Effect of Rosemary and Marjoram Extracts on Oxidative Stability of Refined Sunflower Oil

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□ ABSTRACT □

The purpose of this study is to evaluate the effect of rosemary (*Rosemarinus officinalis L.*) and marjoram extracts (*Origanum majorana L.*) on thermal stability of sunflower oil. Refined sunflower oil free of additives and supplemented by herbal extracts and butylated hydroxytoluene (BHA) at the concentration of (200 ppm) then subjected to heating in oven at 100 °C for 15, 30, 45, 60 minutes. To track the progress of lipid oxidation: peroxide value (PV), p-anisidine value (p-AV) and TOTOX value were determined. Total phenolic content was determined using Folin-Ciocalteu colorimetric method and can be summarized as following: aqueous rosemary extract > aqueous marjoram extract > ethyl acetate rosemary extract > ethyl acetate marjoram extract > ethanolic rosemary extract > ethanolic marjoram extract. All extracts reduced the oxidation indicator values significantly ($p < 0.05$). Ethanolic extract of rosemary had the higher antioxidant activity compared to BHA (200 ppm) and other tested extracts during heating period of sunflower oil. Results show that rosemary extracts and marjoram extracts can be used as a natural antioxidant in edible oils.

Key Words:extract, BHA, sunflower oil, marjoram, rosemary, lipid oxidation.

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تأثير خلاصات إكليل الجبل وخلاصات المردكوش على الثباتية التأكسدية خلال المعالجة الحرارية لزيت دوار الشمس

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□ ملخّص □

كان الهدف من هذا البحث هو تقييم تأثير خلاصات إكليل الجبل (*Rosemarinus officinalis L.*) وخلاصات المردكوش (*Origanum majorana L.*) على الثبات الحراري لزيت عباد الشمس. تم تعريض زيت عباد الشمس المكرر الخالي من الإضافات والمطعّم بخلاصات عشبية وهيدروكسي بوتيل أنيزول (BHA) بتركيز (200 جزء في المليون) للتسخين في الفرن عند 100 درجة مئوية لمدة(15–30–45–60) دقيقة. لتتبع تقدم أكسدة الزيت، تم إجراء التحليلات التالية: قرينة البيروكسيد (PV) وقرينة الأنيزيدين (p–AV) وقرينة الأكسدة الإجماليةTOTOX .تم تحديد المحتوى الفينولي الكلي باستخدام طريقة فولين سيوكالتيو اللونية، كان المحتوى الفينولي الكلي للخلاصات على الترتيب التالي: خلاصة مائية من إكليل الجبل> خلاصة مائية من المردكوش>خلاصة أسيتات إيثيل من إكليل الجبل>خلاصة أسيتات إيثيل من المردكوش>خلاصة إيثانولية من إكليل الجبل>خلاصة إيثانولية من المردكوش. خفضت جميع الخلاصات المضافة لزيت دوار الشمس من قيم مؤشرات الأكسدة بشكل هام إحصائياً ($p<0.05$) ، أعطت الخلاصة الإيتانولية من إكليل الجبل نشاط مضاد للأكسدة أعلى منBHAومن باقي الخلاصات المدروسة خلال فترة تسخين زيت دوار الشمس. أظهرت النتائج أنّ خلاصات إكليل الجبل وخلاصات المردكوش يمكن استخدامها كمضادات أكسدة طبيعية في زيوت الطعام.

الكلمات المفتاحية:خلاصة، BHA، زيت دوار الشمس، مردكوش، إكليل الجبل، أكسدة الدسم

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INTRODUCTION

Lipid oxidation is a major cause of deterioration during the storage and heating of edible oils. Oils and fats are unstable compounds at high temperature, a serial of complex reactions such as: oxidation, hydrolysis and polymerization occur during heating and influence on the quality of oil[1]. Edible oils contain polyunsaturated fatty acids (PUFA). In relation to the chemical structure, fatty acids present double bonds that make them prone to oxidative reactions, that lead to sensory and chemical changes and a reduction in nutritional value, additionally these reactions produce undesirable compounds such as dimers, polymers and cyclic monomers that might be potentially toxic [2, 3]. Therefore, to stabilize the oil and retard oxidative degradation during heating, several synthetic antioxidants, such as tert-butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are frequently used. However, several studies have showed that these synthetic antioxidants may have harmful effects on health, that leads to limit their usage in food industry[4]. Growing consumer awareness about these bad health effects has forced the food industry to either lower the amount of synthetic substances or replace them with natural alternatives[5]. Phenolic compounds are an excellent natural alternative to synthetic antioxidants. The great interest in phenolic compounds has been rapidly grown in recent years. Phenolic compounds have antioxidant and anti-inflammatory activity and might reduce the risk of many diseases such as cardiovascular disease and cancer[6-9]. In addition, the presence of phenolic compounds could effectively inhibit thermo-oxidative degradation of oils as well as the formation of toxic thermo-oxidative degradation compounds such as acrylamide and heterocyclic amines[10, 11].

