Preventive effect of cinnamon essential oil on lipid oxidation of vegetable oil

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Original Article

Abstract

BACKGROUND: Lipid oxidation is the main deterioration process that occurs in vegetable oils. This process was effectively prevented by natural antioxidants. *Cinnamomum zeylanicum* (Cinnamon) is rich with antioxidants. The present study was conducted to evaluate the effect of cinnamon on malondialdehyde (MDA) rate production in two high consumption oils in Iranian market.

METHODS: Chemical composition of cinnamon essential oil was analyzed by gas chromatography-mass spectroscopy (GC-MS). 200 µl each oil, 50 µl tween 20, and 2 ml of 40 Mm AAPH solutions were mixed and the prepared solution was divided into four glass vials. Respectively, 50 µl of 500, 1000 and 2000 ppm of cinnamon essential oil were added to three glass vials separately and one of the glass vials was used as the control. All of the glass vials were incubated at 37° C water bath. Rate of MDA production was measured by thiobarbituric acid (TBA) test at the baseline and after the 0.5, 1, 2, 3 and 5 hours.

RESULTS: Compounds of cinnamon essential oil by GC-MS analysis such as cinnamaldehyde (96.8%), alpha-capaene (0.2%), alpha-murolene (0.11%), para-methoxycinnamaldehyde (0.6%) and delta-cadinen (0.4%) were found to be the major compounds. For both oils, maximum rate of MDA production was achieved in 5th hours of heating. Every three concentrations of cinnamon essential oil significantly decreased MDA production ($P < 0.05$) in comparison with the control.

CONCLUSION: Essential oil of cinnamon considerably inhibited MDA production in studied oils and can be used with fresh and heated oils for reduction of lipid peroxidation and adverse free radicals effects on body.

Keywords: Cinnamon, Essential Oil, Lipid Peroxidation, Vegetable Oils

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Introduction

Recently, there has been observed the increased demand for vegetable oil used both, for technical and food purposes.[1] Vegetable fats contain polyunsaturated fatty acids. These fats are prone to oxidation.[2] The free radical activity and the extent of tissue damage are related quantitatively to the amount of lipid peroxide level in the blood.[3] Malondialdehyde (MDA) is one of the end products of lipid peroxidation and extent of lipid peroxidation is measured by estimating MDA levels most frequently.[4] Increased serum level of MDA has been reported in cardiovascular,[5] neurological and other diseases.[6] Oxidation of vegetable oils has a direct influence on consumer acceptance and adversely affects lipids, proteins, carbohydrates, pigments and fat-soluble vitamins, causing development of off-flavor, loss of nutritional value, discoloration and the production of potentially toxic compounds.[7]

A substantial administration of oxidized vegetable oils in diet may lead to aggravation of free

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radical processes. It is believed that such a diet, similar to excessive consumption of products rich in cholesterol, results in development of vascular lesions, leading to atherosclerosis and then to diseases of the cardiovascular system.[2] Furthermore, researches revealed high consumption of antioxidant substances reduces the risk of developing circulatory system diseases.[8] It would seem crucial to find out whether additions of antioxidant substances to food results in reducing the disadvantageous changes caused by the consumption of oxidized vegetable oils.[9,10] In the food industry, lipid oxidation was inhibited by synthetic antioxidants such as butyl hydroxyanisol, butyl hydroxytoluene, terc butyl hidroxiquinona (TBHQ), and Propyl gallate. The use of those compounds has been questioned in many studies in terms of their safety due to risks of causing heart diseases and carcinogenesis. Thus, in the European continent and other countries such as Japan, Canada, and the United States, the use of certain synthetic antioxidants in foods is not permitted.[11-13]

Since lipid oxidation is a critical factor for food quality and prolonged shelf-life of edible oils, many studies have concentrated on prevention of lipid oxidation by natural antioxidants.[14-16]

It has been established that the oils and extracts from *Cinnamomum zeylanicum* L. (Cinnamon) possess a distinct antioxidant activity, which is especially attributed to the presence of phenolic and polyphenolic substances.[17,18] Barks of *cinnamomum* plants are used as spice and herbal medicine. This plant has been employed as a folk remedy to treat several diseases, disorders and ailments.[18-20] Since long time ago, cinnamon and ginger have been used to treat dyspepsia, gastritis, blood circulation disturbance and inflammatory diseases in many countries.[21] Cinnamon is widely been consumed as spices and food preservation. It is added to food products in the form of essential oils and various extracts.[22] In a study by Mancini-Filho,[23] it was reported cinnamon extracts can be used as food antioxidant together with the improvement of food palatability.

