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Stabilization of Soybean oil by Rosmarinus officinalis L. extracts during accelerated storage

Nabi Shariatifar*[1], Gholam Reza Jahed[1], Fahimeh Tooryan[2] Mohammad Rezaei[1]

[1]Department of Environmental of Health, School of Public Health, Tehran University of Medical Sciences (TUMS), Tehran, Iran
[2] Department of Food Hygiene, Faculty of Veterinary Medicine, University of Amol, Mazanderan, Iran

***Corres.author: nshariati@tums.ac.ir, Telephone number: 02188954914**

Abstract: Some of the plants and their extracts are added to the diet not only aromatic but also as a preservative. This research was performed to determine antioxidant activity of ethanolic and water extract Rosmarinusofficinalis on soybean oil. Antioxidant capacity of extracts of Rosmarinus officinalis was assessed by 1,1 diphenyl-2- picrylhydrazyl (DPPH) and Beta- carotene. Peroxide values (PVs) and thiobarbitoric acid-reactive substances (TBARs) be evaluated different concentrations (0,200, 400 and 800 ppm) of ethanolic, water extracts and β hydroxyl toluene (BHT, 100 ppm) that added on soybean oil and incubated for 35 days at 65°C. Peroxide values (PVs) and thiobarbitoric acid-reactive substances (TBARs) levels were measured every week during the time period of the study.Values were compared among groups in each incubation time using ANOVA test. Results showed that DPPH and β- carotene- linolic acid assay findings on the Rosmarinus officinalis ethanolic and water extract were lower than of synthetic antioxidant, BHT. Therefore, during the incubation time, Rosmarinus officinalis ethanolic and water extracts lowered PVs and TBARs levels when compared to the control ($p<0.001$).
Keywords: Rosmarinus officinalis, Extract, Antioxidant activity and Soybean oil.

1. Introduction:

In recent times, the search for antioxidant activity has been shifted to plants. However, the major part of the search has focused mainly on higher plants while little attention is given to lower plants with possible properties of antioxidant (1).

Most medicinal plants are useful in treating disease in the body and in most cases; the antioxidant activity attributed to some plants is beyond belief. Use from medicinal plant in the world as a preservative has been used since ancient times. Antioxidant activity of some plants, including herbs is due to the presence of hydroxyl groups in their compounds of phenolic (2).

Free radicals reduce the liquid membrane, loss of enzyme receptor activity and damage to membrane protein, leading to death (3). These free radicals are involved in chronic disease such as cancer, heart disease, cataracts, neurodegenerative diseases, epilepsy & degradation of essential fatty acids (4,5).

Antioxidants are considered to play an effective role in preventing the development of some chronic diseases. Synthetic antioxidants used in food, however, due to their toxicity and carcinogenicity are

discouraged. Therefore, due to the toxicity and carcinogenicity of synthetic antioxidants, using of antioxidants of natural origin is recommended by scientists (6)

Rosmarinus officinalis(Vent.) Boissis a genus of plant in the Lamiaceae family. Rosmarinus officinalis species are generally known under the name ‘‘Rosmary” in Iran and are commonly used as flavoring agentand medical plant. Rosmarinus officinalis is a persistent plant of about 1.5-2m high, with flowers of white, pink, purple or deep blue which grow on friable loam soil with good drainage in an open, sunny position places in the Asia and Mediterranean (7). This plant is a worldwide known folk medicine for improving memory, inflammation, nociceptive etc. The chemical investigation of the genus showed the presence of phenolic diterpenes, flavonoids, tannins and phenolic acids (8).

Duo to plant grows in many Provence’s in Iran and as result it can be obtained very cheap. It has been widely used in Persian folk medicine. It may be a good available natural source of antioxidant and a good preservative for food stuffin in order to prevent spoil of food consignment and also improving quality of food products.

Therefore, the aim of this study was to evaluate antioxidant activity of plant extracts in soybean oil as a food system.

2. Experimental Procedure

2.1. Samples and reagents

Aerial parts of Rosmarinus officinalis were collected from Fars province, Iran. A voucher specimen for this plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Tehran University Medical Science, Tehran, Iran. The plants were dried in a dark place at room temperature. Dried leaves were powdered using an electrical device and stored at refrigerator (4°C) until use. Refined, bleached and deodorized soybean oil without any antioxidants was taken from the company of manufacturing (Behshahr, Tehran, Iran).