Extracts from herbs and spices such as rosemary and marjoram are a rich source of natural antioxidants. Their properties are determined by the presence of phenolic compounds, which may act as antioxidants by scavenging of free radicals. Natural antioxidants mostly come from herbs and spices, which contain many phytochemicals that can slow down lipid oxidation[12].

Among herbs reported to have antioxidative activity, rosemary (*Rosmarinus officinalis L.*), where rosemary extracts are widely used in food. The antioxidant properties of rosemary extracts have mainly contributed by the presence of phenolic compounds. The most important antioxidant constituents of this plant species are carnosic acid, carnosol and rosmanol[13]. Several studies have shown that adding rosemary extracts to the frying oil can inhibit the decomposition of polyunsaturated triacylglycerols (TG), inducing the formation of polar compounds and polymers. It seems that, these extracts are more effective for oil oxidation inhibition than synthetic antioxidants such as (BHA) and (BHT)[14].

Sweet Marjoram (*Origanum majorana L.*) is another plant that had high antioxidant capacity due to the polyphenolic compounds; marjoram is useful as health complement and in food preservation. Traditionally, marjoram has been used as a remedy against asthma, indigestion, headache, and rheumatism[15].

RESEARCH IMPORTANCE

The importance of the research lies in the possibility of using rosemary and marjoram as natural antioxidants in food, due to their richness in phenolic compounds which are safe and have an effectiveness close to synthetic antioxidants.

Therefore, the aim of our study is to evaluate the ability of rosemary and marjoram extracts to prevent and delay sunflower oil oxidation during heating at 100 °C.

MATERIALS AND METHODS

Instruments

Rotavapor (BÜCHI Rotavapor R-200), Spectrophotometer (Jasco V-530 UV), Laboratory oven (CARBOLITE), Water bath Ultrasonic (K & H Industries), Analytical balance (RADWAG, AS 220/C/2).

Chemicals

Refined sunflower oil (RSO) was obtained from local store in Homs, Syria. Folin-Denis reagent and p-Anisidine Reagent were purchased from Sigma- Aldrich, Switzerland. Sodium Carbonate was obtained from BDH, England. Gallic acid was purchased from Biotech LTD, India. Ethyl acetate from CARBOLITE, n-hexane, ethanol (75%), Sodium Thiosulfate from TEKKIM. Glacial Acetic Acid, Potassium Iodide, Butylated Hydroxy Anisole (BHA) was obtained from Qalikema Fine Chemicals Pvt.Ltd.

Methods

Extraction:

Fresh leaves of Rosmarinus officinalis L. and Origanum majorana L. were harvested from Homs, Syria, then washed by water and dried in air at room temperature away from sunlight. Leaves were grounded by blender and stored in the refrigerator until analysis.

Organic extracts: The extraction was performed according to the method described by Ali *et al*. (2022)[16] as following: 100 ml of solvent (either ethanol 70%, or ethyl acetate) was added to 10 gr of leaves powder, the mixture was ultra-sounded for 30 min at room temperature then filtrated by whatman paper. The filtrate was concentrated using a rotary evaporator to remove the solvent. The extracts were collected, then freeze-dried, finally stored at -20 °C in darkness glass containers in the dark until analyzed.

Aqueous extracts: The aqueous extracts was prepared according to the method described by De Mejía and Ramírez-Mares, (2002)[17] with some modifations as following: 120 ml of distilled water was heated until 60 °C then added to 10 gr of herbal powder, the mixture was kept at room temperature for 3 min then cooled by ice path then filtrated by whatman paper. The filtrate residue was freeze-dried and stored at -20 °C in dark glass containers in the darkness until analysis.

Freeze-dried extracts were weighed to calculate extraction yield using the following formula:

Extraction yield (%) = $(W2/W1) \times 100$

Where W1 is the weight of the plant sample powder, W2 is the weight of the freeze-dried extract.

Total Phenolic Content (TPC):

Total phenolic contents were determined according to the method described by Aldiab (2018)[8, 18, 19] as following: 0.1 ml of extract (1 mg/ml in ethyl acetate) and 1 ml of Folin Ciocalteu reagent (1:1 in distilled water) were mixed, after 5 min, 1 ml of 2% sodium carbonate Na2CO3 was added and the mixture was left at room temperature for 30 min. The absorbance was measured with a spectrophotometer at 750 nm against the blank. The blank was prepared by replacing the extract sample with ethyl acetate and then repeating the same previous steps. The experiment was carried out three times. Total phenolic contents were calculated from standard curves of gallic acid (y=1.7985x+0.0016, R2=0.9973), and then expressed as mg GAE/ g dried extract weight.

Sample Preparation:

Sample preparation was according to the method described by Dias *et al*. (2015) [20] as following: The extract was dissolved in ethyl acetate (1:1 extract-ethyl acetate, w/v). The dissolved extracts where added to oil to achieve the concentration 200 ppm of phenolic compounds, the mixture of dissolved extract and oil was homogenized with vortex for 10 min. Samples were coded as shown in Table (1). SFO (The oil without additives) is considered as a negative control.

