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Study of Antioxidant Properties of Anacyclus Pyrethrum Root Extract

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Abstract

There are several methods to prevent oxidation of oils, one of which is the addition of synthetic antioxidants. However, due to the fact that synthetic antioxidants have undesirable effects, such as the effect of mutagenicity and carcinogenicity in human body, it is necessary to investigate the sources of natural antioxidants in order to replace synthetic compounds. In this study, methanolic extract of Anacyclus pyrethrum root were extracted. Extracted extract were added to sunflower oil at different concentrations (200, 400, 800 and 1600 ppm) and the amount of phenolic compounds and free radical inhibitory activity as well as oxidative stability of oil at 65° C for three days was investigated by measuring the TBA and finally compared with the synthesis antioxidant BHT at 200 ppm. In general, the results showed that by increasing concentration of Anacyclus pyrethrum root extract in sunflower oil from 200 to 1600 ppm in one fix time, the amount of phenolic compounds and inhibitory activity of free radicals increased while the TBA index decreased.

Keywords: Natural Antioxidants, Anacyclus Pyrethrum Root, Sunflower Oil, Phenolic Compounds.

Introduction

Today, one of the problems of the food industry is the use of different compounds of kinetic as a preservative that potential risks of each of these compounds have proved for human health. Unfortunately, synthetic antioxidants are still in use in the oil industry. In this research, we have used indigenous and valuable sources, such as Anacyclus pyrethrum root, and we have investigated antioxidant effect of its extract for valuable oil stabilization, called sunflower, and finally we compared with the synthetic antioxidant BHT.

Anacyclus pyrethrum is a small herbaceous plant with a height of 20 to 25 centimeters, its root has low branches and small and numerous stems. Its lower leaves are wide and the upper cups have a right appearance and narrow and various cuttings and also covered with fluff and it is green to blue (Zargari, 1992). The root of the plant has a small amount of tannin, 57%. It is an inulin and a yellow material, and the oldest, there is another substance called Poly Turin. Poly Turin is obtained in the form of needle-shaped crystals, melting at a temperature of 70 °. It is slightly soluble in water, but it is soluble in organic solvents.

The origin of oil at the stage of its synthesis is from vegetable sugars. These sugars are placed in organs forming oil or fat such as the oil seed by leaves and subsequently synthesized from the stored proteins. The oil in the cell's plasma is as very fine droplets and emulsion which are visible as dispersed droplets with increasing concentration. The formation of oil is primarily function of controlled genes and, in the second, influenced by environmental factors. Generally, vegetable oils have iodine indexes of 200-80 and are in liquid state at laboratory temperature.

Vegetables oils are used as valuable and natural ingredients in human and animal nutrition, health-medical consumptions to industrial applications such as use as lubricants and / or persistent and clean raw materials for fueling. The main sources of vegetable oils are oilseeds such as soybeans, sunflowers, canola, corn, cottonseed, peanuts, as well as some tree fruits and their kernels such as palm and olive. Triglycerides are as the main compounds of vegetable oils, fatty acids in triglycerides have a large number of carbons, and these acids may be saturated or unsaturated. Shahsavari et al. (2008) investigated the antioxidant effect of essential oil of Zataria multiflora boiss in soybean oil. The essential oil showed a good antioxidant effect and after supplementary tests, it can be used as a natural antioxidant in some foods (Shahsavari et al., 2008). Goli et al. (2005) investigated the antioxidant effect of extracts of pistachio green skin in soybean oil. The results showed that the concentration of 600 ppm of the extract with the concentration of 200 ppm of synthetic antioxidants had the highest antioxidant effect. Mir Ahmadi et al. (2005) investigated the effect of polyphenols in tea leaf extract in preventing oxidation of sunflower oil.

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The results of this study showed that the aqueous extract extracted from Iran's green tea leaf has a more antioxidant properties than BHT, BHA and alfatucophorol in sunflower oil. Due to the solubility of the polyphenolic compounds of the extract in water, its extraction is relatively simple, and for this purpose, wastes of tea factories may naturally be used to reduce cost (Mirahmadi et al., 2005).