Therefore, this experimental study aimed to assess the effect of cinnamon extract on MDA production rate in two high consumption solid oil (A) and liquid oil (B) in Iranian market.

Materials and Methods

Chemicals and Reagents

2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) were obtained from Sigma (Sigma Chemical Co., USA). HCl, n-butanol, pyridine, tween-20, thiobarbituric acid (TBA) were purchased from Merk (Merk Chemical Co., Germany).

Vegetable oils

As illustrated in table 1, it is used vegetable oils. For used oils were selected as "A" and "B". In this study, rate of lipid peroxidation in these oils was investigated.

Plant Material and Preparation of Cinnamon Essential Oil

Bark of cinnamon was purchased from a local herbal grocery from the Isfahan, Iran. The bark was washed thoroughly with distilled water (ddH_2O) to remove the dust or any other extraneous material and was dried in the shade and finely was powdered with an electric grinder.

Prepared powder was subjected to steam distillation for 4 hours using a Clevenger-type apparatus to produce the essential oil. After extraction, the essential oil was separated from water using ethyl ether and was dried with anhydrous sodium sulfate.[24]

Chemical composition of the cinnamon was analyzed by GC-MS on a Finnigan MAT Incos-50 instrument mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph, equipped with a DB-5 fused silica capillary column (25 m × 0.25 mm, film thickness 0.25 µm). The GC operating conditions were as follows: carrier gas, helium with a flow rate of 1.5 mL/min; the oven temperature was programmed 5 min isothermal at 60° C and then from 60°-280° C at 4° C/min; injector and detector temperatures, 280° C; volume injected, 0.1 µL of the oil; split ratio, 1:25. The MS operating parameters were as follows: ionization

Table 1. Specification of the used oils

Oil name	Components and characteristic	
A (solid oil)	Sunflower and soybean	
	Total saturated fatty acid	Max. 30%
	Total unsaturated fatty acid	Min. 70%
	Energy (1 g)	9 Kilocalories
B (liquid oil)	Olive oil	
	Total saturated fatty acid	15%
	Total unsaturated fatty acid	85%
	Energy (1 g)	9.1 Kilocalories

potential, 70 eV; ionization current, 2 A; ion source temperatre, 150° C; resolution, 1000.[25]

Preparation of Cinnamon Essential Oil Stocks

One ml essential oil was used for procurement of 100 ml stock. For preparation of 500, 1000 and 2000 ppm of cinnamon essential oil, and 50 µl of stock was added to 25, 100 and 200 ml ddH_2O, respectively.

Preparation of AAPH Stock and Lipid Peroxidation

0.271 g of AAPH powder were added to ddH2O for preparation of 250 ml AAPH stock in 40 mM concentration and it was maintained at 4-5 °C.

Preparation of Oil Emulsion in Water

50 µl tween 20 was mixed to 0.2 ml (200 µl) oil and 2 ml ddH2O and were mixed by vortexing for 5 minutes. Prepared emulsion was stabled at laboratory temperature for 24 h.[26]

Estimation of MDA in TBA Method

MDA, a lipid peroxidation marker (an end product of lipid peroxidation) was measured by the thiobarbituric acid method. MDA reacted with TBA during lipid peroxidation and yielded a reddish color, which peaked at 532 nm. Color rate indicated MDA concentration.[27]

1 n HCl and 0.67% TBA in a ratio of 1:1 were added to each of oil. The sample was vortexed and heated in a 95° C water bath for 15 minutes. After cooling for 10 minutes, 2 mL of n-butanol-pyridine solution was added. The sample was mixed thoroughly, and centrifuged at 2,000 rpm for 15 minutes. The fluorescence of upper layer was measured by a spectrophotometer at 532 nm.

Identification the Effect of Cinnamon Essential Oil on Lipid Oxidation

200 µl each oil, 50 µl tween 20, 2 ml of 40 Mm AAPH solution were mixed by vortexing. Prepared solution was divided into four glass vials. Respectively, 50 µl of 500, 1000, and 2000 ppm of cinnamon essential oil were added to three of the glass vials separately and one of the glass vials was used as the control. All of the glass vials were incubated at 37° C water bath. Rate of MDA production was measured at the baseline and after the 0.5, 1, 2, 3 and 5 hours. Each experiment was performed in six repetitions.[28,29]

Statistical Analysis

Statistical evaluation was conducted using SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis test was performed for data analysis. Intergroup comparison difference was evaluated using the Dunn's test. Intra-group comparison was carried out by Friedman test. $P < 0.05$ was considered as statistically significant level.

