Examination of the effect of mint extract on sunflower oil stability

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Abstract— This study aimed at extracting phenolics of mint as natural antioxidant sources using cold solvent method and examining their antioxidant properties through determining the stability duration in sunflower oil. Mint extracts were extracted using cold solvent method by two solvents, methanol and acetone. Extraction efficiency was determined and the phenolics values of the extracts were measured using folin - ciocalteu method. Antioxidant effect of different concentrations (0.5, 0.3, 0.1 and 0.05%) of phenolics of mint extracts on retarding oxidation in sunflower oil was examined through Rancimat and peroxide index tests. The results showed that the type of the solvent affected significantly on phenolics extraction efficiency. The result of this study revealed that the extraction efficiency and the phenolics content which extracted using methanol were higher as compared to the extraction using acetone. The results obtained by examining antioxidant property of mint extracts in sunflower oil revealed that increasing the concentration of the extracts had a significant effect on improving antioxidant property.

Keywords-. Oxidation, antioxidant, mint extract .

Introduction

Oxidation of oils cause undesirable smell and flavor in foods produces and its results decrease nutritional value in food due to formation of poisonous compounds in foods (1). Thus, applying the matters capable of retarding oxidation and improving the stability is of great importance. The most common chemical antioxidants in food industry including BHT, BHA, TBHQ and Propyl gallate have been confirmed to be carcinogenic and having negative effect on human health (2). Today, use of medicinal herbs and their active compounds as natural antioxidant sources and as substitutes of chemical antioxidants are considered by researchers (3).
There are several methods for extraction from plant tissues. The type of used method depends on the type of plant tissue, the type of dissociated matter and its resistance to heating thus selecting proper method may result in increased antioxidant concentrations. Various extraction methods have significant effect on extraction efficiency and the compounds present in the extract (4). Extraction by using solvents is one of the most common methods for extraction. In this method the plant and the solvent are in contact with each other for certain duration. After extraction, the solvent is removed (5). In another study kanatt et.al. (2007) reported that mint leaves contained natural phenolic and flavonoid antioxidants. Murica et.al. (2004) stated that mint, dill and ginger improved stability of oils such as sunflower, corn, olive as well as butter and margarine against oxidation at $110^{\circ}C$. These findings seem to be consistent with the results of other research conducted by marinova and Yanishlieva (1997). They reported that mint extract could retard autoxidation of sunflower oil at $110^{\circ}C$. Fecka *et al*. (2008) in their research on phenolic compounds of mint and basil reported that the highest phenolics content was found as rosmarinic acid.
Sunflower oil is extracted from sunflower oil (Helianthus annuus) seeds through mechanical pressure or applying solvents or a combination of both two methods. This type of oil presents a very mild and attractive taste conserving natural flavor of foods. Also its mild and attractive yellow color makes it more acceptable. Sunflower oil is valuable oil for salads, cooking, and frying due to these desirable properties. It contains unsaturated oleic acid and high percentage of linoleic acid which are healthy and beneficial, reducing cholesterol level. Because of low linolenic acid level (about 0.5%), it has longer shelf life as compared to high linolenic acid contained oil such as soybean oil (6). It is essential to replace synthetic antioxidants by natural ones to improve stability of sunflower oil. Since mint among Iran's indigenous plants, are easily available and inexpensive, and their extracts are easily extracted by using organic solvents. The objective of this study was to extract mint extracts by organic solvents methanol, aceton, and to examine and compare the antioxidant activity of mint extracts through evaluating of sunflower oil stability.

MATERIALS AND METHODS

Materials

Mint plant obtained from research field of medicinal herbs research center of Jahad Daneshgahi (Karaj, Iran). Antioxidant free sunflower oil was provided by Pars Ghoo vegetable oil company (Tehran, Iran). The used solvents and other chemicals were obtained from Merc KGaA (Darmstadt, Germany).

Extraction

Cold solvent method was applied to extract mint extract. Mint plant were dried, then powdered and passed through 40 No. mesh. Then, the powdered plant and the solvents separately mixed at 1:10 ratio in a shaker at ambient

temperature for 24h. Then, they were filtered through filter paper using vacuum pump and at the next stage, the obtained extracts were distilled at vacuum by rotary apparatus to remove the solvents. Vacuum was applied at 25 mm Hg at 50-55° C inorder to minimize damage phenolic compounds. Eventually the solvent was removed with the help of residual nitrogen azote and then, the obtained extracts refrigerated in dark glass containers (7).

Determination of extraction efficiency

Extraction efficiency was determined using three solvents methanol and acetone in duplicates.

$$\text{Extraction efficiency} = \frac{extract\ \ weight}{powder\ \ weight} \times 100$$

Determination of total phenolics content

Total phenolics content of mint extracts was determined using Folin–Ciocalteu**.** In this method folin reagent in alkaline solution in the presence of phenolics compounds is reduced and producing blue color in the solution. Color intensity at 765 nm wavelength was measured by optical spectrometer (8).

