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Determination of the Antioxidant Activity and Stability of Chamomile (*Matricaria chamomilla* L.) Extract in Sunflower Oil

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Abstract: Antioxidants prevent the reaction of free radicals with biomolecules and can promote the nutritional values and physiological properties of foodstuffs. There are synthetic and natural kinds of antioxidants and nowadays there is a trend to replace the natural kinds instead of the synthetic kinds. Chamomile (*Matricaria chamomilla L.*) is a well known and valuable medicinal plants that is used widely in Iranian traditional medicine. The antioxidant effect of essential oil of Chamomile had proofed and this study shows the antioxidant activity of chamomile extract. The extract was obtained by the mixture of equal volume of water and ethyl alcohol (1:1). The antioxidant activity and stability was investigated with three methods, DPPH˙ free radical scavenging system; determine of the peroxide and thiobarbituric acid numbers. The antioxidant activity of the extract were determined in 0.2, 0.4, 0.6, 0.8 and 1 mg/ml concentrations by measuring of peroxide and thiobarbituric acid numbers in a crude sunflower oil as a greasy food. The antioxidant activities of the extracts were valuable and were raised by increasing of the extract concentrations. Results show that chamomile extract can be use as a natural antioxidant in oily foods as a complementary material.

Key words: *Matricaria chamomilla* L. · Peroxide number · TBA number · Antioxidant · Sunflower oil

INTRODUCTION

Chamomile is sometimes known as "the plant doctor", because it is thought to help the growth and health of many other plants, especially ones that produce essential oils. It is thought to increase production of those oils, making certain herbs, like mints (spearmint, sage, oregano) and basil stronger in scent and flavor [1-3].

Chamomile tea is also thought to be useful to suppress fungal growth, for example, misting it over seedlings may prevent damping off [4]. The main chemical components of the chamomile extraction oils are α-pinene, β-pinene, camphene, sabinene, myrcene, 1,8- cineole, y-terpinene, caryophyllene, propyl angelate, butyl angelate, chamazulene, a-bisabolol, bisabolol oxide A, bisabolol oxide B and bisabolone oxide A [5,6].

Some compounds such as apigenin 7-O-glucoside and various acylated derivatives of apigenin 7-O-glucoside have identified in chamomile [7-10].

Oil oxidation is a free radical chain process leading to the deterioration of oil and lipid containing materials [11,12].

Recently, the interest in natural antioxidants has been increased since the application of the most widely used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propyl gallate (PG) has been questioned because of possible toxic and carcinogenic components formed during their degradation [15, 16]. Phenolic compounds are the main class of natural antioxidants [17-19].

Chamomile (*Matricaria chamomilla L.*) is one of the popular ingredients in herbal teas. This herb has been traditionally used for medicinal purposes such as selective COX-2 inhibitor with anti-inflammatory activity [20], antimicrobial action, antioxidant action, antiplatelet action, chemopreventive action [21, 22].

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According to the published papers, the research on active constituents in chamomile as an antioxidant and a medical plant is widely carried out [23-25].

The conjunction of chamomile with sedative drugs such as analgesics, benzodiazepines, or alcohol may be contraindicated [26-28].

Chamomile (*Matricaria chamomilla* L.) essential oil has been reported as a natural antioxidant [29].

MATERIALS AND METHODS

Materials: The powder of Chamomile (*Matricaria chamomilla L.*) were obtained from the fresh harvest in region of Shiraz, Iran. A sample of crude sunflower oil was obtained from Ghoo Oil Company, Tehran, Iran. All chemical compounds and solvents were purchased from Merck Company, Germany.

Preparation of Chamomile Extracts: For preparation of Chamomile extract, 10g of dried sample were extracted twice with 200ml of ratio of ethyl alcohol and water (1:1) and mixed at room temperature for 1h and then heated at 80°C for 1 hr. The extract was filtered through Whatman No. 1 and combined followed by concentration using a rotary evaporator at 50 ^{0}C and finally weighed to determine the yield [30,31].

Sample Preparation: Inhibition effect of the oil substrate was achieved by adding of the certain methanolic solution of the antioxidant to a weighed oil sample. Samples contain 0.2, 0.4, 0.6, 0.8 and 1 mg/ml of the antioxidant in the crude sunflower oil, without antioxidant, were prepared. The samples and a control sample, crud sunflower oil without antioxidant, were located in 60°C for certain of period times. The peroxide and Thiobarbituric acid number of samples were measured in 0, 8, 16, 24 and 32 days.

Determination of Antioxidant Activity: Antioxidant activity was determined by the methods to determine of peroxide and thiobarbituric acid numbers.