According to the specification of the bad effects of synthetic antioxidants that have toxic effects on consumers, it disrupts the activity of liver enzymes and leads to a variety of cancers. In the world, their application is limiting, so identifying antioxidant from available and inexpensive sources and determining the effects of their stabilization on oils under various conditions are one of the important goals of this research.

Hence in this research, relatively common abundant, inexpensive and available antioxidant natural resources such as root extract of Anacyclus pyrethrum are added to the sunflower oil, which is one of the most important sources of vegetable oil, and its stabilization effects during keeping conditions in four concentrations will be compared to sunflower oil containing BHT antioxidants.

Materials and Methods

Sunflower oil without antioxidants was prepared from Khorasan three-flowered vegetable oil factory and was stored at 4 ° C until the test time. The Anacyclus pyrethrum root was prepared from a local market of Sabzevar city from a type of variety and its waste parts were separated and immediately dried after washing.

Extraction of Anacyclus pyrethrum root extract by maceration method:

For extracting extract, the roots cleaned with the mill (Convode model 100CG) were crushed and after sifting, it was mixed with methanol solvent at a ratio of 1: 10 and placed in a hot plate at 250 rpm for 24 hours and then straightened with Watten filter No. 1. Now by rotary evaporator (LABORATA4000 model) was concentrated in 40 ° C and finally the extracts were dried by vacuum drier at 45 ° C and placed in a sealed container and impervious to air at 4 ° C until using (Burits & Bucar, 2000).

Measurement of Phenolic Compounds:

Calibration curve drawing:

First, 0.4 g of dried gallic acid was dissolved in 10 ml of methanol and then distilled in 100 ml volumes. A mother solution was prepared, which the values 0,1,2,3,5,2, 10,20 ml of the solution was transferred to 100 ml balloons to plot the calibration curve and each was reached to distill water in a volume of 100 ml. These solutions contained concentrations of 0, 50, 100, 250, 500, 1000, 2000 mg / L glycolic acid. Then, they were taped into foil test tubes, 0.5 ml of gallic acid solutions, 2.5 ml of folin ciocalteu reagent (diluted to prepare folin ciocalteu reagent with distilled water 1 to 10%), and after 10 minutes, 2 ml sodium carbonate 7.5% was added. After an hour at room temperature, absorbance was read at 760 nm. Finally, the absorption curve against the concentration of gallic acid (mg / L) with a coefficient of 0.99 was obtained:

$$Y=0.0034x+0.1059$$

Where X is the absorbance read at 760 nm and Y is the phenolic compounds in mg / ml (1).

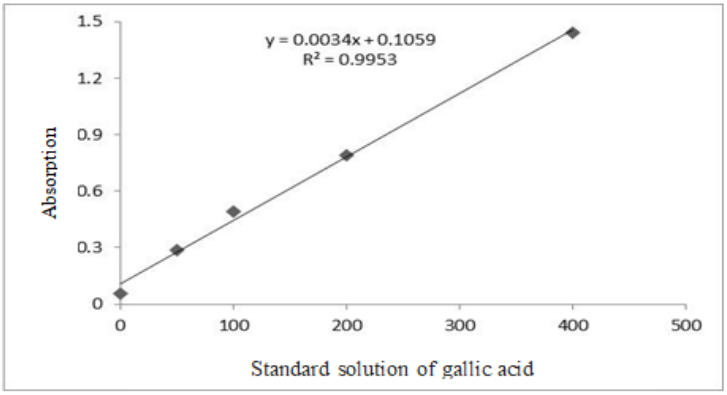

Figure 1. Calibration curve of concentration of phenolic compounds against the absorbance read at 760 nm

Measurement of the phenolic compounds of the sample:

