Stabilization of soybean oil during accelerated storage by essential oil of *ferulago angulata* boiss

Ehsan Sadeghi¹ · Aidin Mahtabani² · Alireza Etminan³ · Farahnaz Karami¹

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Abstract This study has been considered effect of *Ferulago angulata* essential oil on stabilizing soybean oil during accelerated storage. The essential oil was extracted by Clevenger-type apparatus. For analysis of the essential oil, GC/MS was used. Main components of the essential oil were monoterpene and sesquiterpene hydrocarbons. The essential oil of *F. angulata* at four concentrations, i.e. 125 (SBO-125), 250 (SBO-250), 500 (SBO-500) and SBO-Mixture (60 ppm TBHQ +60 ppm essential oil) were added to preheated refined soybean oil. TBHQ was used at 120 ppm as standard besides the control. Antioxidant activity index (AAI), free fatty acid (FFA) content, peroxide value (PV) and p-anisidine value (p-AnV) were served for appreciation of efficacy of *F. angulata* in stabilization of soybean oil. Results from different tests showed that SBO-mixture had highest effect and followed by SBO-TBHQ, SBO-250, SBO-125, SBO-500 and Ctrl. These results reveal *F. angulata* is a strong antioxidant and can be used instead of synthetic antioxidant.

Keywords Soybean oil · *Ferulago angulata* · Antioxidant activity · Essential oil

Introduction

Edible oils are composed of unsaturated fatty acids and provide essential fatty acids for our body (Sarkar et al. 2015). Lipid oxidation poses off flavor in edible oils and decreases their nutritional value (Aardt et al. 2004). Soybean oil has higher content of poly unsaturated fatty acids and more prone to oxidation. For stabilizing of oils and fats, synthetic antioxidants were served as food additive, but recently researches have revealed that these compounds may be harmful for human (Kurahde and Waghmare 2014). Due to these problems, there is a tendency to replace synthetic antioxidants with natural compounds.

Ferulago is in the family of *Apiacea* (umbelliferae), sub-family of *Apiodeae* and subtribe of *Ferulinae* (Mozafarian 1983). The plant *Ferulago* consists of 35–40 species that 8 species grow in Iran and 3 of them are endemic (Khalighi Sigaroodi et al. 2006). *F. angulata* (Schlecht) Boiss (in Iran it is named Chavir) found in north of Iran and Iraq and east of Turkey. It has two subspecies: subsp. *Angulata* (Schelecht) and subsp. *Carduchorum* (Boiss and Hausskn) (Rechinger 1987). *F. angulata* has the height of 50–160 cm, flowers with yellow color and thin leaves (Mozafarian 1983; Zargari 1981). *Ferula*, *Prangos* and *Ferulago* species are used in folk medicine for their tonic, digestive, sedative, aphrodisiac properties and from ancient times have used in treatment of intestinal worms and hemorrhoids in Turkey (Khalighi Sigaroodi et al. 2005). For many years, it has been used as an additive to animal oil for its stability specifically in Kermanshah province and beside preservative effects, poses pleasant taste and odor in oil. Antioxidant effect of *F. angulata* in vitro has been proved by radical of DPPH. Azarbani et al. showed that methanol and aqueous extracts of *Ferulago. angulata* have scavenging activity on DPPH (Azarbani et al. 2014). It has antimicrobial and antibacterial properties. In Taran et al. study,

✉ Farahnaz Karami
farahnazk83@yahoo.com

¹ Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran

² Department of Food Science and Technology, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran

³ Department of Plant Breeding, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran

among different bacteria, *Staphylococcus aureus* and *Listeria monocytogenes* had high sensitivity to essential oil of *F. angulata* (Taran et al. 2010). In this study, *F. angulata* was added to refined, bleached, and deodorized soybean oil. This oil has essential fatty acids and it is biggest source of vegetable oil in the world (Medina-Juarez et al. 1998). Therefore, efficacy of this essential oil on stability of soybean oil studied.

Materials and methods

Materials

Soybean oil (SBO) as refined was supplied from Agro-industry Complex & Vegetable Oil of Mahidasht, Kermanshah, Iran. Gathering of *F. angulata* plant was carried out from Dalahoo Mountains at summer 2013, Kermanshah province in west of Iran. For analysis, the reagents and chemical materials were of analytical grade and were purchased from Germany Merck. TBHQ was prepared from Sigma Chemical Co (St. Louis, MO, USA).