Results

GC-MS analysis of cinnamon chemical composition is illustrated in table 2. Components are as follows: cinnamaldehyde (96.8%), alpha-capaene (0.2%), alpha-murolene (0.11%), para-methoxy-cinnamaldehyde (0.6%), and delta-cadinen (0.4%).

MDA concentration was measured at the baseline and after the 0.5, 1, 2, 3 and 5 hours. Lipid peroxidation was determined based on rate of MDA production in TBA methods in all the samples by the spectrophotometer.

As can be seen in figure 1, maximum rate of MDA concentration obtained in 5th hours of experiment in oil A. Production significantly ($P < 0.05$) reduced with used concentrations of cinnamon essential oil (500, 1000 and 2000 ppm) as compared to the control group in both oils.

As figure 2 illustrates, impact of various concentrations of cinnamon essential oil on changes of MDA rate in oil B were similar to oil A.

Table 3 illustrates intra-group comparison of cinnamon essential oil effects on MDA production in oils A and B. There was no statistically significant difference between the studied groups (control and three case subgroups).

Table 2. GC-MS analysis of chemical composition of *Cinnamomum zeylanicum L.*

Name of compound	Retention time (min)	Ratio of compound in essential oil (%)
Cinnamaldehyde	15.47	96.80
Alpha-capaene	17.90	0.20
Alpha-murolene	21.68	0.11
Para-methoxycinnamaldehyde	22.72	0.60
Delta-cadinen	22.40	0.40

GC-MS: Gas chromatography-mass spectroscopy

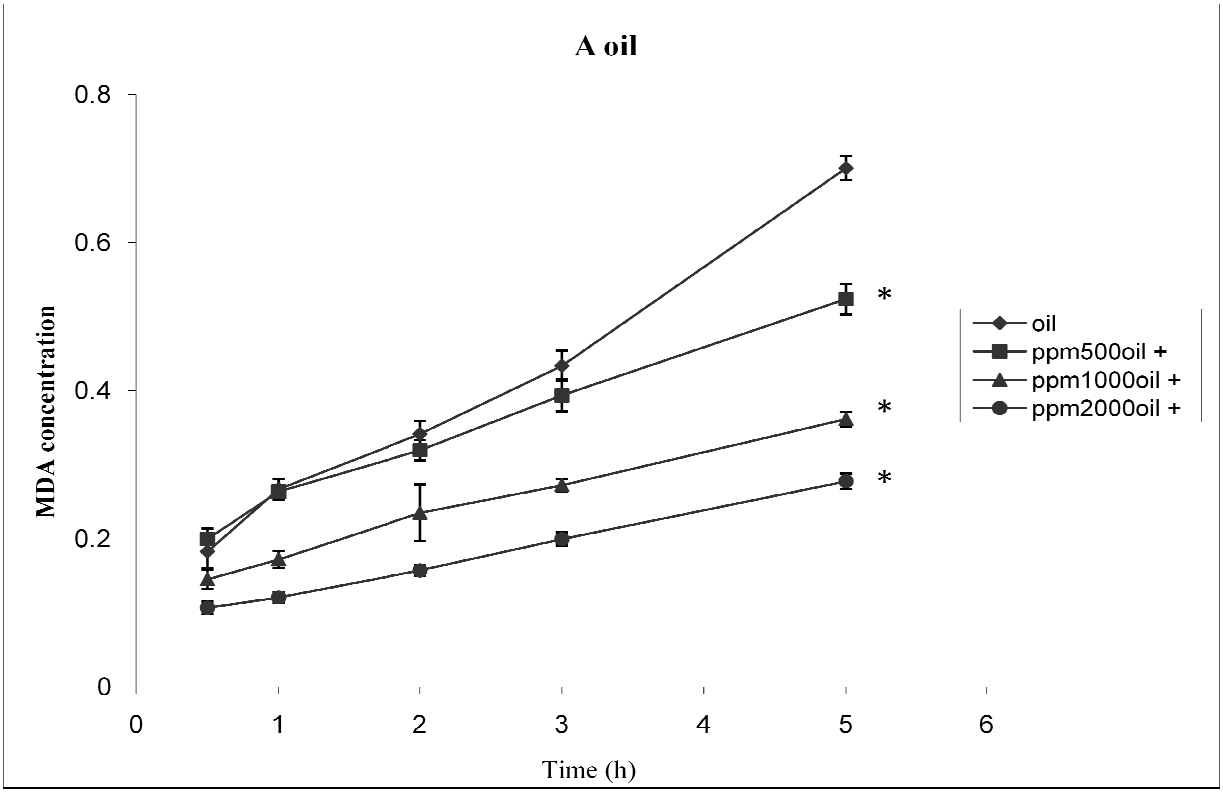

Figure 1. Effect of cinnamon essential oil on malondialdehyde (MDA) production in oil A
*Significant difference between three concentrations of cinnamon essential oil in comparison to the control ($P < 0.05$)