At first, standard solutions were prepared from root extract of Anacyclus pyrethrum with solvent (methanol) that extraction is done by it with different concentrations of 200,400,800, 1600 ppm and control sample. Then 0.5 Ml standard solution of root extract of Anacyclus pyrethrum, 2.5 ml of folin ciocalteu reagent 0.2 normal (for the preparation of the folin ciocalteu reagent, this concentrated solution was diluted 1 to 10 with distilled water) was added into 50 ml balloons, and after 10 minutes, 2 ml of sodium carbonate solution 7.5% was added and reached to the final volume with methanol. After two hours at room temperature, the absorbance was read at 760 nm. From the calibration equation (for gallic acid as standard), the total

amount of phenolic compounds based on gallic acid was determined in percent (Stoilovaet al., 2007)

Measuring Free Radical Inhibitory activity (DPPH) Sample:

Evaluation of antioxidant activity was measured by investigating the free radical inhibitory activity (DPPH). 2 and 2, diphenyl-1-picrylhydrazyl (DPPH) is a stable radical combination with purple color recovered by electron or hydrogen (Antioxidant compounds) is converted to a yellow diphenyl-1-picrylhydrazyl. In this method, as a stable radical compound from (DPPH) was used as a reagent. So, 2 ml of different concentrations of the extract in ethanol were added to 2 ml of 0.004% (DPPH) solution in ethanol. After 90 minutes of darkness at room temperature, optical absorption of sample at 517 nm was read by a spectrophotometer (Milton Roy model made in American). The percentage of inhibition of free radicals (DPPH) was calculated using the formula below.

$$I\% = \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100$$

In this formula, A_{Blank}shows the optical absorbance of negative control that does not have an extract and A_{Sample}expresses the optical absorbance of the various concentrations of the extract. In this experiment, the BHT synthetic antioxidant is used for comparison (Seabury, 2002).

Calculating the Thiobarbituric Acid Index:

To measure the index of thiobarbituric acid in a 250 ml flask, one gram of sample, one ml of 0.75% solution of thiobarbituric acid and 2 ml of a 35% solution of trichloroacetic acid were added. The resulting mixture was boiled in boiling water for 20 minutes. After this time, the mixture was centrifuged at 3,000 rpm for 3 minutes. The aqueous phase was removed by syringe and transferred to a spectrophotometer. The absorbance of the sample was read by spectrophotometer at 532 nm. In this way, the absorbance of sample at the wavelength was considered as the index of thiobarbituric acid (. Nemat, 2013).

Statistical Analysis

To evaluate the results, a completely randomized statistic design was used. The data were analyzed using MstatC software and comparison of the means with each other and with the control sample was done using Duncan test at alpha probability level of 0.05 with this software. Also, Microsoft Excel software was used to plot the graphs.

Discussion and Results

Evaluation of the Phenolic Compounds of Anacyclus pyrethrum Root Extract

Results of variance analysis (Table 1) showed that change in Anacyclus pyrethrum root extract concentration significantly affected the amount of polyphenolic compounds measured ($p < 0.05$). The results of the comparison of mean has shown the effect of different concentrations of the Anacyclus pyrethrum root extract on the amount of phenolic compounds present in the extract measured by the Folin test and the standard curve equation of gallic acid in Fig. 3.1. As it can be seen, by increasing Anacyclus pyrethrum root extract concentration from 200 to 1600 ppm, the amount of phenolic compounds in the extract increased, which leads to an increase in the antioxidant properties of the extract. According to the results, by increasing concentrations of this extract, the amount of these antioxidant compounds increased from 19.41ppm at concentrations of 200 ppm to 103.6 ppm at 1600 ppm of the Anacyclus pyrethrum root extract and this increase was significant in all concentrations of the extract, at 95% probability level ($P < 0.05$) compared to the control sample with 10.45 Ppm polyphenolic compounds.

