]. This can be affected by refining process conditions and the type of canola oil. For the extract, the present result was also more than others' results [27]. However, the tocopherol levels of canola oil was almost similar to those for olive oil reported in the literature [28].

Table 1. The initial chemical characteristics of CO

Fatty acids Amount [%]	Amount[%]
16:0	5.024±0.45
	0.66±0.32
16:1	2.60±0.05
	64.08±0.59
18:0	15.52±0.29
	2.143±0.11
18:1	0.223±0.11
	0.312±0.001
18:2	59.321±10.79
	12.452±3.42
18:3	
PV [meq O 2 /kg oil]	
AV[mg KOH/g oil]	
TT [mg tocopherol/kg oil]	
TP[mg/kg oil]	

Color is a physical parameter widely used to evaluate rancidity development of oil for commercial and domestic purposes [29].Oil discolorations during heatingare signs of chemical decomposition and polymerization.The longer the heating period, the larger the changes in color [30]. Discolorations during heating are shown in Figure 1. the TBHQ sample has lower changes in colorthan other samples. It shows that TBHQ performed better in preventing the decomposition process and also formation of hydroperoxide and conjugated dienes.According to the results, the color index is improper for evaluating oil stability, since the polar combinations in oil might not be enough to throw the oil away. However, based on its discoloration intensity, it becomes dark and can be deemed rancid.

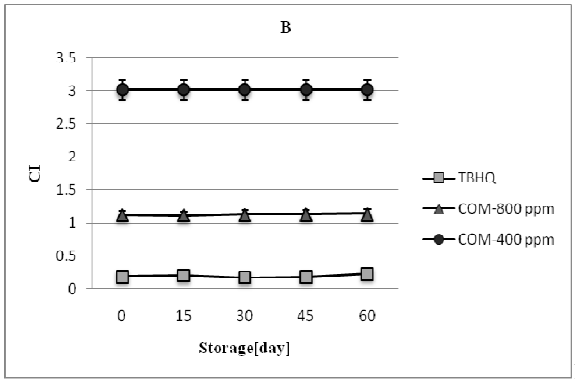

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Figure 1. Color Index of Canola oil stabilized with TBHQ and the COM (400, 800 ppm) during storage

The CDV is considered an important parameter for studying the oxidative deterioration and the antioxidant effects of extracts on oil [31]. During the oxidation,di- or poly-unsaturated fatty acidsmay lead to double-bond trans and double conjugated bond products.These combinations are rarely developed in oils with lowerpolyunsaturated fatty acids (PUFAs) like linolenic acid [32,33]. Table 2 ,under storage conditions, an increasing trend can be seen in CDV associated with both concentrations, compared to the canola oil sample containing TBHQ. However, the 800ppm extract had a protective effect on the stability of oil. The high amount of unsaturated fatty acids and contact with oxygen can be the reasons for this increasing trend. In the studies by Abdelazim Mohdaly et al.[34] and Fawzy Ramadan and Wahdan[35], the effects of potato skin extracts and sugar beet pulp on soybean and sunflower oils were analyzed. Moreover, the effects of black cumin (Nigella sativa) and coriander seed (Coriandrum Sativum) extracts on corn oil were studied. The results of these studies also indicated an increasing trend in CDV under storage conditions. This increasing trend can be due to their

contact with oxygen during storage and heating, development of peroxides during the initial oxidation phase, and the high PUFA content in canola oil(particularly linolenic acid which is decomposed into conjugated hydroperoxide).

The hydrolysis of oil leads to the formation of mono- and di-glycerids of fatty acidsand glycerol. Acid value (mg KOH/g oil), which is used to analyze decomposition of oil within hydrolytic reactions, has an increasing trend, and its variations are dependent on the initial volume of this parameter and heating duration [30,2]. Increases in AV in storage conditionswere almost steady for both concentrations, as shown in Table2, in which the variations were less but more steady than the control sample. Statistically, there was no significant difference. There is no sign of a significant change from the beginning to the end of the process in none of the three analyzed oils