Peroxide Method: This measurement was accomplished according to the AOCS method. In a container 250 ml, added to 3g of each samples to 30 ml of a solution of acetic acid and chloroform (1:1) and mixed to prepared a suitable solution, then added 0.5ml saturated solution of KI and stirred it for one minute, 30ml stilled water was added and titrated with 0.01N sodium thiosulphate, the yellowish color was disappeared. 0.5ml starch glue indicator was added to the solution and blue color was appeared the titration was continued until the blue color was disappeared. The peroxide number (PV) was calculated when S-S_0 was difference between amount of consumption thiosulphate for samples and control:

$$PV = (S-S_0) \times N \times 1000/W$$

Where: N - The thiosulphate solution Normalization;
W - the weight of samples (g).

Thiobarbituric Acid Method (TBA): In addition of the peroxide number, the thiobarbituric acid number (TBA) is a complement test to determine of the antioxidant activity. The thiobarbituric acid number measures the Malon dialdehyde (MDA) per one kilogram oil. This measurement was accomplished according to the Sidewell method. In a container 250 ml, dissolved 1g of each samples to 10ml of carbon tetrachloride and added 10 ml thiobarbituric acid solution (aqueous solution of 0.67% thiobarbituric acid in the same volume of glacial acetic acid) then stirred it for 2 h and centrifuged for 5 min. with 1000 rot/min. after this step the aqueous layer was separated and heated in boiling water bath for 1h. Finally, the absorption values (E) was measured at 532 nm wavelength.

TBA Was Calculated as Bellow Equation:

$$E = e/(d.\ a)$$

Where:
e - Measurement absorption;
d - Cell thickness;
a - weight of sample (g).

DPPH$^{\bullet 0}$ Method: This measurement was carried out according to the Brand - Williams method. In this method prepared seven samples from 1 to 7 mg/ml of the antioxidant and the free radical of DPPH^0 reacted with the antioxidant and produced yellowish color and reduced the absorption value in wavelength to 517nm. Then added 0.5 ml of various concentrations of extracted oil to 2 ml of 6×10^{-5} methanolic solution of the DPPH^0 free

radical and stay in room temperature for 1h.The absorption of solution was read in 517nm. The control sample was a solution of 0.5ml methanol in 2ml solution of the $DPPH^0$. This test was repeated for 3 times. Radical Scavenging Activity (RSA) was determined in below:

$$\% RSA = [1- ((A_{control} - A_{sample}) / A_{control}) \times 100]$$

Where:

$A_{control}$ - The control absorption;
Asample - The sample absorption.

EC_{50} is explained the anti-radically activities which is the percent of the extraction that be able to neutralized 50% of the initial $DPPH^0$ free radical.

Statistical Analysis: All determinations were carried out in three triplicate and data were subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the MSTATC software. Significant differences between means were determined by Duncan's multiple range test. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Figure 1 illustrate the rate of increase in the anti-radically activities and decrease in the reminded $DPPH^0$ free radical with arise of the chamomile extract concentration from 1 to 7 mg/ml. EC_{50} for chamomile extract is 5.52 ± 0.15 mg/ml.

Figure 2 show the rate of decreasing of the remained $DPPH^0$ in 60 min. for the various extract condensations. Radical scavenging activity rise with increasing in extract condensations and to be constant after 60 min. There is no descending in the control sample. Therefore this explains the remained $DPPH^0$ has inversion related to the antioxidant radical scavenging activity.

Table 1 show the average of peroxide number (meq O_2/kg oil) of the various samples of Chamomile extracts and the control sample in 0, 8, 16, 24 and 32 days.

There are the punctual differences among the control sample and the different concentration of extracts and it is clear that the peroxide numbers are depend on the sample concentrations. Increase the concentration makes decrease the peroxide number and increase the antioxidant effects.

These numbers illustrate that Chamomile extracts have antioxidant effects. For example Figure 3 illustrate the peroxide number in 32th day and show the punctual differences in the various condensations.

In addition of the peroxide number, the thiobarbituric acid number (TBA) is a complement test to determine of the antioxidant activity. Table 2 is related to the average of the thiobarbituric acid number (meq/kg oil) of the various samples of Chamomile extracts and the control sample in 0, 8, 16, 24 and 32 days.

There are the punctual differences among the control sample and the different concentration of extracts.

The TBA numbers are depend on the sample concentrations such as the peroxide numbers and increase the extract concentration makes decrease the TBA number and increase the antioxidant effects.

Table 1: The peroxide number (meq O_2/kg) of samples in the five days

Sample	0 day	8^{th} day	16^{th} day	24^{th} day	32^{th} day
C-0.2	0.43 ± 0.12	14.56 ± 0.03	35.65 ± 0.25	45.76 ± 0.30	60.37 ± 0.67
C- 0.4	0.43 ± 0.12	13.59 ± 0.15	32.76 ± 0.65	42.45 ± 0.25	54.45 ± 0.78
C- 0.6	0.43 ± 0.12	13.66 ± 0.35	32.45 ± 0.40	40.86 ± 0.72	52.40 ± 0.12
C- 0.8	0.43 ± 0.12	13.60 ± 0.28	28.51 ± 0.24	39.15 ± 0.12	48.56 ± 0.34
C- 1.0	0.43 ± 0.12	12.43 ± 0.05	26.33 ± 0.06	36.14 ± 0.27	44.20 ± 0.24
Control	0.43 ± 0.12	18.54 ± 0.40	38.45 ± 0.07	61.04 ± 0.55	78.76 ± 0.35