Following extraction, 1 gram of the obtained extract was added to 10 ml of deionized water. Then, 5 µl of diluted extract transferred to separate tubes. Then, 1 ml of Folin–Ciocalteu reagent (diluted by double distilled water at 1:10 ratio) and 0.8 ml of 7.5% sodium carbonate solution were added to each tube. After that 10 ml of double distilled was added to the tubes and then mixed thoroughly. After 1h at room temperature, the absorbance at 765 nm was measured. By using graded curve equation (for gallic acid as standard), total phenolic compounds evaluated and were expressed as mg/g of dry extract (9).

Sunflower sample preparation

To examine antioxidant property of mint extracts obtained by methanol, aceton, they were added to 100 gram of antioxidant free sunflower oil, at different concentrations (0.05%, 0.1%, 0.3%, 0.5%) considering their phenolics content.

Rancimat and peroxide tests

All samples were transferred to oven at 75° C to determine peroxide value test. Peroxide value test was determined using official methods and American oil chemist society (AOCC, 1994) method cd 8b – 90 (iodometric methods) (firestone, 1994) in triplicates at 48 h intervals for 10 days for each sample. Oxidation stability duration of all treatments was determined before incubation using Rancimat (Model 743) at 110° C and 20 ml/h air flow.

Measurement of oil fatty acids

Fatty acid composition of sunflower oil was identified and determined using Gas chromatography **(**GC**)**. The samples were prepared as methyl ester derivatives according to the method of Christie by using 0-5 normal sodium methoxide. Then to examine the profiles of fatty acids, a Gas chromatography (model Acme 6000) equipped with flame detector and 60 m column according to AOAC standard No. Ce 1e-91 (10) was used.

Data statistical analysis

Software spss 16 was used to analyze data statistically. Means of concentration and different solvents were compared using Duncan mean comparison at significant level 0.01% of which the best solvent and concentration were selected.

Data Analysis

Data collected from the aforementioned study samples were analyzed based on 0.05% coefficient of error. The data analysis was performed using MINITAB statistical software, release 14.2 (MINITAB Inc., state college, PA and USA).

Results and Discussion

Extraction efficiency

Mean efficiency of extraction of mint extracts by two organic solvents methanol, acetone with different polarities is presented in Figure 1. The results showed that there were significant difference (p<0.01) between extraction efficiency of phenolics of mint with organic solvents methanol, and acetone. Methanolic extract of mint showed the highest efficiency (0.16%). The results obtained from this study showed that the extracts had dark green color and relatively pungent smell. Methanolic extract had the most pungent smell as compare to the other extracted. As show in diagram 1, methanolic extract of mint showed the highest extraction efficiency. The findings suggested that the type of solvent had a significant effect on the extraction yield. The results indicated that the best solvent for extraction of phenolics compounds of mint was methanol. The reason might be the polarity of methanol compared to the other solvents resulting in solved polar phenolics.

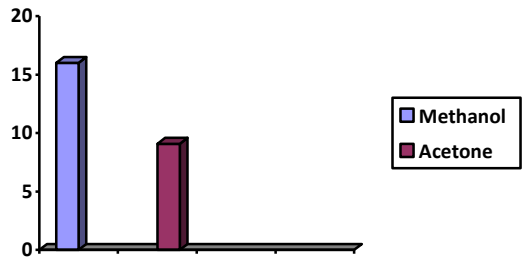

Fig.1: Mean extraction efficiency of mint extracts based on the type of solvent (methanol, Acetone**)**

Total phenolics content of mint extracts

Figure 2. shows the mean of total phenolics content of mint extracts based on gallic acid standard. As the results showed that, there was a significant difference (p<0.01) between phenolics content of mint extracts with different organic solvents. Methanolic extract of mint showed the highest phenolics content at 17.02 mg/g dry weight. This is probably due to the methanol is more polar than the Acetone.

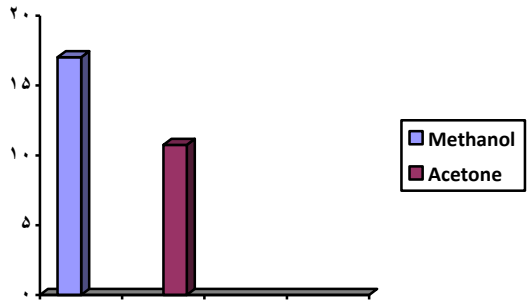

Fig. 2: Mean phenolics content of mint extracts in mg/g dry weight Other fatty acids

Fatty acid composition of sunflower oil

Sunflower oil is valuable oil mainly because of mild taste, clear color, high smoke point, high content of linoleic acid and low amount of linolenic acid. Table 1. indicated that about 90 percent of total fatty acids of sunflower oil consisted of unsaturated oleic and linoleic acids and the others consisted of unsaturated palmitic and stearic fatty acids.