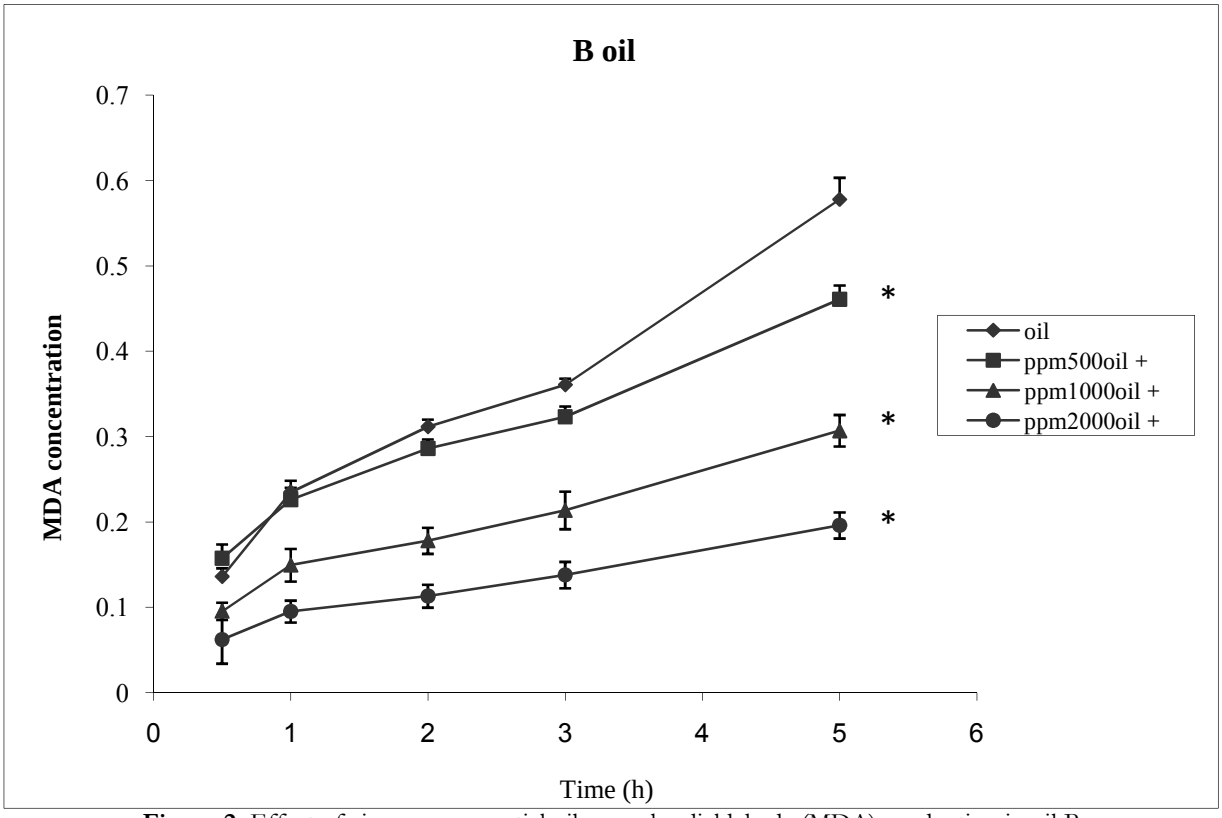

Figure 2. Effect of cinnamon essential oil on malondialdehyde (MDA) production in oil B
*Significant difference between three concentrations of cinnamon essential oil in comparison to the control ($P < 0.05$)

Table 3. Intra-group comparison of cinnamon essential oil effects on malondialdehyde (MDA) production in oils A and B

