Antioxidant potential of rosehip seed extract in stabilisation of flaxseed oil

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The aim of this study was to evaluate the usage of rosehip seed powder (RSP) extract in flaxseed oil to retard lipid oxidation. The acetone extract of the RSP (500 mg kg^{-1}; 1000 mg kg^{-1}) was used. Synthetic antioxidant BHT (200 mg kg^{-1}) was added into the flaxseed oil to evaluate the antioxidant potential of the RSP extract. The peroxide value (PV), TBARS value, and *p*-anisidine value (*p*-AV) of the flaxseed oil were analysed during 12 days of storage. The total oxidation (TOTOX) value was also calculated. The sample with the RSP (500 mg kg^{-1}; 1000 mg kg^{-1}) generally showed lower PV, *p*-AV, and TOTOX value than the control sample and sample with the BHT. These results indicated that the RSP may be used in edible oils to prevent lipid oxidation as a source of natural antioxidants.

Keywords: flaxseed oil, lipid oxidation, rosehip seed powder.

1. INTRODUCTION

Lipid oxidation is the main reason of deterioration in edible oils. Oxidation reactions lead to sensorial and nutritional losses. Products of oxidation reactions cause off-flavour and rancidity. Degradation of fat-soluble vitamins and essential fatty acids reduce the nutritional value. Finally, consumer rejection of the product happens. Therefore, lipid oxidation is generally accepted as a decisive factor determining the shelf life of products [1, 2].

Although synthetic antioxidants have been used to prevent lipid oxidation, their safety has been questioned owing to their possible toxic effects [3]. For this reason, natural sources of antioxidants have been investigated to obtain new natural antioxidants as substitute for synthetic antioxidants. The usage ability of corncob [4], oat malt [5], *Monechma ciliautum* leaf extract [6], black tea and garlic bulbs [7], ajowan [8], mulberry leaves [9], pistachio hull [10], rice bran [11], grape pomace [12], teaw [13], olive leaf [14], pomegranate peel [15], coriander [16], primrose [17], and ginger [18] extracts have been evaluated in edible oils as natural antioxidants.

In food industry, rosehip fruits are processed for numerous food products such as jam, marmalade and nectar. Rosehip seed, the by-product of the rosehip industry, may be an economic source of natural antioxidants. It is rich in antioxidant compounds such as phenolic compounds, tocopherols, carotenoids and ascorbic acid [19, 20]. Flaxseed oil includes α-linolenic acid more than 50 g 100 g^{-1}, and its fatty acid composition makes it susceptible to lipid oxidation.

The aim of this study was to investigate the usage ability of rosehip seed powder extract in flaxseed oil as an antioxidant.

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2. MATERIALS AND METHODS

CHEMICALS

Analytical chemicals and solvents were provided by Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstad, Germany).

MATERIALS

Dried rosehip was obtained from a local market, and their seeds were separated by hand. Cold-pressed flaxseed oil was purchased from Origo Co. (Gaziantep, Turkey).

EXTRACTION OF ANTIOXIDANT COMPOUNDS

Rosehip seed was ground with a grinder (IKA M20, Staufen, Germany). Antioxidants compounds were extracted from the rosehip seed powder (RSP). One gram of the RSP was mixed with hexane (10 mL) to remove lipids, and it was stirred in an orbital shaker for 2 hours. The slurry was filtered through a filter paper. The residual was extracted with extraction solvent (10 mL) in an orbital shaker for 2 hours, and the slurry was filtered through filter paper. The extraction was repeated with solvent (10 mL). The combined extract was stored at -18°C.
Water, methanol (50%), methanol (75%), methanol, acetone (50%), acetone (75%), acetone, ethanol (50%), ethanol (75%), and ethanol were used as extraction solvents.

TOTAL EXTRACTABLE COMPOUNDS

For TEC analysis, 10 mL of the combined extract was dried in an oven at 105°C. Total extractable compounds (%) were calculated.

TOTAL PHENOLIC COMPOUNDS (TPC)

TPC was calculated by using Folin Ciocalteu method [21]. A 100 µL of the extract was mixed with Folin Ciocalteu reagent (500 µL). After 5 minutes, sodium carbonate (1 M, 400 µL), and water (4 mL) were added to the tube. The absorbance at 760 nm was measured after the tubes were stored in the dark for 1 hour. TPC was expressed as mg gallic acid equivalent kg^{-1} RSP.