Extraction

Aerial parts of *F. angulata* dried in ambient temperature and 100 g of them powdered and poured in a flask containing 1200 ml distilled water and distilled with steam by Clevenger-type distillation assembly apparatus for 3 h according to British method. Anhydrous Na_2SO_4 was used for drying of essential oil and it was stored in 4 °C (Ghasempour et al. 2007).

Gas chromatography–mass spectrometry (GC–MS) analysis

The analysis of the essential oil was performed by a gas chromatography model of GC3600 (FID) equipped with GC/MS (HP-5973AGILENT) include a column (thickness of 0.25 μm , 0.25 mm id and length of 30). The oven temperature was set at 50 °C for 1 min and then increased to 200 °C in range of 3.5 °C/min. Analysis from sample injection to achieving final graph longed 14 min. The detector temperature was 250 °C and diluted sample in 1 lit of n-pentane was used in volume of 1 μL and injected in mode of split less. 70 eV used as ionization energy, and helium applied as carrier gas with speed of 1 mL/min. When all of the solvent compounds are excluded, the first component of the essential oil appeared.

For identification of essential oil components, their retention indices with of those normal alkanes that under same condition injected to set and their mass spectra with internal reference in library of mass spectra (Wiley 7.0) and with cases of brought in the sources were compared (Shibamoto 1987).

Sample preparation

Essential oil of *F. angulata* was added to preheated refined soybean oil (at 50 °C for 3 h) at concentrations of 125, 250, and 500 ppm. Synthetic antioxidant tertiary butyl hydroquinone (TBHQ) was used at concentration of 120 ppm. Mixtures of 60 ppm essential oil and 60 ppm TBHQ were used in order to compare synergistic or antagonistic effect on each other. The samples (500 ml) were poured in dark brown bottles and stored in an oven at temperature of 65 °C. Control samples were stored under the same conditions. Researchers reported that one day of storage, in temperature of 65 °C, is even to one month of storage at ambient temperature (Evans et al. 1973). Inspections were carried out in intervals of 6 days for 24 days.

Measurement of peroxide value (PV), free fatty acid (FFA) content and p-anisidine value (p-AnV)

PV, FFA and p-AnV were measured according to the AOCS official methods. For PV determination, AOCS cd 8–53 method (acetic acid-chloroform) was used. In this method, after solving of sample in solution, potassium iodide solution as saturated is added to oil samples to react with hydroperoxides. The liberated iodine (I_2) is titrated with solution of sodium thiosulfate (0.1 N) and starch indicator. The FFA content was measured by AOCS cd3a-63 method. In this method, neutralized solvent mixture by potassium hydroxide (0.1 N) consisting of isopropyl alcohol and toluene is added to oil sample and is titrated with standard alkali and phenolphthalein indicator. To express in terms of free fatty acids as percent oleic, the acid value is divided by 1.99. Determination of P-Anv was carried by AOCS cd18-90 method. 0.5–4 g of sample is dissolved and diluted to volume with isooctane in to a 25 ml volumetric flask and its absorbance is measured at 350 nm, using the reference cuvette filled with solvent as blank. 5 ml of the fat solution and solvent is poured in individual test tubes and 1 ml of the p-anisidine reagent (0.25 g/100 ml glacial acetic acid) added to each tube. After 10 min, the solution absorbance is measured at 350 nm using the solvent as blank. P-Anv is calculated by following equation:

$$\text{p-Anv} = \frac{25 \times (1.2\text{As} - \text{Ab})}{m}$$

As = Absorbance of the fat solution after reaction with the p-anisidine reagent.

Ab = Absorbance of the fat solution and m = mass of the test portion. g (AOCS 1990).

Antioxidant activity index

A Metrohm Rancimat model 679 was used to determine the stability index of control and treated oil samples before

storage. The experiment was performed at temperature of 110 °C according to ISO-6886 (ISO-6886 2006). When volatile acids are formed during oxidation of oil in high temperature and swept out from oil through water, electrical conductivity is changed and this factor is measured by oil stability index (OSI) method (Shahidi and Zhong 2005).