	Group		Time					P
			0	0.5	1	2	3	5	
Oil A	Control		0.034 (0.030, 0.035)	0.178 (0.164, 0.203)	0.267 (0.252, 0.279)	0.336 (0.326, 0.035)	0.435 (0.418, 0.449)	0.705 (0.684, 0.709)	0.001
	Case (concentration of cinnamon essential oil)	500 ppm	0.085 (0.817, 0.880)	0.199 (0.189, 0.212)	0.262 (0.258, 0.272)	0.318 (0.308, 0.330)	0.394 (0.371, 0.416)	0.522 (0.507, 0.541)	0.001
		1000 ppm	0.062 (0.059, 0.063)	0.145 (0.132, 0.157)	0.172 (0.161, 0.182)	0.229 (0.204, 0.257)	0.273 (0.273, 0.279)	0.364 (0.353, 0.369)	0.001
		2000 ppm	0.050 (0.047, 0.052)	0.105 (0.100, 0.114)	0.119 (0.115, 0.127)	0.161 (0.149, 0.162)	0.196 (0.192, 0.209)	0.276 (0.269, 0.287)	0.001
Oil B	Control		0.065 (0.058, 0.073)	0.133 (0.127, 0.147)	0.237 (0.219, 0.248)	0.313 (0.313, 0.318)	0.361 (0.353, 0.368)	0.579 (0.555, 0.600)	0.001
	Case (concentration of cinnamon essential oil)	500 ppm	0.088 (0.085, 0.092)	0.160 (0.141, 0.168)	0.225 (0.218, 0.237)	0.290 (0.290, 0.294)	0.325 (0.312, 0.333)	0.462 (0.448, 0.475)	0.001
		1000 ppm	0.059 (0.057, 0.063)	0.094 (0.087, 0.104)	0.151 (0.136, 0.163)	0.179 (0.166, 0.190)	0.220 (0.189, 0.230)	0.311 (0.292, 0.319)	0.001
		2000 ppm	0.051 (0.050, 0.053)	0.056 (0.038, 0.093)	0.098 (0.085, 0.103)	0.113 (0.099, 0.126)	0.136 (0.127, 0.152)	0.195 (0.184, 0.207)	0.001

MDA: Malondialdehyde
Data shown based on median (Interquartile range)

Discussion

Studies had demonstrated adverse effect of oxidized dietary fats.[30] Cinnamon is rich in antioxidants. These components are additives that delay the onset of oxidative changes in food.[31] Thus, they contribute to food preservation, prevent changes in flavor, and slow rancidity and discoloration processes.[32]

According to the finding of this study, maximum MDA concentration was obtained in 5th hours and using cinnamon essential oil could be significantly reduced ($P < 0.05$) MDA production and lipid peroxidation.

Cinnamon had strong antioxidant activity.[33] Su et al.[34] stated that 50% acetone extract of cinnamon contained high level of phenolic groups. Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation. Cinnamon essential oil was able to reduce lipid peroxidation in the β-carotene-linoleic acid system.[23] They exhibited a protective capacity against irradiation induced lipid peroxidation in liposomes, and quenched hydroxyl radicals and hydrogen peroxide.[35] Extracts on lard and vegetable oils demonstrated that they could stabilize lard against oxidation and showed antioxidative properties when tested on vegetable oils during storage or frying conditions.[35] Faix et al.[36] revealed, significantly lower lipid peroxidation in plasma and duodenal epithelium of chicks fed the diet supplemented with 0.10% of cinnamon essential oil. Other diets containing 0.05% and 0.025% of essential oil had no effect on lipid peroxidation. In their experiment cinnamon, they had no statistically significant effect on the concentration of MDA in the liver and kidney tissue.[36]

These results suggested that the cinnamon essential oils can be used as a food antioxidant together with the improvement of food palatability. Effect of cinnamon essential oil on MDA production was investigated after the 0, 1, 2, 3 and 5 hours in our study; therefore it seems necessary to study this research in longer intervals. Further studies are needed to identify antioxidant activity of other plants and to investigate effects of synergic association these plants for inhibition of oils lipid peroxidation.

The present study supported that cinnamon extracts supplementation in oils reduce lipid peroxidation. Use of various cinnamon extracts concentrations was lead to determine appropriate concentrations for further studies. However this study was limited in a number of ways which deserve careful attention; first, the time of the study

was short. Second, this experiment can be for more oils in different type such as cooking oils, hydrogenated oils and frying oils and compare MDA formation in these oils in present of cinnamon essential oil.

Conclusion

Findings of the present investigation demonstrated that cinnamon essential oil possesses considerable antioxidant capacity and could readily be implemented as a natural preservative, thus reducing or avoiding losses due to oxidative processes. Thus, it appears that this spice can be used with fresh and heated oils. Further studies will be carried out to determine the types of other oils and other spice or plant rich in antioxidant.

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Conflict of Interests

Authors have no conflict of interests.

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