DPPH RADICAL SCAVENGING ACTIVITY

DPPH assay was carried out with the method described by Brands-Williams *et al.* [22]. A 50 µL extract was mixed with DPPH reagent (60 µM, 1450 µL). The absorbance at 515 nm was measured after the tubes were stored in a dark room for 1 hour. DPPH value was expressed as the inhibition percentage of DPPH radi cal, and, it was calculated with the following formula:

$$\text{DPPH value} = \%\ \text{DPPH inhibition} = (A_c\text{-}A_s)*100/(A_c) \quad [1]$$

Where A_c is the absorbance of the control, and A_s is the absorbance of the sample.

FRAP ANTIOXIDANT POWER

FRAP assay was performed with the method described by Benzie and Strain [23]. A 50 µL extract was mixed with FRAP reagent (1450 µL). The absorbance at 595 nm was measured after 20 minutes. FRAP value was expressed as µmole trolox g^{-1} RSP.

CHEMICAL CHARACTERISTIC

The chemical characteristic of the flaxseed oil (free fatty acids, peroxide value, saponification value and iodine value) were determined via AOCS official methods (AOCS Ca 5a-40, AOCS Ja 8-87, AOCS Da 16-48, AOCS Da 15 - 48).

FATTY ACID COMPOSITION

The determination of the fatty composition was described by Ilyasoglu [20]. Shimadzu GC-2010 Plus gas chromatography equipped with a flame ionisation detector, a split/splitless injector and a long capillary column (0.25 mm × 0.20 µm × 60 m, Teknokroma TR-CN100, Spain) was used for the determination of the fatty acids.

OXIDATION STUDY

The Schaal oven test was used to evaluate the antioxidant efficacy of the RSP against lipid oxidation. Acetone extract was used since it exhibited the highest values of antioxidant capacity. Acetone extract of the RSP was added to the flaxseed oil (1 g). The test tubes were stirred in an ultrasonic bath to solve antioxidant compounds in the oil. The test tubes were stored at 60°C in an oven for 12 days. Duplicate samples were taken, and analysed. The peroxide, *p*-anisidine, and TBARS values of the flaxseed oil were analysed with respect to AOCS official methods (AOCS Ja 8-87, AOCS Cd 18-90, AOCS Cd 19-90). RSP (500 mg kg^{-1} and 1000 mg kg^{-1}), and BHT (200 mg kg^{-1}) were used for the oxidation study.
The total oxidation (TOTOX) value was calculated in accordance with the following formula:

$$\text{TOTOX value} = 2\,(\text{PV}) + (\text{p-AV}) \quad [2]$$

STATISTICAL ANALYSIS

The one-way ANOVA test and the LSD test were used to determine significant differences among the samples. Triplicate analyses were done, and mean values and standard deviations were calculated. The SPSS 17.0 software (IBM, New York, USA) was used for data analysis.

RESULTS AND DISCUSSION

EXTRACTS

Methanol, acetone, ethanol and water were used to extract antioxidant compounds from the rosehip seed powder (RSP). The total extractable compounds (TEC), total phenolic content (TPC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) of the extracts from the RSP were investigated to choose the best extraction solvent. It can be seen from Table I, acetone (100%) extract gave the highest extraction yield followed by methanol (100%) and ethanol extracts (100%). Acetone (100%) extract had the highest TPC and DPPH value followed by acetone (75%), and methanol (100%) extracts, and they showed no significant difference ($p > 0.05$). Acetone (100%) extract had the highest FRAP value followed by methanol (100%) and acetone (75%) extracts. Methanol (100%) and acetone (75%) extracts showed no significant difference ($p > 0.05$). The TEC, TPC, DPPH and FRAP values exhibited decreasing trend with increasing water ratio in the solvents. The water extract showed the lowest TPC, DPPH and FRAP values. Acetone (100%) extract was chosen based on the results of the analysis. It is important to select the best solvent to extract the maximum quantity of antioxidant compounds before their applications in foods. Methanol, acetone, and ethanol were the most used solvents to extract antioxidant compounds from the plant materials. Our results revealed that each solvent had a different ability to extract antioxidant compounds from the RSP. The TEC depended on the nature of solvent used, and pure solvents gave higher values than aqueous solutions. The extraction efficiency is related to the solubility of the antioxidant compounds in the extraction solvent. The solubility of antioxidant compounds is known to depend on the solvent polarity. Each solvent has a distinct polarity level. The differences in the TEC among the extracts may be attributed to the solubility levels of the antioxidant compounds in different solvents. A higher TEC gave higher TPC and antioxidant activity, and this may be related to a higher content of antioxidant compounds in an extract. Acetone exhibited higher values compared to the methanol and ethanol. Similar results were reported for guava extract [24].