Peroxide valuecan be used for determining rancidity in oil during the initial oxidation phase. The conjugated double bond and oxygen absorption in unsaturated fatty acids are the major factors generating peroxides[36,37]. It is reported that oxidation products, such as free radicals, peroxides and other oxidation-based products,would developcardiovascular diseases and would raise concerns in terms of rancidity and toxicology [38]. Due to the instability of peroxides, byproducts such as carbonyl and aldehydes are produced, which result in a decrease in PV and also decomposition of hydroperoxides.As seen in Table 3, during storage of oil, the declining trend was observed in Day 30, followed by an increasing trend. This behavior indicates that hydroperoxides are formed slower than being decomposed. The PV in both concentrations is more than that in the control sample, but it is lower than the specified rancidity limit. The same developments were also observed in a study by Bandoniene et al. on the effect of sage and sweet grass extracts on canola oil during 70 days of storage compared to the sample containing BHT with different concentrations [41].This study suggests that higher concentrations of the mentha extract are more effective in preventing peroxideformation. This can be associated to the increase in the antioxidant activities with phenolic compounds.

Table 2.CDV, AV and PV content of CO as affected by TBHQ and the COM (400 ppm, 800 ppm) of Mentha extract during Storage.[a]

storage[day]	CDV[mmol/l]			AV[mg KOH/g oil]			PV[meq O2/kg oil]		
	TBHQ	COM-400ppm	COM-800pm	TBHQ	COM-400ppm	COM-800pm	TBHQ	COM-400ppm	COM-800pm
0	10.913±0.042^{c}	11.830±0.02^{b}	13.220±0.001^{a}	0.185±0.015^{ns}	0.162±0.006^{ns}	0.201±0.006^{ns}	0.391±0.040^{c}	2.571±0.044^{a}	1.700±0.083^{b}
15	13.413±0.046^{b}	12. 793±0.023^{c}	13.563±0.06 ^{a}	0.263±0.015^{ns}	0.213±0.012^{ns}	0.209±0.014^{ns}	0.488±0.094^{c}	2.247±0.065^{a}	2.484±0.093^{b}
30	16.353±0.012^{a}	13.910±0.017^{b}	13. 780±0.017^{c}	0.286±0.006^{ns}	0.218±0.006^{ns}	0.211±0.012^{ns}	0.708±0.043^{ns}	2.063±0.071^{ns}	1.937±0.070^{ns}
45	17.243±0.012^{a}	14.693±0.012^{c}	15.647±0.012 ^{b}	0.289±0.009^{ns}	0.239±0.014^{ns}	0.218±0.006^{ns}	1.173±0.055^{c}	2.474±0.071^{a}	2.262±0.074^{b}
60	21.470±0.02^{b}	17.300±0.017^{a}	17.343±0.012^{c}	0.308±0.028^{ns}	0.272±0.009^{ns}	0.286±0.006^{ns}	1.956±0.042^{b}	2.423±0.072^{a}	2.552±0.069^{a}

TBHQ= canola oil added with 100 ppm, COM=canola oil with Mentha extract

^{ns} not signi ficantly different.

^{a} Means ±SD in each column with different letters differ significantly ($p < 0.05$).

4. Conclusion

Due to the high demand on fried foods throughout the world, several studies have focused on applying natural antioxidants in order to improve oil stability. Most of them have shown that the natural compositions are effective. Various factors affect the stability of oil. The results of these studies have represented the higher effectiveness of the extracts with high concentration in storage. Therefore, high concentrationswere used to analyze stability during storage, and it was found that the extract can retard the oxidative reaction and the formation of initial and secondary products of oxidation during heating and storage of canola oil, similar to theTBHQ synthetic antioxidant.The extract also improved the stability and prevented the formation of conjugated double bonds during the process. As a result, the mentha extract can be almost considered as a rich source of phenolic compositions and as an effective factor in the stability of unsaturated edible oils such as canola oil. In this study, the extract of mentha was adopted as a natural antioxidant source. Therefore, it is suggested that antioxidant components of the extractshould be investigated and analyzed to identify their function in the human body, so that they can be used as alternative natural antioxidants to synthetic antioxidants in order to prolong the shelf life of edible oils and other food products.

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