Ethyl acetate (EA) is an environmentally friendly organic solvent (Green solvent) and considered a low-toxicity solvent due to its rapid hydrolysis to ethanol and acetic acid during metabolism. Its presence in food products is accepted[52].

Table 1: Samples abbreviation

SFO	Sunflower oil without addatives (Negative control)
SFO 1	Sunflower oil + 200 ppm phenolic compounds from aqueuos Marjoram extact
SFO 2	Sunflower oil + 200 ppm phenolic compounds from aqueuos Rosemary extact
SFO 3	Sunflower oil + 200 ppm phenolic compounds from Ethyl acetat Marjoram extact
SFO 4	Sunflower oil + 200 ppm phenolic compounds from Ethyl acetat Rosemary extact
SFO 5	Sunflower oil + 200 ppm phenolic compounds from Ethanolic Marjoram extact
SFO 6	Sunflower oil + 200 ppm phenolic compounds from Ethanolic Rosemary extact
SFO 7	Sunflower oil + 200 ppm BHA (Positive control)

Heating Process:

The prepared oil samples which mentioned in Table (1) were placed in an oven at 100 ^{0}C. An aliquot of 6 ml of each oil sample was taken after (15 – 30 – 45 – 60 min) and then were placed into an ice bath to stop the oxidation reaction.

Peroxide Value Analysis (IUPAC no. 2501)[21]

Peroxide value was determined as following: Approximately one gram of oil sample was mixed with (6) ml solvent (acetic acid: chloroform, 3:2), then 0.1 ml of saturated potassium iodide (KI) solution was added and the mixture was stirred for 1 minute, finally (6) ml of distilled water was added. The mixture was then titrated using (0.01M) Sodium Thiosulfate Pentahydrate solution until the color became light yellow. 0.5 ml of 1% starch was added until the solution color changed to blue. Titration was continued while shaking the flask vigorously until blue color disappeared. The blank was prepared by replacing the oil sample with distilled water and then repeating the same previous steps. Peroxide value can be calculated by the formula[22]:

$$PV = \frac{(S - B) * M * 1000}{g}$$

Where:

Peroxide Value (PV) = mEq peroxide per kg of sample

B = volume of titrant for blank

S = volume of titrant for sample

M = Sodium Thiosulfate molarity

1000 = conversions of unit (g/kg)

g = sample weight (g)

p-Anisidine Value:

p-Anisidine value (*p*-AV) was determined according to Hatem and Sarem (2019)[23]. Briefly, (1) g of oil was weighed into a 25ml volumetric flask, dissolved and made up to volume with n-hexane, The absorbance (A1) of this solution was measured against n-hexane by spectrophotometer at 350 nm. The next step was to take (5) ml of the oil solution (oil with n-hexane) to one test tube (1) and (5) ml n-hexane to a second tube (2).
Next, (1) ml of p-anisidine reagent in glacial acetic acid (0.25% w/v) was added to previous two tubes. The test tubes were shaken and left in darkness for 10 minutes. The difference of absorbance between the last two tubes was recorded as (A2). p-AV was calculated as:

$$AV = \frac{25 * (1.2\ A2 - A1)}{m}$$

A1 and A2 are the absorbances measured as described above; m is the mass of oil.

Totox value

Totox value was calculated by the values determined from peroxide value and anisidine value aforementioned Sun-Waterhouse *et al*. (2011)[24]. The formulation for Totox values was shown below:

Totox value = 2 × PV + AV

Statistical analysis

All results were presented as mean ± standard deviation. The differences of oxidation indexes negative control, positive control and samples of oil with phenolic extracts were tested by Students' t-test. Differences were considered to be significant at p value < 0.05. All statistical analyses were performed using the Microsoft Excel 2016 Software.

RESULTS AND DISCUSSION

Total phenolic compounds determination

Table 2: Total phenolic content in Rosemary and Marjoram extracts

Extracts	Conc.(mg GAE/g Dried Extract)	Yield [%±SD]
Aqueous Rosemary Extract	320.5±0.002	12.32±0.21
Aqueous Marjoram Extract	243.2±0.014	17.11±0.25
Ethyl Acetate Rosemary Extract	220.3±0.003	6.72±0.18
Ethyl Acetate Marjoram Extract	104.8±0.002	4.53±0.31
Ethanolic Rosemary Extract	92.5±0.002	5.13±0.14
Ethanolic Marjoram Extract	89.2±0.001	6.31±0.15

Results are expressed as mean ± SD, n=3

Total phenolic contents (TPC) in various extracts of Rosemary and Marjoram are presented in table (2). The phenolic contents were in the order of aqueous rosemary extract> aqueous marjoram extract> ethyl acetate rosemary extract > ethyl acetate marjoram extract > ethanolic rosemary extract > ethanolic marjoram extract. Results in Table (2) show that the

best solvent to extract phenolic compounds from studied plants is water, this result agrees with El-Maati *et al*. (2012)[25], who found that water was the best solvent for phenolic comounds extraction from Rosemary followed by ethanol 80% and ethyl acetate, while Aldiab (2018)[19] found that alcoholic solvent (methanol/water mixture) can extract phenolic compounds from herbal teas better than water.