Table 1. Summary of the results of the analysis of variance analysis of the effect of Anacyclus pyrethrum root extract on the amount of phenolic compounds

Changes source	Degree of freedom	Sum of Squares	Mean Square	F-alue	p-value
Treatment	4	20358.14	5089.55	698.73	<0.0001p
Error	10	72.84	7.28	-	-
C.V	4.98%	-	-	-	-

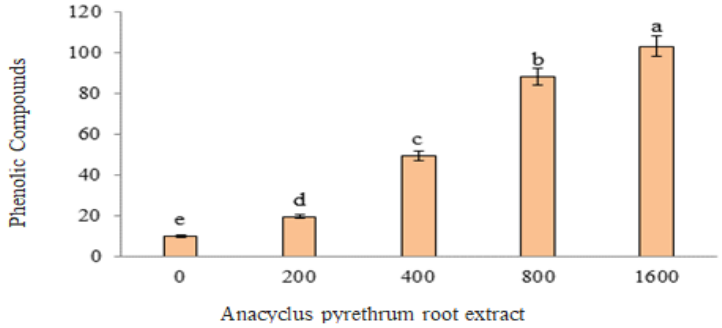

Figure 2. Comparison of the mean of phenolic compounds in different concentrations of Anacyclus pyrethrum root extract

Investigating the Inhibitory Power of Free Radicals (DPPH) Anacyclus pyrethrum Root Extract

Results of one-way ANOVA (Table 2) indicated that the effect of different concentrations of the Anacyclus pyrethrum root extract on the activity of free radical inhibitory activity was significant ($p < 0.05$). Comparison of the means by LSD test showed that by increasing Anacyclus pyrethrum root extract concentration, the inhibitory power of the free radicals of the extract significantly increased from 69.66% at a concentration of 200 ppm to 86.95% at the concentration 1600 ppm (Figure 3). In fact, this result was due to the fact that the ability of the extract to inhibit free radicals is concentration-dependent and increases by increasing the concentration of the extract of its anti-radical activity. According to the results, samples of 400 and 800 ppm of the extract had an antiradical activity of 79.87% and 86.96% respectively, which was not statistically significant ($p > 0.05$).

By comparing the inhibitory activity of different concentrations of the Anacyclus pyrethrum root extract, with the synthesis antioxidant BHT with a concentration of 200 ppm and antidiabetic activity equal to 49.12%, it was found that all concentrations of the Anacyclus pyrethrum root extract in the range of 200 to 1600 ppm had a higher anti-radical activity than the synthesized antioxidant BHT at 200 ppm, indicating the high antioxidant power of the plant extract compared to synthetic antioxidants such as BHT (Fig. 3.2).

Table 2. Summary of the results of the analysis of variance of the effect of Anacyclus pyrethrum root extract on the amount of DPPH-free radical inhibitory activity

Changes source	Degree of freedom	Sum of Squares	Mean Square	F-value	p-value
Treatment	4	2708.87	677.22	191.30	<0.0001p
Error	10	35.40	3.54	-	-
C.V	2.56%	-	-	-	-

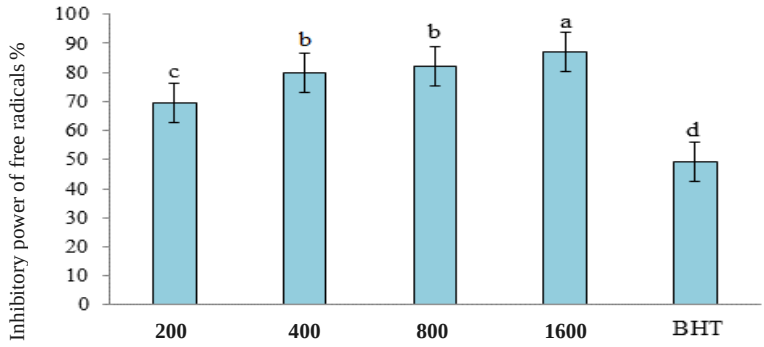

Figure 3. Changes in inhibitory activity of free radicals in different concentrations of the Anacyclus pyrethrum root extract compared to the synthetic antioxidant BHT

Index of Thiobarbituric Acid (TBA)

The results of analysis of variance (Table 3) showed that changes in the TBA index of sunflower oil stored in oven at 65 ° C were significantly influenced by Anacyclus pyrethrum root extract and time of storage ($P < 0.05$). According to the results, the initial value of the TBA index of sunflower oil measured before placing in oven was 0.072 mg malondialdehyde per kilogram, which increased the values of the index of thiobarbituric acid in the fixed treatment by increasing the storage time of the samples under oxidation conditions.