Statistical analysis

The experiment was performed in a completely randomized design with three replications. Analysis was done by one-way ANOVA and significant differences ($p < 0.05$) were calculated using Duncan's multiple range test.

Results and discussion

Chemical composition of the essential oil

The yield of essential oil of aerial parts in *F. angulata* was 0.5 % w/w. The components of the essential oil were identified by GC–MS. Sixty constituents containing 96.84 % of total essential oil were identified (Table 1). The constituents of the essential oil, majority were Monoterpene and Sesquiterpene hydrocarbons. The main components of aerial parts are shown in Table 1 and they include Cis-Ocimene (30.17 %), α -Pinene (15.4 %), Trans- β -Ocimene (5.7 %), γ -Terpinene (5.57 %), Germacrene-D (5.03 %), Limonene (4.88 %), Bomyle Acetate (4.57 %), Myrcene (3.62 %), Camphene (2.41 %), Noe-Allo-Ocimene (1.87 %), β -Phellandrene (1.84 %), α -Terpinolene (1.7 %), Bicyclogermacrene (1.29 %) and δ -Cadinene (1.18 %).

Antioxidant activity index

For measurement of Antioxidant activity index (AAI), IP (induction period) of stabilized sample is divided to control IP (Emmons and Peterson 1999). This index was 2.352, 1.826, 1.167, 1.125 and 0.943 h for TBHQ, SBO-Mixture, SBO-125, SBO-250 and SBO-500, respectively. According to these results, induction period is not increased with concentrations. Each antioxidant may be having highest effect up to certain concentration and after that it is effectless (Dziedzic and Hudson 1994). However, these observations are different from the PV and AnV exams. Therefore, we can say that although the OSI (Oil Stability Index) test is beneficial for evaluating quality of fats and oils, but at higher temperatures, it is not appropriate for counting antioxidant activity because natural antioxidants are volatile

Table 1 Chemical composition of the *F. angulata* essential oil

NO.	Compounds	RI ^a	%RA ^b
1	Cis-Ocimene	1031	30.17
2	α -Pinene	937	15.4
3	Trans- β - Ocimene	1040	5.7
4	γ -Terpinene	1053	5.57
5	Germacrene-D	1487	5.03
6	Limonene	1120	4.88
7	Bornyl Acetate	1275	4.57
8	Myrcene	983	3.62
9	Camphene	950	2.41
10	Noe-Allo-Ocimene	1120	1.87
11	β -Phellandrene	977	1.84
12	α -Terpinolene	1063	1.7
13	Bicyclogermacrene	1501	1.29
14	δ -Cadinene	1523	1.18
15	δ -3-Carene	1011	0.71
16	Germacrene-B	1123	0.63
17	p-Cymene	1018	0.56
18	Spathulenol	1576	0.54
19	α -Terpinene	1013	0.53
20	α -Phellandrene	1003	0.52
21	α -Copaene	1383	0.33
22	Geranyl Isovalerate	1650	0.3
23	Methyl Eugenol	1374	0.3
24	Trans-Caryophyllene	1427	0.28
25	β -Bourbonene	1393	0.28
26	Linallol	1085	0.26
27	β -Cubenone	1384	0.23
28	α -Amorphene	1269	0.22
29	Terpinene-4-ol	1168	0.17
30	β -Elemene	1342	0.16
31	α -Cadinol	1562	0.15
32	β -Bisabolene	1040	0.14
33	Borneol	1151	0.14
34	Epi- α -Cadinol	1257	0.13
35	Cis-Jasmone	1114	0.12
36	γ -Elemene	952	0.11
37	Cuparene	998	0.1
38	α -Cadinene	987	0.1
39	Ledene	926	0.09
40	α -Terpineol	1178	0.09
41	Tricyclene	1582	0.09
42	Verbenene	1131	0.09
43	Palmitic acid	1531	0.08
44	Trans-Sabinene hydrate	970	0.07
45	α -Muurolene	1480	0.07
46	Pinocarvey Acetate	1684	0.06
47	Iso-Spathulenol	1298	0.06
48	α -Humulene	1459	0.06
49	Butanoic Acid, 3- methyl	1449	0.05

Table 1 (continued)

NO.	Compounds	RI ^a	%RA ^b
50	γ -Curcumene	1478	0.05
51	Trans- β -Farnesene	1112	0.05
52	α -Cedrene	1424	0.04
53	Elemol	1500	0.03
54	α -Campholeneal	1436	0.03
55	p-Cymene-8-ol	1016	0.03
56	Geraniol	1237	0.03
57	Carvacrol	1083	0.03
58	α -Cubebene	1008	0.03
59	Myrtenal	1285	0.02
60	Methyl cyclohexane	1125	0.01
Total			96.84

^a Retention indices relative to normal-alkanes on HP-5973 capillary column

^b Relative area (peak area relative to the total peak area)

components and maybe exclude from oil by air flow during experiment (Gordon 2001).