The aqueous extract form rosemary showed the highest TPC of (320 mg GAE/g Dried extract). Dorman *et al*. (2003)[26] reported that aqueous extract from rosemary had the highest total phenolic content among four other lamiaceae herbs (rosemary, oregano, sage and thyme) which was (185 mg GAE/g) and thus it is lower than our result. The lowest TPC was in ethanolic extract which was slightly lower than that reported by Aljabri,(2020)[27] where he found that TPC of rosemary ethanolic extract was (116.7 mg GAE/g).Tohma *et al*.(2021)[28] also found that TPC of rosemary ethanolic extract (104.6 ± 2.5 mg GAE/g).

For marjoram, the highest TPC was in aqueous extract (243.2 mg GAE/g Dried extract) and the lowest TPC was in ethanolic extract (89.8 mg GAE/g Dried extract). These results were close to Duletić-Laušević *et al*.(2018)[29] who reported that aqueous extract of marjoram originating from Greece had higher TPC (111.10 mg GAE/g) than ethanolic extract (83.25 mg GAE/g).

The differences in TPC between our results and those in the literature can be attributed to several factors including the preparation conditions of extracts[19, 30], geographical origin, the part of the plant used, the stage of development at the time of harvest, and the type of solvent used [13, 15, 31-34].

Extraction yield in our study was within the range (5.13% - 17.11%), this result was in agreement with El-Maati *et al*. (2012)[25] who studied five herbs (thyme, clove, sweet marjoram, rosemary and ginger), and found that the extracts yield with different solvents (water – ethanol – ethyl acetate- hexane) varied from 1% to 18.5%.

Variation of the extraction yields of different extracts is attributed to differences in polarity of compounds found in plants[35].

Chemical Oxidation Indicators From Frying Process:

Peroxide value (PV):

Table 3: peroxide values

Sample	Time (min)				
	0	15	30	45	60
SFO	3.07±0.16	7.88±0.14	13.00±0.42	16.28±0.82	22.02±0.57
SFO 1	2.72±0.23	5.28±0.13^{abc}	8.06±0.27^{ab}	10.81±0.92^{a}	14.63±0.91^{ab}
SFO 2	2.85±0.12^{a}	4.83±0.08^{ab}	8.09±0.91^{abc}	9.58±0.40^{ab}	13.94±0.29^{ab}
SFO 3	2.81±0.09^{a}	5.71±0.15^{ab}	8.80±0.20^{ab}	13.20±0.80	17.44±0.60^{a}
SFO 4	2.92±0.05	5.59±0.34^{abc}	8.23±0.23^{abc}	12.36±0.21^{a}	16.24±0.23^{ab}
SFO 5	2.85±0.11	3.98±0.18^{abc}	6.31±0.37^{ab}	8.50±0.08^{ab}	11.31±0.18^{ab}
SFO 6	2.82±0.04	3.25±0.24^{abc}	5.92±0.12^{abc}	7.15±0.12^{ab}	10.56±0.96^{ab}
SFO 7	2.61±0.11	3.86±0.11^{ab}	7.11±0.28^{ab}	8.81±0.37^{ab}	11.36±0.34^{ab}

Note: Results are expressed as mean ± SD, n=3, ^{a}: $p<0.05$, ^{ab}: $p<0.01$, ^{abc}: $p<0.001$. $^{a, ab, abc}$:is the comparison between different oil samples and control sample.it considered as significant

As primary products, peroxides and hydroperoxides are formed in oils through autoxidation during the oxidation process [36]. The PV is one of the most widely used indicators for the measurement of peroxides. The influence of antioxidants on PV in sunflower oil samples during heating is shown in Table (3). SFO showed maximum PV (22.02 meq/kg), while a significant reduction occurred with the addition of rosemary and marjoram extracts after 60 min of heating at 100 ^{0}C. Among these antioxidants, rosemary ethanolic extract remained the most effective and had the lowest PV(10.56 meq/kg). The low PVs of samples treated with herbal extracts were due to their antioxidant ingredients. These results are in agreement with previous findings, which showed that plant extract rich in polyphenols reduce the formation of peroxides in frying oils [36-39]. Zeb *et al*. (2019)[40] found that the addition of spinach extract to sunflower oil leads to a significant reduction in PV after 1 hour of frying at 160 ^{o}C. Guo, Q *et al*. (2016)[37] also found that rosemary ethanolic extract reduce PV of palm oil during frying even more than synthetic antioxidant BHA.

Jun *et al*. (2001)[41] found that phenolic compounds in marjoram have superoxide anion radicals scavenger effect. Soldo *et al*. (2019)[42] attributed the antioxidant effect of rosemary extracts to their high content of carnosic acid, rosmarinic acid and carnosol. These results may explain the ability of rosemary and marjoram extracts to reduce peroxide value.