2.2. Preparation of stripped soybean oil

The method described by Yoshida (1993) with some modifications to remove the tocopherols from soybean oil by column chromatography using alumina. Antioxidants (essential oil and BHT) were added to soybean oils free from tocopherols(9).

2.3. Preparing extracts

First, ethanol extract was prepared according to the method proposed by Shyamala et al . For this purposes, 10 g of powdered leaves of Rosmarinus officinalis were extracted in soxhlet apparatus with 100 ml of ethanol (HPLC grade) until the extraction solvent became colorless. The extraction was repeated twice at the same condition (9).

Second, water extract was prepared by means of a Percolator. Leaf samples were with distilled water in a Percolator apparatus until the extracted water became colourless.

Extracts were filtered and evaporated to dryness in vacuum. The crude extract was weighted and kept in a closed dark glass bottle and stored at 0- 4° C until use.

2.4. In vitro antioxidant activity

2.4.1. DPPH assay

The scavenging activity of the stable 1,1-diphenyl-2-picrylhy-drazyl (DPPH) (Sigma, Aldrich) free radical was determined by the method described by Cuendet et al.

Briefly, 50 µL of the extracts (various concentrations) were added to 5 ml of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm(10). The radical-scavenging activities of the samples were calculated as percentage of inhibition according to the following equation: %DPPH radical scavenging = [(control absorbance (blank) - sample absorbance)/ (control absorbance)] ×100

All tests were done in triplicate. Values (mean ± SD) of the extracts were compared with those of BHT using student’s t-test. A p-value less than 0.05 were statistically considered significant.

2.4.2. β-Carotene–linoleic acid assay

β--carotene bleaching assay was carried out according to the method developed by Dapkevicius et al . Stock solution of β-carotene–linoleic acid was combined as follows: 0.5 mg β-carotene (Merck, Germany) was dissolved in1 ml of chloroform (HPLC grade) and then 25 µl linoleic acid (Sigma, USA) and 200 mg Tween 40 (Merck,Germany) were added. After the evaporation of chloroform, 100 ml of oxygen saturated distilled water was added with vigorous shaking. Then, 2500 µl aliquots were dispensed into the test tubes, 350 µl of the extract (2 g/L) was added and the emulsion system incubated for 48 h at room temperature. The same procedure was performed for both BHT (as positive control) and blank. In turn, absorbance spectra of the mixtures were obtained at 490 nm (11). Afterward, Anti oxidative capacities of the extracts were compared with those of BHT and blank. Further, all inhibition percentages were compared using with 95% confident interval. All tests were done in triplicate.

2.5. Anti-oxidative activities of the extracts in Soybean oil

Anti-oxidative effects of the extracts on lipid per-oxidation were evaluated in soybean oil according to the method described by Duh. Each sample (50 ml) was transferred to a series of capped glass test tubes (12). Then, Rosmarinus officinalis extracts (0, 200, 400, and 800 ppm) and BHT (100ppm) were added to the test tubes and put in a dark oven at 65 ℃. The stability of oil to oxidation was evaluated each week over a 5-week period by analyzing the peroxide values (PVs) and $TBAR_S$ levels.

2.5.1. Peroxide value was measured by American Oil Chemists' Society (AOCS) methods AOCS cd 8-53 Official Method (AOCS,. 1981).

First, a known weight of sample (3 g) was dissolved in glacial acetic acid (30 ml) and chloroform (20 ml). Then saturated KI solution (1 ml) was added. The mixture was kept in the dark for 15 min. After adding distilled water (50 ml), mixture was titrated against sodium thiosulphate (0.02 N) using starch as an indicator. A blank titration was paralleled to treatment and the PVs (meq of oxygen/kg) were calculated using the following formula: peroxide value = 1000 S × N/W. In this formula, S is the volume of sodium thiosulphate solution (blank corrected) in ml; N is the normality of sodium thiosulphate solution (0.02 N) and W is the weight of oil sample (gram)(13).

2.5.2. Thiobarbituric acid-reactive substances ($TBAR_S$) were determined weekly, using the method of AOCS (AOCS,. 1998).