FLAXSEED OIL

The chemical characteristic and fatty acid composition of the studied flaxseed oil are presented in Table II. The main fatty acid found in the flaxseed oil was linolenic acid (54 g 100 g^{-1}) followed by oleic (23 g 100 g^{-1}) and linoleic (14 g 100 g^{-1}) acids.

OXIDATIVE STABILITY

The acetone extract obtained from the rosehip seed powder was added to the flaxseed oil. Two concentrations were studied: 500 mg kg^{-1} and 1000 mg kg^{-1}. BHT, synthetic antioxidant, was studied as well. The BHT concentration was 200 mg kg^{-1} (legal limit). The control sample and samples with BHT and rosehip powder extract (RSP) were stored for 12 days, and their PVs, TBARS values, and *p*-AVs were determined. The PVs of the samples are presented in Table III. The PVs of the samples showed an increasing trend during storage. The PV of the control sample generally exhibited higher values than the samples with RSP. The PV of the sample with RSP (500 mg kg^{-1}) had a significant lower value than the control sample from the 4^{th} day to the 8^{th} day ($p < 0.05$), while the PV of the sample with RSP (1000 mg kg^{-1}) had a significant lower value than the control sample from 4^{th} day to 12^{th} day ($p < 0.05$). The PV of the sample with BHT

Table I - The values of TEC, TPC, DPPH radical scavenging activity and FRAP of obtained extracts

Extract	TEC (g 100 g^{-1})	TPC (mg kg^{-1})*	DPPH (%)**	FRAP (μmol g^{-1})***
Water	1.15±0.06^{d}	289±50^{d}	4.6±0.1^{d}	9.8±1.1^{f}
Methanol				
50%	1.03±0.09^{d}	983±85^{c}	9.4±0.7^{cd}	18.5±1.5^{ef}
75%	0.85±0.15^{d}	1073±155^{bc}	22.5±2.6^{bc}	25.6±1.1^{e}
100%	2.79±0.10^{ba}	2142±267^{a}	40.2±2.7^{a}	62.8±3.9^{b}
Acetone				
50%	1.07±0.07^{d}	1400±65^{b}	26.4±2.3^{b}	37.9±3.8^{d}
75%	1.73±0.02^{c}	2267±206^{a}	44.0±1.1^{a}	58.6±4.4^{b}
100%	3.09±0.43^{a}	2308±165^{a}	45.3±4.5^{a}	70.3±0.9^{a}
Ethanol				
50%	1.02±0.12^{d}	1105±71^{b}	15.1±3.0^{c}	19.8±0.7^{ef}
75%	1.03±0.06^{d}	1267±140^{b}	16.5±3.7^{c}	27.0±4.8^{e}
100%	2.64±0.05^{b}	1752±282^{ba}	31.5±4.5^{b}	50.3±4.9^{c}

*gallic acid equivalent; **50 mg/mL of extract; ***trolox equivalent.
Different letters within each column present significant difference ($p<0.05$)

generally showed higher values than the sample with RSP.

PV shows primary oxidation products. The flaxseed oil with the RSP exhibited a lower PV compared to the control sample, indicating that the RSP was effective in inhibiting primary oxidation products. In the literature, similar results were reported on the addition of corncob, tea, garlic, mulberry leaves, pistachio hull, pomegranate peel and ginger extracts in edible oils [4, 7, 9, 10, 15, 18].

The TBARS values of the samples are presented in Table IV. The control sample and samples with BHT and RSP generally showed similar values. There were no significant difference among the samples ($p > 0.05$), except 1st day. The TBARS values of the sample with BHT and RSP (500 mg kg^{-1}) had a significant lower value than the control sample at 1st day ($p < 0.05$).

TBARS value is an indicator of secondary oxidation products. Fluctuations in the TBARS values were observed during the storage. These findings may be attributed to the decomposition of malonaldehydes to organic alcohols and acids, which are not measured by the TBARS test. Moreover, the losses in the secondary oxidation products, particularly volatile ones may be resulted in a reduction in the TBARS values [25].