The PV of all samples in our study increased throughout the heating time. This result agrees with Navab *et al*. (2012)[43] who found that frying of canola oil at 160 ^{o}C leads to increase PV in all tested sampels during the first 6 hours of frying.

p-anisidine Value (*p*-AnV)

Table 4: *p*-anisidine values (*p*-AnV)

Sample	Time (min)				
	0	15	30	45	60
SFO	1.94±0.02	3.36±0.01	5.99±0.05	7.99±0.20	9.55±0.10
SFO 1	1.95±0.04	2.56±0.06^{ab}	4.09±0.08^{ab}	5.39±0.06^{ab}	6.90±0.04^{abc}
SFO 2	1.93±0.03	2.47±0.11^{abc}	4.63±0.27^{a}	4.94±0.07^{ab}	6.18±0.05^{abc}
SFO 3	1.99±0.06	2.90±0.08^{a}	4.77±0.09^{abc}	6.47±0.09^{ab}	7.36±0.48^{a}
SFO 4	1.87±0.06	2.90±0.05^{ab}	4.32±0.14^{abc}	5.87±0.05^{ab}	7.01±0.02^{abc}
SFO 5	1.79±0.05^{a}	2.09±0.04^{abc}	3.50±0.09^{abc}	4.61±0.06^{abc}	5.27±0.11^{abc}
SFO 6	1.87±0.08	1.97±0.04^{abc}	3.26±0.05^{abc}	4.08±0.08^{abc}	5.08±0.10^{abc}
SFO 7	1.91±0.10	2.29±0.05^{ab}	3.25±0.05^{abc}	4.64±0.06^{abc}	6.03±0.09^{abc}

Note: Results are expressed as mean ± SD, n=3, ^{a}: p<0.05, ^{ab}: p<0.01, ^{abc}: p<0.001. $^{a, ab, abc}$:is the comparison between different oil samples and control sample.it considered as significant

As oil deterioration progress, the formed peroxides convert into the secondary lipid oxidation products (aldehydes and ketones). *p*-AnV is a common indicator to determine the secondary lipid oxidation products. Aldehydes are generated during the secondary lipid oxidation, and can react with *p*-anisidine reagent (0.25% in glacial acetic acid), forming yellow colored solution. A lower *p*-AnV indicates that less rancid oil is produced [37]. In this study, an increase in*p*-AnV throughout the heating period in all tested samples was noted Table (4). Negative control sample showed the highest anisidine values (9.55),

whereas SFO6 showed the lowest values (5.08). All addatives of rosemary and marjoram extracts as well as BHA reduced *p*-AnV of oil significantly ($P < 0.05$). In this study, the highest inhibitory effect on the generation of secondary oxidation products was observed in rosemary ethanolic extract. Rosemary ethanolic extract contains carnosic acid, carnosol, and a large amount of different phenolic compounds that have antioxidant properties. It was supposed that there should be synergy effect between different phenolic compounds[44]. El-Gammal *et al*. (2010)[45] found that adding marjoram methanolic extract to palm oil at concentration of 200 ppm reduces *p*-AV significantly ($p < 0.05$) after 12 hours of heating at 80 ^{o}C. Another study found that adding rosemary extract to soybean oil reduce *p*-AV about 60% compaired to negative control after 6 hours of frying at 180 ^{o}C[46] .

TOTOX Value

Table 5: Totox values

Sample	Time (min)				
	0	15	30	45	60
SFO	8.07±0.30	19.12±0.28	31.99±0.88	40.55±1.46	53.60±1.18
SFO 1	7.34±0.47	13.12 ± 0.21^{abc}	20.21 ± 0.45^{ab}	27.02 ± 1.87^{a}	36.17 ± 1.79^{ab}
SFO 2	7.61±0.27	12.10 ± 0.23^{ab}	20.81 ± 2.07^{abc}	24.09 ± 0.84^{ab}	34.05 ± 0.60^{ab}
SFO 3	7.56±0.23	14.33 ± 0.37^{ab}	22.37 ± 0.49^{ab}	32.87 ± 1.70^{a}	42.25 ± 1.00^{ab}
SFO 4	7.74±0.15	14.08 ± 0.66^{ab}	20.78 ± 0.58^{abc}	30.58 ± 0.46^{a}	39.48 ± 0.46^{ab}
SFO 5	7.49±0.17	10.06 ± 0.39^{abc}	16.13 ± 0.66^{ab}	21.61 ± 0.21^{ab}	27.89 ± 0.46^{abc}
SFO 6	7.51±0.04	8.43 ± 0.44^{abc}	15.10 ± 0.30^{abc}	18.38 ± 0.26^{ab}	26.20 ± 1.86^{abc}
SFO 7	7.01±0.30	10.01 ± 0.18^{abc}	17.46 ± 0.52^{ab}	22.26 ± 0.78^{ab}	28.75 ± 0.76^{ab}