Table 3- Summary of the results of the analysis of variance, the effect of different concentrations of Anacyclus pyrethrum root extract on the TBA index of sunflower oil during storage in an oven at 65 ° C

Changes source	Degree of freedom	Sum of Squares	Mean Square	F- value	p- value
Anacyclus pyrethrum	5	0.0623	0.0124	3.02	0.022
Storage time	2	0.0488	0.0244	5.93	0.0059
Interaction	10	0.0021	0.0002	0.05	-0.982
Error	36	0.148	0.0041		
C.V	14.48%	-	-	-	-

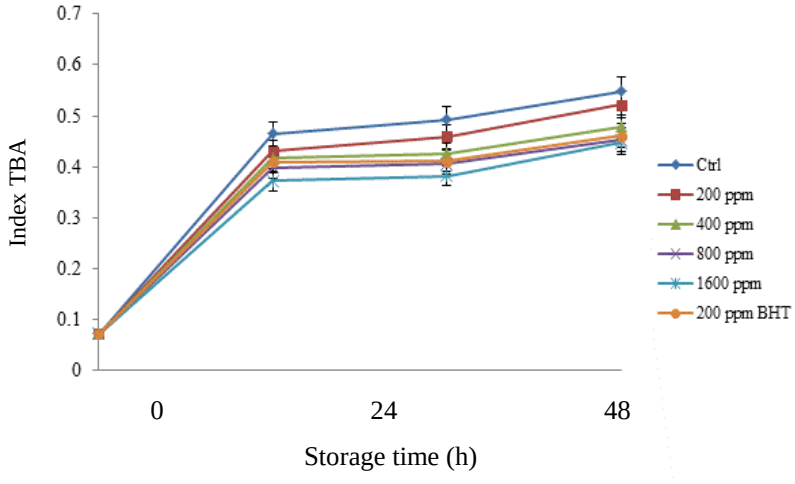

Figure 4. Changes in the index of thiobarbituric acid of sunflower oil stored in oven at 65 ° C influenced by the effect of Anacyclus pyrethrum root extract and antioxidant BHT during storage

As it can be seen, the slope of increasing this index during the first 24 hours of storage was higher, and at the next 48 and 72 hours, the changes in the TBA index of sunflower oil was done with less slope. According to the results after 24 hours of oven for samples containing 200, 400, 800 and 1600 ppm of extract, 200 ppm of synthetic antioxidant BHT, and the control sample, the TBA index from the initial value 0.072 reached to 0.465, 0.431, 0.417, 0.398, 0.372, 0.409 mg malondaldehyde per kilogram, then increased by a gentle slope up to 72 hours, which in most cases was not significant.

Conclusion

Therefore, in this study, Anacyclus pyrethrum root extract was used as a suitable substitute for synthetic antioxidants in order to use plant antioxidants. Based on the results, the concentration of 1600 ppm of the Anacyclus pyrethrum root extract than the other concentrations of the extract (200, 400, 800 and control) due to higher values of antioxidant compounds, in terms of total phenolic compounds and inhibitory activity of free radicals was more effective and had a more inhibitory effect compared to the synthetic antioxidant BHT at 200 ppm. The results of oxidative stability analysis of oil containing different concentrations of extract also showed that the 1600 ppm concentration of Anacyclus pyrethrum root extract was significantly affected by reduction of TBA index in comparison with other concentrations and synthetic BHT antioxidants at 200 ppm level.

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