This procedure allows the direct determination of TBARS in oils and fats without preliminary isolation of secondary oxidation products. Oil sample (50-200mg) was solubilized in10 ml of 1-butanol, mixed with 10ml of 0.2% TBA in 1-butanol, incubated 2h in a 95^{oC} water bath and cooled for 10 min under tap water. The absorbance was measured at 532 nm against a corresponding blank (reaction with all the reagents and treatments except the oil). The standard curve was determined by the $TBAR_S$ reaction of a series of aliquots (0.1-1ml) of 0.2 mM 1, 1, 3, 3-tetra-ethoxypropane (Merck,Germany) prepared in 1-butanol. The results were expressed as µmolmalonaldehyde (MDA)/ g of oil (n=3)(14).

2.6. Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SPSS software program version 16 (one way ANOVA, Tukey). The level of significance was considered ($p<0.05$).

Result and Discussion:

Table 1- In vitro antioxidant activities of, Rosmarinusofficinalis water and ethanol extractsand BHT in DPPH assay. Values (mean ± SD) were expressed as IC50.

Sample	DPPH (µg/ml)
water	8.1±0.35
Ethanol extract	7±0.35
BHT	5.1±0.25

Table 1 shows the antioxidant activity of the ethanol and water extracts Rosmarinus officinalis, compared with that of the BHT-containing.

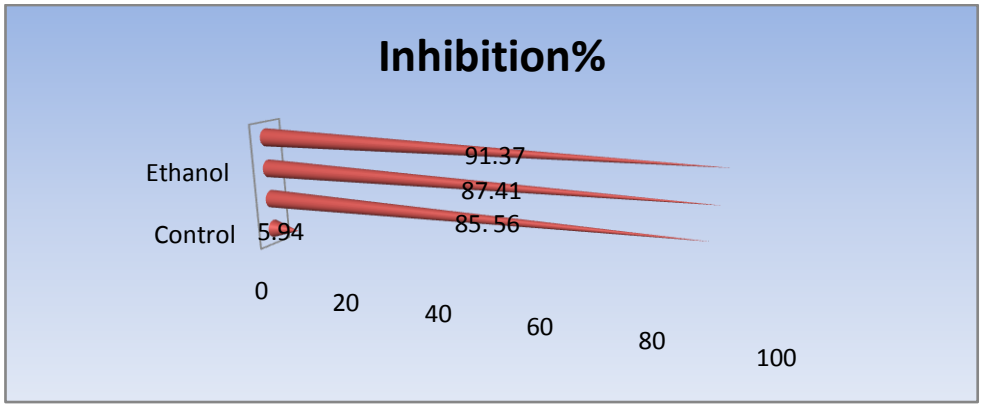

Fig. 1.Antioxidant activity Rosmarinusofficinalis extracts defined as inhibition percentage through β- catroten –linoleic assay

Fig 1 shows inhibition on lipid per oxidation in response to extracts. Ethanol and water extracts effectively inhibited the linoleic acid oxidation as much as 87.41% and 85.56%, respectively. In this regard, with the same concentration, water extract showed lower

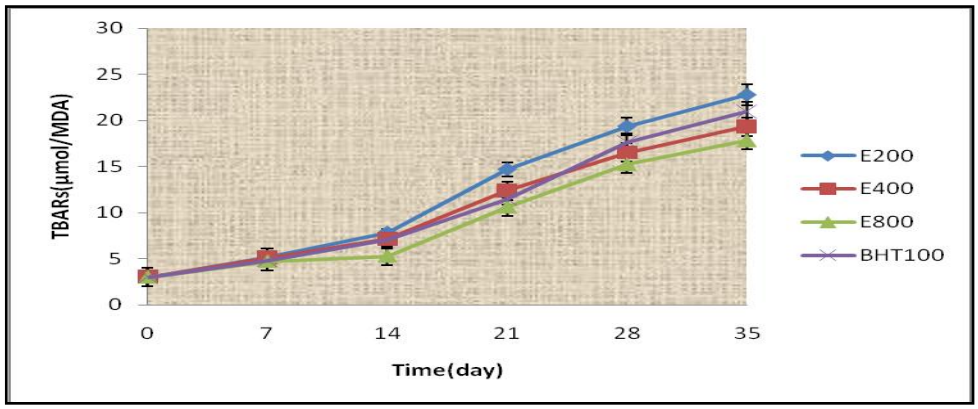

Fig 2-Effect of Ethanol extracts and BHT on TBARs of soybean oil over a 35-day incubation at 65 ºC. Values were expressed as mean ± SD of three experiments in three separate experiments