Note: Results are expressed as mean ± SD, n=3, ^{a}: $p<0.05$, ^{ab}: $p<0.01$, ^{abc}: $p<0.001$. $^{a, ab, abc}$:is the comparison between different oil samples and control sample.it considered as significant

TOTOX value measures primary and secondary oxidation products, reflecting the initial and later stages of the oil oxidation. Therefore, it provides a better estimation of the progressive oxidative deterioration of the oil[47] . Lower TOTOX value indicates a greater stability of oil sample against oxidative rancidity[48]. The results of TOTOX values are shown in Table (5). TOTOX value increased over the time in all tested samples, where after 60 min of heating at 100 ^{o}C the TOTOX values were: SFO (53.60±1.18) >SFO3 (42.25±1.00) >SFO4 (39.48±0.46) >SFO1 (36.17±1.79) >SFO2 (34.05±0.60) >SFO7(28.75±0.76) >SFO5(27.89±0.46) >SFO6(26.20±1.86). However, the addition of phenolic extracts to oil samples caused stability in comparison to negative control sample. Our results were in agreement with Abbas Ali *et al.* (2016)[49] who found that TOTOX value of corn oil increase with increasing heating time in microwave. Many researchers studied the effect of phenolic extracts on lipid oxidation reducing. Okhli *et al*. (2020)[50] found that ethanolic extract from Citrus medica L was more effective than BHA in reducing TOTOX value of sunflower oil during 5 days of heating at 65^{o}C. The rosemary ethanolic extract was able to reduce TOTOX of soybean oil to (17.02) after 20 days of heating at 60 ^{o}C while TOTOX value of negative control sample was (141.06)[20].

Unexpectedly, the ethanolic extracts had the strongest antioxidant action, although it had the lowest phenolic content, which could be related to a mixture of polar and nonpolar phenolic compounds that have a synergistic antioxidant effect. Rosemary ethanolic extract contains high amounts of carnosic acid, carnosol which explain its strong antioxiant activity. One study found that total phenolic content in herbs´ extracts did not always reflect to its antioxidant effectivity in lipids[19]. Ethanolic extract of mint, containing 15% polyphenol higher than thyme extract, was the weakest antioxidant activity[51].

CONCLUSIONS

Our study found that marjoram and rosemary are rich sources of phenolic compounds that have antioxidant effect on sunflower oil and thus, can delay lipid oxidation. We also found that aqueuos extracts from marjoram and rosemary were the highest in phenloic compounds but did not show the strongest antioxidant activity.

The results of our study also showed that heating sunflower oil at 100 ^{o}C led to an increase in all oxidation indicators (PV, *P*-AV, TOTOX) throughout the heating time, while the addition of phenolic extracts led to decrease these values, All extracts in our study showed an antioxidant activity in varying degrees, but the ethanolic extracts of rosemary and marjoram had the higher activity and preceded the synthetic antioxidant (BHA) when added in the same concentration (200 ppm).

References

1. Zhang Q, Saleh AS, Chen J, Shen Q. Chemical alterations taken place during deep-fat frying based on certain reaction products: A review. Chemistry and physics of lipids. 2012;165(6):662-81.
2. Juárez MD, Masson L, Samman NC. Deterioration of partially hydrogenated soybean oil used in deep fat frying of a meat food. 2007.
3. Leclercq S, Milo C, Reineccius GA. Comparison of antioxidants to prevent oxidation of sulphur flavour compound in sunflower oil. Flavour and fragrance journal. 2008;23(5):333-9.
4. Viana da Silva M, Santos MRC, Alves Silva IR, Macedo Viana EB, Dos Anjos DA, Santos IA, et al. Synthetic and natural antioxidants used in the oxidative stability of edible oils: an overview. Food Reviews International. 2021:1-24.
5. Olmedo R, Ribotta P, Grosso NR. Decrease of chemical and volatile oxidation indicators using oregano essential oil combined with BHT in sunflower oil under accelerated storage conditions. Journal of food science and technology. 2019;56(5):2522-35.
6. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews. 1998;56(11):317-33.
7. Al Asaad N, Al Diab D. Antioxidant Activity and Phenolic Content of Eight Mediterranean Fruit Juices. International Journal of Pharmaceutical Sciences and Nanotechnology. 2016;9(3):3299-304.
8. Nezam A, Al Diab D, Hasan N. In-vitro Anti-inflammatory activity of Total Phenolic content of some fruit juices in Syria. Research Journal of Pharmacy and Technology. 2021;14(7):3685-8.
9. Aldiab D., Usage of Phenolic Extract of Grape Waste as Natural Antioxidant for Milk Proteins, Journal of Chemical and Pharmaceutical Sciences., 2018, 11, 196-200.