Table II - Properties of flaxseed oil

Properties	
Chemical properties	
Free fatty acids (g 100 g^{-1})	1.9±0.1
Peroxide value (mEq kg^{-1})	4.6±0.2
Iodine value (g 100g^{-1})	177±1
Saponification value (mg g^{-1})	188±1
Fatty acid (g 100g^{-1})	
Palmitic acid	6.0±0.3
Stearic acid	3.3±0.1
Oleic acid	22.7±0.4
Linoleic acid	14.0±0.1
Linolenic acid	54.0±0.7

The *p*-AVs of the samples are presented in Table V. Firstly, the *p*-AVs of the samples increased, and then decreased. The *p*-AVs of the samples again increased. The *p*-AV of the control sample generally exhibited higher values than the samples with BHT and RSP. The *p*-AV of the sample with RSP (500 mg kg^{-1}) had a significant lower value than the control sample at 3rd day ($p < 0.05$), while the *p*-AV of the sample with RSP (1000 mg kg^{-1}) had a significant lower value than the control sample at 3rd and 8th day ($p < 0.05$). The *p*-AV of the sample with BHT generally showed higher values than the sample with RSP.

p-AV is used to measure secondary oxidation products. The flaxseed oil with the RSP generally had a lower p-AV compared to the control sample. This finding indicated that the RSP extract may inhibit secondary oxidation products. Similar reports were found in the literature. The addition of corncob, leaf from *Monecha ciliatum*, sorghum, and primrose meal extracts in edible oils lowered the p-AV [4, 5, 26, 27]. A decrease in the p-AV was observed, and this finding may be related to the losses of secondary oxidation products by volatilization or chemical reactions [28].

The TOTOX values of the samples are shown in Table VI. The TOTOX values of the samples exhibited increasing trend. The TOTOX value of the sample with RSP (1000 mg kg^{-1}) was significantly lower than the control sample from 3rd day to 12th day ($p < 0.05$). The TOTOX value of the sample with RSP (500 mg kg^{-1}) was significantly lower than the control sample from 3rdday to 8th day as well ($p < 0.05$). The TOTOX value of the control sample and sample with BHT generally showed no significant difference ($p > 0.05$). TOTOX value is used to evaluate the total oxidative stability of oils. The flaxseed oil with the RSP exhibited more total oxidative stability than the control sample and sample with BHT according to the results of TOTOX values.

The flaxseed oil including the RSP generally had lower PV, *p*-AV, and TOTOX value than the control sample, indicating that the RSP may retard oxidation in the flaxseed oil. The RSP (1000 mg kg^{-1}) generally gave better results than the RSP (500 mg kg^{-1}). It can be interpreted that the antioxidant efficacy of the RSP depended on the concentration and was enhanced with increasing concentration. Similar findings were reported for corncob, *Monecha ciliatum*, and pistachio hull extracts [6, 10].

The RSP extract was found to be more efficient in lowering the PV and p-AV than the BHT. In literature, similar findings were available on the use of some plant extracts in edible oils. Mulberry leaves, corncob, and peanut skin extracts were shown to have more antioxidant efficacy in inhibiting primary oxidation products than the BHT. BHT was also found to be less effective in retarding the formation of secondary oxidation product than the corncob, and peanut skin extracts [4, 9, 29].

The flaxseed oil with RSP generally had lower PV, *p*-AV, and TOTOX values than the flaxseed oil with BHT, revealing that the RSP may be more effective than synthetic antioxidant. The ability of plant extracts to retard lipid oxidation has been determined, and their usages have been suggested [4, 18]. In addition to those extracts, the RSP may be proposed as a source of natural antioxidants.

In conclusion, our findings revealed that the usage of the RSP in the flaxseed oil may prevent lipid oxidation. The antioxidant efficacy of the RSP showed an increase with an increase in the concentration. Moreover, the RSP may exhibit more antioxidant efficacy than synthetic antioxidants. Therefore, it may have possible usage in edible oils as a natural antioxidant.

Table III - PVs of the samples

Days	Samples			
	Control	BHT	RSP (1)	RSP (2)
0	4.56±0.17^{a}	4.56±0.17^{a}	4.56±0.17^{a}	4.56±0.17^{a}
1	5.27±0.54^{a}	5.15±0.40^{a}	5.07±0.30^{a}	5.25±0.24^{a}
2	7.58±0.18^{a}	7.11±0.33^{a}	7.21±0.21^{a}	7.87±0.18^{a}
3	10.39±0.37^{a}	10.55±0.78^{a}	9.77±0.07^{a}	8.74±0.55^{a}
4	13.58±0.23^{a}	13.44±0.09^{a}	11.84±0.24^{b}	10.46±0.01^{c}
6	18.71±0.81^{a}	19.08±0.34^{a}	12.99±0.21^{b}	8.74±0.55^{b}
8	20.41±0.01^{a}	19.41±0.46^{a}	17.64±0.74^{ba}	13.76±1.02^{b}
12	38.19±2.89^{a}	36.36±1.14^{a}	36.23±0.04^{a}	19.46±1.03^{b}

1: 500 mg kg^{-1}; 2: 1000 mg kg^{-1}.
Different letters within each row present significant difference ($p<0.05$).