Table 1: Sunflower Oil Fatty Acid Profile

Type of fatty acids	Value (%)
C14:0 (Myristic acid)	0.18
C16:0 (palmitic acid)	6.73
C16:1 (palmitoleic acid)	0.11
C18:0 (stearic acid)	3.49
C18:1 (oleic acid)	24.32
C18:2 (linoleic acid)	63.12
C18:3 (linolenic acid)	0.29
C20:0 (arachidonic acid)	0.24
C20:1(eicosenoic acid)	0.14
Other fatty acids	1.38
Total saturated fatty acids	10.64
Total unsaturated fatty acids	87.98

Sunflower oil is prone to oxidation during processing and storage because of its high degree of unsaturation**.** Therefore, it is essential to use antioxidants to control oxidative rancidity.

Examination of antioxidant effect of mint extracts on sunflower oil

Mean peroxide value of sunflower oil samples at different concentrations of methanolic, acetonic extracts of mint over storage at 75° C for 240 h indicated in Tables 2. The results showed that the type of solvent, concentration and duration had significant effects on peroxide value variation.

The results obtained from peroxide index of oil samples contained mint extracts over storage demonstrated that mint extracts were more effective antioxidant. Tables 2 showed that peroxide value of all samples increased gradually over time (240 h). As shown in Tables 2 oxidative rancidity was declined as the concentration of mint extracts increased probably due to increase of phenolics content. Moreover, control sample (sunflower oil without antioxidant extracted) showed the highest peroxide index as compared to the other samples contained antioxidant extracts and sunflower oil samples contained the highest concentration of methanolic mint extract (0.5%) showed the lowest peroxide index. This is because, the methanolic extract was not able to extract more phenolics compounds as compare to the other solvents while phenolics mint extract could control primary products of oxidation which improved by increased concentration of mint extract. The results obtained from Rancimat test confirmed the results obtained by this test. Duration of oxidation stability in control sample and different oil samples contained 0.5% natural mint extracts by different solvents at 110° C using Rancimat test was evaluated and the results presented in Figure 3.As shown in Figure 3, the highest stability duration in sunflower oil treatments were belong to samples contain 0.5% methanolic mint extract which increased the oxidation stability of sunflower oil by 6.14 h due to its more antioxidant phenolics. Figure 4 shows the typical chromatogram of the highest stability duration of sunflower oil.

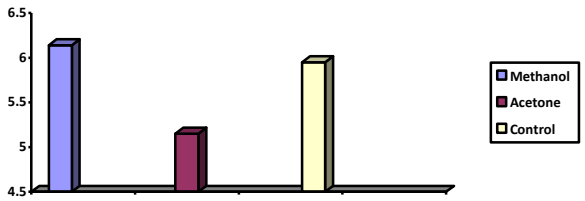

Fig. 3: Comparison of oxidation stability duration of control sample with different samples of sunflower oil contained 0.5% natural mint extracts extracted by solvents methanol, acetone at 110° C.

Table 2. Mean peroxide index of different treatments of sunflower oil containing acetonic and methanolic mint (meq/kg) extracts at 75° C.

	Solvent*	Level of mint extract*	0 hour	48 hour	96 hour	144 hour	192 hour	240 hour
	Control	0	0	11.25	14.25	25.26	33.80	41.48
	Acetone	0/05	0	11.00	14.00	24.00	32.40	39.40
		0/1	0	10.23	12.87	23.84	29.98	36.98
		0/3	0	8.75	9.11	22.16	25.66	32.66
Sun flower oil		0/5	0	7.50	8.39	21.00	25.00	31.00
	methanol	0/05	0	11.00	13.32	21.00	32.00	39.00
		0/1	0	10.00	11.88	20.14	28.97	35.97
		0/3	0	5.31	6.33	18.00	25.26	32.26
		0/5	0	4.99	6.19	17.00	21.35	28.35

[a]The values are given as mean of triplicate.
* Significant ($p < 0.01$).

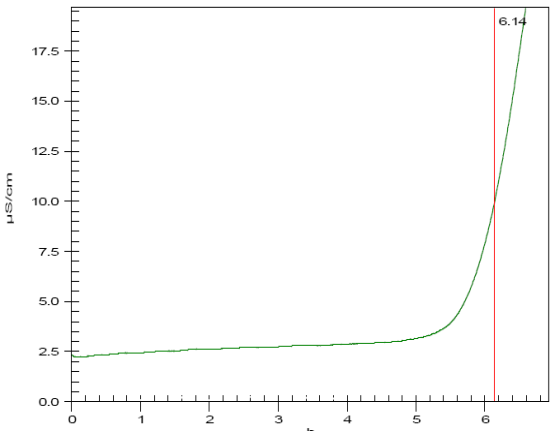

Fig. 4: Typical chromatogram of oxidation stability of methanolic mint extract in sunflower oil evaluated by Rancimat at 110° C.

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