10. Cheng K. W., Shi J. J., Ou S. Y., Wang M.,Jiang Y., Effects of fruit extracts on the formation of acrylamide in model reactions and fried potato crisps, Journal of Agricultural and Food Chemistry., 2010, 58, 309-312.
11. Cheng K-W, Wu Q, Zheng ZP, Peng X, Simon JE, Chen F, et al. Inhibitory effect of fruit extracts on the formation of heterocyclic amines. Journal of agricultural and food chemistry. 2007;55(25):10359-65.
12. Brewer M. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Comprehensive reviews in food science and food safety. 2011;10(4):221-47.
13. González-Minero FJ, Bravo-Díaz L, Ayala-Gómez A. Rosmarinus officinalis L.(Rosemary): An ancient plant with uses in personal healthcare and cosmetics. Cosmetics. 2020;7(4):77.
14. Jaswir I, Man YBC, Kitts DD. Use of natural antioxidants in refined palm olein during repeated deep-fat frying. Food research international. 2000;33(6):501-8.
15. Bouyahya A, Chamkhi I, Benali T, Guaouguaou F-E, Balahbib A, El Omari N, et al. Traditional use, phytochemistry, toxicology, and pharmacology of Origanum majorana L. Journal of Ethnopharmacology. 2021;265:113318.
16. Ali ZS, Muhammad D, Zrieki A. In vitro Assessment of sun protection factor (SPF) and Antioxidant activity of Viola odorata extracts. Research Journal of Pharmacy and Technology. 2022;15(2):655-60.
17. de Mejía EG, Ramírez-Mares MV. Leaf extract from Ardisia compressa protects against 1-nitropyrene-induced cytotoxicity and its antioxidant defense disruption in cultured rat hepatocytes. Toxicology. 2002;179(1-2):151-62.
18. Al-Diab Dima, Al-As'sad Nour, Determination of Phenolic Compounds Levels and Antioxidant Activity in Some Local Functional Juices, Tishreen University Journal for Research and Scientific Studies-Heath Sciences Series, 2015,VOL.(37) NO.(1)
الدياب د، الأسعد ن، تحديد سويات المركبات الفينولية وفعاليتها المضادة للأكسدة في بعض العصائر الوظيفية المحلية، مجلة جامهة تشرين للبحوث والدراسات العملية، سلسلة العلوم الصحية 2015 ,V.47,N.1
19. Aldiab D., Effect of preparation conditions on phenolic content and antioxidant activity of various teas and herbal teas, Journal of Chemical and Pharmaceutical Sciences., 2018, 11,222-226.
20. Dias LS, Menis ME, Jorge N. Effect of rosemary (Rosmarinus officinalis) extracts on the oxidative stability and sensory acceptability of soybean oil. Journal of the Science of Food and Agriculture. 2015;95(10):2021-7.
21. Paquot C., Hauntfenne A., IUPAC standard methods for the analysis of oils, fats and derivatives,Bioresour Technol., 1987, 97, 1365-1371.
22. Zrekah G, Aldiab D, Abboud A. Determination of Protein and fat oxidation levels in imported infant formula available in Syria. Int J Pharm Pharm Sci. 2015;8:169-72.
23. Hatem O, Sarem Z. Investigation of Lipid Oxidation in commonly consumed Fish oil supplements in Syrian market. Research Journal of Pharmacy and Technology. 2019;12(11):5333-7.
24. Sun-Waterhouse D, Zhou J, Miskelly G, Wibisono R, Wadhwa S. Stability of encapsulated olive oil in the presence of caffeic acid. Food Chemistry. 2011;126(3):1049-56.
25. El-Maati MFA, Labib S, Al-Gaby A, Ramadan M. Antioxidant properties of different extracts from five medicinal plants. Zag J Agric Res. 2012;39:1-13.