Free fatty acids content (FFA)

Free fatty acids produced in oil as a result of triglyceride hydrolysis (Frega et al. 1999). FFA content increased with storage time for all the samples. In all stages, control sample exhibited the highest FFA, But SBO-TBHQ, SBO-125, SBO-250, SBO-500 and SBO-Mixture showed relatively equal FFA content until 6th day, but at 12th, 18th and 24th days, FFA content at SBO-Mixture almost became equal to SBO-TBHQ (Fig. 1). SBO-125, SB-250 and SBO-500 had equal FFA to each other and slightly higher than SBO-TBHQ and SBO-Mixture. These results reveal that SBO-Mixture (*F. angulata* at 60 ppm with 60 ppm TBHQ) is as effective as TBHQ and more effective than concentrations of essential oil over longer storage period and perhaps *F. angulata* has synergistic effect to TBHQ and tocopherol in soybean oil. But totally, oil acidity is not change very much during oxidation.

Peroxide value (PV)

Peroxides are the first products of oxidation and decomposed in final stages of oxidation (Dobarganes and Velasco 2002; Melton 1983). Peroxide value was 4.7–7 meq/kg for stabilized samples after 24 days of storage, while for control samples maximum PV was 10.26 meq/kg (Fig. 2). At all stages, control sample showed highest PV and followed by SBO-500, SBO-125, SBO-250, SBO-TBHQ and SBO-Mixture, respectively. PV of SBO- 500 was higher than SBO-250 and SBO-125, maybe due to same reason that

Fig. 1 Free fatty acid content (FFA) of soybean oil in control and experimental group during the period of study

mentioned in section of antioxidant activity index. At all concentrations, essential oil controlled peroxide value and has good effect in stabilization of oil. For all samples, a regular increase in PV was observed as a function of storage time until 12th day, followed by a decrease on the 18th day for all stabilized samples. This perhaps is due to decomposition of peroxides in final stage of storage (Riuz et al. 2001). After the 6th day, there was a tremendous rise in PV of control sample, which rose up to 24th day of analysis. After 18th day, PV of all stabilized samples gradually increased. PV of SBO-Mixture was comparable to that of TBHQ at all storage periods initially; but increased after the 18th

Fig. 2 Peroxide value (PV) of control and experimental group during the period of study

Fig. 3 p-AnV in control and experimental group during the period of study

day. For SBO-Mixture, SBO-250, SBO-125 and SBO-500, maximum PV was 5.93, 6.06, 6.38 and 7 meq/kg, which is lower than rapeseed oil stabilized by a number of natural antioxidants, sunflower oil stabilized with garlic extract, sunflower oils stabilized with kenaf seeds extract, roselle seeds extract and roselle extract (Bandoniene et al. 2000; Iqbal and Bahnger 2007; Nyam et al. 2013). According to these data, essential oil is a strong antioxidant for stability of soybean oil.

P-anisidine value (p-AnV)

The p-anisidine value (p-AnV) method measures secondary products of oxidation (Doleschall et al. 2002). For all samples, p-AnV was determined for 24 days (Fig. 3). The observed results for p-AnV relatively were according to same order for PV. For stabilized samples, p-AnV was 9.13–10.88 and for control sample, it was 12.64 after 24 days of storage. After the 12th day, there was a tremendous rise in p-AnV of control sample, which rose up to 24th day of analysis. Stabilized samples to 18th day had not noticeable differences, but at 24th day their p-AnV became significant. SBO-Mixture until 18th day was better than SBO-TBHQ. *F. angulata* decreased p-AnV at all concentrations.

Conclusion

Our observations showed that essential oil of *F. angulata* is a potent antioxidant for stabilizing soybean oil and can be used instead of synthetic antioxidant.

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