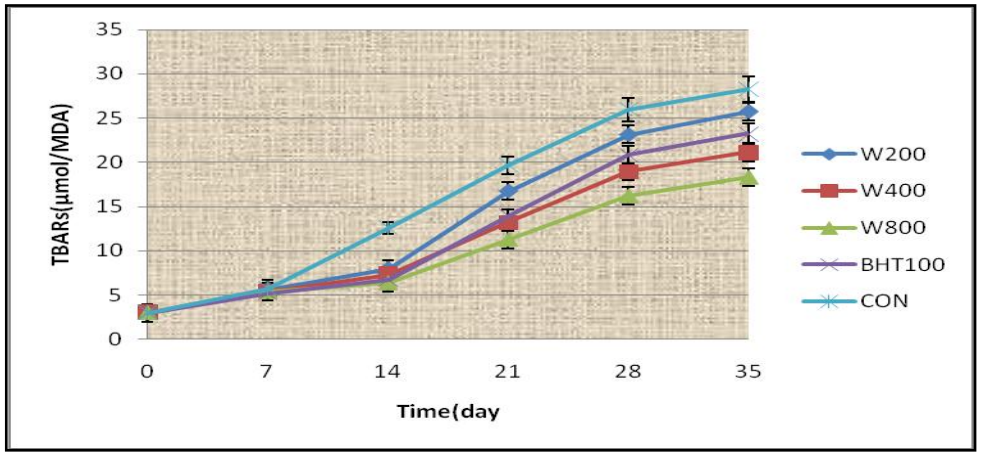

Fig 3- Effect of Rosmarinusofficinalis Water extract and BHT on TBARs of soybean oil over a 35-day incubation at 65 ºC. Values were expressed as mean ± SD of three experiments in three separate experiments.

Fig 2,3 shows the TBARs of the ethanol and water extracts Rosmarinus officinalis with concentration (0,200,400 and 800) showed more antioxidant activity in comparison with control group after 7, 14, 21 ,28 and 35 days ($P<0.001$).

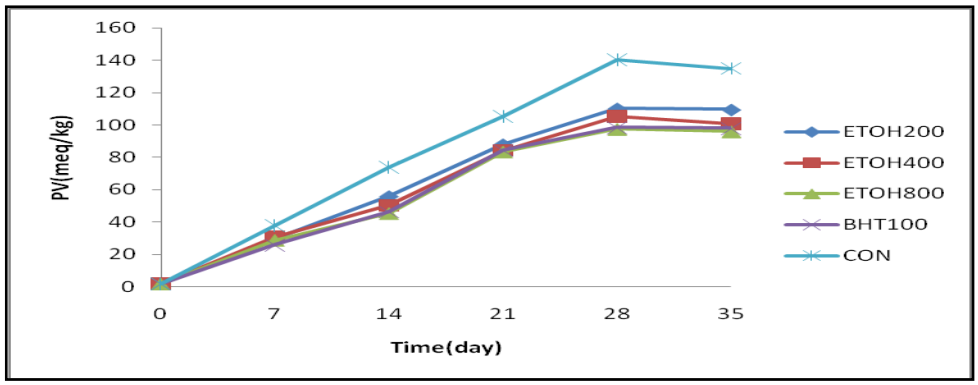

Fig 4-Effect of RosmarinusofficinalisExtracts and BHT on PV of soybean oil over a 35-day incubation at 65 ºC. Values were expressed as mean ± SD of three experiments in three separate experiments.