Table IV - TBARS values of the samples

Days	Samples			
	Control	BHT	RSP (1)	RSP (2)
0	0.48±0.06^{a}	0.48±0.06^{a}	0.48±0.06^{a}	0.48±0.06^{a}
1	0.62±0.01^{a}	0.45±0.06^{b}	0.46±0.03^{b}	0.56±0.08^{ba}
2	0.60±0.05^{a}	0.51±0.01^{a}	0.50±0.06^{a}	0.55±0.06^{a}
3	0.51±0.00^{a}	0.53±0.04^{a}	0.51±0.05^{a}	0.56±0.02^{a}
4	0.49±0.03^{a}	0.46±0.05^{a}	0.45±0.06^{a}	0.52±0.02^{a}
6	0.46±0.01^{a}	0.51±0.02^{a}	0.48±0.02^{a}	0.51±0.12^{a}
8	0.50±0.04^{a}	0.46±0.01^{a}	0.51±0.03^{a}	0.56±0.01^{a}
12	0.57±0.01^{a}	0.58±0.03^{a}	0.54±0.01^{a}	0.52±0.07^{a}

1: 500 mg kg^{-1}; 2: 1000 mg kg^{-1}.
Different letters within each row present significant difference ($p<0.05$)TBARS value expressed as mg malonaldehyde eq/g oil

Table V - p-Anisidine values of the samples

Days	Samples			
	Control	BHT	RSP (1)	RSP (2)
0	4.16±0.45^{a}	4.16±0.45^{a}	4.16±0.45^{a}	4.16±0.45^{a}
1	4.82±0.42^{b}	7.06±0.36^{a}	6.80±0.54^{a}	3.30±0.15^{b}
2	6.25±1.03^{a}	3.87±0.62^{a}	5.40±0.98^{a}	3.76±0.44^{a}
3	5.27±0.53^{a}	5.01±0.02^{a}	3.91±0.02^{ba}	2.33±0.39^{b}
4	2.95±0.21^{b}	5.77±1.02^{a}	2.14±0.38^{b}	1.83±0.38^{b}
6	7.99±0.26^{ba}	5.41±1.07^{b}	9.35±0.17^{a}	6.14±0.70^{b}
8	16.17±1.94^{a}	15.23±1.89^{a}	11.58±1.34^{ba}	8.74±1.02^{b}
12	23.83±1.66^{a}	28.87±2.14^{a}	30.77±3.96^{a}	28.70±4.18^{a}

1: 500 mg kg^{-1}; 2: 1000 mg kg^{-1}
Different letters within each row present significant difference ($p<0.05$)

Table VI - TOTOX values of the samples

Days	Samples			
	Control	BHT	RSP (1)	RSP (2)
0	13.28±0.79^{a}	13.28±0.79^{a}	13.28±0.79^{a}	13.28±0.79^{a}
1	15.13±1.38^{ba}	17.06±0.36^{a}	16.87±0.20^{a}	13.79±0.21^{b}
2	21.32±0.79^{a}	18.06±1.08^{a}	19.80±0.68^{a}	19.46±0.70^{a}
3	25.96±1.03^{a}	26.09±1.08^{a}	23.39±1.69^{ba}	19.77±1.16^{b}
4	30.09±0.12^{b}	32.55±0.90^{a}	25.79±0.05^{c}	22.74±0.37^{d}
6	45.35±1.38^{a}	43.52±1.55^{a}	35.27±1.82^{b}	31.57±1.97^{b}
8	57.09±2.03^{a}	54.07±5.95^{a}	47.02±0.28^{ba}	36.35±2.46^{b}
12	100.25±5.71^{a}	101.59±3.77^{a}	103.33±4.09^{a}	67.77±5.63^{b}

1: 500 mg kg^{-1}; 2: 1000 mg kg^{-1}
Different letters within each row present significant difference ($p<0.05$).

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