26. Dorman HD, Koşar M, Kahlos K, Holm Y, Hiltunen R. Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. Journal of agricultural and food chemistry. 2003;51(16):4563-9.
27. Aljabri M. Composition and antioxidant activities of rosemary (Rosmarinus officinalis) extracts. EurAsian Journal of BioSciences. 2020;14(1):2179-85.
28. Tohma S, Günal-Köroğlu D, Turan S, Ramadan MF. Efficacy of rosemary (Rosmarinus officinalis L.) powder and extracts in the protection of refined and stripped hazelnut oil. Rendiconti Lincei Scienze Fisiche e Naturali. 2021;32(3):585-98.
29. Duletić-Laušević S, Aradski AA, Kolarević S, Vuković-Gačić B, Oalđe M, Živković J, et al. Antineurodegenerative, antioxidant and antibacterial activities and phenolic components of Origanum majorana L.(Lamiaceae) extracts. J Applied Botany and Food Quality. 2018;91:126-34.
30. Nasser S.,AL-Diab D., Study of Some Affecting Factors on Phenolic Compounds Levels and Their Antioxidant Activity in Some Functional Juices,Tishreen University Journal for Research and Scientific Studies-Heath Sciences Series., 2018, 40(2),283-297.
ناصر س. الدياب د . دراسة بعض العوامل المؤثرة على سويات المركبات الفينولية وفعاليتها المضادة للأكسدة في بعض العصائر الوظيفية، مجلة جامعة تشرين للبحوث والدراسات العلمية – سلسة العلوم الصحية، 2018 ,V.4, N.5
31. Boix YF, Victório CP, Defaveri ACA, Arruda RDCDO, Sato A, Lage CLS. Glandular trichomes of Rosmarinus officinalis L.: Anatomical and phytochemical analyses of leaf volatiles. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology. 2011;145(4):848-56.
32. Wang B, Ma L, Yin L, Chen J, Zhang Y, Dong L, et al. Report: Regional variation in the chemical composition and antioxidant activity of Rosmarinus officinalis L. from China and the Mediterranean region. Pakistan journal of pharmaceutical sciences. 2018;31(1):221-9.
33. Lakušić D, Ristić M, Slavkovska V, Lakušić B. Seasonal variations in the composition of the essential oils of rosemary (Rosmarinus officinalis, Lamiaceae). Natural product communications. 2013;8(1):1934578X1300800132.
34. Muñoz Centeno LM. Plantas medicinales españolas: Rosmarinus officinalisL.(Lamiaceae)(romero). 2002.
35. Jayaprakasha GK, Singh R, Sakariah K. Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro. Food chemistry. 2001;73(3):285-90.
36. Iqbal S, Bhanger M. Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chemistry. 2007;100(1):246-54.
37. Guo Q, Gao S, Sun Y, Gao Y, Wang X, Zhang Z. Antioxidant efficacy of rosemary ethanol extract in palm oil during frying and accelerated storage. Industrial Crops and Products. 2016;94:82-8.
38. Buta N, Popa N, Roman L, Bordea G, Bordea A, Bordea N, et al. The antioxidant effect of Melissa officinalis extract regarding the sunflower oil used in food thermal applications. J Agroalimentary Processes Technol. 2013;19:276-9.
39. Zhao B, Lan T, Li H, He Y, Wu D, Chen Z. Antioxidation activity of Moringa oleifera Lam. leaves extract on soybean oil during both storage and thermal treatment. Journal of Food Processing and Preservation. 2019;43(8):e13975.
40. Zeb A, Ullah F. Effects of spinach leaf extracts on quality characteristics and phenolic profile of sunflower oil. European Journal of Lipid Science and Technology. 2019;121(1):1800325.

41. Jun WJ, Han BK, Yu KW, Kim MS, Chang IS, Kim HY, et al. Antioxidant effects of Origanum majorana L. on superoxide anion radicals. Food Chemistry. 2001;75(4):439-44.
42. Soldo B, Andelic I, Nikolov N, Skroza D, Simat V, Ljubenkov I, et al. The Effect of Selected Herb Extracts on Oxidative Stability of Vegetable Oils. Croatica Chemica Acta. 2019;92(3):1C-C.
43. NAVAB DF, Ghavami M. The effect of temperature and time on the production and decomposition of hydroperoxides in canola and soybean Oils. 2012.
44. Romano CS, Abadi K, Repetto V, Vojnov AA, Moreno S. Synergistic antioxidant and antibacterial activity of rosemary plus butylated derivatives. Food chemistry. 2009;115(2):456-61.
45. El-Gammal R, Ramadan A-H. EFFECT OF PHENOLIC COMPOUNDS EXTRACT FROM SOME HERBS AND SPICES ON THE STABILITY OF PALM OIL DURING DEEP FRYING. Journal of Productivity and Development. 2010;15(3):309-25.
46. Saoudi S, Chammem N, Sifaoui I, Bouassida-Beji M, Jiménez IA, Bazzocchi IL, et al. Influence of Tunisian aromatic plants on the prevention of oxidation in soybean oil under heating and frying conditions. Food chemistry. 2016;212:503-11.
47. O'Keefe SF, Pike OA. Fat characterization. Food analysis: Springer; 2010. p. 239-60.
48. Shahidi F, Wanasundara UN. Methods for measuring oxidative rancidity in fats and oils. Food lipids: Chemistry, nutrition and biotechnology. 2002;3:387-403.
49. Abbas Ali M, Hadi Bin Mesran M, Abd Latip R, Hidayu Othman N, Nik Mahmood N. Effect of microwave heating with different exposure times on the degradation of corn oil. International Food Research Journal. 2016;23(2).
50. Okhli S, Mirzaei H, Hosseini SE. Antioxidant activity of citron peel (Citrus medica L.) essential oil and extract on stabilization of sunflower oil. OCL. 2020;27:32.
51. GRAMZA-MICHALOWSKA A, Sidor A, Hes M. Herb extract influence on the oxidative stability of selected lipids. Journal of Food Biochemistry. 2011;35(6):1723-36.
52. Silva Y, Ferreira TA, Celli GB, Brooks MS. Optimization of lycopene extraction from tomato processing waste using an eco-friendly ethyl lactate–ethyl acetate solvent: a green valorization approach. Waste and Biomass Valorization. 2019;10(10):2851-61.