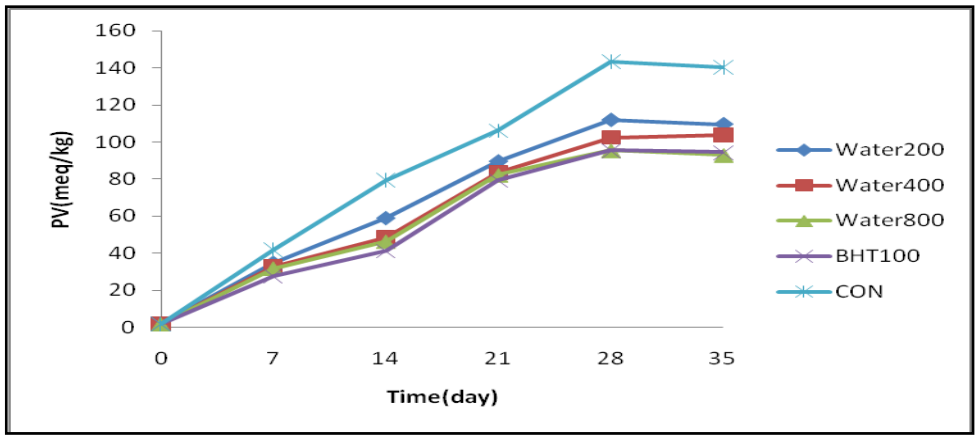

Fig 5- Effect of RosmarinusofficinalisExtracts and BHT on PV of soybean oil over a 35-day incubation at 65 ºC. Values were expressed as mean ± SD of three experiments in three separate experiments.

Fig 4,5 shows the PV of the ethanol and water extracts Rosmarinus officinalis with concentration (0,200,400and 800)showed more antioxidant activity in comparison with control group after 7, 14, 21 ,28 and 35 days ($P<0.001$).

Free radicals are molecular fragments which can cause damage to human cells. Damage to human cells can lead to chronic diseases such as cancer, heart disease, Alzheimer's and inflammation. Throughout history, man has used many different forms of therapy for the relief of this disease, and medicinal herbs are highlighted due to their popular use. To the best of our knowledge, there are no reports on antioxidant activity of Rosmarinus officinalis extracts.

Extracts Rosmarinus officinalis were subjected to assay for their possible antioxidant activities using DPPH and β-caroten/linoleic acid assay methods and PV, TBARs value in soybean oil. The Rosmarinus officinalis extracts showed a good antioxidant activity in DPPH method. Moreover, of extracts showed a good antioxidant activity in the β-caroten/linoleic acid method. In the method β-caroten/linoleic the antioxidant activity level of a substance is determined by measuring oxidation products of linoleic acid that simultaneously attack β-caroten, resulting in bleaching of its characteristic yellow colour (15,16). Percent inhibition of linoleic acid oxidation of the extracts is shown in Figure 1.

In both ethanol and water extracts, increasing the concentration of extract resulted in increasing the anti oxidant activity. For example in high concentrations of ethanol and water extracts (800 mg/l), the antioxidant activity reached 87.41% and 85.56%, respectively. The best percentage of antioxidant activity was observed for BHT (92.69 %).

The antioxidant activity may be due to the presence of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, free carboxylic groups and other active substances (17).

According to the findings, the Antioxidant activity of Rosmarinus officinalis is due to their compounds (18-21).

Effects of Antioxidant of various plant extracts in vegetable oil have been reported, so far. For example (22) compared antioxidative effects of garlic extract with BHT and BHA (synthetic antioxidant) in sunflower oil. They showed that BHT made higher inhibition to primary oxidation of oil than the extract.

Mariana-Atena Poiana compared antioxidative effects of Grape seed extract to butylated ydroxytoluene in sunflower oil in the condition Convective and Microwave Heating. They showed that BHT led a higher inhibitory effect on primary oxidation of the oil than the extract, nevertheless their abilities for inhibiting the secondary oxidation were similar (23).

In this research, we showed that extracts of water and ethanol Rosmarinus officinalis are able to inhibit both primary and secondary oxidation of soybean oil is due to storage period. While PVs and $TBAR_S$ levels of the soybean oil in the control group showed a rapid increase in 35 days of incubation, a slight increase was shown in the supplemented oil samples with water and ethanol extracts.

Conclusion

On the basis of the results found in this study, ethanolic extract showed strong antioxidant activities against various oxidative. Furthermore, ethanolic extract can be used as a source of natural preservatives in food products as it is easily accessible and it activity compared to the other samples was considerable. Therefore it can be a convenient and satisfactory alternative to the synthetic preservatives used in the food industry today.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed equally to the extraction procedures and preparation of the paper, samples collection, statistical analysis and preparation of the paper. All authors read and approved the final form of the manuscript.

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