Data are the averages of the three repetitions ± standard deviation

Table 2: The TBA number (meq MDA/kg oil) of samples in the five days

Sample	0 day	8^{th} day	16^{th} day	24^{th} day	32^{th} day
C-0.2	0.000	0.068 ± 0.01	0.114 ± 0.01	0.216 ± 0.01	0.448 ± 0.01
C- 0.4	0.000	0.059 ± 0.00	0.107 ± 0.01	0.133 ± 0.01	0.432 ± 0.02
C- 0.6	0.000	0.053 ± 0.00	0.089 ± 0.01	0.145 ± 0.00	0.376 ± 0.01
C- 0.8	0.000	0.050 ± 0.01	0.085 ± 0.00	0.110 ± 0.02	0.269 ± 0.01
C- 1.0	0.000	0.041 ± 0.01	0.073 ± 0.00	0.129 ± 0.01	0.230 ± 0.00
Control	0.000	0.093 ± 0.02	0.119 ± 0.02	0.243 ± 0.03	0.586 ± 0.02

Data are the averages of the three repetitions ± standard deviation

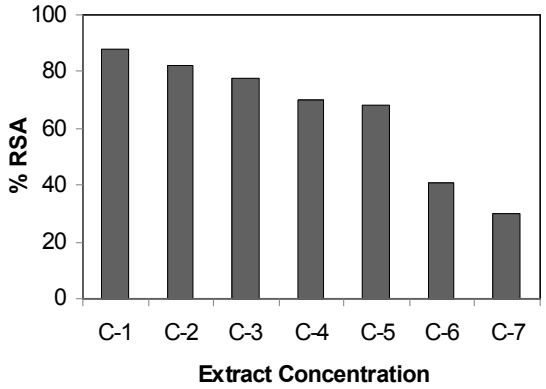

Fig. 1: Relationship between radical scavenging activity with Chamomile extract condensation. C-1 to C-7 are extract condensations from 1 to 7 mg/ml.

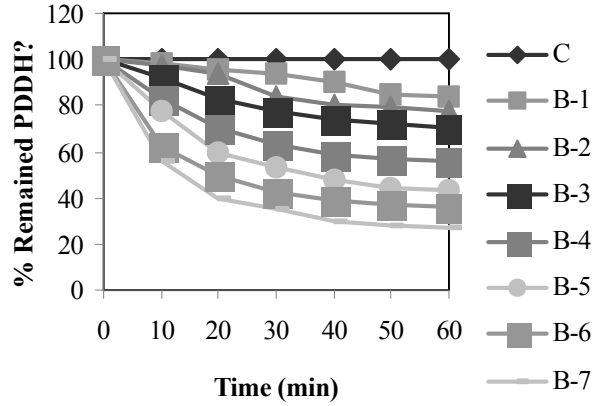

Fig. 2: Trend of the reduce percentage of remained $PDDH^0$ in 60 min with Chamomile extract concentration. B-1 to B-7 are extract condensations from 1 to 7 mg/ml.

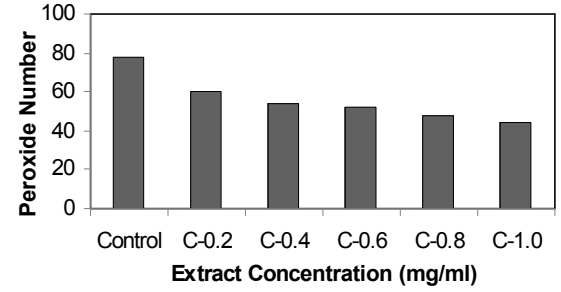

Fig. 3: Relationship between the peroxide number with the Chamomile extract condensation.

These numbers illustrate that Chamomile extracts have antioxidant effects. To more realization the results of the 32th day are explained in the Figure 4. This chart illustrates the TBA number in 32th day and shows the punctual differences in the various condensations.

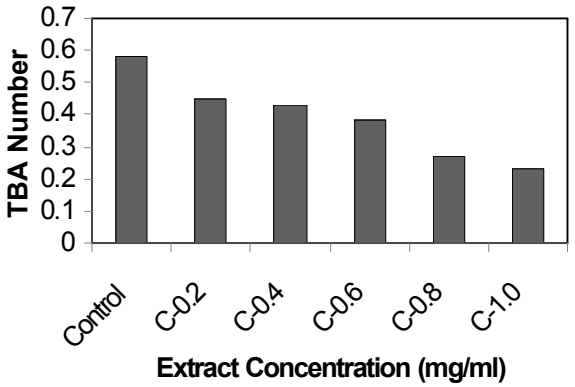

Fig. 4: Relationship between the TBA number with the Chamomile extract concentration

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